MySAB Biorefinery

Final Scientific / Technical Report

DOE Award Number: **DE-EE0002878**

Name of Recipient: **Myriant Lake Providence, Inc.**

Project Title: **BER- Myriant Succinic Acid Biorefinery (MySAB)**

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Executive Summary

Myriant Corporation (Myriant) has successfully produced the bioproduct succinic acid by the fermentation of glucose at a commercial scale operation in Lake Providence, Louisiana. The MySAB facility (Myriant Succinic Acid Biorefinery) came on stream in May 2013 and has been producing product since then.

The MySAB facility is a demonstration-scale plant, capable of utilizing sorghum grits and commercially available dextrose, to ferment glucose into succinic acid. A downstream processing train has demonstrated the ability to produce an industrial, a standard and a polymer grade product. It consists of cell separation, membrane filtration, continuous chromatography, polishing to remove ionic and color bodies impurities, and final evaporation and crystallization. A by-product of the process is ammonium sulfate which is sold as a liquid fertilizer product.

Since 2007 when development work began in the Woburn, Massachusetts R&D laboratories, the succinic acid bio-process has evolved through:

- Process development (microbiology, fermentation, and downstream) – R&D development laboratories.
- Piloting efforts at Fermic S.A. de C.V., Mexico City, Mexico – upstream and downstream processes
- Design, construction, commissioning, and commercial production operations at the MySAB facility

Additionally, Myriant became a wholly-owned subsidiary of the PTT Global Chemical Plc., Thailand, in late 2015, their investment into and support of Myriant goes back to 2011. The support of PTT Global Chemical Plc. helped to improve the upstream and downstream processes, and produce significant metric ton quantities of high quality bio-based succinic acid. The product has gone into a number of commercial markets worldwide for customer applications, development and production.

The experience base gained via operations at the MySAB facility since May 2013, along with continued R&D development efforts involving Microbiology, Fermentation, and Downstream processes, at both the Woburn, Massachusetts and PTT Global Chemical Plc. Thailand laboratories, positions the company well for future production at the plant and commercialization of new bio-based products. This will be especially important and valuable as the green chemistry business climate continues to take root and flourish.

1.0 Project Introduction

In 2009 Myriant applied for and was later awarded a grant under the Recovery Act DE-FOA-0000096 for the purpose of a demonstration of Integrated BioRefinery. Myriant proposed to use its proprietary process to produce a high-value added bioproduct from renewable organic sources. In 2009, Myriant summarized the project as follows:

> The Myriant process is built around a genetically modified organism engineered to efficiently produce succinic acid from bio-derived sugars. In the 55.1 dry tons/day (50 dry tonnes/day) demonstration-scale facility to be located at our existing facility in Lake Providence, Louisiana, Myriant will produce succinic acid using grits (from grain sorghum, a renewable feedstock). The U.S. Department of Energy (DOE) has qualified grain sorghum as an acceptable feedstock for Topic Area 4. The Myriant process works on a wide variety of renewable feedstock-based sugars (including cellulosic). Grain sorghum was selected as the bio source material because of its immediate availability for use in the facility and its low biomass handling costs. Myriant's business plan proposes the use of renewable and/or cellulosic sugar feedstocks for commercial-scale development. This application is under Topic Area 4.
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> Biologically produced succinic acid qualifies as an acceptable "bioproduct." The production of bio-based succinic acid is cost effective when compared to succinic acid, which is presently produced from petroleum-based feedstocks, because it is not dependent on chemical manufacturing price volatility driven by use of petroleum feedstocks. Succinic acid was listed by the DOE as one of the most viable chemicals for further development as a bioproduct derived from renewable feedstocks suitable for commercial production. Myriant's proprietary process that works with organisms to control the fermentation process was developed by a world-class team of genetic and biochemical engineers and scientists, who have already commercialized other similar processes at full production scale. This process uses less energy per ton of succinic acid produced than its petroleum-based alternative. The process has significant greenhouse gas benefits both in its net consumption of carbon dioxide and in its reduction in the use of petroleum-based fuels. At commercial scale, the technology is expected to produce succinic acid at a cost 16 to 19 percent lower than the current market price for this product. The demand for succinic acid is up to one billion pounds per year. The process also produces ammonium sulfate, a marketable and valuable coproduct.

Myriant succeeded in completing construction of the facility and reached mechanical completion by April 2013. Plant startup began in May 2013 and first product was produced on May 30, 2013.

1.1 Benefit to the Public

- The project had an immediate effect on direct employment in Louisiana, employing 250 EPC workers and 60 direct jobs at the plant in production, QA / QC, maintenance and administration.
- The production of succinic acid from renewable feedstocks displaces petroleum based feedstocks for commercial products and reduces lifecycle greenhouse gases "GHG" emissions.
- This demonstrates the economic model to transition legacy manufacturing and first generation biofuels facilities to next generation bio-refineries.
- Production of succinic acid is a "High Value" bio-based chemical derived from renewable feedstocks. Succinic acid was at the top of DOE's list as the most viable chemicals for further development as a bio-product derived from renewable feedstocks suitable for commercial production.

1.2 Project Goals and Objectives

Myriant's objectives are to drive down costs of production while increasing revenues for renewable and sustainable bio-products, in order to spur petroleum and energy independence, job growth, and climate change solutions.

- To produce renewable succinic acid.
- To scale-up and demonstrate Myriant's proprietary succinic acid process.
- To supply large volume of bio-based succinic acid to the market, in order to secure long term off take agreements.
- To use less energy per ton of succinic acid produced than its petroleum based alternatives.
- To show significant GHG benefits in the process's net consumption of CO2 and in the reduction of petroleum use.
- To develop a necessary data package to conform to the requirements for project finance of the commercial facility.
- To validate performance of the proposed technology at demonstration scale and replicate operational data previously achieved in Myriant's pilot plant facility.
- To validate key process metrics (fermentation and separation yield, productivity, and chemical consumption) at scale and in commercially configured equipment at an industrial scale.
- To provide continuous operational data at a scale needed to lower the technical risks associated with proceeding to commercial scale plants.
- To proceed rapidly to demonstration scale and commercial scale production.
- To demonstrate that Myriant has a sound business and technology strategy to deploy succinic acid on a commercial scale and market succinic acid at commercial volumes.

2.0 Designed Process and Anticipated Technology Risks

Myriant has developed strains of genetically modified *Escherichia coli* organisms that can selectively produce lactic acid, acetic acid, formic acid, or succinic acid, depending on the genotype of the bacterial strains. The raw material feedstock used is grain sorghum in the form of grits. As part of the commercialization process, the feedstock materials are planned to include sugars from other lignocellulosic feedstocks. Commercial plants are planned to be built using the best available renewable feedstock source for a particular region in the future, with the first commercial facility designed to run on grain sorghum grits. Myriant's process has been proven to work on both grain sorghum grits and lignocellulosic bagasse material.

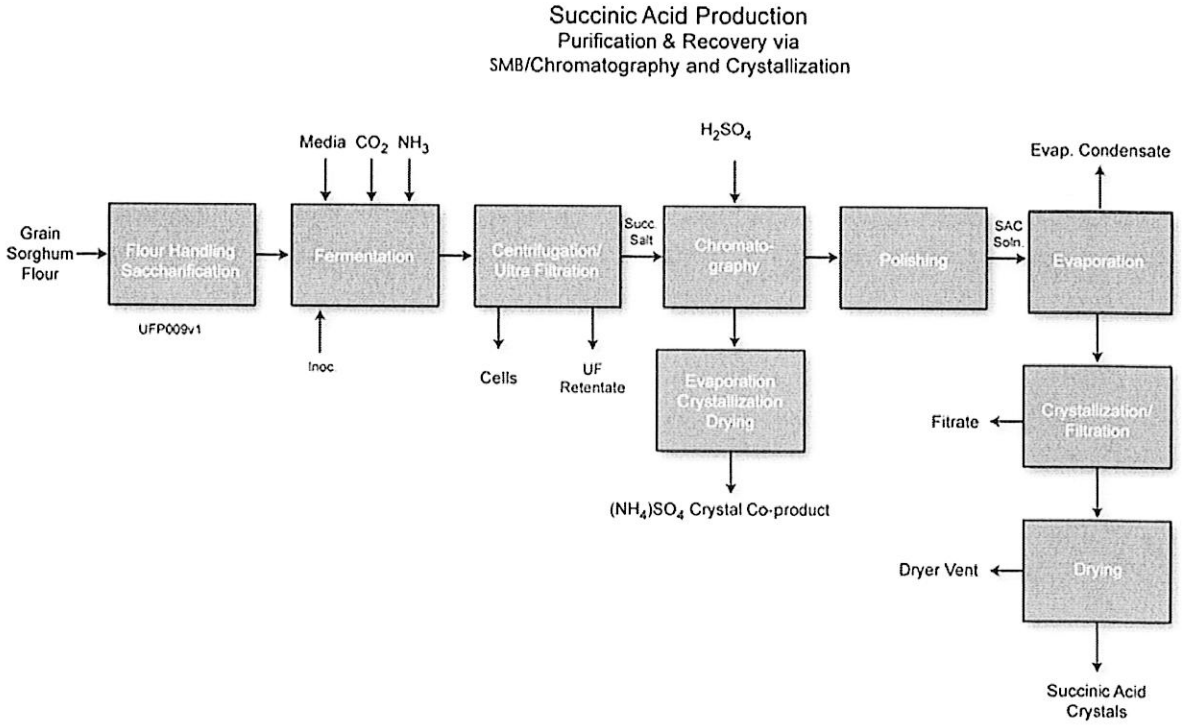

Figure 2.1 MySAB block flow diagram as originally proposed.

Key processes include fermentation, separation, and product recovery. Myriant's proprietary fermentation technology produces a broth with a high concentration of succinate salt.

2.1 Propagation and Fermentation

The purpose of the propagation operation is to grow an active fermentation-inoculum from a frozen stock of modified *E. coli* bacteria. The fermentation-inoculum will then be added to the fermentation vessel to convert glucose to succinic acid (SAC).

2.2 SAC Separation

This operation separates the fermentation-inoculum from the product solution. The separated cells are no longer viable after the inactivation step and are forwarded to the waste water treatment plant for processing. The resulting wastewater treatment biosolids are used for land application.

2.3 SMB Chromatography

The purpose of this operation is to separate the succinic acid product from the ammonium sulfate coproduct. The cell-free filtrate from the filtration unit containing ammonium succinate salt is acidified with sulfuric acid, generating the succinic acid and ammonium sulfate in solution. The ammonium sulfate passes through while the succinic acid is retarded and subsequently eluted into different streams with succinic acid going into extract and ammonium sulfate into raffinate.

