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CRADA Final Report
For
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PRODUCTION OF CHEMICAL DERIVATIVES FROM RENEWABLES

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Final CRADA Report* ORNL96-0407

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Argonne National Laboratory[b]
Pacific Northwest National Laboratory[c]
National Renewable Energy Laboratory[d]
and
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DISCLAIMER

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CONTENTS

INTRODUCTION AND PURPOSE

The purpose of this Cooperative Research and Development Agreement (CRADA) between Lockheed Martin Energy Research Corp., (LMER), Argonne National Laboratory (ANL), National Renewable Energy Laboratory (NREL), and Battelle Memorial Institute, operator of Pacific Northwest National Laboratory (PNNL), (collectively referred to as the "Contractor"), and Applied Carbochemicals, Inc. (Participant) was to scale-up from bench results an economically promising and competitive process for the production of chemical derivatives from biologically produced succinic acid. The products that were under consideration for production from the succinic acid platform included 1,4-butanediol, γ-butyrolactone, 2-pyrrolidinone and N-methyl pyrrolidinone. Preliminary economic analyses indicated that this platform was competitive with the most recent petrochemical routes. The Contractors and participant are hereinafter jointly referred to as the "Parties."

Research to date in succinic acid fermentation, separation and genetic engineering 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. At the time, the current need was to provide the necessary laboratory follow-up information to properly optimize, design and operate a pilot scale process. The purpose of the pilot work was to validate the integrated process, assure "robustness" of the process, define operating conditions, and provide samples for potential customer evaluation. The data from the pilot scale process was used in design and development of a full scale production facility.

A new strain, AFP111 (patented), discovered at ANL was tested and developed for process use at the Oak Ridge National Laboratory (ORNL) and ANL. The operability and product formation are attractive for this strain and effort was being directed at process development and optimization.

Key to the transition from the fermentative production unit operation to the chemical catalysis is the "clean-up" of fermentation broth, succinic acid formation from the salt, and succinic acid concentration. These steps are accomplished by a two-stage membrane ED separation process developed at ANL. Although the current process is well developed, possible modifications and optimization may be called for as development work continues in both the fermentation and catalysis areas.

Research to date performed at PNNL has demonstrated that succinic acid can be converted to value added chemicals such as 1,4-butanediol, γ-butyrolactone, N-methyl pyrrolidinone, and 2 pyrrolidinone with high conversion and selectivities. Continued research will be performed in catalyst development and reaction condition optimization to move this work from the bench scale to the pilot scale.

All development of the process was guided by the NREL technoeconomic model.The model showed that direct aqueous phase catalysis of succinic acid to 1,4-butanediol, γ-butyrolactone, and N-methyl pyrrolidinone provided significant economical advantages in the market, the margin, and the return on capital investment over existing petrochemical processes for production of these compounds. The model also provided the baseline for evaluating current

laboratory research. As data from the bench and pilot work were made available the model was modified and appropriate sensitivities ran to determine impact of the process changes and optimization.

The report will present the planned CRADA tasks followed by the results. The results section has an overall project summary follwed by more detailed reports from the participants. This is a nonproprietary report; additional proprietary information may be made available subject to acceptance of the appropriate proprietary information agreements.

CRADA TASKS

Technical Objectives

The overall goal of the CRADA was to transition a promising bench scale technology to production via a pilot plant demonstration process. This goal was met by obtaining the following key objectives:

(1) Developed the current AFP111 based fermentation process to a 500 liter fermentation size and maintained the critical parameters as dictated by the most current NREL economic analysis.
(2) Develop catalyst and reaction-condition optimization necessary to move from the bench scale to the pilot scale using targets based on the most current NREL economic analysis.
(3) Based on data generated in (1) and (2) developed, planned and performed a pilot demonstration project to validate the proposed integrated process, assure "robustness" of the process, define operating conditions, and provided samples for potential customer evaluation.
(4) Using data from the pilot scale process designed and developed a full scale production facility.

Project Tasks and Deliverables

The CRADA research effort consisted of 9 tasks, described below, designed to meet the above objectives over a 30-month period of performance. ORNL coordinated the Contractors' efforts in liaison with the Participant.

Task 1: Marketing Study -- The Participant performed a marketing study of bulk chemicals. The Participant used internal and contracted resources to determine size and distribution of market and establish a market penetration strategy. An initial market study was completed within 6 months after the effective date of CRADA approval, although the strategy evolved as the catalytic work, described below, changes to potential product slate for a proposed production facility.

Task 2: Pilot Plant Studies -- The Participant provided identification and contracting of pilot plant studies. Based on results generated in the 1st half of 1996 and through input of consultants, the Participant designed a pilot program, contracted, and performed the pilot work to provide data for full scale plant design. The pilot demonstration program began 6 months after the effective date of CRADA and was completed 17 months after the effective date of CRADA approval.

Task 3: Plant Design -- The Participant developed plant design based on pilot results and market penetration strategy.

The Participant used the data developed in Tasks 1 and 2 to develop a plant design (with the construction contractor) for full scale plant construction. This was a decision point for the continuance of the project based on previous results at 20 months.

Task 4: Screening Programs -- The Participant developed a screening program for succinic acid producing organisms to provide other potential production strains with enhanced characteristics. The participant was considering contract research to provide this on-going screening program for succinic acid producers.

Task 5 Biocatalysts and Bioreactor Developments -- ORNL led the research in the growth of AFP111 on inexpensive nutrients. (a) Determined appropriate feedstocks for pilot plant scale-up work as provided by the Participant (b) optimized growth and production characteristic of AFP111 fermentation at the bench scale in conjunction with ANL; (c) performed 500-liter scale cultivation of AFP111: (d) increase stability/rate of AFP111 through process design optimization; (e) preliminary report on simultaneous fermentation and separation; and (f) support of pilot and scale-up efforts.

Task 6: Biocatalysts Development -- ANL provided the research effort in succinic acid production and other strains, to include (a) optimize growth and production characteristic of AFP111 fermentation at the bench scale in conjunction with ORNL; (b) bench scale - physiological studies - AFP111; (c) ANL modified AFP111 by metabolic engineering resulting in the elimination of ethanol side products; and (d) support of pilot and scale-up efforts

Task 7: Improved Purity Separation -- ANL was responsible for the development and evaluation of primary purification process for (a) the succinic acid broth generated by new organism, APP 111; (b) provided optimization testing based on pilot results, when needed; and (c) support for pilot and scale-up efforts

Task 8: Catalysis -- PNNL focused research on the development, characterization, and optimization of new and existing catalysts for the aqueous phase conversion of succinic acid, succinimide, and/or diammonium succinate to 1,4-butanediol, γ-butyrolactone, N-methyl pyrrolidinone, and 2-pyrrolidinone. Catalyst evaluation was based on conversion, selectivity, weight hourly space velocity, catalyst lifetime, and catalyst cost. Tests were both batch and continuous. The research effort was broken into 4 Subtasks as listed below:

1. Catalytic conversion of succinic acid to γ-butyrolactone.

2. Catalytic conversion of γ-butyrolactone to 1,4-butanediol.

3. Catalytic conversion of diammonium succinate and/or succinimide to 2-pyrrolidinone.

4. Catalytic conversion of 2-pyrrolidinone to N-methyl pyrrolidinone.

In addition to the four Subtasks described above PNNL tested for actual ion exchange polishing steps on purified SA from ED and fermentation to determine the impact on BDO/GBL catalysis and performed further catalysis tests based on pilot results.

Task 9: Economic Evaluation and Updates -- NREL updated BDO/GBL with revised data, improved processes and provide sensitivities. Furthermore, NREL estimated production costs for NMP and 2-pyrrolidinone.

Duration of Entire Project -- The project duration was 30 months.

RESULTS

The results are presented in six sections: The first section is the overall results of the project. This is followed by individual reports from ORNL, ANL, and PNNL including a summary of ACC current commercialization efforts as of 11/98.

Results Summary

A 1997 R&D 100 honored a multi-laboratory effort that combined the expertise and experience of four DOE national laboratories in support of an industry partner. The Argonne National Laboratory, National Renewable Energy Laboratory, Oak Ridge National Laboratory and Pacific Northwest National Laboratory worked together to help Applied CarboChemicals Inc. of Alto, MI demonstrate an integrated process for chemical manufacturing.

The hybrid biological/chemical process uses corn syrup as the feedstock to make succinic acid. The acid can then be converted into current commodity chemicals used in a wide assortment of products used to make clothing fibers, paints, inks, food additives, plastics and polymers. These products are currently made from petroleum.

This novel consortium developed a proprietary strain of bacteria to produce the succinic acid biologically, demonstrated the fermentation at 500-L scale, developed advanced separation technologies to isolate the products and adapted chemical catalytic processes to convert the succinic acid into useful manufacturing chemicals. Analysis showed that the process is cost-competitive with existing chemical processes.

This project grew out of the Alternative Feedstocks Program which directed a team of national laboratories to determine and develop alternative commodity chemical platforms based on renewables. After several industrial workshops and reviews, and initial economic estimates by NREL, succinic acid was chosen as a target platform chemical. In this exploratory effort, ORNL focused on fermentation and separations and was the team leader, ANL emphasized metabolic engineering and membrane separations, PNNL on downstream catalytic conversion, and NREL on economic analysis. After a year of effort, the baseline technology was marketed to over six potential industrial partners by a collaborative team from ORNL, PNNL, and NREL. A CRADA was established with Applied Carbochemicals in 5/95. Since then the process has been refined and demonstrated at the 500-l scale. Several patents have been issued or submitted including several joint patents.

