

Cultivation of black soldier fly larvae on almond byproducts: impacts of aeration and moisture on larvae growth and composition

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Abstract

BACKGROUND: The increasing production of almonds worldwide has resulted in the significant generation of byproduct streams that require end uses. One potential use for byproducts is for cultivation of additional food sources including insects. Studies were performed to determine if black soldier fly larvae (*Hermetia illucens* L.) could be cultivated on almond byproducts (hulls and shells) and to examine the effect of aeration and moisture on larvae growth and hull composition.

RESULTS: Increasing aeration from 0.04 to 0.36 mL min⁻¹ g dry weight⁻¹ tripled the harvest weight of larvae and increased larvae yield by a factor of five. Larvae calcium content increased by 18% with an increase in aeration from 0.04 to 0.95 mL min⁻¹ g dry weight⁻¹. Moisture content also affected harvest dry weight and yield; increasing moisture content from 480 g kg⁻¹ (wet basis) to 680 g kg⁻¹ increased harvest weight by 56% and yield by a factor of 2. Variables did not affect larvae methionine and cysteine content. Low moisture content and aeration rate yielded an environment that supported microbial consumption of hulls over larvae consumption and growth.

CONCLUSIONS: The results demonstrate that almond hulls are a suitable feedstock for larvae production under controlled management of moisture content and aeration.

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INTRODUCTION

Almond production in the USA has increased by 62% in the last 10 years due, in part, to global consumer demands for plant-based food sources.¹ Production of almonds in 2016 was nearly 1 million metric tons.² The cultivation and processing of almonds results in byproduct streams that include hulls and shells: on average 1 kg of almonds yields 2 kg hulls and 1 kg shells.³ While hulls can be used for stockfeed,⁴ in California livestock production was largely flat between 2006 and 2015 while almond yields grew by approximately 14% during the same period.⁵ Furthermore, some byproduct streams such as shells are not suitable for animal feed due to the cellulose and lignin content in shells. As almond production continues to grow and as policies are implemented to mandate the diversion of organic waste from landfills,⁶ it is critical that alternative uses of byproducts be developed to ensure almond production systems are environmentally sustainable.

There have been several studies examining the use of byproducts as alternatives or supplements to livestock feed. These have included ethanol production from hull sugars, anaerobic digestion to biogas, and as a nutrient supplement in soil.^{7–10} Another potential use of byproducts is for the cultivation of insects.¹¹ Interest in

insect production has grown recently due to concerns regarding the sustainability of feed sources for fish and poultry.^{12–15} There is also growing evidence that current methods of livestock production will be incompatible with the natural resources needed to support both livestock and a projected 2050 world population of 9 billion people.¹⁶ Insects have nutritional quality that is similar to, or exceeds, existing sources of protein and can use less water, land, and energy to produce.^{13,14,17,18} There are also many types of insects that can grow on organic wastes suggesting further environmental benefits.^{19–25} From a sustainability perspective, the

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most promising feedstocks for insect cultivation are those that originate from low-value food processing residues.¹⁸

Black soldier fly (BSF) larvae (*Hermetia illucens* L.) have potential as a substitute for common commercially available protein sources^{13,26,27} and there have been several reports on the cultivation of black soldier fly larvae (BSFL) on organic wastes. For example, Nguyen *et al.* (2015) observed that BSFL could be grown on a variety of organic waste streams including pig manure, kitchen waste, fruits, and vegetables, and rendered fish.¹⁹ In their study, kitchen waste yielded the heaviest BSFL compared to the control (poultry feed) and other wastes while fruits and vegetables had the greatest level of waste consumption by BSFL. Diener *et al.* studied the feasibility of growing BSFL on municipal organic waste²⁸ and demonstrated a prepupae production rate of 252 g m⁻² day⁻¹ (wet weight) and waste reduction ranging between 65.5% and 78.9%.

Composting of vegetable and food waste produces high amounts of a liquid fraction called leachate, which is high in nutrients and organic compounds. Popa and Green studied the cultivation of BSFL on leachate²⁵ and reported that systems containing BSFL reduced the chemical oxygen demand in leachate to a greater extent than systems without larvae. The system with BSFL neutralized leachate acidity and removed volatile organic acids, amines, and alcohols.

Sprangers *et al.* (2017) measured the protein and amino acid content of larvae fed on chicken feed, vegetable waste, biogas digestate, and restaurant waste.²³ They observed that the protein content of prepupae varied between 399 and 431 g kg⁻¹ dry matter among the substrates. The amino acid profiles and mineral composition of the prepupae also varied between the substrates. Their results suggested that BSFL produced on organic waste could be a source of protein, essential amino acids, and minerals; however, further work would be needed to understand the impact of waste composition on BSFL production and quality to ensure provision of a consistent product.

There are reports of BSF larvae production on a variety of wastes; none has considered byproducts from the almond industry. In addition, very few have directly examined the impact of managing aeration and moisture content on cultivation. The main objective of this research was to begin to determine the effect of engineering variables on the cultivation of BSF larvae and the adjunct decomposition of almond byproducts. A second objective was to test the impact of cultivation variables on larvae composition relevant to poultry feed, namely the methionine, cysteine, and calcium content in larvae, to explore their potential use as a feed supplement for poultry layers. Our results show that both aeration and moisture content can have significant effects on larvae production and the decomposition of hulls, and suggest there are opportunities for engineering the larvae production environment to achieve improvement in larvae growth and conversion of organic wastes to insect biomass. We also demonstrate that almond hulls can be used as a feedstock for insect production with proper management of aeration and moisture.

