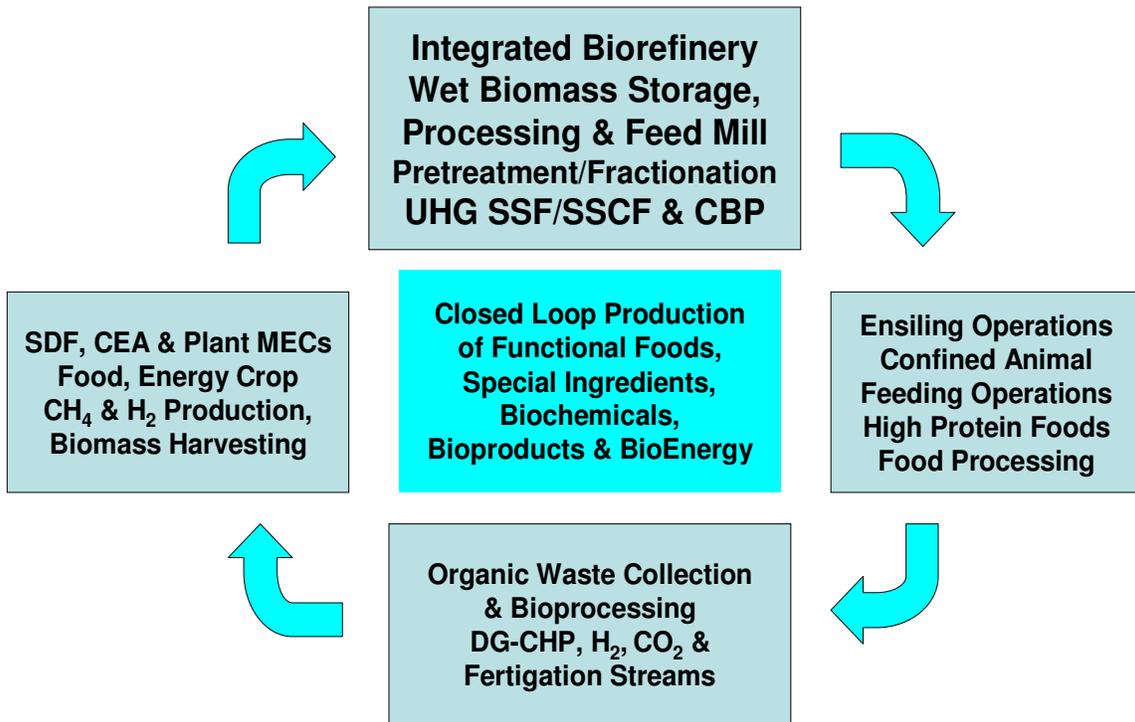


**Ag-Energy Parks & Municipal BioEnergy Complexes**  
 DOE Advanced Biofuels, Integrated Biorefinery & Joint USDA Biomass RFPs



*Figure 1. Comprehensive Nutrient-Energy Management:  
 Sustainable Closed Loop BioSystems Architecture*

**Executive Summary**

Surpassing peak petroleum production and dwindling petroleum reserves, the largest transfer of wealth in global history (\$7 trillion annually for the US and rapidly growing for industrial countries such as China, India and Brazil), impending food and energy crises, global warming, pollution, and national security reveal the necessity for economical production of renewable energy. Due to current dependence on importing fossil fuels by industrial nations, lack of access to these resources would literally shut down industrial economies.

In addition to the need for displacing fossil fuels, the majority of synthetic fertilizers used for production of food and energy crops, and the chemical industry which uses large volumes of petroleum products including production of hydrogen, sulfur, and industrial chemicals used to manufacture plastics, fertilizers, explosives, dyes, solvents, and adhesives in industrial countries, are primarily produced from petroleum products. Similar to the need for developing renewable energy resources, recycling waste and comprehensive nutrient-energy management is essential for production of organic fertilizers in order to transition to bioeconomies and invalidate the food vs. biofuel/bioenergy argument.

“Burning fossil fuels such as coal and oil releases CO<sub>2</sub> which is a major cause of global warming (Yat et al, 2008). With only 4.5% of the world’s population, the US is responsible for about 25% of global energy consumption and 25% of global CO<sub>2</sub> emissions (Yat et al, 2008). The average

price of gasoline in 2005 was \$2.56 per gallon, which was \$0.67 higher than the average price of gasoline in the previous year (Yat et al, 2008). Yet, in June 2008, the average price of gasoline in the US reached \$4.10 per gallon (Weekly Gasoline Prices, DOE). Conversion of abundant lignocellulosic biomass to biofuels as transportation fuels presents a viable option for improving energy security and reducing greenhouse gas emissions (Wyman, 1999). Unlike fossil fuels, which come from plants that grew millions of years ago, biofuels are produced from plants grown today. They are cleaner-burning than fossil fuels, and the short cycle of growing plants and burning fuel made from them does not add CO<sub>2</sub> to the atmosphere. It has been reported that cellulosic ethanol and ethanol produced from other biomass resources have the potential to cut greenhouse gas emissions by 86% (Wang et al, 2007). Lignocellulosic materials such as agricultural residues (e.g. wheat straw, sugarcane bagasse, corn stover, soy hulls), forest products (hardwood and softwood), and dedicated crops (switchgrass, salix) are renewable sources of energy. These raw materials are sufficiently abundant and generate very low net greenhouse emissions. Approximately 90% of the dry weight of most plant materials is stored in the form of cellulose, hemicellulose, lignin, and pectin (Yat et al, 2008; [Kumar et al., 2009](#)).

In addition to contributing to GHGEs, the US economy is currently dependent upon importing a large majority of fossil fuels from economically and politically unstable countries which are hostile towards US citizens and groups from which are engaged in global terrorism. Loss of these fossil fuel imports could literally shut down the US economy and weaken national security.

In a lecture entitled “Day of Reckoning: America’s Fiscal Future,” former US comptroller David Walker has stated: “For the first time in history, life for our children and grandchildren does not look better than it is for us. This is not acceptable, this is not American. The solution is involvement.” (King, 2009) A paradigm shift from the current fossil fuel processes to a system in which energy and food are produced in synergistic fashion, natural resources are conserved, and pollution and GHGEs are dramatically reduced, is necessary in order to insure the well being of future generations.

Innovative low-cost storage, pretreatment, and fractionation of crop residues and forest waste products, economical processing of organic waste, industry integration, and state-of-the-art technology allows for producing ethanol for as little \$0.15/gal from low moisture crop residues and woody biomass, and for less than \$0.50/gal from wet biomass resources. For integrated biorefineries, ag-energy parks, and municipal bioenergy complexes which incorporate closed loop biosystems architecture, biomass can be produced for less than \$30/tonne. High value coproducts such as functional foods, nutraceuticals, special ingredients, and industrial biochemicals (which are not solely dependent upon commodity markets) can be produced synergistically with bioenergy resources. In addition to liquid biofuels, bioelectricity can be generated for less than \$0.04/kWhr and biomethane or biohythane (for displacing natural gas) for less than \$3.00/MBTU.

### ***Processing High Moisture Biomass Feedstocks***

Sustainability of the bioenergy industry, as a major source of renewable energy, is dependent upon economically processing low-cost “high moisture” biomass feedstocks. Energy crops, crop residues and organic waste including food processing waste, animal waste, sewage, municipal waste, and waste paper provide an abundance of bioenergy feedstocks which do not compete with production of food. For integrated bioenergy complexes and ag-energy parks, production of

bioenergy is synergistic with production of functional foods, special ingredients, and industrial biochemicals.

### ***Integration of Confined Animal Feeding Operations***

For example, integration of confined animal feeding operations (CAFOs) such as dairies, feedlots, swine and poultry operations and biorefineries allows for feeding nonfermentable portions of biomass including protein-rich fermentation residues, wet distillers grains (WDG) directly to livestock and poultry. This avoids energy intensive drying and nutrient loss due to heating, volatile organic compound (VOC) emissions, transportation and marketing, all of which can reduce feed costs by up to 50% while reducing pollution and increasing net energy gains. The removal of carbohydrates from biomass produces quality protein feedstuffs which are three times higher in amino acids and other nutrients than whole corn or whole forages. The yeast in the distiller's grains and residues acts as a natural feed additive, allowing for replacing ionophores, antimicrobials and antibiotics for natural production programs. Exogenous enzymes inserted into feedstuffs for ruminants as well as monogastric species enhances digestibility of high fiber diets consisting of distillers grains, fermentation residues, forages (cellulose, hemicellulose, lignin, omega-3 fatty acids), providing a complete balance of macro and micro nutrients.

Balanced nutrient ratios contained in feed rations insure that livestock receive optimal nutrition. This results in maximizing production via healthy livestock with strong immunization systems and superior fertility without the use of steroids and artificial growth promotants. Balanced nutrition subsequently results in production of functional foods high in essential nutrients (including omega-3 fatty acids DHA, EPA; GLA; and micro nutrients) which are often lacking in conventional food products. Marker assisted selection indexes which focus on economically relevant traits (net feed efficiency [NFE], meat tenderness, marbling, yield, fertility) can be included in functional SNP panels providing parentage identification, accelerated genetic improvement, and instant traceability via integration with radio frequency identification (RFID) technology. RFID technology integrated with SNP panels provides DNA fingerprinting enabling instant traceability from consumer plate to producer. SNP-RFID technology also provides producer opportunities for participation in branded meat programs, providing premium pricing which encourages continued genetic development by producers who retain ownership via vertically coordinated programs.

### ***Processing Organic Waste***

Animal waste from CAFOs is a significant contributor to pollution via nutrient run-off according to EPA. However, animal waste contains valuable nutrients in optimal ratios via balanced rations. Animal waste and other sources of organic waste can be efficiently collected and mixed with biomass and processed into organic fertilizers and biomethane for closed loop biomass production. Ion exchange resin technology can be utilized to accurately sample and sense nutrient ratios in effluents from state-of-the-art high solids anaerobic digesters (HSAD). These effluents can be mixed with urea-sulfuric acid to provide nutrient-rich fertigation streams for year round production of biomass via precision agriculture including controlled environment agriculture (CEA). These organic fertilizers provide value added sources of micronutrients which can be used to produce functional foods in addition to bioenergy.

### ***Balanced Nutrients & Functional Foods***

“Twenty–eight essential vitamins and minerals play key roles in the metabolism of proteins, carbohydrates, and fats, as well as in the structure of the human body (e.g. vitamin K in bone matrix, calcium in bone tissue). This is also true for similar metabolic processes utilized by plants and animals including food crops, pets, and livestock. Many micronutrients are also important antioxidants (e.g. vitamins C and E) or act as cofactors for antioxidant enzymes (e.g. selenium in glutathione peroxidase). Several trace minerals, though not considered essential, are being studied for their roles in nutrition. Examples include silicon for bone health and vanadium for stimulation of glucose transport. Although vitamin deficiency diseases (e.g. pellagra) are not widespread, suboptimal micronutrient intake is common.

Recent studies reveal that 10% to 75% of Americans take in less than the recommended dietary allowance for many micronutrients (e.g. zinc, folate, iron, vitamin B<sub>6</sub> and B<sub>12</sub>), and between 5% and 50% of Americans consume less than half the required daily allowance for many micronutrients. While what constitutes sufficient intake is controversial for some nutrients, it is clear that a surprisingly large number of people and domestic livestock are undernourished for certain micronutrients, even as they are over-nourished with respect to macronutrients. Insufficient micronutrient intake has short–term and long–term implications for disease risk. As an example, immune function is adversely affected by poor intakes of nearly every essential vitamin and mineral. Thus, diets lacking essential micronutrients may theoretically, at least, affect health over the short term by impairing resistance to viral or bacterial infection. Among longer term problems, a lack of nutrients required for DNA methylation and gene stability may increase the risk for certain cancers ([Nutrition MD](#)).”

### ***Optimizing Nutrient-Use Efficiency & Valued Added Organic Fertilizers***

Essentially, micronutrients increase the efficiency of metabolic processes that enhance the nutrient use efficiency (NUE) of macronutrients by plants ([Yara](#)) and animals, including consumers. The abundance of balanced nutrients including micronutrients, produced from animal waste and other organic waste resources provide for year round production of high value crops via CEA. CEA includes state-of-the-art hydroponic greenhouses and new generation algal bioreactors that are predicted to annually produce over 50 times the volume of biofuels produced by terrestrial crops. Similar to anaerobic digestion (AD) & biogas refining, infrastructures for SDF, hydroponic greenhouses, and new generation algal bioreactors may be financed by financial institutions utilizing tax-exempt bonds ([U.S. Treasury-IRS, 2004](#)) for solid waste disposal, and trading carbon credits for reduction of NH<sub>3</sub> (via lobbying efforts) and GHGE via the [CCX \(Chicago Climate Exchange\)](#).

For example, cattle normally retain less than 20% of their dietary nitrogen intake revealing that as much as 80% of dietary nitrogen is excreted in animal waste (Bierman et al., 1996). Depending on their diet, cattle excrete approximately 60 to 80% of their N in urine and 20 to 40% in feces (Van Horn et al., 1996; Bierman et al., 1999). Fecal nitrogen is 50% organic nitrogen and 50% NH<sub>3</sub>; however, urine contains up to 97% urea nitrogen, which is readily converted by microbial urease to NH<sub>3</sub> shortly after excretion (Muck and Richards, 1980; Mobley and Hausinger, 1989; Mobley et al., 1995, [Varel et al., 1999](#); [Varel, 2002](#)). Though diet formulation for livestock can reduce a considerable amount of the nitrogen contained in animal waste, a comprehensive nutrient-energy management approach is necessary in order to optimize NUE for livestock and crop production while simultaneously reducing NH<sub>3</sub>, particulate matter and GHGE including CH<sub>4</sub> and N<sub>2</sub>O from agricultural production.

In regards to nitrogen conservation, confined cattle operations typically lose 75% of the nitrogen excreted by animals under current management systems (Vanderholm, 1985; Eghball and Power, 1994; Bierman et al., 1999; [Varel, 2002](#)). “This volatilization of NH<sub>3</sub> and related compounds contributes to offensive odor emissions. The nitrogen from feedlot manure in the US is valued at approximately \$111 million if it is available as a fertilizer (Eghball and Power, 1994). Besides this nitrogen loss, animal waste can create other problems that include nutrient enrichment of soil and water, GHGE, odorants, and transmission of pathogenic microorganisms (Mackie et al., 1998; McCrory and Hobb, 2001; [Varel, 2002](#)).” In 2008-9, various forms of fertilizer nitrogen have been selling for over \$1,000/ton. Hence, allowing for inflation and the growing demand for nitrogen and biomethane/natural gas, the current value of nitrogen contained in animal excreta (from feedlot, dairy, swine, poultry, aqua-farming, sheep, equine, etc.) probably exceeds well over \$2 Billion annually in the US alone. Due to relatively high prices for natural gas, which is the major feedstock for NH<sub>3</sub>/urea production, domestic nitrogen manufactures have been closing down in response to low priced urea products imported from third world countries. Hence, similar to our dependence on importing fossil fuels, the US is also becoming dependent upon importing NH<sub>3</sub>/urea fertilizer. Conserving nitrogen in animal waste will reduce the need for energy intensive manufacturing of fertilizer nitrogen and substantially reduce US dependence on importing fossil fuels and fertilizer nitrogen. Displacement of synthetic fertilizer via conservation of nutrients in animal waste substantially reduces GHGE resulting from production of commercial fertilizer and dramatically reduces associated energy costs. Each ton of fertilizer requires the consumption of 40,000 cu ft of natural gas ([USDA Guide To Achieving Energy Efficiency Through Conservation](#)). Since 1,000 cu ft of gas releases 116 lb of CO<sub>2</sub>, a ton of fertilizer nitrogen releases about 2.1 metric tonnes of CO<sub>2</sub> ([NRCS Conservation Practices Contribute to Energy Efficiency and Fuel Savings](#)). Producing 400 bu corn requires about 400 lb of fertilizer nitrogen utilizing conventional cropping systems.

“Fertilizer nitrogen application to agricultural land comprises more than 50% of global reactive nitrogen attributed to human activity ([Smil, 1999](#)). Although the manufacture and application of nitrogen consumes considerable fossil fuel energy and contributes to agriculture’s share of CO<sub>2</sub>, it is also responsible for enhanced primary productivity and net carbon sequestration. When considering the impact of fertilizer nitrogen on GHGE, attention has increasingly focused on the effect that management of fertilizer nitrogen has on the emission of N<sub>2</sub>O. N<sub>2</sub>O is a very potent GHG with a forcing potential about 296 times greater than CO<sub>2</sub>. In addition to their effect on global warming, nitrogen oxides produced from photochemical reactions of N<sub>2</sub>O in the stratosphere are involved in the destruction of the ozone layer. Although fertilizer nitrogen is an essential component of nearly all agricultural systems, the addition of reactive nitrogen to soils and the emission of N<sub>2</sub>O via soil microbiological processes (nitrification and denitrification) makes agriculture the largest source (62%) of total N<sub>2</sub>O emissions in the US. Because N<sub>2</sub>O is a potent greenhouse gas, total agricultural N<sub>2</sub>O emissions comprise an estimated 1/3 of projected US carbon sequestration potential in arable lands. Programs that encourage best-management-practices (BMP) [including comprehensive nutrient-energy management via AD, SDF and precision agriculture] in prescribing nitrogen dosage to crops that insure an economic optimum return can also reduce total atmospheric N<sub>2</sub>O load without resorting to punitive regulation ([Walters, 2006](#)).”

Since production of commercial fertilizer is energy dependent, the more crops that are produced using commercial fertilizer the more nitrogen and other synthetic fertilizers are required for production. This creates a vicious cycle of inflation by consuming an increasing amount of fossil

fuels. However, up to 75% of nitrogen contained in animal waste that is normally lost via NH<sub>3</sub> volatilization ([Varel, 2002](#)) can be conserved and efficiently utilized as a value added fertilizer via urease inhibitors, nitrification inhibitors, and SDF. Common nitrogen compounds such as urea, which are utilized as surface-applied fertilizers, can undergo transformation.

Transformation is the change in the chemical form of a compound. These transformations, which can be either chemical or biological, are important because different forms of a nutrient have varying degrees of environmental risk. For example, urea nitrogen in manure can be transformed to NH<sub>3</sub> when applied to the soil. If this transformation takes place at the soil surface, the NH<sub>3</sub>, which is a gaseous form of nitrogen, can be lost into the atmosphere. With AD, SDF, and chemigation, NH<sub>3</sub> production is reduced, nitrogen is conserved, and the bioavailability of various forms of nitrogen are enhanced and stabilized. In addition, nutrient rich compounds are delivered directly to the root zones of plants beneath the soil surface where they are readily utilized in inorganic forms. Hence, plant absorption and NUE is enhanced while NO<sub>3</sub> leaching and denitrification are reduced or virtually eliminated through precision agriculture which synchronizes delivery of nutrients with plant growth stages (maturity).

Efficient collection and processing of animal waste and crop residues, including state-of-the-art nitrogen stabilization and AD, conserves up to 94% of organic nitrogen while simultaneously reducing GHGE (Trooien & Hills, 2007). In addition, 25%-40% less fertilizer and nutrients may be required for crop production due to increasing NUE via chemical amendments for increasing bioavailability of phosphorus and nitrogen via SDF, chemigation, and precision agriculture ([Burt, 1995](#)). Many of the liquid fertilizers processed from AD effluents are virtually pathogen free ([Bioplex Research Center](#); [Ruhnke et al.](#)) and qualify for natural and organic programs. These nutrient-rich liquid fertilizers ([Lamm, et al., 1998](#)) allow for doubling plant populations and crop yields, similar to hydroponics, while increasing production efficiencies and reducing crop production costs via SDF, precision agriculture, and relay cropping systems ([Brown, 2006](#)).

### ***Economics of Comprehensive Nutrient-Energy Management***

From an economic perspective, integration of CAFOs with anaerobic digestion and innovative crop production can increase revenues by up to \$855 per AU (animal unit) while providing much needed diversification for sustainable agricultural practices. The following illustrates the savings available for current and future carbon credits. The values highlighted in red are subject to quantifying those particular emissions:

(US-EPA Air Emissions Inventory 2005)

- About 7.3 MBTU of biomethane annually via anaerobic digesters=about \$60
- Enough nutrients to produce 200 bu of corn annually=about \$685
- About 465 lbs of organic compost annually=wholesale value about \$5
- Each bovine AU produces about 1580 lbs of CO<sub>2</sub>=about \$80
- GHGE Reductions Credits for AD= about 2.5 Carbon Credits per AU @ \$4/credit=\$10
- Potential GHGE ammonia (NH<sub>3</sub>) credits = about 1.26 carbon credits per AU @ \$4/credit=about \$5.04
- Potential GHGE CH<sub>4</sub> credits via NFE (Nkrumah et. al., 2006) & Diet (McGinn et al., 2004) = about .89 carbon credits per AU @\$4/credit=about \$3.53 (GHGE)
- Potential GHGE N<sub>2</sub>O credits via NFE & Diet= about 1.48 carbon credits per AU @\$4/credit=about \$5.94
- **Additional Annual Income per AU for CAFOs = about \$855**

The above are estimates based on averages for beef feedlot cattle. Lactating dairy cows will increase the carbon credits available per AU, but decrease the rate of improvement for NFE. As illustrated above, carbon credits and potential NH<sub>3</sub> credits provide significant economic incentives for invoking positive change which increase sustainability of livestock production while simultaneously reducing emissions.

Feed costs for CAFOs can be reduced by as much as 50% by mixing nutrient rich DG co-products with crop residues (Kalscheur and Garcia, 2006; Trenkle, 2007). Integration of organic waste and cellulosic processing with livestock production, biomethane production and innovative on-farm crop/feedstock production eliminates competition between food and biofuel and reduces feed purchases for livestock producers.

Comprehensive nutrient-energy management and innovative crop production combined with conservation farming allows for reducing crop production costs by over \$270/acre, i.e. by over 50% in comparison with conventional corn crop production. Based on the NRCS-USDA energy website (NRCS), efficient nutrient management, anaerobic digestion and medium-low pressure irrigation systems via SDF (sub-surface drip fertigation) will result in the following economic savings per acre for either direct or indirect crop production by livestock operations which utilize animal waste for innovative crop production:

***Savings per acre: Comprehensive Nutrient-Energy Management for Crop Production***  
(US-EPA Air Emissions Inventory 2005)

- Conservation tillage results in saving 3.5 gallons of diesel, valued at \$2.50 per gallon=\$8.75
- Conservation tillage carbon credits=\$0.16
- Conserving animal waste nutrients plus 25% less nutrients required for SDF=\$106
- Carbon credits for conserving nitrogen (Walters 2006) and CO<sub>2</sub>=about \$1.68
- **Potential GHGE carbon credits for conserving nitrous oxide=about ? (McSwiney & Robertson 2005)**
- medium-low pressure SDF system=about \$20
- 30-60% improvement in water conservation via SDF (4x 8 gallons diesel)=\$80-120
- Carbon credits for reducing diesel/power for pumping water=about \$1.80
- SDF and precision agriculture for conserving nutrients=\$13
- Pesticide management and reduced herbicides with high density planting and SDF=\$40
- **Total savings per acre = \$270-\$310<sup>1, 2</sup>**

The result of the above reductions in production costs and additional revenue streams provide unprecedented efficiencies of operation for integrated livestock operations which take advantage of on-farm production of livestock feeds, biofuel feedstocks and agricultural crops. In addition to producing CH<sub>4</sub> from organic waste, by producing corn and other crops from nutrient-rich anaerobic digester effluents, corn can be produced for about \$0.60/bu. In terms of processing the entire corn plant, this is equivalent to producing 20.36 tonnes/acre at a cost of \$11.30/tonne. This would allow for producing ethanol from corn plant biomass and relay triticale crops for less than

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<sup>1</sup> Typical costs for producing corn per acre, about \$500 (after adjusting for fuel and fertilizer price increases), minus \$270 in conservation savings=about \$230 total production costs per acre!

<sup>2</sup> [http://cta.ornl.gov/bedb/feedstocks/Grains/Corn\\_Production\\_Costs\\_and>Returns\\_by\\_region\\_2002-2003.xls](http://cta.ornl.gov/bedb/feedstocks/Grains/Corn_Production_Costs_and>Returns_by_region_2002-2003.xls)

\$0.50/gal for integrated operations while simultaneously reducing emissions and providing high value protein feedstuffs for animals.

Eventually, environmental lobbying efforts will result in NH<sub>3</sub> and carbon credits (for reduction of energy costs and emissions associated with N fertilizer production) being available for conserving N via reduction of NH<sub>3</sub> and N<sub>2</sub>O emissions for livestock production similar to GHGE credits for reduction of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O emissions for crop production. In the US and Canada, carbon credits are currently traded voluntarily over the CCX (Chicago Climate Exchange) for \$4 compared to \$27 for countries which have adopted the Kyoto Protocol as mandatory. As indicated above, about \$4/acre can be earned via carbon credits from crop production. However, the value of these environmental credits could be increased by 7 fold through negotiation and/or participation in the Kyoto protocol. An additional \$15/AU in credits could potentially be earned by CAFOs. Carbon credits and tax exempt bonds are utilized by financial bankers as collateral for financing construction of AD facilities and SDF systems. These systems process animal/solid wastes (including ruminant digestion of crop residues), into biofuel feedstocks and livestock feeds. Eventually, NH<sub>3</sub> and carbon credits for CH<sub>4</sub> and N<sub>2</sub>O will also be available for financing FIS equipment for enhancing NFE and reducing emissions. In addition to AD and biogas improvements, through lobbying efforts tax exempt bonds will become available for comprehensive nutrient-energy management for environmental improvements such as financing construction of SDF, hydroponic greenhouses and new generation algal photobioreactors. For integrated operations, CAFOs provide an abundant source of nutrients for production of both conventional crops and alternative feedstocks for renewable fuel production while simultaneously reducing emissions.

#### ***Closed Loop Production of Lignocellulosic Biomass: Precision Agriculture & SDF***

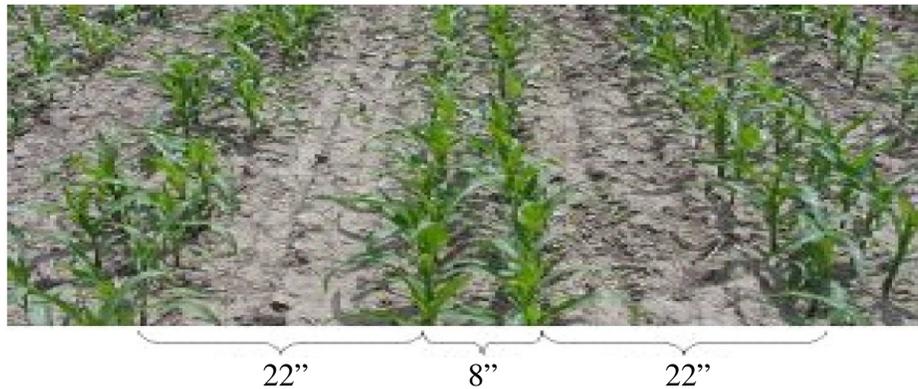
Lignocellulosic biomass from terrestrial plants, energy crops, and crop residues requires cleaning, reduction of particle size, pretreatment and fractionation in order to remove contaminants, degrade structural carbohydrates, remove non-fermentables, and thus optimize fermentation efficiency. Pretreatment processes increase surface area of particles, break the lignin bonds to cellulose, and reduce the crystalline structure of cellulose in terrestrial plant biomass. This is necessary in order to enhance enzymatic hydrolysis prior to fermentation. Enzymatic hydrolysis is preferred over acid hydrolysis in order to reduce production of toxic compounds.

Most lignocellulosic feedstocks including energy crops such as switchgrass and miscanthus will contain about 10-13% protein and less than 25% lignin. Sweet sorghum bagasse contains about 20% lignin and water hyacinth contains 18% crude fiber (including lignin) which could increase ethanol yields for these particular feedstocks. In terms of annual biomass yields, sweet sorghum can seasonally produce 12 tons per acre, and switchgrass/miscanthus can seasonally produce up to 16 ton per acre.

However, SDF and high density relay cropping systems for corn and triticale can produce 24 tons of grain and lignocellulosic biomass per acre. This is based on consistently producing 400 bu/acre corn crops and processing the majority of corn biomass at integrated biorefineries. SDF could also be utilized to improve crop yields for switchgrass, sweet sorghum, and other energy/food crops. SDF integrates subsurface drip irrigation (SDI) ([Rogers and Lamm, 2009](#); [Lamm, 2009](#)) and precision agriculture including chemigation via comprehensive nutrient-

energy management and precision delivery of optimal nutrient ratios synchronized with plant growth stages.

### **SDF, Chemigation & High Density Relay Cropping Systems**



*Figure 2. Twin Row Crops with 30" Lateral Drip-tube Spacing*

#### ***Improving Nutrient Sampling & Sensing***

In order to utilize nutrients efficiently for crop production, accurate soil, manure and water testing needs to be regularly performed prior to nutrient/fertilizer applications of crops. However, conventional soil sampling and chemical extraction by soil testing labs does not account for the dynamic nature of inorganic and biological soil chemistry. As a result, fertilizer applications are often under or over applied which has both economic and environmental consequences. Over application of N fertilizers results in  $\text{NO}_3$  leaching which contribute to nitrification of ground-water aquifers and surface water.

The same conditions for preventing  $\text{NH}_3$  volatilization subsequent to urea fertilizer applications applies to comprehensive animal waste management. Reducing urea hydrolysis will reduce  $\text{NH}_3$  volatilization. With SDF, pH and temperature can be kept relatively low. Hence, little potential exists for  $\text{NH}_3$  volatilization. At pH 7.5, less than 7% of the urea/ $\text{NH}_4$  is actually in the form of  $\text{NH}_3$  over the range of temperatures likely for field conditions (Bock and Kissel, 1988; Fenn and Kissel, 1976; Ernst and Massey, 1960; Ferguson, et al., 1984). In many soils, SDF and chemigation via urea-sulfuric acid (N-pHURIC fertilizer combinations) allows for economically regulating the pH below 7.5 for irrigation water and soil, specifically for the root zones of crops via SDF in order to enhance bioavailability of nutrients, increase nutrient use efficiency, and reduce GHGE.

#### ***Ion Exchange Resin Technology***

In contrast to conventional soil sampling and chemical extraction, ion exchange resin capsules provide for accurately assessing N availability as influenced by mineralization, immobilization, N fixation by legumes, and denitrification. The function of the resin capsule and various applications have been sufficiently proven through a variety of laboratory and field studies ([Yang, et al., 1991](#); [Dobermann, et al., 1994](#); Skogley, 1994; [Skogley and Dobermann, 1996](#); [Skogley, et al., 1996](#); [Skogley and Warrington, 1999](#)). Ion exchange resin technology can be integrated with the DRIS (diagnostic recommendation integrated system) foliar analysis for

comprehensive nutrient-energy management. Similar to resin capsules, DRIS technology has been scientifically validated for enhancing foliar analysis and integration with soil analysis to provide precision delivery of nutrients as well as diagnose both nutritional and non-nutritional problems (Walworth and Sumner, 1987; Walworth and Sumner, 1988).

***Nitrification & Denitrification***

Nitrification is the biological [oxidation](#) of NH<sub>4</sub> with oxygen into [nitrite](#) followed by the oxidation of these nitrites into [nitrates](#) to increase N availability for plants. Denitrification has the opposite effect of nitrification, i.e. the conversion of nitrate into gaseous N compounds N<sub>2</sub> and N<sub>2</sub>O, thus decreasing plant-available N. These complex biological processes along with mineralization and immobilization are primarily responsible for the dynamic nature of soil N biochemistry.

Unlike conventional soil sampling and chemical extraction, ion exchange resin capsules allow for accurately assessing soil N biochemistry, allowing for the synchronization of nutrient delivery with specific plant growth stages. When necessary, soil organic matter (OM) and identification of soluble soil organic constituents will be evaluated using carbonaceous resin capsules. Sorbed quantities of labile carbon are accurate predictors of soil organic carbon (Johns & Skogley, 1994). Using carbonaceous resin capsules for soil OM testing eliminates toxic dichromate waste which is produced by the Walkley-Black test.

***High Moisture Feedstock Processing & Relay Cropping Systems***

During ethanol biorefining processes, substantial water is added to dried corn and corn stover which consists of about 10-15% moisture. The idea of reducing corn production costs, conserving water during harvesting of corn, and subsequently biorefining of high moisture corn feedstocks is economically attractive. Siouxland Energy & Livestock, an integrated biorefinery and cattle feedlot owned by corn producers in Iowa, has determined that harvesting and processing 19% moisture corn reduces production costs by over \$0.92/bu based on current corn, labor and energy prices.

**Table 1. Cost Savings on Producing & Processing 5,000 bushels of Wet Bunker Corn**

<b>Process</b>	<b>@ \$2.27/bu</b>	<b>@ \$4.00/bu</b>
Planting a Fuller Season Corn	\$0.076	\$0.134
Wet Grain Expense	(\$0.028)	(\$0.049)
Reduction of Handling Costs	\$0.05	\$0.088
Labor Cost	\$0.025	\$0.044
Cost of Drying	\$0.125	\$0.22
Misc. Storage Costs	\$0.05	\$0.088
Field Loss Reduction Due to Early Harvest	\$0.076	\$0.134
Storage Cost	\$0.09	\$0.159
<b>Total Savings</b>	<b>\$0.46/bu</b>	<b>\$0.9156</b>

*Adapted from Data Obtained from Siouxland Energy & Livestock, 2003*

With corn futures reaching as high as \$8.50/bu in 2008, and energy costs trending up due to increased demand for natural gas and liquid fuels which have more than doubled in price. Natural gas is utilized for drying and liquid fuels are utilized for transportation. Hence, the above production and handling costs for corn have increased by almost two fold since 2003.

In addition to reducing production costs, harvesting high moisture corn allows for either planting a fuller season and/or utilizing relay cropping systems. Relay cropping systems are often referred to as double cropping, such as harvesting high moisture corn or corn silage followed by triticale.

Relay cropping and precision agricultural production of high density crops allows for maximizing efficiency of food and energy crop production. It also reduces the volume of water required for biorefining since high moisture feedstocks contain almost 70% water compared with dried feedstocks which contain only about 10-15% water.

### ***Processing Crop Residues***

Agricultural crop residues are another source of organic matter rich in carbohydrates which is available for processing into value added products and biofuels. The low nutritional values of cellulosic crop residues (corn stalks, cereal grain straw, soy hulls and sugar beet pulp, etc.) compliment the high fat and protein content of distillers grains (DG). The feeding of crop residues to ruminant livestock and subsequent processing of animal waste via state-of-the-art anaerobic digestion is much more energy efficient than directly processing crop residues as feedstocks for production of renewable fuels. Contrary to using crop residues as cellulosic feedstocks for ethanol biorefining which depletes soil organic matter, feeding a portion of crop residues to livestock and then processing animal waste into value added liquid fertilizers for conventional irrigation systems and sub-surface drip fertigation (SDF) restores soil organic matter (SOM) and essential micronutrients to the soil. The loss of micronutrients from agricultural soils, due to lack of using balanced fertilizer and restoring SOM, is resulting in a loss of these nutrients in food products for consumers. This lack of balanced nutrients in livestock feeds and food products affects our general health and lowers immunity for both livestock and consumers.

**Table 1. Cellulose, Hemicellulose & Lignin Contents in Crop Residues & Organic Waste**

Lignocellulosic Material	Cellulose (%)	Hemicellulose (%)	Lignin (%)
hardwood stems	40-55	24-40	18-25
softwood stems	45-50	25-35	25-35
nut shells	25-30	25-30	30-40
corn cobs	45	35	15
corn stover including cobs	36.1	29.2	17.2
soy hulls	76-86.2% total carbohydrates including pectin		
grasses	25-40	35-50	10-30
paper	85-99	0	0-15
wheat straw	30	50	15
sorted refuse	60	20	20
leaves	15-20	80-85	0
cotton seed hairs	80-95	5-20	0
newspaper	40-55	25-40	18-30
waste papers from chemical pulps	60-70	10-20	5-10
primary wastewater solids	8-15		
solid cattle manure	1.6-4.7	1.4-3.3	2.7-5.7
coastal bermudagrass	25	35.7	6.4
switchgrass	45	31.4	12
swine waste	6.0	28	na

Adapted from ([Kumar et al., 2009](#)).

As listed in the above table, hardwoods have greater amounts of cellulose, whereas wheat straw and leaves have more hemicellulose (Sun and Cheng, 2002). In addition, the ratios between various constituents within a single plant vary with age, stage of growth, and other conditions (Perez et al, 2002; [Kumar et al., 2009](#)). Though pectin is found in most terrestrial herbaceous plants, it is usually in relatively small quantities. In human digestion, pectin goes through the small intestine more or less intact. Pectin is thus a soluble [dietary fiber](#). Consumption of pectin has been shown to reduce blood cholesterol levels. The mechanism appears to be an increase of viscosity in the intestinal tract, leading to a reduced absorption of cholesterol from bile or food. In the large intestine and colon, microorganisms degrade pectin and liberate short-chain fatty acids that have positive influence on health ([prebiotic](#) effect). In [ruminant](#) nutrition, depending on the extent of lignification of the cell wall, pectin is up to 90% digestible by bacterial enzymes. [Ruminant](#) nutritionists recommend that the digestibility and energy concentration in forages can be improved by increasing pectin concentration in forages and fermentation residues.

Cellulose crystallinity, accessible surface area for the particular biomass feedstock, protection by lignin, and sheathing by hemicellulose all contribute to the resistance of cellulose in biomass to hydrolysis. The biomass pretreatment and the intrinsic structure of the biomass itself are primarily responsible for its subsequent hydrolysis. The conditions employed in the chosen pretreatment method will affect various substrate characteristics, which, in turn, govern the susceptibility of the substrate to hydrolysis and the subsequent fermentation of the released sugars. This includes the release of toxic components which inhibit hydrolysis and fermentation resulting from intense pretreatment processes. Therefore, pretreatment of biomass is an extremely important step in the synthesis of biofuels from lignocellulosic biomasses, and there is a critical need to understand the fundamentals of various processes, which can help in making a suitable choice depending on the structure of the biomass substrate and the hydrolysis agent ([Kumar et al., 2009](#)). Ammonia ensiling of wet biomass feedstocks followed by aqueous ammonia-ethanol pretreatment-fractionation platforms provide a low cost and efficient pretreatment process for most herbaceous feedstocks, while woody biomass feedstocks (hardwoods, softwoods, nut shells) low in moisture may be ideal for hybrid thermochemical-fermentation processes which can produce ethanol for as little as \$0.15/gal. ([Chambers, 2009](#))

#### ***Soaking in Ethanol & Aqueous Ammonia (SEAA)***

“A new process for pretreatment of lignocellulosic biomass was developed to improve hemicellulose preservation in solid form. In the SEAA process, an aqueous ammonia solution containing ethanol is used. Corn stover was treated with 15 wt% ammonia at 1:9 solid–liquid ratio (by weight) at 60 °C for 24 h with ethanol added at 1, 5, 20, and 49 wt% (balance was water). The extents by which xylan was solubilized with no ethanol and with ethanol added at 1, 5, 20, and 49 wt% of the total liquid were 17.2%, 16.7%, 14.5%, 10.4%, and 6.3% of the original xylan, respectively. Thus, at the highest ethanol concentration used the loss of hemicellulose to the liquid phase was reduced by 63%. The digestibility of glucan and xylan in the pretreated corn stover samples by cellulase was not affected by ethanol addition of up to 20 wt%. The enzymatic digestibility of the corn stover treated with 49 wt% ethanol added was lower than the digestibility of the sample treated with no ethanol addition.

Based on the above results, 20 wt% was found to be the optimum ethanol concentration for use in the SEAA process for pretreatment of corn stover. With optimum ethanol concentration at 20 wt%, the carbohydrate-rich solid sample obtained from the SEAA treatment contained 51.4% glucan, 26.7% xylan, and 6.2% lignin, which were almost 100% glucan retention, 89.6% xylan

retention, and 70.1% lignin removal, respectively. The SEAA was proven to be an effective method to produce the carbohydrate-rich solid cake, which can be used for production of fuels and chemicals. It is anticipated that the ethanol used in the pretreatment process could be recovered and recycled about six times before the original ethanol stream is exhausted. Hence, an ethanol make-up stream is required to maintain the 20% ethanol in the aqueous ammonia solution (Kim et al., 2009).” The ethanol that is not recycled during the SEAA process would be distilled and sold as fuel so that this process would not significantly decrease ethanol yields.

### Universal SEAA Pretreatment-Fractionation Platform

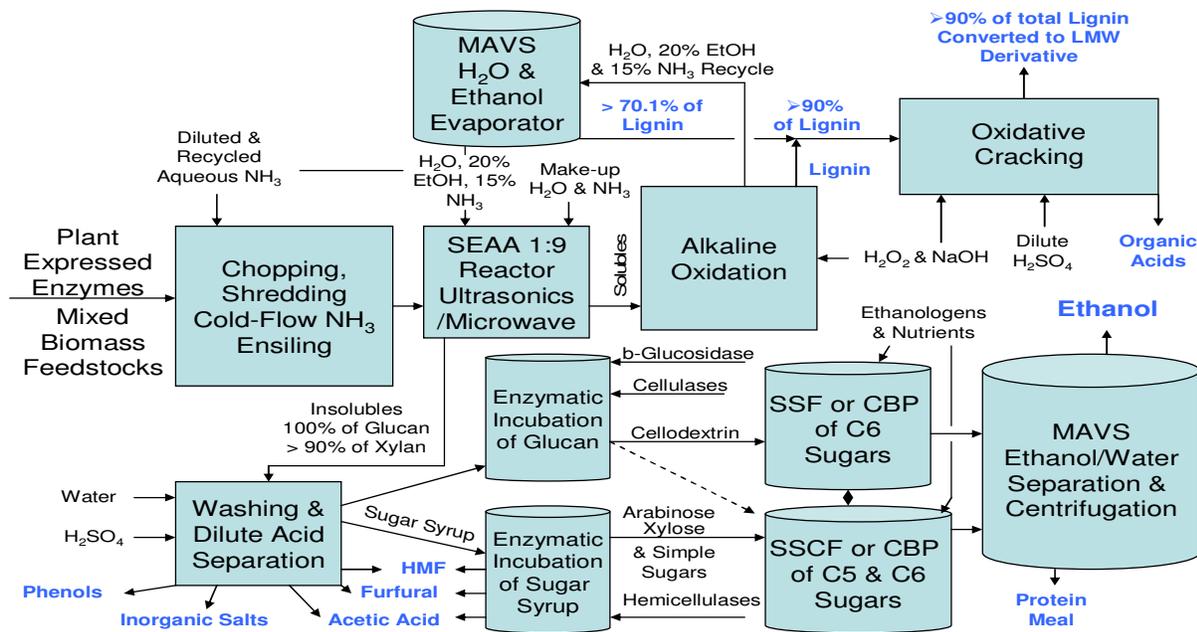


Figure 3. Integration of SEAA, Alkaline Oxidation & Oxidative Cracking of Lignin

As utilized in pretreatment and fractionation processes, dilute acid is ideal for liquefying and separating hemicellulose from cellulose, and preparing lignin for cracking. Dilute acid can also be integrated for extracting furfural, organic acids and sugar syrups (which contain primarily xylose, arabinose and mannose) from hemicellulose.

Ensiling feedstocks with ammonia and enzymes, reducing particle size via ultrasonics or microwave, and incorporation of the alkaline oxidation step could allow for increasing xylan separation and total lignin extraction to over 90%. Pure low molecular weight lignin produced by oxidative cracking would allow for generating additional revenue streams via development of high value chemical products for industries such as the bioplastics markets.

#### Alkaline Oxidation

This pretreatment process combines alkaline and oxidative pretreatments. Pretreatment with  $H_2O_2$  in an alkaline environment or combining it with a preceding alkali treatment-step (ammonia steeping) provides an effective pretreatment of lignocellulosic biomass feedstocks. In weak alkaline media,  $H_2O_2$  only selectively acts on phenolic compounds originated from partial scission of lignin, causing its degradation without affecting the cellulosic fraction. Only the

lignin and hemicellulose are solubilized. This treatment removes approximately 50% of lignin in wheat straw and corn stover. Sugarcane bagasse treated with 2% H<sub>2</sub>O<sub>2</sub> at 30 °C for 8 h solubilized about 50% of lignin and most of the hemicellulose. Subsequent saccharification by cellulase at 45 °C provides 95% conversion to glucose in 24 h (Sun and Cheng, 2002). H<sub>2</sub>O<sub>2</sub> to substrate ratio of 0.25 g per gram of substrate at 25 °C, pH 11.5, were the optimum pretreatment conditions. The lignin degradation products were not found to be toxic either for saccharification or fermentation. Reuse of the solvent up to six times after the initial pretreatment is possible.

“Pretreatment of two different softwood-based lignocellulosic wastes (newsprint and Kraft pulp mill sludge) was investigated. Pretreatment was conducted with aqueous ammonia and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), two delignifying reagents that are environmentally benign. Three different treatment schemes were employed: ARP, mixed stream of aqueous ammonia and H<sub>2</sub>O<sub>2</sub>, and successive treatment with H<sub>2</sub>O<sub>2</sub> and aqueous ammonia. In all cases there was a substantial degree of delignification ranging from 30 to 50%. About half of the hemicellulose sugars were dissolved into the process effluent. Retention of cellulose after pretreatment varied from 85 to 100% for newspaper feedstock and from 77 to 85% for the pulp mill sludge. After treatment with aqueous ammonia alone (ARP), the digestibility of newspaper and the pulp mill sludge was improved only by 5% (from 40 to 45% for the former and from 68 to 73% for the latter), despite a substantial degree of delignification occurring after the ARP process. The lignin content thus did not correlate with the digestibility for these substrates. Simultaneous treatment with H<sub>2</sub>O<sub>2</sub> and aqueous ammonia did not bring about any significant improvement in the digestibility over that of the ARP. A successive treatment by H<sub>2</sub>O<sub>2</sub> and ARP showed the most promise because it improved the digestibility of the newspaper from 41 to 75%, a level comparable to that of alpha cellulose (Kim et al, 2000).”

#### ***Oxidative Cracking for Production of High Value Lignin Derivatives***

“Precipitated hardwood lignin (PHL) is a major byproduct in the biomass to ethanol process. Oxidative cracking of PHL by hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in aqueous medium was investigated as a means to produce potentially useful chemicals. The cracking reaction takes place at moderate temperatures (80–160°C), giving mono- and dicarboxylic acids as the main products. The yields of these products are in the range of 30–50% of initial lignin. The reaction mechanism and the product distribution are dependent upon the reaction conditions, especially the pH value. The reaction under strong alkaline condition proceeds well even at low reaction temperatures (80–90°C). Under acidic conditions, higher temperatures (130–160°C) are required to attain the same degrees of cracking. The reaction patterns of the oxidative cracking reaction involve the cleavage of lignin ring, aryl ether bond, or other linkages within lignin. By using the findings of this investigation and those of previous work, we have illustrated the reaction pathways for degradation of PHL under alkaline and acidic conditions. Aldehydes and aromatic acids are intermediate products in the oxidative degradation of lignin. However, they were produced only in trace amounts owing to rapid degradation induced by hydrogen peroxide ([Xiang and Lee, 2000](#)).

#### ***Pulsed-Electric-Field Pretreatment for Biomass & Organic Waste***

Though ammonia ensiling may reduce the need for further pretreatment of wet biomass, there are several electronic, electroporation, electromagnetic pulse, and ultrasonic treatments which are being developed for processing corn slurries, lignocellulosic biomass and organic waste. These technologies are also being utilized to provide quantum fracturing of nutrient-rich fertigation streams for biomass production, most notably algae production. This technology could also be of

benefit for treatment of fertigation streams used for hydroponic greenhouse production in conjunction with plant microbial electrolysis cells. In 2009, the following was published for a pulsed-electric-field pretreatment process for biomass.

“Pulsed-electric-field (PEF) pretreatment involves application of a short burst of high voltage to a sample placed between two electrodes. When an electric field is generated between two parallel-plate electrodes, the field strength ( $E$ ) is given by  $E = V/d$ , where  $V$  is the voltage and  $d$  is the distance between the two electrodes. PEF pretreatment can have serious effects on the structure of plant tissues. When a high-intensity, external electric field is applied, a critical electric potential is induced across the cell membrane, which leads to rapid electrical breakdown and local structural changes of the cell membrane, the cell wall, and therefore the plant tissue. The electric field results in a dramatic increase in mass permeability and, in some cases, mechanical rupture of the plant tissue. The electric field pulses most commonly applied are in the form of exponential-decay or square waves.

Application of high-intensity electric field pulses from nanoseconds to microseconds in duration leads to the permeabilization of biological membranes. Based on this phenomenon, many practical applications of high electric fields for reversible or irreversible permeabilization of various biological systems have been studied in the fields of medicine and bioscience (Chang et al, 1992; Ho and Mital, 1996; Knorr and Angersbach, 1998; Lynch and Davey, 1996; Zimmermann et al, 1996; Angersbach et al., 2000). The permeabilization of plant membranes to improve mass transfer of metabolites is currently of interest to the food industry. Initial efforts mainly focused on reduction of endogenous microbial load of food products (Jayaram et al., 1993). More recently, there have been reports on the application of PEF pretreatment for inactivation of enzymes (Giner et al, 2001). Pulsed electrical fields have also been used on vegetable tissues to improve mass-transfer processes such as diffusion of soluble substances (Taiwo et al, 2005), juice extraction (Eshtiaghi and Knorr, 2002; Bazhal et al, 2001), and dehydration (Rastogi et al, 1999). In applying PEF pretreatment to plant processing, the electric field strength (voltage/distance), the number of pulses, and the treatment time are the most important factors. Typically, the plant tissue is placed or transported between two electrodes, and the electric discharges are applied in the form of pulses. A typical setup consists of a pulse generator, treatment chamber, data acquisition and control system, and material-handling equipment. Field strengths applied for irreversible plant tissue permeabilization are usually above 1.0 kV/cm. Pulse durations are in the microsecond range.

In biomass-to-fuel conversion, the biomass needs to be treated so that the cellulose in the plant fibers is exposed. Pretreatment with PEFs can facilitate this process. Using high field strengths in the range of 5-20 kV/cm, plant cells can be significantly ruptured. By applying electric pulses with high field strengths, PEF pretreatment can create permanent pores in the cell membrane and hence facilitate the entry of acids or enzymes used to break down the cellulose into its constituent sugars. In the case of the chemical modification of plant tissue, particularly in lignocellulose hydrolysis, appropriate chemicals might need to be transported into the tissue to aid in cell-wall breakdown and digestion and pretreatment with PEFs is important to facilitate the process. Two advantages of PEF pretreatment are that it can be carried out at ambient conditions and energy use is low because pulse times are very short (100  $\mu$ s). Furthermore, the actual PEF process itself does not involve moving parts, so that the equipment is not complex.

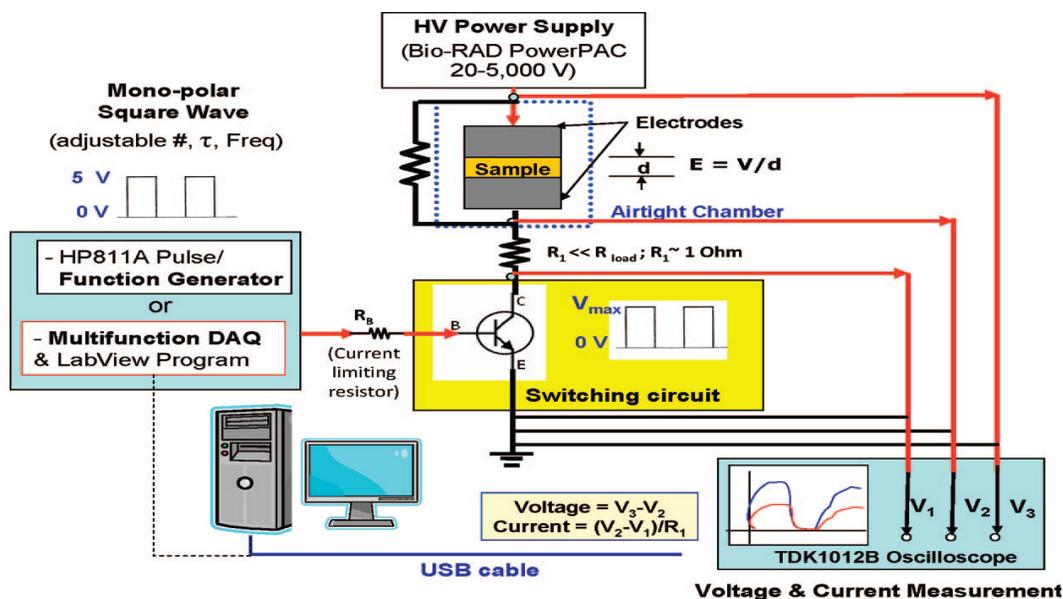


Figure 4. Schematic of Pulse-Electric-Field (PEF) System for Pretreatment of Biomass & Organic Waste.

Kumar et al. (2009) have designed and fabricated a PEF system for the treatment of woodchips (Southern pine) and switchgrass samples. A schematic of the PEF system is shown in Figure 4. The PEF apparatus consists of a high-voltage power supply, a function generator, a switching circuit, and a sample holder. The switching circuit consists of a transistor that is driven by the function generator. The function generator feeds a pulse of desired shape and width to the switching circuit. The switching circuit is turned on when the pulse is applied to it and transfers the high voltage supplied by the power supply across the sample holder. Therefore, high-voltage pulses of desired shape and width can be applied to the sample using the function generator and switching circuit. Switchgrass samples were treated with 2000 pulses of 8 kV/cm with a pulse width of 100  $\mu$ s and a frequency of 3 Hz. Neutral red dye uptake experiments were performed on fresh and PEF-treated samples to study the effects of PEF treatment. Switchgrass samples were stirred in an aqueous solution of neutral red, and the concentration of dye in water was measured as a function of time using a UV-vis spectrophotometer. An example of experimental dye-uptake results is illustrated in Figure 5.

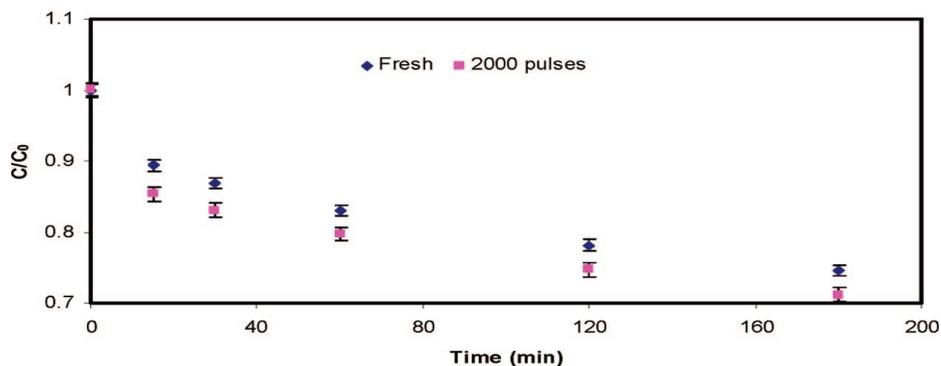


Figure 5. Neutral Red Uptake Comparison for Fresh And PEF-treated (2000 pulses @ 8 kV/cm) Switchgrass Samples

Switchgrass samples treated with 2000 pulses of 8 kV/cm showed faster dye uptake than fresh samples, suggesting a positive effect of PEF pretreatment on switchgrass samples. Pulsed-electrical-field-treated samples can show similar characteristics for enzymes used for hydrolysis and can increase the hydrolysis rate. Further treatments with high electric fields and higher numbers of pulses are planned to study the effects of PEF treatment on wood chip samples ([Kumar et al, 2009](#)).

### ***Synthetic Biology & Consolidated Bioprocessing***

Qteros uses its fast-acting Q microbes to convert the recyllose into cellulosic ethanol. According to the company, one ton of recyllose feedstock can make 120 to 135 gallons of ethanol. Qteros' microbe – found in the soil near the Quabbin Reservoir — is a voracious bacteria which increases the efficiency of cellulosic ethanol production by eliminating the need for exogenous enzymes. The research has been supported in part by a grant from the Binational Industrial Research and Development (BIRD) Foundation. The BIRD Foundation funds joint efforts between Israel and the United States, and their financial support has resulted in the very successful collaboration of Qteros' and ACT's technologies.

The relatively recent discovery of the Q microbe, [Clostridium phytofermentans](#) (synthetic strains for which are currently being developed by [Qteros](#)); and [Clostridium thermocellum](#) strains which are being developed by [Mascoma](#); and also by the [Bioconversion Science and Technology](#) group at the Oak Ridge National Laboratory (DOE-ORNL) are all very promising for commercializing consolidated bioprocessing (CBP). “Qteros' Q Microbe™ process has demonstrated world-class ethanol outputs of 70 grams per liter (9 w/v%) in a single-step using an industrial, pre-treated biomass feedstock. These unprecedented yield results far surpass the 50 grams per liter considered to be the threshold for commercial production of cellulosic ethanol. Coupled with the previously observed bioconversion yields of greater than 90% ([Qteros Report, 2009](#)),” these yields are slightly superior to the 7 w/v% reported by Mascoma via [Clostridium thermocellum](#).

### ***Membrane Assisted Vapor Stripping (MAVS)***

When membrane assisted vapor stripping (MAVS) technology is utilized in place of conventional (fractional or steam) distillation and molecular sieve technologies, the energy required for separating ethanol from water can be decreased by up to 50%. For production of 99.5% anhydrous ethanol, fractional distillation and mol sieves can comprise up to 40% of total energy consumed at a corn ethanol plant. For integrated biorefineries, total energy consumption can be reduced by over 50% by utilizing state-of-the-art MAVS technology, thermal integration, converting organic waste to biomethane, and implementing combined heat and power (CHP) applications. For production of 50-65% ethanol and 35-50% water (hydrous ethanol blends) for use with catalytic and plasmatron reforming technologies, the energy required for ethanol-water separation can be reduced by over 80%.

