**C/ORNL/99-0552**

Chemical Technology Division

**CRADA Final Report**
**For**
**CRADA Number** ORNL99-0552

# PRODUCTION OF SUCCINIC ACID FROM LIGNOCELLULOSIC HYDROLYSATES

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Prepared by the
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For the U. S. Department of Energy
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# PRODUCTION OF SUCCINIC ACID FROM LIGNOCELLULOSIC HYDROLYSATES

**Final ORNL CRADA Report #C/ORNL99-0552**
**ANL CRADA Report #C09801701**

Oak Ridge National Laboratory[a*]
Argonne National Laboratory[b]
and
Applied Carbochemicals, Inc.[c]

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* Managed by UT-Battelle, LLC. under contract DE-AC05-00OR22725 with the U.S. Department of Energy.

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## CONTENTS

## EXECUTIVE SUMMARY

Renewable feedstocks derived from agricultural or forest products offer a potential alternative to fossil feedstocks for the production of chemicals. Initial efforts to establish this new technology often focus on developing processes that convert glucose derived from corn wet milling into the desired products. However, the use less expensive, more complex feedstocks derived from other biological materials could provide significant improvements in the economics of a process, making them more competitive with existing petrochemical processes. The use of lignocellulosic hydrolyzates could decrease feedstock sugar costs by 50-70% compared to corn glucose.

Our goal was to modify or develop microbial strains capable of succinate production from xylose and to test the utility of this new modified microbes for production of organic acid (e.g., succinic acid) from industrial lignocellulosic hydrolyzates as well as to improve on the prior bio-based succinic acid process. The approach was to develop an improved xylose-metabolizing E. coli mutant and to test the expression of utility of this new modified microbes for production of organic acid (e.g., succinic acid) from industrial lignocellulosic hydrolyzates. The team would also improve on the prior bio-based succinic acid process by focusing on the critical high cost steps in separation.

ORNL tested the base case strain (ATCC ATCC 202021; US Pat # 5770435) and subsequent derived strains for xylose metabolism and for fermentation of mixed sugars and actual hydrolyzates. Arkenol provided compositions and actual lignocellulosic hydrolysates. ANL worked on strain development, modification, isolation and identification of critical mutations. The E.coli, ATCC 202021, was not capable of xylose metabolism, but a subsequent mutant was, eliminating the need to genetically engineer insertion of xylose genes. Another better xylose metabolizing mutant was isolated (E. coli, AFP 184, patent pending). This was also tested on actual lignocellulosic hydrolyzates; Arkenol, Inc., provided rice straw hydrolysate as a representative feedstock. We also determined the critical genes (e.g., *ptsG*) for the enhanced succinate production.

ACC worked on economics, new markets developments, scale-up and improvement of the separations. ACC reduced the process costs by modification of the separation process from membranes to crystallization reducing the separations cost by over half. ACC has shown other markets for deicers, ester solvents, and succinic acid itself. Economic estimates showed this process to be competitive with petrochemical-based production of the same products and that hydrolyzates would further decrease the cost. ACC has recently completed a fermentation demonstration run at 150,000L using glucose.

Key technical achievements were:

- Production of succinic acid from lignocellulosics (mixed xylose and glucose)
  - All AFP three strains can produce SA from xylose/glucose mixtures
    - AFP 184 is best in yield and concentration (patent pend.)
    - *ptsG* mutant is key in shift to good SA production
  - All convert actual Arkenol hydrolyzates w/o inhibition
  - Xylose fermentation is linked to glucose conversion
- Investigation of the use of spent medium for cell growth and succinic acid production
- ACC Separations by improved crystallization to eliminate high energy membrane step

## INTRODUCTION AND PURPOSE

The purpose of this Cooperative Research and Development Agreement (CRADA) between UT-Battelle, LLC (UTB) and Argonne National Laboratory (ANL), (collectively referred to as the "Contractors"), and Applied CarboChemicals, Inc. (Participant), is to add and test new metabolic activities to existing microbial catalysts for the production of succinic acid from renewables. In particular, we seek to add to the existing organism the ability to utilize xylose efficiently and simultaneously with glucose in mixtures of sugars or to add succinic acid production to another strain and to test the value of this new capability for production of succinic acid from industrial lignocellulosic hydrolysates. The Contractors and Participant are hereinafter jointly referred to as the "Parties."