The Biologically Derived Succinic Acid (BDSA) process produces succinic acid by fermenting glucose sugar from corn. After separation and purification, the succinic acid is used as a chemical intermediate that is converted into chemical feedstocks, which are then used to make a wide assortment of products. The BDSA process will compete with petrochemical routes of production by providing a lower-cost means of obtaining commodity chemicals such as

1,4-butanediol (BDO), tetrahydrofuran, gamma-butyrolactone, *n*-methyl pyrrolidinone, 2-pyrrolidinone, and succinic acid itself from renewable resources.

This revolutionary new process converts corn into a cost-efficient, environmentally friendly source of the chemicals used to make polymers, clothing fibers, paints, inks, food additives, automobile bumpers, and an array of other industrial and consumer products. Applied CarboChemicals, a specialty chemicals company, is commercializing this process (a biological method for producing an organic acid) to manufacture chemical feedstocks from renewable farm crops at a significantly lower cost than that obtained with conventional petroleum-based methods. A cooperative research and development agreement is in place between four national laboratories and Applied CarboChemicals.

Using a novel microorganism in fermentation, the new process also promises to reduce reliance on imported oil and to expand markets for domestic agriculture. The new process produces succinic acid by fermenting glucose sugar from corn, separating and purifying the acid, and using it as an intermediate to produce BDO, tetrahydrofuran, *n*-methyl pyrrolidinone, and other chemical feedstocks (shown in Figure 1 of the appendix) that are used to make a wide assortment of products. Existing domestic markets for such chemicals total almost 1 billion pounds, or more than $1.3B, each year. Use of the BDSA process could displace petroleum consumption by an equivalent amount. Our process would require 1 to 2 pounds of corn for each pound of chemical produced (the required quantity of corn depending on the specific end product). Applied CarboChemicals expects to build a plant that can produce 50 to 100 million pounds of chemicals annually by the year 2003, with plans to increase capacity as the market grows at a projected rate of 6% per year.

Detailed process and economic assessments have been performed and reviewed by several industries. In addition to the economic benefits afforded by the BDSA process (i.e., expanded markets for corn and other renewable feedstocks and employment growth within agriculture and related industries), the energy savings could also be significant. For example, the savings created by a single combined biological and chemical plant producing chemical components could heat 80,000 single-family homes for an entire year. Use of the BDSA process thus represents a significant opportunity to conserve valuable petroleum resources.

This research effort, which culminated in the process depicted in Figure 2 of the appendix, includes significant contributions by all of the DOE laboratories involved.

* Fermentation

  The BDSA process uses a novel robust microorganism to convert corn-derived glucose to succinic acid at very high yields. Argonne National Laboratory (ANL) has received a patent for this microbe (U.S. Patent #5,770,435). Oak Ridge National Laboratory (ORNL) researchers have developed the fermentation process. ORNL has received a patent concerning the optimal use of the ANL microbe for high-yield, high-productivity fermentation (U.S. Patent #5,869,301).

* Recovery/Purification
  ANL has tested process conditions for a two-stage desalting and water-splitting electrodialysis, which concentrates, purifies, and acidifies the succinic acid, allowing the base to be recycled to the fermentation (where it is used for neutralization). This process eliminates the formation of gypsum, a typical undesirable neutralization by-product that must be shipped to landfills for disposal. This prevented approximately 1 mole of gypsum which would be produced for each mole of acid in other biological methods.

* Catalytic Conversion
  Pacific Northwest National Laboratory (PNNL) researchers have several invention disclosures concerning novel catalysts for the conversion of succinic acid to commodity chemicals, the final step in the conversion process. Innovations have included direct catalysis in the purified aqueous broth and methods to deal with the presence of fermentation by-products such as acetic acid.

ALTERNATIVE FEEDSTOCKS PROGRAM CRADA FINAL REPORT

Task 1: Marketing Study

ACC obviously will be a primary user of the technology. Other planned primary users will include manufacturers of GBL, BDO, THF, 2-P and NMP, as well as users of succinic acid and other organic acids. Secondary users will include manufacturers of products which use these chemicals as feedstocks. The marketing study was completed and indicated difficulties in penetration of the THF markets due to new capacity which had just been brought on line. Other strategies were explored.

ACC plans to introduce succinate to markets where price has historically been a barrier to entry. A low cost route to succinate from the proposed fermentation process will broaden the application range of succinate. The current petrochemical-based route to succinate through maleic anhydride is relatively expensive, limiting succinate to low volume, high margin product applications such as pharmaceuticals, specialty inks and dyes, and food preservatives. A significant reduction in the current pricing levels of succinate will enable customers in a broader range of industries to benefit from the high performance capabilities of succinate and its derivatives. As shown in task 9 below, the manufacturing cost for succinate by fermentation is only one third of that in the petrochemical route.

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.

Additional R&D activities will involve the further optimization of the fermentation and separation processes to reduce the cash costs of manufacturing succinate to threshold levels. Optimization strategies are focused on the improvement of carbon yields, volumetric productivities and separation efficiencies and costs. Another area of interest to the company is the development of a capability to co-ferment xylose and glucose to succinate. The rationale for this investment is to enable ACC to utilize a broader range of feedstocks outside of a dependence on corn-derived glucose. This development would need to be integrated with the state-of-the-art acid hydrolysis process (i.e., Arkenol, Inc.) to provide an inexpensive source of hydrolysates to fermentation operations, consisting of fermentable C5 and C6 sugars.

Task 2: Pilot Plant Studies

ACC currently has a plan to test the fermentation process in a 10,000-liter pilot plant. Optimization of the cell growth media and the utilization of carbon dioxide and hydrogen will be performed by ACC before the design and implementation of the planned 10,000-liter pilot plant runs. Pursuant to successful pilot demonstrations, ACC's engineering partners will develop the engineering design for the company's first commercial facility. These engineering specifications will enable ACC to determine whether to build a greenfield operation or to acquire and retrofit a mothballed fermentation facility. A plant retrofit will reduce siting and permitting costs, engineering and construction costs, and outside battery limit infrastructure costs associated with new plant construction. Additionally, a retrofit will shorten the time required for plant start-up. It was also believed that much of the 500-L data and the large-laboratory scale efforts performed at ORNL, ANL, and ACC using media and succinate broth would allow some of the integrated pilot testing to be deferred. There also were some delays in completion of task 5 which slowed the pilot design.

ACC did perform a series of new separation and production specification tests in 1998 using succinate broth provided by ORNL.

Task 3: Plant Design

A complete plant design was developed based on the technoeconomic assessment performed in Task 9. This plant design was redone and completely vetted by a reputable engineering design firm. This included a through reevaluation of the cost estimates. ACC has contracted for further engineering design work and for the plant contract with another engineering design firm.

Task 4. Establish Screening Program

ACC has established a microbiological laboratory near Pittsburgh. They have further modified and screened for improved microbial strains. One of these potential improved strain was tested at ORNL in late 1999 under a separate WFO.

Task 5. Fermentation Process Development Tasks

Executive Summary (ORNL, Nghiem)

A fermentation process was developed for the national laboratories' proprietary *Escherichia coli* strain AFP111. This strain later was assigned the ID No. ATCC 202021, when the patent on its creation was issued. The original medium used for the fermentation process development was the nutrient-rich Luria-Bertani (LB) medium. The developed process consisted of two stages. In the first stage, the cells were grown under aerobic conditions until the critical cell mass had been achieved. The air then was turned off to force the cells to switch to anaerobic metabolism, which caused the immediate production of succinic acid at high rates. A patent was issued on the developed process (U.S. #5,869,301).

After thorough discussion with the industrial partner, Applied CarboChemicals, Inc. (ACC), AFP111 was selected for the development of a commercial fermentation process for succinic acid production. Laboratory-scale one-liter fermenters were used throughout the process development efforts. An inexpensive fermentation medium was developed. In this medium, the only organic nutrient source was 25% (v/v) light steep water (LSW); other components included glucose and simple inorganic salts such as potassium phosphate, ammonium sulfate, and magnesium sulfate. Optimization of the fermentation process parameters then was performed in the 25% (v/v) LSW medium. The parameters included the type of bases used for pH control, pH, and length of the aeration stage. The following results were obtained.

- Among the four bases used for pH control, which were sodium carbonate, ammonium carbonate, sodium hydroxide, and ammonium hydroxide, sodium carbonate gave the best succinic acid production.
- The optimum pH was found to be between 6.25 and 6.5.
- The length of the aeration stage should be from 4 to 8 hours.