Depending on efficiency of ethanol and water separation from various fermentation broths containing different ethanol concentrations (starch vs. lignocellulosic biomass processing), achieving optimal energy efficiency may require separate water-ethanol removal systems, particularly for production of hydrous vs. anhydrous ethanol.

The MAVS hybrid system utilizes a stripping column which provides a high alcohol recovery and a low effluent alcohol concentration. This is accomplished while the vapor compression

membrane component enables the efficient recovery of latent and sensible heat from both the retentate and permeate streams via the membrane system. No rectification column is used. The actual costs associated with the compressor and membrane components of the MAVS system in Figure 6 for recovering 1 MGY of ethanol from a 5 wt% ethanol feed stream using a 35 °C stripper were determined to be as low as \$0.11 gal-EtOH<sup>-1</sup> (\$0.036 kg-EtOH<sup>-1</sup>) and the associated energy usage was about 3 MJ-fuel equiv kg-EtOH<sup>-1</sup> (Vane and Alvarez, 2008).

### Membrane Assisted Vapor Stripping (MAVS)

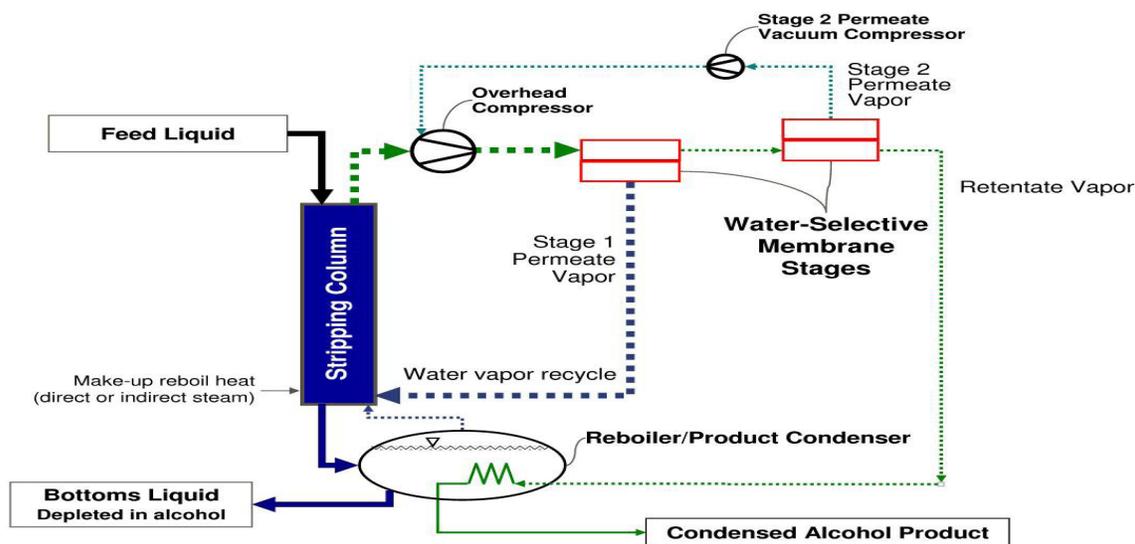


Figure 6. Schematic diagram of a hybrid ethanol-water separation process (Vane, 2008)

The MAVS process has been estimated to require as little as 8.9 MJ-fuel/kg-EtOH to produce 99.5 wt% ethanol from a 1 wt% ethanol feed stream, and only 2.5 MJ-fuel/kg-EtOH for a 5 wt% ethanol feed stream (Vane and Alvarez, 2008; Vane et al., 2008). Comparable heat integrated distillation-molecular sieve systems would require at least 19.2 and 6.2 MJ-fuel/kg-EtOH for 1 and 5 wt% ethanol feed streams, respectively. To produce a 99.5 wt% ethanol product from a broth containing 11.5 wt% ethanol the MAVS process would require as little as 2.2 MJ-fuel/kg-EtOH (Vane et al., 2008). Thus, the MAVS hybrid ethanol separation process yields energy savings of at least 54% compared to the benchmark distillation-adsorption process. Côté *et al.* have proposed a hybrid distillation-vapor permeation process which replaces the rectification column and molecular sieve components of the conventional ethanol-water distillation-adsorption system with a vapor permeation dehydration system – such a process is referred to as the Siftek™ Dewatering System (Cote et al, 2007; Cote et al., 2008). Compression of the water rich permeate vapor from the first of two membrane stages allows for recovery of the latent heat from that vapor stream as heating steam in the reboiler of the beer column. (Cote et al., 2008; Vane, 2008)

Reductions in fuel-equivalents usage of 50% or more for MAVS vs. distillation + sieves - independent of feed concentration are commonly observed. Since this refers to fuel-equivalents, it should translate directly into GHGEs reductions of 50% or more for that part of the separation process. This type of GHGE, associated with the production process is occasionally referred to as "upstream GHGEs".

MAVS data have been compiled for separating 5 wt% ethanol (circa 6 vol%). Distillation + sieves would require between 6.2 and 7.2 MJ-fuel/kg-EtOH to produce 99.5 wt% ethanol. Estimates for the MAVS system would require 2.5 MJ-fuel/kg-EtOH for the same product purity. That assumes 90% fuel-to-heat boiler efficiency for distillation, 33% fuel-to-electricity efficiency, and 75% compressor efficiency. Varying some membrane parameters and process pressures/temperatures, the MAVS number can be as low as 2.2 and as high as 3.1 MJ-fuel/kg-EtOH. Though data has not been compiled for an 8 wt% ethanol scenario (circa 10 vol%), it is estimated that distillation + sieves would require 4.5 to 5.5 MJ-fuel/kg-EtOH and would require 2 to 2.5 MJ-fuel/kg-EtOH for producing a 99.5 wt% ethanol product via MAVS technology.

“The hybrid stripping-vapor permeation MAVS approach is predicted to be more energy efficient and cost effective than distillation for the recovery of ethanol from ethanol–water mixtures and ultimate production of fuel-grade ethanol. The hybrid system takes advantage of the high ethanol recovery and low effluent concentrations offered by a stripping column with the high selectivity and ability to return the stripping phase made available by the vapor permeation system. Within the stripping options studied, steam stripping had the lowest potential energy usage and cost with estimated fuel-grade ethanol production costs of US\$0.16 gal<sup>-1</sup> from a feed containing 5 wt% ethanol and US\$0.37 gal<sup>-1</sup> from 1 wt% ethanol. These are both well below the current (December 2007) price of ethanol in the US of ca \$2.00 gal<sup>-1</sup>.<sup>43</sup> Thus, even low concentration ethanol waste streams could be upgraded economically to fuel-grade ethanol using the hybrid MAVS technology. For example, if considered in the context of wastewater treatment, the MAVS technology would cost US\$5 to treat 1000 gallons of 1 wt% ethanol wastewater. This same volume of wastewater would yield 12.6 gallons of fuel grade ethanol worth US\$25, thereby more than paying for the treatment. Other separations which can be performed with the MAVS system include other alcohol–water systems and even mixtures of organic solvents in which efficient stripping column VLE and selective membranes can be combined advantageously ([Vane and Alvarez, 2008](#)).”

[Membrane Technology & Service \(MTS\)](#)'s Liquid Separation Process Development Group supports the commercialization of PerVap® pervaporation systems technology for bioethanol production, food processing, and water treatment operations. In addition, the group develops liquid separation applications for solvent-resistant membranes in the food processing, petrochemical, and refining industries. MTR's BioSep™ group, together with Dr. Leland M. Vane at the U.S. EPA Cincinnati Laboratory, has developed several novel membrane-distillation hybrid processes for bioethanol production. The membrane units in these processes use either vapor permeation or pervaporation. The processes are simple in design, offer significant separation process energy savings (more than 50%) and are cost competitive with conventional distillation-molecular sieve technology. BioSep™ processes are especially attractive for use in situations where the ethanol concentration in the fermentation step is low, such as cellulose-to-ethanol and algae-to-ethanol. A demonstration plant is being constructed in collaboration with a cellulose-to-ethanol producer; start up is scheduled for early fall 2009. Biobutanol is not currently produced as a biofuel for economic reasons, including the low concentration of butanol in the fermentation broth and the complexity of the conventional separation process to separate the three components – acetone, butanol and ethanol (ABE) – from the fermentation broth. In a new project funded by DOE, MTR is addressing separation issues by developing a low-cost, low-energy hybrid membrane-distillation separation process. Pervaporation/vapor permeation steps use membranes to concentrate and dehydrate the ABE mixture. The proposed process could

save up to 87% of the energy that would be consumed to recover bioethanol and biobutanol by conventional separation techniques.

Vaperma has announced a partnership with UOP for marketing their Siftek technology ([Ethanol Producer Magazine, Mar, 2009](#)) which utilizes Siftek technology. The Siftek™ Dewatering System is similar to the MAVS system, though the latter has advantages according to patent applications which were recently published. One of the biggest differences is the source of the driving force for membrane transport. As the patent application defines it, MAVS compresses the overhead vapor from the stripper to generate the feed vapor for the membrane process. The Siftek design does not compress the feed to the first membrane unit, it uses a vacuum pump on the permeate side of the membrane unit to generate the driving force. In most cases, the stripping column in MAVS will be under a vacuum and the temperature of the column can be controlled by changing the vacuum pressure. In Siftek, the stripping column has to be fairly hot and above ambient pressure to deliver a reasonable membrane feed pressure and, therefore, driving force. The MAVS technology decouples the feed pressure to the membrane unit from the pressure in the stripping column which can be advantageous, particularly in order to prevent killing organisms, deactivate enzymes, or precipitate out proteins, etc.

### ***High Temperature Fuel Cells, Thermal Integration & Fractionation Platforms***

Biomethane can be utilized as a hydrogen-rich feed for high temperature fuel cells which provide distributive generated combined cooling and heating (DG-CHP) and cogeneration of hydrogen at over 90% total energy efficiency for integrated operations. Hydrogen can then be utilized to produce ammonia for storing wet biomass via ensiling, low cost and low temperature biomass pretreatment via aqueous ammonia-ethanol steeping, and clean fractionation platforms. Acid-free pretreatment and clean fractionation can provide over 97% pure cellulose, hemicellulose and lignin fractions which enhances efficiency of hydrolysis. Lignin, which is not fermentable, can be developed into high value derivatives for use in the industrial biochemicals market or converted into hydrocarbon fuels. Nutraceuticals and derivative chemicals found in lignocellulosic biomass -- such as omega-3 and GLA supplements, furfural, HMF, xylitol, D(-) lactic acid, succinic acid, other organic acids and low molecular weight lignin – are giving birth to new high value derivatives which can be utilized in food, bioplastics, medical and industrial manufacturing. The remaining cellulose and hemicellulose can then be exposed to separate pre-hydrolysis, state-of-the-art simultaneous saccharification-fermentation of pentose and hexose sugars, consolidated processing, and state-of-the-art distillation (membrane assisted vapor stripping), allowing for optimizing efficiency and yields for integrated biorefineries. This is particularly true for production of hydrous ethanol, a hydrogen-rich energy carrier which is compatible with existing petroleum infrastructures. Hydrous ethanol containing as much as 50% water can be converted into hydrogen-rich fuels (via catalytic and plasmatron reforming technologies) which contain almost as much energy as neat gasoline, but with a much smaller environmental footprint and a substantially greater net-energy gain based on well to wheel analyses.

In addition to using ammonia as a disinfectant and value added source of free amino nitrogen for ethanologens during fermentation at integrated biorefineries, ammonia can also be utilized to produce synthetic nitrogen fertilizers for supplementing organic fertigation streams. For integrated operations which implement closed loop biomass, liquid organic fertilizers can more than double conventional crop yields in conjunction with precision agriculture including sub-surface drip fertigation (SDF) and high density relay cropping systems capable of producing over

50 tonnes per acre of food and energy crops (biomass) compared to 8 tonne/acre which is based on the current US national average of 160 bu/acre of corn.

### ***Year Round Production of Biomass via Controlled Environment Agriculture***

Anaerobic digester effluents and thermal integration using waste heat from the biorefinery reduces production costs for year round operations while simultaneously producing biomass and food crops year round via CEA including hydroponic greenhouses and algal bioreactors.



***Figure 7. Year Round High Density Production via CEA & Closed Loop Production***

In contrast to terrestrial plants, CEA applications can produce substantial volumes of biomass per acre:

- Duckweed, over 453 tonnes of lignin-free biomass via separate hybrids containing up to 45% DM protein or 75% starch ([Cheng & Stomp, 2009](#))
- Water Hyacinth, over 333 tonnes of lignocellulosic biomass
- Algae, over 441 tonnes of lignin-free biomass (containing up to 60% lipids)
- Cyanobacteria, produce ethanol directly as a waste product of metabolism (Algenol, etc.)

Similar to duckweed, water hyacinth and algal biomass could be produced using nutrients contained in wastewater such as anaerobic digester effluents from processing animal waste. Capturing carbon dioxide (CO<sub>2</sub>) emissions from the integrated biorefinery operations allows for CO<sub>2</sub> enrichment via CEA which can substantially increase biomass yields for duckweed, water hyacinth and algae with potential to achieve production of up to 500 tonnes per acre via year round production. Hydroponic greenhouses for production of duckweed, water hyacinth and hybrid algal bioreactors can potentially utilize photoheterotrophic production to maximize bioenergy potential in conjunction with plant microbial electrolysis for value added production of either biomethane or biohydrogen. Equal portions of water hyacinth and algal biomass can be mixed with animal waste and other sources of organic waste for state-of-the-art anaerobic digestion and processing into biogas (biomethane and biohydrogen), high value compost, and liquid organic fertilizers with energy conversion efficiencies rivaling that of fermentation technologies.

CO<sub>2</sub> is produced during fermentation, during anaerobic digestion, combustion processes, and in the exhaust of high temperature fuel cells which utilize biomethane and bioethanol feeds. For example, a 100 MGY ethanol biorefinery annually produces 468,900 tonnes of CO<sub>2</sub> from

fermentation alone. Generally speaking, for each tonne of ethanol produced, a tonne of CO<sub>2</sub> is also produced. Utilizing this for CO<sub>2</sub> enrichment in conjunction with nutrient rich fertigation streams for CEA can increase biomass production by over 62%. Based on producing 1.9 tonnes of biomass from each tonne of CO<sub>2</sub> that is recycled results in annual production of an additional 890,910 tonnes of biomass. Based on production of 125 gal/tonne of lignocellulosic biomass, this would result in production of an additional 111 MGY of ethanol. Based on 60% oil content of algal biomass and 90% extraction, this would result in production of 138 MGY of biodiesel and either 44.5 MGY ethanol or 356,364 tonnes of starch-protein meal for use in livestock feedstuffs.

In contrast to terrestrial plants, CEA applications for water hyacinth can produce up to 333 tonnes of lignocellulosic biomass per acre, and 441 tonnes of lignin-free duckweed (containing up to 50% starch [biorefinery feedstock] or 40% quality protein [animal feed]) and algal biomass (containing up to 60% lipids) per acre via year round production. CO<sub>2</sub> enrichment via CEA can substantially increase biomass yields for water hyacinth and algae with potential to achieve production of 500 tonnes per acre. For hydroponic greenhouse applications, recycling 50% of CO<sub>2</sub> produced by an integrated biorefinery could allow for producing 815,182 tonnes of lignocellulosic feedstocks.

### ***Municipal BioEnergy Complexes***

In addition to processing animal waste, municipal solids (sewage and food processing waste, etc.) can also be used as an economical biofuel feedstock. On 6 Oct 2009, [Qteros](#), a Marlborough, MA based advanced biofuels company, and [Israel-based ACT](#) announced a joint development project to use feedstock produced from [municipal wastewater solids and turn it into a cellulosic ethanol product](#).

ACT takes the solid sewage from municipal wastewater plants — a process it spent six years developing — and recycles it into a feedstock called recyllose. Recyllose feedstock has a high cellulose content and low moisture, which in turn makes ethanol production more efficient, according to a Qteros statement. Recyllose happens to be low in lignin which is not fermentable and which inhibits conversion of lignocellulosic biomass to ethanol. The low lignin content in recyllose improves cellulosic plant efficiency by 20%, compared to higher lignin content of other feedstocks.

Municipal BioEnergy Complexes which simultaneously produce biomethane, carbon dioxide, and nutrient rich algal biomass from municipal waste water can be integrated with state-of-the-art biodiesel refineries. Similar to the production capacity for each AU to produce biomethane and crops, the same is true on a *per capita* basis. Including municipal wastewater, food processing, and other industrial waste resources, the organic waste produced *per capita* is equivalent to about 9-17 cu. ft. of biogas per day. Depending on the efficiency of organic waste collection and the anaerobic digester technology utilized, biogas usually consists of between 50-65% biomethane (CH<sub>4</sub>), 34-49% carbon dioxide (CO<sub>2</sub>), and less than 1% hydrogen sulfide (H<sub>2</sub>S) and ammonia (CH<sub>3</sub>). Each cu. ft. of biogas, based on 65% biomethane, is equivalent to about 650 BTU ([King County Fuel Cell Demonstration Project. In cooperation with the U.S. Environmental Protection Agency and FuelCell Energy Inc.](#)).

## **Diesel BioEnergy Complex Closed Loop Food, Biofuels & Clean Power Generation**



The primary advantages of using electrochemical conversion technology of high temperature fuel cells over thermal combustion processes of coal and natural gas turbines are the increases in total energy efficiency for CHP applications. It is considerably more energy efficient to produce heat as a byproduct of power generation than *visa versa*. Though rankine heat engines can utilize thermal energy via high temperature fuel cells to produce additional power, for integrated biorefineries this source of heat may be more efficiently utilized to provide process steam for biorefining. Fuel cells also provide dramatic reductions in GHGEs and air pollution. For fuel cells operating on 99.9% pure biomethane and/or ethanol feeds, NO<sub>x</sub> emissions are essentially eliminated. In a comparison of high temperature fuel cells operating at 90% total energy efficiency with conventional natural gas or coal CHP systems operating at 49% total energy efficiency ([CHP for the Ethanol Industry, EPA](#)), GHGEs are reduced by over two fold. In addition to federal incentives which reduce capital investment costs, EPA provides a list of [state incentives and funding opportunities for CHP](#) applications.

### ***Maximizing Biogas & DG-CHP-H<sub>2</sub> Production***

In addition to processing animal waste, the relatively high protein and lipid content of animal offal from slaughter/packing processes and lignin-free cellulose content of duckweed and algal biomass can potentially increase quantity and quality of biogas production by 2-3 fold. The maximum estimate for biogas produced per AU is based on daily production of 100 SCF at 80% methane from animal waste via state-of-the-art animal waste collection, pretreatment, and thermophilic HSAD technology. This is equivalent to producing 29 MBTU per AU. However, average biogas production per AU is currently only 10 MBTU. This is due primarily to inefficient collection of animal waste followed by inadequate processing. In addition to processing animal waste, mixed vegetative or animal substrates high in lipid concentrations such as animal tallow, carbohydrates and protein from food processing waste, offal and algal biomass can produce biogas containing as much as 79% methane ([Ghose et al., 1979](#); [Busch et al., 2009](#)). Processing equal volumes of animal waste, algal biomass and water hyacinth via anaerobic digestion would allow for more than tripling biogas production and subsequent DG-CHP-H<sub>2</sub> for integrated biorefineries.

Up to 93% of methane in lipid-rich feedstocks can be recovered ([Cirne, et al., 2006](#)) via mesophilic or thermophilic anaerobic digesters ([Angelidaki et al., 1990](#)). Thermophilic digesters typically decrease hydraulic retention time (HRT) by 20% and provide pathogen free organic fertilizers and nutrient-rich fertigation streams for use in closed loop production of high value functional foods and biomass. Some studies reveal that utilizing the same biomass feedstock produced on dry lands, anaerobic digestion can produce more energy than that which results from production of biodiesel via conventional FAME processing ([Gunaseelan, 2009](#)). For low cost production and processing of aqueous feedstocks such as algae and water hyacinth, energy gains would be much greater while avoiding capital and energy intensive biorefining of advanced biofuel feedstocks. Hence, algal biomass rich in carbohydrates and lipids appears to be an ideal feedstock for mixing with animal waste substrates for low cost production of biomethane.

With 90% of energy inputs at a typical ethanol plant coming from natural gas or coal, displacing these fossil fuels with renewable biomethane could capture over \$6 million per year in carbon credits and production premiums for producing 50 MGY of ethanol at integrated biorefineries.

With the demand for price-volatile natural gas, which is expected to increase 66% over the next 25 years, biomass and production of biomethane which displaces natural gas is a long-term, physical hedge that biofuel producers can implement to manage risk and increase net energy gain. Using biogas also reduces the carbon footprint and boosts local economies. There are multiple sources of funds available to assist with financing, revealing that these operations can be funded primarily with government money and very little equity.

### ***Federal Funding Opportunities***

Federal funding options include an “open-loop” biomass investment tax credit that can be used to recover 30% of capital costs and a combined heat and power (CHP) investment tax credit can be used to recover 10%. There are also closed loop investment tax credits available and a new market tax credit available to capture \$2 million for every \$10 million in capital expenditures. For loan guarantees, the US DOE offers 100% loan guarantees on 20% equity and the USDA offers loan guarantees of up to \$25 million. The Rural Energy for America program offers up to \$25 million in loan guarantees or \$500,000 grants, and has money available for completing feasibility studies. There is also the opportunity to depreciate the assets over five years and many states have additional incentives available ([Biomass Magazine, Jun 2009](#)). Federal incentives are also available for replacing gas-turbine combustion systems with innovative high temperature fuel cells that provide distributive generated combined cooling heating and power (DG-CCHP) and operate on refined biogas or biomethane feeds as described above.

### ***Reducing Energy Consumption***

In addition to providing process heat which comprises 92% of total energy consumption for conventional dry mill ethanol plants, the electrical power requirement (8%) is 0.75 kWhr/gal of ethanol ([Mueller and Cuttica, 2007](#)). This is equivalent to requiring 8.6 MW per 100 MGY for conventional ethanol production. Based on reducing electrical power requirements for integrated biorefineries to 5 MW per 100 MGY of ethanol produced, up to 86% of the power produced, (e.g. 32 MW per 100 MGY) from animal waste and food processing waste can be sold to the local power grid. CHP providing 90% total energy efficiency provides sufficient process steam and heat for closed loop systems which simultaneously produce biofuels, bioenergy, food, biochemicals and bioproducts. Closed loop architecture results in reducing or eliminating economic dependence on importing petroleum products.

In comparison with gas turbines, energy efficiencies can be vastly improved for integrated biorefineries and bioenergy complexes which capitalize on economies of scale for converting biomethane to combined cooling, heating and power (CHP) via high temperature fuel cells. This requires refining biogas for use of nitrogen-free and sulfur-free biomethane feeds for molten carbonate (MCFC) or phosphoric acid (PAFC) fuel cells providing distributed generated CHP (DG-CHP) with total energy efficiencies exceeding 90% via thermal integration. This is the most efficient use of this bioenergy resource which is produced from organic waste. Refined biogas allows for closed loop production at integrated biorefineries which provides unprecedented efficiencies of operation, ushering in a paradigm shift to distributed bioeconomies in which wholesome functional foods, nutraceuticals, pharmaceuticals, bioproducts (including bioplastics), industrial biochemicals and bioenergy (including biogas, biofuels and bioelectricity) are produced simultaneously. External energy inputs account for over 20% of biorefinery production costs for conventional starch processing and distillation technologies, and as much as 38% for processing lignocellulosic feedstocks. For integrated biorefineries, the use of fossil fuels and grid power could essentially be eliminated.

### ***Reducing Green House Gas Emissions***

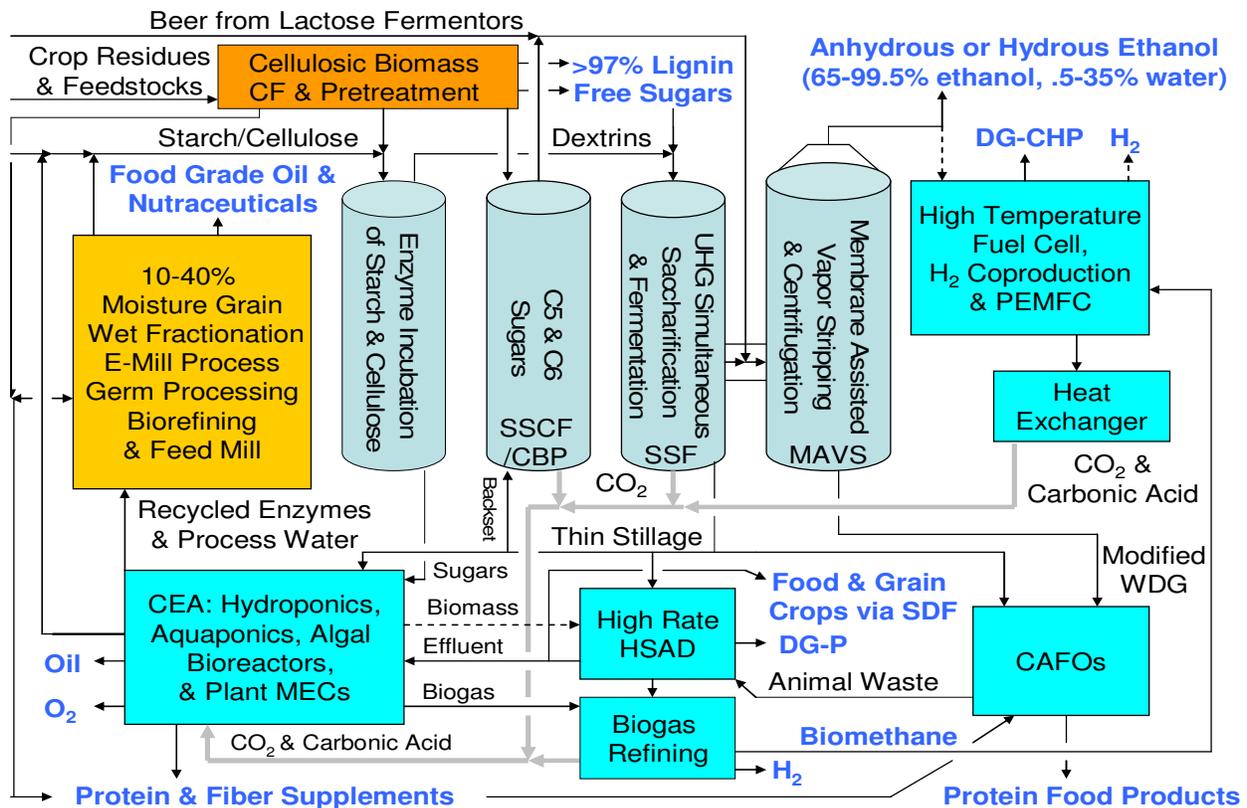
According to US Department of Energy studies conducted by the Argonne Laboratories of the University of Chicago, one of the benefits of cellulosic ethanol is that it reduces greenhouse gas emissions (GHGEs) by 85% in comparison with reformulated gasoline. In addition to reducing GHGEs, recycling these valuable resources dramatically enhances industrial production, increases the efficiency of renewable energy technologies, and replenishes oxygen to the atmosphere via incorporation of closed loop biomass.

The “well to wheel” analysis referred to above reveals that conventional corn ethanol provides 20-30% reductions in GHGEs compared with gasoline. Cellulosic ethanol's favorable profile stems from using lignin, a coproduct used to either fuel the biomass conversion process or provide valuable biochemicals for production of bioplastics which displace petroleum products. Lignin is a renewable fuel with no net greenhouse gas emissions. Cellulosic ethanol reduces fossil fuel use by 90% ([Wang, 2005](#)). A similar reduction in GHGEs for corn ethanol production is achieved by feeding nutrient-rich ethanol coproducts (DGs and fermentation residues) to animals. Animal waste is then processed into biomethane and organic fertilizers which displace natural gas and petroleum derived chemicals and synthetic fertilizers.

### ***Product Diversification via Integrated Biorefineries***

Nutraceuticals and derivative chemicals found in lignocellulosic biomass -- such as furfural, HMF, xylitol, D(-) lactic acid, succinic acid, other organic acids and low molecular weight lignin -- give birth to new high value derivatives which can be utilized in food, bioplastics, medical and industrial manufacturing. For example, lignin derivatives can be utilized to produce high value bioplastics. One of the biochemicals used to produce furfural can be used in the construction of artificial bones that the human body is unlikely to reject in the medical field. The biochemical derived from precursors of furfural retails for \$1,000/lb, as opposed to ethanol which retails for about \$0.20/lb. Similarly, omega-3 fatty acids obtained from algal biomass retail for over \$4,500/gal (\$600/lb). This is in comparison with biodiesel which retails for about \$3-\$4/gal (\$0.40-\$0.53/lb). The nutraceutical astaxanthin, a super antioxidant also obtained from potentially producing and processing green microalga *Haematococcus pluvialis* biomass or the red yeast *Phaffia rhodozyma* ([Nghiem et al., 2009](#)) at integrated biorefineries retails for over \$1,700/lb. Production of omega-3s and astaxanthin (nutraceutical and pharmaceutical grade products) represent an exponential increase (150-850 orders of magnitude) in value in comparison with liquid biofuels.

## **Ethanol BioEnergy Complex Closed Loop Production of Functional Foods, Bioproducts & BioEnergy**



*Figure 9. Ultra High Gravity Starch SSF, Split-Stream SSCF & CBP  
Integration of High Temperature Fuel Cells & MFC/MEC BioEnergy Technologies*

Obviously, there are strong economic and environmental advantages for integrating production of high value foods and other coproducts with production of commodity biofuels, all of which displace petroleum products and reduce economic dependence on fossil fuels. This can be accomplished via integrated biorefineries which simultaneously reduce pollution and GHGs.

### ***Integrated Biorefinery Platform***

The above concepts invalidate the food vs. fuel argument and illustrate how production of food and biofuels are synergistic via integrated biorefineries and bioenergy complexes which incorporate closed loop architecture. Pollution and GHGs are virtually eliminated via efficient processing of corn stover and other second generation lignocellulosic feedstocks which comprise an abundance of crop residues and organic waste. Third generation algal biomass feedstocks (which are lignin free and have limited cellulose in cell walls) require little or no pretreatment and can be more economically processed than lignocellulosic feedstocks. Vertically integrated production of biomass feedstocks and bioenergy via integrated biorefineries which incorporate closed loop architecture is the best risk management strategy available for coping with volatile pricing of commodity markets.

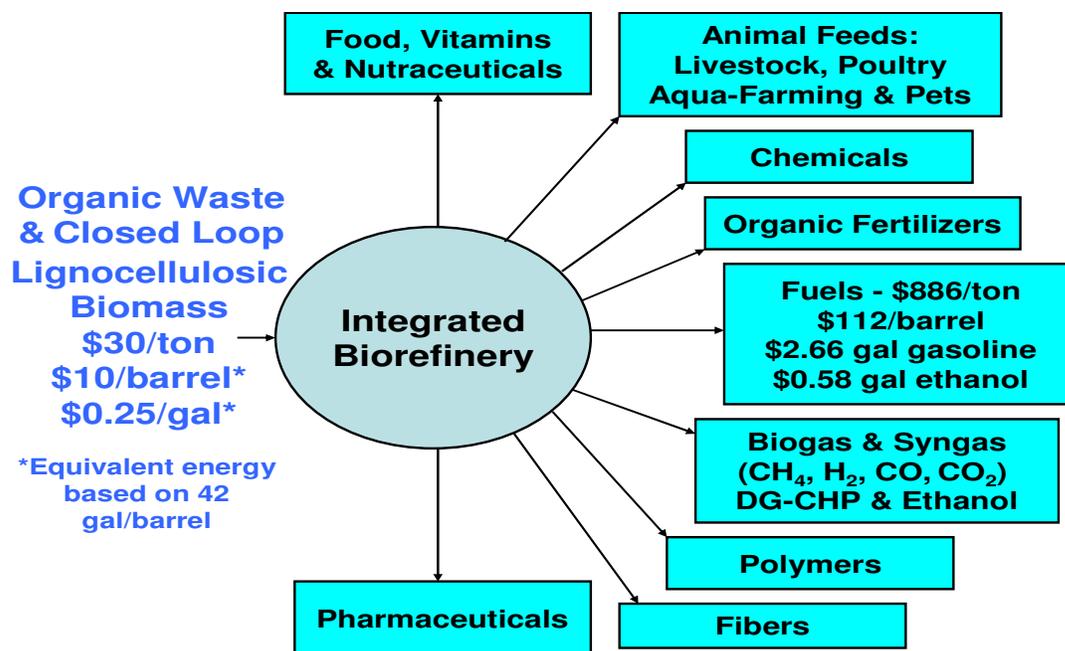


Figure 10. Integrated Biorefinery Platform Providing Diversification & Flexibility

### ***Comprehensive Nutrient-Energy Management***

Comprehensive nutrient-energy management via integrated biorefineries allows for comfortably reaching the US mandate for biofuels while simultaneously producing high quality foods and bioproducts including functional foods (aquaculture, poultry eggs, swine, beef and dairy products enriched with nutraceuticals), vitamins, nutraceuticals, pharmaceuticals, organic fertilizers, adhesives, surfactants, and bioplastics for displacing petroleum products, all of which are biodegradable. This is possible through development of economical separation technologies and biorefining platforms which are very similar to those employed at petroleum refineries, but which process an abundance of renewable agricultural byproducts and organic waste instead of limited supplies of crude oil. In order for production of biofuels to be sustainable, high value functional foods, nutraceuticals, pharmaceuticals, and other high value coproducts must be simultaneously produced in order to offset costs of producing feedstocks and biorefining. In addition to providing diversification and flexibility for maximizing revenue streams, closed loop production at integrated biorefineries allows for achieving unprecedented efficiencies of operation, the revenues of which are not subject to the volatility of commodity markets regarding cost of feedstocks and energy resources which currently comprise over 90% of production costs.

As illustrated in the flow chart for an integrated biorefinery (Figure 11), fermentation of pentose sugars contained in hemicellulose could be conducted via split-stream feeds of dextrin/cellodextrin and hemicellulose substrates for optimizing efficiency of production. Research reveals that hexose sugars substantially improve fermentation efficiency and yields for processing pentose sugars. As suggested above, thin stillage from starch and cellulose fermentations are used as nutrient-rich backsets for fermentation of pentose sugars contained in hemicellulose. Thin stillage from pentose fermentations would then be utilized along with anaerobic digester effluents (which process organic waste including food processing waste and animal waste), carbonic acid and CO<sub>2</sub> as value added fertigation streams which qualify as organic fertilizers. These fertigation streams would be used for closed loop production year

round via controlled environment agriculture (CEA). Plant microbial electrolysis cells would be integrated with CEA including hydroponic greenhouses and algal bioreactors, providing thermal integration and simultaneous production of biomethane and/or biohydrogen. Biomethane would be utilized to produce grid-connected DG-CHP via high temperature fuel cells operating at over 90% total energy efficiency.

### ***Leveraging Existing Ethanol Infrastructure***

Utilizing fractionation and high temperature fuel cell DG-CHP & H<sub>2</sub> cogeneration technology, a 60 MGY ethanol plant can be converted into a 182 MGY Ethanol BioEnergy Complex capable of producing 218 MW of grid connected distributive generated power, 292 MBTU/hr for process steam and thermal integration, and 47,692 tons H<sub>2</sub> or 269,937 tons NH<sub>3</sub>. Total energy produced by DG-CHP & H<sub>2</sub> cogeneration for an Ag-Energy Park Complex amounts to 14,917,986 MBTU. In addition to the DG-CHP & H<sub>2</sub>, production of E100 (anhydrous ethanol) would be equal to 19,667,798 MBTU; hydrous ethanol 3% water (HE100-93) would be equal to 26,626,783 MBTU; catalytic/plasmatron reforming technology would allow for producing 54% more HE100-65 fuel consisting of 32,204,224 MBTU; and catalytic reforming technology for HE100-50 fuel would increase volume of fuel by 100%, consisting of 48,729,513 MBTU. The DG products from the ethanol biorefinery would allow for annual production of 111.56 million lbs of natural beef/dairy products rich in omega-3 fatty acids and GLA. Two to three times larger volumes of enriched meats (functional foods) from poultry and swine (which have higher feed to conversion ratios than cattle) could be produced from distillers grains and algae meal products. Animal waste along with equal portions of water hyacinth and algae substrates produced via closed loop biomass would provide 80% methane biogas and value added organic fertigation streams for year round production of biomass and either biomethane or biohydrogen via CEA and plant MEC technologies. The biomethane and/or biohydrogen produced by the plant MECs would in addition to the biomethane produced via anaerobic digestion.

### ***Closed Loop Systems Architecture & Integrated Biorefineries***

Closed loop systems architecture including precision agriculture and SDF would allow for internally producing feedstocks that would be processed via low energy ammonia ensiling of wet biomass feedstocks followed by aqueous ammonia-ethanol pretreatment and fractionation. The lignin fraction could be combusted via fluidized bed boilers to provide additional process steam and thermal integration, or it could be processed into high value low molecular weight derivatives for the bioplastics and industrial biochemical industries. The result of fractionation and increasing fermentation efficiency by up to 70%, implementing closed loop architecture, and increasing volume of biofuel production by up to 100% using hydrous ethanol as a hydrogen-rich energy carrier dramatically reduces production costs while simultaneously increasing product sales for integrated biorefineries. Hence, not including amortization of capital investments, it is theoretically possible to produce biofuels for less than \$0.50 gal, provide grid connected power for less than \$0.04 kWh, produce all natural functional foods for less than 50% of current production costs, and provide a myriad of high value coproducts including nutraceuticals, pharmaceuticals, and industrial biochemicals at integrated biorefineries.

[BioEnergy International](#) and [Verdezyne](#) are developing technologies for the next-generation of integrated biorefineries. BioEnergy claims that its unique patented microorganisms and proprietary process technology will transform the current hydrocarbon-based manufacturing processes across a variety of industries enabling the replacement of a barrel of crude with plant-based sugars to make everything from plastics and fabrics to transportation fuels. Discoveries

include D(-) lactic acid, succinic acid, butanol, butanediol, propanediol, adipic acid, and HPA. Production of D(-) lactic acid has already been commercialized and commercialization of succinic acid is currently under development for use in the bioplastics industry and also for replacing a host of petro-chemicals in other industries. BioEnergy is a good example of how private companies, universities, and federal research labs in the US are collaborating, enabling them to become global leaders in the development and commercialization of the next generation biorefineries. These integrated biorefineries will produce high-value bio-based chemicals and fuels from renewable feedstocks through the use of biocatalysts including synthetic biology.

## **Introduction**

### ***Processing High Moisture Biomass Feedstocks***

Fresh biomass in its natural form (whether it is comprised primarily of plant matter or organic waste) usually contains over 70% moisture. Due to the energy intensive process of removing water from biomass, high moisture feedstocks are most efficiently processed via biochemical conversion technologies in contrast to conventional thermochemical conversion technologies (such as pyrolysis and gasification followed by a series of catalytic, water-gas shift and hydrogenation reactions, etc.). However, an innovative catalytic fast pyrolysis technology ([Carlson et al., 2008](#)) which eliminates production of tar may eventually become economical for converting low-moisture biomass feedstocks such as lignin, wood chips, and straw from cereal grain crops such as wheat and barley into bioenergy and industrial biochemicals. Integrated biorefineries may actually utilize a combination of biochemical and thermochemical conversion technologies (for producing process steam from combustion of lignin) in order to efficiently process a wide variety of low and high moisture biomass feedstocks via closed loop systems architecture. Closed loop systems conserve water, energy, agricultural lands, and nutrients, thus optimizing production efficiencies while simultaneously reducing pollution and greenhouse gas emissions (GHGs).

A current example of processing low moisture feedstocks is illustrated using [ZeaChem](#) technology. Using existing technologies this company has developed a hybrid process of biochemical and thermochemical processing that preserves the best of both technology approaches from yield and efficiency perspectives. After fractionating dry biomass (hard or softwood chips, corn stover, switchgrass), the sugar stream consisting of pentose and hexose sugars is sent to fermentors where an acetogenic bacterial process is utilized to convert sugars to acetic acid without producing CO<sub>2</sub> as a byproduct. In comparison, traditional yeast fermentation produces one molecule of CO<sub>2</sub> for every molecule of ethanol produced. Thus the carbon efficiency of the ZeaChem fermentation process is nearly 100% vs. 67% for conventional yeast fermentations (systems architecture which recycles CO<sub>2</sub> for closed loop biomass can also approach 100% carbon efficiency for integrated biorefinery operations). The acetic acid is converted to an ester which can then be reacted with hydrogen to make ethanol. To get the hydrogen necessary to convert the ester to ethanol, ZeaChem takes the lignin residue from the fractionation process and gasifies it to create a hydrogen-rich syngas stream. The hydrogen is separated from the syngas and used for ester hydrogenation and the remainder of the syngas is combusted to produce steam and power for the process.

### ***Integration of Biochemical & Thermochemical Processes***

The net effect of combining biochemical and thermochemical processes is that about 2/3 of the energy in the ethanol comes from the sugar stream and 1/3 comes from the lignin steam in the form of hydrogen. At an expected Nth plant yield of 135 gallons per dry ton (based on no moisture in biomass feedstocks), the process is nearly balanced with the necessary steam and power generated from the non-hydrogen portion of the syngas stream. ZeaChem claims that they can produce 2,000 gal/acre from poplar trees with production costs as low as \$0.15/gal for ethanol based on paying \$20/ton for dry wood feedstocks FOB the biorefinery ([Chambers, 2009](#)). Unfortunately, the key to this hybrid process which utilizes gasification is the requirement for dry biomass feedstocks which are mechanically converted into wood chips. Removing moisture from biomass, mechanical processing, water gas-shift and hydrogenation reactions are all energy intensive operations which are often inefficient. Hence, economical processes for

processing wet biomass via engineering of recombinant biocatalysts will continue to be required for most biorefinery feedstocks in order to maximize production efficiencies.

### ***Closed Loop Biosystems Architecture***

Biosystems architecture is essential in order to maximize production efficiencies for simultaneously producing food and biofuels while achieving sustainability for biorefining and bioenergy industries. Several technologies allow for increasing efficiency of producing hydrolysate/sugar substrates from biomass and subsequent refining in order to optimize yields. These include;

- reducing particle size of substrates to increase surface area of feedstocks (thus enhancing enzymatic and microbial access)
- economical storage and pretreatment of wet biomass such as ammonia ensiling and enzyme inoculation
- low-cost and acid-free fractionation platforms
- plant expressed enzymes
- novel hydrolysis and fermentation technologies
- symbiotic microbial cultures
- efficient organic waste processing
- high temperature fuel cells (DG-CHP) which operate on biomethane produced from organic waste resources including biomass (solar fuel cells)
- state-of-the-art water-ethanol membrane separation technologies (MAVS technology)
- similar membrane technologies for separation of biochemicals and toxic components in biomass substrates used for fermentation.

Fermentation technologies include ultra high gravity (UHG-increased starch/sugar concentrations), improved enzymes, optimization of nutrient processing and delivery for microbes, controlled fermentation environments including the use of innovative biofeedback systems, ongoing development of synthetic microbes, and use of antibiotic-free disinfectants and sanitizing processes between fermentation batches.

### ***Biorefinery Sanitation Processes***

An example which illustrates the importance of systems architecture is choice of disinfectants for long term use at biorefineries. Bacterial infections compete with ethanologens for processing of sugars and produce acetic acid. Acetic acid inhibits yeast functioning and thus decreases efficiency of ethanol fermentations. In addition to using disinfectants, optimizing fermentation environments for actively growing yeast and reducing fermentation times via UHG naturally competes with bacterial growth, thereby reducing production of acetic acid. Choice of incompatible disinfectants to combat bacteria and other contaminants between batches and during fermentations can result in inefficient fermentations and contamination of biofuels, distillers grains (DG), fermentation residues, and other bioproducts and biochemicals produced as high value coproducts for integrated biorefineries. Coproducts which are not entirely subject to the volatility of commodity markets are essential for diversification and sustainability of the biorefining and bioenergy industries.

Compatible disinfectants which are available for use at integrated biorefineries include chlorine dioxide [ClO<sub>2</sub>] for low ethanol level blends ([Phibro Ethanol Performance Group, Apr 2009](#)). For mid to high level ethanol blends, ammonia [NH<sub>3</sub>], quaternary ammonium compounds, and ozone

[O<sub>3</sub>] reduce bacteria growth and subsequent production of contaminants. For example, current ASTM specs tightly regulate the amount of inorganic chlorides permitted in E10 ethanol-gasoline blends to 1 ppm (1 mg/kg). E85 has the same limit of 1ppm. Regulations on chloride limits in denatured fuel alcohol are 10 ppm, thus making all ethanol blends which utilize ClO<sub>2</sub> as disinfectants consistent with the 1 ppm limit in E10, but inconsistent with E85 limits. Chloride must also be removed before using ethanol in fuel cells.

In addition to using disinfectants which may contaminate biofuels, about 55% of ethanol producers use antibiotics to eliminate bacterial growth during fermentations. This is a primary concern for ethanol producers. In contrast to using antibiotics, antibiotic-free fermentations and disinfectants eliminate concerns for contamination of protein-rich DG and fermentation residues used in animal feeds which provide substantial revenue streams as coproducts of biorefineries. Several alternative options which do not contain antibiotics or other potential contaminants are available. For example, in contrast to using ClO<sub>2</sub>, NH<sub>3</sub> is a biological agent which is considered a harsh disinfectant. Hence, aqueous NH<sub>3</sub> solutions may be ideal for use in industrial fermentation applications. NH<sub>3</sub> simultaneously kills bacteria and provides free amino nitrogen (FAN) as a value added nutrient for biocatalysts. FAN is an essential nutrient which optimizes metabolic function of ethanologens and when used properly does not contaminate DG or fermentation residues which are used in livestock feeds. In fact, NH<sub>3</sub> is often used to enhance the quality of low-value ruminant feeds which contain lignocellulosic fiber. Similar to providing FAN nutrients for microbes during ethanol fermentations, NH<sub>3</sub> enhances microbial fermentations which occur in the rumen of cattle and sheep.

### ***Renewable Ammonia***

Renewable ammonia can be produced as a coproduct of hybrid anaerobic digesters (bacterial fermentation) equipped with an aerated-submerged biofilter for *in situ* ammonia removal. Removing ammonia enhances solids destruction and increases biomethane production by 26% from food processing waste during anaerobic digestion ([Wang et al, 2003](#)). For integrated biorefineries and municipal bioenergy complexes which process organic waste and/or algal biomass, ammonia is produced as a coproduct of methane and CO<sub>2</sub>. The concentration and volume of ammonia produced is usually a result of excess of protein in substrates, e.g. less than or greater than an ideal carbon to nitrogen ratio of 30:1. Depending on the efficiency and volume of ammonia removal, the removed ammonia could be utilized as a value added nutrient for fermentations and as a disinfectant between batches. This process could substantially reduce biorefinery production costs via closed loop systems architecture. However, since ammonia (gas) is originally in the form of ammonium (liquid) during the anaerobic digestion process, the most efficient and practical way to remove ammonia/ammonium cations may be through the continual removal of effluent via high rate anaerobic digesters such as the induced blanket reactor (IBR) technology and modified high solids anaerobic digestion (HSAD). In this way, ammonium is utilized as a value added nutrient contained in fertigation streams for closed loop biomass production.

Large scale renewable ammonia can be produced from biohydrogen using biomethane as a feedstock for steam reforming or using electrolysis (water electrolysis or microbial electrolysis cells - MECs) as a source of hydrogen in conjunction with nitrogen derived from process air (the atmosphere contains almost 80% inert N<sub>2</sub>) at integrated biorefineries ([Wikipedia, Ammonia Production](#)). Alternatively, high temperature fuel cells can coproduce hydrogen from biomethane via integration of proton exchange membrane technology. Electric efficiency of the fuel cell is

reduced from 47% to 37% for the same amount of fuel utilized. For MCFC-H<sub>2</sub> units, 7.21 MBTU/hr fuel feeds (methane or ethanol) would produce .8 MW of power generation, 1200 lbs/day of H<sub>2</sub>, and 1.34 MBTU of heat recovery per MW of power generation for thermally integrated operations.

The Haber-Bosch process is the industry standard for large scale catalytic synthesis of ammonia from elemental hydrogen and nitrogen gas (which elements are abundant and inexpensive reactants). In the presence of osmium and uranium catalysts, unreactive gaseous nitrogen (N<sub>2</sub>) and hydrogen (H<sub>2</sub>) are combined into ammonia at a temperature of about 500 °C and a pressure of approximately 204 atmospheres (atm) [3,000 pounds per square inch (psi)]. Although the Haber-Bosch process has been modified since its early development, mostly to improve processing efficiencies, it remains the most popular technology utilized by ammonia production facilities. Since conventional ammonia production depends on producing hydrogen from natural gas, the price for purchasing ammonia is positively correlated to the price of natural gas. However, the Haber-Bosch process requires production on an economy of scale in order to be sustainable which may or may not be economical for integrated biorefineries depending on the size of the operation and commodity prices for natural gas, ammonia and synthetic nitrogen fertilizers.

While the use of ammonia and ammonium nitrogen as a fertilizer has brought enormous benefits, the loss of fertilizer nitrogen to the environment has detrimental side effects including reduced biodiversity and the formation of marine algal blooms which result in devastating hypoxia. Nitrogen compounds from nutrient loading, nutrient leaching into the soil contaminate groundwater aquifers and undermine the quality of drinking water. Ammonia and nitrous oxide emissions contribute to air pollution and climate change, affecting quality of life and well being of entire ecosystems ([Erisman et al., 2008](#)).

Future scenarios suggest that such problems will become more extreme, with potential for doubling fertilizer use predicted over the coming century. This demand is partly driven by the growing requirement for "nitrogen-hungry" biofuels in addition to feeding growing world populations. These environmental challenges highlight the need for a new invention, as transforming as the Haber-Bosch process that will benefit both society and the global environment. The Haber-Bosch process is perhaps the most significant invention of the 20th century, yet it has many side effects. Ecological scientists now claim that we need a new invention that provides similar technological advances, but without the environmental impacts. Processing organic waste into renewable biomethane and organic fertilizers for closed loop production of functional foods and bioenergy provides a potential solution. However, small amounts of synthetic nitrogen fertilizers produced from ammonia will probably still be required for enhancing organic fertilizers in order for closed loop production of functional foods and energy crops to be sustainable. Urea sulfuric acid is injected into fertigation streams to optimize soil pH and increase bioavailability of nitrogen and sulfur compounds for plant absorption.

Biohydrogen produced by plant MECs and cogeneration of hydrogen via high temperature fuel cells provide the opportunity for closed loop production of ammonia at integrated biorefineries. The simultaneous production of DG-CHP and cogeneration of biohydrogen from biomethane feeds is substantially more efficient than synthesizing hydrogen from natural gas feedstocks. In the US, almost 80% of ammonia is utilized primarily to produce nitrogen fertilizers for production of food and energy crops, followed by production of plastics, synthetic chemicals,

and explosives. Ammonia is also utilized in the pharmaceutical industry. Due to low natural gas prices and inexpensive labor in third world countries, the US has become increasingly dependent upon importing ammonia and nitrogen fertilizers since the year 1992 ([USGS-Nitrogen, 2004](#)).

### ***Ammonia as a Hydrogen-rich Energy Carrier***

Ammonia is an effective hydrogen-rich energy carrier for efficiently storing and transporting hydrogen. In conjunction with producing ammonia for \$300/metric tonne and using MCFC or solar power inputs costing \$0.214/kW-h, producing hydrogen via ammonia hydrolysis requires 95% less energy and is 71% less costly to produce than using water electrolysis. Renewable electricity can be produced for \$0.07/kwh. Ammonia electrolysis is 8.2% less costly than producing hydrogen via natural gas reforming. Ammonia hydrolysis also has potential for use in wastewater treatment/remediation processes ([Botte, 2006](#)).

Ammonia is an ideal fuel feed for high temperature solid oxide fuel cells (SOFCs) which can cogenerate hydrogen. As with most fuel cells, H<sub>2</sub>S needs to be reduced in order to avoid sulfur poisoning of fuel cell stacks. This can be accomplished by optimizing efficiency of digestion and removing residual H<sub>2</sub>S in biogas via adsorbent beds or other means. Ammonia SOFC technology reduces production costs by using ceramic materials and simplifies operation of fuel cells. Direct use of NH<sub>3</sub> as a feed eliminates costly fuel processing equipment. SOFCs operate at higher temperature ranges (650-1,000 °C) than either MCFCs or PAFCs, and are generally more efficient than other fuel cell designs. Higher operating temperatures eliminate the need for using precious metal catalysts and capital intensive reforming for a variety of fuel feeds ([Patel & Petri, 2006](#)).

### ***USDA GRAS Labeling***

In regards to fermentations which are enhanced by the free amino nitrogen (FAN) content of ammonia, utilizing microbes, chemicals and biological agents which are US Federal Drug Administration (FDA) approved via the Center for Veterinary Medicine (CVM) as “generally recommended safe” (GRAS) is critical for production of high value foods, special ingredients, nutraceuticals, and pharmaceutical grade coproducts at both conventional and integrated biorefineries. In conjunction with GRAS labeling, in 2008 the FDA began testing distillers dried grains with solubles (DDGS) and found residues from four types of antibiotics in the ethanol coproduct which is sold as a livestock feed. The antibiotics found included erythromycin, tylosin and virginiamycin. These three types of antibiotics are the same as the ones that are used to treat human and animal disease. Feeding of livestock rations using distillers products which contain “excessive amounts” of these types of antibiotics can lower immune response to disease in animals and humans ([Ethanol Producer Magazine, Aug 2009a](#); [Ethanol Producer Magazine, Aug 2009b](#)).

### ***Characteristics of Processing Lignocellulosic Biomass***

When lignocellulosic biomass is broken down into individual components via fractionation and hydrolysis, cellulose and hemicellulose are converted to mixtures of sugars (e.g., pentoses including xylose and arabinose, hexoses including glucose and galactose, oligosaccharides, and polysaccharides). Plant cell-wall lignocellulose contains, in decreasing order of relative importance, glucose, xylose, arabinose, galactose, and various other minor sugars. Individually these sugars are most suitable for fermentation only when inhibitors (such as acetic acid produced during hydrolysis) have been removed. “Current best pretreatment processes are reasonably efficient at making the cellulose, hemicellulose, and lignin matrix amenable to

enzymatic hydrolysis and fermentation, but they also release a number of toxic compounds into the hydrolysate which inhibit the growth and ethanol productivity of fermentation organisms. Conditioning methods designed to reduce the toxicity of hydrolysates are effective, but add to process costs and tend to reduce sugar yields, thus adding significantly to the final cost of production. Reducing the cost of cellulosic ethanol production will likely require enhanced understanding of the source and mode of action of hydrolysate toxic compounds, the means by which some organisms resist the actions of these compounds, and the methodology and mechanisms for conditioning hydrolysate to reduce toxicity.” ([Pienkos and Zhang, 2009](#))

The pentose:hexose (C5:C6) ratio from different biomass substrates can range from ca. 70:30 to 15:85 ([University of Georgia Research Foundation](#)). After pretreatment, neutralization, and conditioning, fermentation can be initiated by addition of specific lignocellulosic enzymes (carboxymethyl cellulase [CMCase], endo-1,4-beta-D-glucanase, beta-1,4-glucanase, beta-1,4-endoglucan hydrolase, cellodextrinase, beta-glucosidase, alpha-glucuronidase, endo-beta-1,4-xylanase, beta-xylosidase, alpha-L-arabinofuranosidase, acetylxylan esterase, and feruloyl esterase) in conjunction with a biocatalyst (ethanologens including synthetic microbes) in a process called simultaneous saccharification and fermentation (SSF) (Gauss et al., 1976; Philippidis et al., 1993; Olofsson et al., 2008). Commercial enzymes or dilute acid processes are currently used to complete the depolymerization process in which cellulose (glucan), xylan and arabinofuran polymers are converted to simple sugars. These simple sugars are subsequently fermented to ethanol by microorganisms ([Mielenz et al, 2009](#)). However, enzymes currently represent upwards of \$0.20-\$0.30/gal in production costs for ethanol.

Eventually, synthetic *S. cerevisiae* and select bacterial ethanologens may be able to produce super enzyme cocktails which function under cool or moderate temperatures, thus reducing enzyme costs and paving the way for low energy bioconversion via SSF, simultaneous saccharification and cofermentation (SSCF), and consolidated bioprocessing (CBP) ([USDA-ARS, Superenzymes & Synthetic Yeasts](#)). Low processing temperatures also preserve protein in fermentation coproducts which are used as nutrient-rich animal feedstuffs. “An approach was designed to saturate potential integration sites in the yeast genome with gene cassettes of biomass-degrading enzymes. Yeast cointegrated with mixed gene constructs of alpha-amylase and glucoamylase resulted in the expression and secretion of these enzymes in active forms. The recombinant yeast acquired the capacity of starch hydrolysis with over 75% conversion. Yeast engineered with cellulase, xylanase, and ferulic acid esterase genes individually and their combinations also produced active enzymes capable of breaking down respective substrates.” ([Wong et al., 2009](#))

Since xylose constitutes roughly one-third of the available sugar in biomass, efficient utilization is essential to good process economics. While most companies are hoping to exploit genetically engineered microorganisms to ferment xylose, [Trillium Fiberfuel](#)'s unique approach uses an existing industrial enzyme to convert xylose into xylulose, a different pentose sugar that is fermentable by brewers yeast and other microbes. The difficulty of using this technology approach is that the conversion of xylose to xylulose (called isomerization) occurs at a different temperature and pH than fermentation. Xylulose is a ketopentose, a monosaccharide containing five carbon atoms, and including a ketone functional group. It has the chemical formula C<sub>5</sub>H<sub>10</sub>O<sub>5</sub>. In nature, it occurs in both the L- and D-enantiomers.