EXPERIMENTAL

Acquisition and processing of almond hull feedstock

A pollinator variety of almond hulls and shells (hereafter referred to as hulls) was obtained from a processor in Chico, CA, USA, in June 2017, and used as larvae feedstock. Prior to acquisition, the material had been stored outdoors under a roof for approximately 6 months. The hulls and shells were ground using a hammer

mill with a 6.35 mm screen and then stored in airtight plastic bags. The as-received moisture content of the material was 18% (dry basis). Composition analyses were done by IEH Laboratories in Modesto, CA, USA, and methionine and cysteine content were measured at the VetMed Amino Acid Testing Laboratories (Davis, CA, USA) using an AOAC official method.⁴¹ The calcium, total sugar, total starch, total nitrogen, total carbon, acid detergent fiber, neutral detergent fiber, and acid detergent lignin were measured by JL Analytical Services Inc (Modesto, CA, USA). Calcium was measured using EPA Method 6010C through inductive coupled plasma-atomic spectrometry.²⁹ Total sugar was measured using AOAC 980.13.³⁰ Total starch was determined using an enzymatic-colorimetric method.³¹ Total nitrogen was measured using AOAC 990.03 through sample combustion, which converts all organic and inorganic substances into combustion gases and detected using gas chromatography.³⁰ Total carbon was measured using AOAC 993.13.³² Neutral detergent fiber was measured using NFTA 5.1, where a neutral detergent solution is used to dissolve pectin, protein, sugars, and lipids separating the fibers of cellulose, hemicellulose and lignin.^{33–35} The acid detergent fiber and acid detergent lignin were measured using AOAC 973.18 through acid titration and alkaline titration methods.³⁶ The material was reported to have 0.48 g kg⁻¹ (dry weight basis) methionine, 0.72 g kg⁻¹ cysteine, 2.6 g kg⁻¹ calcium, 143 g kg⁻¹ total sugar, 560 g kg⁻¹ total starch, 6 g kg⁻¹ total nitrogen, 434 g kg⁻¹ total carbon, 253 g kg⁻¹ acid detergent fiber, 370 g kg⁻¹ neutral detergent fiber, and 86 g kg⁻¹ acid detergent lignin.

Cultivation bioreactors

Bioreactors, approximately 750 mL in volume, were constructed using 6 mil clear poly tubing with a diameter of 25.4 cm (Four Star Plastics, Beltsville, MD) and included Teflon luer lock fittings, O-rings, and securing nuts at the inlet and outlet for air supply and exhaust (Fig. 1). Poly tubing was secured to the inlet and exhaust using luer lock fittings. To ensure uniform forced aeration through the bed of feedstock, the aeration inlet fitting was joined by PVC tubing with a 6.4 mm inner diameter (Nalgene 180 Clear PVC Tubing, Thermo Fisher Scientific, Waltham, MA) connected to a 6.35 mm plastic tee with 6.4 mm diameter black porous soaker drip line with securing nuts at both ends. To prevent larvae from escaping through the exhaust, a filter was constructed using a 19.1 mm segment of poly tubing with porous foam inserted into the end. Bioreactor ends were sealed using a vacuum heat sealer (FoodSaver FM2000-FFP, Newell Brands, Hoboken, NJ, USA).

BSF larvae rearing

Approximately 1500 mL bioreactors with four exhaust ports were used to rear larvae. Porous stones were placed on exhaust ports to prevent small larvae from escaping. Eggs were purchased from Symptom Black Soldier Fly (College Station, TX, USA). Eggs and larvae (from eggs that hatched during shipment) were received deposited onto corrugated cardboard slabs. Slabs were placed inside bioreactors on a layer of chicken feed (Purina Premium Poultry Feed Layena Crumbles, Purina Animal Nutrition LLC, Shoreview, MN, USA) at a moisture content between 450 and 500 g kg⁻¹ wet basis. Larvae-rearing reactors were supplied with house compressed humidified air at 40 mL min⁻¹ and incubated at 28 and 30 °C for 5–10 days. Prior to inoculation onto hulls, larvae were manually separated from feed and weighed. Samples of larvae were collected for moisture content measurement and average weight.

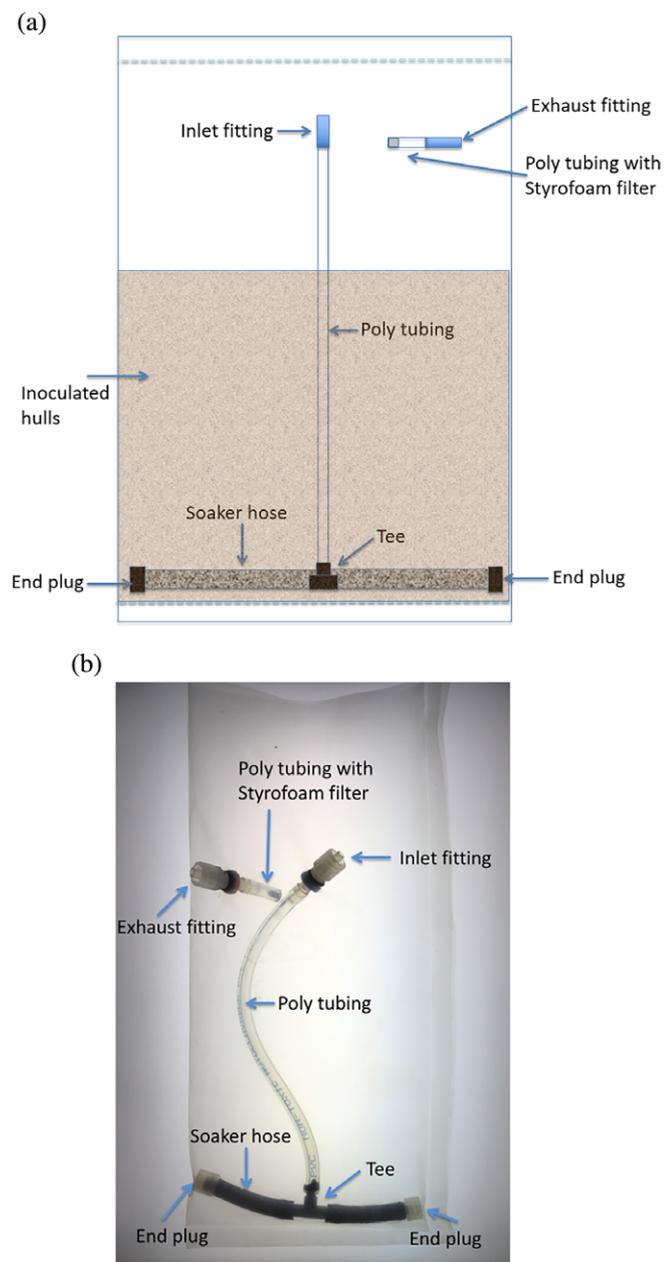


Figure 1. Schematic and picture of a cultivation bioreactor.

