MAKING SMART FERMENTATION DECISIONS II: TOOLS FOR PREDICTION AND PREVENTION OF PROBLEM FERMENTATIONS

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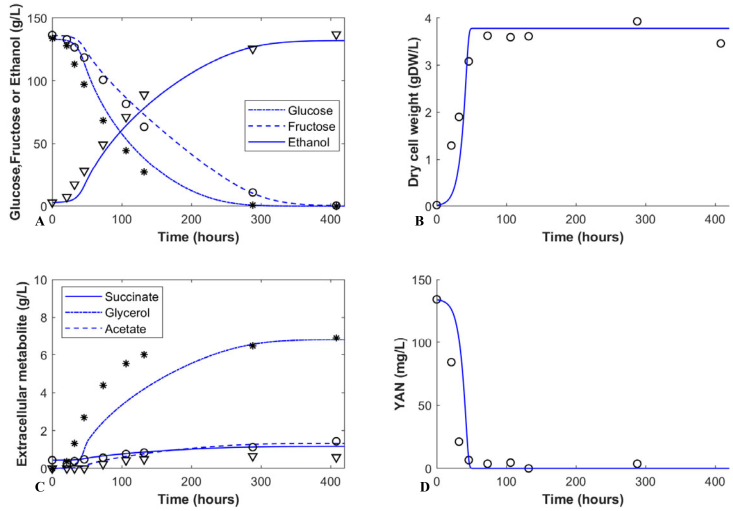
Predicting and Preventing Problem Fermentations

Juice composition (especially sugar and nitrogen)
Fermentation temperature
Mixing in red and white fermentations
Yeast strain differences

Wine Fermentation Kinetics



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JUICE COMPOSITION

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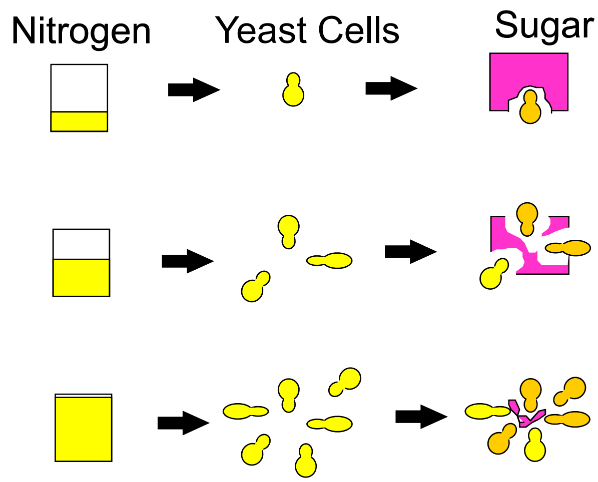
Model for the role of nitrogen in wine fermentation kinetics



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Mechanistic Kinetic Model Based on N Utilization

Viable Biomass (X_v) : $$\frac{dX_v}{dt} = \mu X_v - k_d X_v$$

$$\mu = \frac{\mu_{\max} N}{K_N + N} \qquad k_d = k_d^{/} E$$

Nitrogen (N) : $$\frac{dN}{dt} = -\frac{\mu X_v}{Y_{X/N}}$$

Cramer et al., *Biotech. Bioeng*, 2002

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Model (continued)

Sugar (S) : $$\frac{dS}{dt} = -\frac{\beta X_v}{Y_{E/S}}$$

Ethanol (E) : $$\frac{dE}{dt} = \beta X_v$$

$$\beta = \frac{\beta_{\max} S}{K_s + S}$$

Cramer et al., *Biotech. Bioeng*, 2002

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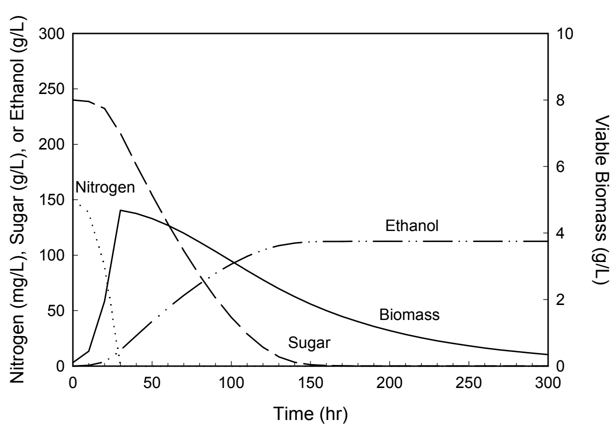
Model Prediction of Normal Kinetics



Cramer et al., *Biotech. Bioeng*, 2002

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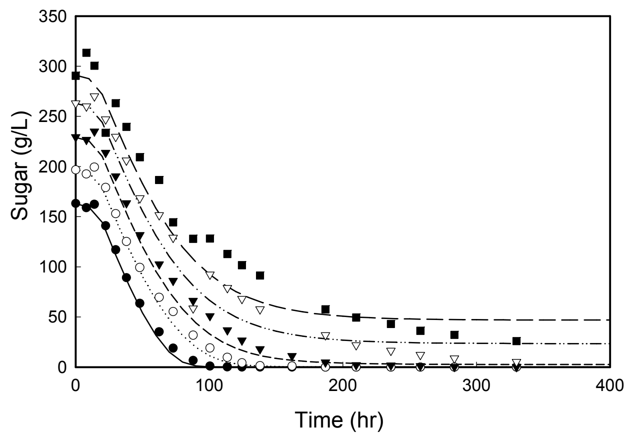
Increasing sugar increases risk of stuck fermentations