2.4 Ammonium Sulfate Recovery

The ammonium sulfate collected from the continuous chromatography system raffinate stream is fed to a package evaporator system. The ammonium sulfate is then prepared for sale as a co-product from the plant.

2.5 SAC Evaporation

This unit operation consists of a package evaporation unit. This evaporator concentrates the product succinic acid to saturation. The concentrated succinic acid stream is further processed in a crystallizer to produce slurry which is then filtered. The evaporated water from both evaporation units is condensed and recycled.

2.6 SAC Crystallization / Filtration / Drying / Packaging

This unit operation consists of a package crystallization unit, crystal centrifuge, fluidized bed dryer, and size classification sieve. The purpose of this operation is to remove residual mother liquor, dry and sieve the solid succinic acid in crystalline form. The filtrate from the centrifuge is collected and recycled to the succinic acid evaporator feed tank. The crystalline succinic acid product is dried, sorted by size and packaged in the unit.

2.7 Technology Risks

All processing steps proposed for the MySAB project are commercially available in similar or equivalent applications. For the areas discussed below, some greater elements of risk exist and a discussion of how this can be mitigated by the experience of technical and project management team. The starting point for managing these risks is proper specification of process criteria as supported by the pilot (and subsequently demonstration plant) data.

Cellular Propagation and Media Preparation: are well established processes at the scales proposed – technology risk is low. The steps to propagate the organism to the required volumes for the commercial process are already practiced commercially for related processes. Medium has been optimized based on our previous experience in scaling up commercial processes to support effective organism growth while using low value nutrients, media and substrates.

Fermentation Technology: is well established at the scales proposed in the demonstration plant (100,000 gallons), and in many industrially similar processes at scales of 200,000 to 300,000 gallons. Key aspects of scale-up which will be addressed in this project and with which our project and technical team has extensive experience include:

Size, Heat Transfer, Sanitary Design: As fermentation volumes increase, heat liberated during fermentation can exceed the heat transfer capabilities of jacketed vessels and external/internal cooling configurations are required. Additionally, larger fermentor sizes can involve increased hydrostatic head, increased gas component partial pressures and concentrations, mixing concerns, differing CIP and SIP procedures, and other factors that make fermentor scale-up issues an important factor to be addressed with a well-planned and methodical approach.

Risk Mitigation – Pilot Scale-up Runs: Scale-up from 1,000 gallon reactors to 100,000 gallon reactors will be mitigated by preparing additional pilot fermentation batches at the 10,000 gallon scale. The mass and heat transfer data from the 10,000 gallon scale operations will be carried forward to the design of the 80,000 gallon demonstration plant reactors.

Risk Mitigation – Flexible Design: As demonstrated at the demo plant, final details of the commercial plant will depend on the learned experiences to maximize fermentor size and minimize cost in a manner which will ensure technical success, at the commercial scale. This robust approach is also supportive of Myriant's pipeline of future bio-products which may have varying characteristics that are organism and process specific.

Succinic Acid Separation and Purification: Myriant's separation and purification process has been piloted together with the fermentation process. The technologies incorporated in Myriant's process consist of off the shelf unit operations that are in commercial usage in various applications including biochemical and sugar applications. Key aspects of this development include:

SMB Process Optimization: The resin selection and processing parameters are optimized to minimize capital cost, improve selectivity for product recoveries, and minimize the dilution of effluent and raffinate streams. Scale-up and modeling is well established by the industry and has been tested at the pilot plant. The technology risk is rated at low to medium risk as the separation characteristics of the fermentation broth have proven to perform well in the pilot plant.

By-product Management: Minor organic byproducts are currently produced by the fermenting organism, at this time primarily acetic acid / acetate. The ion exchange / chromatography process has been optimized to direct this stream to go with either the raffinate or eluent streams. Over time acetic acid levels have been greatly reduced and are expected to be further minimized. When present, this byproduct is easily managed by removal in the evaporation step, but it does cause an organic waste stream to build up and will continue to be addressed.

Final Product Polishing: - Ion exchange polishing is a well-established technology and will mitigate any purification risks from the SMB chromatography separation step. This technology has been used in various industries for control of color in the final product as needed.

3.0 MySAB Design Basis

The design basis established at the Fermic SA de C.V. Mexico City, Mexico in the pilot plant studies was used by the EPC contractor in developing the design basis for the MySAB Plant.

3.1 Production Capacity

Thirty (30) million pounds of industrial grade succinic acid per year (3,754 lb/h).

3.2 Design on-stream time

The plant is designed to operate for 7,992 hours per year (333 production days). Redundant equipment was installed only for critical equipment, though space was left in most places for the ability to add additional redundant equipment at a later time.

3.3 Site

The Port of Lake Providence, LA, on approximately 40 acres of land, was previously intended to be a dry mill ethanol plant. Existing site pilings for ethanol plant were utilized to the maximum reasonable extent. No specific allowances were made for future site expansion.

3.4 Inputs

The design basis feedstock(s) for this facility are sorghum grits that will be subsequently saccharified with enzymes to produce an aqueous glucose feed stream. Capability for 3rd party produced sorghum syrups or 95% D.E. dextrose feedstock directly into fermentation is also provided.

3.5 Sorghum

Annual tons of feedstock: 24,975 short tons of ground dehulled sorghum grain.

Grain: USDA #2 Grain Sorghum

Dehulled / Decortified to remove the pericarp / corneous and to expose the endosperm

Component	Specification
Water	7 – 13 %
Starch	70 – 75 %
Fat	3.0 – 4.0 %
Fiber	1.3 – 1.8 %
Protein	9.5 – 11.5 %
Ash	0.5 – 1.8 %

Table 3.1 - Sorghum grits composition

3.6 95 DE

95% Refined Liquid Dextrose Corn Syrup is a low ash, demineralized corn syrup with a high dextrose content.

Chemical and Physical Properties

Dextrose Equivalent (DE)	95 - 99
Refractive Index (20°C)	1.4632 – 1.4656
Refractive Index (20°C)	1.4584 – 1.4607
Refractive Index (45°C)	1.4584 – 1.4607
Total Solids (%)	70.5 – 71.5
Moisture (%)	28.5 – 29.5
Sulfated Ash (%)	0.05 max
pH (1:1)	3.5 – 5.0
Sulfur Dioxide (ppm)	2 max
Conductivity (30% DS)	65 micromhos
Calories/100g	284

Table 3.2 - 95 Dextrose chemical and physical properties

3.7 Control Philosophy

Major package units were built with local control systems. A Siemens PCS-7 supervisory control system was provided for the balance of plant with capability to communicate and interact with local controls via marshalling panels.

4.0 Process Overview

4.1 Introduction

This document specifies the process design for a 30 million lb/yr succinic acid (SAC) demonstration plant, built in Lake Providence, Louisiana, to run on 'alternate feedstock's' as part of an award by the U.S. Department of Energy (DOE) to Myriant. Alternative feed stocks include sorghum grain, sorghum flour (milo), other cellulosic sugars derived from biomass such as corn stover, sugarcane bagasse and forest residues (wood chips), as examples. The design basis feedstock(s) for this facility are sorghum grain that will be milled and subsequently saccharified with enzymes to produce an aqueous glucose feed stream. Alternatively, dextrose from corn wet milling commonly known at 95DE may be processed. An aerial photograph of the plant is shown in Figure 4.1:

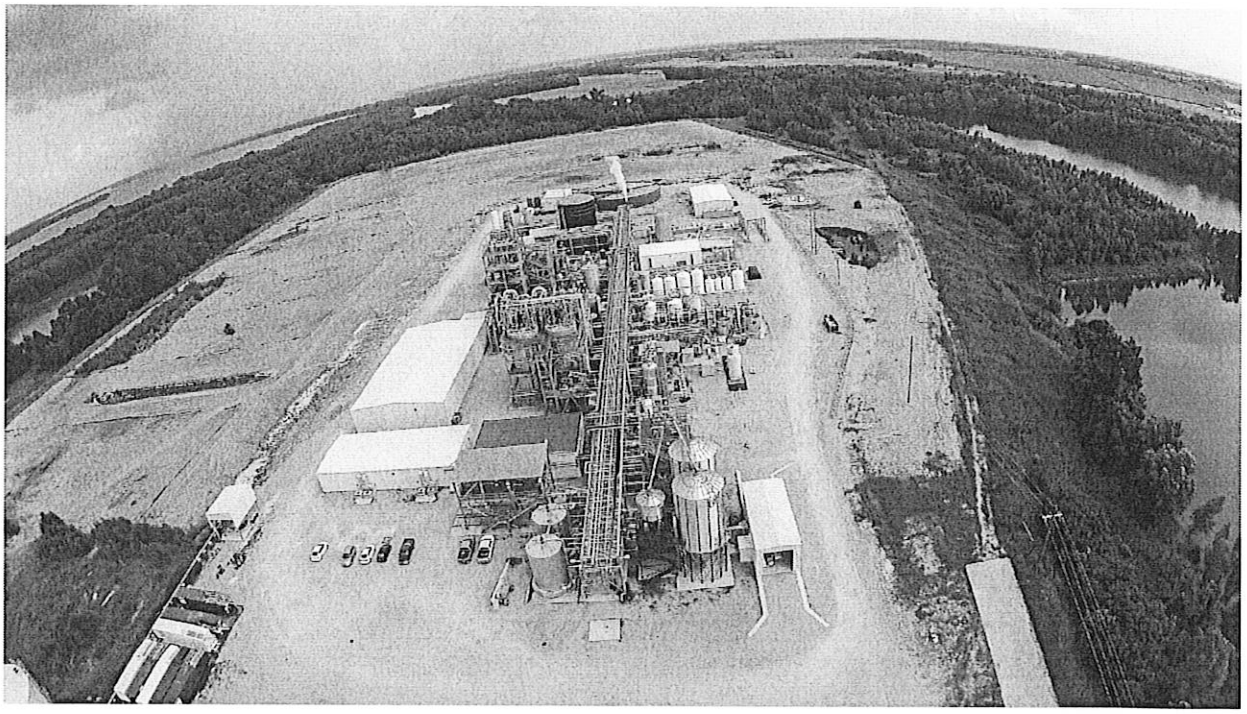

Figure 4.1 - Aerial photograph of MySAB facility.

Desired products from this facility are up to 30 million lb/yr of industrial grade succinic acid (SAC 99.5% SAC purity). Ammonium succinate is produced during fermentation from glucose at near-neutral pH, with the non-genetically modified organism, Bacterium *Escherichia coli* KJ122 (biocatalyst). Base-reagent solution consisting of ammonium hydroxide is used for neutralization during the biochemical fermentation reaction. Succinic acid is prepared from the ammonium succinate in the separation and purification process units downstream of fermentation.

The process is split into three main sections: a) Pretreatment where milled sorghum grains are digested with acids and enzymes to produce glucose, or 95 DE dextrose preparation; b) Fermentation including the seed train where inoculum is grown and succinate is produced biochemically; and c) Downstream separation and purification (DSP) where succinate is converted to succinic acid.