Feedstock preparation and incubations

Feedstock was amended with distilled water and nitrogen prior to incubation. Urea was added to distilled water to achieve a target carbon to nitrogen (C/N) ratio of 29 in the wetted material. The mixtures were allowed to equilibrate at 4 °C overnight and three random samples were collected to measure pH and moisture content. Table 1 lists the C/N ratio, moisture content, aeration rate, inoculation density of larvae and average larvae size for each experiment. The C/N ratio and moisture content ranges were selected because these were within the levels known to be ideal for the aerobic decomposition of plant residues by microorganisms.^{37,38}

Five different aeration rates of 0.04, 0.08, 0.36, 0.65 and 0.95 mL min⁻¹ g dry weight⁻¹ were tested to determine the effect of increasing aeration rate on larvae growth, composition, yield, and hull consumption, and to perform statistical comparisons

Table 1. Parameters for experiments

Variables	Aeration experiment	Moisture experiment
Carbon to nitrogen ratio	29	29
Moisture content (g kg ⁻¹ wet basis)	580	480–680
Inoculation density (g dry weight larvae kg dry weight hulls ⁻¹)	2.4	1.5
Average larvae weight in inoculum (mg dry weight/larvae)	2.5	0.57
Aeration rate (mL min ⁻¹ g dry weight ⁻¹)	0.04–0.95	0.63
Incubation temperature (°C)	26	28

of larvae growth, yield, and hull consumption at set aeration rates. Three replicate bioreactors were prepared for each of the aeration-rate treatments except for 0.95 mL min⁻¹ g dry weight⁻¹, which only had two bioreactors due to a faulty airflow meter, which did not supply adequate airflow.

For the moisture experiment, six different initial feedstock moisture contents of 480, 550, 570, 590, 620, 660 and 680 g kg⁻¹ (wet basis) were tested in individual bioreactors to determine if increasing moisture content had an impact on larvae growth, composition and yield, and hull consumption. The moisture contents tested were equivalent to 630, 720, 750, 780, 820, 870, and 890 g kg⁻¹ of the hull fiber saturation point, respectively. Each level of moisture content tested had one bioreactor incubated with larvae and hulls and one bioreactor with hulls and no larvae.

Larvae were cultivated with constant humidified air and temperature. Reactors were incubated at 26–28 °C and aerated using house compressed air. Air was humidified by bubbling it through distilled water and metered to each bioreactor with polycarbonate rotameters (5 to 50 mL min⁻¹, Dwyer Instruments, Michigan City, IN). The cultivation studies ran for 14 days.

Larvae harvest and analysis

At the end of the experiments larvae were manually separated from feedstock, counted, rinsed with distilled water, and allowed to dry for 2 h in a beaker with paper towels. Total larvae wet weight was recorded. Larvae were stored at –20 °C and then freeze dried (Labconco 4.5 Liter Freeze Dry System, Marshall Scientific, Hampton, NH) for 3 to 5 days. To prepare freeze-dried larvae for analysis, they were frozen in liquid nitrogen and homogenized with an oscillating ball mill (MM400, Retsch Inc., Newtown, PA, USA). The calcium content of milled larvae and the moisture content were measured at the UC Davis Analytical Laboratories (Davis, CA, USA) using nitric acid digestion^{39,40} and vacuum drying, respectively. Methionine and cysteine content were measured at the VetMed Amino Acid Testing Laboratories (Davis, CA) using an AOAC official method.⁴¹ Average larvae weight at harvest was calculated by dividing the total dry weight of larvae by the number of larvae harvested.

Spent hull analysis

At the end of each experiment, samples of spent hulls were analyzed for moisture content gravimetrically by drying samples at 101–105 °C in a convection oven for 24 h. To test for pH, 3 g of spent hulls and 27 mL distilled water were added to a 50 mL polypropylene tube (Sarstedt Inc., Sparks, NV). The mixture was shaken for 1 h (MaxQ 2000, Thermo Fisher Scientific, Waltham, MA),

