Demonstrating Bisabolene Production at 1.8 L Scale

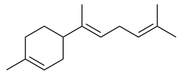
L. Sandoval[1], F. Tachea[1], J. Wong[1], P. Coffman[1], H. Burd[2,3], B. Ozayden[2,3], G. Murray[2,3], J. Keasling[2,3], J. Gardner[1], J. Baez[1], and T.S. Lee[2,3]

[1]Advanced Biofuels Process Demonstration Unit, Lawrence Berkeley National Laboratory, Emeryville, CA;
[2]Joint BioEnergy Institute, Emeryville, CA; [3]Lawrence Berkeley National Laboratory, Berkeley, CA

Introduction

Bisabolane has been identified as a potential biosynthetic alternative to D2 diesel fuel. Researchers at JBEI have engineered *S. Cerevisiae* for the production of bisabolene, bisabolane's immediate precursor, by the introduction of bisabolene synthase from *A. Grandis*. In order to produce large enough quantities of bisabolane for engine testing and to better understand the challenges that arise at larger production scales, JBEI and the Advanced Biofuels Process Demonstration Unit (ABPDU) have developed a fed-batch fermentation process for bisabolene production for eventual scale-up to production scale. Presented here are preliminary results from 1.8 L fed-batch fermentations conducted at ABPDU with a discussion of challenges in scaling up the process.

Molecular Structure of Bisabolene:



Fermentation Control Parameters

Strain:	*S. cerevisiae* CEN.PK2 – JBEI-4734
Fermentation Mode:	Fed-Batch
Batch Medium:	900 ml defined medium with 15 glucose & 5 g/L galactose
Feed Medium:	900 ml defined medium with 500 g/L glucose & 5 g/L galactose
Batch/Feeding Time and Rate:	48 hours / 310 hours at ~0.06 ml/min (~43.2 g glucose/day)
Extractant:	180 ml of decane added 1 hour after Inoculation 5 g/L heptadecane Internal Standard
Dissolved Oxygen (DO):	A1/A2 – 30% controlled by agitation & aeration
	A3/A4 – 5% controlled by agitation & aeration
Temperature and pH:	30ºC and 5.0 using 7N NH_4OH

Table 1. DO Cascade for reactors controlled at 30% and 5%

PID Out Put →		-100	-80	-60	-40	-20	0	20	40	60	80	100
5%	Agitation (RPM)	177	250.5	309.3	338.7	368.1	412.2	456.3	500.4	588.6	691.5	765
	Aeration (L/hr)	3	3	3	6	12	15	21	27	45	60	75
30%	Agitation (RPM)	177	250.5	324	397.5	426.9	471	515.1	573.9	632.7	691.5	765
	Aeration (L/hr)	60	60	75	90	105	111	117	123	129	135	135

Dissolved Oxygen Controlled at 5% Resulted in Ethanol Accumulation

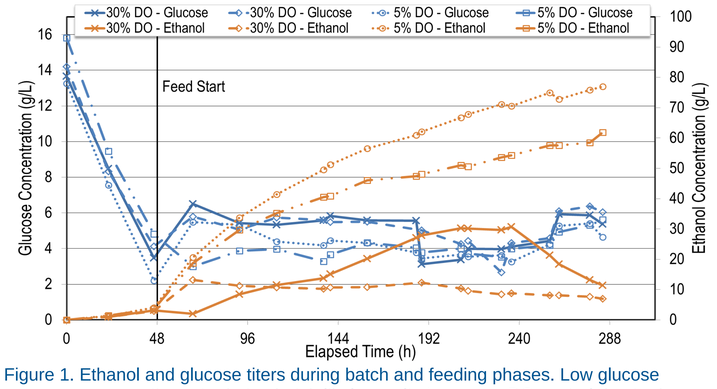
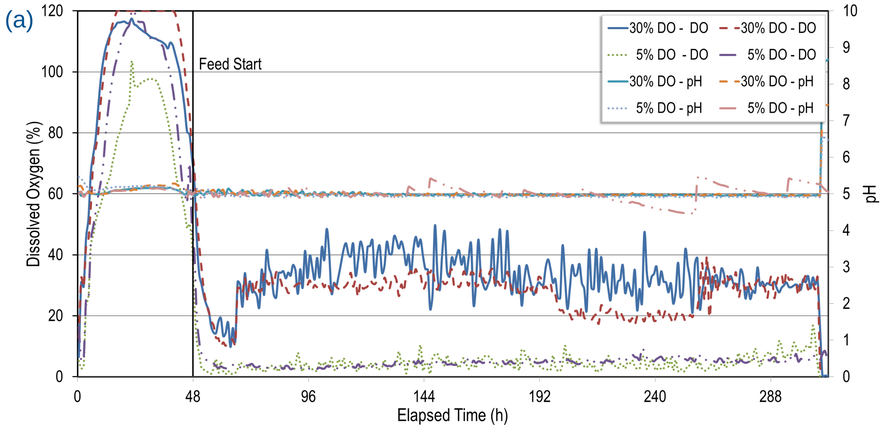
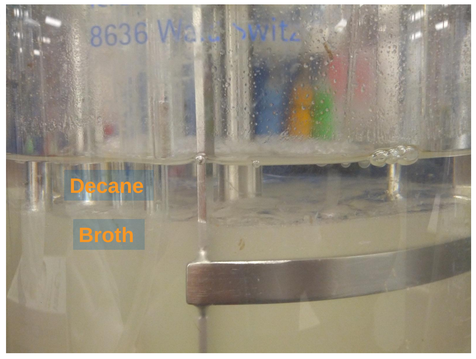


Figure 1. Ethanol and glucose titers during batch and feeding phases. Low glucose consumption rate during batch likely results from poor DO control due to decane.

