Bio-based Chemicals from Low-cost Lignocellulosic Sugars

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Milestones: Myriant utilizes proprietary technology to advance the development of low-cost cellulosic sugars for the sustainable commercialization of high-value bio-based chemicals. The following milestones have been achieved by Myriant in recent years:

- In 2009, Myriant was awarded $50 million from the US Department of Energy to support the construction of a 30 million pounds succinic acid facility in Louisiana and the commercialization of the lignocellulosic succinic acid program.
- From 2010 to 2011, Myriant conducted a pilot campaign to produce 24 tons of succinic acid from a variety of feedstocks and to obtain scale-up data for its commercial scale succinic acid production facility with expected construction completion in fall 2012.
- The nominated technology has led to the publication in 2011 of the International Patent Applications number WO2011/130725 A2 (October 20, 2011) and WO2011/123154 A2 (October 6, 2011).
- From 2009 through 2011, Myriant's team identified and characterized genetic changes in the chromosome of the production strain which result in improved hemicelluloses hydrolysate fermentation.

The nominated technology is eligible for the **small business award**.
The nominated technology focuses on **"the use of greener synthetic pathway"** area.

The development of Myriant's core technology is taking place within the U.S. Myriant's corporate headquarters and research facilities are located in Massachusetts. Myriant's 30-million pounds annual capacity succinic acid facility is located in Louisiana.

Abstract:
Myriant successfully developed its proprietary process for the production of drop-in chemicals and replacement chemicals, including succinic acid and lactic acid, from low cost non-food, lignocellulosic feedstocks. Myriant currently has several *E. coli* platforms capable of generating high titers of organic acids from clean sugars and most importantly from lignocellulosic hydrolysate sugars. Life cycle studies performed on Myriant's bio-succinic acid technology as compared to petroleum derived succinic acid showed a potential of a higher than 50% reduction in green house gases. Myriant's bio-succinic acid, a four carbon molecule, and bio-lactic acid, a three carbon molecule, will be used as drop-in and replacement chemicals in their current petroleum based markets. In 2009, Myriant was awarded $50 million from US Department of Energy, which supports the company's construction of a 30-million pounds capacity bio-succinic acid facility in Lake Providence, Louisiana and the commercialization of its lignocellulosic bio-succinic acid program.

Science and Innovation

Lignocellulosic feedstocks are non-edible plant material composed primarily of the polysaccharides cellulose, hemicelluloses and lignin. This material is present in agricultural and forest resources, including corn stover, wheat straw, rice straw, sugarcane bagasse, forest thinning, switch grass, sorghum and others. Most fermentation routes to produce industrial chemicals are developed using only glucose as the main source of carbon. Due to the high cost of glucose, the volatility of the sugar markets, and competition of pure sugars with the food and feed industry, as compared to lignocellulose feedstock, the use of inexpensive and abundant feedstocks creates a greater advantage in the production of organic chemicals.

E. coli platforms are used to create a wide variety of high-value building block chemicals by metabolizing saccharides, glycerin, and carbon dioxide. By opening or closing specific metabolic pathways during the conversion process, a myriad of important chemical building blocks are selectively created. The Myriant Corporation (Myriant) research and development team is experienced in identifying and commercializing economically attractive pathways which can open new markets.

Figure 1 represents the building blocks that could be created by using *E. coli* as biocatalyst. Illustrated in light green are the chemicals on which Myriant is currently focusing its efforts.

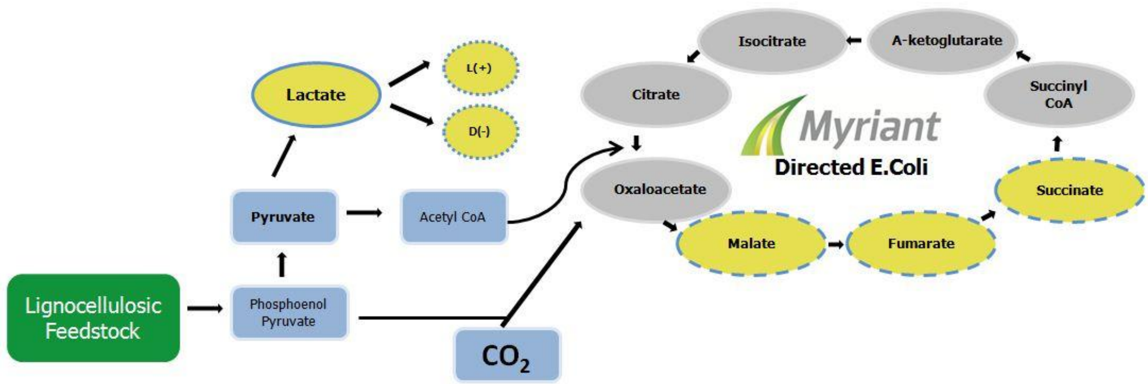


Figure 1 Myriant's *E. coli* platforms are used as biocatalysts for the production of building blocks.