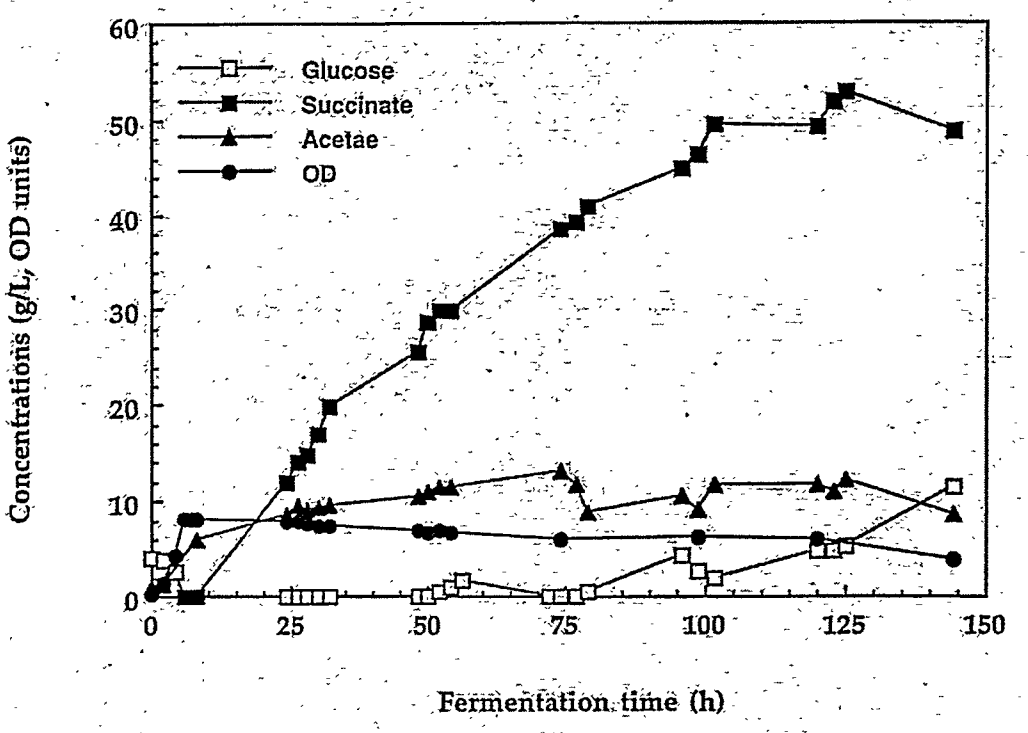
The developed fermentation process was scaled up in 75-liter and 500-liter fermenters. At least three runs were made under the same conditions at each scale. Highly reproducible results were obtained at both scales. The results also confirmed those obtained at the laboratory scale. The confirmation of the laboratory-scale results clearly demonstrated the scalability of the developed fermentation process.

To improve the overall process productivity, a modification was made to the originally developed process. An invention disclosure was submitted and enabling details were eliminated herein. In this modification, specific conditions were employed to cause cell settling which in turn increased the cell concentration by almost two times. Higher cell concentration resulted in a 1.6X increase of the overall process productivity.

5-1. FERMENTATION HIGHLIGHTS

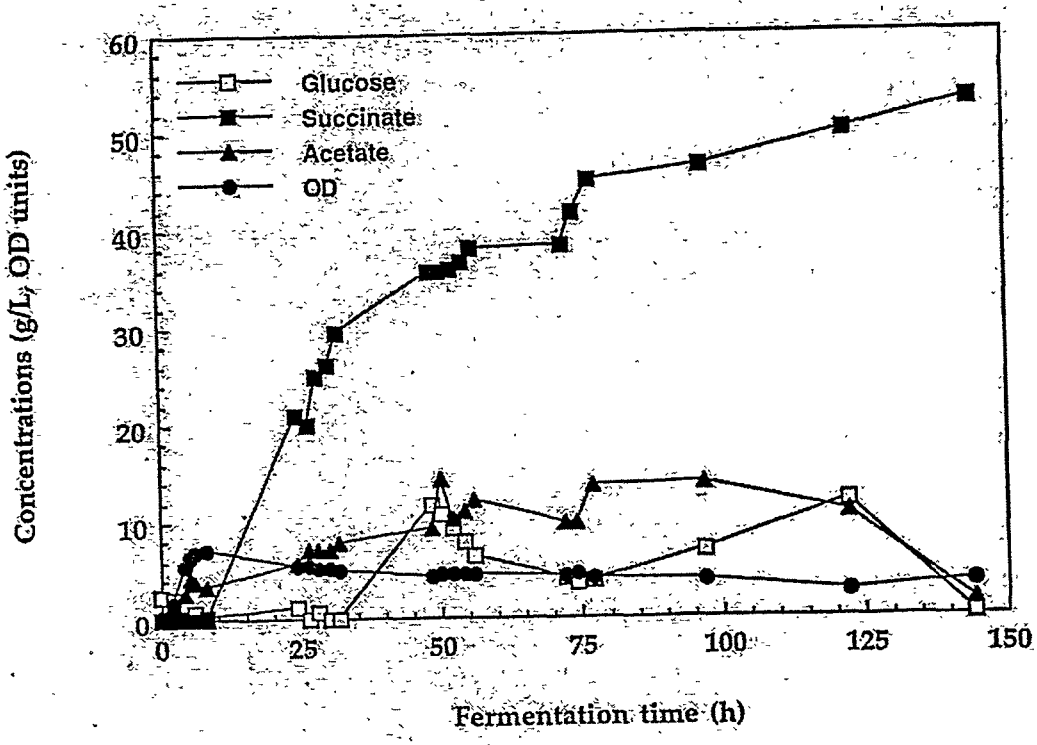
- **Fermentation results in LSW media.** In the first experiment using the LSW medium, the concentration of this nutrient source was 50% (v/v). To maintain pH at 7.0, a solution of 1.5 M Na_2CO_3 was used. The aerobic stage lasted six hours. Maximum succinic acid concentration of 51 g/L was obtained at 99 hours. This was the highest succinic acid concentration ever obtained with the AFP111 strain. It also was considerably higher than the concentration normally obtained with the previously used succinic acid-producing strain, *Anaerobiospirillum succiniciproducenss*, i.e., 30 g/L. At 144 hours, the yield was 0.62 g succinic acid/g glucose consumed. See Fig. 1.

Figure 1



When the LSW concentration was reduced to 25% (v/v), the initial rate of succinic acid production was significantly increased. This was clearly indicated by the improvement of the productivity at 24 hours from 0.50 g/L-h for 50% LSW to 0.87 g/L-h for 25% LSW. Succinic acid concentration again reached above 50 g/L. At 144 hours, succinic acid accumulated to 53 g/L and the yield was 0.66 g succinic acid/g glucose consumed. See Fig. 2.

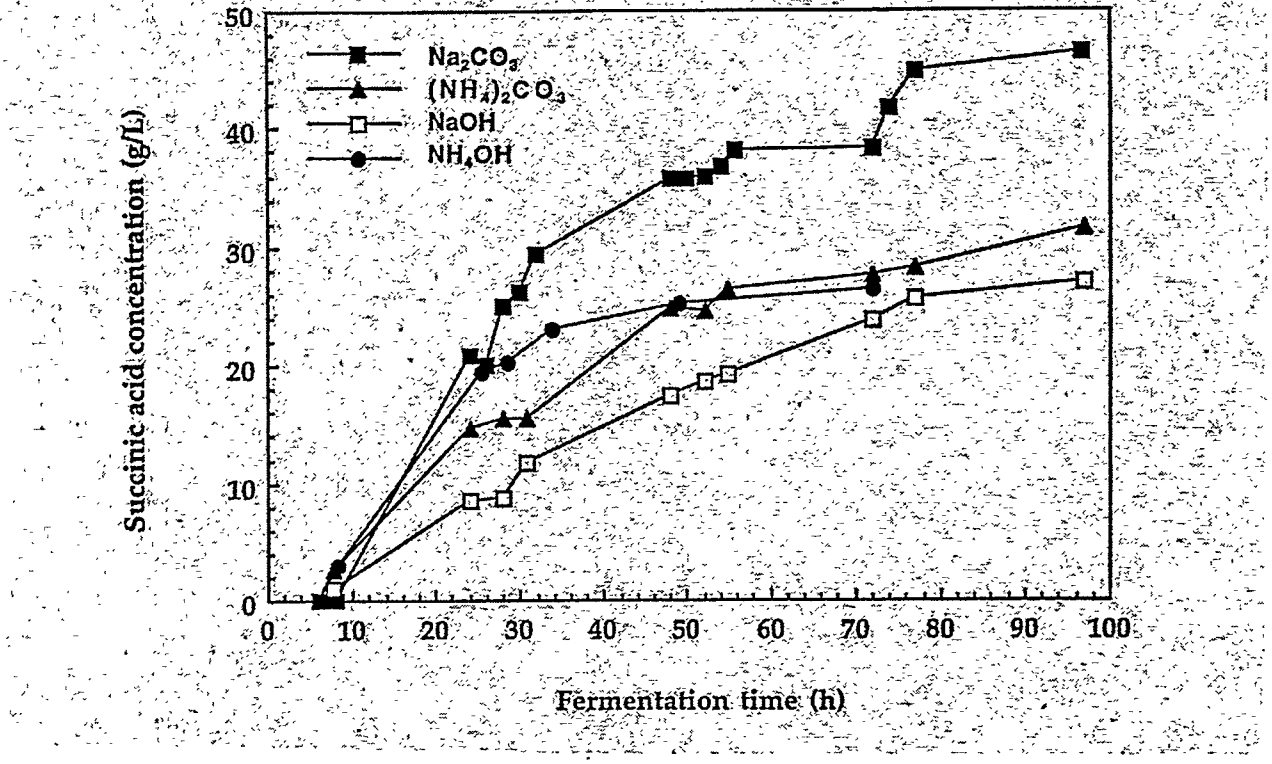
Figure 2



- **The effects of bases used for pH control.** The use of two non-carbonate bases, NaOH and NH_4OH, for pH control was studied in the LSW medium. For each base, two experiments were performed. In one of them, pure carbon dioxide gas was sparged into the fermenter at 0.05 vvm during the succinic acid production stage. Carbon dioxide sparge was not applied in the control experiment. The results clearly indicated the requirements of carbonate ion for succinic acid production. In both cases, without carbon dioxide sparge, succinic acid concentration was well below 10 g/L, whereas with carbon dioxide sparge, 24 g/L was obtained with NaOH and 33 g/L with NH_4OH. The succinate/acetate molar ratio was slightly affected by the type of bases used for pH control. Whereas this ratio was 2.3 when Na_2CO_3 was used, it dropped to 2.0, 1.8, and 1.6, when NaOH, NH_4OH, and $(NH_4)_2CO_3$, respectively, were used.