Research to date in succinic acid fermentation, separation and genetic engineering has resulted in a potentially economical process based on the use of an *Escherichia coli* strain AFP111 with suitable characteristics for the production of succinic acid from glucose. Economic analysis has shown that higher value commodity chemicals can be economically produced from succinic acid based on preliminary laboratory findings and predicted catalytic parameters. The intial target markets include succinic acid itself, succinate salts, esters and other derivatives for use as deicers, solvents and acidulants. The other commodity products from the succinic acid platform include 1,4-butanediol, γ-butyrolactone, 2-pyrrolidinone and N-methyl pyrrolidinone. Current economic analyses indicate that this platform is competitive with existing petrochemical routes, especially for the succinic acid and derivatives.

This research builds on a previous CRADA project which included the Parties. The goal of the earlier CRADA project (ORNL 96-0407) was to develop and demonstrate from bench results an economically promising and competitive process for the production of chemical derivatives from biologically produced succinic acid. AFP111, a new strain discovered at ANL, has been scaled to 500-L scale using a process developed at the Oak Ridge National Laboratory (ORNL) with corn glucose as the feedstock. The key need here is to further lower the costs by the utilization of a cheaper feedstock (lignocellulosics from renewables or wastes).

The report will present the planned CRADA objectives followed by the results. The results section has a combined biocatalysis and fermentation section and a commercialization section. This is a nonproprietary report; additional proprietary information may be made available subject to acceptance of the appropriate proprietary information agreements.

## TECHNICAL OBJECTIVES

The overall goal of the CRADA is to develop and test the feasibility of a modified microbe for production of succinic acid from both hexose and pentose sugars (e.g., glucose and xylose, respectively). We will improve the formation of succinic acid from mixtures of sugars by strain AFP111 and its variants through optimization of fermentation conditions and genetic engineering of rate-limiting genes. The initial focus will be on metabolism of xylose and arabinose, including the possible limitation to uptake and metabolism, then on cellobiose utilization. This goal is to be met by obtaining the following key objectives:

1. Metabolic engineering: 1a) Re-expression of gene(s) in E.coli AFP111 most need to be amplified to improve co-utilization of pentoses. 1b) Development of similar succinate mutant from robust xylose fermenter.
2. Fermentation: 2a) Evaluation of the existing strain, AFP111 and its known variants for fermentation of xylose and mixed sugars in fed-batch process. 2b) Evaluation of fermentation and succinic acid production of new variants. 2c) Testing of actual industrial lignocellulosic hydrolysates on the best available microbes.
3. Economic and market analysis of the process with process improvements.

The original CRADA tasks and milestone are shown in the appendix. As seen in the results, all major objectives were met; however some tasks did change during the course of the project.

ANL led the efforts on strain development. ORNL led the fermentation efforts. Davison (ORNL) was the overall project lead. ACC worked on the market development and process scale-up and commercialization. We had conference calls involving the principal investigators from ANL, ORNL, and ACC. ACC provided economic and marketing assessments. ACC utilized a subcontractors and strategic partner to perform some process development and scale-up. Another strategic partner assisted in the process economics. Arkenol, Inc was a nonsignatory partner to this research. They provided in-kind support of actual lignocellulosic hydrolyzates and the compositional analysis.

ORNL is managed now by UT-Batelle, LLC; it was managed by Lockheed Martin Energy Research when the CRADA was signed. As of May 2003, Applied Carbochemicals, Inc. has changed its name to Diversified Natural Products.

## CRADA RESULTS

### Biocatalysis Development (Metabolic Engineering and Fermentation)

#### Summary

The objective of the CRADA "Production of succinic acid from lignocellulosic hydrolysates" was to establish a proprietary strain for the efficient production of succinic acid from inexpensive agricultural feedstocks. Initial research focused on engineering *E. coli* strain AFP111, developed under an earlier CRADA for the production of succinic acid from glucose, to allow metabolism of the mixtures of sugars found in lignocellulosic hydrolysates. An actual hydrolysate was provided by Arkenol, Inc, to allow real-world evaluation of the strain's performance. Evaluation of AFP111 showed it was unable to metabolize xylose, the major pentose sugar found in lignocellulosic hydrolysates. A mutant of AFP111, called AFPX1, was obtained that had regained the ability to metabolize xylose. This strain was able to convert the mixed sugars in the Arkenol hydrolysate to succinic acid, but only at moderate rates.

