CRADA Final Report Form

Date: 06/18/2020

PI: Deepti Tanjore

CRADA AWD No.: AWD00001946 **CRADA FP No.:** FP00004616

LBNL Report Number: LBNL-2001338

OSTI Number: _____

1. Parties: ZymoChem, Inc.
   (Identify Parties to the CRADA)

2. Title of the Project: Development of a Novel Bioprocess for Synthesizing and Purifying Adipic Acid

3. Summary of the specific research and project accomplishments:
   (Were key major goals of the CRADA achieved?)

   a) Process development & optimization for the anaerobic production of adipic acid at the 2 L scale: aerobic fermentation (cell growth) at the 50 L scale (*stage 1*) followed by cell harvest via centrifugation, washing of cells and resuspension of cells, and then fed-batch high cell density (HCD) anaerobic resting cell biotransformation (RCB) at the 2 L scale (*stage 2*).

   b) Process scale-up for the aerobic fermentation at the 300 L scale (*stage 1*) followed by a fed-batch HCD anaerobic RCB at the 50 L scale (*stage 2*), in turn producing ~1 kg of the production strain's target bioproduct (non-purified).

   c) Recovery of adipic acid-hexamethylenediamine (AA-HMDA) studies done via wiped film evaporation. The final products from both the process development and scale-up studies were delivered to ZymoChem, Inc.

4. Deliverables:

Deliverables met	Party (LBNL, Participant, Both)	Delivered to Other Party?
Process optimization for the anaerobic production of adipic acid at the 2 L scale (both at batch and fed-batch phase)	LBNL	Yes
Process scale-up for the aerobic fermentation at the 300 L scale (*stage 1*) followed by a high cell density (HCD) anaerobic resting cell biotransformation (RCB) at the 50 L scale (*stage 2*)	LBNL	Yes
Recovery of AA-HMDA studies with wiped film evaporator	LBNL	Yes
Delivered final product to Zymochem, Inc	LBNL	Yes

5. Identify (list below) and attach all publications or presentations at conferences directly related to the CRADA: N/A

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

7. A final abstract suitable for public release:

   Through DOE's Bioenergy Technologies Office and the Energy Efficiency and Renewable Energy Small Business Voucher (SBV) Pilot, ZymoChem, Inc received support to work with the ABPDU to accelerate the development of a novel adipic acid (AA) bioprocess by evaluating technologies to demonstrate the following:

   1) Process development of AA via ZymoChem's carbon-conserving (C^2) pathway in 2L bioreactors, using industrial feedstocks, and as a salt with hexamethylenediamine (HMDA), i.e., the industrially desired form of AA as AA-HMDA is the precursor for nylon 6,6 synthesis.
   2) Pilot scale aerobic fermentation at the 300 L scale (*stage 1*) followed by a high cell density (HCD) anaerobic resting cell biotransformation (RCB) at the 50 L scale (*stage 2*).
   3) Demonstration of a viable recovery process at the 50 L scale for purifying the product (i.e., AA-HMDA salt) and deliver multiple kg-quantity batches of purified product for further testing.

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

ZymoChem is a synthetic biology startup whose mission is to develop processes for producing chemicals from bio-based feedstocks with superior economics compared to current petroleum-based processes.

9. Financial Contributions to the CRADA:

DOE Funding to LBNL	$200,000
Participant Funding to LBNL	$0
Participant In-Kind Contribution Value	$50,000
Total of all Contributions	$250,000