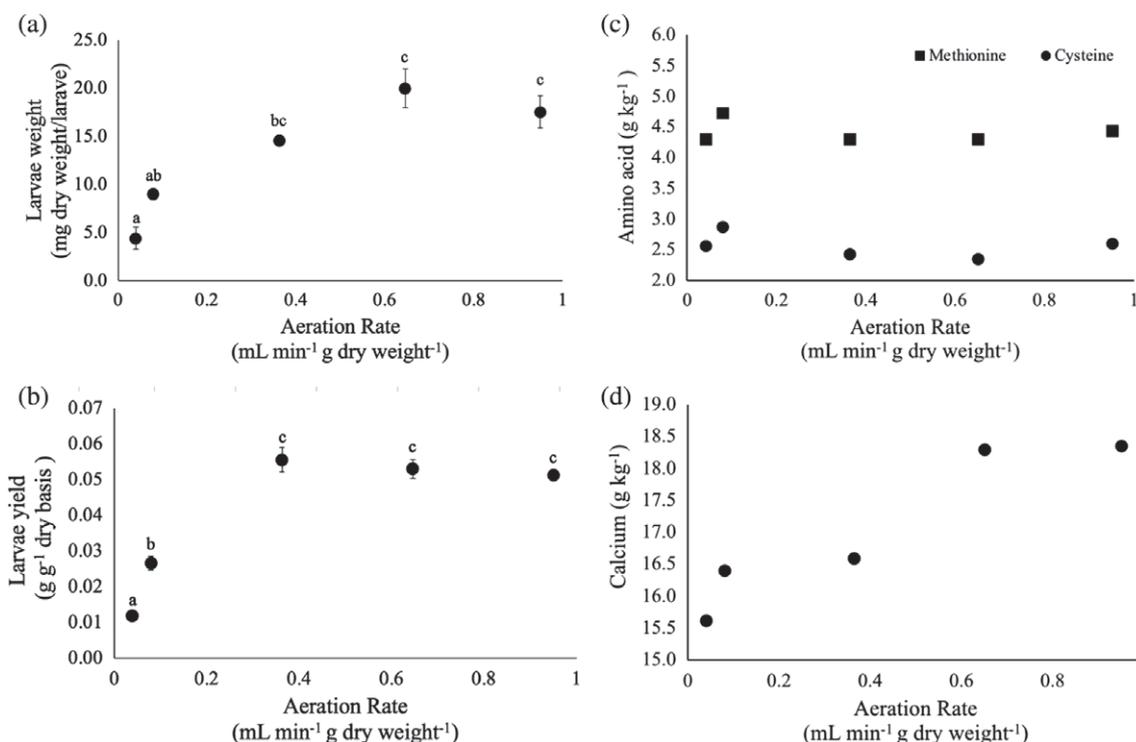


Figure 2. Larvae (a) harvest dry weight, (b) yield, (c) methionine and cysteine content, and (d) calcium content after cultivation on almond hulls for 14 days at varying aeration rates. For harvest dry weight and yield, each point represents the mean harvest weight from three bioreactors with the exception of 0.95 mL min⁻¹ g dry weight⁻¹, which only had two bioreactors. Different letters above points indicate significant differences between treatments ($P < 0.05$). For amino acid and calcium content each point represents analysis of larvae pooled from three bioreactors with the exception of the treatment at 0.95 mL min⁻¹ g dry weight⁻¹ which consisted of pooled larvae from two bioreactors.

and then the supernatant was collected to measure pH (Accumet model 20, Thermo Fisher Scientific, Waltham, MA, USA).

Data analysis

The larvae yield was calculated as the change in larvae dry mass per change in hull dry mass (Eqn (1)). The hull consumption was calculated as the change in hull dry mass per initial hull dry mass (Eqn (2)).

$$\text{yield} = \frac{\text{final larvae dry weight} - \text{initial larvae dry weight}}{\text{initial hull dry weight} - \text{final hull dry weight}} \quad (1)$$

$$\% \text{hull consumption} = \frac{\text{initial hull dry weight} - \text{final hull dry weight}}{\text{initial hull dry weight}} \times 100\% \quad (2)$$

Responses for yield, hull consumption, larvae dry weight at harvest and final larvae composition were analyzed as a function of moisture content and aeration. Linear and nonlinear regression analyses and Tukey's HSD tests were performed using JMP-IN software (version Pro 12, SAS, Cary, NC, USA). The significance level was set at 0.05. Larvae weight at harvest and yield as a function of aeration rate were fit to a logistic model (Eqn 3).

$$y = \frac{M}{1 + e^{-k(x-x_0)}}$$

where, M = predicted maximum value of response, k = slope of curve, x_0 = inflection point of sigmoid.

RESULTS AND DISCUSSION

Aeration

Increasing aeration from 0.04 to 0.36 mL min⁻¹ g dry weight⁻¹ tripled the harvest dry weight of larvae (Fig. 2(a)) and increased yield by a factor of five (Fig. 2(b)). Increasing aeration beyond 0.36 mL min⁻¹ g dry weight⁻¹ did not significantly change either the harvest weight of larvae or yield ($P > 0.05$). Nonlinear regression of the growth and yield data as a function of aeration rate showed a very good fit to a logistic model (Table 2) and estimated maximum values for harvest dry weight and yield as 19.1 mg/larvae and 0.0054, respectively. The models predicted that 95% of the maximum larvae harvest weight and yield would be achieved when aeration is 0.57 and 0.05 mL min⁻¹ g dry weight⁻¹, respectively. Differences in these predicted values were due to the contribution of hull consumption, which also varied as a function of aeration, to yield.

Average methionine and cysteine contents of harvested larvae were 4.7 and 2.8 g kg dry⁻¹, respectively (Fig. 2(c)). Increasing aeration rate did not significantly affect the content of either amino acid in larvae based on linear regression of each amino acid content as a function of aeration rate ($P > 0.05$). Linear regression of the larvae calcium content as a function of aeration rate identified a significant first-order effect of aeration rate on calcium content ($P < 0.05$); levels increased from 16 g kg dry⁻¹ at an aeration rate of 0.04 mL min⁻¹ g dry weight⁻¹ to 18 g kg dry⁻¹ at 0.95 mL min⁻¹ g dry weight⁻¹ (Fig. 2(d)).

During the 14-day experiment, hull consumption averaged 29% of the dry weight for aeration treatments between 0.08 and 0.95 mL min⁻¹ g dry weight⁻¹; however, consumption was significantly lower ($P < 0.05$) for the lowest aeration rate of 0.04 mL min⁻¹ g dry weight⁻¹ where consumption was approximately

Table 2. Parameters from nonlinear regression of larvae specific growth and yield data to a logistic model (Eqn (3))

Independent variable	Parameter, units	Estimated value (standard error)	R ²
Larvae harvest weight, mg dry weight/larvae	M, mg/larvae	19.1 (1.5)	0.97
	k, mL ⁻¹ min g dry	6.9 (2.3)	
	x ₀ , mL min ⁻¹ g dry ⁻¹	0.150 (0.048)	
Larvae yield, unitless	M, unitless	0.0536 (0.0009)	0.98
	k, mL ⁻¹ min g dry	31.7 (5.9)	
	x ₀ , mL min ⁻¹ g dry ⁻¹	0.079 (0.004)	

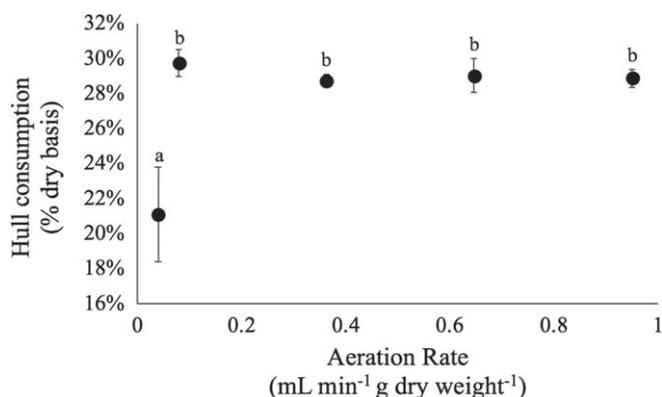


Figure 3. Mean hull consumption after larvae cultivation on almond hulls for 14 days at varying aeration rates. Each point represents the mean hull consumption associated with three bioreactors with the exception of 0.95 mL min⁻¹ g dry weight⁻¹, which only had two bioreactors. Different letters above points indicate significant differences between treatments ($P < 0.05$).