Later work, initiated in response to the desire of Applied CarboChemicals to evaluate alternative hosts for succinate production, transferred the mutations that allowed succinic acid production in AFP111 into a different lineage of *E. coli* selected for its vigorous growth. In this case, the parental strain already metabolized xylose. Introduction of the mutations generated a strain, AFP184, that produced succinic acid from the Arkenol hydrolysate significantly better than did AFPX1. The strain produced succinic acid efficiently and reproducibly in the fermenter using only industrial grade medium components containing the lignocellulosic hydrolysate as a source of sugars and corn steep liquor, a byproduct of corn wet milling as a source of nitrogen and other nutrients.

Finally, new strains were constructed in both lineages using entirely non-reverting, knockout mutations to give strains AFP400 and AFP404. A patent application covering the new strains has been filed. The mutant strains were determined to be non-recombinant (not a GMO, genetically modified organism) due to the traditional methods (not genetic engineering) used and thus eliminated a regulatory concern.

#### Details

Renewable feedstocks derived from agricultural or forest products offer a potential alternative to fossil feedstocks for the production of chemicals. Initial efforts to establish this new technology often focus on developing processes that convert glucose derived from corn wet milling into the desired products. However, the use less expensive, more complex feedstocks derived from other biological materials could provide significant improvements in the economics of a process, making them more competitive with existing petrochemical processes. In this CRADA, we developed new bacterial strains that can take

advantage of the cheaper sugars available in hydrolysates of lignocellulosic material for the production of succinic acid, a precursor of established building blocks of many commercially used polymers. Arkenol, Inc., provided rice straw hydrolysate as a representative feedstock. Table 1 lists the typical composition of rice straw before hydrolysis.

Table 1. Composition of rice straw.

| Feedstock component | Dry wt% |
|---|---|
| Glucan | 38.9 |
| Mannan | 0.0 |
| Galactan | 0.5 |
| Xylan | 20.4 |
| Arabinan | 3.4 |
| Lignin | 13.5 |
| Extractives | 5.3 |
| Ash | 18.0 |

After hydrolysis and removal of the solid lignin and ash present in the material, the major sugars present in the Arkenol hydrolysate are glucose and xylose, in approximately a 2:1 ratio, with traces of arabinose.

*E. coli* normally produces a mixture of products from glucose, but strain AFP111, created by addition of three mutations into strain W1485, converts glucose efficiently to succinic acid (Fig. 1). Two of the mutations block the enzymes that normally produce formate and lactate. The third mutation, that allows succinate formation, is in the *ptsG* gene. This gene encodes a protein involved both in the transport of glucose and in the regulation metabolism of mixtures of sugars. It represses the use of other sugars when glucose in present, but because it is absent AFP111 should be able to metabolize lignocellulosic hydrolysates well. However, AFP111 was unable to grow on xylose. Literature reports indicate that *E. coli* strains that have been cultured in the laboratory for many years often lose their ability to metabolize xylose, and that in some cases this ability can be regained simply by forcing the strain to grow on xylose exclusively. Plating of AFP111 on xylose medium resulting in spontaneous revertants that were able to grow efficiently on xylose. Several of these were purified and characterized more extensively. All were found to metabolize xylose similarly, and one, designated AFPX1, was studied further. It was shown to convert both the xylose and glucose present in rice straw hydrolysates (Fig. 2).

Figure 1. Metabolism of glucose by W1485 and AFP111.