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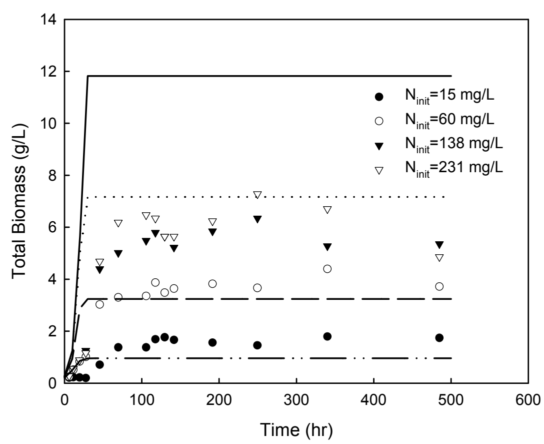
The Effect of Increased Initial N on Biomass



Cramer et al., *Biotech. Bioeng*, 2002

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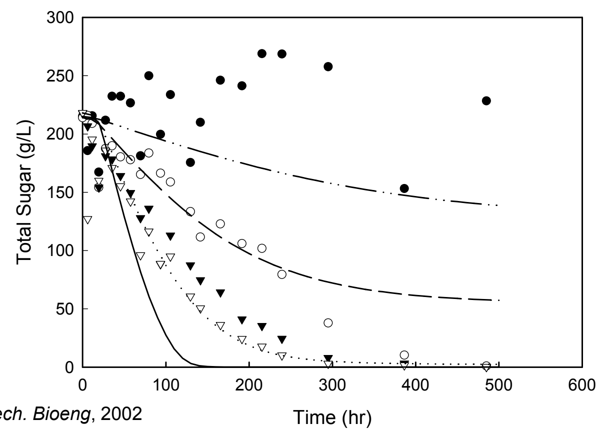
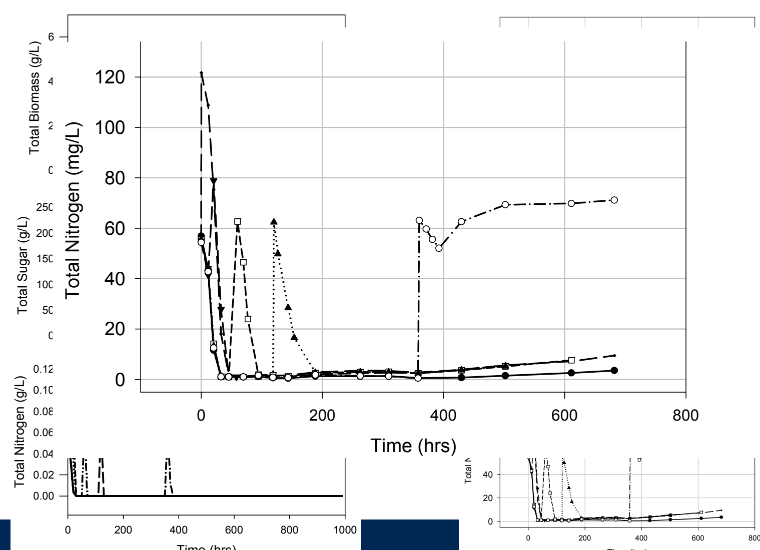
Reduced nitrogen increases risk of stuck fermentations



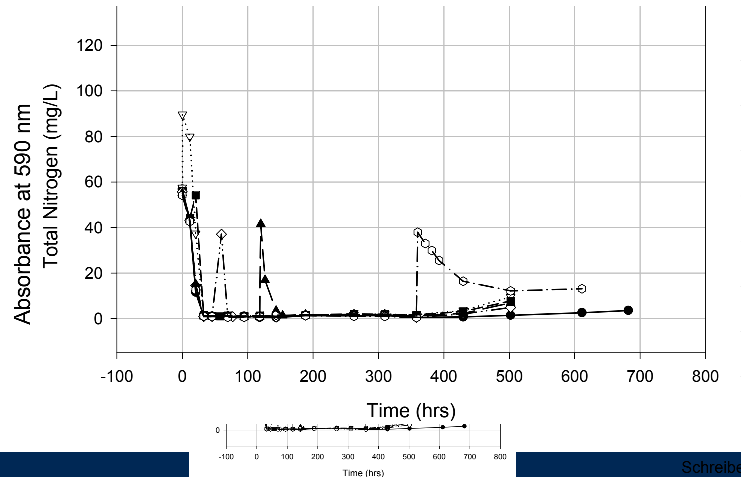
Cramer et al., *Biotech. Bioeng*, 2002

Can fermentations be saved with nitrogen additions?