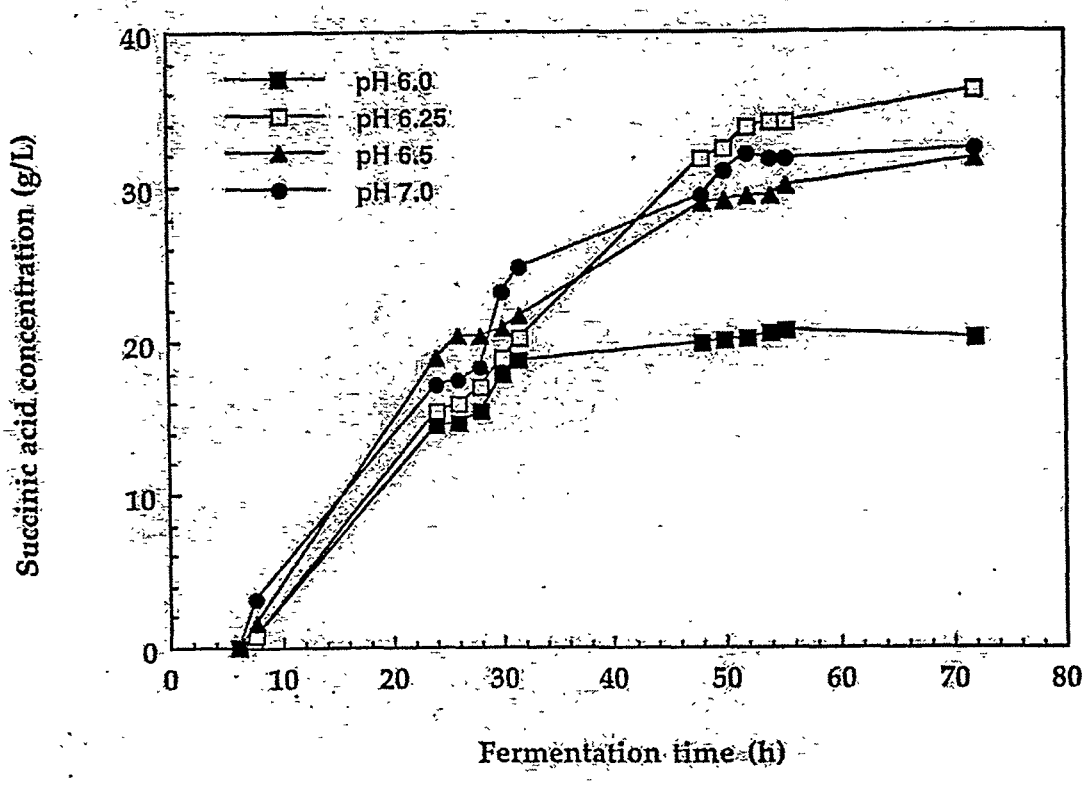
Four bases, Na_2CO_3, $(NH_4)_2CO_3$, NaOH, and NH_4OH, were compared using the LSW medium. In the cases of NaOH and NH_4OH, pure carbon dioxide gas was sparged into the fermenter at 0.05 vvm. Carbon dioxide sparge was not applied when the other two bases were used. Of the four bases examined, Na_2CO_3 gave the best results. The initial rate of succinic acid production when this base was used was 1.24 g/l-h compared to 0.96, 0.75, and 0.47 g/l-h, for NH_4OH, $(NH_4)_2CO_3$ and NaOH, respectively. At 72 hours, Na_2CO_3 also gave the highest succinic acid concentration of 46 g/L. When the other bases were used, the succinic acid concentrations at 72 hours were about 25 g/L. See Fig. 3.

Figure 3



- **The effects of pH.** In the experiments, which were performed to study the effects of pH, NH_4OH was used for pH control. Four pH values, 6.0, 6.25, 6.5, and 7.0, were examined. There were no significant differences among the initial succinic acid production rates observed at all four pH values studied. However, at pH 6.0, succinic acid production abruptly dropped to a very low rate after its concentration reached 19 g/L. The overall succinic acid production results obtained at pH 6.5 and 7.0 were almost the same whereas those obtained at pH 6.25 were slightly better. Maximum succinic acid concentration of 36 g/L was obtained at pH 6.25 compared to 32 g/L obtained at pH 6.5 and 7.0. The yields at all four pH values varied from 0.60 to 0.67 g succinate/g glucose consumed. pH also did not strongly affect the succinate/acetate molar ratio, which varied from 1.6 to 1.8. Optimum pH seemed to be between pH 6.25 and 6.5. See Fig. 4.

Figure 4



- **The effects of length of the aerobic stage.** There was no net cell growth during the aerobic stage. Therefore, the length of the aerobic stage was effectively the same as the age of the cells, which were used for succinic acid production. To study the effects of cell age on succinic acid production, experiments were performed in which the aerobic stage was maintained for 4, 6, 8, 10, and 24 hours. In these experiments, the pH was maintained at 7.0 with NH_4OH and carbon dioxide gas was sparged into the fermenter at 0.05 vvm. The cell specific productivity decreased with cell age, from 0.31 g succinate/h-g dried cell weight (DCW) for 4-hour old cells to 0.11 g succinate/h-g DCW for 24-hour old cells. The cell concentrations at the end of the aerobic stage, however, increased more gradually with the length of the aerobic stage. Consequently the linear succinic acid production rate went through a maximum of 1.03 g/L-h when the length of the aerobic stage was 10 h and then decreased to 0.61 g/L-h when this period was extended to 24 hours. The succinic acid concentrations obtained at 48 hours did not vary significantly when the length of the aerobic stage was between 4 and 10 hours. However, it dropped by almost 50% when the length of the aerobic stage was increased to 24 hours, simply because the production rate was lower and the production time was significantly shortened. The succinate yields were steady around 0.6 g succinate/g glucose consumed when the length of the aerobic stage varied from 4 to 10 hours. However, it dropped to 0.28 g succinate/g glucose consumed when the length of the aerobic stage was increased to 24 hours. The succinate/acetate molar ratio varied with the length of the aerobic stage. This ratio was highest at 2.3 when the aerobic stage was maintained for 8 hours. When

shorter aerobic stages of 4 and 6 hours were maintained, the succinate/acetate molar ratio decreased to 1.7 and 1.5, respectively. A higher succinate/acetate ratio will reduce the recovery and purification cost of succinic acid. However, a longer aerobic stage will increase the aeration cost. Therefore, a complete cost analysis of the process will be needed to determine the optimum length of the aerobic stage. The results obtained in this study suggested that the length of the aerobic stage should be between 4 and 8 hours. See Table 1.

Table 1. The effects of length of the aerobic stage on succinic acid production

Length of the aerobic stage (h)	4	6	8	10	24
OD at aerobic/anaerobic transition	5.3	7.5	6.6	9.5	12.4
Linear succinic acid production rate (g/L-h)	0.74	0.98	0.80	1.03	0.61
Specific productivity* (g succinate/g DCW-h)	0.31	0.29	0.27	0.24	0.11
Succinic acid concentration at 48 h (g/L)	24.9	25.1	27.5	25.9	14.5
Acetic acid concentration at 48 h (g/L)	7.7	8.5	6.1	7.1	4.0
Succinate yield at 48 h (g succinate/g glucose consumed)	0.66	0.62	0.61	0.64	0.28
Succinate/acetate molar ratio at 48 h	1.7	1.5	2.3	1.9	1.9

*Determined for the linear production period

- **Process scale-up.** The scale-up experiments were performed for two purposes: a. to test the scalability of the fermentation process developed in the laboratory-scale fermenters, and b. to provide actual fermentation broth to the researchers at the Argonne National Laboratory who performed recovery and purification studies. In all of the scale-up experiments, the 25% (v/v) LSW medium was used, the pH was maintained at 7.0 with NH_4OH, and carbon dioxide gas was sparged into the fermenters at 0.05 vvm. The experiments were first performed in a 75-liter and then in a 500-liter fermenter. At least three experiments were conducted under the same conditions at each scale. The results showed a very high degree of reproducibility. These results also confirmed the results obtained in the laboratory-scale one-liter fermenters. In fact, the scale-up results were significantly better than those obtained in the laboratory-scale fermenters. It was probable that the larger fermenters were operated under higher pressure, which increased

the solubility of the sparged carbon dioxide gas in the fermentation broth. Higher dissolved carbon dioxide concentrations could have resulted in improved succinic acid production. See Figs. 5 and 6.