In-lieu of EPC contractor plant performance guarantees Myriant elected to work with the individual vendors to guarantee unit performance.

4.1.1 Feedstock Preparation

Glucose is the essential food medium which the Escherichia coli *(E. coli)* ferment to produce succinic acid. The more general term glucose syrup is often used synonymously with corn syrup, since glucose syrup is in the United States most commonly made from corn starch. Glucose syrup is mainly produced by adding alpha amylase to a mixture of starch and water. The enzyme breaks the starch into oligosaccharides, which are then broken into glucose molecules by adding the enzyme glucoamylase. The original facility design anticipates manufacturing glucose on-site from sorghum grits as well as operating from pre-manufactured glucose syrup.

Pre-manufactured glucose syrup ("corn syrup") at ~70% concentration arrives at the facility essentially ready to feed to fermentation. When corn syrup is used in the processing facility, much of the front end equipment is bypassed and vessels are re-purposed for the operating change. The enzymes for breaking down carbohydrates and the accompanying temperature, pH and requisite retention time are not relevant. All of the tanks and process equipment necessary for glucose production are bypassed or repurposed to hold, heat and deliver the glucose syrup to the fermentation areas.

4.1.2 Sorghum Grits Processing

MySAB has installed the capability to receive and process sorghum grits. However, grits have not been processed at the MySAB facility. Sorghum grits as feedstock, however was proven to be a suitable feedstock during pilot phase process testing.

Feedstock (sorghum grain) will be received from trucks and stored in the grain silo. The grain is in the form of sorghum grits, a dehulled grain product. Grits are pneumatically conveyed to a day bin and transferred to a grits / water mixer unit. In the grits/water mixer water is added to make up 30 wt% slurry. Ammonia (equivalent of 8.1 lb/hr of 20% w/w ammonia water) is also added to break down the granular structure of the flour and expose the starch. Exposure time is determined by the retention time in the slurry tank. A small amount of alpha-amylase enzyme is added in the flour water mixer to initiate liquefaction and reduce the high viscosity during gelatinization.

The slurry is then pumped through a hydroheater where the temperature is increased to approximately 240°F. The heated material is then sent to a cook tube where retention time is provided for the gelatinization process to occur. The slurry then enters a cook flash tank where the pressure is reduced by flashing off some steam and non-condensables. The slurry then continues through a cooler and then to the liquefaction tank. The flash steam is condensed in the cook flash condenser and the condensate is also sent to the liquefaction tank.

In the liquefaction tank, pH of the slurry is adjusted to 5.7 using 96-98% w/w sulfuric acid and more alpha-amylase enzyme is added to the slurry in order to facilitate the liquefaction process, this step also reduces the viscosity. The liquefied slurry is pumped to a cooler, then to the sulfuric acid mixer where the pH is reduced to the necessary saccharification pH 5.

In the saccharification tank, glucoamylase is added to release the individual glucose monomer molecules from the liquefied mixture of dextrin (oligomers). This is a batch process and utilizes two saccharification tanks. The slurry is held for 15-20 hours to facilitate the saccharification process before it is pumped to through a cooler and cooled for fermentation.

Solids in the saccharified slurry are removed by separation with a centrifuge and the concentrated glucose solution (approximately 220 g/l) is stored in tanks.

4.1.3 Seed Fermentation

The purpose of the seed fermentation is to grow active biomass. Seed fermentation is done in 3 stages in successively larger vessels.

4.1.4 Production Fermentation

The result of each seed batch is used as the innoculum for the next size seed fermentor. This series of seed-train batches is continued until the last seed fermentor is large enough to support the production fermentation. The batch from the final propagator is the innoculum for the production fermentation where glucose is converted to succinic acid (SAC).

The initial medium is transferred via sanitized piping to the production fermentor. In parallel, Trace Metal Solution "TMS" is added to the fermentor under sanitary conditions. In parallel to that, an initial dosing of sterilized sorghum syrup (~220 g/L) or sterilized 95 DE (500 g/L) is added to the production fermentor. After transfer to the production fermentor, all transfer piping needs to be sanitized using Cleaning-In-Place (CIP) and Steaming-In-Place (SIP) according to the plant cleaning concept.

4.1.5 Fermentation Broth Filtration

Fermentation broth containing ammonium-succinate is stored in the harvest tank. Fermentation broth containing ammonium succinate is pumped continuously to the cell separators, to minimize time between completion of fermentation and separation of cell mass.

Removal of the bacterium *Escherichia coli* KJ122, precipitated proteins, insoluble solids and high molecular weight compounds are needed to produce cell free broth for the downstream processing units. This is accomplished by passing the fermentation broth through two disc-stack cell separators.

4.1.6 Ultrafiltration

The ultrafiltration system is used to remove all soluble high molecular-weight compounds from the cell free broth. During storage of the ammonium succinate broth in tank before feeding to the UF, the tank contents can be held at ambient temperature since the ammonium succinate salt is highly soluble in water.

4.1.7 Acidification

The permeate from the ultrafiltration unit is continuously transferred through a static mixer and acidified to yield succinic acid and ammonium sulfate. This step is needed, to enable salt-acid separation with the SMB chromatography further downstream.

4.1.8 SMB Chromatography

In this step, succinic acid in the filtrated and conditioned fermentor broth is separated into the free acid and ammonia-salt fractions and further processed downstream for separate purification or concentration. The simulated moving bed (SMB) is a chromatographic technique used to separate succinic acid from salts, residual sugars and other components in ultra-filtered and degassed fermentation broth that would be difficult or impossible to resolve otherwise. This increased separation is brought about by a valve-and-column arrangement that is used to lengthen the stationary phase indefinitely.

4.1.9 Nanofiltration (NF)

The succinic acid stream from the SMB unit is processed through a nanofiltration unit before sending to polishing.

4.1.10 Polishing

Polishing uses ion exchange chromatography to capture any remaining charged impurities and color in the product stream. It is comprised of three steps, cation exchange, anion exchange, and carbon adsorption.

4.1.11 Succinic Acid Crystallization

Succinic acid is concentrated in the evaporation and crystallization unit. The evaporation step utilizes a multi-effect evaporator that has multiple evaporation chambers, each chamber is held at a progressively lower pressure than the last. Steam is supplied to the first effect of the evaporator, which is held at the highest pressure. Because the boiling point of water decreases as pressure is reduced, water vapor boiled off from the first effect can be used to heat the subsequent effects held at a lower pressures. As a result, the use of a multi-effect evaporator reduces the overall usage of the plant steam.

The concentrated succinic acid stream from the evaporator is slowly cooled down by evaporative cooling in the crystallizer. This generates a suspension of succinic acid crystals, which will be pumped continuously with a transfer pump to the centrifuge that will separate the crystals and mother liquor. At the end of this unit succinic acid crystals are recovered.

Industrial grade succinic acid has a 99.5 wt% purity. The Technical Grade SAC is produced via a single pass through the evaporator and crystallizer. It is further dewatered, dried and packaged in super sacks.

4.1.12 Clean In Place (CIP) System

The automated CIP system provides for cleaning vessels, equipment, and piping in order to minimize problems that can arise due to fouling and microbial contamination. A hot (180°F), 3-5% caustic solution removes residues (primarily, protein) which collect on the walls of vessels and in piping. It also washes out and eliminates any pockets of bacteria in equipment and piping. In order to conserve water usage, process condensates are used for rinsing and makeup of the caustic solution. The same system is used for tank cleaning with spray nozzles, and also cleans exchangers and piping by circulation.
The CIP system consists of a rinse water tank and a dilute caustic tank and the header systems (CIPS – CIP supply and CIPR – CIP return). A single header system supplies CIP solution to the spray nozzles

mounted in tanks. This system should be capable of supplying 120 gpm of condensate/caustic solution at a pressure of 60 psig to the spray nozzles. In order to have precise control over the amount of rinse used, the entire CIP cycle for each piece of equipment should be controlled by a flow meter and flow totalizer on the respective system. In this way, it is possible to accurately calculate how much water is needed to displace process fluids and to prevent either high biological oxygen demand process fluids or caustic from going to the effluent. It also prevents the dilution of the process or the caustic cleaning solution with excess rinse water.

4.2 Detailed Descriptions of Key Unit Operations

4.2.1 Glucose Preparation

Sorghum syrup or 95 DE is pumped continuously from the Syrup Tank. It is sterilized using a High Temperature Short Time (HTST) system that uses medium pressure steam for heating the syrup to a minimum of 286°F, and then cooling to 104°F by cooling water. The syrup is continuously fed to the Intermediate Vessel, GL20A (nominal volume 11,900 US gal).

The Intermediate Vessel serves as a feeding tank for the circular feeding ring line for the main fermentation system as well as for the initial dosing of the seed fermentors. The ring line is always in operation (operating temperature 102°F), while a dead end header is on standby and sterile. It is crucial to keep the glucose feeding sterile, therefore, the line is switched over every seven days to the standby system and cleaned and sterilized following the CIP cleaning procedure.

4.2.2 Trace Metals and Media Preparation

The trace metal solution is prepared in a batch vessel (nominal volume 106 US gal) for both seed trains as well as for the main fermentors. The trace metal solution is required for optimum fermentation conditions. The trace metals will be added to treated well water and phosphoric acid is added by a chemical metering pump to enhance dissolving of the metals.

The medium solution for production fermentation is prepared in a batch vessel (nominal volume 1,400 US gal). The media solution is prepared with treated well water and sterilized with direct steam injection at a temperature of 250°F.

4.2.3 Base Solution Preparation

Ammonia has many uses throughout the facility. It is used for pH adjustment and as a source of nitrogen in fermentation. It is also used for its chemical properties in the polishing area. Anhydrous ammonia is delivered to the facility where it is mixed with water to form an aqueous ammonia solution often referred to as ammonia water. The ammonia water is used for various processes throughout the facility. Concentrated ammonium hydroxide solution (20 wt%) is used as a neutralization medium in the seed fermentors.

4.2.4 Seed Fermentation

There are two seed fermentation trains with two fermentors in each train. All seed fermentors are designed to achieve good mixing conditions. The seed fermentors are designed to operate with a working volume at 60% of tank volume, leaving a headspace of 40% for safety.

High aeration in the seed fermentors may lead to foaming and creates a lower density cultivation media. Therefore antifoam agent is added to the fermentors manually as needed. The seed fermentors are designed as pressurized vessels with a maximum design pressure of 29.5 psig for seed fermentation 2 and 43.5 psig for seed fermentation 3. Appropriate under and overpressure safeguards are installed in the seed fermentors.