Schreiber, MS Thesis

The form of nitrogen makes a difference



Schreiber, MS Thesis

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Lessons learned on nutrient status (sugar and nitrogen)

High sugar will make other factors more critical

You need enough nitrogen to give you a reasonable fermentation speed

You can supplement nitrogen during the fermentation—until it isn't taken up by cells

Complex forms of nitrogen may work better later in the fermentation

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FERMENTATION TEMPERATURE

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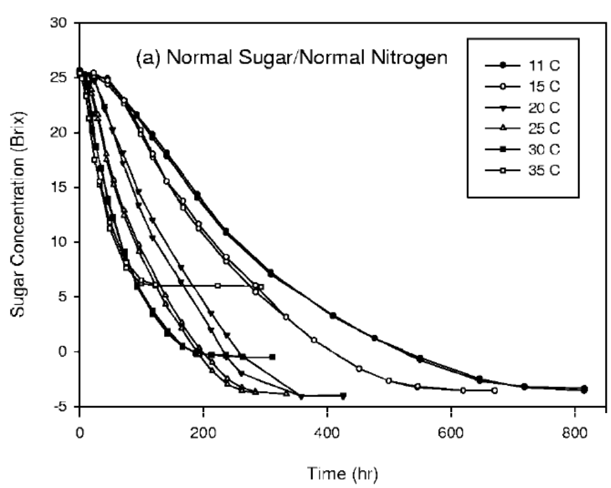
The effect of temperature on fermentation kinetics



Coleman et al., *Applied and Environmental Microbiology*, 2007

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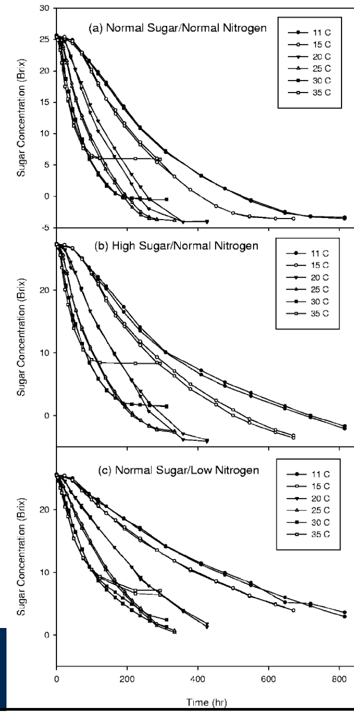
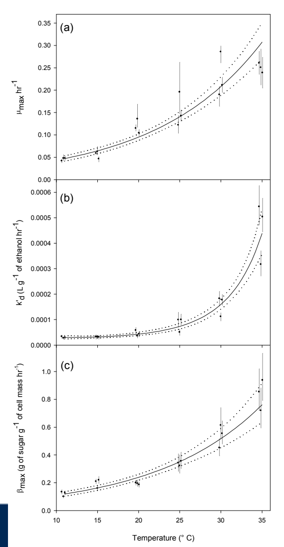
Low nitrogen limits the temperature range of a successful fermentation



Coleman et al., *Applied and Environmental Microbiology*, 2007

Inactivation is highly temperature dependent



μ_{max}

K_d (about 15 x higher at 35 C)

β_{max}

Coleman et al., *AEM*, 2007

Lessons Learned on Fermentation Temperature

Fermentations go from sluggish to normal to stuck as fermentation temperature is increased

Temperature is even more important with low initial nitrogen

Everything speeds up with higher temperature, but especially cell inactivation

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MIXING IN RED AND WHITE FERMENTORS

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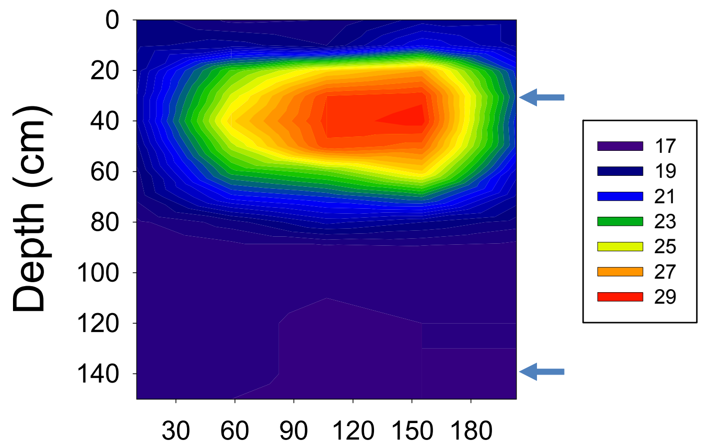
The cap in a red fermentor can get really hot!



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Developing a mathematical model for red wine fermentations based on fundamental knowledge

$$\frac{dX_A}{dt} = \mu X_A - k_d X_A;\ where\ \mu = \frac{\mu_{max} N}{K_N + N}\ \ and\ k_d = k'_d E$$

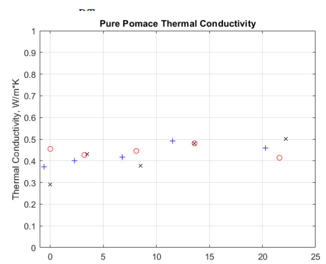
$$\frac{dN}{dt} = -\frac{\mu}{Y_{X/N}} X_A$$

$$\frac{dS}{dt} = -\frac{\beta}{Y_{E/S}} X_A;\ where\ \beta = \frac{\beta_{max} S}{K_S + S}$$

dE

$$\frac{Dc_i}{Dt} = \nabla \cdot (D_i \nabla \cdot c_i) + R_i$$

$$\rho\left(\frac{\partial \mathbf{u}}{\partial t} + \mathbf{u} \cdot \nabla \mathbf{u}\right) = -\nabla p + \nabla \cdot \left[\mu(\nabla \mathbf{u} + (\nabla \mathbf{u})^T)\right] + \nabla \cdot \left[\left(\lambda - \frac{2\mu}{3}\right)(\nabla \cdot \mathbf{u})\mathbf{I}\right] + \rho \mathbf{g}$$