21% (Fig. 3). The pH of the residual hull biomass ranged between 8.17 and 8.45 (Table S1) and was not significantly affected by aeration rate based on linear regression of the results as a function of aeration rate ($P > 0.05$).

To the best of our knowledge, there are no published reports on the direct impact of aeration on the growth of BSF larvae. However, studies on the growth of other insect larvae as a function of substrate bed depth have been done.⁴² In these prior studies, larvae obtained oxygen through migration and via oxygen diffusion from the bed surface; larvae migrated to a particular substrate depth during cultivation to access oxygen. The authors noted that while larvae migrate to obtain nutrients for growth, the anaerobic conditions that likely occurred with increasing depth deterred larvae migration as bed depth increased. Anaerobic conditions in larvae cultivation systems arise when oxygen consumption rate by larvae and microorganisms exceeds oxygen transport by diffusion or forced aeration (this study). In the present study, larvae harvest weight decreased when aeration fell below 0.36 mL min⁻¹ g dry⁻¹; however, hull consumption was only affected when aeration dropped below 0.08 mL min⁻¹ g dry⁻¹. The results indicate the presence and activity of microorganisms in the cultivation environment that consumed resources. The lower harvest weight of larvae at the two low aeration rates tested was likely due to a combination of the low oxygen supply and oxygen consumption associated with microbial activity. The results highlight the importance of considering both larvae and microorganism growth in the provision of oxygen during BSF larvae cultivation.

Larvae calcium content increased with increasing aeration. Black soldier fly larvae calcium content has been reported to vary with growth substrate.²³ In the present study, hull composition likely varied over the course of the experiment, with soluble sugars consumed early during cultivation followed by starch and hemicellulose. The consumption of growth substrate by microorganisms may have altered the substrate composition and/or release of calcium from hulls, and impacted larvae uptake of calcium. Work is needed to further elucidate the effects of growth substrates and cultivation conditions on larvae composition.

The much lower hull consumption at the lowest aeration tested may have been due to oxygen limitations to both larvae and microorganisms. This observation is consistent with other research that has investigated the impact of oxygen concentration on aerobic decomposition rates. Richard *et al.* observed decreasing decomposition rates of food waste as gas-phase oxygen concentration dropped below 2%.⁴³ While oxygen was not measured directly in our experiments, the trends in decomposition suggest that it was the limiting nutrient in the lowest aeration treatment.

Moisture content

The as-received, wet-basis moisture content of the hulls was 15.5%, so water amendment of hulls was imperative for the growth of larvae and for the biochemical reactions necessary for the breakdown of hulls by microorganisms and larvae. Experiments were done to examine the growth of larvae and the consumption of hulls by larvae and microorganisms as a function of moisture content. These experiments included incubations with and without larvae to measure hull consumption by microorganisms alone and to determine if larvae enhance the breakdown of hulls. Increasing the initial hull moisture content from 480 to 680 g kg⁻¹ wet basis increased the harvest dry weight from 5.8 to 8.7 mg larvae⁻¹ (Fig. 4(a)) and yield from 0.05 to 0.11 g g⁻¹ (Fig. 4(b)). Linear regression of the harvest dry weight and yield data as a function of moisture content showed significant first-order effects of moisture content (Table 3) for both larvae harvest weight and yield.

Average methionine and cysteine contents of harvested larvae were 5.2 and 3.1 g kg dry⁻¹, respectively (Fig. 4(c)) and the average calcium content was 25.3 g kg dry⁻¹ (Fig. 4(d)). There was no significant effect of increasing moisture content on the content of either amino acid or calcium in larvae based on linear regression of the results as a function of moisture content ($P > 0.05$).

Hulls were consumed by microorganisms and larvae (Fig. 5), and reactors without larvae showed visible evidence of fungal growth (data not shown). On average hull consumption was greater in incubations that contained larvae than in incubations without larvae; the average difference between hull consumption with and without larvae was 3.7%. The consumption of hulls significantly

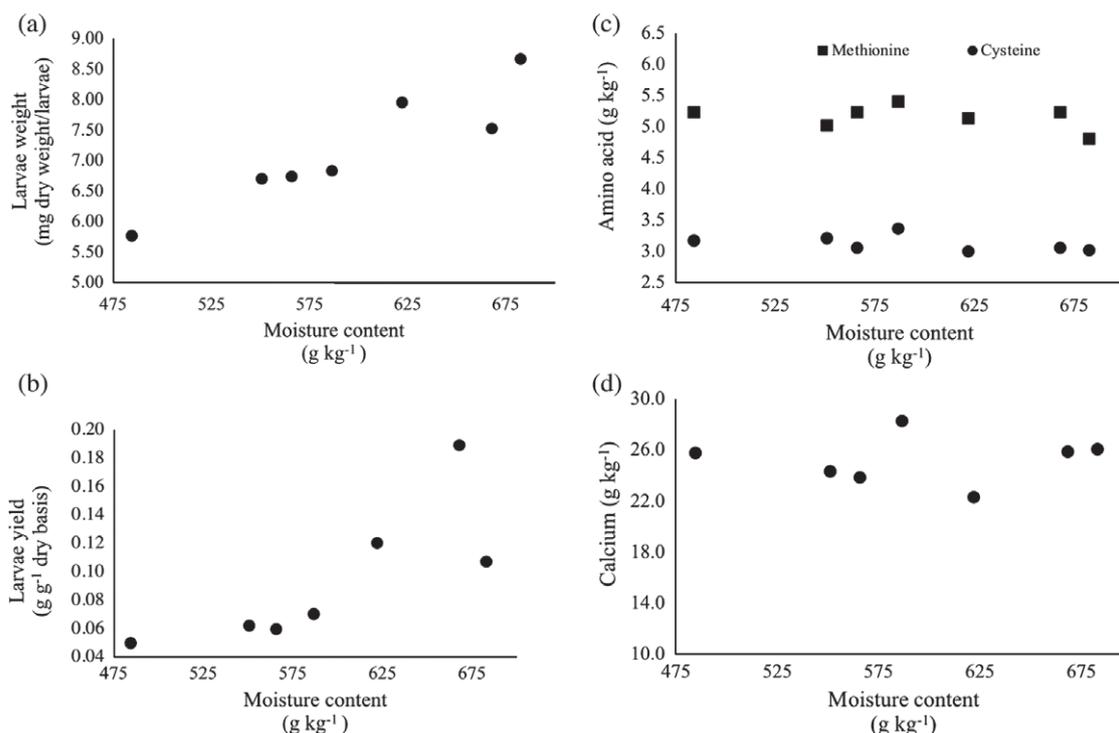


Figure 4. Larvae (a) harvest dry weight, (b) yield, (c) methionine and cysteine content, and (d) calcium content after cultivation on almond hulls for 14 days at varying initial hull moisture contents. Each point represents the results from one bioreactor.