However, for separate hydrolysis and fermentation of xylose, this technological obstacle could potentially be overcome by fractionation platforms which cleanly separate hemicellulose from lignin and cellulose.

### ***Optimizing Yield & Productivity***

Because the unit value of chemical products (including ethanol) derived from biomass is generally low while the potential market is large, the economic viability of such processes depends on optimizing yield and productivity. Yield is the quantity of product produced per volume of biomass processed, while productivity is the rate at which the product is generated. Achieving high yield requires that all biomass components are efficiently converted to desired products, while high productivity requires that complex conversions occur as rapidly as possible. Though this concept is relatively simple to grasp, most ethanol producers have failed to achieve this goal. However, the goal can be accomplished via fractionation platforms for both starch and lignocellulosic feedstocks which allow for separate SSF of relatively pure hexose and pentose sugars, or via SSCF using optimized ratios of C5:C6 sugars which are processed from various biomass feedstocks.

Split-stream hydrolysates (cellodextrin/dextrin and hemicellulose) can be utilized to optimize fermentation efficiency of pentose sugars via UHG SSF and CBP. This can be economically accomplished by simply using the thin stillage from UHG SSF of dextrin and cellodextrin substrates as value added backsets for fermentation of pentose sugars at integrated biorefineries. This is possible since 18 v/v% ethanol achieved during UHG SSF represents only about 70% conversion efficiency of total sugars. Hence, 30% of carbohydrates/sugars remain in the thin stillage for use as a value added backset for processing pentose sugars via subsequent UHG SSCF and CBP processes. These types of technologies are continually being developed for integrated biorefineries to efficiently convert wet biomass (including organic waste resources) into value added bioenergy and high value coproducts. Coproducts include functional foods, nutraceuticals, pharmaceuticals, healthcare and medical products, bioproducts, and industrial biochemicals. Bioenergy resources include biogas (biomethane and biohydrogen), bioelectricity, and liquid biofuels including hydrous ethanol.

The above technologies dramatically enhance efficiency of starch and cellulose SSF technologies and may reduce batch fermentation times by up to 50% (to 27 hours) while producing 18 v/v% ethanol via improved strains of *Saccharomyces cerevisiae*. The key to success in development of ethanologens is in developing biocatalysts which are capable of either SSF or CBP. Since conventional enzymes which are required for acceleration of hydrolysis function best at about 60 °C, several scientific groups are focused on developing thermophilic microbes while others are attempting to develop mesophilic enzymes. Research efforts are also focused on developing economical fractionation platforms for optimizing separate hydrolysis and fermentation of hexose and pentose sugars in order to optimize fermentation efficiency.

### ***Development of Ethanologens via Synthetic Biology***

Industrial recombinant strains of *S. cerevisiae* (424A-LNST [Purdue University], D<sub>5</sub>A [DOE-NREL], [Microbiogen's natural industrial yeast strains](#), and [GMAX-L](#) [ARS-USDA]) and a variety of mesophilic and thermophilic ethanologens (*Geobacillus thermoglucosidasius*, *Zymomonas*, recombinant *Escherichia coli*, Xylose assimilating yeasts, homofermentive and heterofermentive *Lactobacillus* and *Clostridium*) which are being developed via synthetic

biology show much promise in terms of tolerance to acetic acid and temperature for use with UHG SSF-SSCF and CBP.

The GMAX-L cellulosic *S. cerevisiae* strain is under development at ARS-USDA to use xylose and glucose to produce ethanol from cellulosic hydrolysate, and then subsequently produce biodiesel fethyl esters from corn oil and ethanol at the same biorefinery. Esters are compounds formed by the reaction of an acid and an alcohol, with the elimination of water. It is made using vegetable oils, animal fat, algae, and even recycled cooking greases. The GMAX-L yeast strain produces 10-15% more ethanol than a glucose-utilization strain. *S. cerevisiae* engineered to simultaneously metabolize both xylose and glucose can produce more ethanol than the next best fungal or bacterial ethanologens. Additional genetic engineering of recombinant *S. cerevisiae* as a cellulosic ethanologen is ongoing in order to express genes that will allow processing of arabinose and lactose sugars. The GMAX-SSF recombinant *S. cerevisiae* strain is ideal for UHG SSF which currently produces about 14 grams of ethanol per liter of broth produced via initial fast through-put experiments ([Hughes et al., 2009a](#); [Hughes et al., 2009b](#); [Hughes et al., 2009c](#); [Hughes et al., 2009d](#); [Hughes et al., 2009e](#)). Since industrial recombinant *S. cerevisiae* strains are currently producing up to 20 v/v% ethanol, eventually GMAX-SSF strains could potentially achieve similar ethanol concentrations. In addition, a novel yeast engineering strategy has been employed which involves metabolically engineering thermotolerant yeasts to express lignocellulose deconstructing genes which provides the capability of processing both pentose and hexose sugars via CBP ([Christopher et al., 2009](#)). GRAS labeling, the “Crabtree effect” ([Thomson et al., 2005](#); [De Deken, 1966](#)), production of relatively high ethanol concentrations (17-23 v/v%) for efficient fermentations and tolerance to organic acids, relatively high temperatures, and low pH make development of industrial recombinant *S. cerevisiae* strains ideal for SSF, SSCF and CBP applications ([Gibbons and Hughes, 2009, slide 20](#)).

Chemical engineers at the University of California Riverside led by Wilfred Chen have been developing cellulosomes for distillers yeast. They have demonstrated the functional display of a miniscaffoldin on the *Saccharomyces cerevisiae* cell surface consisting of three divergent cohesin domains from *Clostridium thermocellum* (t), *Clostridium cellulolyticum* (c), and *Ruminococcus flavefaciens* (f). Incubation with *Escherichia coli* lysates containing an endoglucanase (CelA) fused with a dockerin domain from *C. thermocellum* (At), an exoglucanase (CelE) from *C. cellulolyticum* fused with a dockerin domain from the same species (Ec), and an endoglucanase (CelG) from *C. cellulolyticum* fused with a dockerin domain from *R. flavefaciens* (Gf) resulted in the assembly of a functional minicellulosome on the yeast cell surface. The displayed minicellulosome retained the synergistic effect for cellulose hydrolysis. When a  $\beta$ -glucosidase (BglA) from *C. thermocellum* tagged with the dockerin from *R. flavefaciens* was used in place of Gf, cells displaying the new minicellulosome exhibited significantly enhanced glucose liberation and produced ethanol directly from phosphoric acid-swollen cellulose. The final ethanol concentration of 3.5 g/liter was 2.6-fold higher than that obtained by using the same amounts of added purified cellulases. The overall yield was 0.49 g of ethanol produced per g of carbohydrate consumed, which corresponds to 95% of the theoretical value. This result confirms that simultaneous and synergistic saccharification and fermentation of cellulose to ethanol can be efficiently accomplished with a yeast strain displaying a functional minicellulosome containing all three required cellulolytic enzymes ([Tsai et al., 2009](#)).

The relatively recent discovery of a super bug, *Clostridium phytofermentans* (*Q Microbe*), synthetic strains for which are currently being developed by [Qteros](#); and [Clostridium](#)

*thermocellum* strains which are being developed by [Mascoma](#) and by the [Bioconversion Science and Technology](#) group at the Oak Ridge National Laboratory (DOE-ORNL) are both very promising for commercializing CBP. “Qteros’ Q Microbe™ process has demonstrated world-class ethanol outputs of 70 grams per liter (9 w/v%) in a single-step using an industrial, pre-treated biomass feedstock. These unprecedented yield results far surpass the 50 grams per liter considered to be the threshold for commercial production of cellulosic ethanol. Coupled with the previously observed bioconversion yields of greater than 90% ([Qteros Report, 2009](#)),” these yields are slightly superior to the 7 w/v% reported by Mascoma via [Clostridium thermocellum](#).

In addition to *Clostridium*, bacterial strains such as *Paenibacillus* ( JDR-2 and XynA<sub>1</sub>) provide a novel system for methylglucuronoxylan utilization, thus improving efficiency of extracellular depolymerization and intracellular metabolism of hemicellulose (glucuronoxylans) for development of CBP via synthetic ethanologens ([StJohn et al., 2006](#); [Bi et al., 2009a](#); [Bi et al., 2009b](#); [Nong et al., 2009](#)), and also production of lactic acid via synthetic microbes ([Bi et al., 2009c](#)). A bacterial strain *Geobacillus thermoglucosidasius* (Gth M10EXG) is currently being developed by a consortium led by Sandia National Laboratories. This strain grows optimally at about 60 °C and fermentation enzymes required for saccharification of hexose and pentose sugars are already present in this strain. Gth M10EXG is tolerant to 10 v/v% ethanol in comparison with most other native bacterium which are tolerant only to 4 v/v% ethanol. In addition to ethanol, this strain produces acetate and lactate from pentose and hexose sugars which makes it a good candidate for SSF if it can be engineered for higher ethanol tolerance. Gth M10EXG has the necessary prerequisites for metabolic engineering ([Keasling et al., 2006](#); [Sapra et al., 2009](#)).

Competition between private companies like Qteros and Mascoma, and publications by academic institutions, scientific consortiums and government research groups (DOE & USDA) will continue to increase efficiency of biomass conversion via continually improving efficiency of pretreatment, fractionation, and separation processes in addition to synthetic biology for development of UHG SSF-SSCF and CBP technologies. Similar to the microbial communities present in the rumen of cattle and sheep, symbiotic microbial cocktails may eventually be developed for UHG SSF, SSCF & CBP.

Producing 18 v/v% ethanol in 27 hour fermentations for starch feedstocks via SSF can be accomplished with current technology by focusing on cultivating actively growing strains of *S. cerevisiae*, e.g. “budding yeast.” This subsequently allows for increasing volume of feedstocks processed and ethanol produced by over 70% without increasing the size of fermentors. Thin stillage from processing starch can then be utilized as nutrient-rich backsets for separate hydrolysis and fermentation of cellulose (hexose sugars) and hemicellulose (primarily pentose sugars) at integrated biorefineries which incorporate clean fractionation of lignocellulosic feedstocks. This allows for substantially increasing fermentation efficiency and yields for integrated biorefineries.

### ***Inhibition of Enzyme Adsorption During Hydrolysis***

“Working at high solids (substrate) concentrations is advantageous in enzymatic conversion of lignocellulosic biomass as it increases product concentrations and plant productivity while lowering energy and water input. However, for a number of lignocellulosic substrates it has been shown that at increasing substrate concentration, the corresponding yield decreases in a fashion

which cannot be explained by current models and knowledge of enzyme-substrate interactions. This decrease in yield is undesirable as it offsets the advantages of working at high solids levels.

The decreasing conversion at increasing solids concentrations was found to be a generic or intrinsic effect, describing a linear correlation from 5 to 30% initial total solids content (w/w). Insufficient mixing has previously been shown not to be involved in the effect. Hydrolysis experiments with filter paper showed that neither lignin content nor hemicellulose-derived inhibitors appear to be responsible for the decrease in yields. Product inhibition by glucose and in particular cellobiose (and ethanol in simultaneous saccharification and fermentation) at the increased concentrations at high solids loading plays a role but could not completely account for the decreasing conversion. Adsorption of cellulases was found to decrease at increasing solids concentrations. There was a strong correlation between the decreasing adsorption and conversion, indicating that the inhibition of cellulase adsorption to cellulose is causing the decrease in yield.

Inhibition of enzyme adsorption during hydrolysis products appear to be the main cause of the decreasing yields at increasing substrate concentrations in the enzymatic decomposition of cellulosic biomass. In order to facilitate high conversions at high solids concentrations, understanding of the mechanisms involved in high-solids product inhibition and adsorption inhibition must be improved.” ([Kristensen et al., 2009](#))

### ***Hydrous Ethanol as a Hydrogen-rich Energy Carrier***

Hydrous ethanol is a hydrogen-rich energy carrier which is compatible with existing gasoline infrastructures and provides more energy than gasoline via either catalytic or plasmatron reforming technologies. Reforming technologies for hydrous ethanol allow for combusting hydrogen fuels in legacy engines with unprecedented energy efficiency. In addition, combustion of midlevel hydrous ethanol-gasoline blends (HE15-HE25) provide superior fuel efficiency, cost competitiveness, life cycle analysis (LCA) and GHG profiles in comparison with combustion of neat gasoline. This is due to the fact that water and ethanol are oxygenates which increase the octane rating and provide a cooling effect for intake charges which enhances combustion efficiency and reduces nitrogen oxide (NOx) emissions for gasoline blends (oxygenated hydrocarbon fuels). The superior latent heat of vaporization of water and ethanol also enhance combustion efficiency of gasoline blends in the form of oxygenated hydrocarbon fuels. Whether utilized in gasoline blends or utilized as a hydrogen-rich energy carrier in which water and ethanol are reformed into a hybrid fuel containing oxygen and hydrogen-rich vapors, hydrous ethanol blends and hydrous ethanol containing as much as 50% water are superior to combustion of neat hydrocarbon fuels.

Optimal fuel and energy efficiency is achieved by integration of internal combustion engines (ICEs) in transportation vehicles with bioelectricity, e.g. the development of “hybrid bioelectric vehicles.” This can be accomplished by using biomethane and hydrous ethanol as dual feeds for high temperature fuel cells which produce distributive generated combined cooling, heating and electric power generation (DG-CHP) at integrated biorefineries and bioenergy complexes. In a recent comparison, “bioelectricity outperformed ethanol across a range feedstock, conversion technologies, and vehicle classes.” The research scientists also showed an average of an 81% increase in fuel efficiency (kilometers/miles per unit of energy) and 108% more GHG offsets (per unit area of cropland used) for bioelectric vehicles (BEVs), verses ethanol powered vehicles (ICV; internal combustion vehicles) ([Ricciardi, 2009](#); Campbell et al., 2009). Though the

increases in fuel efficiency and decreases in GHG offsets would be less for hydrous ethanol blends, reforming hydrous ethanol to hydrogen for use in legacy ICEs, and future direct ethanol fuel cells (DEFCs), there are obvious advantages for utilizing electric hybrid technologies. Hybrid electric-ethanol blend combustion technology is currently available for vehicles in today's economy provided efficient electricity storage, round-trip energy conversion and smart grid technology can be employed.

Hydrous ethanol is typically produced using biocatalysts for SSF, SSCF & CBP. Synthetic microbes utilized as biocatalysts are continually being developed and improved via genetic engineering (systems biology) in order to increase toleration to organic acids, heat, ethanol and nutrient concentrations for optimizing SSCF and eliminating unit processes and byproducts. Along with innovative membrane assisted vapor stripping (MAVS) technology which reduces energy consumption by over 50% for ethanol-water removal, these integrated technologies will allow for continually reducing production costs by increasing efficiency of production. Traditional starch and lipid feedstocks in combination with algal biomass and lignocellulosic biomass feedstocks possess the potential to literally replace petroleum products. Production of bio-based products (including bioenergy, special ingredients, and industrial biochemicals) at integrated biorefineries (such as [Solazyme](#) [algal feedstocks produced from sugar and cellulosic feedstocks]; [LS9](#) [sugar and cellulosic feedstocks], [Amyris](#) [sugar and cellulosic feedstocks]; [Lignol](#) [sugar and cellulosic feedstocks]; [BioEnergy International](#) [sugar and cellulosic feedstocks]) will eventually result in annually generating over a trillion dollars in gross domestic products (GDP) in the US. This will subsequently eliminate economic dependence on importing crude petroleum from the Middle East and improve national security.

### ***Leveraging Existing Ethanol & Petroleum Infrastructures***

Existing agricultural, biofuel and petroleum infrastructures can be leveraged to produce renewable energy with unprecedented production efficiencies for bioeconomies which are environmentally friendly and synergistic with production of food. In conjunction with processing organic waste (including animal waste from integrated CAFOs), this can be accomplished via closed loop biomass production (including water hyacinth and algae) using precision agriculture which can:

- more than double conventional crop yields while conserving irrigation water, reducing energy intensive pumping of irrigation water, and reduce use of synthetic fertilizers which are energy intensive to produce and result in increasing pollution and GHGs;
- provide economical harvesting, storage and pretreatment of wet biomass feedstocks, allowing for relay cropping systems and maximizing annual crop yields per acre;
- provide integrated biorefinery platforms which economically incorporate fractionation and innovative technologies including synthetic biology;
- provide closed loop biosystems architecture for integrated biorefineries and bioenergy complexes.

### ***Processing Crop Residues at Integrated Biorefineries***

Though organic waste and closed loop production of lignocellulosic and algal biomass show much promise as low-cost feedstocks for future biorefineries, starch obtained from corn, also referred to as maize (*Zea mays* L. ssp. *mays*), is currently the predominant feedstock for producing ethanol in the US. However, less than half of total corn plant biomass is currently being utilized in the food and biorefining industries. In addition to processing starch contained in

corn grain, processing corn stover (crop residue consisting of stalks, leaves and cobs) and structural fiber of the corn kernel (both pericarp and endosperm fiber) can almost double sugar production and ethanol yields per tonne of corn biomass produced. This increase in ethanol production is possible by harvesting a portion of corn stover biomass (rich in carbohydrates) from crops utilized directly for food (sweet corn); indirectly for food, animal production, nutraceuticals (yellow dent corn); and for biofuels (also yellow dent corn) via production of ethanol, food grade corn oil, nutrient-rich germ meal, and modified distillers grains (MDG) coproducts. Nutrient-rich germ meal and MDG obtained from processing corn plant biomass (which are utilized as value added animal feedstuffs) are actually higher in nutrients than whole corn, and lower in fiber and fat than conventional distillers grains (DG) due to utilizing wet fractionation which incorporates enzymatic milling (patented E-Mill process).

With almost 86 million acres planted in corn in 2008, stover is currently the most abundant source of lignocellulosic biomass in the US and has been recognized as the most promising source of biomass for the bioenergy industry in the near-term. Harvesting 75% of corn stover associated with 200 bu/acre yields (approximately 50% corn and 50% stover as harvested) could result in processing 328 million tonnes of stover into 27.5 billion gallons per year (BGY) of cellulosic ethanol. Though the actual grain-to-stover ratio for corn may vary up to 57.5:42.5 for dry matter (DM), the above numbers are based on processing 50% of total corn biomass as stover DM which provides an ethanol yield of 84 gal/tonne of stover (67% conversion efficiency). At 100 gal/tonne of corn stover (80% conversion efficiency) for precision agriculture and mature biorefining, US ethanol production could increase to almost 66 BGY.

The goal of the ethanol industry is to eventually reach 95% conversion efficiency which would provide an average yield of 119 gal per tonne of lignocellulosic biomass. The US government target (Renewable Fuel Standard II or RFS2) for the year 2022 is 35 BGY, 20 BGY of which is to be produced from non-food feedstocks. As illustrated from the above numbers, this can be most efficiently accomplished by producing ethanol from crop residues, e.g. non-food portions of crops such as corn stover, wheat/cereal straw, soy hulls (lignocellulosic feedstocks), and other agricultural byproducts, municipal and industrial solid waste, sludge from paper manufacturing, and food processing waste. However, a portion of crop residues and organic waste must be utilized as organic fertilizers to replenish soil organic matter (SOM). This is necessary in order to maintain soil fertility and provide sustainable agriculture for bioenergy economies. This is accomplished via closed loop architecture which converts waste resources into organic fertilizers and other value added coproducts.

### ***Processing Corn Fiber at Integrated Biorefineries***

In addition to processing cellulose contained in corn stover, the pericarp and endosperm fiber in corn grain also contains cellulose. After processing the simple sugars and starch (hexose sugars comprised of sucrose, fructose, galactose and glucose) contained in corn, 8.9% of corn is fiber, e.g. lignocellulosic biomass (lignin, cellulose and hemicellulose) containing a large portion of pentose sugars which are not fermentable by conventional distillers yeast (*Saccharomyces cerevisiae*). Utilizing fractionation platforms, the non-fermentable components of lignocellulosic biomass (lignin and perhaps protein) can be separated and efficiently processed into value added foods and bioproducts which displace petroleum products. Fermentable portions of biomass can then be hydrolyzed and fermented more efficiently by conventional distillers yeast, industrial recombinant yeast strains or synthetic bacteria in order to produce ethanol from the entire corn plant including grain fiber and stover.

**Table 2. Composition of Yellow Dent Corn**

Component	Kernel %	Starch	Protein	Oil	Ash/Minerals	Sugars	Fiber
Endosperm	82.9	88.4	8.0	.8	.3	.6	1.9
Germ	11.0	11.9	18.4	29.6	10.5	10.8	18.8
Bran Coat	5.3	7.3	3.7	1.0	.8	.3	86.9
Tip cap	.8	5.3	9.1	3.8	1.6	1.6	78.6
Whole Kernel	100	75.0	8.9	4.0	1.5	1.7	8.9

([Bunge Dry-Milling Products, 2008](#))

Up to 85.6% of the corn kernel can be hydrolyzed and fermented to ethanol. Total carbohydrates in corn are comprised of about 75% starch (glucose hydrolysate), 1.7% simple sugars (sucrose, fructose, and galactose), and 8.9% structural carbohydrates (cellulosic fiber). Corn fractionation allows for removing the non-fermentable portions of the corn kernel prior to fermentation by conventional distillers yeast. However, during fractionation some of the starch is separated with the germ and fiber fractions which results in reducing ethanol yields. Hence, processing the fiber and germ meal fractions via lignocellulosic biorefining and recycling the thin stillage from starch fermentations as backset for lignocellulosic fermentations can potentially result in converting over 90% of carbohydrates in the corn kernel to ethanol. This integrated process can potentially increase corn ethanol yields from a current industry average of 2.75 gal/bu, to 3.2 gal/bu. This is over a 16% increase in corn ethanol yields based on a 90% conversion rate of total carbohydrates which is possible via integrated biorefineries.

The theoretical ethanol yield for corn is 3.48 gal/bu., e.g. 137 gal/tonne ([DOE theoretical yield calculator for biomass](#)). Achieving a 95% conversion rate of total carbohydrates would allow for production of 3.3 gal/bu of corn, e.g. allowing for an additional 4% increase (20% total increase) in comparison with conventional ethanol yields. This may be possible by:

- fractionating corn, e.g. removing germ and fiber prior to conventional starch fermentations using distillers yeast;
- increasing surface area and enzymatic access by reducing particle size via roller mills (use 50% less electricity and produce more uniform particle size in comparison with conventional hammer mills);
- ultrasonic cavitation which can increase surface area and reduce particle size by 20 fold, thus increasing efficiency of enzymatic processing and fermentation (Grewell, 2008; [Khanal et al., 2007](#));
- recycling unfermented hexose and pentose sugars in thin stillage as backset for lignocellulosic fermentations; and
- fermentation of corn fiber via lignocellulosic pretreatment and processing at integrated biorefineries.

### ***Wet Fractionation of Corn***

Various ultrasonic frequencies, microwave and electromagnetic pulse technologies can enhance both fractionation and hydrolysis processes. For switchgrass, sequential ammonia steeping-ultrasonic pretreatment can release about 10% more fermentable sugars than using ammonia steeping alone. However, the net energy balance (additional chemical in free sugars minus energy consumption of the ultrasound process) needs to be evaluated for each specific application (Grewell, 2008; [Khanal et al., 2007](#)). Similar results may be achieved for treating

corn stover via fractionation and UHG SSF and CBP at integrated biorefineries ([Montalbo-Lomboy, et al., 2007](#)). Due to capital investment and energy costs, the intensity of fractionation, pretreatment, and hydrolysis processes need to be economically balanced with production of high value products and corresponding use of distillers products as value added animal feeds and fertilizers for closed loop systems.

Fractionation of corn allows for producing food grade corn oil from the germ fraction prior to fermentation. This is a substantially more efficient process which results in higher oil yields than extracting and refining corn oil after it has been contaminated through exposure to fermentation and distillation processes. Though corn oil could be processed into biodiesel, with a retail value of about \$7/gal it is obviously more valuable as a food grade product. Based on 90% extraction of 4% corn oil feedstocks, 3.6% of total corn grain processed is equivalent to producing 9.5 MGY of either corn oil or biodiesel for a 100 MGY ethanol biorefinery. The energy value of the biodiesel when used as an internal combustion biofuel is 138,700 BTU/gal HHV compared with 84,530 BTU/gal HHV for anhydrous ethanol.

Based on an average ethanol yield of 2.75 gal/bu for the corn ethanol industry, 108.23 gal/tonne of corn is currently produced. This represents 79% of total carbohydrates contained in corn grain. By increasing the conversion efficiency of processing corn grain to 95% for starch in combination with processing 75% of corn stover at 80% conversion efficiency, this would allow for producing up to 206 gal from the same volume of corn biomass. Efficient processing of corn biomass results in increasing total ethanol production by over 90% in comparison with current average yields.

**Optimizing Conversion Efficiency & Energy Content of Corn Plant Biomass**

Economically achieving 80-95% conversion efficiency for lignocellulosic feedstocks will eventually become possible via fractionation platforms employed at integrated biorefineries. In addition, fractionation of corn containing 4% oil in germ and fiber will provide 10.3 gal of food grade corn oil per tonne of corn processed. According to [DOE’s ethanol yield calculator for biomass](#), corn stover (stalks, leaves, cobs) can produce up to 125 gal/tonne. The economic advantages for increasing conversion efficiencies will vary depending on production costs, value of biofuels, value of coproducts, and how efficiently these coproducts are utilized and marketed by the biorefinery. Integrated biorefineries, closed loop biomass, and closed loop biorefinery production optimize production efficiencies and provide substantial economic advantages.

**Table 3. Biofuel Yields for Processing Corn Biomass at Integrated Biorefineries**

<b>Conversion Efficiency</b>	<b>Type of Feedstock</b>	<b>Gal/tonne</b>	<b>v/v % of Biofuels</b>	<b>MBTU HHV</b>
95%	Corn starch (72% content) 2.94 gal/bu	115.7	50.47	<b>9.78</b>
80-95%	Corn fiber (8.9% content) 0.306-.364 gal/bu	12-14.25	5.6-6.4	<b>1.0-1.2</b>
80-95%	75% of Corn stover	75-89	35.2-39.1	<b>6.34-7.52</b>
90%	Corn oil (4% content-7.7 lbs/gal)	10.3	4.8	<b>1.34</b>
Total BioEthanol	203-219 gal anhydrous ethanol from processing 1 tonne of corn plus .75 tonne of stover (equivalent to producing 107-115 gal of biofuels per tonne of corn biomass)			
Total MBTUs for processing 1.75 tonnes at 80-95% stover conversion efficiency (equivalent to producing 9.23-9.92 MBTU/tonne of corn biomass based on 84,530 BTU/gal of				<b>18.46-19.84</b>

anhydrous ethanol @99.5% ethanol plus 130,000 BTU/gal of corn oil)	
Total MBTUs for processing 1.75 tonnes at 80-95% stover conversion efficiency (equivalent to producing 24.47-26.3 MBTU/tonne of corn biomass based on 113,963 BTU/gal of hydrous ethanol @50% ethanol plus 130,000 BTU/gal of corn oil)	<b>42.82-46.02</b>

In terms of producing energy from thermal combustion, MBTUs for biofuels produced increases by 102%, e.g. from 9.78 MBTU/tonne for conventional corn ethanol production to 19.84 MBTU for processing the majority of corn biomass at an integrated biorefinery via anhydrous ethanol at 95% conversion efficiency. For integrated biorefineries which produce hydrous ethanol for use as a hydrogen-rich energy carrier via reforming technologies, MBTUs are increased by 367%, e.g. almost by four fold for 95% conversion efficiency in comparison with conventional production of anhydrous ethanol (from processing only the starch portion of corn plant biomass).

In contrast to producing anhydrous ethanol, reforming hydrous ethanol substantially increases the energy density of this hydrogen-rich energy carrier. Catalytic and plasmatron reforming technology allows for converting 50-65% ethanol and 35-50% water (HE100 containing 50-65% ethanol) to a hydrogen-rich fuel with an energy density (113,963 BTU/gal HHV) similar to gasoline (127,960 BTU/gal HHV). In addition, production of 50-65% ethanol and 35-50% water (hydrous ethanol blends) for use with catalytic and plasmatron reforming technologies reduces the energy required for ethanol-water separation using the MAVS technology by up to 80%. This information reveals that when hydrous ethanol is used as a hydrogen-rich energy carrier, in regards to energy density and net energy gain (NEG), it is superior to either anhydrous ethanol or gasoline. In addition, hydrous ethanol is not as prone to phase separation which results in corrosion as is anhydrous ethanol. Hence, unlike anhydrous ethanol, hydrous ethanol and hydrous ethanol blends are compatible with gasoline infrastructures and legacy ICEs.

In addition to leaving 16-18 inches of corn stubble and root ball, the above concept returns 25% of corn stover harvested back to crop fields in order to replenish SOM. Hence, about 50% of total corn biomass is left in the crop field to maintain soil fertility. Modern combines can be altered to harvest all but a few inches of corn stalks. 25% of stover can then be shredded, chopped and spread onto the crop field to maintain SOM while operating at close to 90% of conventional combine speed. Additional organic matter is restored to the field in the form of nutrient rich effluents which are obtained by feeding DG products to animals and subsequently processing animal waste via state-of-the-art anaerobic digestion. Restoring SOM reduces the volume of synthetic fertilizers required and restores essential micronutrients for balanced growth of future crops. This reduces production costs for sustainable bioenergy economies via closed loop production of biomass.

Integration of organic waste processing, subsurface drip fertigation (SDF), high density cropping systems, and precision agriculture can allow for consistently producing the equivalent of 400 bu/acre corn crop yields in comparison with yields currently averaging less than 160 bu/acre in the US. These improvements in production efficiencies would be in addition to continual advances in developments of hybrids which grow more efficiently, contain plant expressed enzymes, and are more efficiently converted to sugars. Based on the corn plant consisting of 50% corn and 50% stover for 400 bu/acre corn, processing the majority of the corn plant would allow for harvesting 20.32 tonnes of corn biomass per acre, 17.78 tonnes of which can be converted to sugars and fermented to ethanol. Based on producing an average of 114.65 gal/tonne of biomass for advanced processing technologies, this would result in yields of 2,038

gal of anhydrous ethanol (99.5% ethanol), and 91.6 gal of corn oil produced for each acre of corn biomass harvested.

Utilizing plant expressed alpha amylase corn hybrids, such as those developed by Syngenta, can substantially reduce enzyme loading for starch fermentations. In conjunction with genetic selection and genomics, similar development of plant expressed enzymes for corn biomass hybrids will eventually allow for reducing enzyme loading for hydrolysis and fermentation of carbohydrates contained in corn stover. In the meantime, fractionation platforms which remove lignin and allow for separately fermenting hexose and pentose sugars can dramatically reduce enzyme loading and enhance hydrolysis and fermentation efficiency while simultaneously producing high value coproducts.

Corn is an extremely diverse species, both genetically and phenotypically, and therefore able to adapt to a broad range of soil and environmental conditions and production traits worldwide. Until recently, improvement of corn used as forage for animals focused on increasing grain yield as a way to elevate the energy content of the overall forage. Significant progress has been made to improve biomass yield and stover cell-wall traits, primarily as they relate to ruminant animal nutrition. Research is currently being developed to address the potential use of these traits for the biofuels industry without affecting current production of corn ([De Leon and Coors, 2008](#); [Bernardo and Jung, 2008](#)). Re-engineering of plant cell wall composition (Chiang et al., 2005; Dunn-Coleman et al., 2006; Harvey et al., 1988) could make biomass more amenable to hydrolysis ([Chundawat et al., 2006](#)). This will result in increasing both conversion and production efficiencies via ultra high gravity simultaneous saccharification and fermentation (SSF) and consolidated bioprocessing (CBP).

Other researchers are evaluating use of tropical maize as a dedicated energy crop. According to recent research on tropical maize at the University of Illinois, “it appears that maize may prove to be the ultimate US biofuels/biorefinery feedstock. Early research results reveal that tropical maize, when grown in the Midwest, requires few crop inputs such as nitrogen fertilizers, chiefly because it does not produce any ears. It is easier for farmers to integrate into their current operations than some other dedicated energy crops because it can be easily rotated with corn or soybeans, and can be planted, cultivated and harvested with the same equipment US farmers already have. Finally, tropical maize stalks are believed to require less processing than corn grain, corn stover, switchgrass, *Miscanthus giganteus* and many other plants now being studied for biofuel production. Harvested straight from the field with no processing, tropical maize contains 25% or more sugar -- mostly sucrose, fructose and glucose. This could eliminate expensive pretreatments and subsequent hydrolysis of glucan/cellulose and xylan for a portion of the biomass if the crop were used for biofuel production similar to sweet sorghum. Midwestern-grown tropical maize easily grows 14 or 15 feet tall compared to the 7-1/2 feet height that is average for conventional hybrid corn ([University of Illinois, 2007](#)). SDF and closed loop production is ideal for optimizing nutrient-energy management and reducing production costs for tropical maize which grows too tall for irrigating with conventional sprinkler systems.

Similar to processing the whole corn plant (including stover and fiber), processing sorghum, cereal grain crop residues, and soybean hulls can also provide a substantial volume of cellulosic feedstocks in addition to dedicated energy crops and logging residues. Utilizing precision agriculture and high density relay cropping systems provides the potential to annually process well over a billion tonnes of biomass feedstocks (one trillion gallons of ethanol at only 80%

conversion efficiency) in the US at integrated biorefineries. For example, soybean hulls (SBH) are an abundant agricultural crop residue in the US (17 million tons/yr) containing 10% protein. About 66% of the hull is polymeric ‘biomass sugars,’ primarily cellulose, hemicellulose, and pectin. Along with lignin, these sugars can be utilized as value added feedstocks for integrated biorefineries.

Though conventional biomass pretreatment and ethanol fermentation processes destroy the protein to liberate the sugars, researchers at Dartmouth College have developed a process to produce ethanol from SBH while preserving the valuable protein component. The process yields large levels of ethanol and a higher value protein concentrate. Potential for producing up to 450 MGY of ethanol from SBH in the US is possible with minor capital investment at integrated biorefineries which include confined animal feeding operations (CAFOs). Since soybean products are valued by percent of protein content, the protein component of SBH provides the potential for annually producing 2 million tons of quality protein meal (for use in formulating animal feeds) with a market price of about \$300/ton based on 48% protein. ([Mielenz, 2009](#)) Subsequent to wet fractionation of corn, MDG contain 58% protein which provides a complimentary feedstuff for mixing with SBH residues. These feedstuffs are actually higher in quality and value than that of soybean meal and corn gluten meal.

“Soybean hulls were evaluated as a resource for production of ethanol by the SSF process, and no pretreatment of the hulls was found to be needed to realize high ethanol yields with *S. cerevisiae* D5A. The impact of cellulase,  $\beta$ -glucosidase and pectinase dosages were determined at a 15% biomass loading, and ethanol concentrations of 25–30 g/L were routinely obtained, while under these conditions corn stover, wheat straw, and switchgrass produced 3–4 times lower ethanol yields. Removal of carbohydrates also concentrated the hull protein to over 25 w/w% from the original composition at roughly 10%. Analysis of the soybean hulls before and after fermentation showed similar amino acid profiles including an increase in the essential amino acids lysine and threonine in the residues. Thus, eliminating pretreatment should assure that the protein in the hulls is preserved, and conversion of the carbohydrates to ethanol with high yields produces a more concentrated and valuable co-product in addition to ethanol. The resulting upgraded feed product from soybean hulls would likely be acceptable for feeding to monogastric as well as ruminant livestock.” ([Mielenz et al, 2009](#))

As an alternative to the above process, similar to processing livestock feeds SBH could be simultaneously stored and pretreated via ammonia ensiling ([Anderson et al., 2009](#)) and enzyme inoculation at integrated biorefineries. This process would allow for treating SBH along with corn stover, pericarp fiber and endosperm fiber (from wet fractionation of corn), straw from harvesting cereal grains, and other suitable crop residues and food processing waste for use as value added biomass feedstocks and animal feeds at integrated biorefineries. The ammonia pretreated and enzyme enriched biomass containing mixed substrates could then be processed via steeping in an aqueous ammonia-ethanol solution to provide clean fractionation. In addition to enhancing fractionation, ammonia provides a source of free amino nitrogen (FAN) which enhances efficiency of microbial processing during fermentation of ensiled feedstocks. High moisture fermentation residues, MDG (produced from wet fractionation of corn), corn germ meal and SBH protein meal could subsequently be ensiled and fed to ruminants, or processed and fed to monogastric animals via integrated CAFOs. Animal production using these feedstuffs provides quality protein (exceptional amino acid profiles) in foods including beef, dairy, swine, poultry and aqua-farming products.

In addition to providing quality protein feedstuffs for integrated CAFOs, subsequent animal waste can be converted to methane-rich biogas and organic fertilizers. For example, bovine waste slurry from feedlots and dairies is composed of 1.8-2.4% nitrogen ( $N_2$ ), 1.0-1.2% phosphorus ( $P_2O_5$ ), 0.6-0.8% potassium ( $K_2O$ ) and 50-75% organic humus which includes a balance of other essential plant nutrients. Processing animal waste and other forms of organic waste into biogas does not remove the majority of nitrogen, phosphorus, potassium (N-P-K) and other nutrients. Upon completion of anaerobic digestion which produced biogas, these nutrients remain in the biological effluents for use as value added fertigation streams (organic fertilizers in water solution) for production of food crops and closed loop biomass. Each animal unit (AU- consists of 1,000 lb) produces enough nutrients to grow an acre of conventional crop production, e.g. the equivalent of about 200 bu of corn or 22,500 lb of corn plant biomass. On an annual basis, subsurface drip fertigation (SDF), high density crops, relay cropping systems, and precision agriculture can more than double conventional crop yields per acre of land utilized.

For precision agriculture utilizing  $CO_2$  enrichment from waste streams produced by biorefinery operations via controlled environment agriculture (CEA), the efficiency of photosynthesis can be enhanced in addition to providing 24/7 production of biomass via artificial lighting such as low energy LEDs. Biomass yields per acre could be increased exponentially via year round production. In addition, hydroponic greenhouses and algal photobioreactors can capitalize on the electron transport chain of bacteria and symbiotic relationships with aquatic plants/algae via microbial electrolysis cells (MECs).

Though both algae and plants utilize photosynthesis (phototrophic), algae and cyanobacteria (blue green algae) can reproduce with or without photosynthesis, often utilizing a combination of phototrophic and heterotrophic production (photoheterotrophic). Aquatic single celled algae and kelp are distinguished from plants through relatively simple reproductive systems in comparison with more complex reproductive features of both aquatic and terrestrial plants. Whereas plants obtain carbon dioxide from the atmosphere, algae obtain carbon dioxide primarily from water. Hence, providing adequate amounts of carbon dioxide in water mediums used for optimizing algal growth is one of the challenges for algae producers which can limit production.

The primary products of photosynthesis are sugars and oxygen which are utilized to produce proteins, DNA and lipids. Unlike plant life, single celled algae contain no complex structural carbohydrates containing lignin, hemicellulose and cellulose which are produced from sugars. Lignin is not convertible to sugars during conventional fermentation processes utilized by biorefineries or anaerobic digesters. Algae contain no lignin and only a small amount of cellulose is contained in their cell walls. Hence, the majority of the sugars produced by algae are utilized for production of simple sugars (such as glucose) and lipids which are relatively easy to process into biofuels. In contrast to carbohydrates, lipids are long chain fatty acids containing sixteen or more carbon molecules. Lipids are produced by plants and algae by breaking down and reassembling carbon molecules contained in sugars. Lipids are used primarily for the development of membranes required for production of new cells. When under stress which limits growth, algae convert fatty acids into fats (triglycerides) for internal storage of nutrient reserves. Fats are often referred to as oils when harvested and stored at ambient temperatures. Unlike plants which store oils primarily in seeds, algae store fats/oils throughout their cells and are up to 100 times more efficient at producing oils than terrestrial plants.

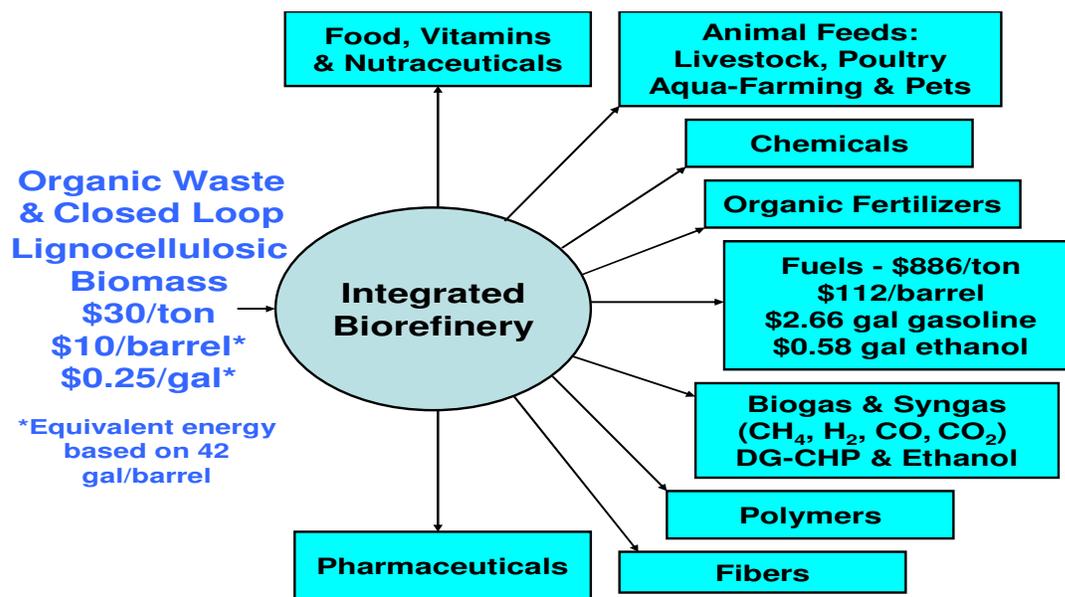
Both plants and algae exude nutrients as they grow and reproduce. Bacteria then feed on the nutrient-rich exudates including carbohydrates and mucilage of plants and algae in the phycosphere, the area immediately surrounding the roots of aquatic plants and cells of phototrophic and heterotrophic microalgae. MECs produce exothermic reactions which simultaneously provide thermal integration for CEA (which lowers heating costs for integrated operations) and produce either biohydrogen or biomethane without inhibiting plant/algal growth.

Heterotrophic production (e.g. microbial fermentation absent of photosynthesis and solar energy) can utilize biological effluents and sugars obtained from low-cost bioenergy feedstocks such as lignocellulosic biomass (crop residues and organic waste) to further increase production efficiencies. Optimized fermentations including the use of synthetic microbes which enable hyper-growth rates and allow for maximizing cell density of microalgae by up to 1,000 fold greater than conventional open-pond or contemporary photobioreactor technologies ([Harrison Dillon, CFO Solazyme](#)). Eventually, hybrid algal bioreactors which economically integrate state-of-the-art LED light arrays ([OriginOil's Helix BioReactor & Dynamic Control System](#)) with symbiotic fermentations may allow for maximizing bioenergy potential via photosynthetic reduction and synthetic microbial cultures capable of metabolizing cellulosic hydrolyzates (both hexose and pentose sugars via CBP). Since cost of purchasing and processing feedstocks (including up to 18% for biomass pretreatment) account for over 70-90% of biorefinery production costs for conventional starch and biomass conversion technologies, closed loop biomass and CEA for advanced biofuel feedstocks provide substantial opportunities to reduce production costs and provide sustainability for integrated biorefineries and bioenergy complexes.

Biogas (consisting primarily of methane and carbon dioxide) produced from organic waste via anaerobic digestion may be economically enriched by filtering it through limewater to remove carbon dioxide, iron filings to absorb corrosive hydrogen sulfide, and calcium chloride to extract water vapor after the other two processes. About one standard cubic foot (SCF) of biogas may be generated from one pound of cattle manure at around 28 °C. This is enough biogas to cook a day's meals for 6 people in India. In terms of thermal energy content for internal combustion engines (ICEs), about 1.7 cubic meters (60 SCF) of biogas (65% methane) is equivalent to 1.55 liters (0.34 gal) of gasoline (115,000 LHV BTU/gal). Theoretically, the manure produced annually by one AU can be converted to biomethane with the energy equivalent of up to 680 liters (180 gal) of gasoline. Typical gasoline ICEs require about 0.5 m<sup>3</sup> of methane per horsepower per hour. Some care must be taken with the lubrication of engines using solely biogas due to the "dry" nature of the fuel and some residual hydrogen sulfide. However, simple conversions of gasoline engines allow for efficiently combusting biomethane. Though ICEs are only capable of achieving total energy efficiencies of about 15%-35%, electric hybrids provide opportunities to increase total energy efficiency to over 50% for the transportation industry.

In comparison with ICEs including gas turbines, energy efficiencies can be vastly improved for integrated biorefineries and bioenergy complexes which capitalize on economies of scale for converting biomethane to combined cooling, heating and power (CHP) via high temperature fuel cells. This requires refining biogas for use of nitrogen-free and sulfur-free biomethane feeds for molten carbonate (MCFC) or phosphoric acid (PAFC) fuel cells providing distributed generated CHP (DG-CHP) with total energy efficiencies exceeding 90% via thermal integration. This is the most efficient use of this bioenergy resource which is produced from organic waste. Refined biogas allows for closed loop production at integrated biorefineries which provides unprecedented efficiencies of operation, ushering in a paradigm shift to distributed bioeconomies

in which wholesome functional foods, nutraceuticals, pharmaceuticals, bioproducts (including bioplastics), industrial biochemicals and bioenergy (including biogas, biofuels and bioelectricity) are produced simultaneously. External energy inputs account for over 20% of biorefinery production costs for conventional starch processing and distillation technologies, and as much as 38% for processing lignocellulosic feedstocks. For integrated biorefineries, the use of fossil fuels and grid power could essentially be eliminated.



*Figure 11. Integrated Biorefinery Platform Providing Diversification & Flexibility*

The above concepts invalidate the food vs. fuel argument and illustrate how production of food and biofuels are synergistic via integrated biorefineries and bioenergy complexes which incorporate closed loop architecture. Pollution and GHGs are virtually eliminated via efficient processing of corn stover and other second generation lignocellulosic feedstocks which comprise an abundance of crop residues and organic waste. Third generation algal biomass feedstocks (which are lignin free and have limited cellulose in cell walls) require little or no pretreatment and can be more economically processed than lignocellulosic feedstocks. Vertically integrated production of biomass feedstocks and bioenergy via integrated biorefineries which incorporate closed loop architecture is the best risk management strategy available for coping with volatile pricing of commodity markets.

According to US Department of Energy studies conducted by the Argonne Laboratories of the University of Chicago, one of the benefits of cellulosic ethanol is that it reduces greenhouse gas emissions (GHGs) by 85% in comparison with reformulated gasoline. In addition to reducing GHGs, recycling these valuable resources dramatically enhances industrial production, increases the efficiency of renewable energy technologies, and replenishes oxygen to the atmosphere via incorporation of closed loop biomass.

The “well to wheel” analysis referred to above reveals that conventional corn ethanol provides 20-30% reductions in GHGs compared with gasoline. Cellulosic ethanol's favorable profile stems from using lignin, a coproduct used to either fuel the biomass conversion process or

provide valuable biochemicals for production of bioplastics which displace petroleum products. Lignin is a renewable fuel with no net greenhouse gas emissions. Cellulosic ethanol reduces fossil fuel use by 90% ([Wang, 2005](#)). A similar reduction in GHGEs for corn ethanol production is achieved by feeding nutrient-rich ethanol coproducts (DGs and fermentation residues) to animals. Animal waste is then processed into biomethane and organic fertilizers which displace both natural gas and petroleum derived fuels and chemical products.

Nutraceuticals and derivative chemicals found in lignocellulosic biomass -- such as furfural, HMF, xylitol, D(-) lactic acid, succinic acid, other organic acids and low molecular weight lignin -- give birth to new high value derivatives which can be utilized in food, bioplastics, medical and industrial manufacturing. For example, lignin derivatives can be utilized to produce high value bioplastics. One of the biochemicals used to produce furfural can be used in the construction of artificial bones that the human body is unlikely to reject in the medical field. The biochemical derived from precursors of furfural retails for \$1,000/lb, as opposed to ethanol which retails for about \$0.20/lb. Similarly, omega-3 fatty acids obtained from algal biomass retail for over \$4,500/gal (\$600/lb). This is in comparison with biodiesel which retails for about \$3-\$4/gal (\$0.40-\$0.53/lb). The nutraceutical astaxanthin, a super antioxidant also obtained from potentially producing and processing green microalga *Haematococcus pluvialis* biomass or the red yeast *Phaffia rhodozyma* ([Nghiem et al., 2009](#)) at integrated biorefineries retails for over \$1,700/lb. Production of omega-3s and astaxanthin (nutraceutical and pharmaceutical grade products) represent an exponential increase (150-850 orders of magnitude) in value in comparison with liquid biofuels.

Obviously, there are strong economic and environmental advantages for integrating production of high value foods and other coproducts with production of commodity biofuels, all of which displace petroleum products and reduce economic dependence on fossil fuels. This can be accomplished via integrated biorefineries which simultaneously reduce pollution and GHGEs.

Comprehensive nutrient-energy management via integrated biorefineries allows for comfortably reaching the US mandate for biofuels while simultaneously producing high quality foods and bioproducts including functional foods (aquaculture, poultry eggs, swine, beef and dairy products enriched with nutraceuticals), vitamins, nutraceuticals, pharmaceuticals, organic fertilizers, adhesives, surfactants, and bioplastics for displacing petroleum products, all of which are biodegradable. This is possible through development of economical separation technologies and biorefining platforms which are very similar to those employed at petroleum refineries, but which process an abundance of renewable agricultural byproducts and organic waste instead of limited supplies of crude oil. In order for production of biofuels to be sustainable, high value functional foods, nutraceuticals, pharmaceuticals, and other high value coproducts must be simultaneously produced in order to offset costs of producing feedstocks and biorefining. In addition to providing diversification and flexibility for maximizing revenue streams, closed loop production at integrated biorefineries allows for achieving unprecedented efficiencies of operation, the revenues of which are not subject to the volatility of commodity markets regarding cost of feedstocks and energy resources which currently comprise over 90% of production costs.

## **Ethanol BioEnergy Complex**

### **Closed Loop Production of Functional Foods, Bioproducts & BioEnergy**



steam and thermal integration, and 47,692 tons H<sub>2</sub> or 269,937 tons NH<sub>3</sub>. Total energy produced by DG-CHP & H<sub>2</sub> cogeneration for an Ag-Energy Park Complex amounts to 14,917,986 MBTU. In addition to the DG-CHP & H<sub>2</sub>, production of E100 (anhydrous ethanol) would be equal to 19,667,798 MBTU; hydrous ethanol 3% water (HE100-93) would be equal to 26,626,783 MBTU; catalytic/plasmatron reforming technology would allow for producing 54% more HE100-65 fuel consisting of 32,204,224 MBTU; and catalytic reforming technology for HE100-50 fuel would increase volume of fuel by 100%, consisting of 48,729,513 MBTU. The DG products from the ethanol biorefinery would allow for annual production of 111.56 million lbs of natural beef/dairy products rich in omega-3 fatty acids and GLA. Two to three times larger volumes of enriched meats (functional foods) from poultry and swine (which have higher feed to conversion ratios than cattle) could be produced from distillers grains and algae meal products. Animal waste along with equal portions of water hyacinth and algae substrates produced via closed loop biomass would provide 80% methane biogas and value added organic fertigation streams for year round production of biomass and either biomethane or biohydrogen via CEA and plant MEC technologies. The biomethane and/or biohydrogen produced by the plant MECs would in addition to the biomethane produced via anaerobic digestion.

Closed loop systems architecture including precision agriculture and SDF would allow for internally producing feedstocks which would be processed via low energy ammonia ensiling of wet biomass feedstocks followed by aqueous ammonia-ethanol pretreatment and fractionation. The lignin fraction could be combusted via fluidized bed boilers to provide additional process steam and thermal integration, or it could be processed into high value low molecular weight derivatives for the bioplastics and industrial biochemical industries. The result of fractionation and increasing fermentation efficiency by up to 70%, implementing closed loop architecture, and increasing volume of biofuel production by up to 100% using hydrous ethanol as a hydrogen-rich energy carrier dramatically reduces production costs while simultaneously increasing product sales for integrated biorefineries. Hence, not including amortization of capital investments, it is theoretically possible to produce biofuels for less than \$0.50 gal, provide grid connected power for less than \$0.04 kWh, produce all natural functional foods for less than 50% of current production costs, and provide a myriad of high value coproducts including nutraceuticals, pharmaceuticals, and industrial biochemicals at integrated biorefineries.

[BioEnergy International](#) and [Verdezyne](#) are developing technologies for the next-generation of integrated biorefineries. BioEnergy claims that its unique patented microorganisms and proprietary process technology will transform the current hydrocarbon-based manufacturing processes across a variety of industries enabling the replacement of a barrel of crude with plant-based sugars to make everything from plastics and fabrics to transportation fuels. Discoveries include D(-) lactic acid, succinic acid, butanol, butanediol, propanediol, adipic acid, and HPA. Production of D(-) lactic acid has already been commercialized and commercialization of succinic acid is currently under development for use in the bioplastics industry and also for replacing a host of petro-chemicals in other industries. BioEnergy is a good example of how private companies, universities, and federal research labs in the US are collaborating, enabling them to become global leaders in the development and commercialization of the next generation biorefineries. These integrated biorefineries will produce high-value bio-based chemicals and fuels from renewable feedstocks through the use of biocatalysts including synthetic biology.

***Synthetic Biology: Integrated BioSystems & Future of Chemical Industry***

Synthetic biology is described as the convergence of molecular biology, information technology and nanotechnology, leading to the systematic design of biological systems. The aim of synthetic biologists is to create an organism from scratch. Microbiologists and teams of molecular biologists and geneticists utilize a computational biology approach similar to that of other types of engineering in the design and construction of biosystems that will support this new technology.

Computational biology is an interdisciplinary field that applies the techniques of computer science, applied mathematics and statistics to address biological problems. The main focus lies on developing mathematical modeling and computational simulation techniques. By these means it addresses scientific research topics with their theoretical and experimental questions without a laboratory. It encompasses the fields of:

- Bioinformatics, which applies algorithms and statistical techniques to the interpretation, classification and understanding of biological datasets. These typically consist of large numbers of DNA, RNA, or protein sequences. Sequence alignment is used to assemble the datasets for analysis. Comparisons of homologous sequences, gene finding, and prediction of gene expression are the most common techniques used on assembled datasets; however, analysis of such datasets have many applications throughout all fields of biology.
- Computational biomodeling, a field within biocybernetics concerned with building computational models of biological systems.
- Computational genomics, a field within genomics which studies the genomes of cells and organisms. High-throughput genome sequencing produces lots of data, which requires extensive post-processing (genome assembly) and uses DNA microarray technologies to perform statistical analyses on the genes expressed in individual cell types. This can help find genes of interests for certain diseases or conditions. This field also studies the mathematical foundations of sequencing.
- Molecular modeling, which consists of modelling the behavior of molecules of biological importance.
- Protein structure prediction and structural genomics, which attempt to systematically produce accurate structural models for three-dimensional protein structures that have not been determined experimentally.
- Computational biochemistry and biophysics, which make extensive use of structural modeling and simulation methods such as molecular dynamics and Monte Carlo method-inspired Boltzmann sampling methods in an attempt to elucidate the kinetics and thermodynamics of protein functions.

This new focus is referred to as systems biology. It is the key to learning how to design and engineer parts, devices and systems of a biological nature from standardized elements that are readily available (referred to as biobricks or bioparts).

Another sector of the field is experimenting with algae to produce oils that can be refined into diesel fuel. Algae cells are fed biomass made from natural substances such as sugar cane in the dark, bio-engineering that allows the algae to make oil without going through the process of photosynthesis. Further into the future, the hope is that the resultant oil can function as a triglyceride oil for the manufacture of other chemical products.

Research in synthetic biosystems and fermentation processes are resulting in revolutionary advances in production of ethanol via consolidated bioprocessing (CBP) and commercial heterotrophic production of algal biomass ([Solazyme](#); [Amyris](#); [General Atomics](#)). These biosystems utilize symbiotic microbial cultures for development of clean and scalable solutions for production of bioenergy, industrial chemicals, and specialty ingredients markets for the food industry.

Synthetic biology will make great strides in improving chemical manufacturing processes when it can develop fermentation that results in the creation of industrial monomers. The value of these monomers will be in their lower cost and the ability to manufacture in smaller volume batches – making them a more valuable commodity than petrochemicals. As well, synthetic biology will allow the substances to be manipulated to provide a high degree of specialization to achieve specific performance goals and product features. The future could become reality in this respect in just two years ([Verdezyne](#); [BioEnergy International](#); [Boswell, 2009](#); [Jenkinson, 2009](#)).

### ***\$85 Million Funding Opportunity for Algae & Advanced Cellulosic Biofuels***

Similar to processing algal biomass and production of “green crude or biocrude” which can be refined via existing petroleum infrastructures, hydrous ethanol is compatible with gasoline and qualifies as an advanced biofuel. As explained in more detail below, catalytic and plasmatron reforming technologies allow for utilizing hydrous ethanol as a hydrogen-rich energy carrier which qualifies it for the following FOA by the DOE. Algae strains high in either lignin-free starch or lipid content can be utilized to produce hydrous ethanol, green gasoline, or biodiesel.

On 16 Jul 2009, the U.S. Department of Energy (DOE) today announced the availability of up to \$85 million from the American Recovery and Reinvestment Act for the development of algae-based biofuels and advanced, infrastructure-compatible biofuels. DOE is seeking to bring together leading scientists and engineers from universities, private industry, and government to develop new methods to bring new biofuels to market in an accelerated timeframe.