Model to predict the spatial distribution of temperature and ethanol

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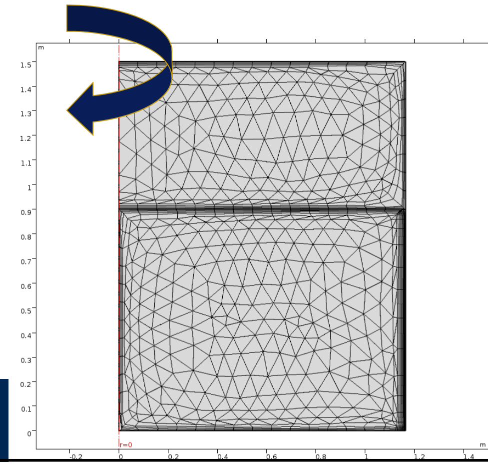
Developing a mathematical model for red wine fermentations based on fundamental knowledge

Solve using COMSOL/Finite Elements Method



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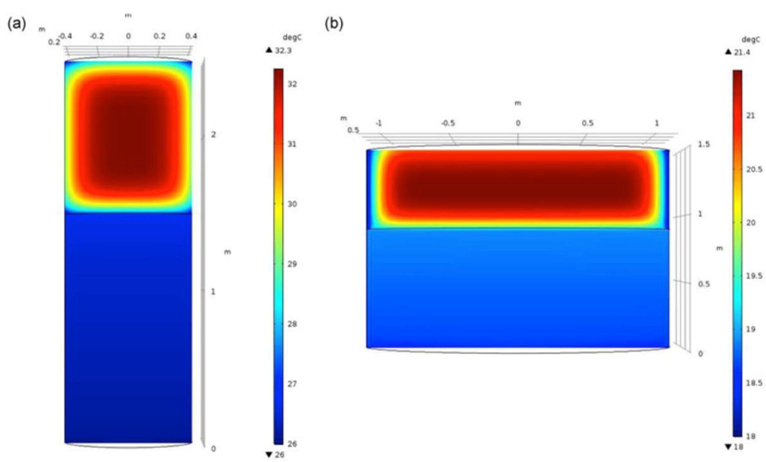
Predictions of Temperature Gradients in Red Wine Fermentors (using COMSOL)



Miller, Oberholster, and Block. Biotechnology and Bioengineering, 2019.

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How do we know the predictions are valid?

TABLE 2 Comparison of model temperature predictions with experimental data

Value	This model	Schmid et al. (2009) (range)
1,400 L Bulk liquid T, C	26.3	26–28
1,400 L Max cap T, C	32.3	24–32
6,400 L Bulk liquid T, C	19.5	18–20
6,400 L Max cap T, C	22.1	22–24

Miller, Oberholster, and Block. Biotechnology and Bioengineering, 2019.

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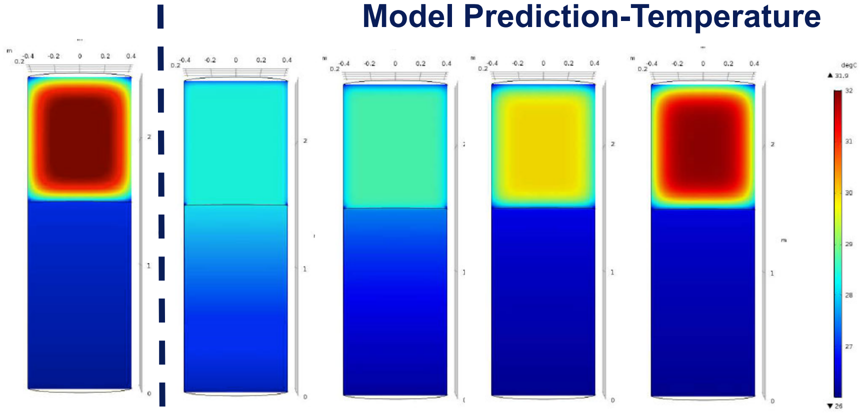
How do we know the predictions are valid?

Model Prediction-Temperature



Pre-Pump | **1 hr** | **2 hr** | **6 hr** | **12 hr**

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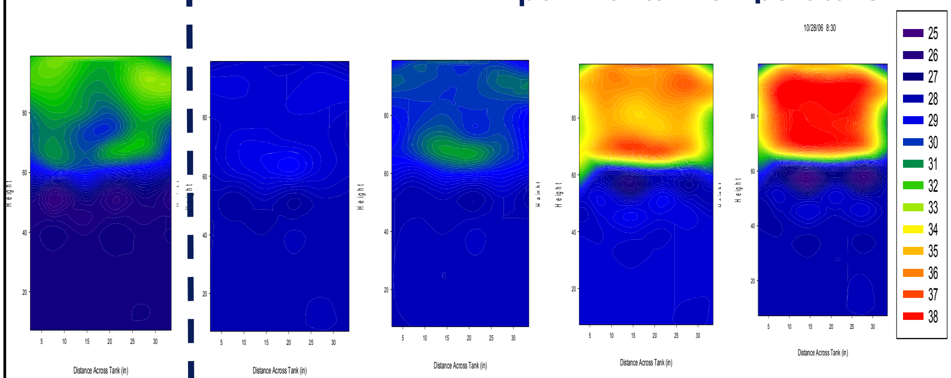
How do we know the predictions are valid?