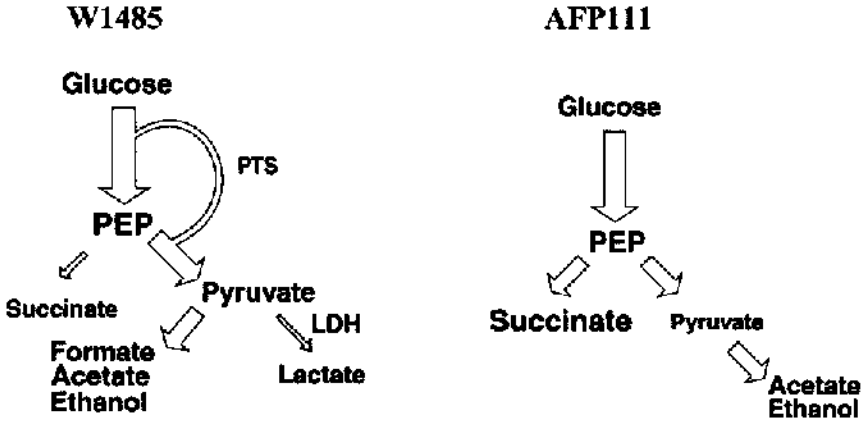
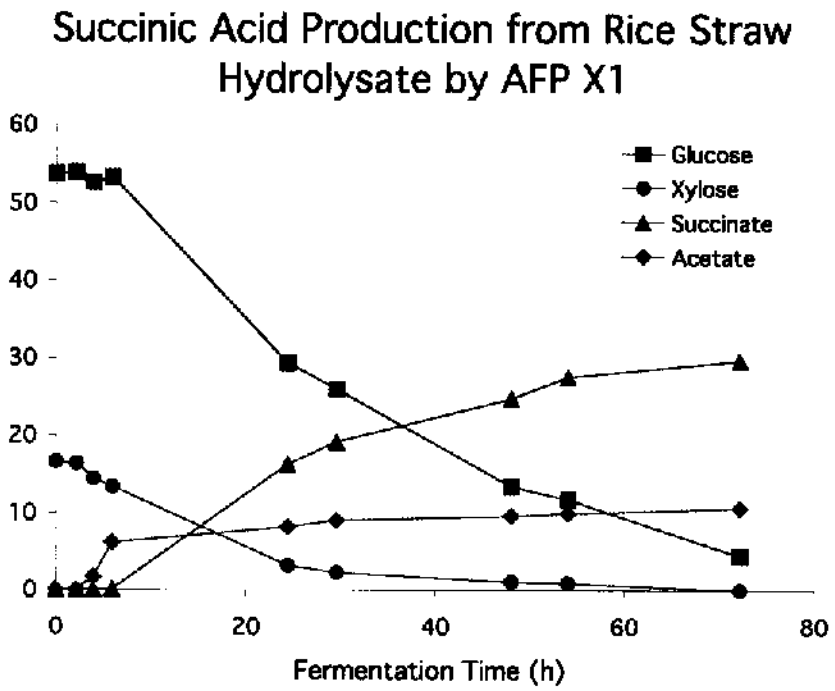


Figure 2. Metabolism of a rice straw hydrolysate by AFPX1.




AFPX1 fulfilled a major objective of the CRADA, generation of a derivative of AFP111 that could convert hydrolysates to succinic acid. However, the rate of succinate formation was slow. In the second year, rather than optimize AFPX1, where the characteristics of the final strain would be limited to some extent by the genetic background of the W1485 lineage, we introduced succinic acid production into a different lineage of *E. coli* that had better growth characteristics. The decision to do this was based in part on the desire, expressed by Applied CarboChemicals, to evaluate alternative, more robust hosts. A wild type strain was obtained from the culture collection of Dr. Edward St.Martin of Argonne National Laboratory. This strain was known to grow very vigorously, and had not been cultured extensively in the laboratory (avoiding the debilitation that can occur when strains are cultured extensively on rich media, such as the loss of the ability to grow on xylose that had occurred in the W1485 lineage). Strain AFP184 was developed by sequentially introducing the three mutations required for succinic acid production into this wild type strain strain (Fig. 3).

AFP184 converted the sugars present in the rice straw hydrolysate to succinic acid rapidly and generated high final concentrations of succinic acid (Fig. 4). The jumps in the concentration of glucose and xylose in this figure reflect additions of more hydrolysate. The rate of formation of succinate by AFP184 was significantly higher than that observed for AFPX1 (compare Figs. 2 and 4). In the initial 24 hours of the production period (starting 6 hours into the experiment), AFP184 made succinic acid at a rate of 1.22 g/Lh compared to 0.7 g/Lh for AFPX1. Experiments using purified glucose as substrate substantiated this difference between the strains (Table 2). In addition, the final concentration obtained was also significantly higher. The yield of succinic acid based on glucose was comparable for the two strains. The improved productivity and titer obtained by AFP184 could improve the economics of a process for production of succinic acid.

Table 2. Metrics for the production of succinic acid from glucose in test tube.

| Strain | SA Titer (g/L) | Productivity (g/Lh) | Yield (g/g glucose) |
|---|---|---|---|
| AFP111 | 51 | 0.87 | 0.95 |
| AFP184 | 72 | 1.00 | 0.91 |

AFP184 possessed additional attributes that could contribute to an industrial scale process to make succinic acid. Because of its vigorous growth, smaller inoculum cultures were needed; the dilute initial culture quickly grew to high density prior to the production phase. AFP184 also remained healthier much further into the production cycle than AFP111, as indicated by the ability to use a production phase culture to inoculate a second production cycle. In contrast, secondary cultures of AFP111 performed poorly. This latter trait could allow development of a continuous or semicontinuous process for succinic acid production using AFP184. Other metrics for AFP 184 are in Table 3.