Table 3. Linear regression of harvest dry weight, yield and consumption data as a function of hull initial moisture content (g kg^{-1} wet basis)

Independent variable	Parameters	Values (standard error)	P value	R ²
Larvae harvest dry weight (mg/larvae)	Slope, $\text{mg g}^{-1} \text{ kg}$ Intercept (mg/larvae)	0.013 (0.002) -0.46 (1.3)	0.002	0.87
Larvae yield (unitless)	Slope, $\text{g}^{-1} \text{ kg}$ Intercept, unitless	3.7×10^{-4} (1.1×10^{-4}) -0.11 (0.07)	0.03	0.72
Hull consumption, with larvae (%)	Slope, $\% \text{ g}^{-1} \text{ kg}$ Intercept, %	-0.09 (0.02) 72 (13)	0.01	0.75
Hull consumption, without larvae (%)	Slope, $\% \text{ g}^{-1} \text{ kg}$ Intercept, %	-0.08 (0.02) 65 (15)	0.02	0.68

decreased ($P < 0.05$) as moisture content increased in both treatments with larvae and without larvae (Table 3). The pH of residual hulls ranged between 8.76 and 8.90 for treatments that contained larvae and between 8.19 and 8.44 for treatments without larvae (Table S1). There was no significant effect of moisture content on final pH ($P > 0.05$).

Very few studies have reported the effect that varying moisture content has on the cultivation of larvae. Cammack and Tomberlin (2017) observed that moisture content had a greater effect on the development and life-history traits of BSF than feedstock.⁴⁴ In this prior study, larvae were fed a synthetic diet at 400, 550, and 700 g kg^{-1} moisture content. The larvae grew to the greatest weight, developed more quickly and required less food when cultivated at 700 g kg^{-1} moisture content than at 550 g kg^{-1} moisture content and were unable to develop on diets at 400 g kg^{-1} moisture. Cheng *et al.* (2017) found that reducing the moisture content of food waste from 800 to 750 g kg^{-1} and then to 700 g kg^{-1} reduced larval growth rate.⁴⁵ The observations from prior research are consistent with the present study; larvae growth increased with increasing moisture content. While moisture content higher than 700 g kg^{-1} could be tested, the impact of increasing moisture content on the reduction in oxygen diffusion would need to be considered. Moreover, as water is a scarce resource in certain regions of the world and increasing hull water content

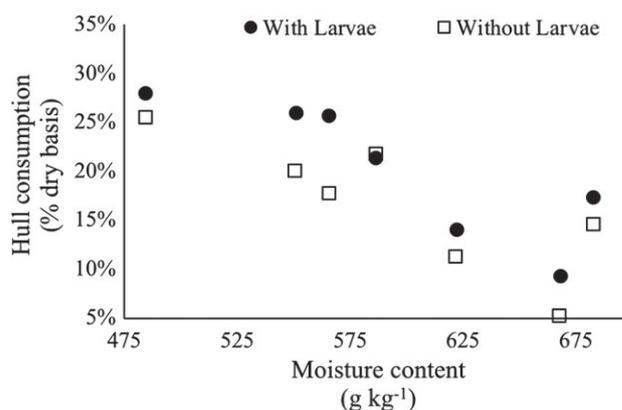


Figure 5. Consumption of almond hulls by larvae and microorganisms after cultivation for 14 days with varying initial hull moisture contents. Each point represents the hull consumption associated with one bioreactor.

would increase the thermal capacity of the cultivation feedstock, increasing water content could substantially increase process costs if substrate heating is required. Future work is needed to elucidate the ideal moisture levels for sustainable production.

The observation of enhanced hull consumption in treatments containing larvae compared to treatments without larvae is

Table 4. BSFL nutritional composition comparison (dry basis)

Growth substrate	Methionine, g kg ⁻¹	Cysteine, g kg ⁻¹	Calcium, g kg ⁻¹	Study
Chicken feed	7.6	2.5	28.7	Spranghers <i>et al.</i> (2017)
Digestate	8.7	2.4	66.2	Spranghers <i>et al.</i> (2017)
Vegetable waste	7.6	2.1	28.7	Spranghers <i>et al.</i> (2017)
Restaurant waste	2.8	2.2	1.2	Spranghers <i>et al.</i> (2017)
Almond hulls	4.7	2.8	16–18	Aeration rate
Almond hulls	5.2	3.1	25.3	Moisture content

consistent with other reports in the literature and supports the use of larvae in an integrated approach to organic waste treatment.²⁴ The decrease in hull consumption with increasing moisture may have been due to a decrease in oxygen diffusion. As moisture content increases, water-filled pores in the hull biomass would also increase and create oxygen diffusion limitations at the pore scale. While a decrease in air-filled pores would not directly impact larvae that grow on the surface of biomass, a decrease in air-filled pores would impact the growth of aerobic, immotile microorganisms. Reduction in microbial activity at moisture contents greater than 650 g kg⁻¹ wet basis has been reported in aerobic decomposition systems.⁴⁶ The results highlight the role of moisture content during larvae cultivation and its impact on potential synergistic and competing microorganisms involved in hull bioconversion.

An important objective of the study was to evaluate almond hulls as a feedstock for BSFL production. Sheppard *et al.* produced 24.4–45.3 g BSFL kg⁻¹ fresh poultry manure (wet basis).⁴⁷ In the current study, larvae production for the aeration experiment ranged between 91 and 303 g BSFL kg⁻¹ hulls while the production for the moisture content experiment ranged between 250 and 270 g BSFL kg⁻¹ hulls (wet basis). The results suggest that hulls are a suitable feedstock for cultivating BSFL compared to poultry manure.