Aeration with low pressure sterile air is started, beginning with a low agitation rate. During the fermentation the dissolved oxygen (DO) level is monitored with a built-in oxygen probe, if necessary, agitation, aeration rate and pressure may be increased to achieve the needed dissolved oxygen level.

Seed fermentation 2 contents are transferred to seed fermentation 3 and the above procedure is repeated. At the completion of fermentation in seed fermentation 3, the inoculum is transferred to one of four primary fermentors.

After draining each seed fermentor by pressurizing the system with plant air, the propagation system with all transfer piping is sterilized by applying Clean-In-Place (CIP) and Steam-In-Place (SIP) procedures.

4.2.5 Main Fermentation

In the glucose fermentation process, the *Escherichia coli (E.coli)* converts the glucose sugars into succinic acid which must be continuously neutralized by a base solution to preserve the pH environment the organism requires to survive. This continuous neutralization provides the method for the *E.coli* to survive and convert most of the sugars into succinic acid in the form of ammonium succinate, which will be separated and recovered in later downstream processes.

Fermentation is where a specially developed strain of the *E. coli* microorganism is used to convert the sugar (glucose) into succinic acid, in the form of ammonium succinate. The feed stream, in the form of available glucose, and the seed stream, in the form of propagated *E. coli*, are combined in the fermentor for the specific purpose of encouraging succinic acid production at optimum capacity.

Fermentation is completed in a fed-batch process using multiple Fermentors. Broth is maintained at the optimum temperature by a cooling jacket that surrounds each fermentor and utilizes cooling tower water to remove heat. Once fermentation is complete, the broth is pumped out of the tank to the harvest tank where it will travel in a continuous flow stream through various mechanical, physical and chemical separation processes designed to isolate, remove, neutralize and/or purify the targeted products, substances, or flow streams.

Once the appropriate initial fermentor fill level is achieved and the level is sufficient to begin agitation, the fermentor is inoculated with the *E.coli* produced in the seed fermentation process.

After the fermentor has been inoculated, the cultivation cycle begins. Sugar is fed into the tank until shortly before the end of fermentation. In addition, a second process stream, often referred to as Base solution, is added separately via pH control loop into the tank to control the broth pH by neutralizing the succinic acid produced by the *E.coli*. Without pH adjustment, the *E.coli* would drive the pH down due to acid production until the culture would die in the environment they created and the fermentation process would stop.

Once fermentation has started, the control system will automatically monitor process operating parameters and make necessary adjustments to ensure proper temperatures, pH, feed rate, agitation and aeration are maintained in order to produce optimum fermentation. The control system alerts the operator if out of range conditions occur. Broth temperature and level control sequences described in the startup are continuously repeated.

Fermentation samples are drawn frequently to monitor broth for the correct fermentation parameters. These parameters include pH, sugar content, ammonium succinate and *E. coli* stress factors. High performance liquid chromatography (HPLC) and YSI (glucose) is recommended for monitoring fermentation parameters.

At the end of the cultivation cycle, the fermentor level will be approximately 85% and all glucose will have been metabolized by the *E.coli*. The fermentor discharge valve is opened to the fermentor broth harvest line. Filtered plant air from the sterile air unit is used to maintain positive pressure while the fermented broth is transferred from the tank into the header. Downstream, the broth is pumped to the harvest tank by the fermentor harvest pumps. The harvest tank is sized to hold the entire contents of the fermentor and serves as a buffer between the batch production cycle and the continuous separation, filtration and condensing processes. Once emptied, the fermentors are cleaned using the central plant CIP System. Steam sterilization may be necessary to combat serious contamination. Initially it was assumed the steaming would occur once per week, adjustments to the schedule would then be made based upon actual plant needs. Following the sterilization process, the tank remains out of service until it has sufficiently cooled to begin the fermentation cycle. After cleaning, the fermentor is then available in sequence to repeat the fermentation cycle.

4.2.6 Clarification – Biomass Separation

The fermentor broth from the harvest tank is first processed through the two biomass separator centrifuges where the broth is separated into two separate process streams: Fermentor Broth Solids (FBS) which is sludge containing the *E. coli* and other suspended solid is separated from Clarified Fermentor Broth (FBC). The FBS are pumped via two large sludge pumps through the biokill pipeline where heat is used to inactivate the *E. coli*. The FBS discharges into the biomass mixer where the FBS stream is mixed with fly ash or another drying agent and moved to storage for disposal. The FBC from the biomass separators is collected in the FBC tank.

4.2.7 Ultrafiltration (UF)

Ultrafiltration (UF) is a separation process that uses a membrane to separate smaller salt and sugar molecules from larger proteins. Once the fermentation broth is centrifuged to remove suspended solids, it is filtered through a membrane ultrafiltration unit to remove proteins. As the feed passes through the successive UF stages, succinic acid will pass through (permeate) the membrane while large protein molecules will be retained. Once the concentrate has reached the desired concentration it is removed from the plant. In this manner, the concentrate and permeate will be continuously removed from the UF system.

Processing conditions will be controlled automatically from the control system with the permeate (i.e. the clarified succinic acid), being sent out as the final product. .

4.2.8 SMB Chromatography

Simulated moving bed chromatography is a variant of high performance liquid chromatography that is used to separate the succinic acid from the fermentor broth. True moving bed chromatography is only a theoretical concept. Instead of moving the bed, the sample inlet and the analyte exit positions are moved continuously, giving the impression of a moving bed. This increased separation is brought about by a valve-and-column arrangement that is used to lengthen the stationary phase indefinitely by the use of multiple columns in series and a complex valve arrangement. This allows switching the sample entry in one direction, the solvent entry in the opposite direction, and changing both the analyte and waste takeoff positions at the appropriate time.

The chromatographic separator system consists of 8 fixed-bed columns arranged in an endless loop. The pipe connecting the columns is referred to as the recirculation pipe. Recirculation pumps provide a continuous internal recirculation flow. Feeds to the system include clarified succinic acid broth and feedwater (VPC) eluent. Each of these feed streams has a "degasser system" before introduction to the SMB. This system is a vacuum pump and a small vessel that is kept at high vacuum. Note that the temperature of the streams will slightly drop through this system. As such the incoming feed streams are heated to a temperature a few degrees above target prior to entering this system.

The columns are filled with resin. Feed and eluent water are introduced into the recirculation loop at appropriate points while equivalent volumes of the succinic acid enriched fraction (extract) and the ammonium sulfate rich fraction (raffinate) are withdrawn. The operation of the separator involves the sequencing of the valves around the recirculation loop. By periodically switching valves in the direction of the internal recirculation flow it is possible to simulate a true moving bed of resin.

The separator operates in a near equilibrated manner. This means that a separated concentration profile is maintained in the system and circulates endlessly around the loop. The internal recirculation direction is from the top of the cells one, to the bottom of cell one, and then on to the top of next cell.

Feed material and eluent water are continuously added to the concentration profile (i.e. sampled into the profile) while extract and raffinate are sampled from the profile. This is important because the system is not stable if quick changes are made to the operating set points like flow or ratios. Therefore any changes must be given time to stabilize before the system is operating at peak performance again. The separated extract and raffinate are never removed in total from the separator. The flow rate in the recirculation lines which carry the equilibrated concentration profile around the separator loop is quite fast. Only a proportion of the recirculation flow passing by an extract or raffinate valve is actually taken off. The internal equilibrated separation profile is therefore unchanged. This near steady state method of operation simplifies control of the separator. The recirculation flow around the loop is maintained to move the desired internal concentration profile at a predicted rate. The inlet and outlet flows are periodically advanced to correspond with the moving concentration profile. The concentration profile is determined by a refractometer.

4.2.9 Nanofiltration

Nanofiltration is a membrane filtration system using pores between 200 and 1000 Daltons. The basic technology behind membrane filtration involves using a semi-permeable membrane to separate a liquid into two distinct streams. Pumping this liquid across the surface of the membrane creates a positive trans-membrane pressure that forces any components smaller than the porosity of the membrane to pass through, forming the permeate stream. Any components larger than the pore size simply cannot pass through, and remain behind in what is called the retentate stream. The surface of the membrane is kept free of blockages by the force of the liquid flow moving parallel to the membrane surface. Small ions pass through while larger ions are retained, which include most organic compounds and color bodies.

The nanofiltration system is a frame-mounted unit with component items and prefabricated piping which are ready for operation when installed and connected to the local up and down stream process equipment, utilities and power supply system. The membrane elements are spiral modules arranged in a number of series-connected circulation loops.

The system is controlled and supervised by the operator via the local PLC operator panel. The cleaning agents for CIP are added automatically by the supplied dosing system or can be manually added by the operator.

4.2.10 Polishing

The polishing train is the 2nd to last step in the downstream process train. Its function is to capture charged proteins, ionic compounds and color, from the succinic acid product stream. Ion exchange through the use of cation and anion resin columns are utilized as well as granulated activated carbon polishing.

4.2.11 SAC Evaporation & Crystallization

The evaporator and crystallization system concentrate the succinic acid using heat and pressure to remove water vapor. Water is recovered and returned to production as process condensate. The concentrated acid is sent on to crystallization, drying and packaging. The evaporator and crystallization system consists of four falling tube evaporator effects, with three associated shell and tube heat exchangers, two crystallizers with associated heat exchangers, a vacuum system and series of pumps, receivers, separators and coolers to condense and remove the condensate and move the succinic acid through the process.

4.2.12 SAC Centrifugation

A centrifuge is used to continuously separate the mother liquor from the wet crystals. The slurry is fed through a stationary feed pipe at the centerline of the basket. The perforated basket will dewater the succinic acid crystals and provide clean water rinsing to remove mother liquor and impurities from the crystals. Dewatering and water rinse depend on centrifugal force to accomplish these task. In addition to the product rinse nozzles, the machines are equipped with wash or CIP nozzles to wash built up crystals from the basket exterior. The dewatered succinic acid crystals are then feed to the dryer system.

4.2.13 SAC Drying & Load out

After the succinic acid has been crystallized and centrifuged, it is dried and conveyed to packaging. The dryer has an air distributor plate welded directly to the fluid bed housing.

The inlet air temperatures should remain fairly constant, as long as the dryer inlet airflow remains constant. The process air flow may vary slightly based upon ambient conditions and the result air density and temperature. The final air flow going to the cyclone is kept relatively constant in order to promote proper operation of the cyclone and scrubber.

The outlet air temperature will vary slightly depending upon bed consistency and percent moisture in the feed. For a constant process air flow, the difference between the inlet and outlet air temperature establishes the water evaporative load within the dryer, and determines the "dryness" and temperature of the final product.