Experimental-Temperature



Pre-Pump **1 hr** **2 hr** **6 hr** **12 hr**

Schmid et al. Australian Journal of Grape and Wine Research, 2009.

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Application of the model to understand the effects of tank size and aspect ratio

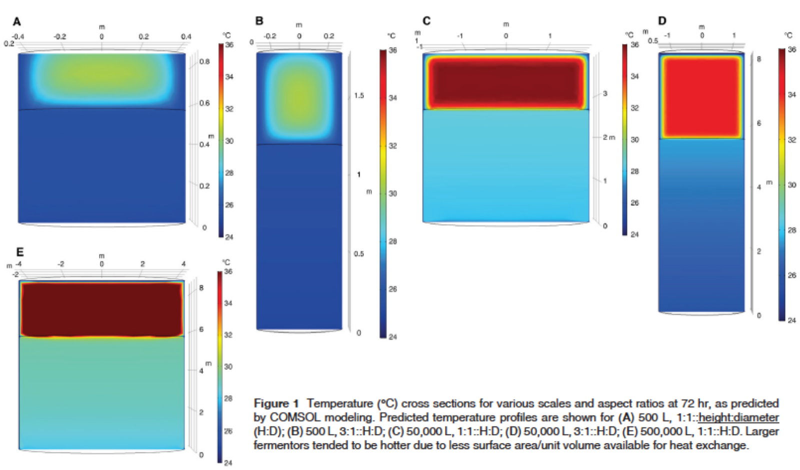

Figure 1 Temperature (°C) cross sections for various scales and aspect ratios at 72 hr, as predicted by COMSOL modeling. Predicted temperature profiles are shown for (**A**) 500 L, 1:1::height:diameter (H:D); (B) 500 L, 3:1::H:D; (C) 50,000 L, 1:1::H:D; (D) 50,000 L, 3:1::H:D; (E) 500,000 L, 1:1::H:D. Larger fermentors tended to be hotter due to less surface area/unit volume available for heat exchange.

Miller, Oberholster, and Block. AJEV, 2019.

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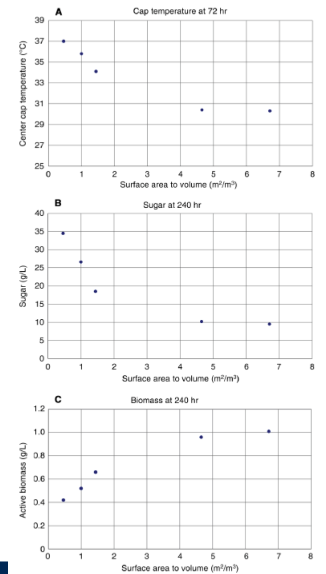
The predicted effects of surface area to volume ratio



Cap Temperature at 72 hr

RS at 240 hr

Active Biomass at 240 hr

Larger Fermentor

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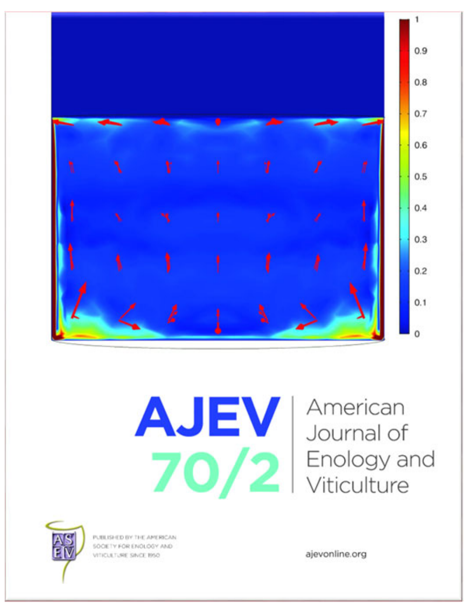

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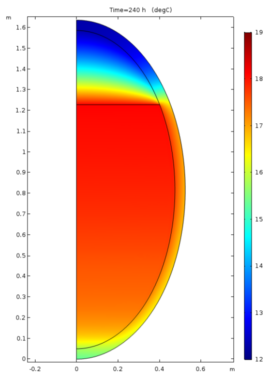
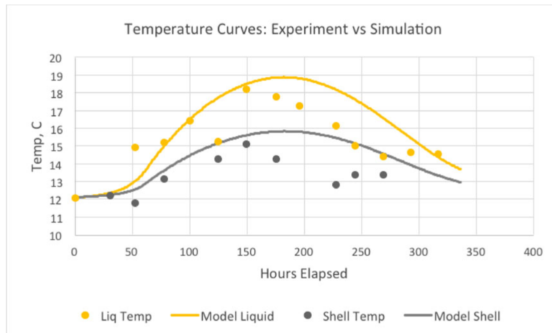
Application to white wine in concrete eggs





Temperature control and mixing are better in a standard jacketed stainless steel tank.

