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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.

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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.