**CRADA Final Report** **Date:** May 2016
**PI:** Todd Pray

**CRADA No. FP2151_____**
**LBNL Report Number_____**

1. Parties: Microvi

2. Title of the Project: "Novel Biocatalytic Platform for Ethanol Production from Lignocellulosic Feedstock"

3. Summary of the specific research and project accomplishments:
(Were the goals of the CRADA achieved? Include relevant information but do not include proprietary or protected CRADA information.)

The goals of the CRADA were achieved by illustrating the scalability of immobilized yeast technology, demonstrating lignocellulosic feedstock consumption by the immobilized cells, and confirming Microvi's proprietary polymer matrix ethanol toxicity tolerance. We conducted fermentations at 2L and 300L scales. For carbon source, we performed pretreatment and saccharification at 100L scale to produce lignocellulosic sugars with glucose and xylose.

| Tasks Achieved | Party (LBNL, Participant, Both) | Delivered to Other Party? |
|---|---|---|
| Small-scale pretreatments/ saccharification testing | LBNL | Yes |
| Analytical profiling | Microvi | Yes |
| Bench-scale pretreatments/ saccharification testing | LBNL | Yes |
| Analytical profiling | Microvi | Yes |
| Complete Final DOE report | LBNL | Yes |

4. Deliverables:

I. Provision of raw materials

Microvi team procured and transferred all raw materials that required for fermentation and deconstruction. We conducted corn stover biomass pretreatment and saccharification at bench and pilot scales to produce enough Lignocellulosic hydrolysate for 2L and 300L scales fermentation. The sugars used for fermentation were derived from pretreated cellulosic biomass where both glucose and xylose were present in significant amounts.

II. 4 ×2L Bioreactor Process profiling and analytics campaign

The 2L bioreactor fermentations in batch mode with 75 g/L Micovi's catalyst loading rate consumed the 52 g/L initial batch glucose concentration within 14 hours while the ethanol concentration was 22.7 g/L. After depleting the initial glucose, xylose concentration decreased from 16.3 g/L to 0.296 g/L within 45 hours. For the case of continuous fermentation, once the glucose and xylose depleted,

the influent and the effluent pumps were started at rate of 0.103 L/hr and the glucose and ethanol concentration in the reactor stabilized around 11 g/L and 14 g/L for 6 days.

III. 300L Scale-up and analytics campaign

The 300 L batch fermentation result indicated that the initial 51.9 g/L glucose was completely consumed within 7 hours; however, the 21.3 g/L xylose was then consumed with a much lower uptake rate. Ethanol production of 22.1 g/L at this scale indicated that Microvi's immobilized catalysts scaled well from 2L to 300L while consuming cellulosic sugars.

5. Identify publications or presentations at conferences directly related to the CRADA?
No publications at the time of this report.

6. List of Subject Inventions and software developed under the CRADA:
(Please provide identifying numbers or other information.)

7. A final abstract suitable for public release:
(Very brief description of the project and accomplishments without inclusion of any proprietary information or protected CRADA information.)

Ethanol manufacturing from lignocellulosic biomass has been challenged with toxic molecules in lignocellulosic hydrolysates, such as HMF, acetic acid, and furfural. Furthermore, ethanol toxicity has hindered fed-batch fermentations that can lead to high titers of the product. The Advanced Biofuels Process Demonstration Unit at Lawrence Berkeley National Laboratory Microvi's proprietary polymer matrix to immobilize yeast with hydrolysate in 2 and 400L fermenters. Lignocellulosic hydrolysate was produced from large-scale alkali pretreatment and enzymatic saccharification (100L) of corn stover. Glucose and xylose from cellulosic sugars were the sole carbon source in the media that contained yeast extract and peptone. Glucose and xylose were completely consumed by immobilized yeast to produce 25.67 and 21.86 g/L ethanol at 2 and 300L scales, respectively. At 2L scale, continuous fermentation was performed by feeding lignocellulosic hydrolysate media and removing fermentation broth at a dilution rate of 0.083 $h^{-1}$ .We illustrated the scalability of this immobilized yeast technology, demonstrated lignocellulosic feedstock consumption by the immobilized cells, and confirmed the Microvi's proprietary polymer matrix ethanol toxicity tolerance.

8. Benefits to DOE, LBNL, Participant and/or the U.S. economy.

Lignocellulosic biomass conversion to ethanol is limited by consumption of C5 sugars and toxicity of ethanol towards yeast. By utilizing Microvi's immobilization technology, we were able to convert lignocellulosic sugars efficiently. Batch and continuous fermentation processes at 2L and 300L scales using Microvi's technology could be useful information for any company that is looking to improve their ethanol production process. The process has reduced footprint of capital and operational costs

due to operation in a continuous mode. This third-party evaluation can be used for marketing and industrial acceptance of Microvi's technology.

9. Financial Contributions to the CRADA:

| | |
|---|---|
| DOE Funding to LBNL | $20,000 |
| Participant Funding to LBNL | $0 |
| Participant In-Kind Contribution Value | $5,000 |
| Total of all Contributions | $25,000 |