Miller, Oberholster, and Block. AJGWR, 2019.

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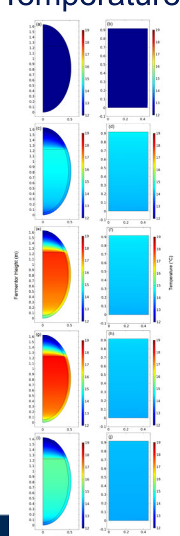
Application to white wine in concrete eggs

Temperature



0 hr

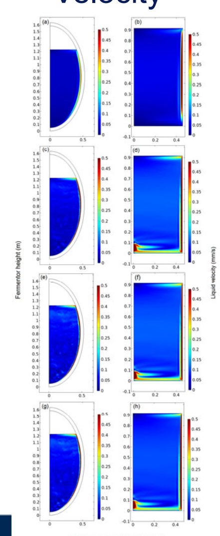
72 hr

120 hr

240 hr

300 hr

Velocity



0 hr

72 hr

120 hr

240 hr

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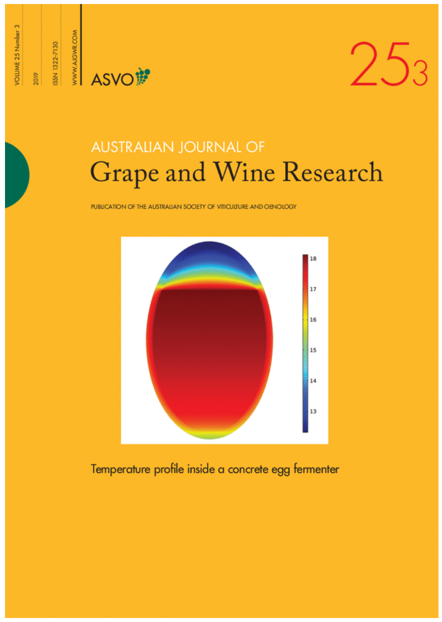

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Inoculation and mixing in white wine fermentors

Depends on dissolution/suspension of yeast and characteristics of juice

- Get less lag from more effective inoculum with mixing
- **Probably accentuated by cold temperatures**
- Want fully mixed fermentation BEFORE filling barrels for completion of fermentation

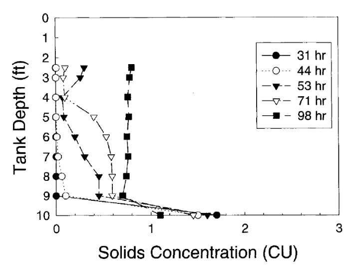

Fig. 3. Evolution of the cell concentration profile during growth phase. The cell concentration measured with the probe at various heights in the pilot fermentor is shown for various times from 31 hours to 98 hours. It can be seen that, after initial settling of the yeast, growth begins from the bottom of the fermentor and evolves upward until the fermentor is homogeneous at approximately the same time that active cell growth ceases.

Vlassides and Block, AJEV, 2000

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Lessons learned for mixing

KNOW YOUR CAP TEMPERATURE!

Your cap could get hot enough to cause a stuck fermentation

Punch downs and pumpovers will cool cap, your jacket will not

The larger and wider your tank gets, the greater this effect

Concrete tanks will get hot without external cooling

Make sure your dry yeast is well hydrated and mixed into the tank prior to barreling down (for barrel fermented whites)

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YEAST STRAIN DIFFERENCES

36 7/29/2019 UCDAVIS

Yeast Strains Exhibit Diverse Fermentation Kinetics

TABLE 2 Fermentation characteristics of *S. cerevisiae* strains used in this study