Figure 5

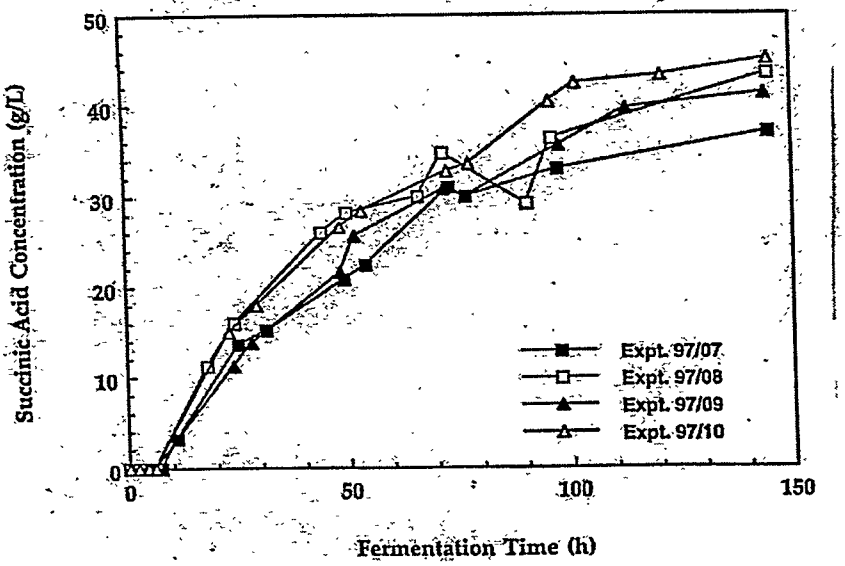
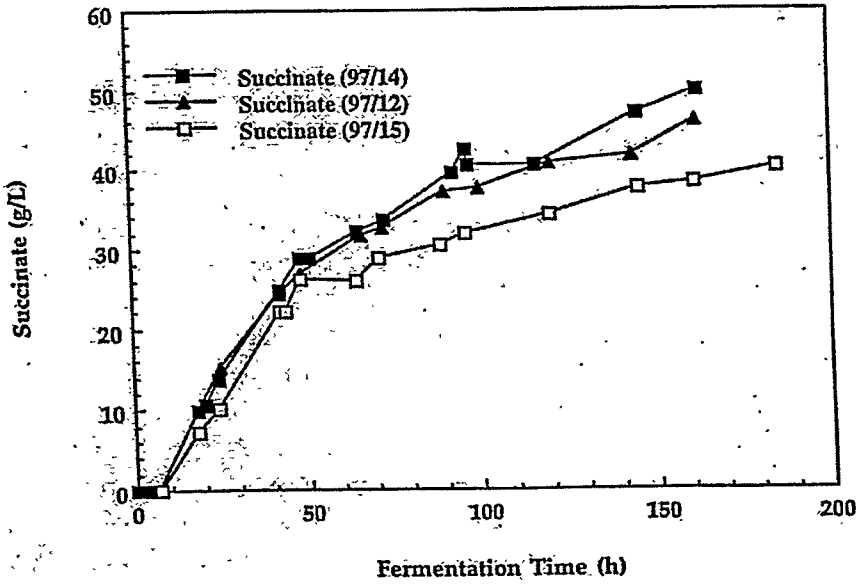


Figure 6



- **Improving process productivity by cell settling.** This process modification to cause cell settling and increase overall productivity is described under an ORNL invention disclosure.

Task 6. Biocatalysts Development
(Argonne National Laboratory, Mark Donnelly, PI.)

Introduction: Prior to establishment of the CRADA, ANL scientists discovered a spontaneous mutant of *E. coli* that produced succinic acid as its major fermentation product. The parental strain (NZN111) was unable to grow anaerobically on glucose due to a pair of mutations in critical genes. ANL's effort in Biocatalyst Development consisted of studying AFP111 in order to 1) optimize its production of succinic acid, 2) improve its fermentation through genetic manipulations, and 3) identify the nature of the mutation through physiological and genetic studies. This last effort strove both to allow extension of the fermentation of AFP111 to other strains and to solidify patent protection.

Subtasks:

a) Optimize growth and production of AFP111 at bench scale (with ORNL)
b) bench-scale physiology studies
c) modify AFP111 for elimination of side product ethanol.
d) support pilot and scale up efforts.

RESULTS:

a) Optimize growth and production of AFP111 at bench scale

Established that optimum pH for fermentation was 6.1-6.5
(with S.-P. Tsai, ANL, within CRADA).

Established that inclusion of H2 in sparge gas enhances yield
(with Eric Johnson, University of Illinois, through contract).

b) bench-scale physiology studies

Discovered that other sugars could be converted to succinic acid by NZN111, the parent strain of AFP111 (with David Clark, consultant, Southern Illinois University).

Observed a lack of preference for glucose compared to parental strain
(which lead to screening procedures that identified the afp mutation).

Applied chromogenic plate tests for distinguishing strains containing the afp mutation from those lacking it.

Established that AFP111 lacked normal catabolite repression (a regulatory mechanism that favors metabolism of glucose)

c) modify AFP111 for elimination of side product ethanol

Generated alcohol dehydrogenase mutants (verified enzymatically) but discovered that they would not grow anaerobically on glucose.

d) support pilot and scale up efforts.

Identified the mutation that confers the afp phenotype - a mutation in the gene *ptsG*, which encodes a protein that transports glucose into the cell and is known to participate in the regulation of sugar metabolism.

Showed that mutation of *ptsG* in other strains also enhances their production of succinic acid significantly.

Established that AFP111 can ferment mixtures of sugars simultaneously to succinic acid.

Discovered that the parental strain, NZN111, also can produce succinic acid anaerobically if first grown to stationary phase aerobically.

Task 7: Improved Purity Separation -
(Shih-Perng Tsai, Argonne National Laboratory)

Separation Summary: The objectives of this task were to:

- Validate the double-electrodialysis primary purification process for the AFP111 fermentation broth,
- Improve the purification process, as needed, and
- Process large volumes of succinate fermentation broths to support fermentation scale up and catalyst development.

During this CRADA, laboratory scale separation experiments were performed using synthetic and AFP111 fermentation broths for several unit operations, including membrane filtration, desalting electrodialysis, water-splitting electrodialysis, ion-exchange, adsorption, evaporation, and crystallization. Options for potential improvement of product purity were evaluated. Several batches of purified succinic acid were produced from AFP111 fermentation broths, and samples were evaluated by PNNL for catalytic conversion and by ACC for another end use.

Separation Highlights:

- The double-electrodialysis process, which consisted of desalting and water-splitting electrodialysis, was found to be suitable for succinic acid purification from the AFP111 fermentation broth. The process performance results for the AFP111 broth, in terms of flux and energy consumption, were better than those obtained earlier for the broth generated by using a different organism.

- A promising nanofiltration process was developed to improve the removal of low-molecular-weight impurities, particularly sugars and acetate. Approaches for further improvement and optimization have been identified.

- A purification processing train, with an activated carbon adsorption polishing step was developed for the ammonium succinate product.

- AFP111 fermentation broths generated in 75-L and 500-L fermenters were successfully processed through the product separation processing trains. Several batches of purified succinic acid were made using different polishing schemes. The relationship between the polishing schemes used and the impurity profiles of the products was established and can be used as a guide for future improvement of product purity.

- A sample of a purified succinic acid product was evaluated by a potential partner of ACC for an end use. The evaluation results were reported to be satisfactory.

- Two batches of purified succinic acid were provided to PNNL for laboratory evaluations of catalytic conversion. Conversion was observed, but a higher succinic acid purity appeared to be required for higher conversion rates. Potential approaches for improved purification have been identified.

Technical Description:

Earlier in this DOE-funded project and prior to execution of this CRADA, a double electrodialysis purification process (hereafter referred to as the base case purification process) was evaluated and validated for primary purification of fermentation-derived succinic acid produced by using the organism *Anaerobiospirillum succiniciproducens*. Compared with the conventional gypsum process for succinic acid purification, the double-electrodialysis process avoids generation of large-volume of salt wastes and is expected to be more economical. Results of earlier separation work of this project have been reported in AFP Quarterly Progress Reports from Fourth Quarter, FY 1993 to Second Quarter, FY 1995.

In this CRADA, new processes were to be developed for succinic acid production using the new succinate-producing organism AFP111 developed by the national laboratory team. Because the fermentation broth compositions are usually affected by the organism and fermentation conditions used, it was necessary to validate the double-electrodialysis (base case) purification process for the AFP111 broth. Work was also performed to improve the base case purification process, particularly in using nanofiltration to improve the removal of low-molecular-weight impurities and in developing a purification processing train for the ammonium succinate product. In addition, batches of purified succinic acid were made from fermentation broths generated in 75-L and 500-L fermenters, by processing the broths through the entire purification processing train with different polishing schemes. Figure 1 shows the summary flowchart for the production of purified ammonium succinate or succinic acid from each batch of fermentation broth, through each unit operation step. Broth # ORNL-96-75-1 was an ammonium succinate broth and the corresponding product was purified ammonium succinate. All other batches of fermentation broths were sodium succinate broths and the products were purified

succinic acid. In addition to experiments shown in Figure 1, nanofiltration and ammonium succinate polishing experiments were also conducted using simulated broths.

The base case double-electrodialysis purification process

The base-case double electrodialysis process for purification of fermentation-derived succinic acid consists of the following sequence of unit operations:

1. Microfiltration: removing cells and other suspended solids from the fermentation broth.
2. Desalting electrodialysis: concentrating and purifying the succinate salt; removing small- and large-molecular-weight nonionic or weakly ionic species, such as sugars, alcohol (if any), amino acids, peptides, and proteins.
3. Chelating ion-exchange: removing multivalent cations, such as calcium and magnesium.
4. Water-splitting electrodialysis: removing 95-99% of cations, such as sodium or ammonium; converting ionized succinate to undissociated succinic acid.
5. Cation-exchange polishing: removing the remaining 1-5% of cations (sodium or ammonium) and some nonionic impurities (by non-ionic interactions).
6. Anion-exchange polishing: removing strong anions (sulfate and chloride) and some nonionic impurities (especially color bodies).