Myriant has developed fermentation technology for the production of organic chemicals, primarily succinic acid and lactic acid, two organic acids identified by DOE as platform chemicals for manufacturing additional green chemicals. For example, bio-based succinic acid can be used as the starting material for manufacturing butanediol and pyrrolidone which are currently derived from petrochemical feedstocks. Currently, Myriant has active R & D programs to develop processes for manufacturing bio-based butanediol, pyrrolidone and acrylic acid.

Myriant currently has several *E. coli* platforms capable of generating high titers of organic acids from clean sugars and most importantly from lignocellulosic hydrolysate sugars. As an example of organic acid production from clean sugars, Myriant has a strain, KJ122, which is a succinic acid production strain. KJ122 was originally developed at the University of Florida under research grants from DOE and USDA and has been exclusively licensed to Myriant.

In 2011, KJ122 was used at Myriant's pilot facility to successfully produce more than 24 metric tons of succinic acid from sorghum flour. This will be the primary strain used at Myriant's succinic acid commercial scale facility in 2013. TG114 is a D-lactic producing strain, which is successfully fermenting sugars at the commercial scale level. TG108 is an L-lactic acid production strain, which has the same fermentation characteristics as the other lactic acid producing strain. TG400 and WH3 are strains being used in the fermentation of lignocellulosic hydrolysates.

KJ122 (*E. coli C*, Δ*ldhA*, Δ*adhE*, Δ*ackA*, Δ*focA-flB*, Δ*mgsA*, Δ*poxB*, Δ*tdcDE*, Δ*citF*, Δ*aspC*, Δ*sfcA*) was genetically engineered for succinic acid production [1, 2]. In order to balance the red-ox potential, this strain has been optimized to utilize a combination of a reductive (higher yielding) and an oxidative (lower yielding) pathway. Succinic acid ($C_4H_6O_4$) is produced from glucose ($C_6H_{12}O_6$), a hexose sugar, as a starting material by the following chemical reaction:

$$7\ C_6H_{12}O_6 + 6\ CO_2 \rightarrow 12\ C_4H_6O_4 + 6\ H_2O$$

The maximum theoretical yield of this reaction is 1.71 moles of succinic acid per mol of glucose or 1.12 gram of succinic per gram of glucose. The higher yielding mass balance is due to a CO_2 fixation step in the reductive pathway. When xylose ($C_5H_{10}O_5$), a pentose, is used for the production of succinic acid the following reaction takes place:

$$7\ C_5H_{10}O_5 + 5\ CO_2 \rightarrow 10\ C_4H_6O_4 + 5\ H_2O$$

The maximal theoretical yield of succinic acid from xylose is 1.43 moles of succinic acid per mole of xylose, or 1.12 gram succinic per gram of xylose. For xylose, in theory, three molecules of xylose are converted to two molecules of fructose-6-phosphate and one molecule of glyceraldyhyde-3-phosphate by the pentose phosphate pathway. These molecules are then fed through the glycolytic pathway to eventually generate the organic acids. For lactic acid, the maximal theoretical yield from pentose sugars is 1.67 moles of lactic acid per mole of pentose sugar, or 1.0 gram of lactic per gram of pentose sugar.

E. coli is naturally capable of fermenting all of the pentose and hexose sugars found in biomass, though not equally well. The production strains had previously undergone significant genetic manipulation and metabolic evolution to enhance the efficiency of hexose sugar fermentation in a clean mineral salts medium. This has reduced their ability to ferment pentose sugars. Strains TG400 and WH3 were isolated based on improved mixed sugars fermentation and improved hydrolysate fermentation.

The desirable traits of biocatalysts for the production of specialty chemicals from biomass are:
1. Capability of metabolizing all sugars in non-food, lignocellulose feedstocks
2. Ability to tolerate the toxins commonly found in lignocellulosic hydrolysates
3. Ability to ferment anaerobically mixed sugars with minimal by-product formation
4. Capability to obtain higher product yields
5. Ability to use simple, inexpensive media with inorganic salts and not to use expensive media like yeast extract during fermentation
6. Robustness with tolerance for high sugars, salts and acids regularly found in hydrolysates and also demonstrating rapid growth without the use of plasmids
7. Excellent knowledge base and genetic tools for the biocatalysts

Most lignocellulosic hydrolysate yields a variety of mixed sugars that includes hexose sugars (glucose and galactose) and pentose sugars (xylose, arabinose, and mannose). Most industrial microorganisms prefer to use glucose first as a carbon source. When this sugar is depleted then they proceed to use other available sugars. This phenomenon related to carbon utilization is referred to as catabolite repression or diauxic growth. This repression needs to be eliminated from the organism for the efficient utilization of all the mixed sugars available in lignocellulosic material which will then lower the cost of chemical production by fermentation.

