# Bisabolene Production Scale Up Work at ABPDU

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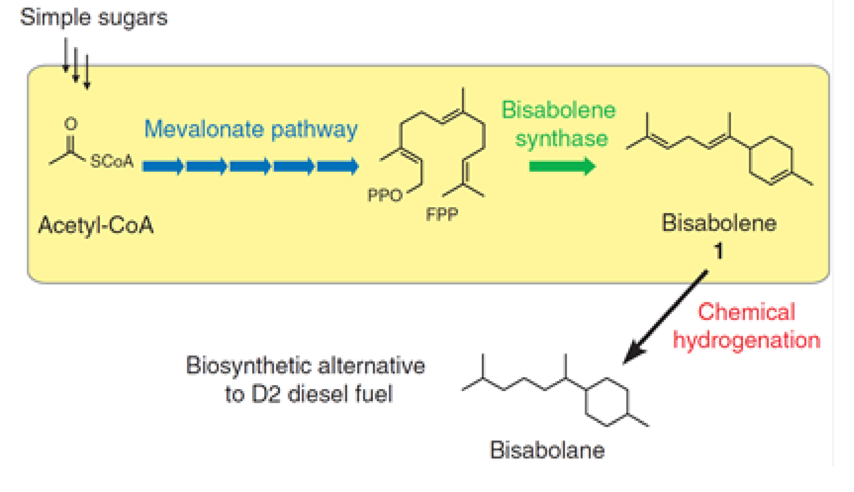
## Introduction

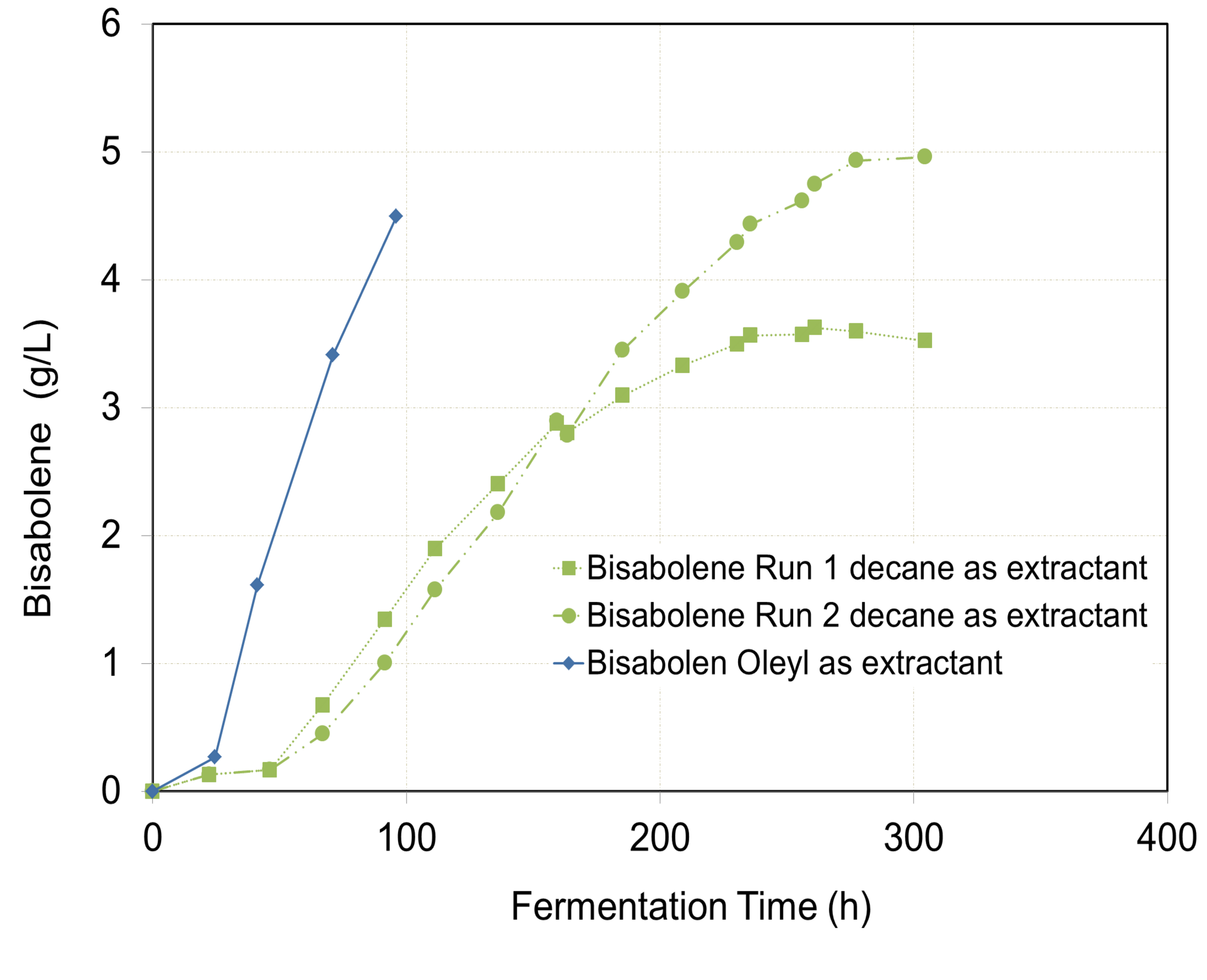
Biosynthic bisabolene is a precursor to bisabolane – a potential renewable diesel fuel alternative. Bisabolane has attractive physical properties including a much lower cloud point (-78°C) than traditional diesel (-35°C) making bisabolane a potential cold weather additive to current fuels.