There are two major limitations of this double-electrodialysis process, regarding the achievable product purity. Firstly, this process is not effective in removing organic acid by-products of fermentation (e.g., acetate in succinate fermentation). Secondly, although the process is capable of removing 90-95% of low-molecular-weight nonionic impurities (e.g., glucose and other unfermented sugars), the remaining levels of such impurities may make the product unsuitable for the intended use. For example, it is commonly recognized that the purity requirements for fermentation products to be used as feedstocks for catalytic conversions are more stringent than for food applications.

The base-case purification process was found to be largely applicable to the AFP111 fermentation broth for succinic acid production. The process performance results for the AFP111 broth, in terms of flux and energy consumption, were better than those obtained earlier for the broth generated by using *A. succiniciproducens*, due to the more favorable fermentation broth compositions and the use of nanofiltration described later. However, the base-case process was not applicable to ammonium succinate production. Also, the aforementioned limitations for product purity remained. Therefore, a purification processing train was developed for ammonium succinate production and a nanofiltration step was added to improve the achievable product purity.

Purification processing train for ammonium succinate production

In addition to purified succinic acid, another target succinate product in this CRADA was an aqueous solution of purified ammonium succinate. The new purification processing train for ammonium succinate production consists of the following sequence of unit operations:

1. Microfiltration: removing cells and other suspended solids from the fermentation broth.

2. Nanofiltration: concentrating ammonium succinate and removing low-molecular-weight nonionic and monovalent impurities (e.g., sugars and acetate)
3. Desalting electrodialysis: further concentrating and purifying the succinate salt; removing small- and large-molecular-weight nonionic or weakly ionic species, such as sugars, alcohol (if any), amino acids, peptides, and proteins.
4. Activated carbon adsorption polishing: removing remaining sugars and proteins.

A laboratory system equipped with a Desalination Systems DK2540 spiral wound module was used for nanofiltration experiments. This module has about 1.8 m^2 of membrane area. This membrane has a molecular weight cut-off of about 150-300 and is reported to have high rejection of divalent anions in the presence of monovalent anions. Nanofiltration experiments were initially carried out using synthetic succinate broths. The membrane successfully permeated acetate and rejected succinate. Succinate rejection was found to increase with retentate pressure and was 97% at 400 psig, 94% at 300 psig, and 84% at 200 psig. Using a sequential nanofiltration and dialfiltration scheme, about 89% of the succinate in the original feed solution was recovered and 77% of the acetate was removed. Additional information was reported in the Fourth Quarter, FY 1996 AFP Quarterly Progress Report.

Development of a new polishing step was necessary for removal of the remaining sugars and proteinaceous impurities from the ammonium succinate solution produced in desalting electrodialysis. In the base-case double electrodialysis process, which generates a succinic acid product, polishing of succinic acid is accomplished by cation-exchange and anion-exchange. This polishing scheme is not applicable for ammonium succinate, which contains succinate in the ionized form, instead of the free acid form. Sugars and proteinaceous impurities are expected to cause side-reactions and tar formation in the catalytic conversion step. Therefore, an alternative polishing step is needed.

Laboratory polishing experiments were carried out in shake flasks and columns, using simulated ammonium succinate solution that contained impurities (sugars, proteins, and inorganic salts). Four non-ionic polymeric resins (XAD-2, XAD-4, XAD-7, and XAD-16 from Rohm & Haas), one weakly basic anionic resin (IRA-94 from Rohm & Haas), and one granular activated carbon (CPG from Calgon Carbon) were tested. CPG activated carbon was found to be the most effective adsorbent in removing sugars and proteins. Additional information can be found in the Fourth Quarter, FY 1996 Alternative Feedstocks Program Quarterly Progress Report.

As shown in Figure 7, this purification processing train was used for the production ammonium succinate from a batch of AFP111 fermentation broth (ORNL-96-75-1). A color-free ammonium succinate solution was produced, which contained 42.3 g/L succinate, 4.4. g/L acetate, 0.1 g/L total reducing sugars, and 0.11 g/L proteins.

Production of purified succinic acid from AFP111 fermentation broth

Four batches of sodium-succinate-containing AFP111 fermentation broth generated at ORNL were processed for production of purified succinic acid. Three batches of broth from the 75-L fermentation runs (Broth # ORNL-97-75-1, ORNL-97-75-2, and ORNL-97-75-3) were processed in the same manner through water-splitting electrodialysis. Each batch was processes

by nanofiltration to remove low-molecular-weight impurities and then by desalting electrodialysis to remove higher-molecular-weight impurities and to concentrate the succinate, followed by water-splitting electrodialysis to convert the ionized succinic acid to undissociated succinic acid. All processing steps proceeded smoothly, except for a membrane fouling incident in one of the desalting experiments. In that experiment, severe membrane fouling caused the run to be terminated. Upon examination of the interior of the membrane stack, the fouling appeared to be deposition of denatured (and insoluble) proteins (from the nutrient source) on the membrane surface. After cleaning using the established protocols, the membranes were reused successfully in subsequent runs, suggesting the fouling was reversible. Therefore, a two-prong approach to control membrane fouling was adopted, which consisted of reducing the protein contents in the broth prior to desalting electrodialysis and cleaning-in-place. In later runs, raising the pH of the broth before microfiltration was found to effectively cause a significant fraction of proteins to denature. The denatured proteins were then removed by microfiltration. This pretreatment reduced the membrane fouling propensity for nanofiltration and desalting electrodialysis. It also appeared to result in higher fluxes for the nanofiltration step.

The water-splitting electrodialysis products still contained small amounts of impurities, and aliquots of them were further purified by using different polishing schemes to evaluate the efficacy of each scheme. Table 2 shows the impurity profiles of purified succinic acid products obtained from different polishing schemes.

Table 2. Impurity profiles of purified succinic acid products obtained from different polishing schemes. TRS: total reducing sugars. All impurity concentrations are expressed as weight % of succinic acid (wt % of SA).

Samples	TRS (wt % of SA)	Proteins (wt % of SA)	Sulfate (wt % of SA)	Lactic acid (wt % SA)
SA-9801	< 0.01	0.45	< 0.01	0.77
SA-9802	< 0.01	0.62	2.34	0.50
SA-9803	< 0.01	0.01	0.56	< 0.01

Although all feeds to the polishing schemes were similar but not identical, the following conclusions can be made:

- The levels of sugars were low for products from all these three polishing schemes.
- Crystallization (used only for SA-9803) was the only polishing step that could effectively remove proteins and lactic acid.
- Anion-exchange polishing (used only for SA-9801) was the only effective means of removing sulfate. Crystallization appeared to remove some sulfate but not very effectively.

- A polishing scheme that consists of cation-exchange polishing, anion-exchange polishing, and crystallization is most promising for the highest product purity.

Subsequently, 200 liters of Broth ORNL-97-500-1 were processed to produce 5.8 liters of the purified succinic acid product SA-9804, which contained 211 g/L of succinic acid in a slurry form (at room temperature). SA-9804 was processed with the same purification train and polishing scheme as SA-9801. However, SA-9804 was found to contain higher levels of impurities. Impurity analyses for SA-9804 found 6.8 wt % lactic acid, 3.1 wt % acetic acid, 0.4 wt % proteins, and 0.02 wt % sulfate, all on succinic acid basis; total reducing sugars were not detectable. The higher level of sulfate was probably due to leakage (breakthrough) in anion-exchange polishing. Extended exposure of the process materials at room temperature, due to repeated manual handling of the materials in between experiments and the longer processing time in each unit operation step, could have caused microbial contamination of the materials and resulted in considerable higher levels of lactic and acetic acids.

Evaluations of purified succinic acid products for end uses

A sample of a crystalline succinic acid product (SA-9701) was evaluated by a potential partner of ACC for an end use. The evaluation results were reported to be satisfactory.

Two succinic acid products (SA-9801 and SA-9804) were evaluated by PNNL for catalytic conversion using two different catalysts (designated here as Catalyst A and Catalyst B). Mixed results were obtained. In the initial test using Sample SA-9801 and Catalyst A, the catalytic conversion test results were comparable with those obtained with pristine succinic acid. Later test results showed lower catalytic activities for Sample SA-9804 with both catalysts, compared with pristine succinic acid. Also, lower catalytic activity was found for Sample SA-9801 with Catalyst B. These results suggest that a higher purity succinic acid product is needed for the catalytic conversion applications. However, these results are also encouraging and suggest that the purity obtained to date may be close to the requirements and a suitable product can potentially be obtained with further optimization of the purification process.

Several approaches to higher product purity can be identified at this time. First of all, continuing improvements in the fermentation process can produce fermentation broths that can allow succinic acid to be purified more easily. Also, as discussed earlier, a polishing scheme that consists of cation-exchange polishing, anion-exchange polishing, and crystallization is expected to give a higher product purity. In addition, processing of large volumes of materials should be conducted in a more integrated manner in suitable facilities with larger scale processing equipment to minimize unnecessary handling and avoid product deterioration.