Spranghers *et al.* (2017) compared the nutritional composition of BSFL reared on four different substrates as summarized in Table 4.²³ The methionine, cysteine and calcium contents of BSFL ranged between 2.8–8.7, 2.1–2.8 and 1.23–66.2 g kg⁻¹, respectively, depending on the substrate. These ranges are comparable to the methionine, cysteine, and calcium contents of BSFL grown on almond hulls and shells reaching 4.7–5.2, 2.8–3.1 and 16–25.3 g kg⁻¹, respectively, depending on the treatment. This indicates that hull substrates achieve BSFL that have a comparable composition to BSFL reared on chicken feed, digestate, vegetable waste, and restaurant waste. The reports from prior studies and current results also suggest there are opportunities to manage the substrate to achieve larvae with specific amino acid and calcium content.

The larvae analyses for this study emphasized measurements that would need to be considered if larvae were to be used as a methionine and cysteine supplement for feed for poultry layers. Additional analyses, such as protein, fat, ash and fatty acids, would be needed if larvae were to be used as a feed source for other animals. Furthermore, because the spent hulls would be a byproduct of larvae production, characterization of spent biomass for potential application in agriculture is warranted.

CONCLUSIONS

The results demonstrate that almond hulls, a major byproduct from the almond industry, can serve as a suitable feedstock for

BSF larvae production. Both aeration and moisture content were shown to have significant impacts on the growth of larvae and the consumption of hulls by larvae and microorganisms. At low levels of aeration rate, the results demonstrated that microorganisms played a larger role in the consumption of hulls than larvae and highlighted the importance of oxygen in supporting larvae growth. Aeration rate also impacted the calcium content of larvae, suggesting opportunities for managing aeration to achieve larvae with specific calcium levels. An increase in moisture content resulted in an increase in larvae growth and decrease in hull consumption. These trends may have been due to a decrease in competition for resources by microorganisms. Overall, the findings suggest that careful management of moisture and oxygen is needed to maximize conversion of hull substrate to larvae. In particular, the impact of the microbial community that co-colonizes the hulls with larvae needs to be considered in the bioconversion system. Additional research is needed to characterize the bioconversion microbiome to elucidate the potential synergies that could be leveraged and competition to be managed between larvae and microorganisms in order to achieve controlled production of larvae and organic waste conversion.

ACKNOWLEDGEMENTS

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

- 1 Ferdman R. The rise of the American almond craze in one nutty chart. *The Washington Post*. 2014 August 6, 2014.
- 2 National Agricultural Statistics Service. *Almonds: Utilized Production, United States 2007–2016*. U.S. Department of Agriculture. Washington, DC. (2017).
- 3 Elahi MY, Kargar H, Dindarlou MS, Kholif AE, Elghandour MMY, Rojas-Hernández S *et al.*, The chemical composition and in vitro digestibility evaluation of almond tree (*Prunus dulcis* D. A. Webb syn. *Prunus amygdalus*; var. *Shokoufeh*) leaves versus hulls and green versus dry leaves as feed for ruminants. *Agroforestry Systems* 91:773–780 (2017).
- 4 Rad MI, Rouzbehan Y and Rezaei J, Effect of dietary replacement of alfalfa with urea-treated almond hulls on intake, growth, digestibility, microbial nitrogen, nitrogen retention, ruminal fermentation,