The partnerships will enable cross-fertilization between multiple disciplines and provide the breadth of expertise necessary to develop new technologies advanced biofuels that can be used in today's fueling infrastructure such as green aviation fuels, green gasoline, and green diesel from a variety of biomass feedstocks. Partnerships may include leading scientists and engineers from universities, private industry, and government, and engage end users and other field experts such as utility specialists and aquaculturists. Effective collaborations will target an accelerated timeframe to bring new biofuels to market.

DOE expects to select two to three partnerships and fund projects over three years. Today's Funding Opportunity Announcement targets two crucial areas:

- Algal Biofuels R&D - The primary objective of this topic area is to develop cost-effective algae-based biofuels that are competitive with traditional petroleum-based fuels.
- Advanced, Infrastructure-Compatible Biofuels R&D is focused on enabling cost-effective conversion of biomass to advanced biofuels including cellulosic ethanol, with particular focus on bio-based hydrocarbon fuels such as green gasoline and green diesel. Such fuels could be transported and sold using today's existing fueling infrastructure.

Responses are due by 14 Sep 2009. The FOA is available at [FedConnect](#), and can be found by searching for Reference Number DE-FOA-0000123 ([DOE Recovery and Reinvestment Web site](#); [DOE Biomass Program Web site](#)).

In addition to the advanced biomass feedstocks and biofuels consortiums, on 31 Jul 2009, as part of the Recovery Act: DOE & the US Treasury announced they are now accepting applications for funding of renewable energy projects placed in service beginning 1 Jan 2009. With the goal of expanding development of renewable energy projects throughout the United States and creating new jobs, the U.S. Department of Energy and the U.S. Department of the Treasury is accepting applications for a program that will make direct payments in lieu of tax credits. The two Departments estimate distributing at least \$3 billion in financial support to approximately 5,000 biomass, solar, wind, and other types of renewable energy production facilities. ([DOE News Release, Jul, 2009](#))

### **Hydrous vs. Anhydrous Ethanol**

Ethanol (C<sub>2</sub>H<sub>5</sub>OH), otherwise known as ethyl alcohol, alcohol, or grain spirit, is a clear, colorless, flammable oxygenated hydrocarbon with a boiling point of 78.5 °C in the anhydrous state. In transportation, ethanol is used as a vehicle fuel by itself (E100), blended with gasoline (E85), or as a gasoline octane enhancer and oxygenate (10% concentration).

Anhydrous alcohol literally means no-water alcohol but in reality the term refers to less than 1% water. This distinction is often used synonymously with 200 proof alcohol. Anhydrous grades of alcohol in the US are usually below 0.3% water, typically below 0.2% water and several grades are certified below 0.1% water. It is also important to note that the term “anhydrous” is not synonymous with the term pure. Pure alcohol denotes an undenatured alcohol, which could be anhydrous or hydrated (190 proof or some other cut or proof).

The term anhydrous is not unique to alcohol products. It is also used for a wide range of high purity solvents, many of which are manufactured by industrial and pharmaceutical companies. Anhydrous ethanol refers to an ethyl alcohol that has a purity of at least 99%, exclusive of added denaturants, that meets all the requirements of the American Society of Testing and Materials (ASTM) D4806, the standard specification for ethanol used as a motor fuel. Industrially, the majority of all ethanol products, whether pure or denatured can be classified as 200 proof or 190 proof. 99% ethanol is actually equivalent to 198 proof and 99.5% ethanol is equivalent to 199 proof. 198-200 proof is considered absolute or pharmaceutical grade ethanol in which water has been almost completely removed from the alcohol.

The term hydrous ethanol is often used as a synonym for azeotropic ethanol. Regarding fermentation which is utilized to produce bioethanol via microbes (yeast or bacteria), azeotropic ethanol is the product of a series of conventional distillation columns which eventually produces a range of 93-96% ethanol and 4-7% water, e.g. 184-192 proof ethyl alcohol. For production of anhydrous ethanol using conventional technology, energy and capital intensive molecular sieves are then utilized to remove most of the remaining 3-6% water.

Conventional distiller's yeast which is utilized to process primarily starch feedstocks produces an average of 17 v/v% ethanol and 83% water. Cellulosic ethanol processes produce as little as 5-6 v/v% ethanol. Separation of ethanol and water currently comprises up to 40% of the total energy utilized by conventional ethanol plants, and considerably more for cellulosic ethanol plants

which produce anhydrous ethanol using conventional distillation and molecular sieve technologies. Hence, the opportunity to reduce energy costs for separation of ethanol from water provides substantial economical and environmental benefits.

Reforming technologies are currently being developed to convert up to 50% ethanol and 50% water to hydrogen rich fuels which contain similar BTU values as those for gasoline, but which produce considerably lower levels of GHGEs and particulate matter. In contrast to anhydrous ethanol, for gasoline blends containing 15% hydrous ethanol, there is no phase separation. Hence, hydrous ethanol is compatible with legacy vehicles and current gasoline infrastructures. Eventually, direct ethanol fuel cells (DEFCs) which run on hydrous ethanol promise to be much more efficient than internal combustion engines. In the meantime, catalytic and plasmatron reforming technologies which convert hydrous ethanol to hydrogen-rich fuels provide a bridge to the more efficient DEFC technologies under development.

### ***Characteristics of Azeotropic Ethanol & Dehydration Processes***

Azeotropic ethanol is a binary homogeneous mixture of ethanol and water in such a ratio that its composition cannot be changed except through azeotropic distillation (dehydration). This is due to the fact that when an azeotrope is boiled, the resulting vapor has the same ratio of constituents as the original mixture of liquids. For example, once the ethanol to water concentration reaches 96.4% through fractional distillation, the vapor from the boiling mixture is also 96.4%. This is due to the fact that the boiling temperature of the binary azeotrope is below that of pure ethanol. Further fractional distillation is therefore ineffective.

Anhydrous (also referred to as absolute) ethanol is a monohydric primary alcohol containing less than 1% water. It melts at -117.3 °C and boils at 78.5 °C. In contrast to anhydrous ethanol, azeotropic ethanol containing 95% ethanol and 5% water boils at 78.15 °C. Contrary to methanol and gasoline, ethanol is miscible (i.e., mixes without separation) with water in all proportions.

Energy and capital intensive azeotropic dehydration technologies such as material separation agents (benzene and other aromatics), mol sieves, and membranes are commonly utilized to reduce water content to less than 1% in order to meet ASTM specifications for anhydrous ethanol in the US. The most common dehydration method is the use of molecular sieves which simply absorb water from azeotropic ethanol. This breaks the azeotrope bond and increases the ethanol concentration, allowing for achieving over 200 proof ethanol (less than 1% water). The molecular sieve is then heated to remove the water and reused. This elaborate azeotropic dehydration process requires drying ethanol twice which is inefficient and results in increasing production costs and greenhouse gas emissions (GHGEs).

### ***Simple Distillation can Economically Produce 93-96% Ethanol Concentrations***

In Brazil, the majority of ethanol biorefineries utilize sugar cane feedstocks and fine tuning of conventional distillation columns to produce 95% ethanol. Though most corn to ethanol biorefineries in the US produce 92-93% ethanol, this could be increased to 95-96% ethanol by simply increasing the efficiency of fractional distillation columns similar to what Brazil producers are already doing. However, the potential opportunity to utilize less than 95-96% ethanol concentrations is technologically feasible and economically attractive, particularly for lower and midlevel gasoline blends.

Extensive engine testing of E100 anhydrous and hydrous fuels containing 7-20% water have been compared. Hydrous ethanol containing 93% ethanol and 7% water performs efficiently in automobile and high performance aviation engines. According to automobile and aviation fuel tests for midlevel to high level blends, 4-7% water in hydrous ethanol can actually enhance thermodynamic efficiency of internal combustion engines. This is particularly true for fuel injected turbocharged engines which capitalize on using oxygenated fuels (hydrous ethanol) with higher compression ratios and higher latent heat of vaporization. This results in increasing engine horsepower, engine torque, and fuel efficiency. Hydrous ethanol also reduces GHGs ([Society of Automotive Engineers, 2007](#); [Green Car Congress, 2008](#); [The Case for Ethanol: Baylor University Institute of Air Science](#); [Ford Lincoln Turbocharged MKR](#); [GM-Saab BioPower FFV Turbocharged Engines](#)).

Hence, azeotropic ethanol blends containing at least 93% ethanol could be sufficient for midlevel gasoline blends from HE10 to HE30 without affecting engine performance. For these blends, fuel efficiency could actually be increased in comparison with anhydrous blends and neat gasoline. Tests have been performed which indicate that fuel efficiency of high-level hydrous ethanol blends containing optimal water content are slightly superior to anhydrous ethanol blends due primarily to water's cooling effect and higher latent heat of vaporization ([Society of Automotive Engineers, 2007](#); [Green Car Congress, 2008](#); [The Case for Ethanol: Baylor University Institute of Air Science](#)). Depending on the denaturant and gasoline used in HE85 blends, fuel efficiency could be improved (vs. anhydrous ethanol) utilizing less than 7% water in hydrous ethanol. Parallel tests of hydrous and anhydrous ethanol blends, such as those currently being conducted in Louisiana, should determine optimal fuel efficiency of midlevel and high level blends correlated with water content.

### ***Independent Production & Blending Pump Distribution vs. Oil Company Monopolies***

US oil companies are using ethanol merely as a blending component in gasoline (in the form of E10) rather than a true alternative transportation fuel (in the form of E85). The major obstacle to widespread ethanol usage continues to be the lack of fueling infrastructure. Only 2,175 of the 161,768 retail gasoline stations in the United States (1.3%) offer E85. These E85 fueling stations are located primarily in the Midwest. According to the U.S. Department of Energy, each 2% increment of U.S. market share growth for E85 represents approximately 3 billion gallons per year of additional ethanol demand.

While alleging an oversupply of corn ethanol, U.S. oil companies, due to a loophole in the Caribbean Basin Initiative, are currently allowed to import thousands of barrels of advanced biofuel ("non-corn ethanol") every month without having to pay the 54-cent-per-gallon tariff.

Oil companies, or affiliates of oil companies, currently have a monopoly on blending fuel ethanol with unblended gasoline. Many states, e.g., Florida, allow only oil companies and their affiliates to blend and receive the 45 cents-per-gallon blender's tax credit. This monopoly impairs fair and healthy competition in the marketing of ethanol blends. Independent U.S. ethanol producers have the legal right, and must be assured the availability of unblended gasoline, to blend fuel ethanol and unblended gasoline to receive the blender's tax credit and be cost-competitive.

In short, independent U.S. ethanol producers do not have bargaining power on either end of the supply chain. Corn ethanol producers are price takers. A comprehensive advanced biofuel

industry development initiative is required to disrupt the status quo and establish fair and healthy competition in the marketing of advanced biofuel blends in our nation.

On June 21, 2008, Louisiana Governor Bobby Jindal signed into law the Advanced Biofuel Industry Development Initiative ("Act 382"). Act 382, the most comprehensive and far-reaching state legislation in the U.S. enacted to develop a statewide advanced biofuel industry, is based upon the "Field-to-Pump" strategy. It is the cost of the feedstock which ultimately determines the economic feasibility of an ethanol processing facility. "Field-to-Pump" does not allow an advanced biofuel producer to fall victim to rising feedstock costs. Non-corn feedstock is acquired under the terms of an agreement analogous to an oil & gas lease. It is not purchased as a commodity. A link exists between the cost of feedstock and ethanol market conditions. Farmers/landowners receive a lease payment for their acreage and a royalty payment based on a percentage of the gross revenue generated from the sale of advanced biofuel. "Field-to-Pump" marks the first time that farmers/landowners share risk-free in the profits realized from the sale of value-added products made from their crops ([Donavon, 2009](#)).

Blending pumps which provide anhydrous or hydrous ethanol blends ranging from E10 to E85 allow ethanol producers to capture the \$0.45/gal blending credit in addition to temporary advanced biofuel credits which can range as high as \$1.00/gal for processing lignocellulosic feedstocks. In addition to leasing land for production of advanced biofuel feedstocks, which indirectly competes with production of food, integrated biorefineries can utilize hydroponic greenhouses and algal bioreactors to produce advanced biofuel feedstocks rich in sugars, carbohydrates or oils depending on production goals. This production model for closed loop biomass and systems architecture for integrated biorefineries can dramatically reduce production costs and eliminate the volatility of commodity markets for biofuels. In addition, high value coproducts (including functional foods and special ingredients) and a variety of biochemicals and bioenergy resources are produced in addition to advanced liquid biofuels, all of which displace petroleum products.

### ***Energy Value for Hydrous vs. Anhydrous Ethanol Blends as Hydrogen-rich Energy Carriers***

It is generally accepted that molecules which contain polar hydrogen bonds such as hydrous ethanol, can store over five fold more latent energy than can hydrocarbon molecules contained in conventional neat gasoline components. The manner in which latent heat of vaporization occurs during the fuel reforming and combustion processes, exactly how that latent energy is stored and the effect that it has on inter-molecular forces and flame front speed, all contribute to explaining the phenomena of combusting hydrous ethanol in internal combustion engines. In addition, for gaseous samples of atoms such as oxygen, hydrogen and carbon, the only form that energy can take is translational motion of atoms. However, in gaseous samples of water and ethanol, energy can take the form of translational motion, rotational motion, and at high enough temperatures, vibrational motions of molecules.

In contrast to the low energy density of hydrogen (10.1 MJ/kg) in gaseous form ( $H_2$ ), hydrogen-rich liquid fuels contain 24.0-34.2 MJ/kg by volume. This represents almost a three-fold increase in energy density for liquid fuels in comparison with hydrogen gas in its natural state at ambient temperature and atmospheric pressure. In addition, liquid biofuels rich in hydrogen such as hydrous ethanol and hydrocarbons produced from biocrude can leverage existing petroleum distribution infrastructures without the expense and low safety factors associated with energy intensive compression of hydrogen including cryogenics.

Though there is a 34% difference in energy content (HHV/LHV) between ethanol and gasoline as utilized in conventional internal combustion engines, hybrid fuels consisting of midlevel ethanol blends actually increase fuel efficiency when compared with neat gasoline. This is due largely to oxygenated hydrocarbons. Research is currently being conducted to more fully understand this phenomena and determine optimal fuel efficiency for midlevel ethanol-gasoline blends (oxygenated hydrocarbons) which are being utilized in both legacy (non-flex fuel) and flex fuel vehicles (FFVs) in Europe and Brazil.

As illustrated below, much of the phenomena associated with both anhydrous and hydrous ethanol-gasoline blends and the use of hydrous ethanol as a hydrogen-rich energy carrier can be explained by intermolecular forces. The primary intermolecular force in this case is provided by hydrogen bonding to oxygen which is associated with the polar molecules of water and ethanol. Each hydrogen atom bond (2.2 electronegativity) to an oxygen atom (3.44 electronegativity) produces a net electronegativity of 1.04. While each polar ethanol molecule contains a single hydrogen-oxygen bond, each polar water molecule (H<sub>2</sub>O) contains two hydrogen-oxygen bonds providing a total electronegativity of 2.08. This is in comparison with a net electronegativity of only 0.35 for each hydrogen to carbon bond contained in hydrocarbon fuels. This represents almost a six-fold increase in electronegativity via hydrogen bond attractions for each hydrogen-oxygen bond in water vs. hydrogen-carbon bonds.

As illustrated above via hydrogen bonding, the intermolecular attractions of polar molecules create relatively strong bonding forces in comparison with neat gasoline components which are not comprised of polar molecules. Hence, the intermolecular attractions between water and ethanol in hydrous ethanol blends allow for storing vast amounts of energy in the form of latent heat of vaporization, particularly in comparison with neat gasoline. On a mass basis, the latent heat of vaporization for ethanol and water is substantially higher than that for neat gasoline. Heat of vaporization is always calculated as a positive value since it is an endothermic process, e.g. energy must be absorbed by molecules in order for vaporization to occur. This is what occurs during the phase change in which heat is continually added while the substance or solution remains at a constant temperature. This stored/potential energy in the form of latent heat of vaporization is subsequently released in an exothermic reaction during combustion of hydrous ethanol. The relatively strong intermolecular forces for homogenous mixtures of hydrous ethanol also prevent detonation, e.g. preignition which results in engine knocking which decreases thermodynamic efficiency. Prevention of detonation for anhydrous and hydrous ethanol blends appears to be partially due to the fact that the relatively strong intermolecular forces of ethanol and water make them less volatile than gasoline components.

In addition to the intermolecular forces created by hydrogen-oxygen bonding, the oxygen contained in water and ethanol molecules enhances the octane rating for “oxygenated hydrocarbon fuels,” thus improving efficiency of thermodynamic combustion and subsequently enhancing fuel mileage.

**Table 4. LHV & HHV for Internal Combustion of Ethanol & Hydrocarbon Fuels**

	Oxygenated renewable hydrocarbons	Petroleum hydrocarbon
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	Hydrous ethanol (4% H <sub>2</sub> O)			Anhydrous ethanol (<1% H <sub>2</sub> O)			Unleaded reg. gasoline (<1% H <sub>2</sub> O)		
	BTU/gal	MJ/gal	MJ/Lt	BTU/gal	MJ/gal	MJ/Lt	BTU/gal	MJ/gal	MJ/Lt
LHV	74,040	78.12	20.64	76,330	80.53	21.28	119,550	126.13	33.32
HHV	81,994	86.51	22.85	84,530	89.18	23.56	127,960	135.00	35.66

(Source: <http://www.eere.energy.gov/afdc/fuels/properties.html>)

Notice that in Table 4 there is a 10% difference between HHV and LHV for ethanol fuels, and a 7% difference for neat gasoline. The HHV (gross energy or caloric content) of a fuel is defined as the amount of heat released by a specified quantity (initially at 25 °C) once it is combusted and the products have returned to a temperature of 25 °C. The HHV takes into account the latent heat of vaporization of water in combustion products, and is useful in calculating heating values for fuels. HHV reflects improvements in thermodynamic efficiency of internal combustion engines. Since the latent heat of vaporization is higher for water and ethanol than it is for neat gasoline components, this explains why there is a larger variance between HHV and LHV for oxygenated hydrocarbons (ethanol blends).

The difference between two heating values depends on the chemical composition of the fuel. In the case of pure carbon or CO<sub>2</sub>, both heating values are almost identical, the difference being the sensible heat content of CO<sub>2</sub> between 150 °C (302 °F) and 25 °C (77 °F). Sensible heat exchange causes a change of temperature. In contrast, latent heat is added for endothermic reactions or subtracted for exothermic reactions during phase changes at constant temperature. An example is heat of vaporization for water which is reflected in the phase change from liquid to vapor/gas.

For hydrocarbons the difference depends on the hydrogen content of the fuel. Hydrous ethanol blends (oxygenated hydrocarbons) containing 7% water (azeotropic ethanol) lower engine operating temperatures due to cooling of the intake fuel mixture with 3-6.5% more water and provide an increase in heat of vaporization when compared to anhydrous ethanol blends. The result is more efficient combustion, cooler running engines, lower exhaust temperatures, and increased longevity of engine life for using hydrous ethanol blends. Due to the cooling effect that water has on intake charge and subsequent combustion, hydrous ethanol blends also reduce NO<sub>x</sub> emissions. In terms of greenhouse gas emissions, NO<sub>x</sub> are 296 times more potent than carbon dioxide.

In addition to the beneficial effects of higher water content in hydrous ethanol, ethanol allows for increasing compression ratios and octane levels in internal combustion engines. Essentially, both water and ethanol increase the octane level of the fuel mixture. The octane number is a measure of the resistance of a fuel to auto-ignition. It is also defined as a measure of anti-knock performance of gasoline or a gasoline component such as hydrous ethanol. Higher octane levels contribute to enhancing the thermodynamic efficiency of combustion engines, which subsequently increases fuel efficiency. The increase in total engine/thermodynamic efficiency results in optimizing fuel efficiency for both ethanol and gasoline. Theoretically, this would allow for achieving a BTU rating somewhere between LHV and HHV for oxygenation of hydrocarbon chains. As indicated above, midlevel blends of hydrous ethanol and gasoline, i.e. hybrid fuels, are actually more fuel efficient and higher in energy (LHV/HHV) than neat gasoline.

### ***Optimal Blend Level for Hydrous Ethanol & Gasoline***

“Previous assumptions held that ethanol's lower energy content directly correlated with lower fuel economy for automobiles. Those assumptions were found to be incorrect. Instead, the new research strongly suggests that there is an ‘optimal blend level’ of ethanol and gasoline - most likely between E20 and E30 - at which cars will get better mileage than predicted based strictly on the fuel's per-gallon BTU content. The 2007 flex-fuel Chevrolet Impala utilized in midlevel blends testing revealed a 15% increase in fuel efficiency using the Highway Fuel Economy Test (HWFET) for E20 in comparison with unleaded regular gasoline. For the same vehicle, the highway fuel economy was greater than calculated for all tested blends, with an especially high peak at E20. The new study, cosponsored by the US Department of Energy (DOE) and the American Coalition for Ethanol (ACE), also found that mid-range ethanol blends reduce harmful tailpipe emissions.” ([American Coalition for Ethanol, 2007](#); [DOE AFDC Intermediate Ethanol Blend Research](#))

Additional studies reveal that E25 may provide optimal fuel efficiency for ethanol-gasoline blends. “As known, ethanol has lower calorific value, higher latent heat of vaporization and higher stoichiometric fuel–air ratio than gasoline. As a result of such properties, it can produce a cooling effect on intake charge. Therefore, engine volumetric efficiency can rise. Due to the leaning effect on intake charge and the lower calorific value of ethanol, the cylinder pressure and temperature may increase by improving combustion and higher volumetric efficiency when gasoline–ethanol blends are used. Research indicates that ethanol addition to gasoline up to 25% raises combustion pressure. Increasing cylinder pressure can result in higher mean indicated work and the mean indicated pressure; therefore, engine power output and thermal efficiency may also increase. Improvements in combustion also result in higher combustion temperatures. Burned gas temperatures at the beginning of combustion were calculated approximately as the adiabatic flame temperature by neglecting the heat losses. For this reason, temperatures predicted at 10° before top center (TC) are higher and close to those predicted at TC. Combustion temperatures calculated at each piston position reached a maximum for the blend of 25 vol% ethanol ([Bayraktar, 2006](#)).”

Subject to conducting parallel tests with hydrous and anhydrous ethanol blends, the same theories should be true for midlevel hydrous ethanol blends. However, the cooling effect of water on the intake charge and heat of vaporization will result in increasing combustion pressure and decreasing exhaust temperatures. This results in increasing fuel efficiency and lowers NO<sub>x</sub> emissions. According to actual fuel tests in Europe, hydrous ethanol blends exceed the fuel efficiency of anhydrous ethanol blends which validates this concept.

This recently discovered phenomena for hybrid ethanol fuels appears to be due to the combined benefits of oxygenation and heat of vaporization in conjunction with capitalizing on the change in chemical and physical properties which occur as a result of mixing water, ethanol, and gasoline. When appropriately combined in hybrid fuels, the chemical reactions of these compounds optimize the efficiency at which internal combustion engines operate. For hydrous ethanol blends, this is accomplished primarily through the total heat of vaporization resulting from combining ethanol and water, which effectively increases net energy gain (NEG). Essentially, the lower energy content of hydrous ethanol is counteracted by an increase in engine performance due to the higher heat of vaporization and higher octane ratings for ethanol and water in comparison with neat gasoline and anhydrous ethanol blends.

### ***Latent Heat of Vaporization, Heat Capacity & Hydrous Ethanol***

Also referred to as enthalpy of vaporization (symbol  $\Delta_v H$ ), the heat of vaporization is the energy required to transform a given quantity of a substance, usually one mole of the substance such as water and ethanol, into gaseous form. It is measured at the normal boiling point of the substances. Enthalpy of vaporization can be viewed as the amount of energy required for overcoming the intermolecular forces and subsequent interactions of atoms and molecules in hydrous ethanol blends.

The heat of vaporization values for water and ethanol contained in hydrous ethanol blends (oxygenated hydrocarbons) are substantially higher than that for the combined hydrocarbon components of gasoline. The polar molecules in liquid water are held together by relatively strong hydrogen-oxygen bonds, and its enthalpy of vaporization ( $\Delta H_{\text{vap}}$  40.7 kJ/mol) represents a 5.4 fold increase in the energy ( $\Delta T_{\text{liquid water}}$  7.52 kJ/mol) required to heat the same quantity of water from 0 °C to 100 °C.

Though enthalpy of heat of vaporization is inversely related to thermal mass ( $C_{\text{th}}$ ), also called heat capacity, the amount of thermal energy released or absorbed is identical. Heat capacity is the ability of a substance to store heat or thermal energy. It is typically measured in units of J/°C or J/K (which are equivalent). If the substance consists of a homogeneous material with sufficiently known physical properties, the thermal mass is simply the amount of material present times the [specific heat capacity](#) of that material. For compounds such as hydrous ethanol, the sum of heat capacities for the individual components such as water and ethanol are used in the calculation.

As an [extensive property](#), heat capacity is characteristic of an object; its corresponding [bulk property](#) is [specific heat capacity](#), expressed in terms of a mass or number of moles or some other measure of the amount of material, which must be multiplied by similar units to give the heat capacity of the entire body of material. Thus the heat capacity can be equivalently calculated as the product of the mass of the substance and the specific heat capacity for the material, or the product of the number of moles of molecules present ( $n$ ) and the molar specific heat capacity ( $C_p$ ).

In regards to internal combustion engines, heat capacity ( $C_p$ ) is the amount of thermal energy stored in water vapor during combustion of hydrous ethanol and hydrocarbon blends. It is measured in terms of joules (J) that can be absorbed, such as during a phase change for enthalpy of vaporization and enthalpy of fusion. Quantitatively, the heat released by exothermic reactions such as freezing to produce ice, or cooling water from boiling (100 °C) to the point of freezing (0 °C), is the same thermal energy value as the heat of fusion (melting ice) and the heat required to warm water. Similarly, condensation releases as much thermal energy as that absorbed by heat of vaporization. Both heat of fusion and heat of vaporization are endothermic reactions.

When discussing latent heat of vaporization, it is important to distinguish the difference between latent heat and sensible heat. These terms merely refer to two different types of effects that can be produced by heat, not two different types of thermal energy. The three basic physical states of matter are solid, liquid, and gas (or vapor). Supercritical fluids (hybrid between gas and liquids) and plasma gases are arguably additional states of matter since they possess different physical properties at higher temperatures. The plasma state of matter is significant for reforming of hydrous ethanol into hydrogen-rich fuels via plasmatron technology.

The physical state of a substance is closely related to the distance between molecules, the number of electrons, and the size of electron clouds. As a general rule, the molecules are closest together in solids, farther apart in liquids, and farthest apart in gases and plasma. Electron activity increases with temperature. Each time water or another substance changes physical state, energy is involved. In the vapor state, water molecules are very energetic. The molecules are not bonded with each other, but move around as single molecules. The heat used in the phase change from a liquid to a gas is called the latent heat of vaporization. It is referred to as “latent” because it is being stored in the water molecules to later be released during the condensation process. Latent heat cannot be sensed since it does not raise the temperature of water or other molecules.

When heat flow to a substance is not reflected in a temperature increase in that substance, energy is being used to increase the distance between the molecules of the substance and to change it from a solid to a liquid or from a liquid to a gas. Latent heat is the energy transfer that occurs due to a change of state from solid to liquid or from liquid to gas. In accordance with the Laws of Thermodynamics, the energy is not lost to entropy, it is merely stored in the substance, in this case water and ethanol, as latent energy. In further accordance with the Laws of Thermodynamics, the energy is released when the substance changes back from gas to liquid (condensation) or from liquid to solid (freezing), since heat flows from the substance (i.e. water and ethanol) during these changes of state (phase change).

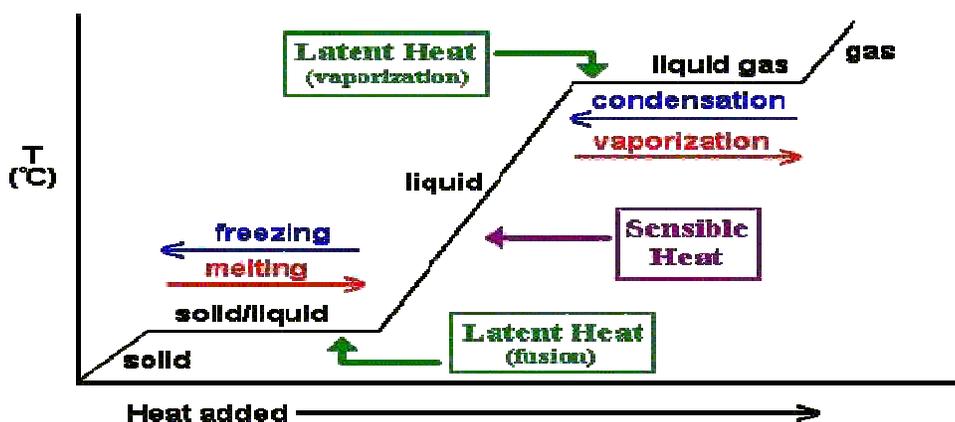


Figure 13. Illustration of latent heat in relation to sensible heat.

The thermal energy of a chemical system or reaction is measured in terms of enthalpy ( $\Delta H$ ). In the case of hydrous ethanol, it is the amount of thermal energy required to produce a phase change for one mole of water or ethanol. For water, the thermal energy required to melt ice is represented by  $\Delta H_{\text{liq}}$  6.02 kJ/mol, while the heat capacity of ice is represented as  $C_{s, \text{ice}}$  2.09 J/g multiplied by °C. The heat capacity if ice represents the amount of energy required to heat ice to 0 °C. Once all of the ice is melted, the amount of thermal energy required to heat water from 0 °C to boiling at 100 °C, is represented as  $\Delta T_{\text{liq water}}$  7.52 kJ/mol. The heat capacity for liquid water is represented as  $C_{s, \text{liquid water}}$  4.18 J/g multiplied by °C. The enthalpy/heat of vaporization for water increases to  $\Delta H_{\text{vap}}$  40.7 kJ/mol after reaching boiling, while the heat capacity of steam is represented as the same as that of ice, e.g.  $C_{s, \text{steam}}$  2.09 J/g multiplied by °C.

Since the mass for one mole of steam/water is 18g, in units of moles the heat capacity of steam is 3.76 kJ/mol (2.09 J/g x 18 g in a mole of H<sub>2</sub>O vapor x 100 °C). The heat capacity of steam is 10.82 fold less than the heat of vaporization. However, specific heat capacities for ice, water and steam should not be confused with the amount of thermal energy required for achieving phase changes. According to the Laws of Thermodynamics, though enthalpy and specific heat capacity use different units of measurement for quantifying energy, enthalpy of fusion and condensation which are the reverse processes of melting and vaporization, must release the same amount of thermal energy as that absorbed ( $\Delta H_{\text{vap}}$  40.7 kJ/mol) through endothermic reactions.

In addition to the strong hydrogen bonds contained in water molecules, the polarity of the OH groups contained in ethanol molecules form hydrogen bridges causing relatively strong attractive forces between molecules in liquid phases. Upon vaporization of hydrous ethanol as a fuel, the distance between the water and ethanol molecules increase such that molecular interactions including physical properties are entirely disrupted. This process accumulates a certain amount of latent (stored) energy. During combustion of these vapors, this explains why the latent heat of vaporization of hydrous ethanol blends is so much higher than that of regular gasoline components and non-alcohol oxygenates like methyl tertiary butyl ether (MTBE) which do not contain OH groups (non-alcohols). High heat of vaporization values are typical for water and alcohols including hydrous ethanol and hydrous ethanol blends (oxygenated hydrocarbons). Compare the differences in values listed for alcohols and water listed in Table 5.

**Table 5. Heat of Vaporization Values for Common Substances**

Compound	Molecular Weight	Heat of vaporization (kJ mol <sup>-1</sup> )	Heat of vaporization (kJ kg <sup>-1</sup> )
<a href="#">Ammonia</a>	17.0	23.4	1374
<a href="#">Butane</a>	58.1	22.4	385
<a href="#">Ethanol</a>	<b>46.1</b>	<b>38.8</b>	<b>842</b>
Gasoline	<b>100-115</b>	<b>34.95 - 40.2</b>	<b>304 - 350</b>
<a href="#">Methane</a>	16.0	8.2	511
<a href="#">Methanol</a>	32.0	35.3	1102
<a href="#">Propane</a>	44.1	18.8	426
<a href="#">Phosphine</a>	34.0	14.6	429
<a href="#">Water</a>	<b>18.0</b>	<b>40.7</b>	<b>2259</b>

(Source: Jo Sijben, senior consultant, [www.process-design-center.com](http://www.process-design-center.com))

Based on mass, the latent heat of vaporization stored in the hydrogen bonds contained in homogeneous mixtures of water and ethanol molecules results in nearly a three-fold increase (304:842 kJ-1 kg<sup>-1</sup>) in latent heat of vaporization (stored energy) for ethanol in comparison with neat gasoline. There is almost a 7 fold increase (304:2259 kJ kg<sup>-1</sup>) in latent heat of vaporization for water in comparison with neat gasoline. This superior latent heat of vaporization is manifested during the combustion of hydrous ethanol and the energy which is subsequently released by the hydrogen bonds of ethanol and water. This exothermic reaction is responsible for increasing combustion/thermodynamic efficiency for anhydrous and hydrous ethanol blends in comparison with neat gasoline.

Since the heat of vaporization for water is valued higher than that for ethanol, the combined heat of vaporization for hydrous ethanol would be valued slightly higher than anhydrous ethanol which contains 3-6.5% less water. There is an exponential increase in latent heat of vaporization in comparison of molar mass (kJ/mol) to kJ/kg, particularly for water (55.5 fold increase) and ethanol (21.7 fold increase) vs. neat gasoline (8.75 fold increase). This is due to the exponential increase in electronegativity of polar molecules which exhibit hydrogen bonding and strong intermolecular forces which are stored in the molecules prior to being compressed, and then released during combustion in piston engines. When water, ethanol and gas are vaporized and combusted in piston chambers, thermodynamic efficiency is dramatically increased since vaporization substantially enhances combustion efficiency.

According to Baylor University, “as far as safety and performance is concerned, hydrous ethanol is a slightly better fuel [than anhydrous ethanol] in every respect (except specific fuel consumption since water does not provide any caloric content). Small quantities of water absorbed in the fuel result in a slight increase in power caused by the higher latent heat of vaporization of the fuel... ([The Case for Ethanol: Baylor University Institute of Air Science](#)).” Baylor University’s Institute of Air Science has extensively tested hydrous ethanol containing up to 20% water. No noticeable change in performance of aviation piston engines was noticed for using up to 7% water for E100. For these experiments, it appears that water was merely added to anhydrous ethanol to develop hydrous ethanol fuels. Phase separation was only noticed for hydrous ethanol blends consisting of less than HE15 (15% hydrous ethanol).

Max Shauck, director of Baylor University’s Institute of Air Science conducted the first Trans-Atlantic flight using ethanol in 1989. Since 2004, over 800,000 hours of flights, primarily for crop dusters, have been logged using hydrous ethanol. Other than a 10-20% loss of range due to the lower compression ratio utilized in conventional internal combustion engines, the Institute of Air Science states that “ethanol is cleaner burning, produces more power, improves detonation resistance, and burns cooler and smoother, thus extending engine life. Additionally, it is considerably more economical than avgas, a fourth of the cost of avgas in Brazil and half the cost in the states, and requires only simple engine modifications for use in aircraft engines. And once an aircraft is converted, it can use either fuel or any combination of the two ([The Future of Aviation Fuels, Baylor University](#)).”

The safe and reliable use of hydrous ethanol in aircraft in the US and Brazil illustrates the improved performance and fuel efficiency available for use in automobile engines. This is particularly true for low and midlevel blends which do not require engine modifications while substantially improving fuel efficiency. Vaporization of liquids with a higher heat of vaporization leads to vapors with lower temperature, provided no extra heat is present in the vaporization chamber. Hence, the engine fuel intake is cooler and subsequently, so is the exhaust of an unmodified engine. In summary, increasing engine performance via oxygenated hydrocarbons such as hydrous ethanol blends appears to translate into higher thermodynamic efficiency.

### ***Water Injection is a Proven Technology for Improving Engine Performance***

Similar to the benefits of using hydrous ethanol blends in conventional internal combustion engines, many water injection systems for high performance engines use approximately a 1:1 ratio of water and alcohol. The water provides the primary cooling effect for air and fuel injection mixtures due to increased density and high heat absorption properties. Water instantly

reduces air intake charge temperatures by 50-200 °F. This provides a substantially cooler and denser air charge for greater expansion of power within the combustion chamber.

It is becoming quite common to find engine builders using 91-93 octane gasoline combined with water/alcohol injection to support engines well over 1000+ horsepower. One top engine builder in the industry has already produced upwards of 1700+ horsepower with a ProCharger supercharger carbureted big block Chevrolet on 93 octane regular unleaded gas using 100% water injection.

### ***Hydrous Ethanol Prevents Fuel from Freezing & Eliminates Cold Starting***

According to HE Blends, the alcohol in hydrous ethanol blends serves as antifreeze for water to temperatures as low as -70 °F (-57 °C), thus eliminating the requirement for cold starting systems. The freezing point for ethanol is -173.2 °F in contrast to -40 °F for gasoline.

The difference in freezing temperature between hydrous ethanol and neat gasoline is another example of how hydrogen bonding alters the physical properties of homogenous mixtures of hydrous ethanol in comparison with anhydrous ethanol and neat gasoline. Similar to the phase change which occurs during boiling of water (latent heat of vaporization), the energy required to change a gram of a substance from the solid to the liquid state without changing its temperature is commonly referred to as heat of fusion. This thermal energy breaks the solid molecular bonds, but leaves a significant amount of energy associated with the intermolecular forces of the liquid state. In the case of hydrous ethanol, this primarily results from hydrogen bonding of polar water molecules and ethanol. The polarity of the hydrogen oxygen bonds in ethanol and water molecules enhances hydrogen bonding which enhances miscibility, forming a homogeneous mixture which reduces phase separation and provides lower freezing temperatures.

Interestingly, the unique properties of polar water molecules reveal that liquid water warming contains 7.52 kJ/mol of heat capacity (latent heat of fusion) while ice contains 6.02 kJ/mol. Thus, liquid water retains 80% of the energy contained in its solid form (ice) during warming in which the temperature of the water remains stable at 0 °C (32 F). After all of the ice is melted, the water temperature increases to 100 °C (212 °F) with heat capacity increasing by 6.76 fold to 40.7 kJ/mol (latent heat of vaporization) during the phase change from liquid to gas. In contrast to water, anhydrous ethanol boils at 78.3 °C and fusion (melting) occurs at 114.1 °C. It is the polar bonding and subsequent hydrogen bonding attractions between ethanol and water molecules which allows for miscibility, i.e. the formation of homogeneous hybrid fuel mixtures which are rich in hydrogen and possess unique physical and chemical properties in comparison with anhydrous ethanol, water, or hydrous ethanol-gasoline blends.

Though engines using from 30% to 100% anhydrous ethanol require cold-starting systems, engines using hydrous ethanol require no cold starting systems. For E85 fuel at temperatures below 11°C (52 °F) a cold-starting system or flexible fuel vehicle (FFV) is required for reliable starting. Such systems are also required to meet US EPA emissions standards.

Hydrous ethanol blends from HE15 to HE90 (15-90% hydrous ethanol) can be utilized in conventional gasoline fuel injection engines or in FFVs. FFV technology allows for optimizing a variety of mid and high level ethanol blends, thus reducing the effects of cold starting and inefficient catalytic converter processing due to lower exhaust temperatures.

### ***Hydrous Ethanol Prevents Phase Separation***

By avoiding lower percentage hydrous ethanol blends in the HE5-HE6 (5-6% hydrous ethanol) range which is chemically unstable, phase separation (which causes corrosion resulting from exposing water to steel pipe lines, etc.) is prevented. This is due to the lower (freezing) and higher heat of vaporization exhibited by the relatively strong intermolecular forces of hydrogen bonds contained in homogenous mixtures of ethanol and water.

For European gasoline, HE10 (10% hydrous ethanol) is sufficient. North American gasoline fuels require HE15 (15% hydrous ethanol). If the hydrous ethanol concentration is beyond 10-15%, water in the mix is taken up by the alcohol, and then water and gasoline are completely compatible. For blends lower than HE15, isobutanol (i-ButOH) can be added to hydrous ethanol blends to eliminate phase separation.

Another simple solution to avoid phase separation is to use higher hydrous ethanol blends. For example, obtaining an EPA waiver and raising the RFS to HE15 to HE30 would benefit all sectors of the ethanol industry including current supply and demand issues which are driving down the price of ethanol and reducing profit margins for ethanol producers.

In the case that phase separation occurs due to mixing blends ranging from HE10 to HE15 with neat gasoline during refueling, excess water will merely precipitate to the bottom of the fuel tank where it will not typically enter into the engine fuel system. Most fuel systems are specifically designed to avoid pumping water which accumulates at the bottom of fuel tanks.

### ***Recent R&D on Hydrous Ethanol***

In principle, one can use a liquid-liquid extraction column to extract ethanol from an ethanol-water mixture into a gasoline stream. This presumes that the two liquids then can be totally separated, but researchers found this was not the case with hydrous ethanol. In the top of the extractor the densities of the two liquids were similar and took a long time to completely disengage, which led to prohibitively large extractor volumes that were operationally too expensive. An idea immediately followed, which was, if we can't get the water out, why not leave it in. 'So what appeared to be a show stopper for extraction promised to be an opportunity for hydrous ethanol blending.

## What's new in blending? – New patented insight

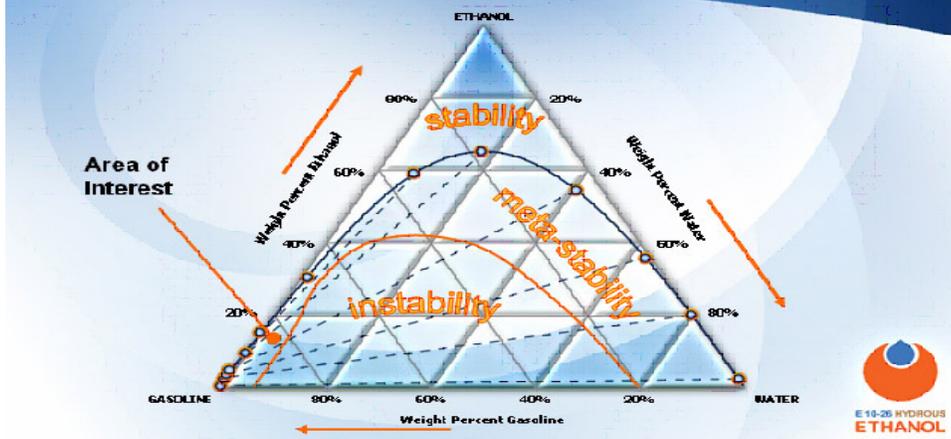


Figure 14. Ternary Diagram of Hydrous Ethanol-Gasoline Blends  
(Obtained from HE Blends website: [www.heblends.com](http://www.heblends.com))

Investigations of ethanol dehydration processes by the [Process Design Center](#) and the subsequent spin-off company [HE Blends](#) led to research work that revises and advances the technical understanding of water tolerances of ethanol/gasoline mixtures and the conditions under which phase separation occurs. This patented discovery reveals for the first time what conventional wisdom has simply overlooked – namely that hydrous ethanol can be effectively used in most ethanol/gasoline blending applications, reducing or eliminating the need for anhydrous ethanol production and distribution.

### ***Hydrous Ethanol is Currently being Utilized in Europe & South America***

Hydrous ethanol blends have passed stringent European Union (EU) standards according to independent laboratory validations conducted by TNO Automotive and SGS Nederland. HE15 in a standard VW Golf 5 FSI meets EU4 exhaust emission limits without engine optimization. The [SGS Group](#) is a global leader and innovator in inspection, verification, testing and certification services. Founded in 1878, SGS is recognized as the global benchmark in quality and integrity. With more than 48,000 employees, SGS operates a network of over 1,000 offices and laboratories around the world including the US.

In addition, [TNO](#) Automotive has been investigating state-of-the-art low blend ethanol fuels, looking at the information available regarding engine performance and exhaust emissions. The focus was on the differences between hydrous and anhydrous ethanol for low-ethanol gasoline blends. Component testing studies were conducted at SGS and TNO for CO, HC, NO<sub>x</sub>, and fuel consumption (CO<sub>2</sub>). These European test results may be utilized to assist with obtaining EPA waivers for midlevel hydrous ethanol in North America. 100% hydrous ethanol blends (HE100) are currently being utilized in German manufactured auto engines and other manufactured engines including FFVs in Brazil and Sweden. In 2007, HE10 & HE15 were commercially introduced in the Rotterdam, Netherlands area under the European BEST project.

In Brazil, anhydrous ethanol is utilized for blending with gasoline in order to prevent phase separation for low level blends. In Sweden and Brazil, HE100 is commonly utilized in German manufactured engines and FFVs produced by a variety of auto manufacturers. Brazil has embraced FFV technology (90% of new cars can utilize E20 to E100 or HE100 blends) to a much larger extent than the US (3% in 2008). Except for Sweden which has also embraced FFV technology, the European Union appears to have adopted FFV technology on a more limited basis than Brazil and the US.

In Brazil, total June (2009) production of ethanol should reach 1.9 billion liters (502 million US gallons), of which 1.4 billion liters (370 million US gallons) will be utilized as hydrous ethanol for flex-fuel vehicles and 500 million liters (132 million US gallons) of anhydrous ethanol for gasoline blending in Brazil.

### ***Further R&D of Hydrous Ethanol***

International research is currently being conducted to continually improve commercial use of hydrous ethanol blends. This includes HE Blends' [International Patent Number WO 2006/137725 A1](#) which covers midlevel hydrous ethanol blends from 1 to 50 wt.% ethanol containing 1 to 10 wt.% water on the basis of the weight of ethanol for use as motor fuels. These motor fuel compositions may contain a second liquid phase that does not form a separate layer, and where no separate liquid phase can be detected by vision, and so meets with the specification that has become known as 'clear and bright'. HE Blends has filed a provisional patent for extending their blending, emulsifiers, and engine optimizing technologies to HE51-HE90. This International Patent pertains to utilizing blends of hydrous ethanol and gasoline in conventional internal combustion engines.

Brazil appears to have patented the use of 100% hydrous ethanol, which usually consists of 95 wt.% ethanol and 5 wt.% water. Though it is questionable whether the above patents would be enforceable due to the lack of proprietary technology involved, ethanol blends containing between 51 to 99 wt.% ethanol and 1-10 wt.% water are not currently patented.

HE Blends is continuing test programs in Europe, including operation and testing of vehicles with hydrous ethanol/gasoline blends. Independent research regarding technological and economical benefits of hydrous ethanol are currently ongoing in the Netherlands, US, Brazil, Sweden, Australia and other countries.

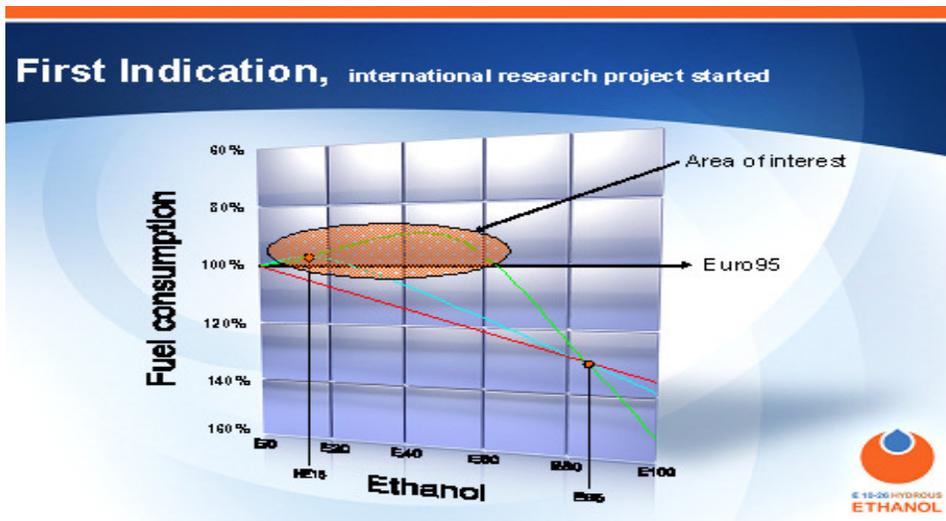


Figure 15. Diagram of Hydrous Ethanol-Gasoline Blend Research Project  
(Obtained from HE Blends website: [www.heblends.com](http://www.heblends.com))

In Europe, the [BioEthanol for Sustainable Transportation \(BEST\)](#) program is testing hydrous ethanol in more than 10,000 ethanol cars and 160 ethanol buses. E85 and E95 fuel stations will be opened in cities throughout Europe. The project started in January 2006 and will continue until the end of 2009.

On 25 Jun 2008, Louisiana enacted into law the [Advanced Biofuel Industry Development Initiative](#). It is the most comprehensive and far-reaching state legislation in the United States, enacted to develop a statewide advanced biofuel industry. Louisiana is the first state to enact alternative transportation fuel legislation that includes a variable blending pump pilot program and a hydrous ethanol pilot program. The initiative will also give an efficiency boost to the ethanol industry in the state by supporting high yielding non-corn crops.

### ***Preventing Phase Separation Eliminates Corrosion of Gasoline Pipelines***

Hydrous ethanol may be the key to ethanol's acceptance in the mainstream fuel pipeline distribution network. Conventional pipelining with anhydrous ethanol picks up water, and when it separates out corrosion is a concern. According to HE Blends, with higher concentrations of wet ethanol able to homogeneously retain up to five times more moisture than dry ethanol blends, the fear of phase separation is significantly reduced. People associate water with corrosion, but in this case the water is dissolved on a molecular level and completely sealed off by hydrocarbons, so these corrosion effects will not occur.

While more studies are needed to look at pipelining 15% or more hydrous ethanol in gasoline blends through petroleum pipeline networks, the phase separation principles should hold steadfast. If a certain higher percentage of hydrous alcohol and gas are premixed, the water-gas-ethanol mix will remain homogeneous—and therefore no phase separation and hence, no corrosion concerns.

### ***Hydrous Ethanol Trials Reveal no Damage to Engine Seals***

Extensive use of hydrous ethanol blends in Europe and Brazil reveal no damage to engine seals. In regards to safety and performance, Baylor University has concluded that “there is no danger

associated with water injection in an engine when using anhydrous ethanol [or hydrous ethanol from water absorption of anhydrous ethanol] as a fuel. Practical evidence of this characteristic of ethanol is the use of anhydrous ethanol in automobiles as a drying agent for fuel tanks in which water is suspected ([The Case for Ethanol: Baylor University Institute of Air Science](#)).”

### ***Hydrous Ethanol Reduces Fuel & Vehicle Costs***

According to HE Blends, eliminating ethanol drying could provide most biorefineries with a 10-20% reduction in production costs. This would be equivalent to about 0.15 € per liter or \$0.30 per gallon based on \$2.65/gal rack price for ethanol.

The 3-6% increase in volume of hydrous ethanol produced would substantially increase net profits for biorefineries. In addition, the reduction of energy consumption at the biorefinery would make ethanol production more sustainable—by 10-30% depending on the drying technology utilized by the biorefinery. An ethanol plant builder (Fagan) based in Minnesota is currently conducting a pro bono study to pinpoint the energy savings that result from skipping the dehydration step. The energy savings would provide opportunities for trading carbon credits as an additional source of revenue for biorefineries producing hydrous ethanol.

Once ethanol blends testing has been completed and environmental (EPA) waivers have been obtained, consumers would benefit from decreased costs of anhydrous ethanol blends, increased fuel mileage, and cleaner burning engines for longer lasting vehicles. For mid-level blends, this can be accomplished without purchasing a flex-fuel vehicle (FFV). The environment would benefit from lower GHGE (both CO<sub>2</sub> and NO<sub>x</sub>) and lower HC particulate emissions from utilizing hydrous ethanol.

### ***Overall Benefits of Hydrous Ethanol as a Transportation Fuel***

The advantages pertaining to the discovery of hydrous ethanol stem primarily from the avoided capital and reducing operation and energy costs by eliminating the energy intensive dehydration process for the hydrous-to-anhydrous ethanol step. A 3-6% product volume increase is achieved while reducing energy consumption and subsequent energy costs.

In summary, a transition from anhydrous to hydrous ethanol for gasoline blending is expected to make a significant contribution to ethanol’s cost-competitiveness, fuel cycle net energy balance, and greenhouse gas emissions profile.

Another well known benefit of water in fuels is the potential to reduce the formation of particle emissions. Today’s discussions about the high level of particle emissions caused by two stroke engines used in scooters and mopeds is an area where hydrous ethanol blends might be a solution reducing those emissions. This potential is addressed and well received by various stakeholders and continues to be investigated.

### ***Hydrous Ethanol’s Contributions to the Renewable Energy Industry***

Rapid expansion of the ethanol industry is creating global supply/demand issues. In some geographical areas, like the US, supply is outgrowing demand. This is having a negative effect on the price of ethanol for producers and sustainability of the ethanol industry. Due to emissions and durability testing requirements, ethanol producers are having difficulty with assessing the economic and environmental impacts of midlevel anhydrous ethanol blends on current auto engines in order to increase blending rates and the RFS.

In contrast to higher percentage anhydrous ethanol blends, HE15 and higher blends can be utilized in legacy vehicles (existing auto engines) as well as FFVs. Once parallel testing has been conducted for midlevel and E85/HE85 anhydrous and hydrous ethanol blends, further fuel efficiency and emissions testing may not be necessary. In addition to raising blending rates and the RFS, the high price of corn and competition between food and fuel is squeezing profit margins of ethanol producers, resulting in global inflation of fertilizers, and reducing food supplies for staple food products including rice, corn, potatoes and wheat. Hydrous ethanol blends could reduce some of this inflationary pressure by increasing efficiencies of production.

A 3-6% increase in ethanol production accompanied by a decrease in energy costs, plus an increase in fuel efficiency, will help to increase ethanol sales and profit margins for ethanol producers. Existing gasoline pipelines will be able to utilize midlevel hydrous ethanol blends as a much more compatible blendstock. This will dramatically reduce transportation costs by allowing petro-refineries and blenders to leverage existing infrastructures for distribution of hydrous ethanol. New high compression turbocharged engines designed for ethanol only, FFV, and ethanol hybrid vehicle technologies allow for utilizing hydrous ethanol in E85 and E100 fuels in conjunction with electric power to provide unprecedented power, fuel efficiency and emissions reductions. Such combinations can substantially reduce and eventually eliminate dependence on fossil fuels.

### ***Hydrous Ethanol as a Hydrogen-Rich Energy Carrier via Reforming Technologies***

Though conventional corn ethanol production can displace less than 25% of gasoline utilized in the US, if the whole plant (including corn stover, e.g. stalks, leaves, cobs) is processed along with crop residues and processing an abundant supply of “free” organic waste into biomass feedstocks, over 50% of current US gasoline consumption could be displaced with bioethanol. While corn produces up to 123 gal of ethanol per tonne via mature starch and fiber biorefining, corn stover can eventually produce up to 113 gal/tonne via mature SSCF and CBP technologies. In addition to using the whole corn plant to increase ethanol production by almost two fold, SDF, CEA, and precision agriculture could increase conventional crop yields by over two fold. In conjunction with energy conservation and development of high compression hydrous ethanol engines utilizing fuel injection, high octane vaporization, hydrogen boosting, and turbocharged engines, fuel efficiency could be increased by over 50%. These efforts could potentially allow for displacing 100% of gasoline in the US with bioethanol via internal combustion engines (ICEs). As illustrated by this concept, unprecedented economic and environmental production efficiencies for food and renewable energy are possible via comprehensive nutrient-energy management, precision agriculture, and closed loop production.

Unlike anhydrous ethanol, hydrous ethanol blends (HE15 or higher) do not phase separate and are compatible with gasoline pipelines and distribution infrastructures. In addition, hydrous ethanol can increase current ethanol production by over 6% for azeotropic blends, reduce production costs by as much as 30% depending on the efficiency of the separation technology utilized, provide higher fuel efficiency, eliminate issues with cold starting, and provide lower freezing temperatures than neat gasoline. Eventually, high compression, direct fuel injection, vaporization and turbocharged engines, hybrid electric technology, and particularly direct ethanol fuel cells (DEFCs) could dramatically increase fuel efficiency for the transportation industry. This is accomplished utilizing hydrous ethanol as a hydrogen-rich energy carrier without the need for expensive and unsafe compression or cryogenic storage technologies

required for pure hydrogen. The water contained in hydrous ethanol actually enhances combustion of ICEs by cooling the intake and providing latent heat of vaporization.

While hydrous ethanol testing via blending pumps has been approved and is underway in the US via the State of Louisiana ([Renewable Energy World, 2009](#)), Ford, GM-Saab, and Lotus are utilizing engine technology which enhances the energy contained in ethanol using E85 in Flex-Fuel vehicles (FFVs). The Lotus Exige 270E Tri-fuel is the most powerful road version yet of the Exige (0-60 mph / 96 kph in 3.88 seconds, a top speed of 158 mph (255 km/h), 270 hp (201 kW / 273 PS at 8000 rpm) and it runs on any mixture of gasoline, bioethanol and methanol. Emerging technologies will allow alcohol fuels such as methanol, already a proven internal combustion fuel, to be made synthetically from CO<sub>2</sub> extracted from the atmosphere. The heart of the Exige 270E Tri-fuel is a Roots-type supercharger (with a sealed-for-life internal mechanism meaning that it does not require the use of the engine's oil) and air to air intercooler attached to the tried and tested 4-cylinder, 1.8 liter 2ZZ-GE VVTL-i engine. Using a development of the supercharger and intercooler package from the Exige S, the Exige 270E Tri-fuel has peak power of 270hp (201 kW / 273 PS) at 8000 rpm, 184 lbft (260 Nm) of torque at 5500 rpm, up by 51 hp (38 kW, 52 PS) or 19% and 25 lbft 45 Nm or 14% over the standard gasoline Exige S. Maximum engine speed is 8000 rpm (8500 rpm transient for up to 2 seconds) ([Autoblog Green](#)). The [Ford Lincoln Turbocharged MKR](#) and [GM-Saab BioPower FFV Turbocharged Engines](#) also produce more horsepower, engine torque, and superior fuel efficiency via E-85 or even E-100 than do gasoline engines.