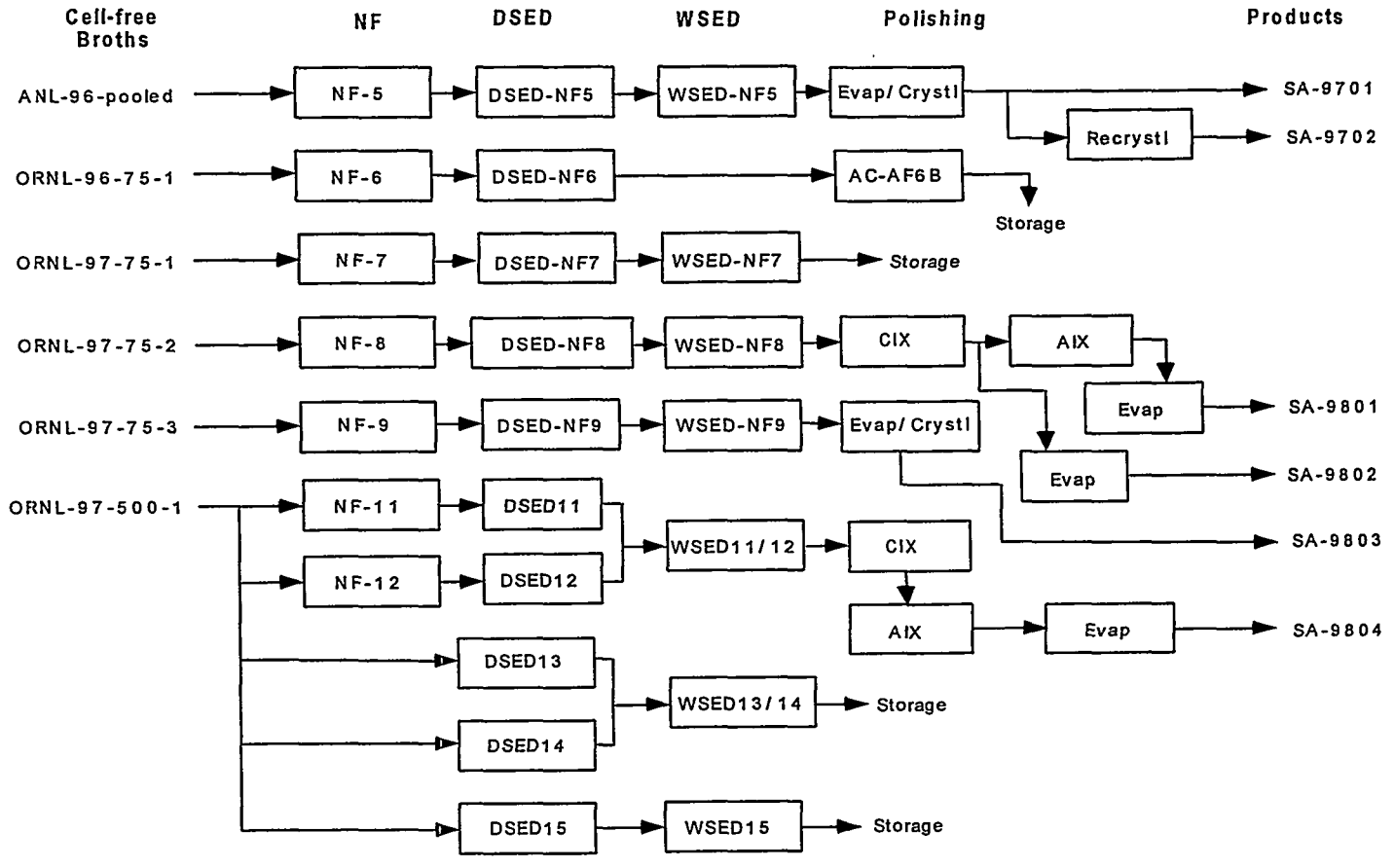


Figure 7. Flowchart of ammonium succinate or succinic acid product purification from AFP111 fermentation broths. NF: nanofiltration; DSED: desalting electrodialysis; WSED, water-splitting electrodialysis; CIX: cation-exchange; AIX: anion-exchange; AC: activated carbon adsorption; Evap: evaporation; Crystl: crystallization; Recrystl: recrystallization. Chelating ion-exchange was performed prior to each WSED step but is omitted here for simplicity.

Task 8: Catalysis
(John Frye, Pacific Northwest National Laboratory)

Catalysis Executive Summary

Researchers at the Pacific Northwest National Laboratory investigated and advanced catalytic technology for converting succinic acid to various commodity chemicals at commercially attractive rates. Novel high activity/selectivity catalysts and innovative processing schemes have been developed for producing GBL, BDO, THF, and Pyrrolidinone-related products in high yields. Two invention disclosures have recently been submitted, and are being reviewed by CRADA partner ACC to determine if they wish to pursue licensing of these technologies. The first is a novel, and potentially patentable, catalyst preparation procedure that was discovered during the course of this work and reported on previously. These new materials are referred to as "textured" catalysts. These inexpensive and highly active catalysts may be useable in a wide range of important chemical processes. The second disclosure involves the discovery of a novel, and likewise potentially patentable, one-step process for the production of mixtures of 2-

pyrrolidinone and NMP. The new process is thought to have several appealing features over a two-step process and, at first glance, attractive economic potential. One significant technical issue remained unresolved at the completion of the CRADA period. This involved the observation that the purified, fermentation-derived succinic acid products (supplied by ORNL and ANL) were converted at much lower rates than were pristine succinic acid solutions. Initial tests on small samples of fermentation-derived materials run in semi-batch tests and using commercial powdered catalysts failed to show any significant difference in conversion rates between the fermentation-derived materials and pristine feedstocks. When new high activity granular catalysts were substituted for the powdered catalysts, the rates observed for the fermentation-derived feedstocks were nearly an order of magnitude lower than those observed for the pristine materials. The fermentation-derived feedstock material is known to contain several percent of residual protein material (by ANL's analysis) that was not successfully removed by the ANL processing scheme. It is suspected that this material may be at least partially responsible for the observed decrease in catalytic activity (either by fouling or poisoning of the catalyst). Unfortunately, by the time that the magnitude of the differences between these materials was fully realized, PNNL's CRADA funding was also nearly exhausted. It was not possible, to undertake additional studies to further elucidate what factor or factors were responsible for the observed differences between the two feed materials or to find an effective way of dealing with the problem.

Catalysis Highlights:

Prior to the formal signing of the CRADA agreement between D.O.E. and Applied Carbochemicals, researchers at PNNL had already made preliminary investigations into the production of GBL, BDO, THF, 2-pyrrolidinone, and N-methyl-2-pyrrolidinone from succinate precursors. Significant progress had already been made at identifying selective catalysts for the selective reduction of succinic acid to GBL in the presence of fermentative by-product acetic acid. While high GBL yields had been successfully demonstrated, catalyst productivities were still below commercially attractive rates. A very promising catalyst and set of processing conditions had been found that was capable of converting GBL to 1,4 BDO at high rates and at nearly 100% selectivity. This was intended to be part of a two-step succinic acid to GBL to 1,4 BDO process. Also, several catalyst systems had been identified that were capable of converting succinic acid to a mixture of GBL, 1,4 BDO, and THF in a single step. Initial investigations had also begun on determining appropriate catalysts and processing conditions for the production of 2-pyrrolidinone from succinimide or diammonium succinate. A very promising catalyst/non-aqueous process was discovered for converting succinimide to a mixed product containing ~ 80% 2-pyrrolidinone and 20%GBL. However, early attempts at preparing 2-pyrrolidinone via aqueous processing considerably lagged behind results reported in the patent literature. A few preliminary investigations had also been made into the production of N-methyl-2-pyrrolidinone in single-step reactions. While several of these reactions yielded the desired NMP product, rates of production were exceptionally low.

Even though the CRADA agreement with Applied Carbochemicals was not officially signed until May of 1996, we were encouraged to begin working with ACC on their research needs somewhat earlier than that. ACC's initial business interest was in the production of 2-pyrrolidinone and related derivatives. They felt that a bio-derived ammonium succinate or succinimide feedstock would allow them to better compete with 2-pyrrolidinone and related derivatives produced by petrochemical routes. The discussion that follows contains what are

considered as the key technical highlights that occurred in the area of catalyst and process development at PNNL in the support of the ACC CRADA.

* Semi-batch reactor tests were used to map reaction pathways and to obtain rate data to guide rational catalyst selection for the production of 2-pyrrolidinone from aqueous ammonium succinate solutions. Rh/C catalysts appeared to offered the best compromise of reaction rate with the least amount of over-reduction of the 2-pyrrolidinone product (therefore displayed the highest product yields). 2-pyrrolidinone yields as high as ~ 82%, and at high conversion rates (in excess of the targeted 1g per hour per gram of catalyst) were achieved within a relatively short time, and were comparable to prior patent literature claims.

* During the course of the semi-batch investigations into the production of 2-pyrrolidinone, it was discovered that the addition of methanol to the reaction mixture would result in a mixed product of 2-pyrrolidinone and N-methyl-2-pyrrolidinone where the combined yield of the two products were in excess of 90%. The over-reduction products, such as N-butyl-2-pyrrolidinone, that were normally observed along with 2-pyrrolidinone in the product solutions were all but eliminated, and were replaced by NMP, an end product in which ACC had already shown great interest. This observation has been written up as a PNNL invention disclosure and the details are not specified herein as proprietary information. A patent application is also in preparation covering this concept.

* Flow reactor studies of the production of 2-pyrrolidinone from aqueous ammonium succinate solutions showed that the Rh/C catalysts, that had worked well in semi-batch tests, were indeed quite susceptible to rapid deactivation in longer duration runs. Significant Rh metal sintering was observed in spent catalyst samples, which is thought to be largely responsible for the large losses of catalytic activity that were observed in the early flow reactor studies. These results led to the investigation of the use of various catalyst promoters, in an effort to try to reduce the tendency of the Rh metal to sinter and lose active surface area over time. New promoted Rh/C catalysts were developed and found to be substantially more active that the previous Rh/C catalysts (~ 60% higher initial catalytic activity than that displayed by the Rh/C catalysts and containing an equivalent amount of Rh metal). These catalysts were later termed "textured" catalysts. These high activity "textured" catalysts were also written up in a PNNL invention disclosure and the details are considered to be proprietary information. Additional work has been conducted on the development of the "textured" catalysts with a commercialization partner and a patent application is in preparation.