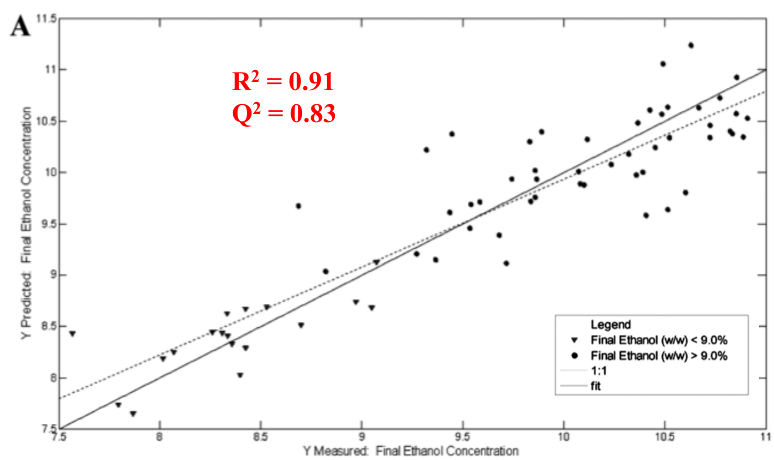
		Mean ± SD			
Strain[a]	UCD accession no.	Max OD_{600}	Final °Brix	% (wt/wt) ethanol	End time point (h)
Enoferm T306	2502	5.89 ± 0.16	0.60 ± 0.36	9.93 ± 0.13	312
ICVK1 (V-1116)	2537	5.84 ± 0.42	0.67 ± 0.61	9.83 ± 0.59	264
Sake A18	612	5.76 ± 0.49	0.10 ± 0.17	10.36 ± 0.90	312
Cepage chardonnay	2061	5.64 ± 0.48	0.43 ± 0.45	10.18 ± 0.50	312
FM16-7	V4	5.61 ± 0.14	0.47 ± 0.32	10.67 ± 0.17	264
Enoferm Simi White	2501	5.51 ± 0.32	0.90 ± 0.79	10.67 ± 0.23	312
EC 1118	777	5.49 ± 0.40	0.33 ± 0.58	10.62 ± 0.37	264
Lalvin Rhone L2226	2545	5.20 ± 0.21	0.30 ± 0.30	10.44 ± 0.29	312
ICV D254	2499	5.03 ± 0.15	0.50 ± 0.36	10.34 ± 0.11	312
C0490GP2-B11	V3	4.99 ± 0.32	1.20 ± 0.53	10.57 ± 0.25	312
M2	906	4.97 ± 0.12	0.36 ± 0.02	9.50 ± 0.21	552
Uvaferm 43	2032	4.78 ± 0.51	1.17 ± 1.11	9.08 ± 0.56	312
Lalvin ICVD47	963	4.78 ± 0.14	0.73 ± 0.87	10.10 ± 0.51	312
Cote de Blanc	2031	4.37 ± 0.18	0.30 ± 0.26	10.55 ± 0.16	360
Bread yeast	668	4.20 ± 0.21	0.27 ± 0.46	10.13 ± 0.38	312
Zymaflore VL-1	2074	4.01 ± 0.20	0.27 ± 0.46	9.69 ± 0.28	384
Premier Cuvee	2212	3.21 ± 0.07	1.17 ± 1.00	8.88 ± 0.31	576
FST 40-27*	1427	3.14 ± 0.17	4.40 ± 0.90	7.96 ± 0.44	408
Montrachet*	522	2.98 ± 0.08	3.63 ± 0.32	7.96 ± 0.14	576
Prise de Mousse*	594	2.93 ± 0.06	2.53 ± 0.86	8.57 ± 0.35	432
CY3079*	2497	2.77 ± 0.08	2.60 ± 0.30	8.46 ± 0.20	576
DV10*	2498	2.68 ± 0.07	2.80 ± 0.26	8.33 ± 0.07	576

[a] *, fermentation was stopped due to slow progress.

Henderson et al., *App. Environ. Micro.*, 2013

37

Lipid Composition Predicts Yeast Biomass and Ethanol Production (using 22 strains)



Henderson et al., *App. Environ. Micro.*, 2013

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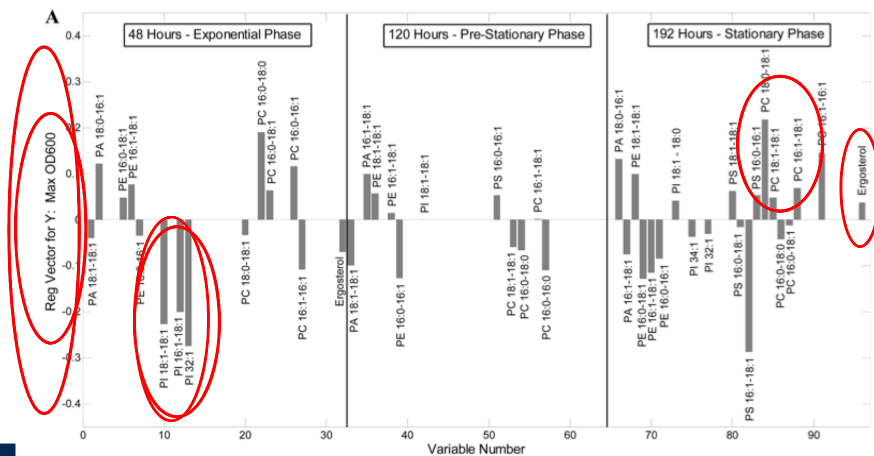
38

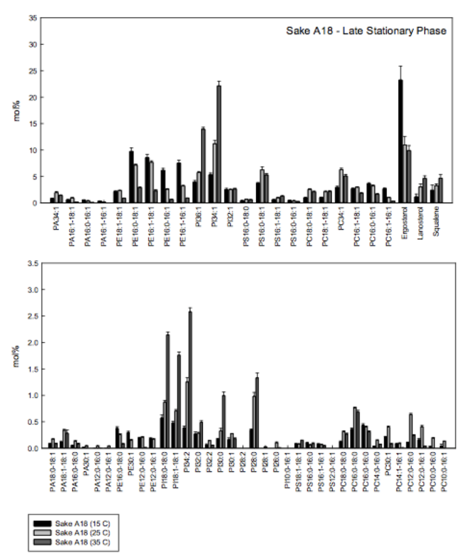
Correlation of Specific Lipids with Cell Growth and Final Ethanol Concentration



Henderson et al., *Applied and Environmental Microbiology* , 2012.