Unlike gasoline, methanol (extremely toxic) and ethanol are fully miscible in water. Butanol will mix with 7-9% water. Gasoline will mix with pre-bonded 4% hydrous ethanol. Hydrous ethanol in a liquid state will combust with up to 50% water. Above 50% water, hydrous ethanol needs to be vaporized before combustion in order to be efficiently utilized as a fuel. Ethanol's compatibility with water is what makes it superior to other fuels. This is possible by reforming hydrous ethanol, which has a relatively low energy density, to a hydrogen-rich fuel which has a relatively high energy density. Ethanol is extremely volatile and has a very high vaporization rate and flame speed. This appears to facilitate splitting water vapor into hydrogen and oxygen inside the combustion chamber of ICEs. This concept is illustrated by the energy density of hydrogen (61,100 BTU/lb HHV; 143 MJ/kg) in comparison with the energy density of ethanol (12,747 BTU/lb HHV; 84,530 BTU/gal HHV; 30 MJ/kg HHV). Hydrogen contains 4.77 fold the energy of ethanol. Each molecule of ethanol (C<sub>2</sub>H<sub>6</sub>O) contains six hydrogen atoms, and each molecule of water (H<sub>2</sub>O) contains two atoms of hydrogen. According to atomic mass units (AMU) ethanol is 12.5% hydrogen, and water is 11% hydrogen. Hence, a gallon of a homogeneous mixture of hydrous ethanol containing 93% ethanol and 7% water would contain approximately .8255 lbs H<sub>2</sub> per gallon according to the atomic mass units (AMU) of ethanol and water molecules:

- $.125 \text{ H}_2 \times 6.55 \text{ lbs/gal ethanol (C}_2\text{H}_6\text{O)} \times .93 = .7614 \text{ lbs H}_2$
- $.11 \text{ H}_2 \times 8.33 \text{ lbs/gal water (H}_2\text{O)} \times .07 = .0641 \text{ lb H}_2$
- HE100-93 (93% ethanol) weight =  $(.93 \times 6.55) + (.07 \times 8.33) = 6.6746 \text{ lb/gal}$
- **Total hydrogen reformed = .8255 lbs H<sub>2</sub> (12.3678%) per gal of HE100-93**

In contrast to ethanol, methanol contains only one carbon atom and four hydrogen atoms. Though the energy density of methanol is only 20 MJ/kg, e.g. 33% less than ethanol when reformed to hydrogen, methanol actually provides more energy due to a higher concentration of

hydrogen. However, methanol is toxic to handle and would require building new distribution infrastructures.

### ***Hydrous Ethanol Reforming Technologies***

“Direct utilization of ethanol at a 35% volume fraction reduces water separation cost to only 3% of the energy of ethanol and coproducts (versus 37% for producing pure ethanol), and improves the net energy gain from 21% to 55% of the energy of ethanol and coproducts. Vaporization and homogenous charge via timed ignition and auto-ignition utilize high compression engines to optimize combustion efficiency/thermodynamics.” A company in Idaho utilizes a catalytic process integrated with electronic ignition via smart plugs in the combustion chamber to dramatically enhance thermodynamic efficiency of internal combustion engines ([SmartPlugs](#)).

The University of California Berkeley and Lawrence Livermore National Laboratory has been developing a homogeneous charge compression ignition (HCCI) engine which runs on virtually any hydrous ethanol blend containing as little as 35% ethanol. “HCCI engines are amenable to a large variety of fuels as long as the fuel can be fully vaporized, mixed with air, and receive sufficient heat during the compression stroke to reach auto-ignition conditions. Stable HCCI operation was obtained for fuels containing up to 40% water. Incomplete combustion and excessive intake temperatures limited the operating range at higher water concentrations. The maximum value of the cumulative heat release profiles decreases with an increase in water concentration. Hydrocarbon and carbon monoxide emissions tend to increase with increasing fuel water content while NO<sub>x</sub> levels are low, which is typical in HCCI engines. However, there are problems related to non-catalytic assisted compression ignition of homogeneous wet ethanol in comparison with the catalytic timed ignition process developed by aqua-diesel utilizing smart plugs and times ignition. If the water enters the engine as a vapor, the benefits of the water in flame temperature control and detonation suppression are lost. Also, without a catalyst to lower the activation energy of the steam-reforming reaction it appears that there is little if any steam reformation in-cylinder if a proper catalyst is not present.

Because of the typically lean fueling rates of the above technologies, the low final flame temperatures result in significantly reduced emissions of oxides of nitrogen (NO<sub>x</sub>). Since the fuel and air in an HCCI engine are premixed and lean, there is no rich combustion (as in a Diesel engine) so particulate matter (PM) emissions are also quite low. HCCI engines also run at high compression ratios (CR) which directly increase the efficiency of the engine and consequently increases the fuel economy by a significant amount ([Martinez-Frias et al., 2007](#); Mack et al., 2009).

Chemical reforming is the processing technique by which the molecular structure of a hydrocarbon is rearranged to alter its properties. This includes increasing energy density. The hydrogen rich content of hydrous ethanol enhances reforming processes utilized by catalysts, plasmatron devices, , and in fuel cell applications. As demonstrated by Dongfeng Motors in China beginning in 2008, maximizing the energy efficiency of ethanol is achieved by blending it with water rather than gasoline. The reforming technology is based on a catalyst technology for use with hydrous ethanol containing 65% ethanol and 35% water (HE100).

In addition to decreasing the energy required for ethanol water separation, fuel efficiency is extended 35-50% through the addition of water. The result is production of hydrogen on demand

from hydrous ethanol. Hence, a gallon of a homogeneous mixture of hydrous ethanol containing 65% ethanol and 35% water would contain approximately .8529 lbs H<sub>2</sub> per gallon according to the atomic mass units (AMU) of ethanol and water molecules:

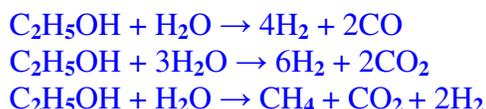
- .125 H<sub>2</sub> x 6.55 lbs/gal ethanol (C<sub>2</sub>H<sub>6</sub>O) x .65 = .5329 lbs H<sub>2</sub>
- .11 H<sub>2</sub> x 8.33 lbs/gal water (H<sub>2</sub>O) x .35 = .3207 lb H<sub>2</sub>
- HE100-65 (65% ethanol) weight = (.65 x 6.55) + (.35 x 8.33) = 7.173 lb/gal
- **Total hydrogen reformed = .8529 lbs H<sub>2</sub> (11.89%) per gal of HE100-65**

Notice that HE100-65 actually produces 3.3% more H<sub>2</sub> than HE100-95. Theoretically, the Aquanol-SmartPlug technology which reforms 50% ethanol and 50% water, results in producing 3.6% more hydrogen than HE100-65 per gallon of HE100-50. In addition, HE100-50 further reduces energy costs associated with ethanol and water separation processes:

- .125 H<sub>2</sub> x 6.55 lbs/gal ethanol (C<sub>2</sub>H<sub>6</sub>O) x .5 = .409375 lbs H<sub>2</sub>
- .11 H<sub>2</sub> x 8.33 lbs/gal water (H<sub>2</sub>O) x .5 = .45815 lb H<sub>2</sub>
- HE100-50 (50% ethanol) = (.5 x 6.55) + (.5 x 8.33) = 7.44 lb/gal
- **Total hydrogen reformed = .867525 lbs H<sub>2</sub> (11.66%) per gal of HE100-50**

Though the percent of hydrogen produced per gallon of HE100-50 is lower than that for HE100-65 due to the higher molecular weight of water, the actual mass of H<sub>2</sub> produced is 3.6% higher which results in higher fuel efficiency achieved from the same volume of fuel.

In addition to the reforming technology based on catalysts, a plasmatron reformer is being developed which produces the following from HE100-65 in terms of v/v%: 60.4% H<sub>2</sub>, 24.13% CO, 6.58% CH<sub>4</sub>, and 8.89% comprised of other combustible hydrocarbon compounds via a series of hydrous ethanol reactions which occur during reforming:



In regards to the functioning of a plasmatron, a special type of plasma electrical discharge boosts partial oxidation reactions between gasoline and air, producing hydrogen-rich gas. Based on energy densities of hydrogen (.604 x 143 MJ/kg), methane (.0658 x 55.6 MJ/kg), sundry hydrocarbon compounds and carbon monoxide is equal to about 94 MJ/kg, or 289,569 BTU/gal HHV for HE100-65. However, less than 39% of HE100-65 is reformed to plasma hydrogen compounds which results in producing approximately 113,963 BTU/gal HHV.

Unlike high compression, direct injection, turbocharged engines designed to combust anhydrous ethanol fuels, the plasmatron module is merely attached to the intake system of existing gasoline engines. Hydrous ethanol fuels with 5% hydrogen can improve combustion efficiency by 15% and reduce NO<sub>x</sub> emissions by 90% in comparison with gasoline ([Hu, 2007](#); [DOE Plasmatron Fuel Converter, 2001](#)). A gallon of HE100-50 contains .867525 lb H<sub>2</sub> while HE100-65 contains .8529 lb H<sub>2</sub>, and HE100-93 contains .8255 lb H<sub>2</sub>. Hence, on a mass basis calculated for the same volume of fuel, HE100-50 would increase combustion efficiency by 1.7% in comparison with HE100-65, and by 4.9% in comparison with HE100-93. This reveals that utilizing the Aquanol-

SmartPlug catalytic and China's DongFeng Motors plasmatron reforming technologies, HE100-50 and HE100-65 provide nearly identical concentrations of hydrogen with HE100-93 relatively close behind. However, in comparison with anhydrous ethanol, HE100-50 and HE100-65 reduce production costs by over 40% and increase volume of hydrous ethanol production by 50% and 35% respectively. This results in enhancing the net energy gain (NEG) for hydrous ethanol by almost two fold in comparison with anhydrous ethanol while reducing the carbon footprint and GHGs profile by over 90% in comparison with gasoline.

In physics and chemistry, plasma is a partially ionized gas, in which a certain proportion of electrons are free rather than being bound to an atom or molecule. The ability of the positive and negative charges to move somewhat independently makes the plasma electrically conductive so that it responds strongly to electromagnetic fields. Plasma therefore has properties quite unlike those of solids, liquids or gases and is considered to be a distinct state of matter. Plasma is produced via electrical discharges in which gases undergo a phase change to form the fourth state of matter.

A special type of plasma electrical discharge using a beveled-gear-electrode discharge boosts partial oxidation reactions between hydrous ethanol and air, producing hydrogen-rich gas. The plasma boosting provided by plasmatron devices facilitates the realization of a rapid response, compact and robust fuel- converter device. The hydrogen-rich gas, produced by conversion of a fraction of the hydrous ethanol, is fed into the vehicle's gasoline engine where it promotes more efficient combustion.

The hydrous ethanol plasmatron technology could be integrated with hybrid electric power trains. Though hydrogen can be combusted to produce energy, because of its high burning temperature, hydrogen combustion in a conventional engine would produce very high levels of NO<sub>x</sub>. However, there are methods for decreasing NO<sub>x</sub> emissions using existing technologies. For instance, catalytic burners can reduce the burning temperature and some burners use diffusion for low NO<sub>x</sub> burning of hydrogen. This is accomplished by using a small portion of hydrogen gas to convert NO<sub>x</sub> to inert nitrogen (N<sub>2</sub>) and water. The State of Louisiana has recently received EPA approval for testing hydrous ethanol blends HE10, HE20, HE30, and HE85 in both legacy and FFVs (HE10 refers to 10% hydrous ethanol, etc.).

### ***Utilizing Hydrous Ethanol in Fuel Cells***

Hydrous ethanol is also ideal for direct ethanol fuel cells (DEFCs). It is the future of liquid biofuels when utilized as a hydrogen-rich energy carrier. DEFCs have achieved a fuel efficiency of over 6,000 miles per gallon in test vehicles. Eventually, DEFCs will provide unprecedented fuel efficiency while emitting primarily water, no NO<sub>x</sub> emissions.

According to FuelCell Energy research trials conducted in the 1990's (provided sulfur and chloride contaminants are removed), water contained in hydrous ethanol actually enhances the steam reforming process for use with high temperature molten carbonate and solid oxide fuel cells. This steam reforming technology allows for replacing coal and natural gas with hydrous ethanol as feeds for fuel cell power plants. High temperature fuel cells provide DG-CHP with the capability of coproducing hydrogen for biorefining processes responsible for production of bioethanol and hydroprocessing of next generation biodiesel.

Ethanol may also be used as a fuel to power [direct-ethanol fuel cells \(DEFC\)](#) in order to produce electricity and by-products: water (H<sub>2</sub>O) and carbon dioxide (CO<sub>2</sub>). DEFCs are a subcategory of [proton-exchange fuel cells](#) where ethanol is not reformed, but fed directly to the fuel cell. Platinum is commonly used as an anode in such fuel cells in order to achieve a power density that is comparable to competing technologies. Until recently the high price of platinum has been cost prohibitive.

A company called [Acta Nanotech](#) has created platinum free [nanostructured anodes](#) using more common and therefore less expensive metals. A vehicle using a [DEFC](#) and non-platinum nanostructured anodes was used in the [Shell Eco-Marathon 2007](#) by a team of students from Offenburg Germany which [achieved an efficiency of 2,716 kilometers per liter of ethanol](#) (6,388 miles per gallon).

With ethanol, power densities as high as 140 mW/cm<sup>2</sup> at 0.5 V have been obtained at 25 °C with self-breathing cells containing commercial anion-exchange membranes. In practice, tiny metal particles are fixed on a substrate in such a way that they produce a very active catalyst.

A polymer acts as electrolyte. The charge is carried by the hydrogen ion (proton). The liquid ethanol (C<sub>2</sub>H<sub>5</sub>OH) is oxidized at the anode in the presence of water, generating CO<sub>2</sub>, hydrogen ions and electrons. The fact that water is used to oxidize ethanol indicates that hydrous ethanol could theoretically enhance performance of DEFCs. After oxidation, hydrogen ions travel through the electrolyte. They react at the anode with oxygen from the air and the electrons from the external circuit forming water.

Though mass production of DEFC technologies is not yet economical for commercial applications, this may change in the near future. Similar to conducting tests of combustion engine fuels, utilizing anhydrous and hydrous ethanol feeds should be compared in order to determine the efficiency of these fuel cell feeds for DEFCs.

### ***Specific Industry & Environmental Benefits of Hydrous Ethanol***

#### ***Ethanol Biorefineries***

- higher net energy balance
- lower production and energy costs due to skipping dehydration step (10-30% reduction in energy costs depending on type of drying technology used)
- lower capital costs for new biorefineries which can eliminate mol sieves
- reductions in maintenance and operation costs
- lower carbon footprint and carbon credits available for generating additional revenues
- decentralized ethanol production
- 3-6% increase in ethanol production yields and sales

#### ***Petroleum Pipelines Industry & Blenders***

- higher net energy balance
- elimination of phase separation for HE15 blends
- reduction/elimination of corrosion caused by phase separation
- absorbs 4-5 times more water than anhydrous ethanol
- more compatible high octane fuel (than anhydrous ethanol) for blending with gasoline

*Consumers (both transportation and small engine/2-cycle industry users)*

- higher net energy balance
- increasing fuel mileage via Hydrous ethanol which increases engine compression ratio and reduces detonation (engine knocking)
- lower costs for purchasing ethanol at the pump
- lower GHGE & HC particulate emissions
- FFV not required

*Automotive & Engine Manufacturers*

- higher net energy balance
- increased thermal efficiency of engines
- use of either existing gasoline engines or FFV engines
- improved fuel mileage
- higher octane fuel and water content extend engine life
- reduce GHGE and HC particulate emissions
- does not affect engine seals
- cold starting systems are not required to down to -70° F (-57° C)

*Fuel Cells & Turbine (Utility) Industries*

- higher net energy balance
- higher water content enhances combustion for turbines and steam reforming for fuel cells
- reduced cost of fuels for generating process steam via CHP technologies
- increased revenues via trading carbon credits

*Environment*

- higher net energy balance
- lower GHGE and HC particulate emissions (calculate total reductions in GHGE for various industry sectors listed above)

*Production of Bioproducts & Biochemicals*

- Higher value food, nutraceutical, and pharmaceutical products made from hydrous ethanol. U.S. Pharmaceutical (USP XV) grade is the water azeotrope of ethyl alcohol and is 95% ethyl alcohol by volume.
- The major use of ethyl alcohol is as a starting material for various organic syntheses:
  - Bimolecular dehydration of ethyl alcohol gives [diethyl ether](#), which is employed as a solvent, extractant, and [anesthetic](#).
  - Dehydrogenation of ethyl alcohol yields [acetaldehyde](#), which is the precursor of a vast number of organic chemicals, such as [acetic acid](#), [acetic anhydride](#), [chloral](#), butanol, [crotonaldehyde](#), and ethylhexanol.
  - Reaction with carboxylic acids or anhydrides yields [esters](#) which are useful in many applications.
  - The hydroxyl group of ethyl alcohol may be replaced by [halogen](#) to give the ethyl halides.
  - Treatment with [sulfuric acid](#) gives ethyl hydrogen sulfate and [diethyl sulfate](#), a useful [ethylating](#) agent.

- Reaction of ethyl alcohol with aldehydes gives the respective diethyl acetals, and reaction with [acetylene](#) produces the acetals, as well as ethyl vinyl ether.
- Treatment of ethyl alcohol with [ammonia](#) produces [acetonitrile](#), which may be reduced to ethylamine. These and other ethyl alcohol-derived chemicals are used in dyes, drugs, synthetic rubber, solvents, extractants, detergents, plasticizers, lubricants, surface coatings, adhesives, moldings, [cosmetics](#), explosives, [pesticides](#), and synthetic fiber resins.

### ***Hydrous Ethanol vs. High Oil Feedstocks Including Algal Biomass***

Using 6-8% high oil corn feedstocks such as that produced by the TOPCROSS® system could double the volume of corn oil and subsequent energy produced at integrated biorefineries. The TOPCROSS® grain production system licensed by DuPont Quality Grains is rapidly gaining popularity as the preferred method of producing high oil corn. The TOPCROSS system may minimize the yield disadvantage associated with conventional high oil corn hybrids, while also enhancing grain nutrient composition ([Thomison, 2007](#); [Thomison et al., 2001](#)). Agronomy and plant genetics professor Ron Phillips and a team of researchers at the University of Minnesota are working on breeding a new strain of corn that has over five times the oil of regular corn, e.g. from 4% oil up to 20% oil. The first generation hybrids contain 12% oil ([University of Minnesota, 2008](#)). In comparison with corn and other oil feedstocks, synthetic algae are being developed with up to 75% lipid content.

Since oil has a 1.0 conversion rate to biodiesel compared to a .511 conversion rate for starch to anhydrous ethanol, and a .568 conversion rate for cellulose to anhydrous ethanol, production of biodiesel provides significant economic advantages over production of anhydrous ethanol. However, an exception to this is the use of hydrous ethanol as a hydrogen rich energy carrier. Catalytic and plasmatron reforming technology allows for converting 50-65% ethanol and 35-50% water (HE100 containing 50-65% ethanol) to a hydrogen-rich fuel with an energy density (113,963 BTU/gal HHV) similar to gasoline (127,960 BTU/gal HHV). Catalytic reforming processes occur in the combustion chamber and be utilized with 50% ethanol in hydrous ethanol fuels. Until catalytic and plasmatron reforming technologies are commercially available, production of corn oil can substantially enhance net energy gain for integrated biorefineries. Corn oil and biodiesel (130,000-138,700 BTU/gal HHV) possess considerably higher energy densities than anhydrous ethanol (84,530 BTU/gal HHV) when combusted without chemical reforming. In addition, the economic value of food grade oil is nearly 3 fold higher than the value of biodiesel or ethanol.

The above example for producing corn oil illustrates the economic advantages provided by integrated biorefineries. The ability to produce biodiesel or food grade corn oil with a current retail value of \$7/gal provides flexibility, diversity, and additional revenue streams, thereby providing sustainability via simultaneous production of biofuels, bioproducts and high quality foods. However, algae oil which is much higher in omega-3s (long chain polyunsaturated fatty acids) than corn oil and hence considerably more valuable as a high value nutraceutical coproduct, is very appealing for production at integrated biorefineries which incorporate algal bioreactors in the form of fermentation vats.

Fats (fatty acids) are chemicals made up of long chains of carbon atoms surrounded by hydrogen atoms. When a carbon chain is attached to the maximum number of hydrogen molecules that it can hold, it is called a saturated fat. When one or more carbon atoms are joined by double bonds,

the fat is unsaturated. These double bonds allow the molecules to be easily broken down and used for a variety of essential body functions.

Omega-3 fatty acids have many different uses in the body. They are essential for the production of new cells and give flexibility to cell membranes, allowing for better absorption of minerals and nutrients. Doctors indicate that modern food processing, pollution, unhealthy lifestyles, and poor eating habits have resulted in 90% of Western populations requiring omega-3 fatty acid supplements.

### **Aqueous Enzymatic Corn Oil Extraction Process**

“An aqueous enzymatic method is being developed by USDA-ARS scientists to extract corn oil from corn germ. The basic steps in the method involve “churning” the corn germ with various enzymes and buffer for 4 hours at 50 °C, and an additional 16 hours at 65 °C, followed by centrifugation and removal of the oil layer from the surface. No hexane or other organic solvents are used in this environmentally benign extraction process. By using oven-dried corn germ samples (6 g) from a commercial corn wet mill, corn oil yields of about 80% were achieved using three different commercial cellulases. A fourfold scale-up of the method (to 24 g of germ) resulted in oil yields of about 90%. Nine other commercial enzymes were evaluated and resulted in significant but lower oil yields. In the absence of enzymes, oil yields of 27 to 37% were achieved. The chemical compositions of hexane-extracted vs. aqueous enzymatic-extracted corn oils were very similar. Paper no. J10906 in JAOCS 81, 1071–1075 ([Moreau et al., 2004](#)).” As new cellulase enzymes are developed for the ethanol industry, aqueous enzymatic oil extraction will become more affordable and economically attractive for commercial applications. ([Dickey et al., 2008](#))

### **Producing Nutraceuticals from Oil in Corn Hulls**

Corn hulls are composed of two major layers: the outer layer, the pericarp, is made up of non-living cell walls, and an inner layer, the aleurone, consists of a single layer of living cells, surrounded by thick cell walls. Commercial corn oil is extracted from corn germ and could thus more accurately be called ‘corn germ oil.’ The compositional differences between corn fiber oil and corn-germ oil (Moreau et al., 1996), with the most significant difference being the much higher levels of phytosterols in corn-fiber oil. Because of these exceptionally high levels of phytosterols, it has been suggested that corn-fiber oil has potential as a cholesterol-lowering nutraceutical product or food ingredient (Moreau et al., 1999).

During the conventional wet milling of corn, the fiber fraction (sometimes called white fiber) contains both ‘fine fiber’ derived from the endosperm (inner cellular fiber), and ‘coarse fiber.’ The coarse fiber is mainly the hull and is comprised of both pericarp and aleurone layers ([Moreau et al., 2000](#)).

Similar to harvesting corn oil from germ, high value food products such as fiber oil can also be produced by processing pericarp fiber. Agricultural Research Service (ARS) scientists have developed a method for preparing corn fiber oil, which allows corn aleurone cells to be recovered from the process. Corn aleurone cells provide higher oil yields than corn fiber. Corn fiber is comprised of about one-third aleurone cells and two-thirds other fibrous material. This means corn aleurone cells contain three times more oil than corn fiber—so about three times more phytosterol-rich oil can be obtained by extracting the same amount of material.

Corn fiber is a byproduct of the wet milling process—a process to remove the starch from corn. Corn fiber is the richest known source of natural phytosterols. Phytosterols block the absorption of dietary cholesterol in the body. Corn fiber oil contains about 10 times more phytosterols than regular corn oil. It can potentially lower serum cholesterol by 10-15%. Corn is the number one crop in the United States in terms of acreage and grain production. ARS research has shown that corn aleurone could be the most valuable part of the corn, thus industrial processing of the technology could prove highly profitable to millers and processors.

This invention could be used to develop nutraceutical products at integrated biorefineries if the volume of aleurone cells produced from corn fiber oils is sustainable based on the value of this product. The current technology lowers production costs from ARS's previous patent (USPN 5,843,499) on corn fiber oil. US Patent Reference: USPN 7,115,295 (Docket #0012.01), "Methods of Preparing Corn Fiber Oil and of Recovering Corn Aleurone Cells from Corn Fiber," which issued on October 3, 2006. Foreign rights are available ([Moreau & Singh, 2006](#)).

Once oils are extracted, both pericarp fiber and germ meal are excellent livestock feed coproducts for use by CAFOs. Ruminants such as cattle and sheep can efficiently process relatively high fiber diets. In addition, new-generation hemicellulase and cellulase enzymes enhance fiber digestion for both ruminants and monogastric species (swine and poultry). These exogenous fibrolytic enzymes allow for digesting high dietary fiber (NDF) without affecting livestock health and animal performance, particularly for monogastric species such as swine and poultry. Pericarp fiber can also be utilized as a fuel for fluidized bed reactors to produce power and process heat/steam for integrated biorefineries.

### **Optimizing Fermentation Efficiency & Production of Coproducts**

In addition to processing corn fiber and germ, corn fractionation allows for increasing efficiency of fermentation by over 70%. Similar increases in fermentation efficiency are obtained from using fractionation platforms for processing corn stover. The increase in production efficiencies is accomplished by removing nonfermentable portions of the corn kernel and corn stover biomass prior to fermentation. Increasing the concentration of starch and cellulose in the fermentor allows for simultaneous saccharification of ultra high gravity (UHG) fermentations. For corn stover, this allows for processing over 30% more biomass. It also results in a 30% increase in high quality coproducts including low molecular weight lignin derivatives and protein rich meal for animal feeding operations. For corn, fractionation results in processing 70% more grain and subsequently producing 84% more ethanol by fermenting both starch and fiber components; 70% more food grade corn oil; and a 50% increase in production of nutrient-rich distillers' grains (DGs) which can be fed at higher (50%) inclusion rates than conventional dried distillers' grains with solubles (DDGSs) products. All of this is accomplished without increasing the size of the fermentors.

The modified distillers' grains (MDG) contain 58.5% protein, 4.5% fat, and 2% fiber. This nutrient-rich animal feedstuff is substantially lower in fat and fiber than DDGS, and higher in protein (including essential amino acids) than either soybean meal or corn gluten meal. This results in increasing digestibility and subsequent inclusion rates in animal diets by over two fold. The increase in feed efficiency results in decreasing feed costs for livestock and poultry production by as much as 50% when MDG and fermentation residues are combined with other low cost feedstuffs (hay and crops residues). Fermentation residues are produced via processing lignocellulosic biomass. The 50% reduction in feed costs is possible by eliminating energy

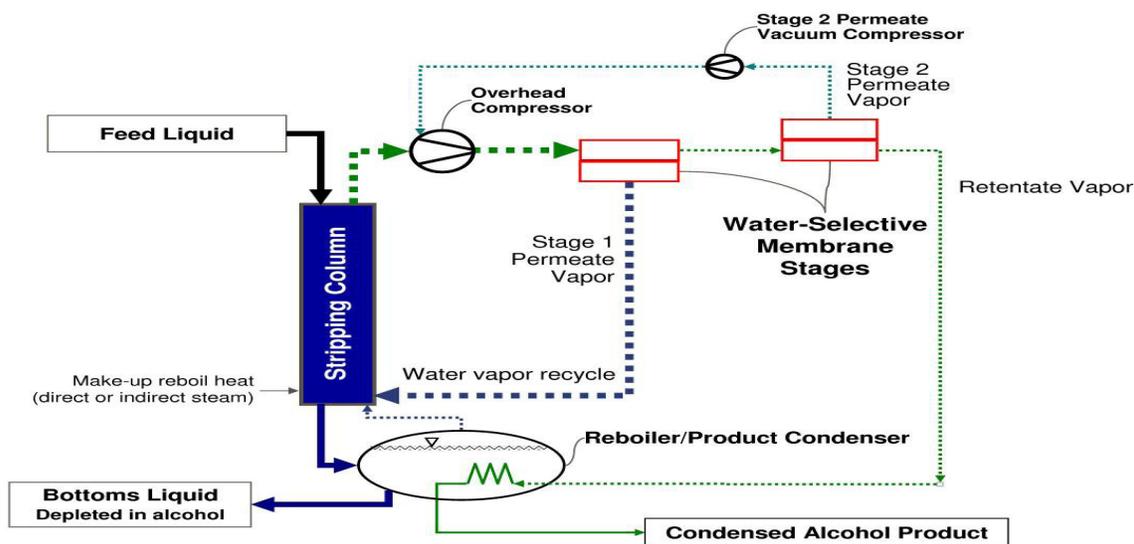
intensive drying, handling, transportation, and marketing required for distribution of DDGS and also for drying protein rich fermentation residues resulting from lignocellulosic biorefining. Drying and regenerative thermal oxidizers for DDGS represent 34% of process heat required at conventional corn ethanol plants ([Mueller and Cuttica, 2007](#)). Enzymatic hydrolysis for starch can eliminate jet cooking and reduce energy intensive hydrolysis, allowing for eliminating an additional 15% of energy costs for processing corn at integrated biorefineries. Eventually, enzymatic processing may allow for similar reductions in energy for hydrolyzing corn fiber and stover.

### **Membrane Assisted Vapor Stripping (MAVS)**

When membrane assisted vapor stripping (MAVS) technology is utilized in place of conventional (fractional or steam) distillation and molecular sieve technologies, the energy required for separating ethanol from water can be decreased by up to 50%. For production of 99.5% anhydrous ethanol, fractional distillation and mol sieves can comprise up to 40% of total energy consumed at a corn ethanol plant. For integrated biorefineries, total energy consumption can be reduced by over 50% by utilizing state-of-the-art MAVS technology, thermal integration, converting organic waste to biomethane, and implementing combined heat and power (CHP) applications. For production of 50-65% ethanol and 35-50% water (hydrous ethanol blends) for use with catalytic and plasmatron reforming technologies, the energy required for ethanol-water separation can be reduced by over 80%.

Depending on efficiency of ethanol and water separation from various fermentation broths containing different ethanol concentrations (starch vs. lignocellulosic biomass processing), achieving optimal energy efficiency may require separate water-ethanol removal systems, particularly for production of hydrous vs. anhydrous ethanol.

### **Membrane Assisted Vapor Stripping (MAVS)**



*Figure 16. Schematic diagram of a hybrid ethanol-water separation process ([Vane, 2008](#))*

The MAVS hybrid system utilizes a stripping column which provides a high alcohol recovery and a low effluent alcohol concentration. This is accomplished while the vapor compression

membrane component enables the efficient recovery of latent and sensible heat from both the retentate and permeate streams via the membrane system. No rectification column is used. The actual costs associated with the compressor and membrane components of the MAVS system in Figure 16 for recovering 1 MGY of ethanol from a 5 wt% ethanol feed stream using a 35 °C stripper were determined to be as low as \$0.11 gal-EtOH<sup>-1</sup> (\$0.036 kg-EtOH<sup>-1</sup>) and the associated energy usage was about 3 MJ-fuel equiv kg-EtOH<sup>-1</sup> (Vane and Alvarez, 2008).

The MAVS process has been estimated to require as little as 8.9 MJ-fuel/kg-EtOH to produce 99.5 wt% ethanol from a 1 wt% ethanol feed stream, and only 2.5 MJ-fuel/kg-EtOH for a 5 wt% ethanol feed stream (Vane and Alvarez, 2008; Vane et al., 2008). Comparable heat integrated distillation-molecular sieve systems would require at least 19.2 and 6.2 MJ-fuel/kg-EtOH for 1 and 5 wt% ethanol feed streams, respectively. To produce a 99.5 wt% ethanol product from a broth containing 11.5 wt% ethanol the MAVS process would require as little as 2.2 MJ-fuel/kg-EtOH (Vane et al., 2008). Thus, the MAVS hybrid ethanol separation process yields energy savings of at least 54% compared to the benchmark distillation-adsorption process. Côté *et al.* have proposed a hybrid distillation-vapor permeation process which replaces the rectification column and molecular sieve components of the conventional ethanol-water distillation-adsorption system with a vapor permeation dehydration system – such a process is referred to as the Siftek™ Dewatering System (Cote et al, 2007; Cote et al., 2008). Compression of the water rich permeate vapor from the first of two membrane stages allows for recovery of the latent heat from that vapor stream as heating steam in the reboiler of the beer column. (Cote et al., 2008; Vane, 2008)

In summary, reductions in fuel-equivalents usage of 50% or more for MAVS vs. distillation + sieves - independent of feed concentration are commonly observed. Since this refers to fuel-equivalents, it should translate directly into GHGEs reductions of 50% or more for that part of the separation process. This type of GHGE, associated with the production process is occasionally referred to as "upstream GHGEs".

MAVS data have been compiled for separating 5 wt% ethanol (circa 6 vol%). Distillation + sieves would require between 6.2 and 7.2 MJ-fuel/kg-EtOH to produce 99.5 wt% ethanol. Estimates for the MAVS system would require 2.5 MJ-fuel/kg-EtOH for the same product purity. That assumes 90% fuel-to-heat boiler efficiency for distillation, 33% fuel-to-electricity efficiency, and 75% compressor efficiency. Varying some membrane parameters and process pressures/temperatures, the MAVS number can be as low as 2.2 and as high as 3.1 MJ-fuel/kg-EtOH. Though data has not been compiled for an 8 wt% ethanol scenario (circa 10 vol%), it is estimated that distillation + sieves would require 4.5 to 5.5 MJ-fuel/kg-EtOH and would require 2 to 2.5 MJ-fuel/kg-EtOH for producing a 99.5 wt% ethanol product via MAVS technology.

“The hybrid stripping-vapor permeation MAVS approach is predicted to be more energy efficient and cost effective than distillation for the recovery of ethanol from ethanol–water mixtures and ultimate production of fuel-grade ethanol. The hybrid system takes advantage of the high ethanol recovery and low effluent concentrations offered by a stripping column with the high selectivity and ability to return the stripping phase made available by the vapor permeation system. Within the stripping options studied, steam stripping had the lowest potential energy usage and cost with estimated fuel-grade ethanol production costs of US\$0.16 gal<sup>-1</sup> from a feed containing 5 wt% ethanol and US\$0.37 gal<sup>-1</sup> from 1 wt% ethanol. These are both well below the current (December 2007) price of ethanol in the US of ca \$2.00 gal<sup>-1</sup>.43 Thus, even low

concentration ethanol waste streams could be upgraded economically to fuel-grade ethanol using the hybrid MAVS technology. For example, if considered in the context of wastewater treatment, the MAVS technology would cost US\$5 to treat 1000 gallons of 1 wt% ethanol wastewater. This same volume of wastewater would yield 12.6 gallons of fuel grade ethanol worth US\$25, thereby more than paying for the treatment. Other separations which can be performed with the MAVS system include other alcohol–water systems and even mixtures of organic solvents in which efficient stripping column VLE and selective membranes can be combined advantageously ([Vane and Alvarez, 2008](#)).”

[Membrane Technology & Service \(MTS\)](#)’s Liquid Separation Process Development Group supports the commercialization of PerVap® pervaporation systems technology for bioethanol production, food processing, and water treatment operations. In addition, the group develops liquid separation applications for solvent-resistant membranes in the food processing, petrochemical, and refining industries. MTR’s BioSep™ group, together with Dr. Leland M. Vane at the U.S. EPA Cincinnati Laboratory, has developed several novel membrane-distillation hybrid processes for bioethanol production. The membrane units in these processes use either vapor permeation or pervaporation. The processes are simple in design, offer significant separation process energy savings (more than 50%) and are cost competitive with conventional distillation-molecular sieve technology. BioSep™ processes are especially attractive for use in situations where the ethanol concentration in the fermentation step is low, such as cellulose-to-ethanol and algae-to-ethanol. A demonstration plant is being constructed in collaboration with a cellulose-to-ethanol producer; start up is scheduled for early fall 2009. Biobutanol is not currently produced as a biofuel for economic reasons, including the low concentration of butanol in the fermentation broth and the complexity of the conventional separation process to separate the three components – acetone, butanol and ethanol (ABE) – from the fermentation broth. In a new project funded by DOE, MTR is addressing separation issues by developing a low-cost, low-energy hybrid membrane-distillation separation process. Pervaporation/vapor permeation steps use membranes to concentrate and dehydrate the ABE mixture. The proposed process could save up to 87% of the energy that would be consumed to recover bioethanol and biobutanol by conventional separation techniques.

Vaperma has announced a partnership with UOP for marketing their Siftek technology ([Ethanol Producer Magazine, Mar, 2009](#)) which utilizes Siftek technology. The Siftek™ Dewatering System is similar to the MAVS system, though the latter has advantages according to patent applications which were recently published. One of biggest differences is the source of the driving force for membrane transport. As the patent application defines it, MAVS compresses the overhead vapor from the stripper to generate the feed vapor for the membrane process. The Siftek design does not compress the feed to the first membrane unit, it uses a vacuum pump on the permeate side of the membrane unit to generate the driving force. In most cases, the stripping column in MAVS will be under a vacuum and the temperature of the column can be controlled by changing the vacuum pressure. In Siftek, the stripping column has to be fairly hot and above ambient pressure to deliver a reasonable membrane feed pressure and, therefore, driving force. The MAVS technology decouples the feed pressure to the membrane unit from the pressure in the stripping column which can be advantageous, particularly in order to prevent killing organisms, deactivate enzymes, or precipitate out proteins, etc.

### ***Integration of CAFOs & Organic Waste Processing***

For each tonne of ethanol produced from corn biomass, a tonne of food and livestock feeds is also produced. Once fed to animals, animal waste from integrated CAFOs is then processed into biogas which provides grid connected DG-CHP. Each tonne of corn biomass including both grain and stover produces 14.27% (314.5 lb) MDG and 12.5% (275.5 lb) fermentation residues from lignocellulosic biorefining. When these biorefinery coproducts are mixed with 50% crop residues and forages, this is sufficient for feeding 59 animal units (AU) per tonne of corn biomass processed. An AU represents 1,000 lb of live weight animals. Hence, a 100 MGY ethanol biorefinery which processes 846,945 tonnes of corn and 677,556 tonnes of corn stover can support feeding 146,973 AU. Based on a 7.0 feed to conversion rate (FCR) for beef cattle, this would result in production of 64,718,697 lb of live weight beef. Based on a 65% dress weight after slaughter and food processing, this would be equivalent to producing 42,067,153 lb of high quality protein beef products. Dairy cows have a FCR of about 7.5 ([Grainger and Goddard](#); Rastani et al., 2001), e.g. they produce slightly less in terms of dairy products per volume of feed intake in comparison with beef cattle. Dairy cattle consume about 3.5% of body weight in comparison with 2% for beef cattle. Based on a 4.0 FCR for swine, and 2.0 FCR for poultry, integration of CAFOs would result in processing 79,280,404 lb of pork and 158,560,808 lb of chicken based on 70% dress weights.

Each AU produces enough organic waste to produce 10-29 MBTU of biomethane depending on efficiency of animal waste collection, food processing waste management, and efficiency of anaerobic digestion technology. Utilizing state-of-the-art high solids anaerobic digesters (HSAD) developed by NREL, it is possible to annually produce 2,204,595 MBTU based on each AU annually producing 15 MBTU. Based on a hybrid fuel cell capable of achieving 60% electric efficiency via a rankine heat engine, this would result in a feed requirement of 5.692 MBTU/hr, e.g. providing a 44.2 MW DG-CHP plant. At 90% total energy efficiency via CHP, 1.7078 MBTU/hr HHV would also be produced per MW, e.g. 75.51 MBTU/hr HHV for provision of process steam, heating digesters, and providing space heat for closed loop biomass via controlled environment agriculture (CEA).

### ***High Temperature Fuel Cells: DG-CHP***

Matrixes for calculating total energy efficiency consisting of electric efficiency and thermal heat recovery reveal the following:

***90% total energy efficiency, 60% electric efficiency with rankine heat engine***

$$(1.0 \text{ MWhr} \times 3.415 \text{ MBTU}) / 5.692 \text{ MBTU} = .60 \text{ electric efficiency}$$

$$0.90 \text{ total energy efficiency} = (1.0 \text{ MW} \times 3.415 \text{ MBTU} + X \text{ MBTU/hr}) / 5.692 \text{ MBTU/hr input}$$

$$\text{Solving for X} = (0.90 \times 5.692) - (1.0 \times 3.415) = 1.7078 \text{ MBTU/hr}$$

***90% total energy efficiency, 47% electric efficiency w/o rankine heat engine***

$$(1.0 \text{ MWhr} \times 3.415 \text{ MBTU}) / 7.21 \text{ MBTU} = .47 \text{ electric efficiency}$$

$$0.90 \text{ total energy efficiency} = (1.0 \text{ MW} \times 3.415 \text{ MBTU} + X \text{ MBTU/hr}) / 7.26 \text{ MBTU/hr input}$$

$$\text{Solving for X} = (0.90 \times 7.21) - (1.0 \times 3.415) = 3.074 \text{ MBTU/hr}$$

The primary advantages of using electrochemical conversion technology of fuel cells over thermal combustion processes of coal and natural gas turbines are the increases in total energy efficiency for CHP applications. It is considerably more energy efficient to produce heat as a byproduct of power generation than visa versa. Though rankine heat engines can utilize thermal integration via high temperature fuel cells to produce additional power, for integrated biorefineries this source of heat may be more efficiently utilized to provide process steam for

biorefining. Fuel cells also provide dramatic reductions in GHGEs and air pollution. For fuel cells operating on 99.9% pure biomethane or ethanol feeds, NO<sub>x</sub> emissions are essentially eliminated. In a comparison of high temperature fuel cells operating at 90% total energy efficiency with conventional natural gas or coal CHP systems operating at 49% total energy efficiency ([CHP for the Ethanol Industry, EPA](#)), GHGEs are reduced by over two fold. EPA provides a list of [state incentives and funding opportunities for CHP](#) applications.

In Jul 2009, DOE announced that a solicitation will seek applications for funding of research, development and demonstration of stationary CHP systems at three power levels. The first level covers "large" systems with greater than 20 megawatts (MW) of electricity output. This area has an estimated total budget of \$30 million—\$15 million from the DOE. Area 2 has an estimated total budget of \$30 million—\$15 million in federal funding and covers "medium" size systems between 1 MW and 20 MW. Area 3 has an estimated budget of \$20 million—\$10 million in DOE cost-share—and covers "small" size systems under 1 MW.

All levels will seek applications that provide plans to perform research and development focused on increasing the efficiency and reducing GHGEs of stationary CHP systems. These systems will be able to replace or reduce natural gas usage as well as minimize the energy and cost penalties of meeting emission regulations. The [Industrial Technologies Program](#) (ITP), part of the Office of Energy Efficiency and Renewable Energy, is DOE's lead organization for CHP Research and Development.

For closed loop production of biomass and biofuels via integrated biorefineries which process organic waste and recycle CO<sub>2</sub>, carbonic acid (produced during heat recovery using condensers) and NO<sub>x</sub> emissions via CEA applications (which provide CO<sub>2</sub> enrichment), energy costs and total GHGEs are essentially eliminated.

In comparison with other prime movers, additional advantages of fuel cell power plants are provision of uninterrupted power, stability to the grid via DG power contribution, and reduction of electric efficiency losses via DC power output. Fuel cells are best suited for providing base-load power needs, but with hybridization can also support peak power needs. The [Emergency Economic Stabilization Act of 2008 \(Section 103\)](#) and the Economic Stimulus Acts of 2009 provide Investment Tax Credits (ITC) of \$3,500/kW up to 30% of capital costs for fuel cell applications. Emissions Reduction Credits (ERCs) are also available for fuel cell applications in some states such as CA, providing an investment credit of \$200,000 per 300 kW of power generation in comparison with combustion technologies. A list of ITCs, ERCs, and [Renewable Energy Certificates \(RECs\)](#) available for each state is available via the [Database of State Incentives for Renewable Energy \(DSIRE\)](#).

CO<sub>2</sub> is produced during fermentation, during anaerobic digestion, combustion processes, and in the exhaust of high temperature fuel cells which utilize biomethane and bioethanol feeds. For example, a 100 MGY ethanol biorefinery annually produces 468,900 tonnes of CO<sub>2</sub> from fermentation alone. Generally speaking, for each tonne of ethanol produced, a tonne of CO<sub>2</sub> is also produced. Utilizing this for CO<sub>2</sub> enrichment in conjunction with nutrient rich fertigation streams for CEA can increase biomass production by over 62%. Based on producing 1.9 tonnes of biomass from each tonne of CO<sub>2</sub> that is recycled results in annual production of 890,910 tonnes of biomass. Based on production of 125 gal/tonne of lignocellulosic biomass, this would result in production of an additional 111 MGY of ethanol. Based on 60% oil content of algal

biomass and 90% extraction, this would result in production of 138 MGY of biodiesel and either 44.5 MGY ethanol or 356,364 tonnes of starch-protein meal for use in livestock feedstuffs.

In addition to processing animal waste, the relatively high protein and lipid content of animal offal from slaughter/packing processes and lignin-free cellulose content of algal biomass can potentially increase quantity and quality of biogas production by 2-3 fold. The maximum estimate for biogas produced per AU is based on daily production of 100 SCF at 80% methane from animal waste via state-of-the-art animal waste collection, pretreatment, and thermophilic HSAD technology. This is equivalent to producing 29 MBTU per AU. However, average biogas production per AU is currently only 10 MBTU. This is due primarily to inefficient collection of animal waste followed by inadequate processing. In addition to processing animal waste, mixed vegetative or animal substrates high in lipid concentrations such as animal tallow, carbohydrates and protein from food processing waste, offal and algal biomass can produce biogas containing as much as 79% methane ([Ghose et al., 1979](#); [Busch et al., 2009](#)). Processing equal volumes of animal waste, algal biomass and water hyacinth via anaerobic digestion would allow for more than tripling biogas production and subsequent DG-CHP for integrated biorefineries.

Up to 93% of methane in lipid-rich feedstocks can be recovered ([Cirne, et al., 2006](#)) via mesophilic or thermophilic anaerobic digesters ([Angelidaki et al., 1990](#)). Thermophilic digesters typically decrease hydraulic retention time (HRT) by 20% and provide pathogen free organic fertilizers and nutrient-rich fertigation streams for use in closed loop production of high value functional foods and biomass. Some studies reveal that utilizing the same biomass feedstock produced on dry lands, anaerobic digestion can produce more energy than that which results from production of biodiesel via conventional FAME processing ([Gunaseelan, 2009](#)). For low cost production and processing of aqueous feedstocks such as algae and water hyacinth, energy gains would be much greater while avoiding capital and energy intensive biorefining of advanced biofuel feedstocks. Hence, algal biomass rich in carbohydrates and lipids appears to be an ideal feedstock for mixing with animal waste for low cost production of biomethane.

With 90% of energy inputs at a typical ethanol plant coming from natural gas or coal, displacing these fossil fuels with renewable biomethane could capture over \$6 million per year in carbon credits and premiums for producing ethanol at 50 MGY biorefinery.

With the demand for price-volatile natural gas expected to increase 66% over the next 25 years, biomass and biogas energy is a long-term, physical hedge that an ethanol producer can implement to manage risk. Using biogas can also help to reduce an ethanol producer's carbon footprint and help to keep more money in the local economy. There are multiple sources of funds available to assist with financing. It's possible that these operations can be funded primarily with government money and very little equity.

Federal funding options include an "open-loop" biomass investment tax credit that can be used to recover 30% of capital costs and a combined heat and power (CHP) investment tax credit can be used to recover 10%. There are also closed loop investment tax credits available and a new market tax credit available to capture \$2 million for every \$10 million in capital expenditures. For loan guarantees, the US DOE offers 100% loan guarantees on 20% equity and the USDA offers loan guarantees of up to \$25 million. The Rural Energy for America program offers up to \$25 million in loan guarantees or \$500,000 grants, and has money available for completing feasibility studies. There is also the opportunity to depreciate the assets over five years and many

states have additional incentives available ([Biomass Magazine, Jun 2009](#)). Federal incentives are also available for replacing gas-turbine combustion systems with innovative high temperature fuel cells that provide distributive generated combined cooling heating and power (DG-CCHP) and operate on refined biogas or biomethane feeds as described above.

In addition to providing process heat which comprises 92% of total energy consumption for conventional dry mill ethanol plants, the electrical power requirement (8%) is .75 kWhr/gal of ethanol ([Mueller and Cuttica, 2007](#)). This is equivalent to requiring 8.6 MW per 100 MGY for conventional ethanol production. Based on reducing electrical power requirements for integrated biorefineries to 5 MW per 100 MGY of ethanol produced, up to 86% of the power produced, (e.g. 32 MW per 100 MGY) from animal waste and food processing waste can be sold to the local power grid. CHP providing 90% total energy efficiency provides sufficient process steam and heat for closed loop systems which simultaneously produce biofuels, bioenergy, food, biochemicals and bioproducts. Closed loop architecture results in reducing or eliminating economic dependence on importing petroleum products.

On June 12, the USDA Rural Business-Cooperative Service published two notices in the Federal Register announcing that funding will be made available for two programs established under Title IX of the 2008 Farm Bill.

One of these programs, the Repowering Assistance Program, is designed to provide payments to biorefineries that replace fossil fuel-derived heat and power with renewable biomass heat and power systems. The bill provides a total of \$35 million in funding for fiscal year 2009, and authorized \$15 million of that funding for fiscal years 2009 through 2012. In order to qualify, a biorefinery must have been in existence at the time the 2008 Farm Bill was passed.

Applications for the Repowering Assistance Program are being accepted between July 1 and November 1. “There is a window of opportunity for biorefinery entities that have an interest in retooling to bring on a biomass energy source versus a fossil fuel energy source to make application,” said Bill Hagy, deputy administrator of business programs rural development with the USDA Rural Business-Cooperative Service. The program is competitive. Procedures and criteria for applying are detailed in the [notice of funding](#) that was published in the Federal Register.

The [second notice](#) published in the Federal Register announced the intent of the USDA to enter into contracts to make payments to Eligible Advanced Biofuel Producers to support and ensure an expanding production of Advanced Biofuels. According to the notice, in order to participate in the program during fiscal year 2009, applications must be received between June 12 and August 11. “What’s unique about this program is there is no competition for entities that apply that are determined to be eligible,” Hagy said. “Any entity that applies that is determined to be eligible that is producing an advanced biofuel and that meets the other criteria of the notice of contract proposal will get a portion of the \$30 million that is available. That will be divided up among the eligible applicants based on their amount of production.”

For more information on programs established under Title IX of the 2008 Farm Bill, refer to the [USDA website](#).

### ***Comprehensive Nutrient-Energy Management***

After extracting methane, CO<sub>2</sub> and nutrient rich biological effluents from anaerobic digesters can be utilized as fertigation streams for closed loop biomass production. Subsurface drip fertigation (SDF) and high density cropping systems can increase conventional corn crop yields by over two fold. In addition to provision of precision irrigation and high density cropping systems, Monsanto's [Genuity](#) division along with other agricultural companies are developing improved hybrids with the goal of doubling crop yields by 2030. This will be necessary in order to keep pace with demands for feeding, clothing and providing bioenergy for growing world populations. CO<sub>2</sub> enrichment and CEA applications such as hydroponic greenhouses and algal photobioreactors can increase food and biomass yields by over 20 fold for integrated operations. For example, up to 60% oil content in algal biomass could be utilized for lignin-free processing of biofuels via either HSAD or biorefining.

A portion of lignocellulosic or algal biomass could also be fed to livestock to further enhance feed efficiency of rations. Each AU produces enough nutrients contained in the biological effluents of anaerobic digesters to produce the equivalent of 200 bu of corn. Based on a 100 MGY ethanol biorefinery supporting 146,973 AU, processing organic nutrients contained in animal waste to biomass would be equivalent to annually producing 29,394,600 bu of corn, e.g. 1,307,019 tonnes of corn and stover via closed loop biomass production. Based on producing 100 gal/tonne for processing corn and 75% of corn stover biomass, this would allow for producing 130 MGY of ethanol and 7.7 MGY of either biodiesel feedstocks or food grade corn oil at integrated biorefineries.

Lignocellulosic biomass consists primarily of polysaccharides, lignin, protein, extraneous substances and ash minerals. The extraneous substance refers to all the non-cell wall materials. Based on their solubility in water and neutral organic solvents, they can be classified as extractives, e.g., terpenes, resins and phenols, and non-extractives (mainly inorganics present in ash minerals), e.g., alkali, alkali earth carbonates and oxalates (Fan et al., 1987).

Polysaccharides, also called glycans, comprise high molecular weight carbohydrates, namely cellulose and hemicellulose. Once lignin and cellulose solid fractions have been separated from soluble hemicellulose via dilute acid, a black liquor containing monosaccharides, oligosaccharides, and polysaccharides is produced. Though there are some hexose sugars contained in the black liquor (primarily mannose and galactose), the majority of polysaccharides contained in liquefied hemicellulose are converted to pentose sugars. Thus, separation processes for liquids and solids allow for processing cellulosic pulp (hexose sugar) and pentose sugars separately or together in optimal ratios in order to optimize the fermentation process. This is accomplished using industrial yeasts and specialized ethanologens which are more efficient at converting xylose and arabinose to ethanol than conventional distillers' yeast.

The 97% pure cellulose can then be processed to ethanol or sold as a high value pulp/fiber. Hydrolyzing lignin free cellulose requires minimal enzyme loading and maximum conversion of polysaccharides and cellodextrin to glucose monosaccharides. Incubation of enzyme complexes and proteases for 4-6 hours prior to fermentation allows the hydrolyzing process to be completed in less than 24 hours. Subsequent fermentation allows for SSF via UHG slurries. If the fermentor temperature can be optimized for yeast and enzymes, it may be possible to ferment cellodextrins with dextrans from starch slurries. This would allow for using conventional distillers' yeast which are capable of reducing fermentation times to 27-30 hours while producing 18 v/v%. This is accomplished by optimizing the fermentation environment including provision of optimal

nutrient ratios for actively growing yeast. Once batch fermentations are concluded, the thin stillage can be utilized as a nutrient-rich backset for pentose fermentations. This allows for converting remaining polysaccharides and glucose sugars to ethanol via additional ethanologens such as industrial yeasts and synthetic thermophiles, thus maximizing ethanol yields for both starch and cellulose substrates via integrated biorefineries in order to achieve over 90% conversion efficiency.

### ***Economics for Anaerobic Digestion of Thin Stillage***

The thin stillage produced by pentose fermentations can be processed via anaerobic digestion to produce biogas which can be refined into biomethane and CO<sub>2</sub>. This option allows for reducing ethanol production costs and also carbon footprints. Eisenmann's patent-pending EtOH-TS anaerobic digestion process technology can be used to process thin stillage, a byproduct of the ethanol production process that is normally recycled to be reused during fermentation, to improve water and energy balance at an ethanol plant and to improve the performance of the plant's fermentation process. The EtOH-TS process produces both biogas—which can be used in place of natural gas for drying distillers grains or used to generate electricity—and also a clean effluent, with fewer solids and organic acids, which can be recycled to be used in the fermentation process at the plant. The process removes 100% of the lactic acid, glycerol, and waste ethanol, as well as 94% of the acetic acid from the thin stillage, which is six times less than the threshold where the acid begins to inhibit yeast. The process also removes as much as 78% of the total solids from the thin stillage, 52% of which are organic solids.

A typical 50 MGY ethanol plant that produces 80 gallons per minute of thin stillage might produce as much as 24 million BTU per hour of biogas using EtOH-TS. The biogas is 62% methane and the load on the ethanol plant's evaporator might be reduced as much as 21%.

Because thin stillage is an acidic byproduct, Eisenmann uses a neutralizer/buffer to control pH. To avoid increasing capital or operating costs, Eisenmann has developed specialized ammonia-adapted microorganisms for use in its anaerobic digesters so that ammonia—a product ethanol producers already store and handle—can be used as the neutralizer. The ammonia used in the digester is recycled and reused in the effluent, providing nutrition for the yeast during fermentation. Micronutrients are also fed to the digester to aid the microorganisms with digestion.

An additional benefit to having a thin stillage anaerobic digestion system, is that during ethanol plant maintenance, the thin stillage tank can be dumped into the anaerobic digester to produce more biogas. The system can be used to process any mix of syrup and thin stillage with a payback period of less than five years ([Biomass Magazine, Jun, 2009](#)). Poet Energy has developed a similar anaerobic digester for processing thin stillage ([Renewable Energy World, Jun, 2009](#)).

Depending on the feasibility for integration of CEA with microbial electrolysis cell (MEC) technology, it may be possible to eliminate anaerobic digestion for thin stillage and use it as a value added fertigation stream along with anaerobic digester effluents for SDF, hydroponic greenhouses and algal bioreactors.

### ***Integrated Biorefineries & Bioenergy Complexes***

Corn stover biomass contains 17-24% lignin which is not fermentable. As indicated above, this portion of biomass can be efficiently separated and purified prior to fermentation via complex lignocellulosic fractionation platforms. Over 97% pure lignin derivatives can be either sold as high value coproducts, or combusted via fluidized bed boiler systems to provide additional CHP for integrated biorefineries. FBRs utilize a relatively low combustion temperature at 850 °C (1560 °F). For combustion of lignin contained in fermentation residues this essentially eliminates nitrous oxide (NO<sub>x</sub>) emissions. The mineral-rich ash produced is relatively high in phosphorus and potassium which allows for use as an organic fertilizer. The 12.4% CO<sub>2</sub> and any NO<sub>x</sub> contained in the flue gas of the FBR (which is utilized to heat boilers to provide process steam for biorefining), can be captured and recycled for enhancing biomass production via CO<sub>2</sub> enrichment and CEA. Based on containing 20% lignin, if the entire volume of lignin obtained from processing corn stover at a 100 MGY integrated biorefinery is combusted via FBRs, this is equivalent to producing 115 MBTU/hr (307 tonne/day x 30% DM x 30 MBTU/tonne / 24 hours). Heat recovery using boilers allows for production of process steam for biorefining and provision of thermal integration, e.g. heating anaerobic digesters and providing space heat for CEA applications.