4.2.14 Ammonium Sulfate Evaporation

In the AMS evaporation unit, dilute ammonium sulfate SMB raffinate generated from the chromatography is condensed to meet the specifications for commercial sale. Ammonium sulfate, $(NH_4)_2SO_4$, is an inorganic salt with a number of commercial uses. The most common use is as a fertilizer. It contains 21% nitrogen as ammonium cations, and 24% sulfur as sulfate anions. The primary use of ammonium sulfate is as a fertilizer for alkaline soils where the ammonium ion is released and lowers soil pH while contributing essential nitrogen for plant growth. The dilute liquid is processed through an evaporation system using plate and frame heat exchangers; the product is increased to 40% concentration prior to being pumped off site to a bulk storage facility.

4.2.16 Clean in place system

Clean in Place (CIP) cleaning is utilized to clean interior surfaces of tanks and pipelines of liquid process equipment. A chemical solution of sodium hydroxide and water is circulated through a circuit of tanks or lines and then returned to a central reservoir allowing for reuse. Time, temperature, and mechanical force are manipulated to achieve maximum cleaning. This method is the most frequently used cleaning method for bio-chemical facilities. The CIP system is designed to provide the cleaning needed to keep the equipment microbiologically clean and to remove residues from heat exchangers, tanks, and pipes.

Each tank serviced by the CIP system is equipped with one or more permanently installed spray nozzles. These nozzles are designed to work in an open tank, where the high pressure works with the spray jets to disperse the cleaning fluids throughout the tank. All equipment serviced by the CIP System require manual and automated isolation valves at each piece of equipment to be set properly prior to beginning the CIP procedure. The first wash and final rinse process utilizes hot process condensate, thereby minimizing fresh water usage. A heated, ~130°F (55°C) dilute caustic solution composed of 2-5% sodium hydroxide and water is used as the cleaning agent. The dilute caustic solution dissolves most residues and is an adequate sanitizing agent for the organic acid production process.

5.0 MySAB Performance

5.1 Performance Test Results

Myriant prepared for and conducted a test run in March 2014 with the goal of demonstrating the MySAB plant's ability and capacity to operate over a 3 day (72 hour) test run period. Test run objectives were:

- achieving sustained operations for 72 hours;
- achieving 70% of its design capacity (measured on an economic basis); and
- providing consumption data for comparison to the pro forma.

Batches of fermentation broth were prepared ahead of time and stored after cell solids removal (centrifuge, UF). This pre-inventorying activity is necessary to prevent the running out of broth before the next fermentation is complete, as downstream equipment are best run continuously. Five fermentations were prepared ahead of time and two additional fermentations were run after downstream processing was started. A total of 561,323 gallons of broth (375,235 lbs of succinic acid) were produced from these fermentations. In steady state production, fermentations will go directly from fermentation to separation, filtration and then through downstream without the storage step.

Downstream unit operations beginning with SMB was started on March 19th. The SMB stopped processing broth early morning March 25th. Solid crystals from SAC ECC stopped dropping 12:30 AM March 26th.

Consumption data was recorded throughout the run of the campaign (from March 19th through March 26th). Consumption data for fermentations was recorded during each batch and not during the window for the recovery process.

The economic pro-forma cost estimates for chemical and energy consumption are calculated on a per pound of succinic acid basis and are compared with values calculated during the 72 hour test.

Overall process yields were estimated during the operating period from December 29, 2014 to January 6, 2015. During this period a total of 102 metric tons of final product were produced. Individual unit performance compared to the design basis and vendor guarantees.

MySAB has produced product meeting the specification in the following table. It is important to note that plant has proven the capability to exceed the product quality specified in the original design basis.

Succinic Acid	
Specificiation	**Limit**
Assay	≥ 99.5 wt%
Moisture	< 0.5 wt%
Fumaric Acid	< 0.1 wt%
Ash	< 0.025 wt%
Sulfur	< 150 ppm
Phosphorous	< 3 ppm
Chloride	< 10 ppm
Iron	< 10 ppm
Arsenic	< 2 ppm
Lead	< 2 ppm
Appearance	White Crystalline Solid

Table 5.1 – MySAB succinic acid specifications.

5.4 Key Performance Data (KPI's) by unit operation

From May 2013 till the end of December 2015, the MySAB plant operated in a campaign mode, where fermentation batches were produced and then processed downstream. This was due to several mechanical issues with process equipment as the plant was started up and attempted to be 'lined out' in a steady state mode of operation. Upon correction of the mechanical issues encountered, the plant did progress towards steady state operation.

Each of the processing areas has technical unit owners that support the process and MySAB Operations team. An important element of support is the formulation of KPI's (key performance indicators), that quantify a process unit's performance in terms of mass balance, energy and chemicals usage, succinic acid product recovery and purification, and product quality metrics.

5.4.1 Fermentation

The fermentation process is well established with supporting documentation, including SOP's and work instructions. Apart from some mechanical issues encountered, the Fermentation train operated reliably well and largely achieved broth titers and yields of succinic acid. These values are in alignment with projected production targets and goals.

5.4.2 Cell separation by centrifugation

The MySAB cell separation area is (2) bowl centrifuges that can operate singularly or in parallel. This flexibility allows for mechanical maintenance and repair on one unit while offline, which has occurred at this unit. Otherwise this process unit is very consistent and reliable in its performance.

5.4.3 Proteins removal by Ultrafiltration (UF)

The MySAB Ultrafiltration (UF) area utilizes commercial UF membranes to remove residual proteins from the upstream process. This crucial step is to further purify the product stream before it is processed by the SMB continuous chromatography unit, to prevent fouling of the chromatography resin by proteins. The UF unit processed during 2014 a considerable range of feed volumes; this is largely due to plant mechanical issues encountered.

5.4.4 SMB continuous chromatography

The MySAB SMB continuous chromatography process applies ion exclusion chromatography to separate succinic acid from the co-product ammonium sulfate. The succinic acid is uncharged while the ammonium sulfate is positively charged via the ammonium cation, and negatively charged via the sulfate anion. The process is the primary separation unit for the downstream train to being to purify the succinic acid product, and remove the ammonium sulfate co-product, which is subsequently concentrated by evaporation.

The SMB process also experienced occasional mechanical issues due to its complexity of design and operation. This led to varying performance at times, of the % recovery of the succinic acid product into the extract stream.

5.4.5 Nanofiltration (NF)

The nanofiltration process is a key unit that applies membrane filtration using spiral membrane elements, for the removal of maltose and color bodies, separating them into its retentate stream. The filtered succinic acid product goes into its permeate stream.

The color bodies are a mixture of organic molecules resulting from the fermentation process, of varying molecular weights and types. It is critical to remove these to a very significant extent prior to application of the nanofiltration permeate stream onto the front end cation columns of the polishing train. If performance is not consistently realized in the nanofiltration unit, it will and has been demonstrated to adversely impact the performance of the polishing train. As mentioned below in the polishing section, essentially complete removal of sugar (maltose) is also critical in nanofiltration, so that it will not continue on to the evaporation and crystallization (ECC) unit. Residual sugars are not removed by any means in the polishing train.

5.4.6 Polishing

Key Performance Indicators (KPI's) that are quantified around the polishing train include the % reduction of each impurity, and chemicals and energy usage.

To produce polymer grade succinic acid, the levels of sugars and nitrogen must be very low If ppm levels of sugars and nitrogen are present in the evaporation and crystallization unit (ECC), and build up over time, there is the risk that the Maillard reaction can take place. This will then produce color bodies which will appear in the final crystalline product.

The polishing train has been fairly successful at achieving impurity removal targets, but not consistently. Some mechanical problems and the competing aspects of removing color bodies, ammonium ion, and amino acids in the front end cation exchange resin beds, have presented challenges to reliably reducing the total nitrogen content in polished effluent. These factors, along with not being able to supply reverse osmosis (RO) quality water in large volumes for the polishing train regeneration needs, have at times caused polished effluent to have an impurities composition set that exceeded that permissible for polymer grade product.

These challenges have been actively addressed and the performance should be acceptable going into the year 2016.

5.4.7 Evaporation and Crystallization (ECC)

The final downstream unit is the ECC (Evaporation and Crystallization) for producing crystalline succinic acid. This unit is well characterized and operated according to thorough SOP's and work instructions. The Key Performance Indicators (KPI's) quantified are:

1. Succinic acid wt. % in the feed to the unit.
2. The wt. % and concentration of acetic acid, other minor organic acids, and sugars in the feed to the unit.
3. The final wt. % of succinic acid in the crystalline product.
4. The number of dry product sacks (1,000 mt nominal quantity) produced in a 24 hr. period.

Root causes for observed vs. design values:

A. The number of dry product sacks produced per 24 hr. period is not at the nameplate design rate. This is a direct function of process downtimes, due to mechanical issues, for other units ahead of the ECC, encountered during the 2013-2015 timeframe. As part of the overall optimization strategy for the plant, these mechanical issues are being addressed to enable continuous operation of the ECC.

B. The succinic acid product in crystalline form largely met its KPI of a design concentration of >99.5 wt. %. Continued operating experience led to optimized control of the purge and mother liquor streams, to avoid buildup of ppm levels of N, sugars, and color bodies, not removed from the polishing train. To consistently achieve polymer grade product, the ECC has to operate continuously, receive a feed stream that is at design conditions, and handle purge and mother liquor streams in a controlled manner.

6.0 Sorghum Grits Fermentation

Due to significant unplanned construction delays and other considerations Myriant elected to utilize 95DE as the preferred feedstock for the plant. However a significant development effort during plant design and construction focused on the evaluation of fermentation performance of grain sorghum derived sugars.

Sorghum grits and starch can be digested with acids and enzymes to produce glucose. The glucose produced from this renewable feed stock is then converted into succinic acid via fermentation with Myriant's proprietary microorganism. Alternative feedstocks for use could include sorghum grain and sorghum flour (Milo).