Henderson et al., *App. Environ. Micro.*, 2013 39

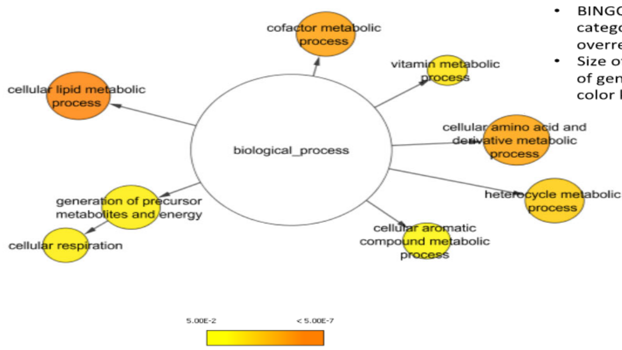
Lipid composition changes with temperature



Henderson et al., *AEM*, 2013.

40

BINGO analysis of transcriptomic data identifies classes of genes most associated with nutrient utilization efficiency



- BINGO determines which gene ontology categories are statistically overrepresented in a set of genes.
- Size of the circles represent the number of genes involved in the process, and the color key represents the p-value

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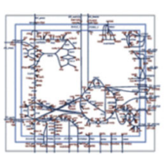
Creating a Genome-Scale Model to Understand Strain Differences

Mathematical representation of a metabolism

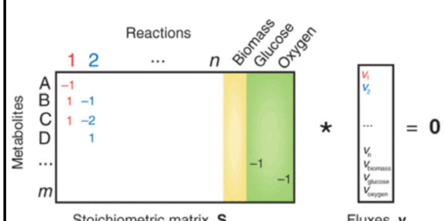


A ⟷ B + C Reaction 1

B + 2C ⟶ D Reaction 2

…

Reaction n



Jeffrey D Orth, Ines Thiele & Bernhard Ø Palsson, Nature Biotechnology (2010)

Yeast 8.3.3

Taxonomy	Template Model	Reactions	Metabolites	Genes
Saccharomyces cerevisiae	Yeast 7.6	3963	2691	1139

Division of Systems and Synthetic Biology, Department of Biology and Biological Engineering, Chalmers University of Technology. https://github.com/SysBioChalmers/yeast-GEM

Modification of Yeast 8.3.3 to fit enological conditions

- No O_2 uptake
- Allow unrestricted uptake of sterols
- Nitrogen limited
- Make the model dynamic

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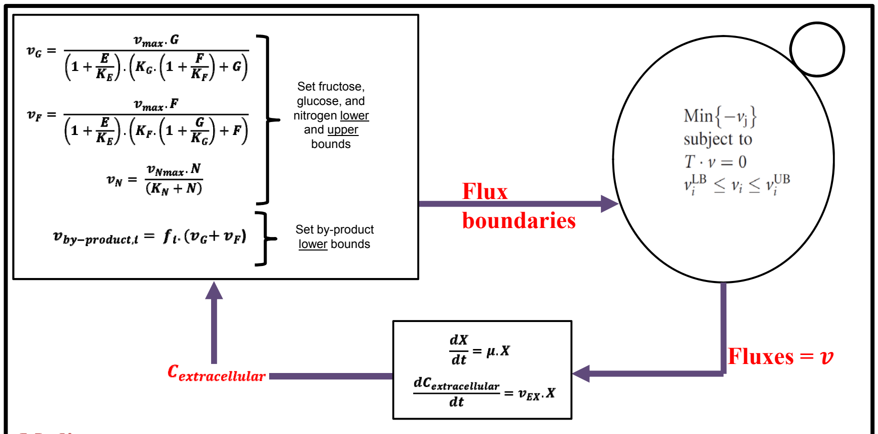
Making the genome-scale model dynamic to predict fermentation kinetics



COBRA Toolbox, (Hierendt et al., 2013) using Gurobi optimizer (http://www.gurobi.com, 2016)

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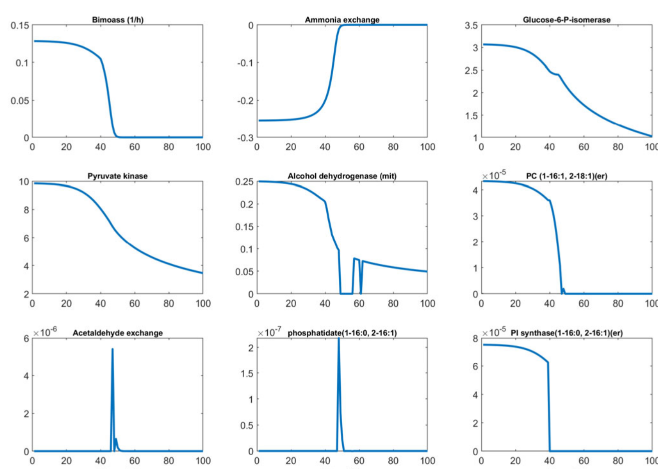
This model allows us to predict what goes on ***inside*** the yeast cell



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Lessons Learned on Yeast Strains

Commercial yeast strains can have very different ethanol tolerance and nutrient utilization efficiency

These traits are a strong function of specific cell membrane lipids

Omics and metabolic modeling will help explain these traits so they can be controlled—not there yet.

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