When combined with the thermal energy provided by 60% electric efficient fuel cells operating at 90% total energy efficiency (1.7078 MBTU/hr per MW), a 44.2 MW power plant would produce 75.5 MBTU/hr via biomethane. This would increase the thermal heat recovery available for integrated biorefineries which process both corn and stover to 190 MBTU/hr for a 100 MGY facility. However, based on a 50% reduction of the 1,720,000 MBTU of steam and process heat annually required for a 100 MGY corn ethanol plant ([Mueller and Cuttica, 2007](#)), the integrated biorefinery described above would require only 98 MBTU/hr. Hence, the large majority of the lignin produced from corn stover could be sold as a high value coproduct. Alternatively, the efficiency of organic waste collection and processing could be enhanced to increase CHP, and/or a 47% electric efficient high temperature fuel cell could be utilized to provide balance of CHP for integrated biorefineries. Either of these latter options would allow for producing sufficient process heat and steam for the integrated biorefinery without requiring the use of FBRs.

## **Ethanol BioEnergy Complex**

### **Closed Loop Production of Functional Foods, Bioproducts & BioEnergy**



resulting from marketing high quality foods and bioproducts, provision of an abundant and free energy resource in the form of organic waste, and trading carbon credits. Unprecedented efficiencies of operation can be achieved while simultaneously reducing pollution and GHG emissions via integrated biorefineries and closed loop production systems which leverage existing corn ethanol infrastructures for processing lignocellulosic feedstocks.

Fiber and germ meal can be utilized as livestock feeds, particularly for ruminants which utilize microbial populations and exogenous enzymes for efficient digestion of fibrous feedstuffs. In addition to use with ensiling, enzymes are aiding in digestion of ethanol coproducts which are included in animal diets. Use of enzymes in fibrous diets such as conventional DDGS can enhance feed efficiency and reduce nutritional concerns for livestock and poultry. Enzymes and the effects of feeding yeast in DG products can essentially eliminate the need for ionophores, particularly for natural production programs. “Evidence suggests that the benefits of exogenous enzymes is synergistic to ruminal endogenous enzymes. This synergy appears to explain why relatively small amounts of enzymes can have such large positive effects on animal productivity (Beauchemin et al., 2003). Enzymes help bridge the gap between actual digestibility of the feed that occurs *in vivo* and the potential digestibility of the feed that would be possible if the conditions are ideal (Beauchemin et al., 2008a; Beauchemin et al., 2008b). In addition to using exogenous enzymes for improving digestibility of livestock feedstuffs, select bacterial inoculations can substantially degrade and improve feed efficiency of fibrous feedstuffs, possibly without extensive use of exogenous enzymes (Biototal Forage Inoculations). Symbiotic bacteria and yeasts, such as those found in DG products, are utilized as probiotics for ruminants.

Fractionation of corn stover biomass and subsequent fermentation of cellulose and hemicellulose fractions is predicted to produce over 84 gal/tonne via fractionation using current technology. This is a 68% conversion rate in comparison with a theoretical yield of 125 gal/tonne for corn stover. The current industry average is less than 60%. Enzymatic hydrolysis via SSF may eventually increase conversion efficiency to 90%, e.g. producing over 112 gal/tonne of corn stover biomass. Consolidated bioprocessing (CBP) may eventually increase conversion efficiency to 95%, e.g. 119 gal/tonne.

Integration of lignocellulosic biorefineries with existing corn ethanol plants will substantially reduce energy requirements and capital investment. It also allows for recycling nutrient-rich thin stillage which contains undigested sugars for value added conversion to ethanol via industrial yeasts. The corn ethanol plant would provide power and additional heat via thermal integration and CHP technology. This is particularly advantageous for future use of lignin in higher value products rather than using it as a combustible fuel to generate process steam.

### ***Producing Ethanol from Food Processing Waste***

Similar to producing ethanol from crop residues, wheat gluten mills and cheese manufacturers produce byproducts which are rich in carbohydrates for use as ethanol feedstocks. Other byproducts from food processing industries provide similar opportunities for integrated biorefineries.

*Wheat Gluten:* Wheat gluten mills have been successfully integrated with ethanol biorefineries. Once the gluten has been extracted from the wheat kernel for production of protein supplements used in the baking industry, the remaining starch portion of the kernel is utilized as a starch rich feedstock for ethanol production. The result of mixing corn and wheat starch produces DG

products that are higher in nutrients for integrated livestock operations than straight corn DG products. DG products increase feed efficiency of livestock by over 20%, which enhances feed to conversion ratio (FCR). When DG products are mixed with crop residues and other high fiber/lignocellulosic feedstuffs, feed costs for CAFOs can be reduced by up to 50%.

*Cheese Manufacturing:* Cheese manufacturing produces 8.6 lbs of whey permeate for every lb of cheese produced. Depending on the cost for food grade lactose, producing ethanol can be more cost effective. The whey permeate consists of about 16% solids, the majority of which is lactose. Lactose is a form of sugar which can be converted into ethanol via fermentation utilizing a specialized form of yeast. Since lactose is a form of sugar, it does not need to be pretreated in slurry tanks like grain feedstocks. Producing ethanol from lactose requires only 30,000 BTU to produce a gallon of ethanol from whey permeate. Each year the United States produces 10 billion pounds of cheese, which results in 86 billion pounds of whey permeate ([Schill 2008](#); [Dubay Biofuels](#)).

A 40 million lb cheese factory requires processing 400 million lb of whole milk annually. Based on a 3.5% average daily feed intake for dairy cows (in contrast to 2% average daily feed intake for beef cattle) for production of 12,000 lb of milk per AU, this would require 58,333 AU to supply 400 million lb of whole milk. Based on a 7.5 FCR after supplementing 's products with additional feedstuffs, a 100 MGY biorefinery could provide enough dairy feed for supplying about four (40 million lb cheese) manufacturing plants with whole milk.

A 40 million lb cheese factory can produce enough whey permeate based on 16% solids to produce 3.18 MGY anhydrous ethanol. The beer from the lactose fermentor can be inserted directly with the beer from the biomass fermentors for ethanol-water separation via integrated biorefineries.

**Table 6. Whey Permeate Products:  
Cheese Factory Producing 40 Million lbs Annually**

400,000,000	lb of whole milk (33,333 AU; 12,000 lb per AU)
40,000,000	lb of cheese
344,000,000	lb of whey permeate with 16% solids (13% lactose)
39,090,909	gal whey permeate
3,179,654	gal anhydrous ethanol (<1 v/v% water)
3,473,610	gal hydrous ethanol (7 v/v% water – HE100)
4,292,533	gal hydrous ethanol (35 v/v% water – HE100)
5,580,293	gal hydrous ethanol (50 v/v% water – HE100)
9,622	tonnes CO <sub>2</sub>
879	tonnes dry yeast
32,836,364	gal recycled water for anhydrous ethanol

Similar to starch fermentation, lactose produces a lb of CO<sub>2</sub> for each lb of ethanol produced. For integrated operations CO<sub>2</sub> can be utilized as a value added nutrient stream for CEA such as hydroponic greenhouses and algal photobioreactors. Dry yeast is approved for use as a protein rich feedstuff for cattle and other livestock/poultry operations.

The 85% water and organic acids contained in the thin stillage of whey permeate fermentation can be utilized as livestock feeds or recycled along with distillation bottoms via anaerobic

digestion. These organic waste streams can be mixed with animal waste and excess thin stillage to produce additional biomethane and nutrient rich fertigation streams for closed loop biomass production.

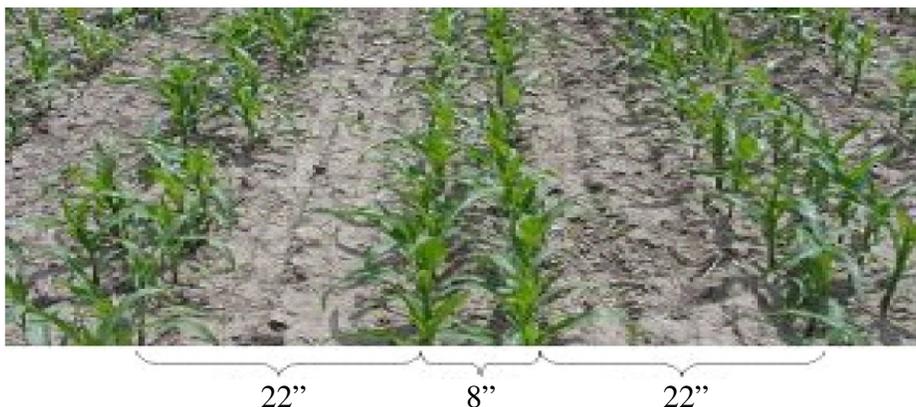
### ***Production of Lignocellulosic Biomass***

Lignocellulosic biomass from terrestrial plants, energy crops, and crop residues requires cleaning, reduction of particle size, pretreatment and fractionation in order to remove contaminants, degrade structural carbohydrates, remove non-fermentables, and thus optimize fermentation efficiency. Pretreatment processes increase surface area of particles, break the lignin bonds to cellulose, and reduce the crystalline structure of cellulose in terrestrial plant biomass. This is necessary in order to enhance enzymatic hydrolysis prior to fermentation. Enzymatic hydrolysis is preferred over acid hydrolysis in order to reduce production of toxic compounds.

Most lignocellulosic feedstocks including energy crops such as switchgrass and miscanthus will contain about 10-13% protein and less than 25% lignin. Sweet sorghum bagasse contains about 20% lignin and water hyacinth contains 18% crude fiber (including lignin) which could increase ethanol yields for these particular feedstocks. In terms of annual biomass yields, sweet sorghum can seasonally produce 12 tons per acre, and switchgrass/miscanthus can seasonally produce up to 16 ton per acre.

However, SDF and high density relay cropping systems for corn and triticale can produce 24 tons of grain and lignocellulosic biomass per acre. This is based on consistently producing 400 bu/acre corn crops and processing the majority of corn biomass at integrated biorefineries. SDF could also be utilized to improve crop yields for switchgrass, sweet sorghum, and other energy/food crops. SDF integrates subsurface drip irrigation (SDI) ([Rogers and Lamm, 2009](#); [Lamm, 2009](#)) and precision agriculture including chemigation via comprehensive nutrient-energy management and precision delivery of optimal nutrient ratios synchronized with plant growth stages.

### **SDF, Chemigation & High Density Relay Cropping Systems**



*Figure 18. Twin Row Crops with 30" Lateral Drip-tube Spacing*

### ***Controlled Environment Agriculture (CEA)***

Anaerobic digester effluents (from processing animal waste and other forms of organic waste) and thermal integration using waste heat from biorefineries reduces production costs for year

round production of food and energy crops. This is accomplished via CEA which includes hydroponic greenhouses and algal bioreactors. In contrast to terrestrial plants, CEA applications can produce the following volumes of biomass per acre:

- over 453 tonnes of lignin-free duckweed via specialized hybrids containing up to 45% protein or 75% starch ([Cheng & Stomp, 2009](#))
- over 333 tonnes of water hyacinth (lignocellulosic)
- over 441 tonnes of lignin-free algal biomass (containing up to 60% lipids)

Ideally, simultaneous production of all three aqueous plants/algae listed above would be preferred for integrated biorefineries in order to provide diversity and abundant sources of lipids, quality protein, and carbohydrates. Whereas lignin-free duckweed species are ideal for use in animal feeds and as biomass feedstocks, water hyacinth and algae can be utilized as either a biorefinery feedstock or processed via AD along with algae and animal waste.

Capturing carbon dioxide (CO<sub>2</sub>) emissions from integrated biorefinery operations allows for CO<sub>2</sub> enrichment via CEA. This can substantially increase biomass yields for duckweed, water hyacinth and algae with potential to achieve production of up to 500 tonnes per acre via year round production. Hydroponic greenhouses for production of duckweed, water hyacinth and hybrid algal bioreactors can potentially utilize photoheterotrophic production to maximize bioenergy potential in conjunction with plant microbial electrolysis for value added production of either biomethane or biohydrogen. Equal portions of water hyacinth and algal biomass can be mixed with animal waste and other sources of organic waste for state-of-the-art anaerobic digestion and processing into biogas (biomethane and biohydrogen), high value compost, and liquid organic fertilizers with energy conversion efficiencies rivaling that of fermentation technologies.

For hydroponic greenhouse applications, recycling 50% of CO<sub>2</sub> produced by an integrated biorefinery could allow for producing 815,182 tonnes of lignocellulosic feedstocks. State-of-the-art fractionation/pretreatment allowing for 80% conversion efficiency would allow for producing an additional 81 MGY ethanol based on a yield of 100 gal per tonne. This could increase total ethanol production to 268 MGY (119 MGY for corn ethanol, 68 MGY for corn stover ethanol, and 81 MGY for CEA for integrated biorefineries). This is based on 80% conversion efficiency. Eventually, 90% conversion efficiency would allow for producing 112 gal/tonne of ethanol for mature SSF and CBP technologies.

This is based on utilizing biomass feedstocks containing 68% polysaccharides and 32% lignin for ash-free biomass. In reality, ash-mineral content will comprise about 3-12% of biomass and will accumulate along with lignin as fermentation residues for biorefining processes which do not utilize clean fractionation.

### **Year Round Production of Biomass via CEA**



Figure 19. Hydroponic Greenhouses & Algal Photobioreactors

### **Duckweed as Ideal Biomass Resource**

According to [Cheng & Stomp, 2009](#), “Some of the duckweed species have morphological and growth characteristics that translate into the attributes of the ideal plant which makes them exceptional candidates for wastewater-based agriculture. The *Lemnaceae* is a monocotyledonous family of aquatic plants with four genera and a total of 37 species: *Spirodela*, *Lemna*, *Wolffia*, and *Wolffiella*. All members of the *Lemnaceae* are small, free-floating, fresh-water plants whose geographical ranges span the entire globe (Landolt, 1986). An example from each genus is shown in Fig. 10a.

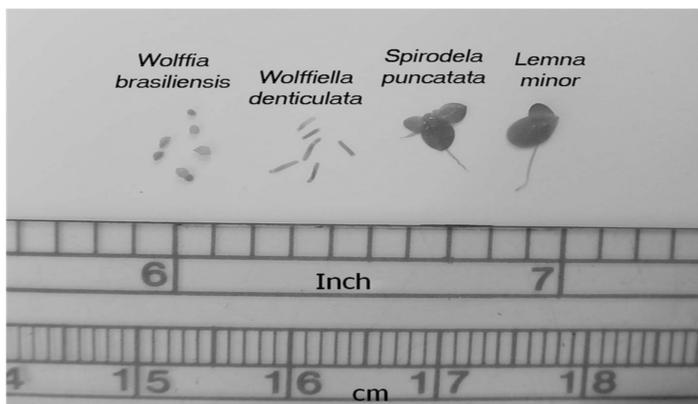


Figure 20. Size of duckweeds from four different genera.

The duckweeds inhabit freshwater ponds and pools, preferring those that are shallow and without flowing water. One species, *Spirodela polyrrhiza*, is the most widely distributed, and has been studied for its use for nutrient capture from wastewater (Sutton and Ornes, 1985). Its wide geographical range means that a native strain of this species can be used to establish a wastewater treatment system at almost any global location thus avoiding the use of an invasive exotic species. Members of the *Lemnaceae* are the most morphologically reduced species of higher plants (see Fig. 10a). *Spirodela* and *Lemna* plants consist of disc-shaped fronds of varying sizes, shapes and thickness depending on species, a hair-like root or roots (the number is species specific) and, when present, one or two flowers. The morphologies of *Wolffia* species are further reduced, with plants consisting of tiny fronds resembling geometric solids (shape is species specific), no roots and rarely single flowers (see Fig. 10a). *Wolffiella* species are the most varied in morphology. Regardless of species, the size of fronds of *Lemna* and *Spirodela* species vary

from less than one to several millimeters in diameter, with roots elongating to no more than several centimeters in length; the fronds of *Wolffia* species are less than 2 mm in diameter.

All *Lemnaceae* species proliferate primarily through vegetative budding of new fronds from parent fronds. Newly budded fronds remain attached to the parent frond to varying degrees. *Lemna* and *Spirodela* species are forming frond clusters of varying number and *Wolffia* species remaining solitary. Although parent fronds are limited in the number of progeny fronds that are produced before the parent frond dies, duckweed cultures achieve near exponential growth rates. Doubling times vary by species and environmental conditions and are as short as 20 to 24 hours and many species have doubling times of 2 to 3 days (Chang et al., 1977; Datko et al., 1980; Venkararaman et al., 1970). Intensive laboratory culture of duckweed has achieved high rates of biomass accumulation per unit time at culture densities of 1–2 kg/m<sup>2</sup> (Landolt and Kandeler, 1987). Greenhouse production levels of 1 kg fresh weight/m<sup>2</sup>wk have been achieved (M. Edelman, personal communication).

In our wastewater treatment research we have achieved a growth rate of 0.2 kg dry weight/m<sup>2</sup>wk (Cheng et al., 2002a). To achieve these growth rates, only low concentrations of nutrients are required. Oron and co-workers (Oron et al., 1988) achieved optimal growth rates at 20 ppm nitrogen utilizing municipal wastewater. Our research with wastewater indicates that high growth rates can be achieved at nitrogen levels less than 10 ppm (Cheng et al., 2002a; Chaiprapat et al., 2005; Cheng et al., 2002b). When duckweed grows the individual fronds and frond clusters repel each other pushing the entire mat across the open water. Thus duckweed proliferation creates a floating photosynthetic surface that both maximizes capture of sunlight per unit area and shades out competing algal growth. Without continual harvesting proliferation creates dense mats of multiple frond layers that float at, or are slightly submerged relative to, the surface of the supporting fluid.

To optimize production continuous harvesting of duckweed biomass is necessary. The floating particulate growth habit of duckweed facilitates harvesting and a variety of methods have been devised to corral and harvest duckweed biomass (Midtling et al., 1991; Sygen et al., 1993). Duckweed's small size gives the plant a large surface area to volume ratio. Duckweed also lacks a waxy cuticle, present on land plants to prevent water loss. Both of these characteristics mean that duckweed can be dried quickly with low energy inputs.

Duckweed growth can be optimized to produce high levels of protein or high levels of starch. The plant's dry weight accumulation varies by species and growth conditions and ranges from 6 to 20% of fresh weight (Landolt et al., 1987; Tillberg et al., 1979). Protein content of a number of duckweed species grown under varying conditions has been reported to range from 15 to 45% dry weight (Chang et al., 1977; Porath et al., 1979; Appenroth et al., 1982). These values place the protein content of dry duckweed biomass between alfalfa meal (20%) and soybean meal (41.7%) (Hillman, 1961). We routinely grow duckweed on dilute swine wastewater and get 30 to 35% protein of dry duckweed. Duckweed starch content is dependent on growth conditions, e. g., pH, phosphate concentration (Tasseron-De-Jong and Veldstra, 1971; McLaren and Smith, 1976) and developmental states controlled by the plant hormones, cytokinin (Tasseron-De-Jong and Veldstra, 1971; McCombs and Ralph, 1972) and abscissic acid (Landolt and Kandeler, 1987; McLaren and Smith, 1976). Starch contents ranging from 3 to 75% have been reported (Landolt and Kandeler, 1987; Reid and Bielecki, 1970). A duckweed starch content of 75% is comparable to corn, whose starch content ranges from 65 to 75% (Lin and Tanaka, 2006).

The possibility to manipulate growth to produce either high-protein or high-starch duckweed provides two opportunities to use duckweed biomass. The high-starch content suggests that duckweed could be used as an industrial feedstock for ethanol production for fuel. The high-protein content suggests that duckweed could be used as the protein component for animal feed. However, to commercialize either of these potential products requires a cropping system that can consistently produce stable duckweed biomass in massive quantities. For the past 10 years, we have been investigating the development of a coupled anaerobic digestion/duckweed system to recover energy and nutrients from swine wastewater and produce large quantities of either high-protein or high-starch duckweed. These studies have led to development of a duckweed cropping system and the applications of duckweed biomass.” ([Cheng & Stomp, 2009](#)) ([Duckweed Physiology, Biochemistry & Molecular Biology](#))

### ***Consolidated Bioprocessing***

In terms of utilizing CBP technology in commercial applications, in May 2009, the [Mascoma Corp.](#) announced that it has made revolutionary technology breakthroughs for development of synthetic microbes.

#### *Thermophilic Bacteria:*

- production of nearly 6 w/v% ethanol by an engineered thermophile, a 60% increase in ethanol concentration from a year ago
- the first report of targeted metabolic engineering of a cellulose-fermenting thermophile, *Clostridium thermocellum*, leading to a reduced production of unwanted organic acid byproducts; and
- selected strains of *C. thermocellum* that can rapidly consume cellulose with high conversion and no added cellulase, and grow on cellulose in the presence of commercial levels of ethanol.

#### *Recombinant, Cellulolytic Yeast:*

- 3,000-fold increase in cellulase expression
- a significant 2.5-fold reduction in the added cellulase required for conversion of pretreated hardwood to ethanol
- complete elimination of added cellulase for conversion of waste paper sludge to ethanol

In February 2009, Mascoma’s pilot facility in Rome, NY began producing cellulosic ethanol with the flexibility to process numerous biomass feedstocks including wood chips, tall grasses, corn stover (residual corn stalks) and sugar cane bagasse.

Both water hyacinth and algae have the potential to simultaneously produce biofuels and animal feeds. For integrated biorefineries, economical protein separation processes can be utilized prior to pretreatment to produce animal feed supplements relatively high in essential amino acids. Similar to corn fractionation, separation and fractionation processes enhance fermentation efficiency for lignocellulosic biomass feedstocks by removing nonfermentables prior to fermentation. Clean biomass separation processes (clean fractionation) also allow for utilizing high value coproducts produced from free sugars (primarily sucrose and fructose), proteins, oils, and lignin in addition to hemicellulose and cellulose more efficiently. Subsequent production of bio-chemicals and bioproducts at integrated biorefineries provides diversification and results in

increasing net profits. Hence, production of biofuels, biohydrogen, chemical feedstocks, and animal feeds can offset the \$100/m<sup>2</sup>, e.g. \$404,700 per acre that is estimated for construction of CEA applications. For example, each ton of hydrogen contains 122 MBTU HHV (based on 61,100 BTU/lb HHV; 51,623 BTU/lb LHV).

When combusted, H<sub>2</sub> produces the equivalent of:

- 5.3 tons bituminous coal (23 MBTU/ton HHV)
- 21 barrels of crude oil (5.8 MBTU/barrel HHV)
- 976 gallons of gasoline (125,000 BTU/gal HHV)
- 119 dekatherms of natural gas (1.027 MBTU/dekatherm HHV)

### **Microbial Fuel Cell (MFC) & Microbial Electrolysis Cell (MEC) Technologies**

MFCs and MECs convert chemical energy available in bio-convertible substrates, directly into bioelectricity, biohydrogen, biomethane, and nutrient-rich biological effluents. To achieve this, various bacteria strains are utilized as biocatalysts to convert organic substrates into electrons, biogas, and ionic solutions which are bioavailable to plants and algae. Bacteria are very small (size approximately 1 μm) organisms (microbes) which can convert organic compounds (primarily carbohydrates and CO<sub>2</sub>) contained in bio-convertible substrates specifically into hydrogen (H<sub>2</sub>) or methane (CH<sub>4</sub>), minerals, water (H<sub>2</sub>O) and electricity via extracellular electron transfer. Microbes use electron transfer to grow and to maintain their metabolism. However, by using MFC and MEC technologies, a portion of this extracellular microbial energy can be harvested in the form of bioenergy without significantly affecting plant growth.

Bio-convertible substrates consist of plant root and micro-algal exudates, nutrient-rich organic waste, and wastewater resources derived primarily from animal excretions, municipal sewage, food processing waste, thin stillage and process wastewater generated by the ethanol industry and other commercial industries. Similar to animal's excreting organic waste, plants and microalgae exude organic matter through their roots and cell membranes. These organic compounds can often be separated into two classes: low-molecular weight compounds, which include amino acids, organic acids, sugars, phenolics and an array of secondary metabolites, and high-molecular weight compounds like mucilage and proteins (Badri and Vivanco, 2009).

### ***Epiphytic Associations of Bacteria with Plant Roots & Microalgae via Exudations***

Similar to plant root exudates, microalgal exudates include carbonaceous materials ([Janse et al., 1999](#)) and high molecular weight compounds ([Galarza et al., 1981](#)). Recent investigations reveal specific populations of bacteria are associated with microalgae. These bacteria live in the nutrient-rich phycosphere (Bell et al., 1974), e.g. the region immediately surrounding microalgae (or phytoplankton in marine environments) and resemble other nutrient-rich interfaces such as the root (rhizosphere) and arial (phyllosphere) surfaces of higher plants. These nutrients are extracellular products exuded by algae communities, and may support extensive populations of bacteria and other microbes resulting in maximizing biomass productivity.

In addition to exudates, the promotion of phycosphere bacterial populations by phytoplankton in marine environments and in algal bioreactor cultures depends on other biochemical interactions (e.g. the production of algal antibiotics) as well as physical properties of the algal surface (e.g. presence or absence of mucilage), both of which vary with the physiological state and species of alga involved. The bacteria population that develops will also depend on broader aspects of the

phycosphere community such as grazing activities by protozoa and the equilibrium between mucilaginous and planktonic organisms ([Sigeo, 2005](#)). Bacteria play important roles in food webs beginning with symbiotic relations to plants and algae at the bottom of marine and terrestrial food chains, especially with regard to nutrient cycling ([Sapp et al., 2004](#)). Specific associations of bacteria with phytoplankton (microalgae) have recently been reported and analyzed ([Sapp et al., 2007](#)). Closed algal bioreactors, a form of controlled environment agriculture (CEA), could be developed to maximize algal biomass and microbial bioenergy production by optimizing factors which affect algae growth and reproduction.

Exudation is part of the rhizodeposition and phycodeposition processes. These ecological processes are major sources of soil and aquatic organic carbon released by plant roots and microalgae (Hutsch, Augustin & Merbach 2000; Nguyen 2003; [Sapp et al., 2004](#); [Sapp et al., 2007](#)). The quantity and quality of exudates are determined by specific plant and microalgae species, the age of an individual species, and external factors like biotic and abiotic stressors. Exudation represents a significant carbon cost to plant species (Marschner 1995), with young seedlings typically exuding about 30–40% of their fixed carbon as root exudates (Whipps 1990). Similar volumes of exudates may be produced by various microalgae and cyanobacteria strains. Root and microalgal exudates contain released ions (i.e. H<sup>+</sup>), inorganic acids, oxygen and water, but mainly consist of carbon-based compounds (Uren 2000; [Bais et al., 2006](#)).

Root and microalgal exudates serve as signals that initiate symbiosis with microbes including rhizobia, mycorrhizal fungi, and plant growth–promoting rhizo-bacteria (Badri and Vivanco, 2009). Micorhizal funji, rhizobia, and plant growth–promoting bacteria exist in the rhizosphere, the millimeters of soil surrounding plant roots where nutrients are exuded. As explained above, for microbial communities and nutrient-rich microenvironments of aquatic species, this area is referred to as the phycosphere. Rhizobia comprise soil and aquatic bacteria that fix nitrogen (diazotrophy) after becoming established inside root nodules of legumes (Fabaceae) ([Bais et al., 2006](#)). Other plant types can then utilize this nitrogen via symbiotic relationships in order to optimize plant growth and electron transfer rates for MFC/MEC applications, primarily via production of aquatic plant species.

Root and microalgal exudates mediate both positive and negative interactions in the rhizosphere and phycosphere microenvironments. The positive interactions include symbiotic associations with the beneficial microbes described above including plant and algae growth-promoting bacteria. Negative interactions include association with parasitic plants, pathogenic microbes and invertebrate herbivores (Badri and Vivanco, 2009). Both positive and negative interactions influenced by plant root exudates and biological effluents can substantially affect the efficiency of MFC/MEC applications, including those integrated with aquatic plant production via CEA operations. Hence, proper management, e.g. regulating temperature, pH, nutrient processing and optimizing nutrient ratios including CO<sub>2</sub>, light arrays, photo periods, and eliminating contaminants for algal bioreactors and hydroponic greenhouses can optimize the rhizosphere, phycosphere, and phyllosphere microenvironments, thus maximizing efficiency of both biomass and bioenergy production.

### ***The Phycosphere & Algal Bioreactor Cultures***

“Although many species of alga/phytoplankton typically have relatively low levels of epiphytic bacteria, particularly when they are in a healthy condition (Brandberg, 1999), this is not true for blue-green algae (cyanobacteria). Various morphological and physiological features of blue-

green algae make them particularly suitable for forming and maintaining high populations of associated bacteria, including the tendency to aggregate as filamentous or globular colonies, and the production by many species of copious amounts of extracellular mucilage. The high nutrient phycosphere surrounding these organisms forms a microenvironment for extensive populations of bacteria, protozoa, and invertebrates ([Sigeo, 2005](#)).”

For closed algal bioreactors there are opportunities to optimize production environments for specific algae strains in order to limit production of antibiotics, and reduce populations of grazers which feed on algae, primarily protozoa and invertebrates. This would result in increasing efficiency of algal biomass production, particularly via integrated biorefineries and closed loop architecture.

### ***Optimizing Efficiency of Bioenergy Production via Integration***

The overall efficiency of bioenergy applications can potentially be maximized via integration of algal bioreactors and MFCs/MECs with high temperature fuel cell technologies such as molten carbonate fuel cells (MCFCs), phosphoric acid fuel cells (PAFCs) and solid oxide fuel cells (SOFCs). Similar to anaerobic digestion (AD), biogas refining, and using methane rich feeds for MCFC-SOFCs, MFC/MEC configurations can convert organic matter into renewable sources of biopower, biohydrogen, biomethane, and CO<sub>2</sub>. In addition, depending on the quality of the substrates utilized, extraction of electrons and CO<sub>2</sub> by MFCs which produce electricity, and production of H<sub>2</sub> and CH<sub>4</sub> from carbohydrates and CO<sub>2</sub> by MECs, may provide nutrient-rich biological effluents for simultaneous production of food and biomass via integration with controlled environment agriculture (CEA) and aquatic plant species.

[Membrane Technology Research \(MTR\)](#) is developing a membrane process that upgrades biogas streams from anaerobic digesters to high quality fuel methane. The process removes water and carbon dioxide from anaerobic digester gas and produces methane suitable for use in place of pipeline natural gas. The current work is being funded by EPA. If successfully developed, the proposed membrane approach will significantly reduce the cost of methane capture from biogas, and accelerate the adoption of anaerobic digestion/biogas utilization technology at livestock farms. Use of biogas methane on or near the recovery site can help meet rural demands for heat and electricity, and offset the costs associated with environmentally responsible waste and greenhouse gas management. MTR is also developing Hydrogen membranes as an economical method to recover and purify hydrogen from a refinery waste gases, reactor purges, and syngas. MTR's hydrogen-permeable VaporSep®-H<sub>2</sub> membranes can provide 90% to 99% hydrogen purity and greater than 90% recovery. MTR's Polaris™ membrane is unique because it is highly permeable to CO<sub>2</sub>, but retains hydrogen. With this advance, it is now possible to use membranes to remove CO<sub>2</sub> from streams containing hydrogen such as gasifier streams, PSA tail gas, and various biorefinery and bioreactor process streams.

Utilizing comprehensive nutrient-energy management for integrated operations which incorporate AD and MFC/MEC technologies, biological effluents can be processed into value added fertigation streams and utilized along with exudates, thin stillage and CO<sub>2</sub> for simultaneous closed loop production of biogas, bioelectricity, and biomass. Biomass can subsequently be converted to liquid biofuels, nutraceuticals, pharmaceuticals, functional foods, biochemicals and bioplastics, etc. Integration allows for achieving unprecedented production efficiencies by reducing transportation and labor costs. Organic matter is converted into value added products while simultaneously reducing/eliminating pollution and GHGs from organic

waste resources. This integrated process allows for biologically removing nitrogen (N) and phosphorus (P). These elements are primary pollutants as a result of nutrient runoff and nutrient loading in water bodies. This over-abundance of nutrients contributes to algae blooms, and subsequently hypoxia which destroys marine ecosystems. The end result of incorporating nutrient-energy management via MFC/MEC applications integrated with CEA, biorefineries and bioenergy complexes is value-added production of biomass, bioenergy, and simultaneous remediation of wastewater resources.

An experiment was conducted proving this principle using Reed mannagrass to demonstrate a plant MFC. A maximal electrical power production of  $67 \text{ mW m}^{-2}$  anode surface was achieved. The system was characterized by: (1) nondestructive, *in situ* harvesting of bioenergy; (2) potential implementation in wetlands and poor soils without competition to food or conventional bioenergy production which makes it an additional bioenergy supply; (3) an estimated potential electricity production of  $21 \text{ GJ ha}^{-1} \text{ year}^{-1}$  ( $5800 \text{ kWh ha}^{-1} \text{ year}^{-1}$ ) in a temperate controlled environment; and (4) carbon neutral and combustion emission-free operation ([Strik et al. 2008](#)).

### ***Bioenergy Efficiency, Advantages & Challenges of Microbial Applications***

Plant MFCs and MECs exploit bioenergy *in situ*. In principle, producing energy directly from live plants and/or algae via the electron transport chain and exoelectrogenic microbes is much more efficient than producing energy from biomass due to proximity of the source of energy. Unlike harvesting solar energy via technologies such as photovoltaic panels during daylight hours, this innovative source of *in situ* bioenergy can be produced 24 hours a day.

The properties of electrolytes in microbial fuel cells (MFCs) are exploited using electrolysis to extract constituent elements and compounds contained within the substrate solutions. This relatively new MEC technology is based on modifying a microbial fuel cell (MFC) in two ways: adding a small amount of voltage ( $>0.2 \text{ V}$ ) to that produced by bacteria at the anode; and excluding access to oxygen at the cathode ([Liu et al., 2005](#); Rozendal et al., 2006). The addition of the voltage makes it possible to produce pure hydrogen or methane gas at the cathode. This MEC/BEAMR system is therefore operated as an anaerobic reactor. The voltage required at the abiotic anode or MEC can be produced using power from a high temperature fuel cell configuration (integration of an MCFC, PAFC, or SOFC), or eventually by using hydrogen gas from the MEC to produce electricity in a low temperature proton exchange membrane fuel cell (PEMFC) configuration.

The technological challenges for plant MFC/MEC configurations are optimization of the rhizodeposition of organic compounds, optimization of biological effluents and  $\text{CO}_2$  resources, optimization of the electricity and hydrogen/methane production of MFCs and MECs, and further integration of the plant in the anode compartment of the MFC and MEC configurations ([Wageningen University, 2009](#)).

### ***Integrated MFC Design Produces Coulombic Efficiency of 69%***

“In 2009, an MFC was utilized to reduce the levels of furfural, 5-hydroxymethylfurfural, vanillic acid, 4-hydroxybenzaldehyde and 4-hydroxyacetophenone while simultaneously producing electricity. An integrated MFC design approach was used which resulted in high power densities for the MFC, reaching up to  $3700 \text{ mW/m}^2$  ( $356 \text{ W/m}^3$  net anode volume) and a coulombic efficiency of 69%. The exoelectrogenic microbial consortium enriched in the anode was characterized using a 16S rRNA clone library method. A unique exoelectrogenic microbial

consortium dominated by  $\delta$ -Proteobacteria (50%), along with  $\beta$ -Proteobacteria (28%),  $\alpha$ -Proteobacteria (14%),  $\gamma$ -Proteobacteria (6%) and others was identified. The consortium demonstrated broad substrate specificity, ability to handle high inhibitor concentrations (5 to 20 mM) with near complete removal, while maintaining long-term stability with respect to power production.” ([Borole et al., 2009](#)) For integration with ethanol biorefineries, use of MFCs for removing fermentation inhibitors has implications for:

- enabling higher ethanol yields at high biomass loading in cellulosic ethanol biorefineries
- improved water recycling
- electricity production of up to 25% of total biorefinery power needs
- integration with closed loop biomass production, providing bioelectricity and thermal integration for CEAs such as hydroponic greenhouses and algal photobioreactors via plant MFCs. ([Borole et al., 2009](#))

### ***Stainless Steel Brush Cathodes for MECs***

“Microbial electrolysis cells (MECs) are an efficient technology for generating hydrogen gas from organic matter, but alternatives to precious metals are needed for cathode catalysts. High surface area stainless steel brush cathodes have been developed which produce hydrogen at rates and efficiencies similar to those achieved with platinum-catalyzed carbon cloth cathodes in single-chamber MECs. Using a stainless steel brush cathode with a specific surface area of  $810 \text{ m}^2/\text{m}^3$ , hydrogen was produced at a rate of  $1.7 \pm 0.1 \text{ m}^3\text{-H}_2/\text{m}^3\text{-d}$  (current density of  $188 \pm 10 \text{ A}/\text{m}^3$ ) at an applied voltage of 0.6 V. The energy efficiency relative to the electrical energy input was  $221 \pm 8\%$ , and the overall energy efficiency was  $78 \pm 5\%$  based on both electrical energy and substrate utilization. These values compare well to previous results obtained using platinum on flat carbon cathodes in a similar system. Reducing the cathode surface area by 75% decreased performance from  $91 \pm 3 \text{ A}/\text{m}^3$  to  $78 \pm 4 \text{ A}/\text{m}^3$ . A brush cathode with graphite instead of stainless steel and a specific surface area of  $4600 \text{ m}^2/\text{m}^3$  generated substantially less current ( $1.7 (0.0 \text{ A}/\text{m}^3)$ ), and a flat stainless steel cathode ( $25 \text{ m}^2/\text{m}^3$ ) produced  $64 (1 \text{ A}/\text{m}^3)$ , demonstrating that both the stainless steel and the large surface area contributed to high current densities. Linear sweep voltammetry revealed that the stainless steel brush cathodes both reduced the over-potential needed for hydrogen evolution and exhibited a decrease in over-potential over time as a result of activation. These results demonstrate for the first time that hydrogen production can be achieved at rates comparable to those with precious metal catalysts in MECs without the need for expensive cathodes.

Using high surface area SS brushes as cathodes in MECs is an important step toward building larger scale reactors that are economical because it avoids the use of expensive catalysts. Platinum currently costs  $\sim \$43,000/\text{kg}$  compared to  $\$4.82/\text{kg}$  for 304 grade SS (25, 26), which translates into a cost of  $\$0.03$  for the half SS brush cathode and  $\$0.15$  for the Pt on the Pt/C cathode used in this study (excluding the cost for the carbon cloth, Nafion binder, and current collector). [Rozendal et al. \(2008\)](#) and [Liu et al. \(2008\)](#) determined that the cathode (including catalyst) accounts for the greatest percentage (47%) of the total capital costs for both MFCs and MECs. Their models predict that cathodes will play a smaller role in the total reactor cost by identifying materials that have substantially lower costs than those currently used. Clearly, the use of SS instead of Pt moves cathode designs closer to that goal.” ([Call et al., 2009](#))

### ***Exploiting MECs via CEA & Aquatic Plant Species***

The idea behind MEC technology is that the protons and electrons produced by bacteria can be recombined at the cathode as hydrogen gas-- a process called the hydrogen evolution reaction (HER). Theoretically, only 0.41 V is needed to produce H<sub>2</sub> from acetate. Since bacteria produce ~0.2 to 0.3 V, only an additional 0.2 V or more is needed to produce hydrogen gas via the MEC/BEAMR technology. This voltage is much less than that needed for water electrolysis, which is about 1.8 V in practice. Though over 2000 °C (3632 °F) is required via steam reforming to split water (separate H<sub>2</sub> and O components from H<sub>2</sub>O), “splitting” organic matter by bacteria is a thermodynamically favorable reaction when no oxygen is available at the cathode. In the MEC process, the cathode is sealed to prevent access to oxygen. When a small boost of voltage is added to the voltage produced by the bacteria, the microbes spontaneously produce hydrogen. Thus, the MEC process is a more efficient means of electrolysis using organic matter at ambient temperatures in comparison with conducting water electrolysis at extreme temperatures. ([Cheng et al., 2007](#); [Cheng and Logan, 2007b](#))

In addition to producing energy from wastewater, mediator-less MECs can also derive energy directly from certain aquatic plants. These include reed sweetgrass, cordgrass, rice, tomatoes, lupines, water hyacinth, and algae. Hence, there is potential for integration of algal bioreactors with MECs for production of biohydrogen and biomethane from organic waste and wastewater resources through electrogenic microbes. By integrating organic waste and wastewater treatment with algae production and CO<sub>2</sub> sequestration via closed loop systems, it is possible to sustainably produce biohydrogen ([Cheng and Logan, 2007a](#); [Cheng and Logan, 2007b](#)) and/or biomethane ([Cheng et al., 2009](#)) at integrated biorefineries.

Current pricing for hydrogen gas is expected to increase to about \$5/kg at the refueling pump for the next few years. In addition to refueling hydrogen vehicles, this is due partially to the demand for hydrogen which is utilized in hydro-cracking processes by oil refineries in order to remove sulfur from petroleum fuel products, primarily diesel. Since 1 kg is equivalent to 2.20462262 lbs, \$5/kg is equivalent to \$2.267/lb (\$5,000/tonne). However, for purposes of calculating revenues based on long term “low cost” production of hydrogen, the following is based on \$110/tonne. This is the cost estimated for large scale hydrogen production via water electrolysis ([Mook, 2008](#)). Based on production of 8093.7 M<sup>3</sup> at 1.1 Nm<sup>3</sup>/day/m<sup>3</sup> ([Cheng and Logan, 2007a](#); [Cheng and Logan, 2007b](#)) via MECs, and .19815 lbs per Nm<sup>3</sup> of H<sub>2</sub>, each acre of hydroponic greenhouses could produce 0.8 tonnes of biohydrogen per day. The actual hydrogen production rate for plant MECs will need to be determined in order to confirm the following scenarios which are based on producing 10% of the H<sub>2</sub> produced by Cheng and Logan (2007a,2007b) utilizing organic waste substrates.

Scenario 1: Hydroponic greenhouses for water hyacinth could annually produce gross revenues of \$130,936 per acre [(333 tonnes biomass per acre x 100 gal/ton x \$2.65/gal rack price for ethanol = \$88,245) + (0.8 tonne biohydrogen per acre per day x 365 days/yr x \$110 from plant MECs = \$32,120) + (333 tonnes x 10% protein animal feed/7.0 FCR x \$1/lb live weight = \$10,571] for an integrated biorefinery utilizing mature technology. Temporary tax credits and production incentives (\$1/gal lignocellulosic ethanol production credit x 333 tons x 100 gal/tonne = \$33,300) + (\$0.51/gal blending credit x 333 tonnes x 100 gal/tonne = \$16,983) provide an additional \$50,283 per acre, e.g. providing adjusted gross revenues of \$181,219. Allowing for 50% of gross revenues (\$65,468) in production costs including labor, maintenance, fertilizers, energy costs and amortization, this would provide adjusted gross revenues of \$115,751 per acre. This results in a 3.5 year return on investment (ROI) (\$404,700 per acre

construction cost/\$115,751 per acre adjusted gross revenue). Increasing selling price of hydrogen to \$5,000 per tonne would provide \$1,460,000 (0.8 tonne biohydrogen per acre per day x 365 days/yr x \$5,000 from plant MECs) in annual revenue per acre solely for hydrogen sales. This would reduce the ROI to less than four months.

Scenario II: Algal photobioreactor. If 40% lipid algal biomass is produced along with MECs, annual revenues would be \$266,253 [(441 tonnes x 114 gal/ton x \$3/gal biodiesel = \$150,822) + (441 tonnes x .60 starch-protein meal/7.0 FCR x \$1/lb. live weight natural beef = \$83,311) + (0.8 tonne biohydrogen per acre per day x 365 days/yr x \$110 from plant MECs = \$32,120)]. Including temporary tax credits and production incentives (\$1/gal biodiesel production/blending credit x 441 tons x 180 gal/ton = \$80,360) provides an additional \$80,360 per acre, e.g. providing adjusted gross revenues of \$346,613. Allowing for 50% of gross revenues (\$133,126) in production costs including labor, maintenance, fertilizers, energy costs and amortization, this would provide adjusted gross revenues of \$213,487 per acre. This results in less than a 2 year ROI (\$404,700 per acre construction cost/\$213,487 per acre adjusted gross revenue). Increasing selling price of hydrogen to \$5,000 per tonne would provide \$1,460,000 (0.8 tonne biohydrogen per acre per day x 365 days/yr x \$5,000 from plant MECs) in annual revenue per acre solely for hydrogen sales. This would reduce the ROI to less than four months.

### Closed Loop Architecture Synergies of Fuel Cell Technologies & Organic Waste Processing

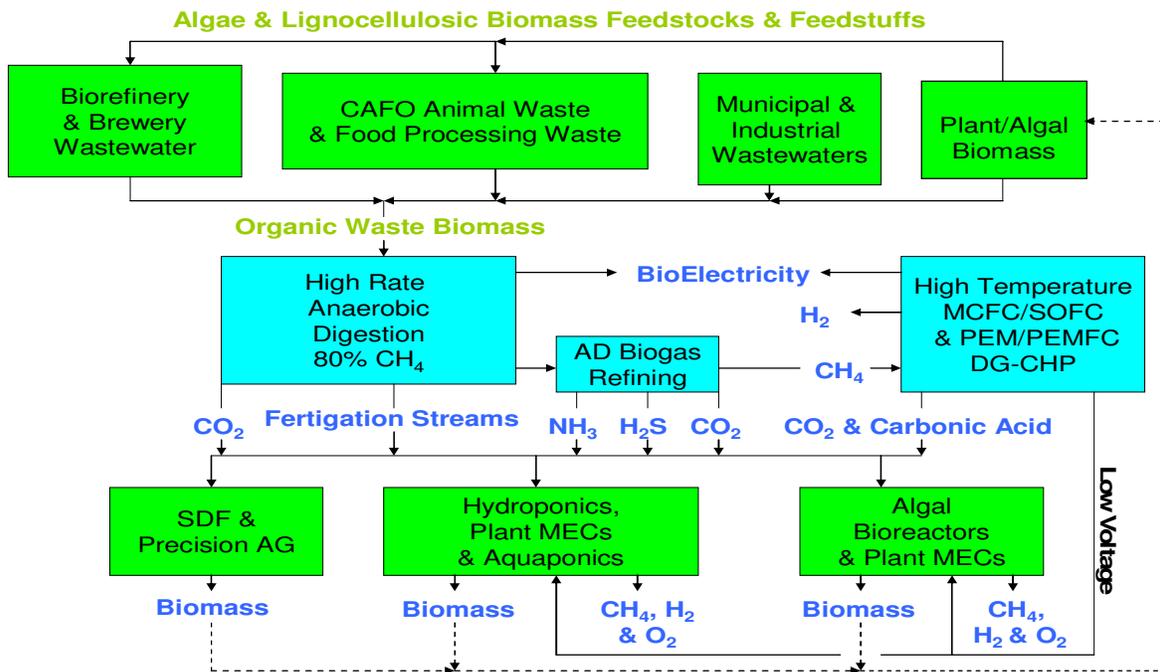


Figure 21. Closed Loop Production of BioPower, Biogas & Biomass.

In summary, the principal concept for aquatic plant MECs is based on the fact that plants produce rhizodeposits, mostly in the form of carbohydrates, and bacteria (electrogenic microbes) convert these rhizodeposits and other available organic resources into electrical energy via plant

MFCs, or biogases via plant MECs. As indicated above, a considerable amount of fixed carbon is used for plant growth but a portion of it, which can range from 30-40% for young plants is exudated through the roots and microalgae membranes as rhizodeposits (Badri and Vivanco, 2009; Whipps 1990; [Bais et al., 2006](#)). These organic molecules are then used as fuel and oxidized back to CO<sub>2</sub> at the microbial anode of an MFC. Plant MECs provide opportunities for converting plant exudates such as organic acids, carbon-containing primary and secondary metabolites, and even CO<sub>2</sub> to CH<sub>4</sub>.

### ***Potential for Efficient & Economical Electromethanogenesis via MECs***

In addition to hydrogen, methane can be directly produced using a biocathode containing methanogens in electrochemical systems (abiotic anode) or microbial electrolysis cells (MECs; biotic anode) by a process called electromethanogenesis. At a set potential of less than -0.7 V (vs Ag/AgCl), carbon dioxide was reduced to methane using a two-chamber electrochemical reactor containing an abiotic anode, a biocathode, and no precious metal catalysts. At -1.0 V, the current capture efficiency was 96%. Electrochemical measurements made using linear sweep voltammetry showed that the biocathode substantially increased current densities compared to a plain carbon cathode where only small amounts of hydrogen gas could be produced. Both increased current densities and very small hydrogen production rates by a plain cathode therefore support a mechanism of methane production directly from current and not from hydrogen gas.

The biocathode was dominated by a single Archaeon, *Methanobacterium palustre*. When a current was generated by an exoelectrogenic biofilm on the anode growing on acetate in a single-chamber MEC, methane was produced at an overall energy efficiency of 80% (electrical energy and substrate heat of combustion). These results reveal that electromethanogenesis can be used to convert electrical current produced from renewable energy sources (such as wind, solar, or biomass) into a biofuel (methane) as well as serving as a method for the capture of CO<sub>2</sub>. Researchers discovered that Archaea, using about the same electrical input for processing of acetic acid, could use the current to convert CO<sub>2</sub> and water to methane without any organic material, bacteria or hydrogen usually found in MECs.



The advantages of utilizing electromethanogenesis in comparison with electrohydrogenesis is that methane is more readily used in today's economy than hydrogen. This is due to leveraging current natural gas infrastructures and high temperature fuel cells which are designed to run on methane-rich feeds for production of bioelectricity. In this example, biomethane is utilized as a hydrogen-rich energy carrier that can leverage existing natural gas infrastructures, but more importantly can be utilized as a renewable fuel for distributed generation of combined heat and power. For closed loop systems total energy efficiency can approach 90% for integrated biorefineries via a carbon neutral process. A portion of the power, CO<sub>2</sub> and waste heat produced by the high temperature fuel cell is utilized to provide electrical current, space heat and nutrients for integration of plant MECs and controlled environment agriculture (CEA) for aquatic plant species such as algae and water hyacinth.

As alluded to above, transforming electrons into methane has the advantage of producing a fuel that can easily be stored or transported. Compression, transport in pipes, and storage of methane involves mature technologies, and thus methane production by electromethanogenesis could immediately be integrated into existing energy infrastructures. The efficiency of CO<sub>2</sub> capture

demonstrated by electromethanogenesis is high compared to other methods and thus may be useful for recycling carbon. As listed above, electrochemical reduction of CO<sub>2</sub> using electromethanogenesis has an electron capture efficiency of 96%, compared to 10-57% using metal catalyzed electrodes for methane production (Jitaru, 2007).

The use of Archaea bacteria for producing methane via electromethanogenesis provides an additional route for biofuel production accompanied with CO<sub>2</sub> capture, without the need for precious metal catalysts. The use of a methanogenic biocathode enables methane production from any electrical source, although renewable energy sources including fuel cells running on biogas would provide the greatest advantages for sustainable bioenergy systems.

The production of methane by electromethanogenesis will likely not displace existing methods of biomethane production from organic matter using anaerobic digesters, especially for high-strength wastewaters. MECs will likely be more appropriate for treatment of relatively dilute wastewaters such as nutrient-rich biological effluents utilized as value added fertigation streams for integration of MECs with CEA. The above research project reveals that microorganisms can be used in the form of biofilm on the cathode of an MEC to produce methane gas from electrical current at rates much greater than those possible via hydrogen gas evolution from a noncatalyzed electrode (Cheng et al., 2009).

### Municipal BioEnergy Complex Closed Loop Modular DG-CHP On-Demand

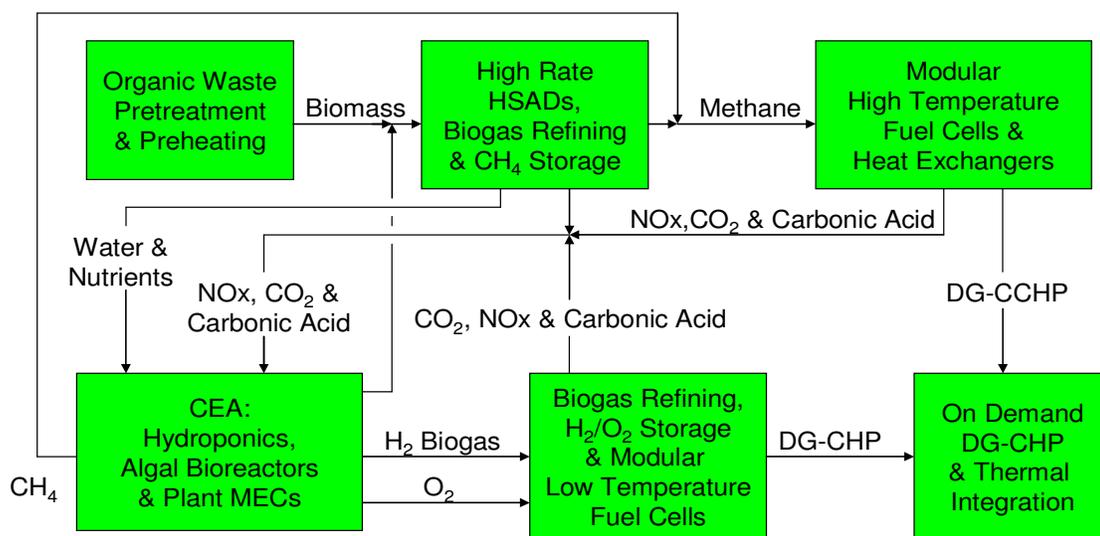


Figure 22. Integration of Organic Waste Processing, Algal Bioreactors & Plant MECs.

The opportunity to simultaneously produce biomethane and biohydrogen via MECs in addition to third generation biomass feedstocks such as algae and water hyacinth would substantially increase sustainability of the biofuels industry, particularly for integrated biorefineries and bioenergy complexes. If next generation algal bioreactors and hydroponic greenhouses are designed to accommodate plant MECs, methane and/or hydrogen biogas could be produced in addition to biofuels and bioelectricity via integrated biorefineries and bioenergy complexes which incorporate closed loop architecture.

In summary, through photosynthesis by micro-organisms (algae) as well as by higher plants, solar energy is converted to chemical energy stored in biomass or chemical compounds. Mixed microbial communities provide the possibility to exploit this chemical energy by converting it to useful energy (carriers) such as biogas and bioelectricity.

### ***Comprehensive Nutrient-Energy Management***

MFCs function efficiently under mild conditions relative to other types of fuel cells. Operating temperatures range from 20-40 °C. However, optimal yields of biogas and biopower are achieved between 35-40 °C (mesophilic) with the capability of producing over 80% total energy efficiency. With thermal integration for controlled environment applications, total energy efficiency could exceed 90%. This is two to three fold more energy than conventional internal combustion engine (ICE) and turbine engine technologies are capable of producing. Essentially, MFCs & MECs produce about two to three times more energy than they consume. MFCs are suitable for small scale applications such as potential medical devices fueled by glucose contained in blood, or larger applications such as wastewater treatment plants, ethanol biorefineries, and breweries which produce organic waste that can be used as fuel for MFCs.

The electrolyte utilized in MFCs is water containing free ions that behave as an electrically conductive medium. Because electrolytes generally consist of ions in solution, particularly for use in batteries and fuel cells, electrolytes are also known as ionic solutions. Electrons complete the circuit by traveling to the cathode, where they are taken up by oxygen and hydrogen ions to form water. Thus, the air cathode utilizes oxygen to produce water while the anode biofilm oxidizes organic matter in an anaerobic environment which optimizes power production (Coulombic efficiency) and produces CO<sub>2</sub> at the anode. The water that is produced at the air-cathode can be mixed with essential plant nutrients which remain after processing organic matter, comprising nutrient-rich biological effluents. These biological effluents may be processed into value added fertigation streams, which along with capturing carbon dioxide emissions as a value added fertilizer, may be utilized for closed loop production systems for food and biomass.

### ***Advantages of MFC & MEC BioEnergy Technologies***

- Biocatalysts (communities of microbial organisms capable of either exocellular or extracellular electron transfer) provide for efficiently converting organic matter, particularly abundant sources of organic waste substrates contained in wastewaters, into bioelectricity, biohydrogen, and biomethane.
- Bioelectricity is produced directly from organic waste, crop residues, biorefinery wastewater, and other forms of cellulosic biomass contained in domestic and industrial wastewaters ([Cheng and Logan, 2007b](#); [Huang and Logan, 2008a](#); [Huang and Logan, 2008b](#); [Rezaei et al., 2008](#)). This is in contrast to conventional aeration processes which actually consume 1.5% of current power generation.
- MFC & MEC technologies can be used to process an abundant supply of organic waste from CAFOs, food processing, and municipal wastewaters while simultaneously reducing pollutants via using value added fertigation streams and carbon dioxide for production of food & bioenergy via closed loop systems.
- Operating at ambient or mesophilic temperatures, it is possible to increase total energy efficiency (production of biopower, biomethane and biohydrogen) by two to three fold in

comparison with internal combustion and turbine engines while eliminating emissions. H<sub>2</sub> is produced from electrons (current) via exoelectrogenic bacteria while CH<sub>4</sub> is typically produced by methanogenic bacteria from acetate and other organic compounds. However, methane has recently been produced by electromethanogenic bacteria via MECs in a process called electromethanogenesis without requiring precious metal cathodes ([Cheng et al., 2009](#)).