**Table 3. Metric for production of succinic acid from glucose and from hydrolysate in 1-L fermentation at 72h.**

| **Glucose as carbon source** | **AFP111** | **AFP184** |
|---|---|---|
| SA Titer (g/L) | 36 | 48 |
| Yield (g SA/g G consumed) | 0.67 | 1.04 |
| **Hydrolysate as carbon source** | | |
| SA titer (g/L) | 30 | 49 |
| Yield (g SA/g G+X consumed) | 0.58 | 1.13 |

Figure 3. Construction of AFP184 from a wild type *E. coli* strain.

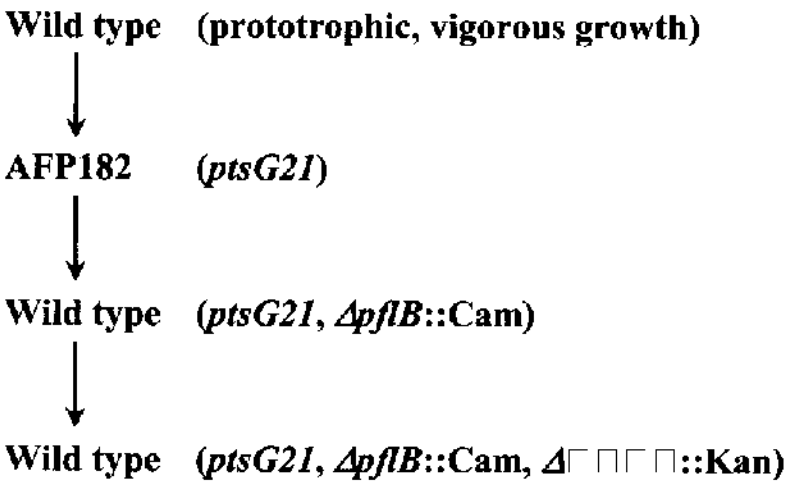
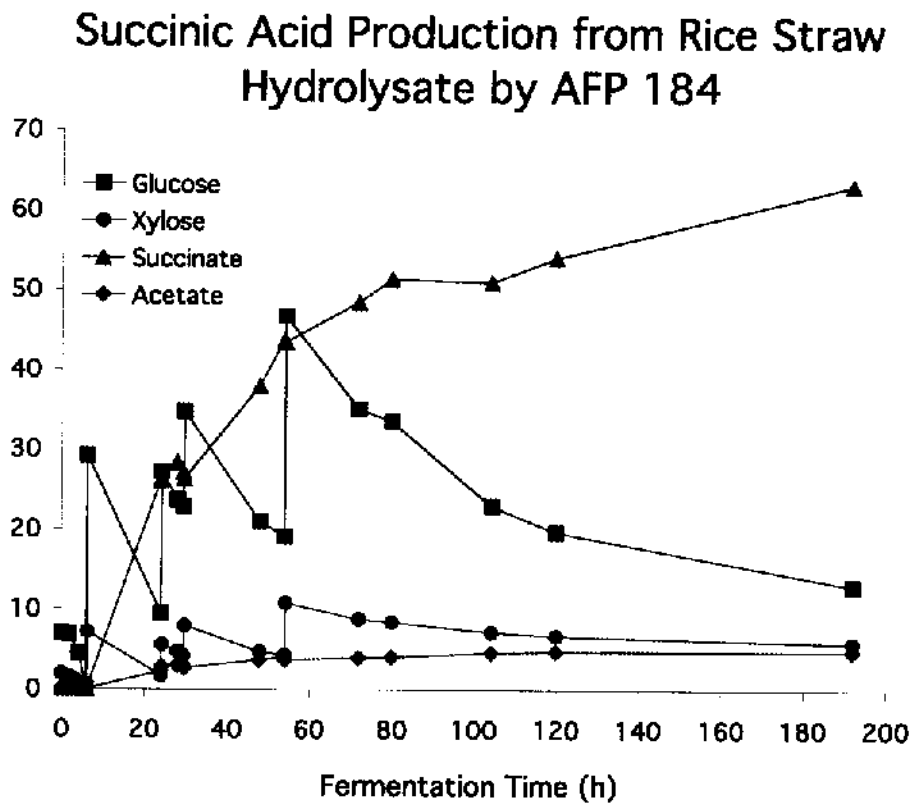


Fig. 4. Conversion of rice straw hydrolysate to succinic acid by AFP184.




