National Laboratory Field Work Proposal
Final Report

Project Title: 1,3-Propanediol via Fermentation-Derived Malonic Acid

Covering Period: February 15, 2001 to June 26, 2002

Date of Report: August 15, 2002

Laboratory: Pacific Northwest National Laboratory, P.O. Box 999, Richland, WA 99352

FWP/OTIS Number: DE-AC06-76RL0 1830 #41721

Other Partners: National Corn Growers Association (NCGA)

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Executive Summary:
The objective of this project was to develop a new process for production of the chemical, 1,3-propanediol (PDO) from agriculturally-derived biomass (glucose). The effort involved development of a new fermentation organism and process for the production of malonic acid and a process to then convert that acid to PDO via a catalyst. As a result, the project scope of work was divided into two primary areas of research, fermentation process development and catalytic process development. Although a substantial effort was made in both areas, the technical process goals were not attained. The perceived technical hurdles remaining to meet those goals were judged to be high and requiring a much larger research investment than originally proposed. Therefore, the project was terminated midway through the scheduled time commitment.

The project evaluated a number of fungal organisms for malonic acid production and identified several candidates. Preliminary tests demonstrated very low levels of malonic acid production in shake flask and fermentor. UV mutagenesis was applied to one of the organisms. Among the survivors screened, mutants with apparent improvement in total acid production (as indicated by qualitative color change of a pH indicator) were observed. In subsequent testing, it was determined that these putative mutants did not produce malonate. No direct selection for malonic acid overproduction is available; in fact, the pathway is unknown, and no economically feasible malonic acid specific detection method is available. The research required to achieve the fermentation yield goals is beyond the scope of this project.

Heterogeneous catalysis for hydrogenation of malonic acid as originally proposed could not be achieved after testing a number of catalyst materials. Evaluation of modified malonic-based substrates, such as esters, was similarly unsuccessful. Initial attempts at the use of homogeneous catalysts, at more mild processing conditions, led to improved PDO production but at levels well below the technical goals and with higher processing costs expected with this type of processing.

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Project Objective:
The objective of this project was to develop a new process for production of the chemical, 1,3-propanediol (PDO) from agriculturally-derived biomass (glucose). The goal was to develop a new fermentation organism and process for the production of malonic acid in high yields and then convert that acid to PDO at high yield via a catalyst.

Background:
PDO is used to make a new class of polymers with enhanced functionality. The market for PDO is currently about 100 million pounds and is growing very rapidly, as new products are developed to capitalize upon the functionality of the polymers that can be synthesized from PDO. This market offers a significant opportunity to develop new, cost-competitive processes that utilize renewable feedstocks to produce PDO. Introduction of such processes would avoid the use of more petroleum, provide substantial energy savings (10 to 19 trillion Btu), and afford significant market penetration for the burgeoning bio-products industry. Corn-derived glucose is a logical consideration for a renewable feedstock to produce PDO, as corn refiners are a concentrated, low-cost source of biomass that can achieve the production scales necessary to match the projected demand for PDO. Research conducted at PNNL indicates that it is possible to develop a new fermentation system exploiting proprietary collections of filamentous fungi to convert corn-derived glucose to an organic acid intermediate that can then be converted to PDO using one of PNNL's proprietary catalysts. The proposed program supported development of a new fermentation organism to produce malonic acid at high yields, as well as engineering design to develop fermentation processes to produce malonic acid at suitable scale and purity. The program also supported development and demonstration of the catalysis processes to convert the malonic acid to PDO. Finally, the program supported scale-up engineering and demonstration of the processes at an appropriate pilot scale.

Results:
The discussion in this heading is limited as the project was completed under a Cooperative Research and Development Agreement (CRADA) and technical results are CRADA protected information.

<u>Fermentation Process Development:</u>
An analytical method employing ion chromatography was established to measure malonate in culture filtrate and for monitoring the bioprocess experiments in the fermentors. Based on a preliminary observation that some fungi may be producing malonate a review of the literature was performed.

An in-house collection of Basidiomycetes and other isolates representing the two other major groups of fungi, the Zygomycetes and Ascomycetes, were screened for malonic acid production. Isolates of the basidiomycete *Phanerochaete chrysosporium* were acquired and added to the collection because malonic acid was reported in the literature to be produced concurrent with the production of lignin degrading enzymes. Malonic acid was detected in culture filtrates of several fungi including Phaneroachaete.

Phanerochaete was selected for additional experiments to improve the yield of malonate. Several experiments were run using P. chrysosporium in small fermentors. These experiments confirmed the presence of malonic acid. The same time course of malonate synthesis was observed in the fermentor and shake flasks.