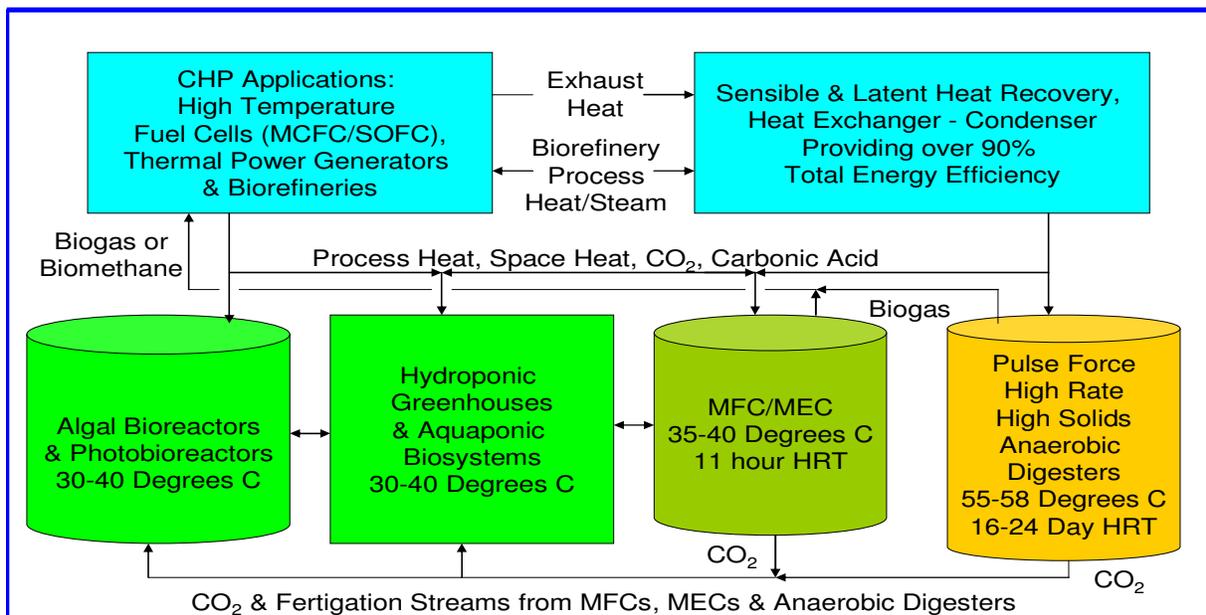
- Hydraulic retention time (HRT) for organic waste processing via MFC is estimated to be about 11 hours ([Liu et al., 2008](#)) vs. 4-10 days for high rate anaerobic digestion (AD), and 16-30 days for conventional AD. HRT for MFCs depends on loading rate and amount of electrode surface area per volume.
- Odors from animal waste can be reduced by over 99% via MFCs ([Kim et al., 2008b](#)).
- Integration of MFC/MEC and high temperature MCFC or SOFC technologies can be utilized to provide thermal integration for mesophilic MFC/MEC applications via DG-CHP for bioenergy complexes; and coproduction of biohydrogen, biomethane, and biohythane. Using biomethane as a feed, the MCFC can also provide coproduction of biohydrogen (via PEM technology) from either biomethane or biogas.
- MEC applications produce a biogas consisting of either 99.5% hydrogen gas (trace amounts of CO<sub>2</sub> and CH<sub>4</sub>) or 99.5% methane for use in fuel cells. This eliminates the need for removing H<sub>2</sub>S and siloxane from MEC biogas for use in high temperature fuel cells.
- Similar to engineering microbes for improving production of ethanologens, microbial engineering could eventually enhance efficiency of MFC and MEC applications via engineering of exoelectrogens, i.e. microbes which are capable of exocellular electron transfer ([Xing et al., 2008](#); [Zuo et al., 2008a](#); [Ren et al., 2007](#)). (Exocellular refers to electron transfers that are initiated inside a cell but actually take place outside the cell, such as anaerobic respiration by both bacteria and yeast.)
- For thermally integrated operations, such as bioenergy complexes, in temperate climates MFCs & MECs could be co-located in insulated buildings along with CEA, AD, and MCFC applications. This would eliminate transportation costs and allow for thermally regulating mesophilic MFCs and MECs using waste heat recovery from high temperature fuel cells and other integrated biorefinery operations.
- The heat generated from MECs along with latent heat provided by high temperature MCFCs integrated with CEA such as hydroponic greenhouses could essentially eliminate the need for additional energy inputs for year round biomass production in temperate climates.

### ***Comprehensive Nutrient-Energy Management via Integration***

Optimizing nutrient-energy management for integrated operations such as bioenergy complexes will require balancing the volume of organic waste processing via AD & high temperature fuel cells which provide CHP & biohydrogen production, with MFC/MEC applications which produce biopower, biomethane, and biohydrogen. Theoretically, all of these fuel cell technologies can achieve over 90% total energy efficiency via thermal integration and incorporation of closed loop production systems. The 11 hour HRT for MFC/MEC applications and lack of having to remove contaminants from biogas are strong economic advantages over AD & high temperature fuel cell technologies. However, mesophilic MFC/MEC applications require energy in the form of heat to reach maximum efficiency for biopower production. High temperature fuel cells can provide thermal integration via DG-CHP configurations which

efficiently recover waste heat. Carbonic acid produced from condensation during heat recovery processes, space heat and carbon dioxide from MCFC exhaust can be utilized for enhancing biomass production via CEA applications such as algal bioreactors.

### Thermal Integration & Comprehensive Nutrient-Energy Management Enclosed Insulated Structure for Temperate Climates



*Figure 23. Achieving over 90% total energy efficiency via thermal integration.*

The quality of the biological effluents from MFC & MEC applications, which can possibly be used as value added fertigation streams for closed loop production, are critical economic factors which need to be determined for bioenergy complexes. Based on \$6/bu corn and biological effluents produced by conventional anaerobic digester systems, the quantity of the biomass that can be produced is worth about 10-12 times the value of the biomethane which is extracted from organic waste. Since MFC/MECs efficiently utilize organic acids contained in thin stillage and brewery wastewater (Feng et al., 2008; Hawkes et al., 2007; Min and Logan, 2004; Logan, 2004), that could be the most efficient application for integration with ethanol biorefineries and breweries. In addition to providing thermal integration for mesophilic MFC/MECs, high temperature fuel cells could efficiently provide low levels of power required for MEC applications via closed loop production systems.

There are a variety of organic substrates and municipal/industrial waste streams which can be utilized as feeds for MFCs. For example, thin stillage and brewery wastewater which remains after microbial (primarily yeast) fermentation processes produce ethanol, is relatively rich in COD/BOD, contains organic acids which are optimal feeds for MFCs, and is preheated for optimizing power production via integration of MFCs.

The scientific breakthrough leading to development of MFC/MEC technologies is translated into applied biotechnology by using modern membrane and electrode materials that are compatible with biofilm growth and operation at ambient or near ambient (mesophilic) temperatures. This is

accomplished via thermal integration which maximizes production of bioenergy. Optimizing density of the biofilm at the anode increases electron transfer to the anode which maximizes power generation ([Microbial Fuel Cell Organization, 2008](#); [Biodesign Institute, Arizona State University, 2008](#); [Marcus & Rittman, 2008](#)).

### ***Algal Bioreactor Technology, Bioenergy, Nutraceuticals & Functional Foods***

Algae oil is relatively pure and doesn't include fats contained in most plant-based oils. Unlike algal oil, fats freeze at low temperatures and cause clogging which is detrimental to the biodiesel industry for many FAME processing technologies. Some forms of algae are 60% oil by mass. Compared to current biodiesel feedstocks, algae can produce over 30 times more oil per acre than palm trees, and 400 times more than soybeans. Producing algae doesn't require using productive farmland and it can be integrated with biorefineries and organic waste processing. This allows for utilizing CO<sub>2</sub> and other nutrients which typically contribute to pollution via nutrient run-off and algae blooms which subsequently result in hypoxia. Research is underway to reduce and/or mediate production costs of algal biomass via development of innovative technologies and diversification, e.g. simultaneous production of high value nutraceutical and pharmaceutical products at integrated biorefineries via closed loop architecture.

Nutraceutical grade algae oil (omega-3 fatty acid supplements) rich in essential fatty acids (EFAs) including GLA currently retail for over \$4,500/gal in comparison with \$2-4/gal for biodiesel. Production costs for biodiesel currently range from \$30-\$80/gal depending on technologies utilized. Recent improvements in separation processes, adding value via closed loop production, and innovative catalytic conversion processes will continually reduce production costs for algae oil and biodiesel. In regards to nutraceuticals and pharmaceuticals, the lack of omega-3 fatty acids in Western diets is responsible for doctors and nutritionists recommending that their patients take daily omega-3 and GLA supplements.

Enriching omega oils for the pharmaceutical and nutraceutical industries is possible via low temperature fractionation, a process known as crystallization. The low temperature enables saturates and mono-unsaturates to be transformed into crystals which facilitates the physical separation of the polyunsaturated stream. The removal of most saturates and monounsaturates leaves polyunsaturates in higher concentrations. The oils can then be deodorized for maximum palatability.

The key advantage in using crystallization is that the resulting enriched triglyceride or phospholipids have undergone no chemical manipulation, and so the natural state of the oil is maintained. It also allows the enriched oil to be marketed as natural, and is 'label friendly'. The technique can be applied to any marine or botanical oil, and will enrich EPA, DHA, GLA, & Free Fatty Acids.

Equateq, the developer of the crystallization technology, splits its business between pharmaceutical and nutraceutical clients. Concentrations for the pharmaceutical industry are about being "super pure", whereas the nutraceutical industry is concerned with providing customized blends. All of Equateq's ingredients are manufactured to pharmaceutical standards. Essentially, crystallization offers high-dose omega-3 potential ([www.equateq.com](http://www.equateq.com)).

### ***Preventing Rancidity of Omega-3s with Antioxidants***

EPA and DHA are long chain fatty acids that are highly unsaturated, and this makes them prone to rapid 'peroxidation' or rancidity, if the oils are exposed to air over extended periods. This can be prevented by the addition of the appropriate mixture of antioxidants added to the oils in the correct concentrations. Pharmax has developed a mixture of natural mixed tocopherols, called the Multox system, which provides maximum protection for all Pharmax omega oil products.

Pharmax has established the effectiveness of the Multox system by measuring the stability of all products under normal usage conditions. For instance, with Finest Pure Fish Oil kept at room temperature, one teaspoon was removed every two days and analyzed for evidence of peroxidation. This provides the most difficult test for any product because, as the bottle becomes depleted of oil, the residual oil is exposed to progressively more oxygen. The test confirmed that the Multox system is effective in preventing peroxidation.

Omega-3 oils, phospholipids, and astaxanthin (super antioxidant) obtained from algae along with chlorella (green algae) and spirulina (blue-green algae or cyanobacteria) provide opportunities for integrated biorefineries to substantially increase net profits via simultaneous production of high value nutraceuticals, pharmaceuticals, and functional foods in addition to biofuels and bioenergy. Though mass production of algae via bioreactors (based on an economy of scale) is expected to require 4-12 years for commercial development of the biofuels industry, production of nutraceuticals and health-food supplements could be extremely profitable using existing algae bioreactor technology. Once algae oil is extracted, it would merely be purified and packaged for retail distribution since conversion to biodiesel would not be necessary for nutraceuticals. Integration of nutraceutical production with biorefineries could dramatically reduce production costs and provide diversification of revenues and long term sustainability for integrated biorefineries.

A portion of the algae starch-protein meal which remains after oil extraction along with oil residues which are not extracted, could be included in total mixed rations for natural and organic livestock and poultry production. The algae meal could be mixed with nutrient-rich distillers' coproducts for enhancing the omega-3 and micronutrient concentrations in functional foods such as enriched eggs, dairy, pork, chicken and beef products ([Martek Animal Nutrition Products](#); [Martek Omega-3 Products and Supplements](#)). Production of omega-3 oils, GLAs, vitamins, and minerals from algae (vs. extracting these same nutraceuticals from cold water fish or krill) provides both ecological and economical advantages over ocean harvesting. In addition, commercial fisheries are rapidly dwindling and pollution of ocean waters is increasing contamination of fish with mercury, other heavy metals, dioxins, and PCP, etc. All natural functional foods which are enriched with essential algal vitamins and minerals would be privately branded and sold as high value coproducts along with biochemicals, biofuels and bioenergy via integrated biorefineries, Ag-Energy Park Complexes & Municipal Bioenergy Complexes.

Single cell microalgae can reproduce in as little as a few hours and are capable of converting CO<sub>2</sub> and nutrients from organic waste via photosynthesis more efficiently than terrestrial plants. Though it varies according to type of microalgae, algal biomass consists of about a 50:9:1 carbon:nitrogen:phosphorus (C:N:P) ratio. However, organic waste is carbon limited, e.g. it is comprised of a 20:9:1 ratio which provides only 40% of the carbon required for efficiently utilizing the available nitrogen and phosphorus for production of algal biomass ([Lundquist](#),

[2008](#)). This represents the potential for a 150% increase in biomass production via CO<sub>2</sub> enrichment. CO<sub>2</sub> enrichment has been demonstrated to increase efficiency of biomass production for terrestrial C3 carbon fixation plants such as strawberries by over 62% ([Bushway and Pritts, 2002](#)), and for citrus trees by up to 80% ([Kimball et al., 2007](#)). For algal photobioreactors which utilize controlled environment agriculture (CEA), each ton of CO<sub>2</sub> along with nutrient-rich biological effluents can produce up to 1.9 tons of algal biomass. For lignin-free algae which are considerably more efficient at utilizing photosynthesis and carbon fixation than terrestrial plants, biomass production yields are substantially higher via CO<sub>2</sub> enrichment.

Some algae specialists and engineers who are designing photobioreactors claim that vertical algae curtains will eventually be capable of annually producing enough biomass to produce 100,000 gal/acre of biofuels (ethanol and biodiesel) ([Valcent VertiGrow Technology](#)). However, other engineers claim that 50,000 gal/acre is the theoretical limit ([Zemke et al., 2008](#)).

In contrast to production of green algae or diatoms, some cyanobacteria species, also referred to as blue-green algae, reproduce much faster and naturally produce ethanol without harvesting the algal biomass. The cyanobacteria produce ethanol for about a year before having to be replaced. No biorefining or distilling is required. This simplifies production of ethanol from cellulosic biomass ([Brown 2008a](#); [Brown 2008b](#); [Algenol Biofuels](#)). In 2008, NREL was proceeding with a project that aims to engineer cyanobacteria (a form of prokaryotic algae) to divert biosynthetic pathways away from glycogen synthesis and toward lipid synthesis. Cyanobacteria are not currently considered to be good candidates for high density biofuel production because they typically produce carbohydrates as storage products rather than lipids. This project takes advantage of advanced methods for genetic manipulation available for cyanobacteria. Other projects being conducted by NREL are focusing on accurate analysis and lipid production of various algae strains, and genome mapping.

There are several synthetic algae and cyanobacteria technologies currently being developed and commercialized, some of which are capable of directly producing ethanol as an excretion in salt water environments. For example, [Algenol Biofuels](#) is commercially installing blue-green algae photobioreactors with Direct to Ethanol™ technology which are currently producing 6,000-10,000 gal/acre with goals of reaching 40,000 gal/acre via low cost production in arid environments. The obvious advantage of this direct to ethanol technology is the lack of biorefining that is required. However, the excreted bioethanol still requires separation from water and salts. The lifespan of the cyanobacteria is about two years, after which the bacteria is recycled into organic fertilizers for use in closed loop production. Another company, [Joule Biotechnology](#), claims they can produce ethanol from a photobioreactor that utilizes only sunlight in conjunction with their genetically engineered microbe.

Due to elimination of biorefining, Algenol can sell ethanol at a price that is cheaper than any other fuel across the US. Algenol is developing industrial-scale production systems to make ethanol from algae on desert land using seawater and vast amounts of CO<sub>2</sub>. Algenol uses cyanobacteria (blue green algae), natural selection, environmental selection, and the tools of molecular biology to produce low cost and environmentally safe biofuels. Biofuels are essentially a form of solar energy. Like all plants, algae uses photosynthesis to convert solar energy into chemical energy stored in the form of oils, carbohydrates and proteins. Algenol's patented technology produces ethanol from four abundant and virtually limitless renewable sources: Algae, sunlight, carbon dioxide and seawater. The outputs from this process are ethanol,

oxygen, freshwater and agricultural fertilizer. Algenol has multiple collaborating laboratories in the US and Europe, with over 60 scientists and engineers working together to improve processes. The internal R&D is expanding, with the U.S. molecular biology labs and the engineering labs both newly built and staffed.

On 30 Jun 2009, Algenol announced they were teaming up with Dow Chemicals, NREL, the Georgia Institute of Technology and [Membrane Technology & Research \(MTR\)](#), to build a commercial scale ethanol plant at Dow's Freeport, TX facility. Dow said it plans to develop the advanced materials and specialty films for the photobioreactor system. Dow is also expected to provide the technology and expertise related to water treatment solutions. Dow said it plans to provide Algenol with access to a CO<sub>2</sub> source for the biorefinery from a nearby Dow manufacturing facility. The CO<sub>2</sub> is expected to serve as the carbon source for the ethanol produced. The result, according to a news release, would be a CO<sub>2</sub> capture process that converts industrially-derived CO<sub>2</sub> into more sustainable fuels and chemicals. The ethanol would be sold as fuel, but Dow's long-term interest is in using it as an ingredient for plastics, replacing natural gas. The process also produces oxygen for use in other biorefinery applications such as enhancing the efficiency of fermentations and fuel cell operation. The exhaust from high temperature fuels cells is mostly CO<sub>2</sub> which could be recycled to make more algae. In summary, Algenol's photobioreactors provide concentrated oxygen, and they convert nearly pure CO<sub>2</sub> into bioethanol for about \$1/gal.

Algal bioreactors are currently being integrated with ethanol biorefineries and organic waste processing which are critical steps for closed loop production. Engineers predict that by utilizing algal bioreactors to convert CO<sub>2</sub> into algal biomass, ethanol biorefinery production can be increased by 30%. For integrated biorefinery operations which collectively utilize CO<sub>2</sub> captured from fermentors, anaerobic digestion, ICEs, and exhaust from high temperature fuel cells, biofuel production can be increased by over 62% ([Bushway and Pritts 2002](#)) with production costs decreasing to as little as \$0.50/gal. for bioethanol. Since lignin-free microalgae grow more efficiently than terrestrial plants, CO<sub>2</sub> enrichment could increase biomass production by over two fold for this single cell aquatic species. Algenol's synthetic cyanobacteria which directly produce ethanol via photobioreactors and Solazyme's heterotrophic-fermentation approach using synthetic algae are the only two algae companies which currently claim to have scalability for commercial applications. Chevron, Bill Gates and the Rockefeller family have invested well over \$100 million in [Sapphire Energy](#) which claims that it will have commercially scalable photosynthetic algae technology which utilizes existing refineries for processing green crude (biocrude) in about 2012. On 14 Jul 2009, Exxon-Mobile Research & Engineering and [Synthetic Genomics](#) announced a \$600 million investment in photosynthetic algae production over a 4-5 year period ([Synthetic Genomics, News Release](#)).

Integration of next generation algal photobioreactors, hydroponic greenhouses, and wastewater processing is very promising, particularly in conjunction with MEC and CEA applications. CO<sub>2</sub> enrichment of wastewater resources substantially enhances algal biomass production while eliminating energy intensive nutrient removal/water reclamation processes required via conventional treatment. Since wastewater is generally carbon deficient (C:N:P ratio is typically 20:8:1) pertaining to maximizing algal biomass production, enriching wastewater with CO<sub>2</sub> substantially enhances efficiency of algal growth for pond or photobioreactor designs which are based on using photosynthesis ([Lundquist, 2008](#)). For integrated biorefineries, the opportunity to implement closed loop production of biomass (either algal bioreactors or hydroponic

greenhouses) substantially enhances NEG for sustainable operations. Algal biomass can be processed directly via anaerobic digesters to enhance biogas and DG-CHP production, or refined to liquid biofuels via integrated biorefineries. Coproducts of biorefining can then be fed to animals, and animal waste can then be processed via anaerobic digestion to provide biomethane, CO<sub>2</sub> and value added organic fertilizers for closed loop biomass.

Theoretically, the photosynthetic conversion efficiency for CO<sub>2</sub> to algae is 12%. This is substantially higher, almost double the average range of 1-7% for terrestrial plants. Depending on organic carbon content consisting primarily of carbohydrates and lipids in a particular algae strain, CO<sub>2</sub> can produce a substantial amount of biomass. Based on a 1.9 conversion ratio of CO<sub>2</sub> to algal biomass and a corresponding increase in CO<sub>2</sub> conversion efficiency of photosynthesis for CEA applications, conservatively recycling 50% of total CO<sub>2</sub> emissions (0.50 x 961,230 tons produced during fermentation, anaerobic digestion and from fuel cell exhaust via closed loop systems for integrated biorefineries) allows for production of 480,615 tonnes of algal biomass for a 60 MGY ethanol biorefinery. Slightly lower volumes of lignocellulosic feedstocks such as water hyacinth (used for ethanol and heterotrophic production of algae) could also be produced via hydroponic greenhouses. In addition to CO<sub>2</sub> enrichment, biological effluents produced from anaerobic digestion of organic waste can be utilized as value added fertigation streams for production of algal biomass and lignocellulosic biomass ([Kebede-Westhead et al., 2004](#)) via closed loop systems.

### ***Characteristics of Algae Production***

Typically, algae are autotrophic (derive cell carbon from inorganic carbon dioxide), photosynthetic (derive energy for cell synthesis from light), and contain chlorophyll. They are also chemotrophic in terms of night time respiration, e.g., metabolism of molecular oxygen (O<sub>2</sub>). Algae utilize photosynthesis (solar energy) to convert simple inorganic nutrients into more complex organic molecules. Photosynthetic processes result in surplus oxygen and non-equilibrium conditions by producing reduced forms of organic matter, i.e., biomass containing high-energy bonds made with hydrogen and carbon, nitrogen, sulfur, and phosphorus compounds. Hence, integration of heterotrophic production with an optimal balance of light for photosynthesis can trigger metabolic reduction which optimizes high-energy bonds in algal biomass. This biological reduction consequently increases bioenergy potential for hybrid algal bioreactors which utilize microbes which are both heterotrophic and phototrophic of natural or synthetic origin.

Redox (shorthand for reduction-oxidation reaction) describes all biochemical reactions in which atoms have their oxidation number (oxidation state) changed. This can be either a simple redox process such as the oxidation of carbon to yield carbon dioxide or the reduction of carbon by hydrogen to yield methane (CH<sub>4</sub>), or it can be a complex process such as the oxidation of sugar in the human body through a series of very complex electron transfer processes.

The term redox comes from the two concepts of reduction and oxidation:

- Oxidation describes the loss of electrons / hydrogen or gain of oxygen / increase in oxidation state by a molecule, atom or ion.
- Reduction describes the gain of electrons / hydrogen or a loss of oxygen / decrease in oxidation state by a molecule, atom or ion.

Though sufficient for many purposes, these descriptions are not precisely correct. Oxidation and reduction properly refer to a change in oxidation number while the actual transfer of electrons may never occur. Thus, oxidation is better defined as an increase in oxidation number, and reduction as a decrease in oxidation number. In practice, the transfer of electrons will always cause a change in oxidation number, but there are many reactions that are classed as "redox" even though no electron transfer occurs (such as those involving covalent bonds).

Cellular respiration, for instance, is the oxidation of glucose (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>) to CO<sub>2</sub> and the reduction of oxygen to water. The summary equation for cell respiration is:



The process of cell respiration also depends heavily on the reduction of NAD<sup>+</sup> to NADH and the reverse reaction (the oxidation of NADH to NAD<sup>+</sup>). Photosynthesis is essentially the reverse of the redox reaction in cell respiration:



In this instance, CO<sub>2</sub> is converted to glucose (carbohydrates contained in algal biomass) and oxygen. This simultaneously reduces CO<sub>2</sub> emissions produced during dark fermentation and cellular respiration.

Fermentation is the process of deriving energy from the oxidation of organic compounds, such as carbohydrates, using an endogenous electron acceptor, which is usually an organic compound. This is in contrast to cellular respiration, where electrons are donated to an exogenous electron acceptor, such as oxygen, via an electron transport chain. Fermentation does not necessarily have to be carried out in an anaerobic environment. For example, even in the presence of abundant oxygen, yeast cells greatly prefer fermentation to oxidative phosphorylation, as long as sugars are readily available for consumption. In contrast to conventional yeast fermentations, dark fermentation of algae, yeast/fungi, and bacteria is the fermentative conversion of organic substrate to algal biomass, biohydrogen or biomethane, and CO<sub>2</sub>. It is a complex process manifested by algae and diverse groups of yeast, fungi and bacteria through a series of biochemical reactions involving three steps similar to anaerobic conversion. Dark fermentation differs from photofermentation because it proceeds without the presence of light.

Biological energy is frequently stored and released by means of redox reactions. Photosynthesis involves the reduction of carbon dioxide into sugars and the oxidation of water into molecular oxygen. The reverse reaction, respiration, oxidizes sugars to produce carbon dioxide and water. As intermediate steps, the reduced carbon compounds are used to reduce nicotinamide adenine dinucleotide (NAD<sup>+</sup>), which then contributes to the creation of a proton gradient, which drives the synthesis of adenosine triphosphate (ATP) and is maintained by the reduction of oxygen.

The term redox state is often used to describe the balance of NAD<sup>+</sup>/NADH and NADP<sup>+</sup>/NADPH in a biological system such as a cell or organ. The redox state is reflected in the balance of several sets of metabolites (e.g., lactate and pyruvate, beta-hydroxybutyrate and acetoacetate), whose interconversion is dependent on these ratios. An abnormal redox state can develop in a variety of deleterious situations, such as hypoxia, shock, and sepsis. Redox signaling involves the control of cellular processes by redox processes.

Redox proteins and their genes must be Co-located for Redox Regulation according to the CoRR Hypothesis for the function of DNA in mitochondria and chloroplasts.

“Organic matter (carbohydrates or sugars produced primarily via photosynthesis) serves as an energy source for non-photosynthetic or heterotrophic organisms (animals, including most bacteria, which subsist on organic matter). Heterotrophic organisms tend to restore equilibrium by catalytically decomposing these unstable organic products of photosynthesis, thereby obtaining a source of energy for their metabolic needs. The organisms use this energy both to synthesize new cells and to maintain old cells already formed (Stumm and Morgan, 1996). From the point of overall reactions, these heterotrophic organisms only act as reduction-oxidation catalysts - they only mediate the reaction (or more specifically the electron transfer). Oxidation may produce several intermediate reduction-oxidation states prior to reaching a fully oxidized state (e.g., inorganic state).

Respiration is the reverse process of growth in which protoplasm undergoes endogenous decay and/or cell lysis and oxidation. Through respiration and decomposition, organic matter is returned to the simpler (vs. complex and unstable) inorganic state. During breakdown oxygen is consumed and carbon dioxide is liberated (Chapra 1997). Although algae respire oxygen in the presence of sunlight, the amount produced via photosynthesis usually exceeds the amount used during daylight.

Light is the most limiting factor for algal growth, followed by nitrogen and phosphorus limitations. Algal productivity is often correlated to levels of nitrogen (N) and phosphorus (P) (See N:P ratio, above), but other nutrients are required including carbon, silica, and other micronutrients. Biomass is usually measured by the amount of chlorophyll *a* in the water column (measurement of gross level of algae) and/or as mass per area for attached species.

Chlorophyll *a* is a photosynthetic pigment that serves as a measurable parameter for all algae production. Quantitative biomass estimates can be made noting that on average 1.5% of algal organic matter is chlorophyll *a*. Qualitative assessment of primary production on water quality can be based on chlorophyll *a* concentrations as illustrated below.

**Table 7. Chlorophyll *a* Concentrations According to Water Quality**

Chlr- <i>a</i> Concentration (g/l)	Water discoloration
<10	no discoloration
10-15	some discoloration, some algal scum
20-30	deep discoloration, frequent algal scum
>30	very deep discoloration, algal matting

Though not true algae, certain strains of cyanobacteria (blue green-algae) can produce an active intracellular toxin, especially when phytoplankton are senescent (the growth phase following maturity and prior to death, characterized by accumulated metabolic products, increased respiration, and loss of dry weight) and decaying.

The intensity, duration, and quality of light influence the dominance of algal species and the structure of algal communities. Likewise, water temperature influences the metabolic and reproductive rates of algae. ([Deas and Orlob, 1999](#))

### ***Advantages of Heterotrophic vs. Phototrophic Production via Algal Bioreactors***

In comparison with conventional algal photobioreactors and open pond systems, closed production systems (including fermentation vats-heterotrophic production) offer more control and can produce algal biomass 24 hours a day. Heterotrophic algae obtain energy from carbon sources such as glucose, while phototrophic algae obtain energy from the sun via CO<sub>2</sub> and photosynthesis.

Specific algal strains can be targeted for production of DHA, EPA, astaxanthin (super antioxidant), and possibly phospholipids for use in both nutraceutical and pharmaceutical grade products. One of the advantages of fermentation vats is that the capital intensive and relative inefficiency of photosynthesis used by conventional photobioreactors is not required. Cell density for heterotrophic production can exceed the density produced by conventional photobioreactors (3-4 g/l to over 150 g/l). In addition, the hyper-growth rate exhibited by heterotrophic production of algal biomass 24/7 can result in increasing the efficiency of producing algae by 1,000 fold in comparison with conventional phototrophic production. Fermentation vats are also simpler to build and operate than the complex construction and operation of sophisticated photobioreactors. However, closed algal photobioreactors which incorporate artificial light arrays and nutrient delivery systems via modular technology are currently under development ([OriginOil](#)) and eventually could be capable of economically producing algal biomass with similar efficiencies as those of fermentation vats.

Current emphasis is being placed on development of algal photobioreactors since it is believed that there are more phototrophic algae than heterotrophic algae (Bold and Wynne, 1985). However, Martek has discovered that numerous species from all algal groups are capable of heterotrophic growth. Advancements in algal molecular biology (Dunahay et al., 1995; Apt et al., 1996; Dawson et al., 1997) have made it possible to genetically transform an obligate phototroph into a heterotroph (Zaslavskaja et al., 2001). The potential now exists to develop synthetic algal strains capable of dual growth modes for either heterotrophic or phototrophic production. Solazyme, Sapphire, and Mascoma are three companies which are leading research in genetic engineering of microbes to consolidate unit processes and reduce production costs for both heterotrophic and phototrophic production systems. While Solazyme and Sapphire focus on synthetic algal oil production, Mascoma and others are focusing on production of ethanol via development of synthetic yeast and bacteria. Symbiotic production systems which simultaneously produce algae and yeast are currently being utilized with unprecedented heterotrophic production efficiencies.

For heterotrophs, fermentors have a number of important advantages over photobioreactors. A large knowledge base has been accumulated over the years for fermentation processes. Modern fermentation vats consist of highly sophisticated hardware which provide large scale availability on a global basis. Fermentation provides relatively low unit operating costs provided an economical source of glucose and nutrients are available.

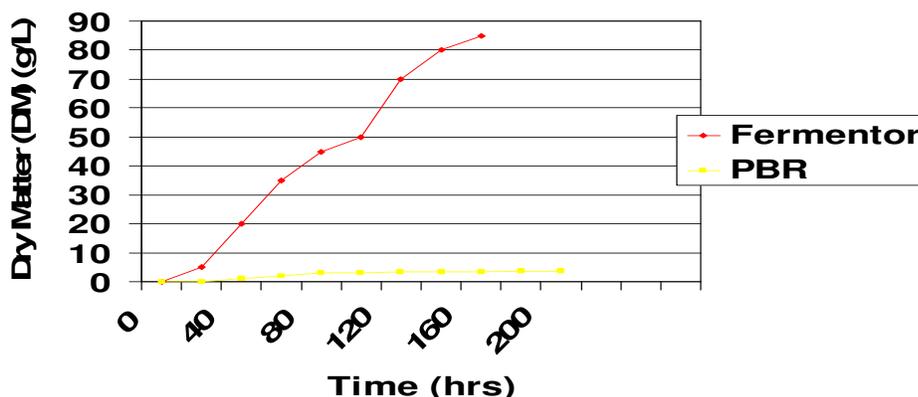
The choice of whether to utilize fermentation vats or photobioreactors depends on several factors, including algal growth mode of species utilized, final end product desired, and anticipated purpose of the alga or algal product. If heterotrophic growth is possible, it will generally be more economical provided an economical source of glucose and nutrients is available ([Behrens, 2005](#)).

**Table 8. Calculation of Energy Cost to Produce a Kilogram of Algal Biomass (DM)**

	Phototroph	Heterotroph	Heterotroph
Energy Source	Light	Starch Glucose	Cellulosic Biomass
Energy Cost	\$0.07/kW-hr	Less than \$0.67/kg	Less than \$0.013/lb
Estimated Cost/kg (DM)	\$11.22	Less than \$0.81/kg	Less than \$0.015/lb
Actual Cost/kg (DM)	Less than \$11.22	Less than \$2.01/kg Less than \$4.43/lb	Less than \$0.016/kg Less than \$0.035/lb
Productivity	$0.4\text{g} \times \text{L}^{-1} \times \text{day}^{-1}$	$5.8\text{g} \text{L}^{-1} \times \text{day}^{-1}$	$5.8\text{g} \text{L}^{-1} \times \text{day}^{-1}$

(Adapted from [Behrens, 2005](#))

According to the production estimates listed in table 8, heterotrophs can produce 14.5 fold more algal biomass per day in comparison with phototrophs. The following chart compares growth rates and corresponding cell densities for production of *Chlorella* over a 180 hour period (7.5 days) which illustrates a 24.3 fold increase in productivity for an unmodified organism.



*Figure 24. Comparison of Chlorella growth in a fermentor and photobioreactor (PBR).*  
(Adapted from [Behrens, 2005](#))

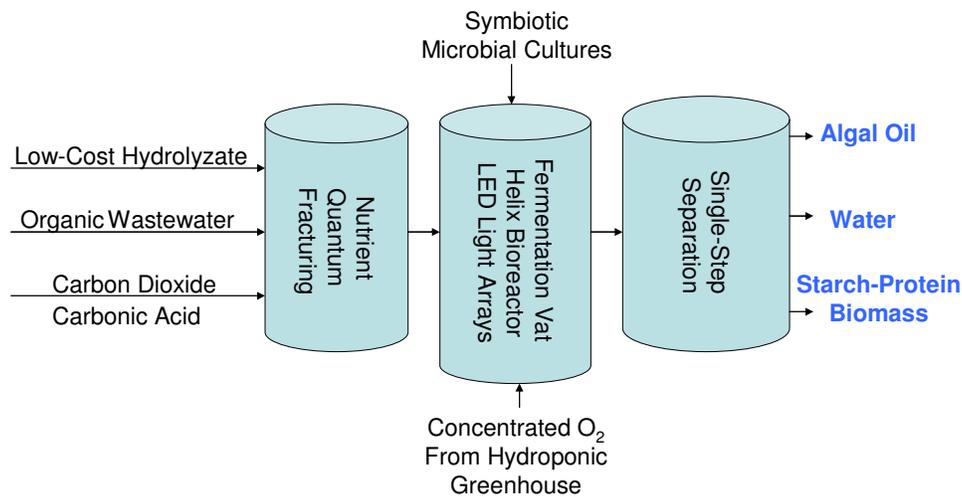
According to Harrison Dillon, Chief Science Officer and co-founder of Solazyme, depending on the algal strain utilized the efficiency of heterotrophic production can exceed phototrophic production by up to 1,000 fold. This dramatic increase in cell density and over-all production rate is partially due to advances in genetic engineering of synthetic algae by companies such as Solazyme and General Atomics. Solazyme is converting high producing phototrophs to heterotrophs, some of which are capable of dual growth modes which can process both hexose and pentose sugars into algal biomass.

While costs of electricity are continually going up in pace with world population growth, production costs for glucose and cellulose have actually decreased dramatically since 2005. This is due to reductions in cost of enzymes and more efficient pretreatment and biorefining processes which are continually being developed. Though LED lighting may offset energy costs for next

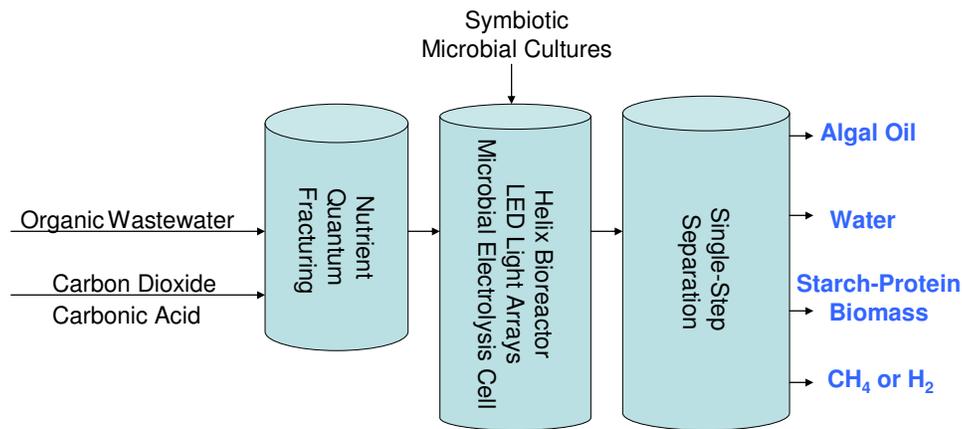
generation photobioreactors, rising energy prices and capital intensive construction will make it increasingly difficult for phototrophic production to compete with efficient heterotrophic production utilizing low-cost sources of starch and cellulosic feedstocks.

Some dual heterotrophic-phototrophic algae are capable of efficiently metabolizing both hexose and pentose sugars. As new and improved synthetic microbial cultures are developed for symbiotic production of algal biomass via the next generation of algal bioreactors, production costs will continue to decrease. Optimizing the fermentation environment including temperature, pH, nutrient processing and nutrient ratios including oxygen and CO<sub>2</sub> maximizes the hyper growth rates of algae. For integrated biorefineries, unprecedented production efficiencies can be achieved via closed loop architecture for both production and processing of biomass.

### Hybrid Algal Bioreactors



*Figure 25a. Dual Heterotrophic-Phototrophic Algae Production Simultaneous Production of High Value Nutraceuticals, Animal Feeds & Biofuels*



*Figure 25b. Phototrophic Algae Production & Microbial Electrolysis Cell Simultaneous Production of High Value Nutraceuticals, Animal Feeds & Biofuels*

For example, algae could be combined with corn or cellulosic hydrolyzates. Quantum fractured CO<sub>2</sub> and nutrient-rich biological effluents could be fed to hybrid bioreactors providing inexpensive LED light arrays such as that provided by OriginOil's Helix Bioreactor. The fermentor temperature would be regulated using waste heat from thermal integration and heat exchangers which would reduce energy costs. In comparison with the cellulosic hydrolyzates utilized, this could provide a 20 fold increase in algal biomass within 48 hours via integration with an ethanol biorefinery. The biological effluents utilized would consist of organic wastewater, thin stillage, and anaerobic digester effluents. These mixed substrates comprised of organic fertilizers, carbohydrates and minerals would provide the majority of algal nutrients in addition to hydrolyzates and CO<sub>2</sub>.

If the corn sugar (primarily glucose) that is now utilized to produce 10 BGY of ethanol was fed to heterotrophic algae (high in carbohydrates vs. lipids) in tanks, a 20 fold increase would increase annual yields to 200 BGY in the US alone. If hexose (primarily starch and cellulose) and pentose sugars (hemicellulose) are processed for both corn grain and stover feedstocks, this could increase annual ethanol yields to over 400 BGY while reducing current production costs by over 50% (refer to table 8). Production of algal biomass solely from crop residues would reduce current heterotrophic production costs for algal biomass by over 99% in comparison with production costs in 2005, e.g. from \$4.43/lb to \$0.035/lb (\$84/tonne). These calculations are based on biomass feedstocks (crop residues such as corn stover and wheat straw) costing \$30/tonne FOB the biorefinery ([Cellulosic Ethanol Feasibility Program](#)). Further reductions in production costs of algal biomass (less than \$54/tonne) can be achieved by using organic waste and waste heat for closed loop biomass production and incorporating efficient pretreatment and fractionation platforms at integrated biorefineries. This allows for simultaneously producing high value coproducts including nutraceuticals, pharmaceuticals, medical products and biochemicals from the lignin and lipid portions in addition to processing the carbohydrates contained in algal biomass.

For precision agriculture SDF high density relay cropping systems, sugar and ethanol yields could be more than doubled by increasing crop/biomass yields per acre. The US average corn crop yield is about 150 bu/acre. By increasing this to the equivalent of 400 bu/acre corn plus triticale biomass, this could increase total biomass produced per acre to over 25 tonnes. Based on the corn plant consisting of 50% corn and 50% stover, processing the majority of the corn plant would allow for harvesting and processing 20 tonnes of biomass per acre. At a 70% conversion rate of carbohydrates to sugars, this would be equivalent to producing 14 tonnes of hexose and pentose sugars. A 20 fold increase in comparison with hydrolyzates obtained from corn biomass would increase total algal biomass to the equivalent of 280 tonnes/acre. This lignin-free biomass containing a thin cellulose wall around the single cell algae could increase ethanol yields by as much as 40% in comparison with terrestrial crops (which contain structural carbohydrates). Based on producing an average of 161 gal/tonne of algal biomass for advanced processing technologies, this would result in yields over 45,000 gal of anhydrous ethanol (99.5% ethanol) per acre (1 trillion gals of ethanol produced annually). 5-10% of the algal biomass would be utilized to grow the next batch. Ethanol production would be in addition to production of algal oil and protein meal products depending on the composition of the particular algae strain.

Processing organic waste resources and in-house production of CHP for closed loop biomass and biorefining will allow for continually reducing production costs for integrated operations. Novel FAME processing of algal oils and organic waste feedstocks (including food processing waste,

crop residues, municipal waste and sewage) high in free fatty acids (FFAs) and water content to biodiesel with excellent cold flow capabilities and no glycerol produced could eventually allow for producing biodiesel for less than \$0.50/gal. In addition, integrated biorefineries and bioenergy complexes could produce bioethanol for less than \$0.50/gal and bioelectricity for less than \$0.04 kW-hr for closed loop systems which incorporate high temperature fuel cells providing CHP via biomethane feeds.

### ***Artemisinin Combination Therapies (ACTs)***

“Artemisinin derivatives are the key active ingredients in Artemisinin combination therapies (ACTs), the most effective therapies available for treatment of malaria. Because the raw material is extracted from plants with long growing seasons, artemisinin is often in short supply, and fermentation would be an attractive alternative production method to supplement the plant source. Previous work showed that high levels of amorpha-4,11-diene, an artemisinin precursor, can be made in *Escherichia coli* using a heterologous mevalonate pathway derived from yeast (*Saccharomyces cerevisiae*), though the reconstructed mevalonate pathway was limited at a particular enzymatic step.

By combining improvements in the heterologous mevalonate pathway with a superior fermentation process, commercially relevant titers were achieved in fed-batch fermentations. Yeast genes for HMG-CoA synthase and HMG-CoA reductase (the second and third enzymes in the pathway) were replaced with equivalent genes from *Staphylococcus aureus*, more than doubling production. Amorpha-4,11-diene titers were further increased by optimizing nitrogen delivery in the fermentation process. Successful cultivation of the improved strain under carbon and nitrogen restriction consistently yielded 90 g/L dry cell weight and an average titer of 27.4 g/L amorpha-4,11-diene.

Production of >25 g/L amorpha-4,11-diene by glucose fermentations followed by chemical conversion to artemisinin may allow for development of a process to provide an economical alternative for production of artemisinin to be incorporated into ACTs.” ([Tsuruta et al., 2009](#); [Amyris](#))

### ***Closed Loop Algae Production Systems for Integrated Biorefineries***

In regards to coproducing high value nutraceutical and pharmaceutical products at integrated biorefineries, algae provide an attractive opportunity in contrast to fish oil. Because of its peculiar taste, odor and complexity, fish oil has significant limitations as a source of DHA & EPA (Barclay et al., 1994). The purification of PUFAs, especially EPA, from low-grade fish oil is difficult and costly (Belarbi et al., 2000). In addition, marine fish stocks are subject to seasonal and climatic variations (Gill and Valivety, 1997). Fish, like humans, are not capable of synthesizing EPA *de novo*. EPA in fish is derived from consuming microalgae in oceanic environments. As uncontaminated fish oil fails to meet the growing demand for DHA & EPA nutraceuticals, alternative feedstocks and more eco-friendly production processes will continue to be sought.

Microalgae contain large quantities of high-quality DHA & EPA and they are considered an ideal source of this essential fatty acid. Some microalgae can be grown heterotrophically on cheap organic substrates such as cellulosic hydrolysates (simple sugars, glucose and possibly xylose), organic acids (acetate), and even organic waste and CO<sub>2</sub> without light. Numerous strategies have been investigated for commercial production of EPA by microalgae. These

include screening of high EPA & DHA yielding microalgal strains, improvement of strains by genetic manipulation, optimization of culture conditions, and development of efficient cultivation systems via fermentation vats ([Wen and Chen, 2003](#)) and next generation heterotrophic ([Solazyme](#); [General Atomics](#); [Amyris](#)) and phototrophic ([OriginOil](#); [Sapphire](#); [Synthetic Genomics](#)) bioreactors. The future of synthetic biology which is being developed by companies such as Solazyme could revolutionize chemical manufacturing by simplifying the application of biotechnology. As indicated above, synthetic biology involves genetic engineering of microbes, including algae, yeast, fungi and bacteria for the purpose of eliminating entire unit processes. Examples are consolidated bioprocessing (CBP) and integrated biosystems.

### ***High Value Algal Coproducts***

*Food Ingredients:* Emulsifiers such as those produced by ADM from vegetable oils (lecithin made from soy or sunflower oil, etc.) for the food industry range in price from a low of \$0.50/lb for a standard fluid lecithin to \$10/lb for a highly purified sucrose ester. Most emulsifiers used as food ingredients would be priced in the \$1-\$5/lb range and would be used to solubilize hydrophobic oils/etc into water-based systems. The revenue generated by these types of high value food products provide diversification. In addition, commercial scale marketing does not usually saturate the market, so specialty food ingredients generally maintain value and provide relatively higher values than commodity based biofuels which currently retail for about \$0.36/lb.

Vegetable and algal oil emulsifiers could also be utilized in the cosmetics and personal care industries in with a higher product price can be absorbed. A nutrient-rich product such as development of fruit drinks and other functional foods which incorporate omega-3s while providing emulsification could add substantial value. ADM has offered to provide testing to determine value for simultaneous emulsification and nutrient enrichment for projects of mutual interest/value which commercialize algal oils. Algal oil appears to be much more promising for processing as emulsifiers than either fish or krill oil. These types of functional ingredients and enriched functional foods high in DHA, DPA, EPA and phospholipids could potentially wholesale for much more than double the price that (\$5-10/lb) current food ingredients/emulsifiers wholesale for. The same proprietary processing of soy and sunflower oils used by ADM could be utilized for processing algal oils.

### ***Developing Algal Bioreactors***

One strategy for continually improving heterotrophic production is integration of yeast/fungi and microalgal production. For instance, yeast gives off large amounts of carbon dioxide when it grows, but needs sugar to maintain rapid growth. Algae need carbon dioxide to accelerate growth, and in return can excrete sugars. Hence, production of yeast and algae could be produced simultaneously via integrated biosystems which benefit from robust synergies.

In addition to algae rich in lipids, a 96% starch variety of algae has been reported. Growing such a starch-rich algae strain in a heterotrophic bioreactor provides opportunities for phenomenal growth rates to be achieved. Ethanol from algae is possible by converting the starch (the storage component) and lignin-free cellulose (the cell wall component) to glucose. Algae are one of the optimal sources for second generation bioethanol due to the fact that they are high in carbohydrates/polysaccharides and have thin cellulose walls.

At a typical corn ethanol biorefinery only about 30-35% of the corn grain is actually converted to ethanol. In addition, a third is converted to CO<sub>2</sub>, and a third remains as dissolved organics and

solids in the whole stillage after distillation (removing water from the ethanol). The stillage contains about 89% water. Most of the solids not held as solubles are centrifuged out and sold as distillers' grains for use as livestock feedstuffs. The remaining thin stillage resulting from removing excess water from the centrifuge (centrate) contains about 6% organic material and is a major waste product of ethanol plants. At conventional ethanol plants, due to relatively high levels of contaminants such as acetic acid, only half of this nutrient-rich thin stillage is recycled as backset into the next batch of corn slurry. The other half of the centrate is disposed of, usually through costly water removal which adds about 10 cents to every gallon of ethanol. The thin stillage wastewater resource subsequently contributes to pollution. For example, a 100 MGY ethanol plant will produce 600 million gallons of waste water centrate which could be utilized to produce algae via 6% mineral solids in solution with water. For integrated biorefineries which incorporate algal bioreactors, waste products and a portion of sugars obtained from cellulosic biomass can be utilized for value added production of algal biomass with unprecedented efficiencies of operation via closed loop architecture.

Fermentation is the process of deriving energy from the oxidation of organic compounds, such as carbohydrates, using an endogenous electron acceptor, which is usually an organic compound (Klein et al., 2005). This is in contrast to cellular respiration, where electrons are donated to an exogenous electron acceptor, such as oxygen, via an electron transport chain. Hence, in contrast to fermentation, cellular respiration and the use of exoelectrogenic bacteria via phototrophic algal strains may be required for efficient integration of plant MECs with algal bioreactors.

Cultures of heterotrophic microalgae do not suffer from the limitations imposed by the need for external light supply and can therefore be grown to much higher densities and give rise to productivities that are orders of magnitude higher than can be obtained in conventional phototrophic cultures. A limited number of heterotrophic species have been able to support growth of oysters and rotifers (Glaude and Maxey, 1994) but all microalgae are not equally valuable as biofuel feedstocks or animal feeds which depends on their digestibility, biochemical composition, cell size and other characteristics. Identification of microalgal species that grow well under heterotrophic conditions and are eaten and digested by filter feeding animals, and design of cultivation processes that result in algae of high energy or nutritional value are therefore of central steps in the process of replacing phototrophic microalgae by heterotrophic species. Microalgal species that are able to grow heterotrophically have been identified from several algal divisions and some species, which for different purposes have been developed into highly productive, high-cell-density cultures, may also be promising candidates as bioenergy feedstocks and 'feedalgae' for integration of aquaculture biosystems via closed loop architecture.

These include the chlorophyte *Chlorella spp.* (Wu et al., 2007; Xiong et al., 2008), the diatom *Nitzschia laevis* (Wen and Chen, 2001), the dinoflagellate *Cryptothecodinium cohnii* (de Swaaf et al., 2003a; de Swaaf et al., 2003b), and the rhodophyte *Galdieria sulphuraria* (Graverholt and Eriksen, 2007; Schmidt et al., 2005). In particular the lipid content of phototrophic microalgae are important for their nutritional value as oyster feed (Wikfors et al., 1996) and *Chlorella spp.*, *N. laevis*, and *C. cohnii* may all accumulate large quantities of lipids rich in poly-unsaturated fatty acids under the right conditions. *G. sulphuraria*, which accumulates starch rather than lipids, can be grown at extremely low pH, a property which may prove valuable in order to minimize contamination of cultures by other microorganisms.

Fermentation does not necessarily have to be carried out in an anaerobic environment, however. For example, even in the presence of abundant oxygen, yeast cells greatly prefer fermentation to oxidative phosphorylation, as long as sugars are readily available for consumption (Dickinson et al., 1999).

Sugars are the most common substrate of fermentation, and typical examples of fermentation products are ethanol, lactic acid, methane and hydrogen. However, more exotic compounds can be produced by fermentation, such as butyric acid and acetone (acetic acid). Yeast carries out fermentation in the production of ethanol in beers, wines and other alcoholic drinks, along with the production of large quantities of carbon dioxide which provide a carbon source for algal bioreactors and MECs.

In contrast to open systems, each closed bioreactor or fermentation vat contains specific algae species or algae cocktails so that desired biomass production can be maximized. Many species of algae and bacteria are symbiotic and when produced in mixed cultures are most prolific. Temperature, pressure, and other environmental conditions are optimized for the specific algae strains utilized for the nutraceutical, pharmaceutical, and functional food industries.

Additionally, fermentation offers flexibility: A row of vats can be used to make biofuels, and then scrubbed and prepped for production of nutraceuticals and pharmaceuticals. The sugar water that is required for algae growth can be economically produced via integrated biorefineries which incorporate ammonia ensiling and enzyme inoculation of crop residues and organic waste as a low-cost pretreatment-hydrolysis process. Ethanol and CO<sub>2</sub> can be utilized as an organic solvent for fractionation processes prior to enzymatic hydrolysis, and also for production of pharmaceutical grade products such as omega-3 fatty acids (DHA & EPA) and phospholipids. The ammonia process is also utilized to remove lignin for subsequently processing it into high value low molecular weight derivatives for manufacturing of bioplastics, etc.

For integrated biorefineries which utilize closed systems, temperature regulated bioreactors and fermentation vats which produce algal biomass 24 hours a day from organic waste, sugar water and CO<sub>2</sub> emissions can increase efficiency of production by up to 1,000 fold in comparison with open pond systems. Heterotrophic algae grown in nutrient rich water in controlled environments becomes many times more concentrated due to a hyper fast growth rate. Chevron is investing in a proprietary fermentation technology for synthetic algae that is being developed by Solazyme. Besides Solazyme, this technology is also being developed in a collaboration between East Kentucky University and General Atomics. Heterotrophic algae grow in the dark and multiply very rapidly when fed sugars or local biomass cellulose converted to sugars.

Corn ethanol biorefineries have abundant access to waste heat, CO<sub>2</sub>, nutrient rich wastewater effluents, and corn sugars for development of integrated biorefineries. It is theoretically possible to combine a tablespoon full of live algae with a pound of corn sugar, and bubble CO<sub>2</sub> through a medium of nutrient rich biological effluents, regulate temperature and pH, and produce over 20 pounds of algae within 48 hours. Utilizing the corn sugar that is now producing 10 billion gallons of ethanol, feeding it and cellulose produced from corn stover to heterotrophic algae in tanks (fermentation vats) would, at only a 30 fold increase in yields, produce over 300 billion gallons of ethanol per year in the US alone.

Out of tens of thousands of strains of algae, thirty two types of heterotrophic algae have been

identified thus far. Some are high in starch. Some are high in oil. Some are high in proteins. Depending on production goals, one could select algae strains accordingly. Coproducts could be produced in addition to biofuels once ethanol or oils are extracted. There are now 172 corn ethanol biorefineries which form a viable framework for a much larger bioenergy/bioeconomy via integration.

In addition to development of heterotrophic algae production, a hybrid bioreactor which utilizes an innovative hybrid lighting system for phototrophic algae production is being developed by OriginOil. Similar to heterotrophic algae production via fermentation vats, for integrated biorefineries waste heat and organic waste can be utilized to regulate temperature and provide essential nutrients for rapidly growing algae in closed biosystems. Hence, closed loop production at integrated biorefineries offer economically competitive alternatives to open pond systems which are subject to varying temperatures and seasonality of photoperiods. Closed systems allow for maximizing rate of algae growth and reducing contamination by invasive species while integrated biorefineries allow for converting waste resources into value added production of algal biomass with unprecedented efficiencies of production.

In conjunction with innovative bioreactor designs, plant microbial electrolysis cells (MECs), and revolutionary separation technologies, it is estimated that algal oil could eventually be produced for less than \$2/gal at integrated biorefineries.

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For integrated biorefineries which utilize closed systems, temperature regulated bioreactors and fermentation vats which produce algal biomass 24 hours a day from organic waste, sugar water and CO<sub>2</sub> emissions can increase efficiency of production by up to 1,000 fold (density of algal biomass) in comparison with open pond systems according to Solazyme's chief science officer and CEO, Harrison Dillon. Chevron is investing in development of a proprietary fermentation technology for synthetic algae (phototrophic algae strains which have been genetically modified for heterotrophic production) which is being developed by Solazyme.

A similar though different hybrid bioreactor which utilizes an innovative lighting system is being developed by OriginOil. For integrated biorefineries, waste heat and organic waste can be utilized to regulate temperature and provide essential nutrients for rapidly growing algae in closed production systems. Hence, closed loop production at integrated biorefineries offer economically competitive alternatives to open pond systems which are subject to varying temperatures and seasonality of photoperiods. Closed systems allow for maximizing rate of algae growth and reducing contamination by invasive species while integrated biorefineries allow for converting waste resources into value added production of algal biomass with unprecedented efficiencies of production.

In conjunction with innovative bioreactor designs, plant microbial electrolysis cells (MECs), and revolutionary separation technologies, it is estimated that algal oil could be produced for less than \$2/gal at integrated biorefineries in the near future. For closed loop systems architecture, processing organic waste and crop residues, and implementing innovative technology, liquid biofuels could eventually be produced for less than \$0.50/gal.

The primary areas of concern for continually improving heterotrophic production of algae are provision of nutrients including CO<sub>2</sub> and O<sub>2</sub>, regulating temperature and pH of fermentors, and sterilization to slow or prevent growth of bacteria. These areas of concern can be economically addressed via comprehensive nutrient-energy management provided by integrated biorefineries and innovative technologies used in closed loop system architecture.

### ***Incorporation of Quantum Fracturing™ & Optimizing Nutrient Ratios***

As explained below, reducing nutrients to micron sized particles via OriginOil's Quantum Fracturing™ or similar technology (microwave or ultrasonics) can substantially enhance bioavailability for microalgae and yeast. Quantum Fracturing, is based on the science of mass transfer and fluid fracturing and addresses some of the challenges of industrializing algae oil production. In the initial Quantum Fracturing step, water, carbon dioxide and other nutrients are fractured at very high pressure to create a slurry of micron-sized nutrition-bubbles, which is then channeled to the algae culture awaiting it in a lower-pressure growth vessel. This process achieves total and instantaneous distribution of nutrients to the algae culture without fluid disruption or aeration. The pressure differentials between the two zones substantially increase contact and exchange between the micronized nutrients and the algae culture for optimizing nutrient absorption.

