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Title

Remediation of recalcitrant and emerging organic pollutants using bacteria and their extracted enzymes

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CRADA Final Report Form
Date: November 20, 2019

PI Todd Pray

CRADA No. FP00007386

LBNL Report Number_____

OSTI Number_____

1. Parties:
   Lawrence Berkeley National Lab
   Allied Microbiota

2. Title of the Project: Remediation of recalcitrant and emerging organic pollutants using bacteria and their extracted enzymes

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

   The CRADA as contracted had two primary goals:

   1. The production of surfactants (glycolipids and glycoproteins) and proteases in the broth during bioreactor-based cultivation of Allied Microbiota's bacterial culture.

   2. The initial development of a method for the isolation of toluene dioxygenase and downstream enzymes from biomass produced during bioreactor cultivation.

   The first goal was met, via completion of a 2L bioreactor campaign at ABPDU optimized for production of surfactants and proteases. Bioreactor performance was documented and a list of future process optimization strategies was provided to Allied Microbiota. Key process improvements included strategies to minimize evaporation, minimize foaming, optimize and standardize seed culture preparation, and supply additional carbon to fermentation via a fed-batch approach. Following completion of the first portion of the work, work at ABPDU was paused awaiting an advance from Allied Microbiota on the CRADA funding. The advance was not received, and the project was ultimately closed out prior to completion of the second phase.

4. Deliverables:

Deliverables met	Party (LBNL, Participant, Both)	Delivered to Other Party?

Technology transfer of Allied Microbiota strains and protocols to ABPDU for further process development.	Allied Microbiota	Yes
Completion of a process development campaign in 2L bioreactors, compilation of data, and preparation of a summary report with process development recommendations.	LBNL	Yes

5. Identify 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:

   Researchers at the LBNL Advanced Biofuels Process Development Unit (ABPDU) engaged in a collaborative research agreement with Allied Microbiota, to demonstrate improved cultivation of thermophilic organisms known to secrete thermally stable enzymes suitable for bioremediation applications. During the course of the project, ABPDU researchers demonstrated a fed batch bioreactor cultivation method that improved process intensity and process consistency, helping to reduce the production cost of these promising enzymes.

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

   Moving the Allied Microbiota process from shake flask cultivation to a more sophisticated bioreactor setup enabled significant process improvements for production of bioremediation enzymes with high activity for remediation of organic contaminants, along with high thermal stability. For DOE, this work contributes to the success of a company providing an innovative solution for bioremediation of sensitive sites, including oil and gas production and storage sites, chemical production and storage facilities, and contaminated sites on government lab. For LBNL, this work provided an opportunity to engage with industry and develop expertise surrounding cultivation of thermophilic organisms with industrial applications. For the broader US economy, this work could ultimately contribute to a successful commercial enterprise while enhancing US expertise in bioremediation, enabling more cost-effective remediation of contaminated areas.

9. Financial Contributions to the CRADA:

DOE Funding to LBNL	$0
Participant Funding to LBNL	$30,500 planned ($14,412 actual)
Participant In-Kind Contribution Value	$194,500
Total of all Contributions	$225,000 planned ($208,912 actual)

** "Proprietary Information" means information, including data, which is developed at private expense outside of this CRADA, is marked as Proprietary Information, and embodies (i) trade secrets or (ii) commercial or financial information which is privileged or confidential under the Freedom of Information Act (5 U.S.C. 552 (b)(4)).*