Initially, development efforts utilized commercially available Dextrose 95, along with sorghum grits, for which the plant was designed to process. These were evaluated in 7L fermentors, in the Woburn, Massachusetts R&D laboratories of the Myriant, in 2010. Subsequent piloting of the lab process was conducted at Fermic S.A. DE C.V., Mexico City, Mexico in 2011. The Myriant successfully scaled up the fermentation of succinic acid on a volume basis by a factor of 10,000x from R&D Laboratory evaluations to its flagship biorefinery in Louisiana, as shown below:

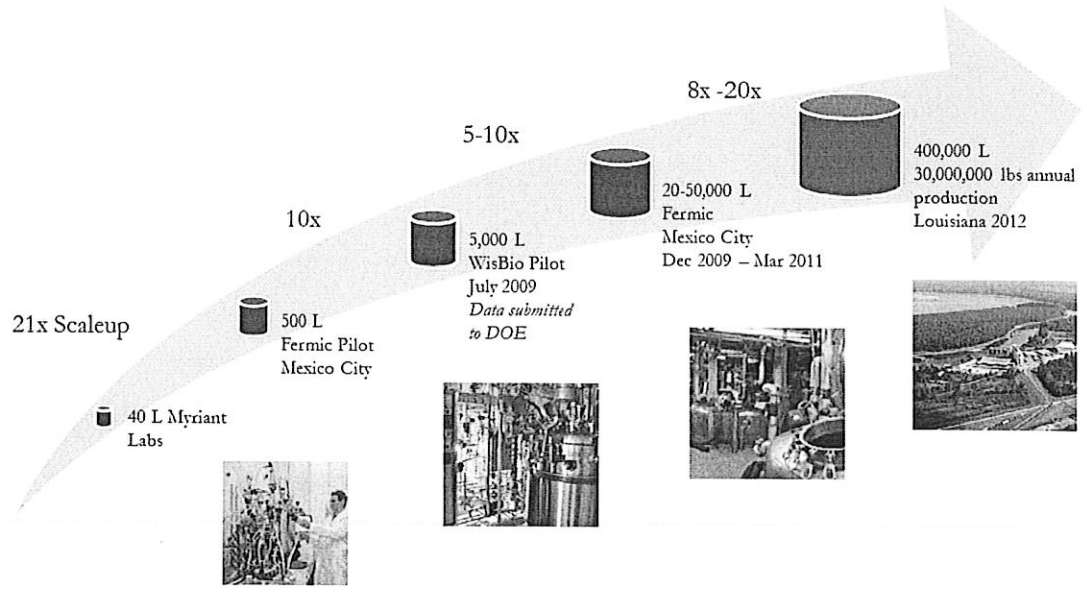

Figure 6.1 – Myriant Fermentation scale-up history

When glucose from 95DE-dextrose and sorghum syrup are approximately the same concentration, the key performance measures of yield (g of succinic acid / g of feed glucose) and resulting titer, are very

similar. The average yield and titer for the sorghum runs are slightly higher due to the higher feed concentrations utilized or added nutrients contained in the sorghum syrup. The standard deviation in yield and titer compared for both glucose and sorghum runs are nearly the same. Thus, it can be concluded that the sorghum feed syrup performed quite comparably to that of the glucose syrup using the KJ122 microorganism.

From the results it can be seen that the performance of the glucose syrup in the lab and pilot scale are very similar in the productivity of succinic acid in g/L – hr. The glucose concentration was higher at the pilot scale which resulted in the higher yield. However, the larger size of fermentor and mechanical configuration at the pilot scale led to a lower succinic acid final titer concentration, compared to lab scale results. The productivity and yield of succinic acid at both scales was nearly identical.

At the lab scale the final succinic acid titer increased when the feed glucose concentration was increased. Thus, the effect of increasing the feed glucose concentration, whether from glucose syrup or sorghum syrup, translated to higher final succinic acid titers for lab scale fermentations.

7.0 MySAB Cellulosic Evaluation

7.1 Summary

Myriant's Succinic Acid Biorefinery (MySAB) in Lake Providence, LA, achieved mechanical completion in early 2013. The MySAB facility is designed to produce succinic acid from a variety of renewable feedstocks including grain sorghum, 95-dextrose (95DE), and other commercially available sugar sources. The U.S. Department of Energy (DOE) supported the MySAB project with a $50 MM cost sharing award as part of the Bioenergy Technologies Office (BETO) Biomass Program. In keeping with the goals of the Biomass Program, the MySAB project included activities to further develop technologies for producing bio-succinic acid and related products from cellulosic feedstock sources. This cellulosic evaluation report presents the results of that program including Myriant's successful strain development, fermentation optimization, sample product generation and purification, and an evaluation of the economic and technical impacts of using cellulosic feedstock to produce bio-succinic acid.

Myriant began this effort with a global search of the leading lignocellulosic feedstock suppliers to determine which potential partners (if any) would be capable of providing a large, high quality supply of cellulosic sugars in a timeframe consistent with start-up operations at the MySAB facility. The search included a commercial evaluation of each potential partner, and a technical evaluation of their cellulosic sugars' qualities. This exhaustive search determined there were no potential suppliers that could meet the scale or timeline requirements in a manner that would allow Myriant to conduct a representative test run (10 days) at an economically viable cost. As a result, a laboratory- and analytical-based program was developed, reviewed, and agreed with the DOE, as an effective way to achieve Myriant's commitment to conduct the cellulosic processing component of the MySAB project.

Over the course of the project life (January, 2010 - present), Myriant conducted extensive testing and development using samples provided by over 30 companies developing cellulosic technologies. This report provides the results of our successful strain development efforts, the screening of the best potential feedstock / strain combinations at larger fermentation scale, and the subsequent scale-up of our fermentation process on the two best identified feedstock sources. These two sources were further evaluated to determine any significant pretreatment requirements, and for optimization of fermentation protocols. Finally, three confirmation runs were conducted in our R&D facility's largest scale fermentors for the production of a crude bio-succinic acid product, and further processing to produce one commercial processed end product – bio-based dimethyl succinate (DMS).

In addition to successfully generating cellulosic based succinic acid products, the cellulosic program also identified the economic differences between using cellulosic-based sugars and the MySAB facility design feedstock(s). Additional required capital and operating costs for cellulosic processing primarily result from increased fermentation times (larger fermentors), greater polishing requirements, and the corresponding impacts on the plant's waste treatment requirements. Capital and operating costs for a cellulosic-based process are estimated to be 3-5% higher than Myriant's current baseline costs.

This study demonstrates Myriant's tremendous progress in developing and confirming the viability of our organisms and fermentation processing of cellulosic sugars. It confirms that as high-quality cellulosic sugars become reliably available in commercial quantities, bio-industrial chemical products may be effectively produced if cellulosic sugar costs are nominally lower than conventional sugars. Production of cellulosic sugars and processing at the demonstration plant scale are now the next steps in the commercialization of biochemical products from advanced feedstocks.

7.2 Cellulosic Program Objectives

The main objectives of Myriant's corporate cellulosic program were to identify the most viable cellulosic sugar feedstocks sources and suppliers, and to be prepared with effective strains, processes, and processing equipment when these sources become commercially cost effective and reliably available. Myriant's succinic acid process is commercially viable based on currently available sugars, however lignocellulosic biomass based sugars remain of great interest to Myriant especially as they are expected to become more available in the upcoming years, and are renewable and are non-food based. Our program continues to plan for the next required steps of scale up as technologies improve and develop.

Myriant conducted an extensive evaluation of the feasibility of using lignocellulosic-based sugars at MySAB to produce bio-succinic acid Myriant's objectives for this program including:

1) Identifying one or more lignocellulosic sugar stream supplier(s)with sufficient capacity to provide MySAB with steady sugar supply for a test period of approximately 10 days;
2) Metabolically evolving Myriant's proprietary organisms to successfully grow on lignocellulosic hydrolysate for the production of bio-succinic acid;
3) Identifying the characteristic differences in sugar feed streams and fermentation broths to better identify and understand downstream separation/purification challenges as compared to the base case utilizing 95-dextrose as the feedstock of choice.

In pursuit of Objective 1, Myriant identified and contacted approximately 40 domestic and non-U.S. potential lignocellulosic sugars technology providers with the following screening criteria:

1. Access to a lignocellulosic feedstocks processing pilot plant with at least one ton per day (TPD) feedstock processing capacity;
2. Ability to run campaigns capable of producing and delivering large quantities (sufficient to support a ten-day test run) of biomass-derived sugars with consistent composition and purity to MySAB in 2013;
3. A business with a proven, scalable technology, bolstered by a strong and stable balance sheet;
4. Sugars hydrolysate capable of fermentation with Myriant's bio-succinic acid fermentation organism and capable of delivering an acceptable ionic impurity profile, and suitable color for downstream purification considerations;

Myriant's considerations included lignocellulosic feedstocks providers employing a variety of biomass pretreatment technologies (e.g. auto hydrolysis, various dilute acids, concentrated acids, solvents, alkaline, pulp & paper fiber and supercritical fluids etc.) as well as those using multiple types of biomass feedstocks (e.g. mixed hardwoods, corn stover, wheat straw, switch grass, sugarcane bagasse,

softwoods etc.). During the early vetting and due diligence process, those lignocellulosic feedstock providers that did not meet the first two criteria identified above were eliminated from consideration.

A short list of companies was then identified based on the overall technology feasibility, business viability and the quality of hydrolysate. Detailed technical and business discussions were held with the shortlisted companies. Myriant personnel visited many of the facilities to conduct on-site discussions, validate claims and assess partnership ability with Myriant.

With a short-list of potential partners identified, the main tasks of the MySAB cellulosic program roadmap then included:

1. Screening lignocellulosic hydrolysate from third party suppliers;
2. Procuring samples and running detailed analytical tests to determine chemical compositions;
3. Fermentation screening in Myriant's proprietary small scale screening equipment ("Fleaker");
4. Scaling up the best potential feedstock and organism match(es) to the 7-liter scale;
5. Optimizing conditions of the best performer for a series of three 20 to 40-liter fermentation scale reproducibility runs; and
6. Using the resulting bulk quantities of broth for crude SAC production, and conversion to the dimethyl succinate final product sample.

7.3 Hydrolysate Screening and Selection

Hydrolysate sugar samples were obtained from all companies meeting Myriant's requirements for analytical testing and fermentation feasibility. Myriant provided desired specification parameters to companies as follows:

Components	Concentration
Sugars (C5 and C6, mixed or separate)	~30 wt%
Acetic Acid	< 1 g/L
Formic Acid	Low ppm
Furfural	Low ppm
Hydroxymethyl (HMF) Furfural	Low ppm
Phenolics from Lignin Degradation	Low ppm

Table 7.1 - Desired hydrolysate specifications when samples from suppliers were requested.

More than 40 hydrolysate samples from short-listed companies were received for analytical testing. Hydrolysates were analyzed for the type and quantity of sugars, biomass degradation products, and/ or other materials that present in the sample. The samples were subjected to small scale fermentation tests with Myriant's *E. coli* bacteria to evaluate the bio-succinic acid production feasibility. Below is a list of the types of compounds analyzed and the techniques used for detection.

Hydrolysate Compositional Analysis	
Analyte	**Method**
C5 and C6 sugar monomers and dimers	HPLC
Organic Acids	HPLC
HMF, Furfural, Phenolics	HPLC
Sulfate, Phosphate, Chloride, Ammonium	Ion Chromatography
Trace Metals	ICP-OES
Amino Acids	HPLC

Table 7.2 - Types of analytes and methods techniques used on hydrolysate samples.

From the short list of suppliers, two were chosen as the suppliers of choice for further development and final testing. The selection decision was based on the following results:

1) Hydrolysates from both companies performed better than samples from all other companies at the Fleaker fermentation scale;
2) One, as a pulp production company, demonstrated excellent partnering viability, existing capital assets, and existing infrastructure including utilities and biomass handling at its facility;
3) One hydrolysate sample also demonstrated the best composition and color characteristics;
4) The other presented excellent partnering and business synergies with Myriant, as they also have an existing DOE-funded biorefinery, as well as an extensive background and deep experience in building corn ethanol facilities.