Two additional strains were also developed in the course of the CRADA research. The original mutation in the *ptsG* gene of AFP111, AFPX1 and AFP184 was a point mutation. As such, it is possible that a secondary mutation could restore a functional PTSG protein, eliminating succinic acid production. To insure that this could not occur, we constructed a knockout of the *ptsG* gene by cloning the unmodified gene and inserting an antibiotic resistance gene into the *ptsG* gene (eliminating the possibility of reversion). The modified gene was introduced into the lineage of both W1485 and the wild type strain by a method that replaced the native gene, generating non-revertable mutations of *ptsG*. The resulting strains, AFP400 and AFP404 (Table 4), contain three non-reverting knockout mutations that, in concert, allow succinic acid production from mixtures of sugars. These strains fermented glucose to succinic acid similarly to their relatives, AFP111 and AFP184, respectively.

Table 4. Triple knockout mutants for production of succinic acid.

| **Strain** | Host | **Phenotype** |
|---|---|---|
| AFP400 | W1485 | Δ*pflB*::Cam, *ldhA*::Tet, *ptsG*::Kan |
| AFP404 | wild type | Δ*pflB*::Cam, *ldhA*::Tet, *ptsG*::Kan |

The final strain, AFP404, represents the best strain developed under the CRADA and is recommended for future process optimization.

**Commericalization (ACC)**

There are three primary aspects to Applied Carbochemicals, Inc. commercialization efforts: process scale-up, economics, and marketing of new product applications.

The DOE/ACC fermentation process route provides an opportunity to manufacture an array of succinate salts, such as sodium, potassium or ammonium succinate, depending on the neutralization strategy chosen. These salts have large volume merchant applications that the company is currently exploiting. Product and market development efforts involve the testing of these succinate salts in target applications against existing chemicals to establish price/performance analysis. Additionally, customer evaluations provide the specifications of the end products which are back integrated into the process development efforts to ensure that the process flows are consistent with consumer demands and cash cost analysis for these end-products can be assessed before commencing the negotiation of the terms and conditions of supply contracts. The company's short-term commercialization strategy focuses on the introduction of succinate salts to high growth core markets, where the price and performance of these salts offer the company sustainable competitive advantages. Process, product and market development efforts will continue to support the initial launch of these products.

***Process Scale Up***

Fermentation: Michigan Biotechnology Institute (MBI) in East Lansing, MI, worked with ACC to refine the process conditions and scale the process up to the 3700 liter scale. This scale up yielded valuable information that enabled the development of the final Standard Operating Procedures enabling us to engage a contract fermentation house for the final scale up and eventual contract manufacturing. The final scale at MBI (3700 liters) would be the volume of the innoculum tank for our commercial scale up, which was conducted by FermPro Manufacturing LP in Kingstree, SC. The FermPro run was conducted at a 150,000 liter scale.

Separations: The separations work was originally carried out at MSU. The separations work is now being conducted under a Phase 2 SBIR through LecTec, in Lansing, MI. ACC has an ownership position in LecTec, which is now functioning as the technology development arm of ACC. Current work is concentrating on finalizing the coupling of the fermentation/separations operations for a smooth transition to the various downstream synthesis operations. Crystallization has replaced membrane electrodialysis as the preferred separation scheme. However, several product lines have been developed for the partially purified salt form.

***Process Economics***

Process Economics were developed by ORNL, ProForma Systems, Raphael Katzen Associates International (Katzen), Inc. and ACC. Katzen rendered the original opinion on the viability of the economics based on data supplied by ProForma, ORNL and ACC and discussions with ACC personnel. Subsequent economic refinements of downstream processing and the incorporation of new, proprietary separation operations were conducted by ProForma Systems and ACC. ACC proprietary improvements and patents in separations, formulation, fermentation, and catalysis lower the project SA cost well below target of <$0.25/lb SA

***Marketing/Market Development***

Activities in Marketing and Market Development have been largely orchestrated by ACC in collaboration with numerous industry partners in the various markets and applications identified by ACC as target markets. ACC has availed itself of multiclient studies as well as the expertise of those already in the

marketplace. Marketing efforts have been conducted on a global basis, and ACC is working with companies all over the world. Figure 5 demonstrates the breadth of the product offering and market applications that are enabled by the succinic acid platform. In particular, R&D and tests should that succinate salts act as a improved, biodegradable, very low corrosion deicer.