In addition to a mixed sugar stream, most lignocellulosic hydrolysate also yields toxins like acetic acid, furfural and 5-hydroxymethylfurfural (HMF) which inhibit the organism from completing the metabolism of sugars to products. Most of these toxins are removed prior to exposing the organism during fermentation in a process called detoxification. The ability of the biocatalyst to tolerate inhibitory chemicals present in hydrolysate is of key importance as the process of detoxifying the material adds unit operations and cost to the production of chemicals [4, 5].

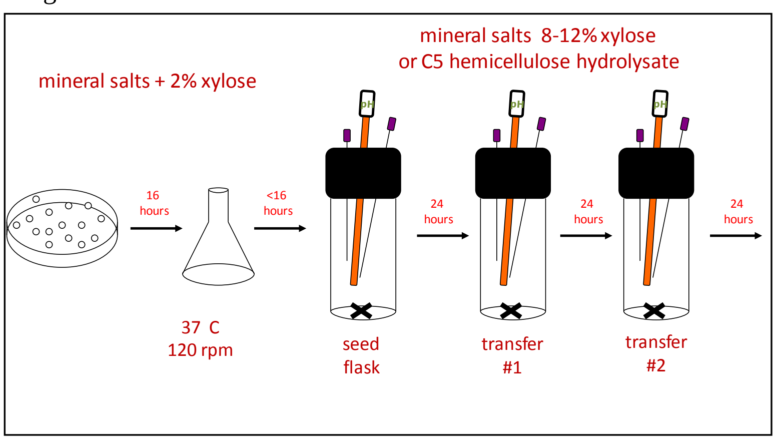
Myriant has evolved biocatalysts for the production of organic acids including lactic acid and succinic acid, which are able to metabolize multiple sugars simultaneously and can ferment on non-detoxified lignocellulosic hydrolysate [3]. Myriant's proprietary strains were used to test various lignocellulosic feedstocks which were treated using a range of methods shown in Table 1. Myriant's strains ability to ferment a wide variety of feedstocks hydrolyzed by a variety of methods will allow the company to choose the most practical and economic source moving forward [4, 5].

Table 1: Lignocellulosic feedstocks and pretreatment methods used for producing sugar streams.

Lignocellulosic Feedstock	**Pretreatment Methods**	**Sugar Concentration Range (mixed sugars)**
Sugarcane bagasse	Various Dilute Acid	
Corn stover	Concentrated Acid	
Forage Sorghum	Organic Solvents	
Poplar	Enzymatic Hydrolysis	
Pine Wood Chips		80-100 g/L
Aspen Wood Chips		
Switchgrass		
Municipal Solid Waste		

A metabolic evolution strategy, as illustrated in Figure 2, was applied to improve xylose fermentation. Myriant's proprietary succinic acid and lactic acid production organisms were genetically designed such that the cells must produce the desired organic acid in order to grow. In all fermentations a mineral salts medium (AM1) [6, 7] was used and supplemented with either xylose or bagasse hemicellulose hydrolysate, and mineral salts and trace elements. Cells were serially transferred every 24 hours to encourage metabolic evolution through growth based selection. Clones with improved fermentation characteristics were isolated throughout and at the end of the selections and were assigned new names.

Figure 2 Metabolic Evolution



Myriant's succinic acid production strains were used to test our ability to ferment lignocellulosic hydrolysates from various feedstocks. Only a sample of the tested feedstocks is illustrated in Figure 3, including sugarcane bagasse, corn stover and switch grass. The feedstocks were pretreated using a variety of methods including several different dilute acid treatments with and without enzymatic hydrolysis, concentrated acid and organic solvent processes. These methods are compared in Figure 3 to fermentations in pure xylose or pure glucose in mineral medium (AM1).