7.4 Downstream Processing

7.4.1 Initial Cellulosic Bio-Succinic Acid Crystal Production

As a screening evaluation to produce bio-succinic acid from cellulosic hydrolysates, initial fermented broth samples produced using lignocellulosic hydrolysate were processed at the laboratory scale to produce a small product sample using fixed treatment beds not reflective of a true commercial processing configuration. The sample resulted in a high purity product (Table 7.3), and confirmed the viability of our fermentation approach to produce cellulosic-based succinic acid.

Figure 7.1 shows a sample of the cellulosic hydrolysate as received and Figure 7.2 shows the final cellulosic mother liquor and crystals, respectively.

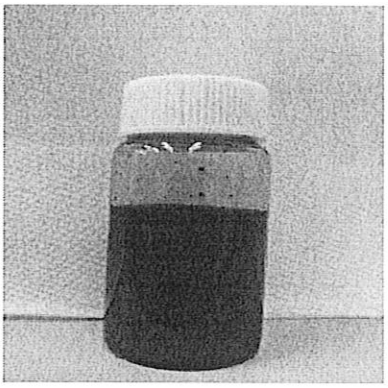

Figure 7.1 - Cellulosic hydrolysate as received.

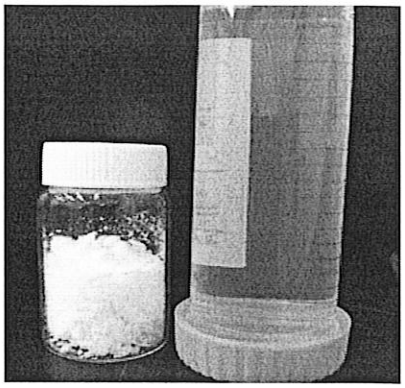

Figure 7.2 - Final cellulosic succinic acid crystals and mother liquor.

The analytical results of the initial sample production via a non-representative processing scheme are provided below.

Certificate of Analysis – 1st Cellulosic SAC	**Result**
Purity	99.4%
Unsaturated Compounds (as Fumaric Acid)	<0.01%
Phosphate	<1.0ppm
Sulfate	2.5 ppm
Chloride	3.2 ppm
Iron	<1.0ppm
Lead	<1.0 ppm
Arsenic	<1.0 ppm
Moisture	0.50%

Table 7.3 - Analytical composition of cellulosic succinic acid crystals.

However, the downstream processing approach used to produce this sample (Figure 7.3) was not fully representative of the MySAB commercial scale equipment or processing scheme.

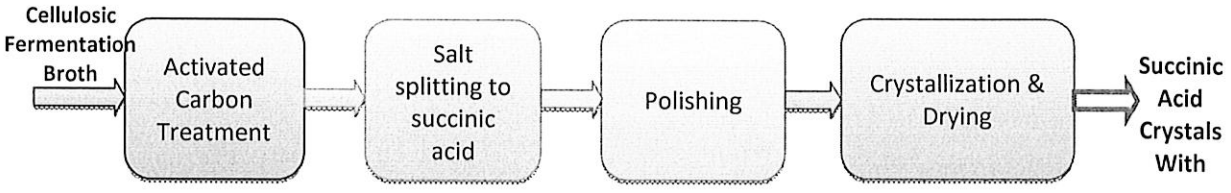

Figure 7.3 - Laboratory scale downstream processing of succinic acid broth from cellulosic sugars.

To prepare a commercially representative process sample would require a timely and expensive piloting effort at multiple third-party vendor sites. For these reasons, Myriant chose to further process our fermentation broth using the "crude bio-succinic acid" production methodology as described below, followed by the esterification process to produce samples of the commercially available end product dimethyl succinate (DMS). This approach is consistent with one of the projected end uses of a MySAB facility customer.

7.4.2 Producing Derivatives of Bio-Succinic Acid

Due to the purity of crude succinic acid crystals obtained from the cellulosic broth which is lower quality compared to higher purity bio-succinic acid crystals produced at MySAB, where there is salt separation equipment, we executed the proposed plan for conversion of crude bio-succinic acid crystals into an ester, specifically dimethyl succinate (DMS).

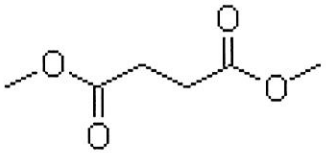

Figure 7.4 - Dimethyl succinate ($C_6H_{10}O_4$) is being used in the medical, chemical, food industries, as well as an intermediate product, for the production of 1,4 butanediol (BDO), which has a large market for industrial use in plastics, elastic fibers and polyurethanes.

The chemical reaction for the production of DMS from succinic acid is as follows:

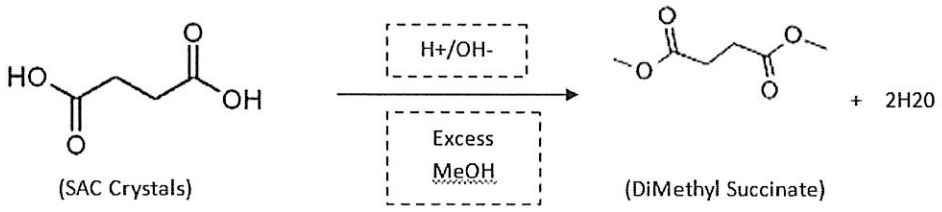

Figure 7.5 - DMS from succinic acid.

Myriant produced esters in a batch process, performed in a round bottom flask, by dissolving crude bio-succinic acid crystals in an excess of methanol and then using a cationic resin as an acidic catalyst due to its ease of separation from the end product. At the end of the reaction, the collecting flask contained an excess of methanol, cationic resin (catalyst), dimethyl succinate, and any unconverted bio-succinic acid. This mixture was passed through a vacuum filter and the filtrate underwent vacuum distillation in order to separate the above mentioned components on the basis of their respective boiling points.

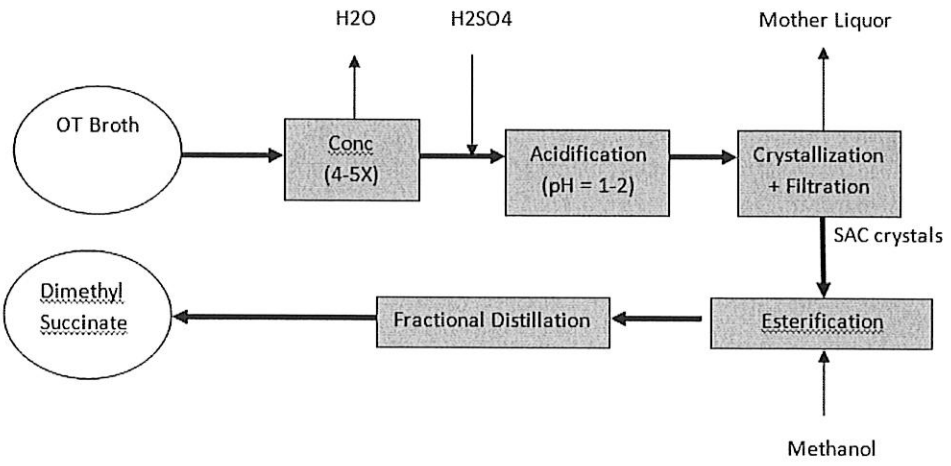

Figure 7.6 - Diagram of the esterification of the production of crude crystals and esterification.

The production of DMS from crude bio-succinic acid had the objective of producing more than five kilograms of crude bio-succinic acid crystals from cellulosic fermentation broth using the method described previously and also to perform a proof-of-concept test for Di-methyl ester production from crude SAC crystals as presented in Figure 7.5.

A detailed mass balance was performed to understand the feasibility of this process. Cellulosic broth from a pool of fermentation runs was clarified and ultra-filtered and then used as feed to the evaporation, acidification, crystallization and esterification process.

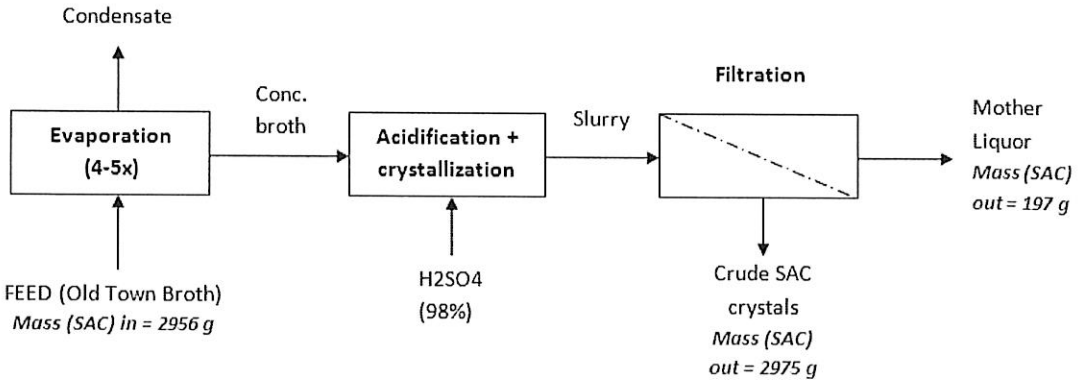

Figure 7.7: Bio-Succinic Acid component mass balance data.

The crude bio-succinic acid crystals that were produced (Figure 7.7) were characterized as follows:

Crude crystal composition	**% values**
moisture %	24.8
Succinic acid %	**45.72**
Ammonium %	4.66
Sulfate %	15.13
Phosphate %	0.49
Potassium %	0.16
Sugars %	0.18
Acetic acid %	1.11
Glycerol %	0.41
accounted	**92.66**

Table 7.4 – Analysis and characterization of crystals

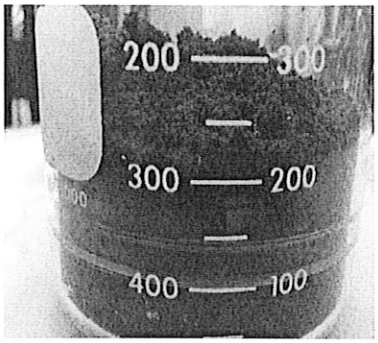

Figure 7.8 - Crude bio-succinic acid crystals with a YI equal to 13.

7.4.3 Esterification of Crude Bio-Succinic Acid Crystals:

A portion of the bio-succinic acid crystals were used for demonstrating the ester preparation concept at laboratory scale. The crystals were dissolved in methanol and the residual undissolved solids, which consists of mostly the inorganic salts (e.g. ammonium sulfate), were separated by filtration. The supernatant from the filtration step, a solution of succinic acid and methanol, was subjected to esterification reaction using sulfuric acid as a catalyst of the reaction mixture. The reaction was performed on a hot stir plate with methanol reflux. The reaction mixture was neutralized with lime after completion to inactivate the sulfuric acid catalyst. The following figure depicts the process steps of esterification reaction performed at laboratory scale.