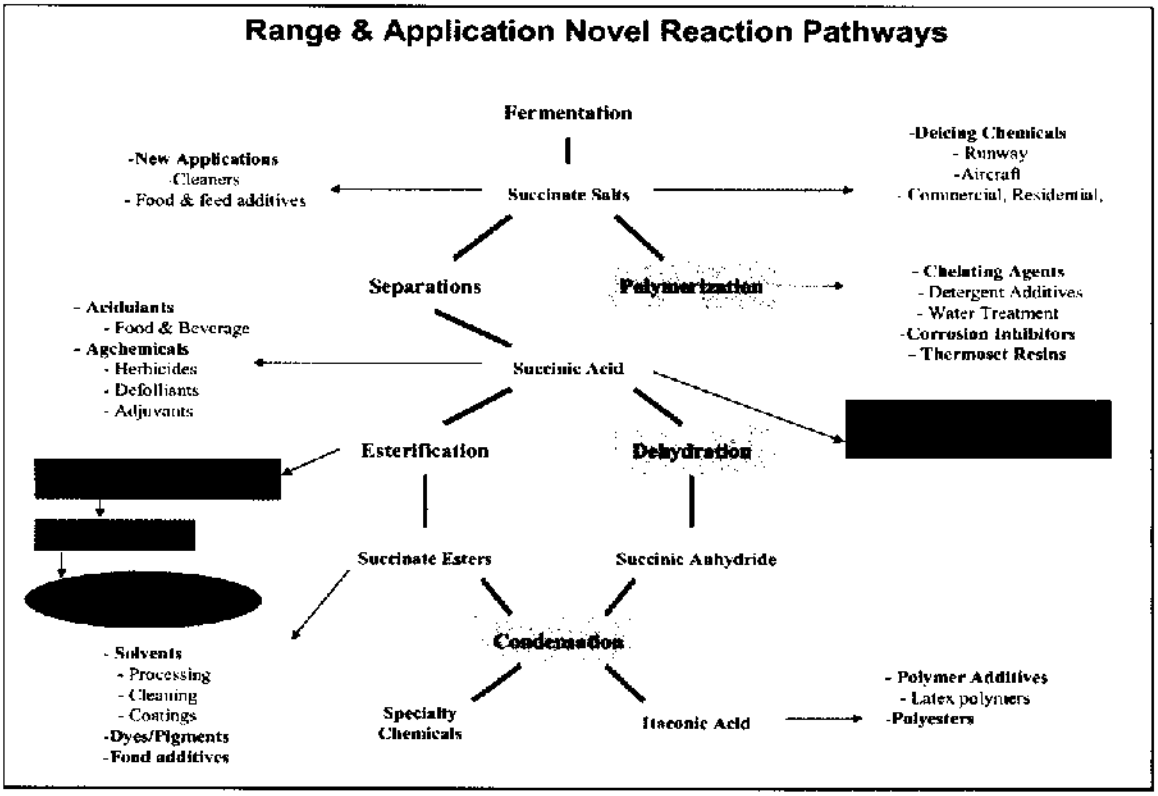



**Figure 5. Succinic Acid as a Platform Molecule**

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## APPENDIX
## SUBJECT PATENTS AND INVENTION DISCLOSURES.

**Subject patents and invention disclosures developed under CRADA:**

Method for Construction of Bacterial Strains with Increased Succinic Acid Production, 6,159,738. 12/12/00 M. Donnelly et al. Argonne National Laboratory

**ACC Patents Related to CRADA**

| | US Patent # and date |
|---|---|
| SEPARATION | |
| Succinic Acid Production and Purification I and II | #5,958,744 (9/28/99) & #6,265,190 (7/24/01) |
| FORMULATION | |
| Enhanced Herbicides I | #6,218,336 B1 (4/17/01) |
| Deicing Compositions and Methods of Use | #6,287,480 (9/11/01) |
| DOWNSTREAM SYNTHESIS | |
| Dicarboxylic Alkyl Phosphate Esters of epoxy succinic acid | #6,229,038 (5/8/01) |

There are also ten pending ACC patents for further product and process improvements

## PREEXISTING INTELLECTUAL PROPERTY

US Patent # 5,770,435 "A Mutant E. Coli Strain, AFP111, with Increased Succinic Acid Production" Mark Donnelly, Cynthia Millard, Lucy Stols - Argonne National Lab. 6/23/98.
Reissued as RE 37,393 9/25/01.