Decane Affects DO Probe Measurements Resulting in Poor DO Control During Batch Phase





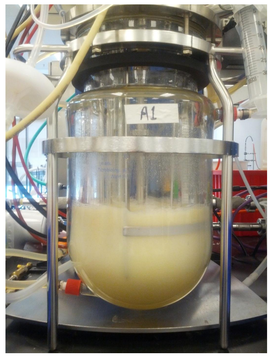


Figure 2. (a) Reactor pH and DO process values show decane overlay during batch phase resulted in unreliable DO readings, once volume increased above the DO probe readings returned to normal (b) Batch volume puts DO probe at the interface of decane and broth (c) Accurate DO reading results in increased agitation and thus mixing of solvent and broth

Oxygen Limitation Inhibits Cell Growth

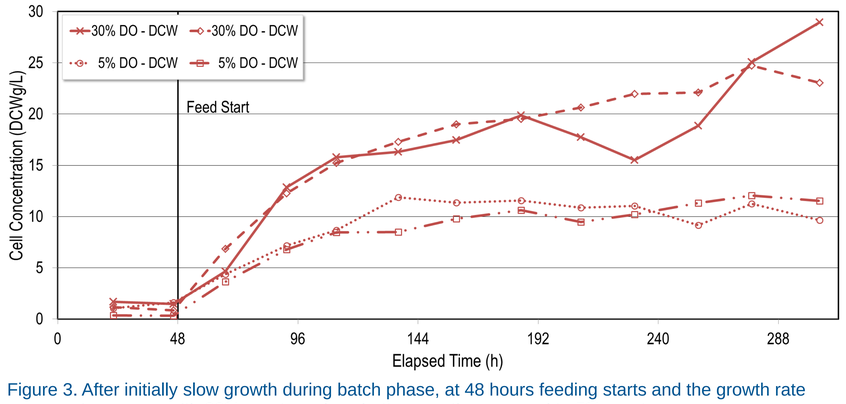


Figure 3. After initially slow growth during batch phase, at 48 hours feeding starts and the growth rate increases, with 30% reactors show better growth compared to 5% reactors

Demonstration of Bisabolene Production Using Heptadecane as Internal Standard

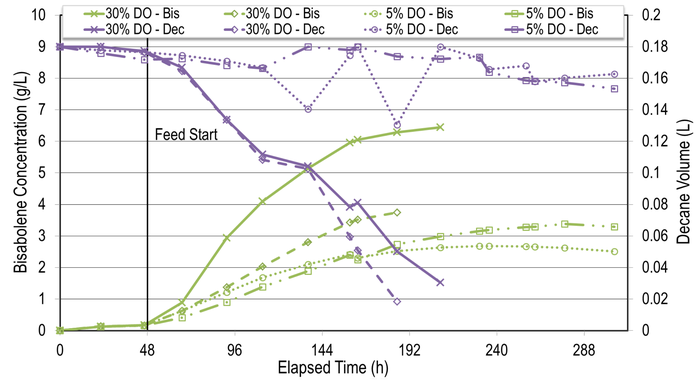


Figure 4. Severe decane evaporation in 30% reactors compared to 5% reactors counteracts higher bisabolene titers observed in 30% reactors

$$V_t^{dec} = \frac{V_0^{dec}[H_0]}{[H_t]}$$

V^{dec} – Decane volume
$[H]$ – Heptadecane concentration

Table 2. Bisabolene yield on glucose

	A1 30%	A2 30%	A3 5%	A4 5%
Bisabolene Yield (g g^{-1} glucose)	0.019	0.013	0.010	0.020

Summary

- Demonstration of bisabolene production at 1.8 L resulted in accumulation levels between 2.5 and 6.5 g/L, with yields comparable or higher than those reported in literature
- Addition of decane overlay during the initial batch phase is both unnecessary and undesirable as bisabolene production is low during this phase. Furthermore, decane has been reported to inhibit cell growth, and its addition resulted in inaccurate DO readings leading to oxygen limitation during batch growth.
- Controlling DO at 30% results in better cell growth and higher bisabolene accumulation, but solvent stripping limits ability to recover product
- Controlling DO at 5% results in lower bisabolene accumulation and high ethanol accumulation, due to oxygen limitation, but mitigates stripping of product and solvent
- In future runs decane should be added after feeding has initiated to prevent erroneously high DO readings leading to oxygen limitation. DO will be controlled at 15% to minimize solvent/product stripping, while preventing oxygen limitation.

Acknowledgements

This work was funded by DOE – EERE (Contract No. DE-AC02-05CH11231)
We would like to thank our JBEI collaborators.