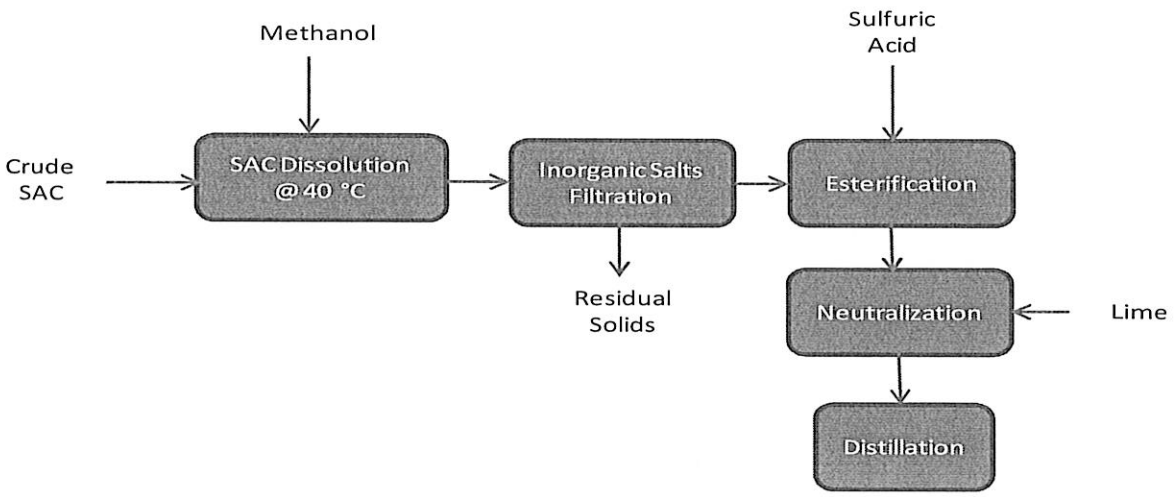

Figure 7.9 - Process steps in crude bio-succinic esterification at laboratory scale.

The pictures of the esterification reaction set up, reaction mixture and the residual solids from methanol dissolution step are shown below.

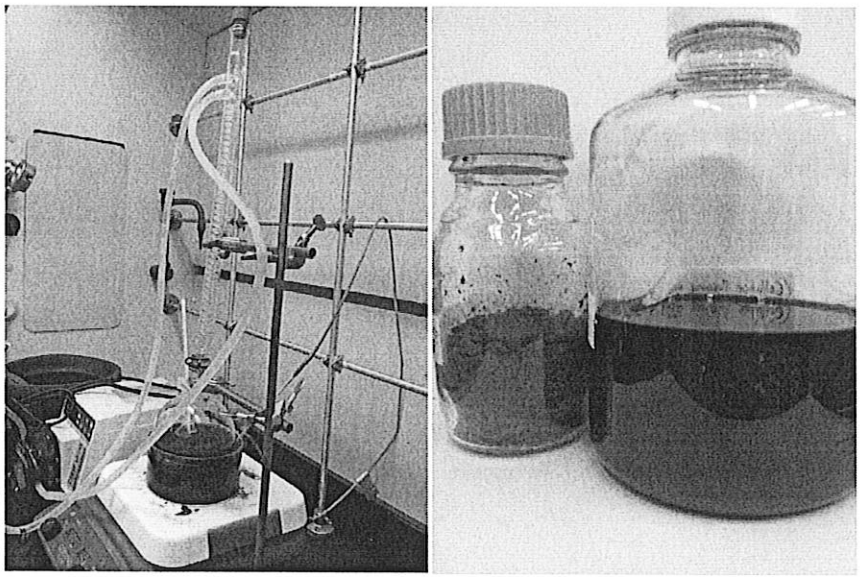

Figure 7.10 - Esterification reaction set-up, residual solids, reaction mixture (left to right).

7.4.4 Recovery of dimethyl succinate by fractional distillation:

The neutralized reaction slurry was subjected to fractional distillation using a lab scale set up in two stages to recover methanol and water rich fraction in the first stage and DMS rich fraction in the second stage following the order of their boiling points. DMS boils at 200°C at normal atmospheric pressure, so vacuum conditions were used to lower the boiling point for separation. The fractional distillation set up is shown in figure 7.11.

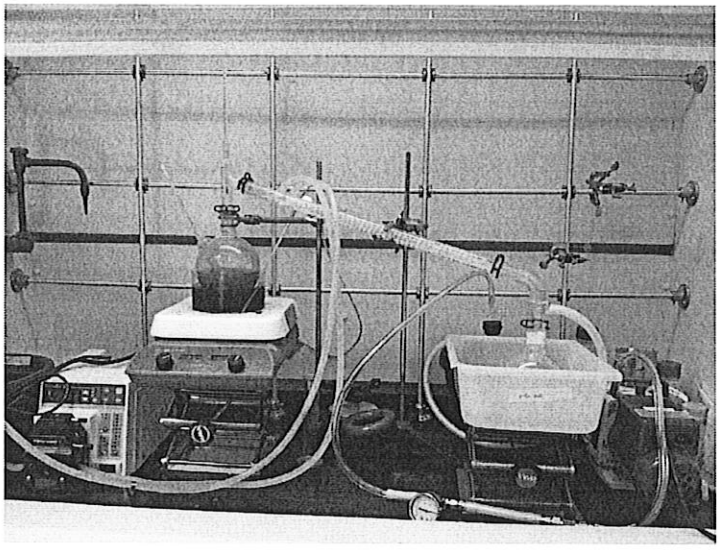

Figure 7.11 - Fractional distillation set-up for DMS recovery

About 30 grams of bio-based DMS was produced, and analyzed for moisture and purity. The analytical data is compared to a commercial DMS assay in the following Table. The high moisture in the Myriant Bio-DMS is attributed to the fact that the distillation was done with only one stage without any fractionation column, and is an artifact of the equipment limitations at lab scale. Myriant has full confidence that at large scale the commercial-grade specification can be obtained with a suitable fractionation column.

	Commercial/Petro-DMS	**Myriant Bio-DMS**
Assay, wt%	> 99.0	99.0
Water Content, wt%	< 0.1	4.7
Color, APHA	< 15	Visually clear & same

Table 7.5 - Comparison of commercial petro-based DMS and Myriant bio-based DMS.

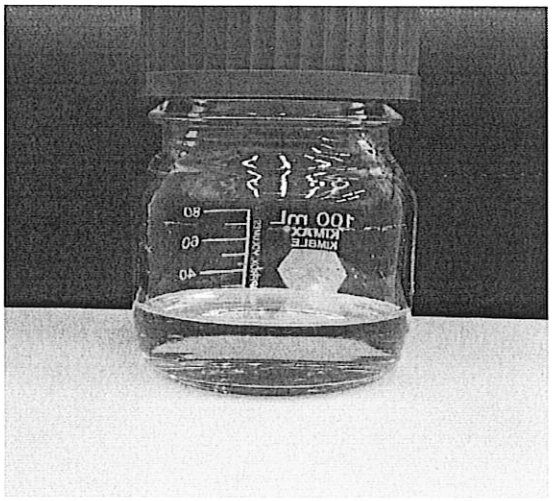

Figure 7.12 - Myriant bio-based DMS produced at Myriant's laboratory.

Summary

Myriant's bio-succinic acid process has been designed as a standalone facility at our Lake Providence, LA MySAB plant, but as the scale of commercial production grows it is clear that the bio-refinery concept may be effectively extended to include supplier(s) of sugars, utilities, waste treatment, and other supporting services such as industrial gases (CO_2) or cogenerated power. Myriant is also considering opportunities for co-location with the users of our bio-succinic acid, as it is further converted to one of its many end uses, or to specific end products being developed by Myriant's applications team. Ammonium sulfate also presents further opportunities for processing to a higher grade crystalline form.

Many of these bio-refineries have started to emerge around the country, initially located near corn wet mills which may supply both conventional ethanol plants, as well as others. These bio-refineries convert the sugars to other biological products such as bio-industrial chemicals, nutraceuticals, and other biofuels. The complexes are expected to evolve further as synergies continue to develop around uses of CO_2, glycerol, and cellulosic sugars.

Ultimately, Myriant expects our larger facilities to be sited at locations selected for the best economic balance between low cost feedstock supply sources, cost effective energy supply (natural gas, power), transportation accessibility to rail and barge, proximity to our main offtake partners, and near areas of the country with a skilled labor force and supporting services.

As this report shows, Myriant has had very significant success in the demonstration of our process on multiple sources of sugars – from conventional sugars, to emerging energy crops, to cellulosic feedstocks. Our MySAB facility is producing bio-succinic acid at a small commercial scale, and with some design modifications it could potentially operate with cellulosic feedstocks; given their commercial availability. A review of the current projects in development indicates that cost effective sources of lignocellulosic sugars remain several years in the future. The sugars would need to be comparable or lower in cost than conventional sugars, available from the specific sources identified in this work that are compatible with our bio-process, and reliably available to support year-round operations.

It is Myriant's hope that within five years the leaders in the cellulosic development field will have achieved their commercialization goals, and our bio-succinic acid process may become a major component of these next generation bio-refineries. Until that time we will continue to develop and improve our processing technologies, expand markets for our products, and develop new green end products that exploit the advantages of renewable feedstocks, reduced greenhouse gas footprint, and potentially better biodegradability.

8.0 Patent Disclosure

Title	Inventor	Date Reported / DOE "S" No.
METABOLILC EVOLUTION OF ESCHERICHIA COLI STRAINS THAT PRODUCE ORGANIC ACIDS	TAMMY GRABAR WEI GONG R. ROGER YOCUM	September 7, 2016 10043070-16-0002
ENGINEERING MICROBES FOR EFFICIENT PRODUCTION OF CHEMICALS	WEI GONG SUDHANSHU DOLE TAMMY GRABAR ANDREW CHRISTOPHER COLLARD JANICE G. PERO R. ROGER YOCUM	NOVEMBER 17, 2010 S-126,498
IMPROVED FERMENTATION PROCESS FOR THE PRODUCTION OF ORGANIC ACIDS	THERON HERMANN JAMES REINHARDT XIAOHUI YU RUSSEL UDANI LAUREN STAPLES	AUGUST 16, 2010 S-126,024
PRODUCTION OF ORGANIC ACIDS FROM XYLOSE RICH HYDROLYSATE BY BACTERIAL FERMENTATION	TAMMY GRABAR WILLIAM HOUSER	September 7, 2016 10043070-16-0001