Scientists at ABPDU have scaled up the biosynthetic production of bisobolene using the *S. cerevisiae* from a bench scale prototype to a 19 liter fed-batch production process which is more indicative of potential commercializable technology. The use of an immiscible organic solvent (oleyl alcohol) in the fermentation roth allows for in-situ product recovery thereby reducing product inhibition and boosting yield. Production at this scale provides clarity on the path forward with bisabolene technology by answering some questions and raising more, particularly about viable recovery strategies.

## Microbial Pathway for Bisabolene Production[1]



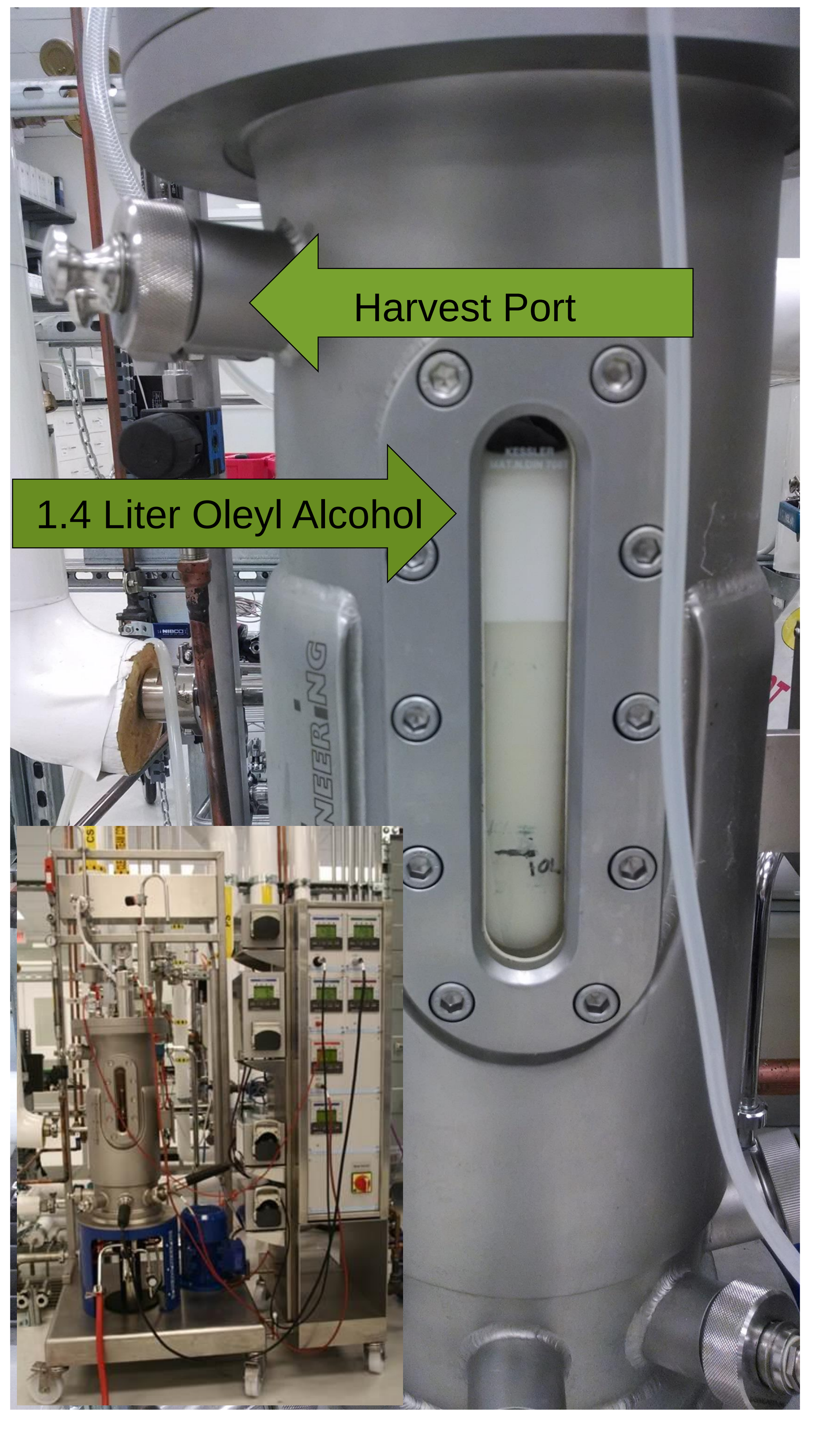