U.S. Patent #5,869,301 A Fermentation Process for the Production of Dicarboxylic Acids. N. P. Nghiem, M. Donnelly, C. Millard **2/9/99**

## Appendix
## Original CRADA Milestones and Decision Points (August 1999)

| | |
|---|---|
| PROJECT YEAR 1 | |
| Fermentation Milestones: | |
| Establish efficiency/limitations of AFP111 in metabolizing mixed sugars. | 6 months |
| Optimize fermentation of mixed sugars by AFP111 | 12 months |
| Report on potential of alternative strains | 12 months |
| | |
| Metabolic Engineering Milestones | |
| Initiate cloning of target gene(s). | 6 months |
| Introduce, express, and measure effect of gene in AFP111 | 12 months |
| | |
| Economic and market analysis | |
| Prepare report on economic and energy assessment for use in DOE metrics | 9 months |
| | |
| Decision Point Milestone: | |
| Is overexpression of genes for pentose metabolism necessary? | 12 months |
| If yes, continue cloning as guided by results. | |
| If no, concentrate on cellobiose and fermentation optimization | |
| | |
| PROJECT YEAR 2 | |
| Fermentation Milestones: | |
| Milestone: | |
| Establish effect of cellobiose genes in the fermenter | 6 months |
| Demonstrate fermentation of real hydrolysate by modified AFP111 | 12 months |
| | |
| Metabolic engineering | |
| Clone, express and evaluate additional gene(s) of pentose metabolism. | 6 months |
| or - Integrate genes of pentose metabolism. | |
| Select variants with improved metabolism of mixed sugars. | 12 months |
| | |
| Economic and market analysis | |
| Prepare marketing report of succinate derivatives | 15 months |
| | |
| PROJECT YEAR 3 | |
| Pilot Plant Demonstration | |
| Running a fermentation at least 500L scale | 36 months |

As seen in the results, all major objectives were met; however some tasks did change during the course of the project.

Appendix
**Funding Support**

The total estimated cost of this CRADA is $505,000 (excluding the Federal Administrative Charges). The Government's estimated contribution, which is provided through the Contractors' contracts with DOE, is $255,000, subject to available funding. The Participant's estimated contribution is $250,000 (with $50,000 of that amount being funds-in to one of the Contractors). Since the Participant is a small business, DOE has waived the billing of $1,500 in Federal Administrative Charges, which increases the Government's contribution to $256,500. The total authorized amount to be expended on this CRADA by the Contractors is $305,000, of which $255,000 is programmatic funds and $50,000 is funds-in from the Participant. The amount of funds, in thousands of dollars, is summarized below for each project year:

| Parties | Project Year 1 | Project Year 2 | Project Year 3 | Total |
|---|---|---|---|---|
| DOE's Contribution via Contractor (UTB) | 45 | 45 | 0 | 90 |
| DOE's Contribution via Contractor (ANL) | 100 | 65 | 0 | 165 |
| Participant (In-Kind Contribution) | 25 | 50 | 125 | 200 |
| Participant (Funds-in to ANL) | 40 | 10 | 0 | 50 |
| Total | 210 | 170 | 125 | 505 |

Research performed prior to formal CRADA brought DOE support to $560k was funded FY98-FY01. The funds-in were never provided to ANL.

**Final Report Certification**

**for CRADA Number 99-0552**

**between**

**UT-Batelle, LLC**

**and**

**Argonne National Laboratory**

The following certification is made for the subject final report:

_____ 1a) The final report contains a claim that information contained in the final report is qualifying as "Protected CRADA Information" (PCI) and any PCI is identified as such in the report;

or

_____ 1b) neither Energy Research nor the participant asserts any claim to information contained in the final report as qualifying as "Protected CRADA Information";

2) that Energy Research and the participant have no objection to public distribution of the final report due to patentable information; and

3) the final report contains no proprietary information.

For the Participant:

_____
(Name)

_____
(Title)

_____
(Date)

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**Applied Carbochemicals, Inc.**

The following certification is made for the subject final report:

_____ 1a) The final report contains a claim that information contained in the final report is qualifying as "Protected CRADA Information" (PCI) and any PCI is identified as such in the report;

or

_____ 1b) neither Energy Research nor the participant asserts any claim to information contained in the final report as qualifying as "Protected CRADA Information";

2) that Energy Research and the participant have no objection to public distribution of the final report due to patentable information; and

3) the final report contains no proprietary information.

For the Participant:

_____
(Name)

_____
(Title)

_____
(Date)