A quantum is the smallest quantity of some physical property that a system can possess. The term is utilized to illustrate how nutrient substrates are processed and delivered into the algal culture in micron (a millionth of a meter) sized particulates. These fractured nutrients which create a cloudy liquid substance are then channeled to the algae culture. This process achieves total and instantaneous distribution of nutrients to the algae culture without fluid disruption or aeration. The pressure differentials between the two zones substantially increase contact and exchange between the micronized nutrients and the algae culture.

Quantum Fracturing reduces particle size and surface area of organic matter. Organic matter must be converted to inorganic forms in order to maximize bioavailability. In contrast to conventional sampling and chemical sensing technologies, ion exchange resin technology can be utilized in conjunction with Quantum Fracturing to optimize nutrient ratios for uniformly processing a variety of nutrient substrates including organic waste, biological effluents in the form of wastewater, sugar water via cellulosic hydrolysates, and CO<sub>2</sub>, thereby optimizing bioavailability and maximizing algal growth and reproduction.

### ***Regulating Temperature & pH for Algal Fermentations***

Heat exchangers which transfer heat from fermentors to anaerobic digesters or to hydroponic greenhouses provide value added opportunities to capture waste heat while optimizing fermentor temperatures and maximizing algae and yeast growth. An economical source of cooled water could also be utilized in fermentor cooling systems. This could be accomplished by processing

condensation produced by the MCFC and combined cooling heating, and power (CCHP) application. pH of fermentors can be monitored and regulated via real time sensors.

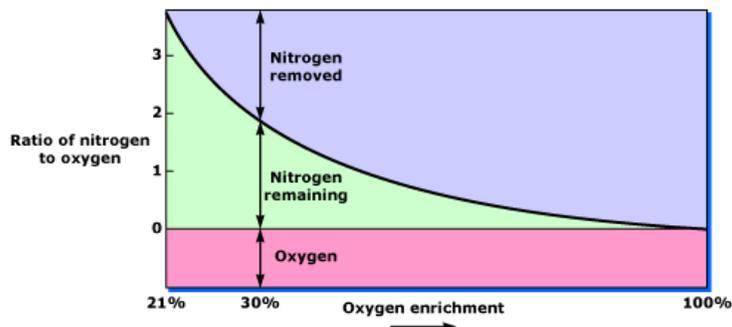
### ***Sterilization for Algal Fermentations***

An abundant source of steam produced by high temperature fuel cells via CHP technologies provides for economical and efficient sterilization of nutrient streams and cleaning fermentors between batches.

### ***Providing Oxygen for Algal, Starch and Cellulosic Fermentations***

Oxygen has a relatively low solubility in aqueous media and providing sufficient O<sub>2</sub> is often the single most limiting factor for achieving a rapid growth rate and a high cell concentration for fermentors. O<sub>2</sub> is generally supplied to a fermentor as compressed air (about 21% oxygen) ([Behrens, 2005](#)). Supplying higher levels of O<sub>2</sub> by blending pure O<sub>2</sub> with air may be economical for integrated operations which have access to an economical source of air with relatively high concentrations of oxygen. Hydroponic greenhouses used to produce lignocellulosic biomass (such as rapidly growing water hyacinth) provide such an opportunity.

Increasing the oxygen content of air brings advantages in any process where inert nitrogen has a ballast effect. The benefit accrues even at only modest enrichment. For example, air with oxygen enriched from 21% to 30% – an enrichment of only 9% – will contain nearly 40% less nitrogen per unit of oxygen.



*Figure 26. Utilizing Membrane Technology for Increasing Oxygen Concentration.*

In a partnership arrangement, [Ube Industries](#) and [MTR](#) have developed and commercialized low-cost micro-membrane modules that are used in conjunction with small vacuum blowers in appliances such as high-end air conditioners. The oxygen content of air is enriched up to 28%. Such units are totally self-contained, operate continuously, require no tanks, and avoid dangerously high oxygen concentrations. The above technology could also be utilized to remove concentrated oxygen from algal photobioreactors and hydroponic greenhouses and subsequently use it for increasing efficiency of both fermentation and fuel cell operation.

In addition, impellers and internal baffles in a fermentor “break” the air-stream, making smaller bubbles and increasing the dissolved oxygen in the fermentor. Longer residence times of oxygen-air bubbles in the fermentor also increases the concentration of dissolved oxygen in the microbial culture. Bubble rate rise is determined by gravity, but the residence time is increased with increased height of fermentors and with increased viscosity ([Behrens, 2005](#)).

## **Light Immersion Technology for Algal Photobioreactors**

Innovative Light Immersion Technology (LIT) developed by [Bionavitas](#) provides a solution for resolving issues with algae self-shading. The self-shading phenomenon results in a layer that limits the amount of algae per acre that can be grown and harvested (Eriksen, 2008). LIT enables the algae growth layer in open ponds to be up to 1.5 meters deep. This represents up to a 30 fold increase in yield over previous methods that produced only 3-5 centimeters of growth. The LIT is cost-efficient, as well as a passive, low-input, net energy positive system which is inexpensive to mass produce.

At the core of LIT is an innovative approach at bringing light to the algae culture in both open ponds and closed bioreactors through a system of light rods which extend deep into the algae culture. By distributing light below the surface “shade” layer and releasing the light in controlled locations, algae cultures grow denser. In external canal systems, the rods distribute light from the sun into the culture. This abundant and free energy source is ideal for generating large amounts of algae for use as biofuels.

In closed bioreactors, the rods evenly distribute more readily absorbed red and blue spectrum light from high efficiency LEDs. While the LEDs increase the cost of production, algae grown in these systems are used for higher value markets such as nutraceuticals.

In order to grow algae in the large-scale, cost-efficient manner needed for biofuels, the technology developers have specifically designed the technology to require as little energy as possible. The company claims that LIT has all of the attributes needed to allow algae to compete with petroleum. The LIT is designed to be independent of the light source, distributing solar as well as artificial light ([Bionavitas News Release, 2009](#)). The distributed lighting system developed by Bionavitas is similar to Origin Oil’s algae production system which utilizes a rotating vertical shaft with very low energy lights arranged in a helix or spiral pattern.

### ***OriginOil’s Dynamic Lighting Process***

On July 15, 2009 – OriginOil released information on their biofeedback process controller which optimizes algae growth and daily harvesting rates. The Dynamic Control System is designed to respond continuously to algae behavior, thus maximizing energy efficiency and growth rates by optimizing light intensity and frequency as algae strains grow to maturity.

The biofeedback system lets the controller know what it needs as it needs it, creating a self-adjusting growth system. At the heart of the system is a programmable controller that receives information from multiple sensor types providing information on the algae culture. The controller, which can be programmed for specific algae strains, responds by sending out commands to change lighting parameters such as intensity, pulsing frequency, and duty cycle. OriginOil’s existing automation of the nutrient delivery process will be integrated in the final version of the Dynamic Control System.

This lighting automation takes advantage of a new generation of low energy Light-Emitting Diodes (LEDs), which greatly improve productivity in dense cultures, and which also deliver the accurate frequency tuning and instant-on capability needed for a true feedback loop. The new Dynamic Control System is being used to test many lighting variations such as concentration, angles, distribution, and pulsing intervals. The data being compiled will allow for continually improving algae production and cell density. ([OriginOil News Release, 15 Jul 2009](#))

### ***OriginOil's Helix Bioreactor Production System***

There are four primary obstacles for mass production of algal biomass for use as a third generation biofuel feedstock via photobioreactor (PBR) technology:

- Algae growth is dependent on a calm fluid environment; it does not like agitation. One of the primary challenges is how to optimally introduce carbon dioxide (CO<sub>2</sub>) and nutrients needed by the growing algae culture without disrupting or over-aerating it.
- Removing excess oxygen for closed photobioreactors.
- Phototrophic algae requires an economical source of light as energy to fuel its growth and oil production. Algae cultivation systems need to cost-effectively and evenly distribute light within the algae culture, preferably 24 hours per day to be competitive with heterotrophic production and/or utilized in hybrid algal bioreactors for heterotrophic-phototrophic production via synthetic microbes which maximize bioenergy via photosynthetic reduction. However, if artificial lighting is utilized in algal bioreactor designs, the relatively low 5-15% efficiency for converting light energy to biomass via photosynthesis must be over-come in order to be sustainable.
- Algae organisms are protected by a tough cell wall. That wall must be cracked - an energy-expensive process - to extract the oil and separate it from water, starch and protein components. The challenge is to maximize oil yield by cracking as many of the algae cells as possible with the smallest amount of energy.

“Algae growth occurs in a Helix BioReactor™, an enclosed vessel where chemical and biological reactions take place. In the initial Quantum Fracturing step, water, carbon dioxide and other nutrients are fractured at very high pressure to create a slurry of micron-sized nutrition-bubbles, which is then channeled to the algae culture awaiting it in a lower-pressure growth vessel, the Helix BioReactor™. This process provides distribution of nutrients to the algae culture without fluid disruption or aeration. The pressure differentials between the two zones substantially increase contact and exchange between the micronized nutrients and the algae culture which results in maximizing nutrient absorption.

A quantum is the smallest quantity of some physical property that a system can possess. The term is utilized to illustrate how nutrient substrates are processed and delivered into the algal culture in micron (a millionth of a meter) sized particulates via fertigation streams. These fractured nutrients which create a cloudy liquid substance are then channeled to the algae culture awaiting it in a lower-pressure growth vessel, the Helix BioReactor™. The pressure differentials between the two zones substantially increase contact and exchange between the micronized nutrients and the algae culture.

Quantum Fracturing utilizes electromagnetic pulse technology to reduce particle size and surface area of organic matter. Organic matter must be converted to inorganic forms in order to maximize absorption/bioavailability. In contrast to conventional sampling and chemical sensing technologies, ion exchange resin technology can be utilized in conjunction with Quantum Fracturing to optimize nutrient ratios for uniformly processing a variety of nutrient substrates including organic waste, biological effluents/wastewater, sugar water via cellulosic hydrolysates, and CO<sub>2</sub>, thereby optimizing bioavailability and maximizing algal growth and reproduction.

The Helix BioReactor™ features a rotating vertical shaft with very low energy LED lights

arranged in a helix or spiral pattern which results in a theoretically unlimited number of algae growth layers. Additionally, each lighting element is engineered to produce specific light waves and frequencies for optimizing growth of algal biomass. The core of the bioreactor design is the innovative helix lighting system, which facilitates the distribution of light at multiple layers. This allows for vertical growth of algae. In addition, artificial light arrays allow for continuous growth of algal biomass 24 hours/day.

The helix structure also serves as the bioreactor's nutrient delivery system, through which the Quantum Fractured nutrients including CO<sub>2</sub>, are uniformly delivered to the entire algae culture, monitored via ion exchange resin technology and continually fine tuned for maximizing growth and reproduction.

Once algae matures, 90% of it is transferred out for harvesting, and the remaining 10% 'green' water is purified and returned to the growth tank. The remaining 10% of algal biomass is used to replenish the Helix BioReactor™, and the process is repeated. With this system, there is no need to re-incubate each batch: The remaining algae culture has already matured and proceeds to re-enter the log phase after each harvest in the continuous process of replenishing the growth environment.

In the extraction unit, flowing algae biomass is first sent through a shielded wave guide system where it receives low-wattage, frequency-tuned microwave bursts, weakening the cell walls. Then Quantum Fracturing is applied to these pre-cracked cells to complete the oil extraction. Using Quantum Fracturing, the amount of energy used to crack the algae is many times less than that of other extraction technologies. Finally, a single step extraction process is utilized to separate algal oil, water and algae starch-protein biomass. Process water is recycled back into the system. The harvested oil is packaged for refining and distribution while the starch-protein portion of algal biomass is utilized to enrich animal feeds for production of nutraceuticals, pharmaceuticals, functional foods and/or utilized to produce ethanol and high value biochemicals for production of bioplastics and other high value bioproducts which displace petroleum products.

The OriginOil System is designed to be modular. It can function as a standalone oil producing system, or it can be connected in a parallel network to produce a large outputs via vertical production systems which reduce footprint and dramatically increase production capacity per acre. OriginOil Systems can be placed anywhere to operate as round-the-clock oil-producing plants. Distributed algal oil production can help transform the current oil and energy industry from a centralized to a distributed model. The ability to generate clean, carbon-neutral energy anywhere can empower industrialization in villages, townships, communities, states and countries. There will be no need to import oil. A new oil can be cleanly manufactured in an industrialized process using the OriginOil System. The oil produced from algae can replace all but the heavier (tarry) fractions of petroleum. As explained above, the algal starch-protein biomass left over after extraction of oil can be utilized for a number of value added products including ethanol, biochemicals, and enriched animal feeds for production of functional foods ([OriginOil Website](#); [OriginOil Investor Presentation, 2009](#); [OriginOil: The New Energy Challenge, 2009](#)).

On 17 Feb 2009, OriginOil signed a Cooperative Agreement with the US Department of Energy's Idaho National Laboratory (INL). The multi-phase research program will focus on

validation and commercial scaling of the company's technology in the production of algae-based fuels by utilizing the state-of-the-art equipment, capabilities, scientists and engineers of the INL. The initial phase, which starts immediately, will focus on the collaborative development of an energy balance model for photobioreactor-based algae systems. OriginOil expects to use this model in the optimization of its algae-to-oil technology as early as the 1st Quarter of 2009. Subsequent phases will center on validation of the OriginOil processes and piloting specific commercial applications ([OriginOil News Release, 17 Feb 2009](#)).

### **OriginOil Announces Breakthrough Process for Live Algae Oil Extraction**

On 27 Jul 2009, OriginOil today announced that it has succeeded in extracting algae oil on a continuous basis without cell sacrifice. This new 'milking' process will join the company's Cascading Production™ technique to create a combined cycle promising new efficiencies.

Live Extraction™, or milking, is inherently efficient because it achieves continuous production of algae oil without destroying the algae cell. Therefore a single algae cell can produce more oil during its lifetime using lower amounts of energy. Unlike other approaches to live extraction, OriginOil's process does not employ expensive consumables such as reverse osmosis membranes; furthermore, it is not limited to oil-bearing algae strains, such as *Botryococcus braunii*, that are known to excrete algae oil naturally.

Algae typically protect their oil behind a tough cell wall. The challenge of live extraction is to harvest the oil without causing permanent damage to the cell. This goal has been achieved in the laboratory at bench scale and is now being scaled up to OriginOil's intermediate 200-gallon tank size. A diagram illustrates the process at [www.originoil.com/technology/live-extraction.html](http://www.originoil.com/technology/live-extraction.html).

The company recently filed for patent protection of the new Live Extraction process, its ninth patent application, entitled "Procedure for Extraction of Lipids from Algae without Cell Sacrifice." Live Extraction works by stimulating the algae cells through specific electrical modulations. The new Live Extraction process will coexist with OriginOil's daily 'destructive' process to create an even more efficient combined cycle.

Aside from any production gains, combining the two processes is desirable because algae cultures must be refreshed regularly to remove waste toxins. Cascading Production supports Live Extraction by removing a percentage of the culture every day, refreshing the environment and giving the algae culture space to grow.

Live lipid extraction is especially beneficial when used with algae that have been genetically engineered for faster growth rate or higher lipid yields. With Live Extraction, OriginOil provides a technology platform for companies that are working to improve algae strains for greater productivity.

### **Algae Oil & Water Separation Process**

For nearly 40 years, it has been widely accepted that if the cost of removing, harvesting and dewatering algae could be reduced to \$50 a ton, algae could become a significant source of fuel. On 23 Mar 2009, AlgaeVenture Systems, a subsidiary of Univenture, demonstrated a disruptive technology that reduces the cost of separating water from algae by more than 99% – from \$875 per ton to \$1.92 per ton ([AlgaeVenture Systems News Release, 23 Mar 2009](#); [YouTube Video of Oil Separation Membrane Technology](#)). Once the algae is dry, the oil can be extracted using mechanical or chemical crushing.

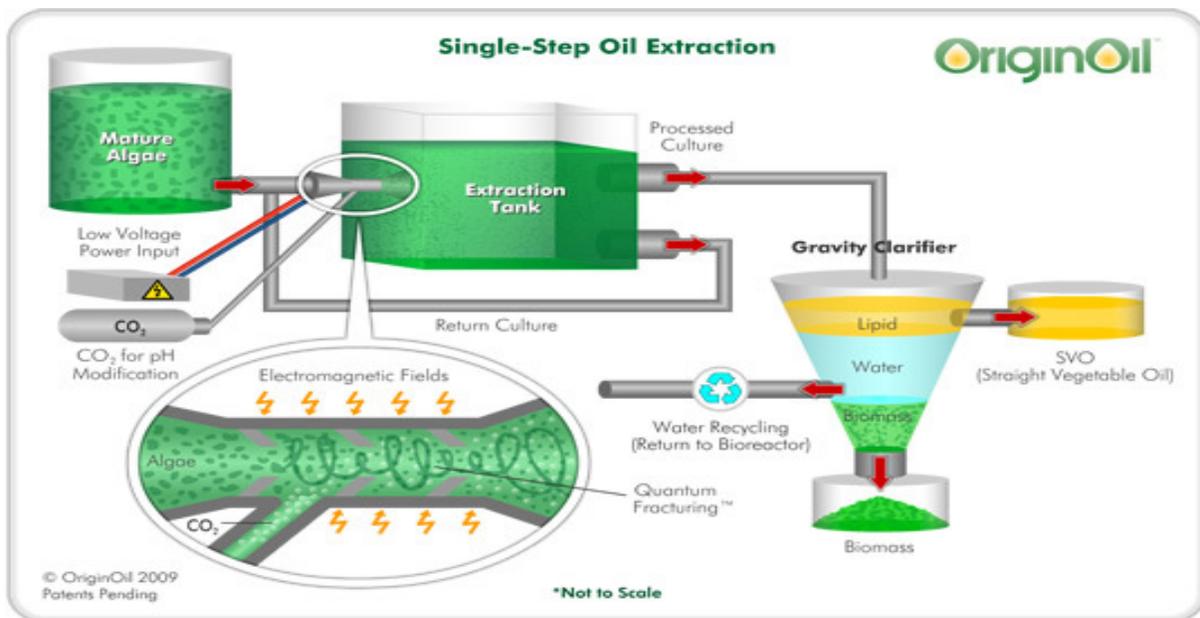
AlgaeVenture Systems is quickly developing a Rapid Algae Farming (RAF) system, which will serve as the platform for the creation of numerous products from algae, specifically nutraceuticals, pharmaceuticals, biofuels and bioplastics. The RAF System is a fully automated network of enclosed photo-bioreactors that efficiently grows and processes algae. The design is fully adaptable, giving it the capability to utilize virtually any species of algae and integrate it with biorefineries. However, there are significant advantages for eliminating the algae drying step through an innovative single-step separation and extraction process developed by OriginOil.

***Single-Step Separation Process is Simpler & More Efficient***

On 20 Apr 2009, OriginOil introduced a new patented single step oil extraction process. Harvesting algae is a challenge. Algae grow suspended in large volumes of water. Once ready for harvest, the algae culture must be concentrated and the oil extracted from each cell. Then, the oil, water and biomass must all be separated for processing. The new OriginOil process achieves all these steps in one pass.

In the process, the company’s Quantum Fracturing™ combines with electromagnetism and pH modification to break down cell walls, thereby releasing the oil within these cells. Algae oil rises to the top for skimming and refining, while the remaining biomass settles to the bottom for further processing as fuel and other valuable products.

**Single-Step Separation & Extraction Process**



*Figure 27. Mature algae culture is injected through the OriginOil device, where Quantum Fracturing™, electromagnetism and pH modification (using CO<sub>2</sub>) combine to break the cell walls. The processed culture then travels into a settling tank, or gravity clarifier, to fully separate into oil, water and starch-protein biomass (OriginOil News Release, 2009).*

Initial testing indicates that the new algae oil extraction process is simpler and more efficient than current systems, without requiring chemicals or significant capital expenditure for heavy

machinery. Conventional algae oil extraction systems use petrochemical or alcohol solvents to extract algae oil. In this process, the largest amount of energy consumption comes from the initial steps of dewatering and drying the algal biomass. The novel OriginOil [Single Step Extraction](#) technology, however, allows the oil to be separated from the algal biomass in water with no dewatering or drying steps. As a result, substantial electrical and thermal energy can be saved, up to 90% depending on the current system utilized.

In addition to integrating this process into its algae production system, OriginOil plans to rapidly commercialize the patent-pending process for use by others in the rapidly-growing algae industry. On 5 May 2009, OriginOil announced a partnership with [Desmet Ballestra](#), an International leader in development of oil and fats technologies for the food, soaps, detergents, surfactants, biochemical and biofuels industries; and a pioneer in commercial algal oil extraction.

This same separation technology may be utilized for extracting corn oil from germ in conjunction with fractionation of the corn kernel for integrated biorefineries without using capital intensive and environmentally unfriendly hexane separation processes.

### **Conversion of Wet Algal Biomass to Biodiesel**

[Algaen \(an algae nutraceutical company\)](#) and [Quartek \(nanotechnology\) Company](#), both based in Greensboro, NC have developed an innovative technology to convert aqueous algae into biodiesel. Aqueous algal biomass is put through a couple of changes that convert it into a solid. A special powder is then added to the solid, the resulting solution is cooled, and the final product is pure biodiesel. The materials used in the process can then be reused.

While the process for creating the fuel has been perfected, what hasn't been honed is the ability for companies to produce it on a mass scale. This has created a race to see which company will be able to make it cost-effective enough that algae-based biodiesel, or some other new kind of biodiesel, can fuel vehicles on the highway. The companies are currently trying to raise money to determine scalability of this technology, e.g. conducting experiments to see if the same process that works in test tubes will work in 60-gallon tanks. ([WXI12, News Release, July 2009](#))

### ***Hybrid Closed Pond Algal Photobioreactor***

“The [Carbon Algae Recycling System](#) project is currently being designed by [Innoventures Canada](#) (I-CAN), a not-for-profit consortium of 10 Canadian research corporations including the Alberta Research Council, Saskatchewan Research Council and Quebec’s Centre de Recherche Industrielle du Québec and Manitoba’s Industrial Technology Centre.

The objective of the project is to create a technically and economically feasible algae conversion system suitable for Canadian weather conditions that can be integrated into large-final emitters such as power plants, refineries and other major carbon dioxide emitters. By providing an algae-based carbon management tool, I-CAN researchers can offer industries the opportunity to mitigate and convert carbon dioxide into value-added products such as biodiesel, ethanol and fertilizer.

The CARS project feeds low-grade waste heat and industrial flue gas directly into man-made ponds to heat and feed the growth of microalgae, which can be harvested and processed into biofuels and other bioproducts.

Stage one of the project was completed in 2008 and focused on identifying the conditions in which algae strains thrived. Ninety-eight percent of the world's commercial algae facilities use an open-pond system for producing algae, while the remaining 2% use photobioreactors. The CARS project has developed a hybrid system.

CARS researchers are now developing a 90 centimeter (cm) deep, in-ground, covered pond that is subject to an intense lighting system that will penetrate every layer of algae and capture approximately 233,000 metric tons of carbon dioxide per year. While traditional ponds are 30 cm deep, the covered pond system helps maintain a consistent temperature and environment and is able to achieve greater results by optimizing light conditions, lowering capital costs and reducing its overall environmental footprint. CARS researchers will test its concepts at a bench-scale level and then will progressively scale up from a small pond to a large pilot pond as the project develops.

The ultimate goal of the project is to produce a demonstration facility that can grow algae commercially year-round and is integrated with a large emitter.” ([Biomass Magazine, April 2009](#)).

### ***Algae Open Pond System***

In Jul 2009, NASA and [Seambiotic USA](#) partnered on a R&D project to convert flu gases from coal combustion at power generation plants to algal biomass via open-pond systems. The agreement will focus on large scale microalgae optimization. Flue gases are filtered and then CO<sub>2</sub> is released into the pond water. This allows for production of algal biomass with an accelerated growth rate over 10 fold greater than other pond systems. Centrifuges are utilized for separating the algae from the water for nutraceutical production. Less energy intensive separation processes are utilized for production of animal feeds and biofuels. It may be possible for the Seambiotic technology to capitalize on the hybrid closed pond algal photobioreactor being developed in Canada as listed above.

### ***Innovative Biodiesel Production: Mixed Bed Catalytic Converter***

Similar to advances in ethanol production, revolutionary improvements are being made in biodiesel production. Diesel fuels can be displaced with new generations of biodiesel and green diesel fuels produced from algal biomass and other oil feedstocks. This is possible through innovative processing of nutrient-rich organic waste resources, precision agriculture and next generation algal bioreactors. Similar to hydrous ethanol, new biodiesel and “green” diesel biofuels are compatible with current petroleum diesel fuels.

One of these new biorefining technologies has been developed utilizing a continuous mixed bed catalytic reactor for processing a variety of low cost oil and tallow feedstocks. The biodiesel is clean burning and has cold flow, life-cycle, and performance characteristics similar to that of diesel. The biorefining process is substantially less capital and energy intensive than conventional FAME processing and hydroprocessing technologies currently utilized for conversion of bio-oils. These bio-oils are obtained from conventional oil feedstocks, algal biomass and even from crop residues utilizing fast pyrolysis. For every BTU of energy input, 6.7 BTU are produced via the continuous mixed bed catalytic converter. The metal-oxide based catalyst process recycles glycerol, does not require water, and is not affected by free fatty acids (FFAs) or water content of feedstocks ([Mcgyan Biodiesel Process, 2009](#)).



to commercialization in the near term. This announcement adds to the more than \$1 billion DOE has already committed to research, development, and demonstration of cellulosic biofuels technology.

Completed applications are due by April 30, 2009. The projects selected will demonstrate commercial viability for producing advanced biofuels from a variety of biomass conversion technologies and non-food feedstocks. See the [DOE press release](#) and the [full text of the funding opportunity announcement](#) at [www.grants.gov](http://www.grants.gov).

Utilizing the above described technologies, the following table illustrates the potential increase in production for conversion of a 60 MGY ethanol plant to an integrated biorefinery which incorporates grain fractionation and processes the entire corn plant. For integrated biorefineries, pretreatment and lignocellulosic processing of pericarp and endosperm fiber can increase ethanol yields from an industry average of 2.75 to 3.30 gal/bu of corn (123.3 gal/tonne), a 20% increase in ethanol yields for conventional ethanol plants which incorporate grain fractionation. This would be in addition to the increase in ethanol production resulting from processing corn stover. Fiber and germ meal can alternatively be utilized as livestock feeds, particularly for ruminants which utilize microbial populations and exogenous enzymes for efficient digestion of fibrous feedstuffs.

**Table 9. Conversion of a 60 MGY Ethanol Plant to a 182 MGY Integrated Biorefinery**

Product	Energy	Unit	60 MGY Plant	MBTU -HHV	Integrated Biorefinery	MBTU -HHV
Corn Ethanol (2.75-2.9 gal/bu) (.08453 MBTU/gal)	E100	gal	(2.75 gal per bu) 60 MGY	5,071,800	182 MGY	15,384,460
UHG SSF	E100	gal	-----		(2.9 gal/bu) 107 MGY	
Corn Fiber (0.30 gal/bu)	E100	gal	-----		11 MGY	
Corn Stover Ethanol 75% of corn stover @ 90 gal/tonne (72% conversion)	E100	gal	-----		64 MGY	
HE100-93 (hydrogen boosting = .1151724 MBTU/gal)	93% Ethanol, 7% water	gal	64 MGY	7,394,068 Up 46%	194 MGY	22,343,445 Up 46%
HE100-65 (hydrogen reforming = .1139628 MBTU/gal)	65% Ethanol, 35% water	gal	81 MGY	9,230,987 Up 82%	245 MGY	27,920,886 Up 82%
HE100-50 (hydrogen reforming = .1628065 MBTU/gal)	50% Ethanol, 50% water	gal	90 MGY	14,652,585 Up 98%	273 MGY	44,446,175 Up 189%
Food Grade Corn Oil (.130 MBTU/gal)	B100	gal	-----	-----	9.71 MGY	1,262,300
DDGS (18 lbs/bu)	-----	tonnes	178,188		-----	
MDG (16 lbs/bu)	-----	tonnes	-----		269,963	
Stover Residues	-----	tonnes	-----		236,218	
Total Animal Feed	-----	tonnes	178,188		506,181	
Low Cost Sup.	-----	tonnes	178,188		506,181	
Total CAFO AUs			107,596		305,650	

HSAD-CH <sub>4</sub> (15 MBTU/AU)	MBTU	AU		1,613,940		4,584,750
HSAD-CH <sub>4</sub> 45 MBTU per AU, .33 algae and .33 water hyacinth	MBTU	AU plus Biomass		4,841,820		13,754,250
MBTU/hr Required 196 MBTU/hr Conv. 98 MBTU/hr Integrated Biorefinery	MBTU		117.6/hr		179.35/hr	
MCFC (47% electric efficiency: 7.21 MBTU/hr/MW) 8.6 MW/100 MGY for corn ethanol plant 5 MW/100 MGY for Integrated Biorefinery 3.412 MBTU/MW	Power	MW	25.6 gross 20.44 net	610,934	72.0 gross 63.0 net	1,883,015
Heat Recovery (43%: 3.074 MBTU/hr per MW)	Heat	MBTU	78.54/hr		193.66/hr	
MCFC (60% electric efficiency: 5.692 MBTU/hr/MW) 8.6 MW/100 MGY for corn ethanol plant 5 MW/100 MGY for Integrated Biorefinery 3.412 MBTU/MW	Power	MW	28.4 gross 23.4 net	699,405 For HE100-65 or HE100-50?	92.0 gross 82.85 net	2,479,799 For HE100-65 or HE100-50?
Heat Recovery (30%: 1.7078 MBTU/hr per MW)	Heat	MBTU	48.5/hr		157.12/hr	
MCFC-H <sub>2</sub> : 37% electric efficiency: 7.21 MBTU/hr fuel feeds (methane or ethanol) produces .8 MW of power generation	Power	MW	76.66/hr		217.77/hr	
Heat Recovery (30%: 1.34 MBTU of heat per MW)	Heat	MBTU	102.72/hr		291.81/hr	
1200 lbs H <sub>2</sub> per MW (33% of Energy Input) 61,100 BTU/lb H <sub>2</sub> (tons H <sub>2</sub> x 5.66 = tons NH <sub>3</sub> via Haber-Bosch)	H <sub>2</sub>	tons	16,789 H <sub>2</sub> (or 95,138 tons NH <sub>3</sub> )		47,692 H <sub>2</sub> (or 269,937 tons NH <sub>3</sub> )	
FBR-20% lignin (7.362 MBTU/tonne at 30% DM)	-----	tonnes	-----	-----	154,580	1,138,023
3% Ash Fertilizer		tonnes			5,669	
Beef Production (dress weight)		million lb	39.27		111.56	

			60 MGY Plant	MBTU -HHV	Integrated Biorefinery	MBTU -HHV
MCFC Power		MBTU		2,300,030		6,533,753
Thermal Heat		MBTU		1,205,769		2,556,271
H <sub>2</sub> (61,100 BTU/lb)		MBTU		2,048,258		5,827,962
Net Energy (E100)		MBTU		5,682,734		19,667,798
Net Energy (HE100-93)		MBTU		8,005,002		26,626,783
Net Energy (HE100-65)		MBTU		9,930,392		32,204,224
Net Energy (HE100-50)		MBTU		15,263,519		48,729,513

Processing equal volumes of animal waste, algal biomass and water hyacinth would allow for more than tripling biogas production and subsequent DG-CHP for integrated biorefineries. The above technologies utilize proven technologies. For MCFCs operating at 60% electric efficiency, this would be equivalent to producing over 276 MW of grid connected power for conversion of a 60 MGY ethanol plant to an integrated biorefinery. In addition, over 471 MBTU of heat recovery would be produced each hour at 90% total energy efficiency. In conjunction with production and processing of algal and water hyacinth biomass, Table 10 illustrates what is possible by recycling GHGEs and incorporation of CEA (such as hydroponic greenhouses and next generation algal photobioreactors). Integration of CEA and MEC could substantially increase biomass yields per acre while simultaneously producing biohydrogen/biopower and providing carbon credits.

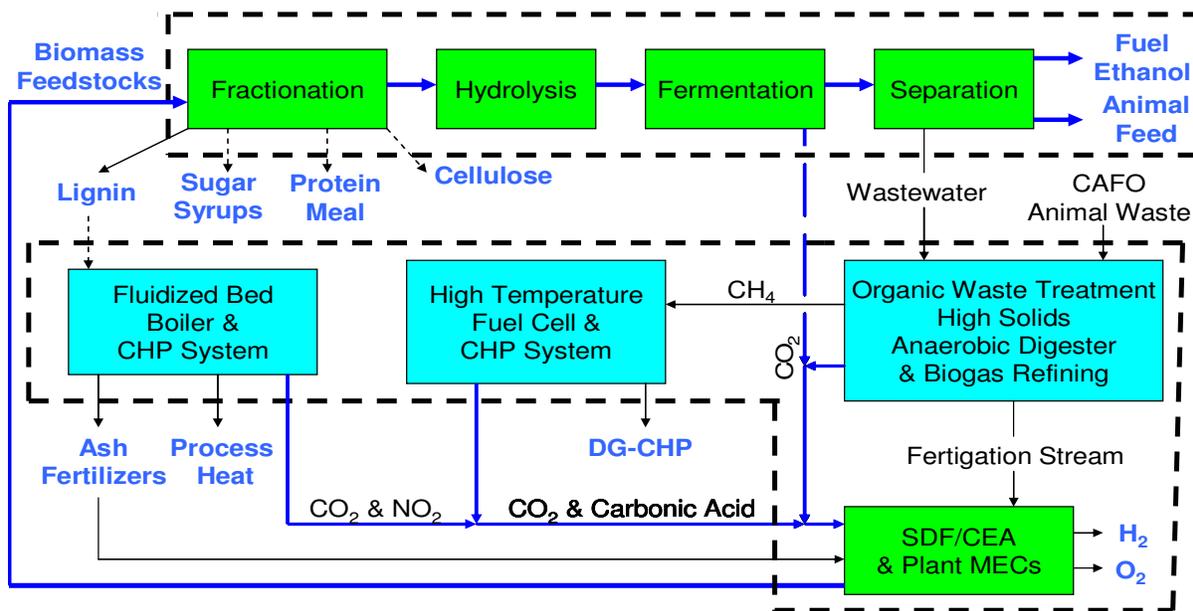
[A new study released by Sandia National Laboratories \(SNL\)](#) and General Motors reveals that the US could produce 90 billion gallons of ethanol per year by the year 2030 (75 billion gallons of cellulosic ethanol and 15 billion gallons from corn); that is 54 billion gallons more than the current federal ethanol mandates aimed for by the year 2022 and roughly 30% of current gasoline usage. US gasoline consumption is expected to reach 180 BGY by 2030. Whereas first generation ethanol has concentrated on food crops that required copious amounts of water and fertilizer in order to be grown, second generation ethanol will rely primarily on agricultural waste and energy crops, which includes switchgrass, corn stover, wheat straw, and woody plants. However, electric hybrids, hydrous ethanol, and direct ethanol fuel cells (DEFCS) will also need to be utilized along with energy conservation in order to eventually eliminate use of fossil fuels.

As illustrated above, hydrous ethanol and hydrogen boosting technology which is available today could increase the energy density of ethanol by 82%. In addition, HE50-HE65 allows for decreasing energy required for separating water and ethanol by up to 80% via MAVS technology. This results in a substantial net energy gain for utilizing hydrous ethanol as a hydrogen-rich energy carrier, particularly for catalytic and plasmatron reforming technologies.

### ***Integrated Biorefinery Emissions Profile***

With the exception of mature FBR technology for combustion of “wet lignin” to provide thermal energy, only biochemical and electrochemical conversion technologies are utilized in the above scenarios. Gasification and fast pyrolysis technologies are not utilized since these technologies are not yet designed to efficiently process “wet biomass” feedstocks. For closed loop production, CO<sub>2</sub> enrichment is utilized to enhance biomass production by over 62% via photosynthesis, employ natural carbon fixation, restore vital oxygen to our environment, and provide year round production utilizing CEA. Optimizing total energy efficiency via fuel cells and recycling waste heat, wastewater, and bioprocessing of organic waste reduce production costs and emissions for integrated biorefineries.

## Integrated Lignocellulosic Biorefinery Emissions Profile



*Figure 31. Recycling up to 80% of CO<sub>2</sub> & NO<sub>x</sub> Emissions via Closed Loop Production*

CO<sub>2</sub> enrichment is also utilized for innovative biohydrogen production via integration of CEA and plant MFCs. Emphasis is on increasing efficiency of corn ethanol production and leveraging existing biorefinery and petroleum distribution infrastructures for production of advanced biofuels. This is accomplished through integration and processing an abundance of “free” organic waste for simultaneous production of food and renewable energy via closed loop systems. This concept provides the most attractive opportunity to eliminate fossil fuels, reduce production costs, implement closed loop production for integrated operations, and reduce GHGEs.

Production of hydrous ethanol containing 7% water can increase total ethanol production by over 6% for integrated biorefineries. Based on the Brazilian experience that 1.2 liters of ethanol can replace 1.0 liters of gasoline, the net contribution to carbon dioxide accumulation by vehicles running on hydrous ethanol is less than about 10% of gasoline ([New Energy Alternative, 2009](#)). For HE100-50, the decrease in CO<sub>2</sub> emissions for ethanol production and vehicle emissions would dramatically decrease.

### ***Integrated Bioenergy Complexes***

Biodiesel and ethanol bioenergy complexes could be integrated. Similar to incorporation of fractionation technologies for corn and biomass, macerators and economic pretreatment technologies for organic wastewater can substantially enhance surface area of volatile solids. This enhances enzymatic processing and microbial digestion which increases organic carbon conversion to methane, enhances solids destruction, and reduces hydraulic retention time (HRT). In bench trials funded by NASA, proprietary biocatalysts have increased solids destruction and methane production by over 25% for mesophilic digestion. Thermophilic systems can reduce HRT by 20%. In addition, thermophilic HSADs can increase total solids destruction from an average of 45% for mesophilic digesters to 65% with a solids loading rate of 45-55%. HSAD

allows for converting up to 90% of organic carbon contained in animal waste, offal, paper waste, food waste, sewage and municipal solid waste (MSW) to biogas containing as much as 80% methane and 20% CO<sub>2</sub>. As illustrated in the following information, optimizing organic waste collection, improving substrate management, and utilizing HSAD can substantially enhance conversion efficiency for production of methane-rich biogas and nutrient-rich biological effluents. This provides the foundation for comprehensive nutrient-energy management and closed loop production via integrated biorefineries.

In addition to the CO<sub>2</sub> produced during fermentation, anaerobic digestion of animal waste would annually produce 93,948 tonnes of CO<sub>2</sub> (.35 CO<sub>2</sub> in biogas x 100 SCF x 250,000 AU x 365 days/15,734.7 SCF per tonne) produced daily in addition to biomethane (80% CH<sub>4</sub> in biogas). This provides a total of 790,382 tonnes of CO<sub>2</sub> from anaerobic digestion, high temperature fuel cells, and that produced during fermentation. Based on a conversion rate of 1.9 tonnes of CO<sub>2</sub> per tonne of algal biomass produced, this would allow for annually producing 1,501,725 tonnes of either algae or plant biomass via photosynthesis and CEA. At 100 SCF produced daily per AU, 2,145,322 tonnes of algae or plant biomass would be produced. This is a 30% increase in production for state-of-the-art thermophilic HSAD technology.

Based on 40% oil, 40% carbohydrates, and 20% protein in algal biomass feedstocks, 1,501,725 tonnes of biomass, this is equivalent to producing about 178 MGY of biodiesel, an additional 68 MGY of ethanol, and 300,345 tonnes of protein feed supplements for livestock production and production of consumer food products. In regards to the potential for utilizing next generation algal photobioreactors, production of lignocellulosic feedstocks such as water hyacinth via hydroponic greenhouses are much closer to being viable for commercial applications.

Annual CO<sub>2</sub> recycled from fermentation of ethanol, anaerobic digestion of organic waste, and from CO<sub>2</sub> contained in fuel cell exhaust for a 100 MGY bioenergy complex is as follows (metric tonnes contain 2,204.62262 lbs; 1.1023 conversion ratio for converting metric tonnes to US short tons).

**Table 10. Carbon Volume & Credits Available via 100 MGY Integrated Biorefinery**

<b>Carbon Credit Source</b>	<b>Volume of Carbon Credits</b>	<b>Trading Value</b>
CO <sub>2</sub> from fermentation of corn biomass	468,900 tonnes	
CO <sub>2</sub> from HSAD of animal waste	116,005 tonnes	
CO <sub>2</sub> from HSAD of algal biomass	closed loop	
CO <sub>2</sub> from FBR	230,130 tonnes	
CO <sub>2</sub> from fuel cell exhaust	257,002 tonnes	
Nitrous oxide credits	776 tonnes	
Total CO <sub>2</sub> and NO <sub>x</sub> credits for recycling	1,072,813 tonnes	
US value via <a href="#">CCX</a> @\$5/metric tonne	1,072,813 tonnes	<b>\$5,364,065</b>
European Union value @\$27 metric tonne	1,072,813 tonnes	<b>\$28,965,951</b>

In addition to capturing CO<sub>2</sub> and nitrous oxides via integrated biorefineries, methane and nitrous oxide emissions would also be reduced for agricultural production. Reduction on these emissions via fuel cells and closed loop systems provide opportunities for Emissions Reduction Credits (ERC).

Production Tax Credits (PTC) for renewable electricity production via closed loop biomass provide \$0.025/kWhr, lignocellulosic ethanol (\$1/gal), biodiesel (\$1/gal), and ethanol blending

(\$0.51/gal) would provide additional incentives and provide additional revenue streams for integrated biorefineries and bioenergy complexes.

After biogas is extracted from animal/organic waste, the biological effluents which remain are rich in nutrients and provide for replenishing soil organic matter (SOM) via closed loop biomass production. Each AU produces enough nutrients to grow approximately 200 bu of corn or 5.6 tons of biomass. Based on feeding 250,000 AU, there is enough nutrients contained in animal waste to produce 50 million bu or 1.27 million tonnes of biomass. This is 142% of the volume of corn grain and stover required to supply the 182 MGY ethanol biorefinery with feedstocks. The additional 13.6 million bu of corn and associated corn stover could increase biorefinery production by 49 MGY of ethanol or the surplus could be utilized to produce corn silage for supplementing animal feeds.

The 13.6 million bu of surplus corn would be equivalent to producing 345,454 tonnes (dry matter) of corn silage or producing substantially larger volumes of water hyacinth or algal biomass (which are much more efficient at utilizing nutrients and faster growing than corn). Biomass production would be in conjunction with CO<sub>2</sub> enrichment via algal photobioreactors and hydroponic greenhouses which provide biomass feedstocks and animal feeds year round. In the example listed below, the surplus corn and triticale bumper crop are ensiled along with other livestock feeds to enhance ruminant digestion and reduce feed purchases for integrated CAFOs. Fertilizer costs can be reduced by up to 80% and water use efficiency can be increased by over 50% by recycling wastewater resources. This includes utilizing thin stillage and process water from integrated biorefineries as nutrient rich water sources for animal production in addition to dilution of animal waste for high rate anaerobic digestion prior to use as value added fertigation streams for SDF, precision agriculture, and CEA applications. When these nutrient rich fertigation streams are utilized along with CO<sub>2</sub> enrichment via CEA applications, biomass yields per acre can be increased exponentially for integrated biorefineries.

For production and processing of lignocellulosic feedstocks such as water hyacinth via hydroponic greenhouses, an additional 101 MGY of ethanol could be produced along with 280 MBTUhr from combustion of lignin-rich fermentation residues via FBR technology. 132,928 tonnes of protein animal feed supplements could also be provided. For production and processing of algal biomass via next generation algal bioreactors, an additional 81 MGY of biodiesel could be produced along with over 450,000 tonnes of starch-protein animal feed supplements. This would allow for increasing the number of AU for the integrated CAFO to 208,349 without purchasing additional feedstuffs. The increase in AU for CAFO operations would increase biogas and organic fertilizer production by 42% and provide closed loop production for integrated biorefineries. In addition, production of biohydrogen via CEA and plant MECs could result in increasing DG-CHP via PAFC applications, providing up to 135 MW and 528 MBTU/hr.

**Table 10. Incorporation of GHGE Recycling via CEA, CO<sub>2</sub> Enrichment & Plant MECs (Additional 81 MGY of Biofuels; 135 MW; 450,000 tonnes starch-protein animal feed)**

<b>Production Scenario</b>	<b>Yields</b>
CO <sub>2</sub> /NO <sub>x</sub> Produced w/o FBR emissions	842,683 tonnes CO <sub>2</sub>
50% Recycled via CEA	429,043 tonnes CO <sub>2</sub>
CEA – CO <sub>2</sub> Enrichment (1.9 tons biomass per ton of CO <sub>2</sub> )	815,182 tonnes

2,448 acres of Hydroponic Greenhouses (333 tonne lignocellulosic biomass [water hyacinth] per acre containing 13% protein) 100 gal/tonne 20% lignin; 30% DM as received 24.54 MBTU/tonne DM; 7.362 MBTU 30% DM 12.5% protein animal feed via fermentation residues	81.52 MGY ethanol 457 MBTU/hr (FBR) 101,898 tonnes protein animal feed 2,179,471 Nm <sup>3</sup> H <sub>2</sub> per day; 8.164 tonnes H <sub>2</sub> produced hourly
MEC: 10% of H <sub>2</sub> Production: 2,448 acres @ 8093.7 m <sup>3</sup> per acre and 1.1 Nm <sup>3</sup> /day/m <sup>3</sup> for organic waste substrates ( <a href="#">Cheng and Logan, 2007a</a> ; <a href="#">Cheng and Logan, 2007b</a> ); 1 Nm <sup>3</sup> H <sub>2</sub> = .19815 lbs H <sub>2</sub> PAFC Power Generation (42% electric efficiency; 90% total energy efficiency) (HHV of H <sub>2</sub> is 61,100 Btu/lb (gross) or 51,623 BTU/lb (net); 3.412 MBTU/MW hr PAFC Heat Recovery (48% of total energy produced) 3.902 MBTU/MW hr	135 MW/hr  528 MBTU/hr 1,849 acres required;
Algal Photobioreactors (441 tonne lignin-free cellulosic biomass per acre; 40% lipids, 30% carbohydrates, 27% protein, and 3% ash minerals) 90% oil extraction, 7.3 lb/gal algal oil	81 MGY biodiesel; 450,000 tonnes starch-protein animal feed for 134,863 AU 1.65-2.2 Million Nm <sup>3</sup> H <sub>2</sub> produced per day; 6.167-8.164 tonnes H <sub>2</sub> produced hourly
MEC: 10% of H <sub>2</sub> Production: 1,849 acres @ 8093.7 m <sup>3</sup> per acre and 1.1 Nm <sup>3</sup> /day/m <sup>3</sup> for organic waste substrates ( <a href="#">Cheng and Logan, 2007a</a> ; <a href="#">Cheng and Logan, 2007b</a> ); 1 Nm <sup>3</sup> H <sub>2</sub> = .19815 lbs H <sub>2</sub> PAFC Power Generation (42% electric efficiency; 90% total energy efficiency) (HHV of H <sub>2</sub> is 61,100 Btu/lb (gross) or 51,623 BTU/lb (net); 3.412 MBTU/MW hr PAFC Heat Recovery (48% of total energy produced) 3.902 MBTU/MW hr	102-135 MW/hr  399-528 MBTU/hr

Pending verification of H<sub>2</sub> production for plant MECs utilizing anaerobic digester effluents vs. processing of raw organic waste, the above calculations for CEA applications are based on only 10% of the volume of biohydrogen produced from bacteria in organic waste.

### ***Emissions Trading & Carbon Credit Revenues***

An early example of an emission trading system has been the SO<sub>2</sub> trading system under the framework of the [Acid Rain Program](#) of the 1990 [Clean Air Act](#) in the US. Under the program, which is essentially a cap-and-trade emissions trading system, SO<sub>2</sub> emissions are expected to be reduced by 50% from 1980 to 2010. Some experts argue that the "cap and trade" system of SO<sub>2</sub> emissions reduction has reduced the cost of controlling acid rain by as much as 80% versus source-by-source reduction.

Trading carbon credits on the Chicago Climate Exchange (CCX), European Union Exchange, or via private trading provides additional revenue streams for integrated operations. The European Union Emission Trading Scheme (or EU ETS) is the largest multi-national, greenhouse gas emissions trading scheme in the world and was created in conjunction with the [Kyoto Protocol](#). It is currently the world's only mandatory carbon trading program.

Total Volume & Annual Revenue for trading Carbon Credits not including reductions of methane or nitrous oxide emissions for agricultural production:

- *25 MW Coal Power Plant Comparison to Fuel Cell & BioEnergy Complex*: 282,399 metric tonnes CO<sub>2</sub> x \$5 = approximately \$1,129,596 in annual revenue is possible via trading carbon credits on the Chicago Climate Exchange (CCX) for an Ethanol

BioEnergy Complex. At \$27/tonne on the EU ETS, \$7,624,773 in annual revenue is possible.

- *25 MW Natural Gas Power Plant Comparison to Fuel Cell & BioEnergy Complex:* 260,760 metric tonnes CO<sub>2</sub> x \$5 = approximately \$1,303,801 in annual revenue is possible via trading carbon credits on the CCX for an Ethanol BioEnergy Complex. At \$27/tonne on the EU ETS, \$7,040,520 in annual revenue is possible.

Reduction on these emissions via fuel cells and closed loop biomass systems provide opportunities for obtaining Emissions Reduction Credits (ERC) which further offset costs for installing high temperature fuel cells. At \$666,667 per MW, this would be equivalent to an ERC of \$16,666,667 for a 25 MW fuel cell power plant, and an ERC of \$48,000,024 for the 72 MW power plant listed above via conversion of a 60 MGY ethanol plant to a 182 MGY integrated biorefinery. This ERC would be in addition to ITCs and PTCs provided for anaerobic digestion, biofuels and the \$25/MWhr for closed loop biomass production. Additional ERCs, RECs, and trading carbon credits can be implemented for reduction of GHGs and production of renewable energy.

An ERC is a credit granted upon request by an emission source who voluntarily reduces emissions beyond required levels of control. An ERC represents the legal ability to emit regulated pollutants in an amount equal to the quantity specified when the ERC was granted. ERCs may be sold, leased, banked for future use, or traded in accordance with applicable regulations established by SWCAA. ERCs are intended to provide an incentive for reducing emissions below required levels, and to establish a framework to promote a market based approach to air pollution control.

A Certified Emission Reduction (CERs) is the technical term for the output of Clean Development Mechanism (CDM) projects, as defined by the Kyoto Protocol. A unit of Greenhouse Gas reductions that has been generated and certified under the provisions of Article 12 of the Kyoto Protocol, the Clean Development Mechanism (CDM). In contrast, Emissions Reduction Units (ERUs) are used for Joint Implementation (JI) under Article 6 of the Protocol. According to Article 12, CERs must be "certified by operational entities to be designated by the Conference of the Parties (COP) serving as the Meeting of the Parties (MOP)".

One Renewable Energy Credit or "REC" represents one megawatt hour (MWh) of renewable energy that is physically metered and verified from the generator, or the renewable energy project. "Renewable Energy Credits" are created when a Renewable Energy project is certified and begins producing renewable energy. Renewable energy projects create green power which helps reduce pollution. Renewable Energy Credits are the group of environmental benefits society benefits from in the production of green power. The green-power (electricity) is sold into the local electric grid where the renewable energy project is located. The REC's are sold separately as a commodity into the marketplace.

### ***Economic Stimulus Act Extends Renewable Energy Tax Credits***

The tax section of the American Recovery and Reinvestment Act of 2009 provides a three-year extension of the production tax credit (PTC) for most renewable energy facilities, while offering expansions on and alternatives for tax credits on renewable energy systems. The extension keeps the wind energy PTC in effect through 2012, while keeping the PTC alive for municipal solid

waste, qualified hydropower, and biomass and geothermal energy facilities through 2013. In addition, a two-year extension of the PTC for marine and hydrokinetic renewable energy systems will keep that tax credit in effect through 2013. The PTC provides a credit for every kilowatt-hour produced at new qualified facilities during the first 10 years of operation, provided the facilities are placed in service before the tax credit's expiration date. For 2008, biomass facilities fueled with dedicated energy crops ("closed-loop biomass"), as well as wind, solar, and geothermal energy facilities earned 2.1 cents per kilowatt-hour, while other qualified facilities earned 1 cent per kilowatt-hour. See pages 33-34 of the American Recovery and Reinvestment Tax Act of 2009 ([PDF 5.9 MB](#)), as well as PDF pages 105-112 (labeled as pages 103-110) of the accompanying joint explanatory statement of the conference committee ([PDF 24.9 MB](#)). [Download Adobe Reader.](#)

Unfortunately, the current slump in business activity means that fewer businesses are seeking tax credits, which means that renewable energy producers are having trouble taking advantage of the PTC. With that in mind, the act also allows owners of non-solar renewable energy facilities to make an irrevocable election to earn a 30% investment credit rather than the PTC. The option remains in effect for the current period of the PTC, that is, through 2012 for wind energy facilities and through 2013 for other qualified renewable energy facilities. See pages 34-36 of the American Recovery and Reinvestment Tax Act of 2009, as well as PDF pages 112-113 of the joint explanatory statement of the conference committee.

Alternately, the facility owner could choose to receive a grant equal to 30% of the tax basis (that is, the reportable business investment) for the facility, so long as the facility is depreciable or amortizable. The grants are also available for renewable energy facilities that would normally earn a business energy credit of 10%-30%, including systems using fuel cells, solar energy, small wind turbines, geothermal energy, microturbines, and combined heat and power (CHP) technologies. To earn a grant, the facility must be placed in service in 2009 or 2010, or construction must begin in either of those years and must be completed prior to the termination of the PTC. For facilities that would normally earn a business tax credit, construction must be completed prior to 2017. The grants will be paid directly from the U.S. Treasury. A separate measure in the act removes limitations on the business credit based on how the systems are financed and also removes a business credit limit on small wind energy systems. See pages 36-39 and 153-158 of the American Recovery and Reinvestment Tax Act of 2009, as well as PDF pages 113-117 of the joint explanatory statement of the conference committee.

### ***Clean Renewable Energy Bonds Expanded by the Economic Stimulus Act***

Two bonding mechanisms for financing renewable energy and energy efficiency systems have been expanded under the tax section of the American Recovery and Reinvestment Act of 2009. The act authorizes the allocation of as much as \$1.6 billion in new Clean Renewable Energy Bonds (CREBs), which are tax credit bonds for financing renewable energy projects. CREBs were previously limited to a maximum of \$800 million. The act also authorizes the allocation of \$2.4 billion in qualified energy conservation bonds, up from the current limit of \$800 million. These tax credit bonds are allocated to states and large local governments to finance a variety of clean energy projects.

Unlike normal bonds that pay interest, tax credit bonds pay the bondholders by providing a credit against their federal income tax. In effect, the new tax credit bonds will provide interest-free financing for clean energy projects. But because the federal government essentially pays the

interest via tax credits, the US Internal Revenue Service must allocate such credits in advance. However, tax credit bonds require the investment of a bondholder that will benefit from the federal tax credits, and those investors may be hard to find during the current business downturn. To try to draw more investment, a separate measure in the tax bill will allow regulated investment companies to pass through to their shareholders the tax credits earned by such bonds. Yet another measure adds a prevailing wage requirement to projects financed with CREBs or energy conservation bonds. See pages 39-41 and 143-149 of the American Recovery and Reinvestment Tax Act of 2009 ([PDF 5.9 MB](#)), as well as PDF pages 100-101, 118-123, and 148 of the accompanying joint explanatory statement of the conference committee ([PDF 24.9 MB](#)).

### **Biomass Research & Development Initiative**

On 30 Jan 2009, the U.S. Departments of Energy (DOE) and Agriculture (USDA) announced up to \$25 million in funding for research and development of technologies and processes to produce biofuels, bioenergy, and high-value biobased products, subject to annual appropriations.

“These projects will be among many Obama Administration investments that will help strengthen our economy and address the climate crisis. A robust biofuels industry – focused on the next generation of biofuels – is critical to reducing greenhouse gas emissions, reducing our addiction to foreign oil and putting Americans back to work,” said Secretary of Energy Steven Chu.

“USDA is committed to research that will support President Obama’s goal of dramatically increasing the production of biofuels in the United States,” said Agriculture Secretary Tom Vilsack. “These grants will help support the development of a sustainable domestic biofuels industry by broadening the nation's energy sources as well as improving the efficiency of renewable fuels.”

USDA and DOE are issuing this joint funding opportunity announcement (FOA) for several types of projects aimed at increasing the availability of alternative renewable fuels and biobased products. The projects will aim to create a diverse group of economically and environmentally sustainable sources of renewable biomass. Advanced biofuels produced from these types of sources are expected to reduce greenhouse gas emissions by a minimum of 50%. The FOA will fund projects in the following three technical areas specified in the Food, Conservation, and Energy Act (FCEA) of 2008:

- Feedstocks development
- Biofuels and biobased products development
- Biofuels development analysis

Award amounts are planned to range from \$1 million to up to \$5 million with project periods up to four years, subject to annual appropriations. Eligible applicants include institutions of higher education, national laboratories, federal research agencies, state research agencies, private sector entities, non-profit organizations, or a consortium of two or more of those entities.

The FOA is available online at [www.grants.gov](http://www.grants.gov). The closing date for pre-applications is March 6, 2009, which must be submitted electronically. A minimum recipient cost-share of at least 20% of total project cost for research and development projects and 50% of total project cost for demonstration projects is required.

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