## Extractant Choice Affects Bisabolene Production



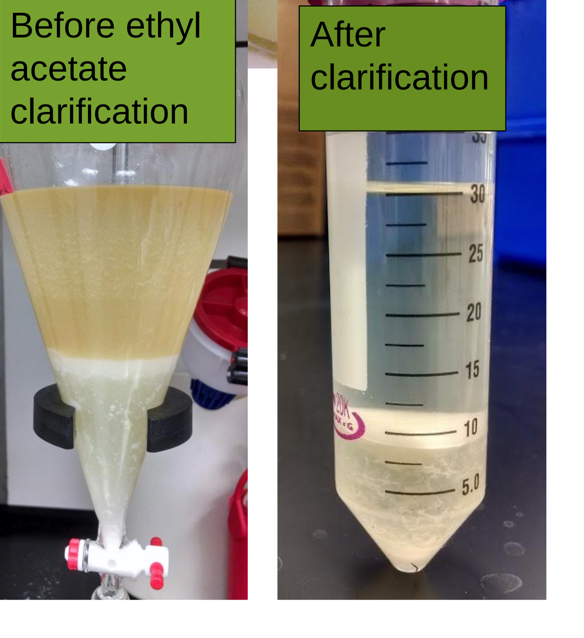

Olyel alcohol is selected based on, low toxicity to cells, degree of stripping, immiscibility with water, high distribution coefficient towards bisabolene and commercially available at low cost.

## 19L Fed-batch Fermentation with In Situ Oylel Alcohol

Strain:
S. cerevisiae CEN.PK2 – JBEI-4734
Batch Medium:
Defined with 15 g/L glucose & 5 g/L galactose
Feed Medium:
Defined with 500 g/L glucose & 5 g/L galactose
Extractant:
Oleyl alcohol, 10% v/v before feed
Dissolved Oxygen (DO):
15% saturation controlled by agitation & aeration
Temperature:
30°C
pH:
5.0 using 4N NH4OH

