Lawrence Berkeley National Laboratory

Recent Work

Title
Production of the gasoline additive 3-methylanisole via fermentation of cellulosic sugars.

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Authors
Sundstrom, Eric
Hubbard, Stephen
Tachea, Firehiwot
et al.

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SUMMARY REPORT

Objective

The objective of this study was to scale-up the biological production of 3-methylanisole (3-MA) via the intermediate m-cresol, a proprietary fermentation pathway developed by Rho Renewables. 3-MA is a promising fuel oxygenate capable of increasing the octane of gasoline when used as a blendstock. The intermediate in this pathway, m-cresol, is a high volume commodity chemical with applications as a flavor and vitamin intermediate, and as a disinfectant. At the initiation of the CRADA, Rho Renewables had demonstrated proof of concept for 3-MA production in yeast at shake-flask scale but had not demonstrated this technology in controlled 2L fermentations with cellulosic sugars. In addition, due to the high volatility of 3-MA, the recovery process was yet to be developed.

ABPDU Project Team: Eric Sundstrom, Stephen Hubbard, Fre Tachea, Deepti Tanjore, and Todd Pray

Rho Project Team: Jianping Sun, Phil Barr

Deliverables

1. Development of xylose-fermenting strain for m-cresol and 3-MA production from cellulosic sugars

Rho Renewables collaborated with the Mukhopadhyay lab at LBNL to be able to produce a xylose-consuming strain that could convert the C5 sugar along with glucose to produce 3-MA. Rho Renewables delivered the strain after testing it at the shake-flask level.

2. Technology transfer for fermentation baselining

Shake-flask and 2L scale fermentations indicated that the yeast provided by Rho Renewables was able to produce about 2 g/L m-cresol, as observed at the collaborators’ facilities.
3. **Cellulosic sugar production**

Dilute alkali pretreatment and saccharification of corn stover was performed to produce cellulosic sugars with glucose and xylose concentrations of about 25 and 4 g/L, respectively.

4. **Demonstration of 3-MA production from cellulosic sugars in 2L fermentations**

Production of 3-MA from xylose was documented using a two strain fermentation, in which m-cresol was produced by a xylose-utilizing yeast strain followed by conversion of m-cresol to 3-MA by a glucose-utilizing strain. Titers of 2.13 g/L were observed for m-cresol when cellulosic sugars were used for fermented. Titers of 200 mg/L were observed for 3-MA captured via extractive fermentation.

5. **Development of capture and analysis techniques to quantify 3-MA production in aerated liquid culture**

Recovery of volatile 3-methylanisole from fermenter off-gas was documented using both *in situ* extractive fermentation and ex-situ solvent traps. Two methods proved successful for capture of 3-MA: use of an external solvent trap containing oleyl alcohol and use of an oleyl alcohol overlay for *in situ* extractive fermentation. This work demonstrates the feasibility of m-cresol and 3-methylanisole production under industrially relevant conditions and helps pave the way for larger scale production.

**Summary**

Researchers at Rho Renewables and the Advanced Biofuels Process Demonstration Unit at Lawrence Berkeley National Lab have successfully demonstrated a system for biological production of m-cresol and 3-methylanisole. Both molecules are valuable commodity chemicals; 3-methylanisole has favorable properties as a fuel additive while m-cresol is widely used as a flavor and vitamin intermediate. Recovery of volatile 3-methylanisole from fermenter off-gas was documented using both *in situ* extractive fermentation and *ex situ* solvent traps. This work demonstrates the feasibility of m-cresol and 3-methylanisole production under industrially relevant conditions and helps pave the way for larger scale production.