Phanerochaete was selected for strain improvement experiments to evaluate the level of effort required to achieve the targeted yield of malonate per liter. Spores were exposed to mutagen. An agar growth medium containing a pH indicator was used to screen mutagenized spores for improved acid production. No improved mutants were found.

Catalytic Process Development:
Relevant process chemistry from the literature was reviewed. Analytical standards were obtained, as far as possible. Analytical methods were identified and reduced to practice.

The malonic acid was tested as a 10% solution in water to model the potential fermentation product. A suite of catalysts and processing conditions were tested. The thermal instability of the malonic acid (a beta-keto organic acid) was problematic. No catalyst tested could produce the desired product, 1,3-propanediol (PDO), even in processing tests conducted at lower temperatures. Neutralization of the acid with caustic had little effect on the processing result.

Low temperature reactivity was investigated with several highly active candidate catalysts at 80°C where the malonic acid (in ~15wt% water solution) was relatively stable toward thermal decomposition. No detectable amount of PDO was produced after 24 hours at reaction conditions. A subsequent test of malonic acid hydrogenation in organic solvent was similarly unsuccessful.

Tests were also performed with a more stable form of the malonic acid (its dimethyl- or diethyl-ester) and improved processing was evident. The methyl diester could be processed neat at up to 250°C with little evidence of decomposing. The intermediate was identified in several of the tests. However, no significant amount of PDO was produced in any of the tests.

The ethyl diester tests gave a minor PDO product in only one case. PDO was found as an intermediate because it continued to react and disappear. Additional tests of PDO at the same hydrogenation conditions with these catalysts confirmed the instability of the PDO product under these conditions.

We have also evaluated the use of homogeneous catalysts, which could have hydrogenation activity for the malonic acid at lower temperature. A literature search indicated that three methods could be applicable palladium phosphine catalysis, borohydride reduction, and ruthenium phosphine catalysis.

Catalysis by palladium phosphines involves initial equilibration of the carboxylic acid with trimethylacetic anhydride to form a mixed anhydride, followed by selective hydrogenation to the aldehyde of the carboxylic acid. A second reduction by another catalyst would be necessary to reduce the intermediate aldehyde to the desired alcohol. In our studies, nonanoic acid was nearly quantitatively reduced to nonyl aldehyde. Indications are that malonic acid was also reduced. However, it was found that the malondialdehyde product was unstable under the reaction conditions, apparently condensing in a bimolecular reaction. Attempts to use a second catalyst to simultaneously hydrogenate the aldehyde as it was formed had limited success.

Borohydride reduction successfully reduced nonanoic and malonic acids to the respective alcohols. Nonyl alcohol was prepared in 74% yield. PDO was produced in 16% yield, but it appeared that more of this product might have been bound as a borate ester. No attempts were made to optimize either reduction.

Open Items:
The CRADA was signed in November 2001 by NCGA, PNNL, and DOE.

Patents: none.

Publications/Presentations:
M. T. Kingsley, R. A. Romine, L. L. Lasure (2002)
Effects of Medium Composition on Morphology and Organic Acid Production in *Phanerochaete chrysosporium,* Poster presentation at the Annual Meeting American Society of Microbiology

Milestone Status Table:

ID Number	Task / Milestone Description	Planned Completion	Actual Completion	Comments
1	Complete proof of concept demonstration with catalyst at >80% yield of 1,3-PDO from malonic acid	12/15/01 new planned completion date is 12/15/02	Terminated	This process has not been straightforward; new ideas in homogeneous catalysis were investigated.
2	Select production organism, define fermentation parameters for >1 g/L malonic acid production	2/15/02 new planned completion date 9/30/02	Terminated	Progress was made, but slowly.
3	Complete competitive process economics to verify energy, environmental, and economic impact	2/15/02 new planned completion date 12/30/02	Terminated	initial model building was supported by NCGA
4	Demonstrate 1,3-PDO synthesis from fermentation product by achieving >80% yield of 1,3-PDO	12/15/02	Not started.	
5	Complete process economics of new technology to support scale up and allow comparison of energy, environmental, and economic impact	10/15/02	Not started.	
6	Confirm pilot demonstration partner to fulfill 50% cost share	10/15/02	Terminated	negotiations were underway with GPC
7	Integrate fermentation, separation, and catalysis processes with fermentation yield at >10 g/L	2/15/03	Not started.	
8	Complete pilot demonstration at scale of 100 g/hr showing >50 g/L malonic acid concentration and overall yield of >60% of 1,3-PDO from glucose	2/15/04	Not started.	