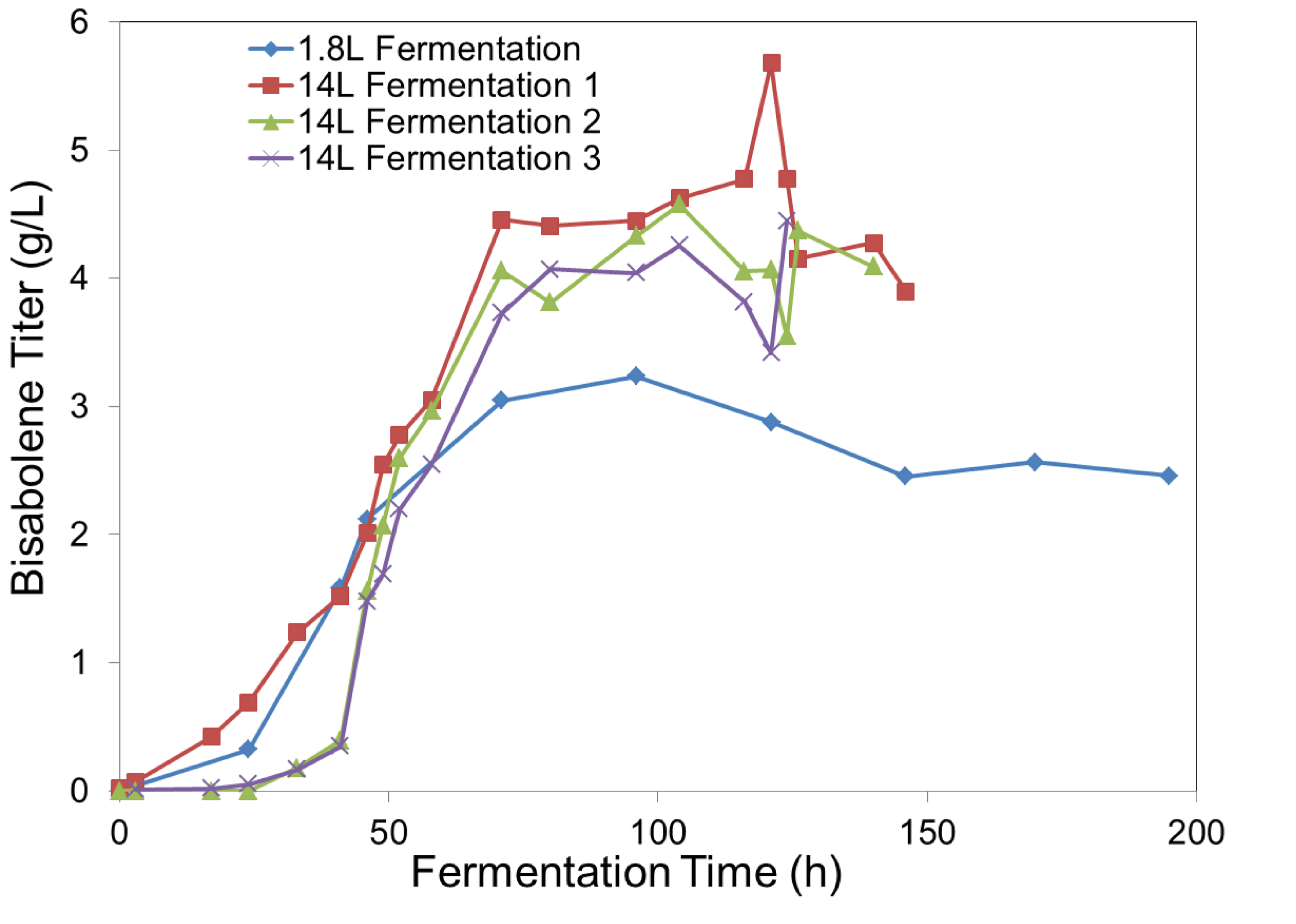



- ❑ Low density in situ oleyl alcohol rises to the top of the reactor.
- ❑ Harvest is performed by pumping water to the bottom of the reactor and collecting fractions as they exit the top port.
- ❑ Bisabolene's boiling point is much lower than oylel alcohol allowing for later distillation of the end product.


- ❑ Recovered organic phase settled (24-72h), a an emulsion of olyel alcohol and cellular components persisted in 2/3rd of recoverable extractant.
- ❑ The emulsion was then broken with additional solvents, ethyl acetate being the most successful.

## Improved Bisabolene Production at 19L Scale




Bisabolene titers and overall productivity reach higher levels in the 19L reactor.

## Conclusion:

- ❑ Using oleyl alcohol over decane as an extractant enhanced productivity and bisabolene yields
- ❑ Product titer was improved in scale up
- ❑ Controlling DO at 15% results in better cell groth and higher bisabolene titers
- ❑ An oleyl alcohol in situ extraction poses challenges due to formation of emulsions which makes recovery product difficult.
- ❑ Returning olyel alcohol to original purity may make the solvent reusable in future production runs.
- ❑ More work is necessary to develop a means of extracting bisabolene in situ and then running full distillation separation.

## Acknowledgements

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1. P. Peralta-Yahya et al. Identifi cation and microbial production of a terpene-based advanced biofuel: nature communications , 2011, (2:483), 1-8