* Significant improvements in the conversion rates of pristine succinic acid solutions to GBL were achieved with "textured" versions of Pd/C catalysts. In semi-batch tests at reaction temperatures of 225°C, productivities as high as ~ 1.7 g per hour per g of catalyst were observed while displaying nearly 100% selectivity to the desired GBL product. While these results are quite acceptable, later work has indicated that the Pd/Zr/C catalyst that was used in these runs may benefit from some further optimization work.

* As alluded to previously, the discovery that the fermentation-derived succinic acid broth (received from ANL) performed unsatisfactorily when used with granular C-supported Pd catalysts was indeed surprising and disappointing. Tests that had been conducted with an Engelhard Escat-11 powdered catalyst failed to show any significant difference between the conversion rates of the fermentation-derived feedstock samples tested earlier and pristine succinic acid solutions. While the exact cause of the problem remains unresolved, it is obvious from what we observed, that ANL's processing scheme did not provide a feedstock of sufficient purity to allow it to be processed economically. It may be that a very inexpensive change in their overall process scheme would solve the problem that we have observed, but at this time we don't know with certainty what it is that they need to fix. This is certainly a broader issue that needs to be addressed when one is contemplating the use of any bio-derived material as a potential feedstock.

Task 9: Economic Evaluation
(Ron Landucci, NREL and New Horizon, and ACC)

These results were obtained from a technical and economic analysis performed by National Renewable Energy Laboratory (NREL) and by New Horizon, Inc. for this project. This has been reviewed by several industrial representatives, updated by Oak Ridge National Laboratory (ORNL) and Energetics, Inc., and reviewed by A.D. Little. The conventional process estimates were based on a plant with a capacity of 105 million lbs/yr BDO. The capital cost of the conventional technology was more than the new technology.

The market for this family of chemicals is expected to continue to expand at a moderate pace of 6-10% per year. The raw material costs for corn sugars were estimated at $0.06-0.08/lb as compared to $0.04/lb for lignocellulosic mixed sugars. With comparable yields and rates (a goal of this near term research), this will decrease the estimated succinic acid production cost below $0.25/lb [Confidential information]. A more aggressive market penetration due to lower costs of lignocellulosic hydrolysates compared to corn sugars could more than double the impact. Therefore, it is very reasonable to expect that in 2020, when at least six units will be in operation, the net energy saving will be 9,810 billion Btu/yr and 252,000 tons waste saved/yr. This is a total market penetration of only about 25% or a capture of half of the new capacity required or twice the replacement of old Reppe plants going off-line.

Below is a nonproprietary summary showing the analysis on the improvement on competitive products or technologies. The primary competitors with the BDSA process are the three currently available petrochemical-based processes outlined above. As indicated in Table 3, the Reppe process, which has been described the most extensively in published reports, has the lowest cost among the competitors ($0.66/lb). The estimated production costs are $1.14/lb for the Kuraray/ARCO process and $0.89/lb for the Davy McKee process. Economic estimates concerning competing technologies are from SRI International reports; those relating to BDSA are from a detailed process flowsheet, based on a plant producing 100 million lb of BDO per year. Pollution and energy estimates are from the SRI reports and from a life-cycle analysis report by PNNL (*Stream-lined Life-Cycle Assessment of 1,4-Butanediol Produced for Petroleum Feedstock Versus Bio-Derived Feedstocks*, PNNL-11213, Battelle Memorial Institute, Pacific Northwest Natl. Lab., June 1996).

Table 3. Comparison of BDSA process with petrochemical-based processes

Process	BDSA	Reppe	Kuraray/ARCO	Davy McKee
Raw material	Corn sugar	Petroleum, natural gas	Syngas	Butane (petroleum)
Estimated production cost	$0.43/lb BDO (detailed estimate)	$0.66/lb BDO (SRI report)	$1.14/lb BDO (SRI report)	$0.89/lb BDO (SRI report)
Raw material cost	$0.20/lb BDO	$0.42/lb BDO	$0.99/lb BDO	$0.67/lb BDO
Energy use (per lb BDO)	36,000 Btu	42,000 Btu	39,000 Btu*	----

*Estimated from utilities cost.

As shown in Table 3, the BDSA process offers significant advantages in two primary areas. First, the process has a significantly lower estimated production cost for BDO, succinic acid, and several other commodity chemicals, as well as lower energy consumption during production. Furthermore, the process is a viable means of producing commodity chemicals from renewable resources. Current corn production could easily supply the 100 to 200 hundred million pounds of corn sugar required for each major chemical plant, and use of this feedstock would decrease petroleum consumption and imports. Although pollution from electricity generated by coal (as opposed to natural gas) could be increased with the use of the BDSA process, the direct environmental impact would be less than that associated with the petroleum-based processes. The BDSA process would also employ less expensive materials of construction, because succinic acid is less corrosive than other chemical intermediates such as maleic acid, which is used in competitive processes.

With established commodity industrial chemicals, 1 to 3% reduction of costs represents an incremental improvement, and improvements of 20 to 50% are dramatic. For example, a 75% improvement could result in a situation in which existing (already capitalized) chemical plants could no longer compete.

Further analysis by ACC and New Horizon of the production cost for succinic acid in our fermentation process, therefore, have significantly lowered the production costs. This assessment also included several process modifications to further decrease costs. The estimated costs in 1998 were $0.20 / lb sodium succinate compared to a petrochemical route of $0.60/lb from butane.

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APPENDIX
SUBJECT PATENTS AND INVENTION DISCLOSURES.

Subject patents and invention disclosures developed under CRADA:

Argonne National Laboratory:

ANL-IN-97-013	Elimination of alcohol dehydrogenase. No patent filed
ANL-IN-97-015	ANL's disclosure related to process patent (ORNL lead) Patent filed by ORNL and allowed.
ANL-IN-97-050	Metabolism of mixtures of sugars by AFP111. Covered under patent for ANL-IN-97-050, filed 4/28/98.
ANL-IN-97-140	Identification of *ptsG* gene as mutation in AFP111. Patent filed 4/28/98 and allowed 6/2000. Not yet issued.
ANL-IN-98-062	Process for succinic acid production with NZN111. Provisional patent filed 12/17/98.

Oak Ridge National Laboratory:

U.S. Patent #5,869,301	A Fermentation Process for the Production of Dicarboxylic Acids.
ERID-0750	An Improved Fermentation Method for the Production of Succinic Acid – Invention disclosure.

Pacific Northwest National Laboratory:

E-1715	"Novel Materials for Use in Heterogeneous Catalysis" (T.A.Werpy / A.H.Zacher / Y.Wang / J.G.Frye,Jr.)
E-1448"	One-Step Preparation of the Mixture of 2-Pyrrolidinone and N-Substituted Pyrrolidinones" (T.A.Werpy / J.G.Frye,Jr. / Y.Wang / S.D.Burton / A.H.Zacher)

PREEXISTING INTELLECTUAL PROPERTY

US Patent # 5,770,435
#ANL-IN-95-055; submitted 11/2/95. "A Mutant E. Coli Strain, AFP111, with Increased Succinic Acid Production"
Mark Donnelly, Cynthia Millard, Lucy Stols - Argonne National Lab
This is new strain with very good production characteristics and improved robustness and reliability; plus potential for higher yields and further improvements..

ESID-1834. "Fermentation Process using E. Coli to produce Succinic acid"
John Nghiem - ORNL
process patent on method to best utilize AFP111. We plan to expand claims as process examples are continuing. perhaps with ANL

#ID E-1413. "Selective Reduction of Organic Acids to Facilitate Separations"
Todd Werpy, John Frye, Yong Wang - PNL
Catalyst selectively reduces Succinic acid to gamma butyrolactone (GBL) without converting the fermentation coproduct, acetic acid into lower value ethanol. Note: all the catalysis are done in aqueous environments - an improvement that simplifies the overall processes.

#ID E-1440. "Highly Selective Catalysts for Conversion of GBL to 1,4-Butanediol"
John G. Frye, Todd A. Werpy, Yong Wang
Catalysts convert GBL to BDO, with above the second stage of efficient two stage catalytic process.

#E-1449. "Selective Reduction of Ammonium Salts, Amides or Imides of Organic Acids to Facilitate Separations"
John Frye, Todd Werpy, Yong Wang - PNL
These catalysts will convert DAS or succinimide into 2-pyrrolidinone. This allow use of cheaper ammonium hydroxide to neutralize Succinic fermentation into Diammonium succinate (DAS) or succinimide.

#E-1448. "Process for Converting 2-Pyrrolidinone to n-Methyl-2-Pyrrolidinone via Reaction with CO2 and H2"
John Frye, Todd Werpy, Yong Wang - PNL
This process allows production on NMP with conditions for addition of CO2 and H2 suing existing catalysts. This may drop cost of NMP (growing market) from ~$2/lb to $0.60/lb.

#E-1354. "Catalytic Upgrading of CO2 via Aqueous Phase Hydrogenation"
John Frye, Todd Werpy, Yong Wang - PNL
Catalysts and process for aqueous phase conversion of CO2 and H2 into other chemicals such as methane, methanol, formaldehyde, formic acid, etc.

#ANL-ID-95-109. "Biodegradable poly(ester carbonate) for plastics applications based upon succinate esters, 1,4-butanediol, and phosgene or phosgene derivatives"
T. Abraham
new polymer from products of the other AFP process; This invention is available but is not of current interest to ACC.

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