- and blood parameters in fattening lambs. *J Anim Sci* **94**:349–358 (2016).
- 5 California Department of Food and Agriculture, *California Agriculture Statistics Review 2015–2016*. Sacramento, CA (2016).
 - 6 California State Legislature Assembly. *Solid waste: organic waste. Assembly Bill 1826*. Sacramento, CA. (2014).
 - 7 Offeman RD, Holtman KM, Covello KM and Orts WJ, Almond hulls as a biofuels feedstock: variations in carbohydrates by variety and location in California. *Indus Crops Products* **54**:109–114 (2014).
 - 8 Holtman KM, Offeman RD, Franqui-Villanueva D, Bayati AK and Orts WJ, Countercurrent extraction of soluble sugars from almond hulls and assessment of the bioenergy potential. *J Agric Food Chem* **63**:2490–2498 (2015).
 - 9 Nitsos C, Matsakas L, Triantafyllidis K, Rova U and Christakopoulos P, Evaluation of Mediterranean agricultural residues as a potential feedstock for the production of biogas via anaerobic fermentation. *Biomed Res Int* **2015**:6 (2015).
 - 10 Valverde M, Madrid R, García AL, Del Amor F, Rincón LF. Use of almond shell and almond hull as substrate for sweet pepper cultivation. Effect on fruit yield and mineral content. *Span J Agric Res* **164** (2013). 164 p.
 - 11 Lundy ME and Parrella MP, Crickets are not a free lunch: protein capture from scalable organic side-streams via high-density populations of *Acheta domesticus*. *PLOS ONE* **10**:e0118785 (2015).
 - 12 Foley JA, Ramankutty N, Brauman KA, Cassidy ES, Gerber JS, Johnston M et al., Solutions for a cultivated planet. *Nature* **478**:337–342 (2011).
 - 13 Al-Qazzaz MFA, Ismail H, Akit H and Idris LH, Effect of using insect larvae meal as a complete protein source on quality and productivity characteristics of laying hens. *Revista Brasileira de Zootecnia* **45**:518–523 (2016).
 - 14 Cullere M, Tasoniero G, Giaccone V, Miotti-Scapin R, Claeys E, De Smet S et al., Black soldier fly as dietary protein source for broiler quails: apparent digestibility, excreta microbial load, feed choice, performance, carcass and meat traits. *Animal* **10**:1923–1930 (2016).
 - 15 Leiber F, Gelencsér T, Stamer A, Amsler Z, Wohlfahrt J, Früh B et al., Insect and legume-based protein sources to replace soybean cake in an organic broiler diet: effects on growth performance and physical meat quality. *Renew Agric Food Syst* **32**:21–27 (2017).
 - 16 Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF et al., Food security: the challenge of feeding 9 billion people. *Science* **327**:812–818 (2010).
 - 17 Bußler S, Rumpold BA, Jander E, Rawel HM and Schlüter OK, Recovery and techno-functionality of flours and proteins from two edible insect species: meal worm (*Tenebrio molitor*) and black soldier fly (*Hermetia illucens*) larvae. *Heliyon* **2**:e00218 (2016).
 - 18 Smetana S, Palanisamy M, Mathys A and Heinz V, Sustainability of insect use for feed and food: life cycle assessment perspective. *J Clean Prod* **137**:741–751 (2016).
 - 19 Nguyen TTX, Tomberlin JK and Vanlaerhoven S, Ability of black soldier fly (Diptera: Stratiomyidae) larvae to recycle food waste. *Environ Entomol* **44**:406–410 (2015).
 - 20 Manurung R, Supriatna A, Eyanthi RR and Ramadhani EP, Bioconversion of rice straw waste by black soldier fly larvae (*Hermetia illucens* L.): optimal feed rate for biomass production. *J Entomol Zool Stud* **4**:1036–1041 (2016).
 - 21 Diener S, Zurbrügg C and Tockner K, Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. *Waste Manag Res* **27**:603–610 (2009).
 - 22 Myers HM, Tomberlin JK, Lambert BD and Kattes D, Development of black soldier fly (Diptera: Stratiomyidae) larvae fed dairy manure. *Environ Entomol* **37**:11–15 (2008).
 - 23 Spranghers T, Ottoboni M, Klootwijk C, Obyn A, Deboosere S, De Meulenaer B et al., Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. *J Sci Food Agric* **97**:2594–2600 (2017).
 - 24 Čičková H, Newton GL, Lacy RC and Kozánek M, The use of fly larvae for organic waste treatment. *Waste Manag* **35**:68–80 (2015).
 - 25 Popa R and Green TR, Using black soldier fly larvae for processing organic leachates. *J Econ Entomol* **105**:374–378 (2012).
 - 26 Lalander C, Diener S, Magri ME, Zurbrügg C, Lindström A and Vinnerås B, Faecal sludge management with the larvae of the black soldier fly (*Hermetia illucens*) – from a hygiene aspect. *Sci Total Environ* **458–460**:312–318 (2013).
 - 27 Bosch G, Zhang S, Ooninx DGAB and Hendriks WH, Protein quality of insects as potential ingredients for dog and cat foods. *J Nutr Sci* **3**:e29 (2014).
 - 28 Diener S, Studt Solano NM, Roa Gutiérrez F, Zurbrügg C and Tockner K, Biological treatment of municipal organic waste using black soldier fly larvae. *Waste Biomass Valorization* **2**:357–363 (2011).
 - 29 EPA Method 6010C, *Inductively Coupled Plasma-Atomic Emission Spectrometry*. United States Environmental Protection Agency, Washington, DC (2015).
 - 30 AOAC. Official Method 980.13, *Fructose, Glucose, Lactose, Maltose, and Sucrose in Milk Chocolate: Liquid Chromatographic Method*. Official Methods of Analysis of AOAC International, 18th edn. Association of Official Analytical Chemists, Gaithersburg, MD (2004).
 - 31 Hall MB, Determination of dietary starch in animal feeds and pet food by an enzymatic-colorimetric method: collaborative study. *J Assoc Off Anal Chem Int* **98**:397–409 (2015).
 - 32 AOAC. Official Method 993.13, *Nitrogen (Total) in Fertilizers*. Official Methods of Analysis of AOAC International. Association of Official Analytical Chemists, Gaithersburg, MD (2005).
 - 33 Van Soest PJ, Robertson JB and Lewis BA, Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *J Dairy Sci* **74**:3583–3597 (1991).
 - 34 Goering HK and Van Soest PJ, *Forage Fiber Analysis (Apparatus, Reagents, Procedures, and some Applications)*. USDA Agricultural Research Service, (1970) Handbook number 379 as modified by D.R. Mertens Washington, DC.
 - 35 Mertens DR. Critical conditions in determining detergent fiber. Proceedings of NFTA Forage Analysis Workshop 1992:C1-C8.
 - 36 AOAC. Official Method 973.18, *Fiber (Acid Detergent) and Lignin in Animal Feed*. Official Methods of Analysis of AOAC International. Association of Official Analytical Chemists, Gaithersburg, MD (2005).
 - 37 Reddy AP, Jenkins BM and VanderGheynst JS, The critical moisture range for rapid microbial decomposition of rice straw during storage. *Trans ASABE* **52**:673–676 (2009).
 - 38 Yu C, Harrold DR, Claypool JT, Simmons BA, Singer SW, Simmons CW et al., Nitrogen amendment of green waste impacts microbial community, enzyme secretion and potential for lignocellulose decomposition. *Process Biochem* **52**:214–222 (2017).
 - 39 Meyer GA and Keliher PN, in *An Overview of Analysis by Inductively Coupled Plasma-Atomic Emission Spectrometry*, ed. by MaDW G. VCH Publishers, New York, NY (1992).
 - 40 Sah RN and Miller RO, Spontaneous reaction for acid dissolution of biological tissues in closed vessels. *Anal Chem* **64**:230–233 (1992).
 - 41 AOAC. Official Method 994.12, *Amino Acids in Feeds. Alternative II, Performic Acid Oxidation with Acid Hydrolysis - Hydrobromic Acid Method*. Official Methods of Analysis of AOAC International. Association of Official Analytical Chemists, Gaithersburg, MD (1997).
 - 42 Eby HJ and Dandy WL, An attempt to mechanize nutrient recovery from animal excreta. *Trans ASAE* **21**:395–398 (1978).
 - 43 Richard TL, Walker LP and Gossett JM, Effects of oxygen on aerobic solid-state biodegradation kinetics. *Biotechnol Prog* **22**:60–69 (2006).
 - 44 Cammack JA and Tomberlin JK, The impact of diet protein and carbohydrate on select life-history traits of the black soldier fly *Hermetia illucens* (L.) (Diptera: Stratiomyidae). *Insects* **8** (2017). <https://doi.org/10.3390/insects8020056>
 - 45 Cheng JYK, Chiu SLH and Lo IMC, Effects of moisture content of food waste on residue separation, larval growth and larval survival in black soldier fly bioconversion. *Waste Manag* **67**:315–323 (2017).
 - 46 Richard TL, Hamelers HVM, Veeken A and Silva T, Moisture relationships in composting processes. *Compost Sci Utiliz* **10**:286–302 (2002).
 - 47 Sheppard CD, Newton LG, Sidney TA and Stan S, A value added manure management system using the black soldier fly. *Bioresour Technol* **50**:275–279 (1994).