Figure 3

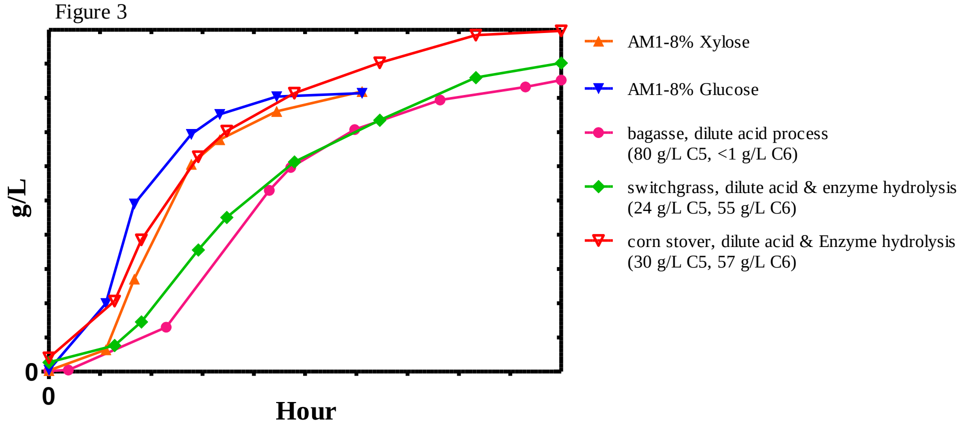


Figure 3 Succinic Acid production comparing Myriant's strain using various lignocellulosic feedstocks and various pretreatment methods.

Shown in Figure 4a and 4b are the lactic acid strains developed in hemicelluloses hydrolysates. Parent strains used pure sugars as carbon source [8, 9, 10], while genetically manipulated and evolved strains (isolates) produced lactic acid from hemicellulosic hydrolysate. It is important to recognize that the strains used to test fermentability of the various feedstocks were not evolved with the selected feedstocks or pretreatment methods used. Further evolution of selected strains will be needed for more improvements.

Figure 4a) Comparison of lactic acid production **between strains metabolically evolved to ferment hemicellulosic sugars and the original strain.** Figure 4b) Isolate **#3 fermentation of bagasse hemicelluloses hydrolysate.**

Figure 4a

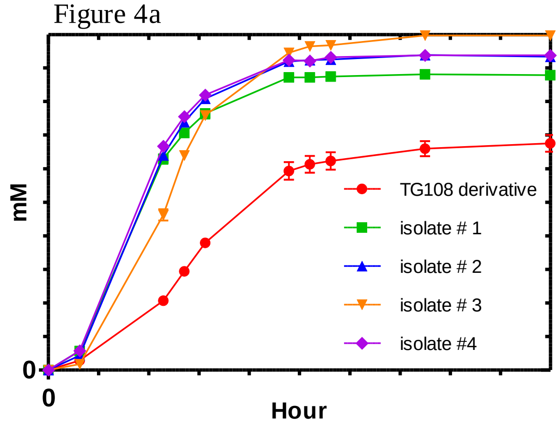
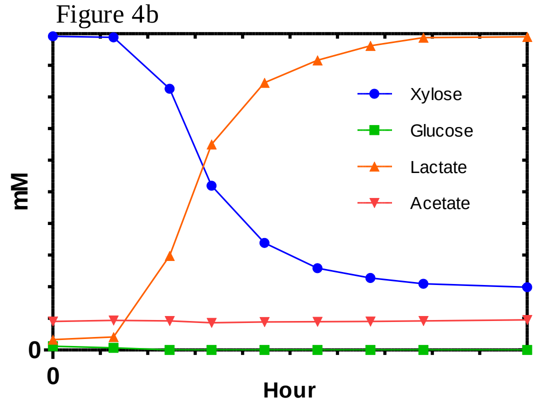


Figure 4b



Human Health and Environmental Benefits

The life cycle analysis was prepared by Myriant and Life Cycle Associates, LLC. Life cycle energy and emissions were calculated utilizing components of the latest default GREET model (version GREET1.8c.0) developed by Argonne National Laboratory to determine the life cycle energy and greenhouse gas (GHG) intensity of the grain sorghum to succinic acid chemical pathway, on a Btu/lb and g/lb succinic acid basis. Assumptions in the GREET tool were used for agricultural and transportation GHG estimates. Myriant's process assumptions for energy use, product yield and chemical use were combined with existing GREET assumptions to model GHG emissions from the succinic acid production process. Life cycle vectors were extracted from the GREET model (a large Excel spreadsheet) and reassembled in a new worksheet using a MOUSE (matrix organization using specific energies) framework. The MOUSE framework uses LCI (life cycle index) vectors extracted from the GREET model to develop customized life cycle analyses for novel production pathways. Each LCI vector is a column of energy and emission values in the same format used in GREET. Figure 5 presents the product pathway system boundary for the life cycle analysis and the system boundary of the production of bio-succinic acid, petro-succinic acid, petro-maleic anhydride, and petro-adipic acid.

The life cycle results for grain sorghum succinic acid production via the Myriant demonstration and commercial scale pathways are presented below in Table 2. The natural gas input for steam production is responsible for the dominant share of life cycle greenhouse gases.

Figure 5 System boundary diagrams for grain sorghum succinic acid and petrochemicals

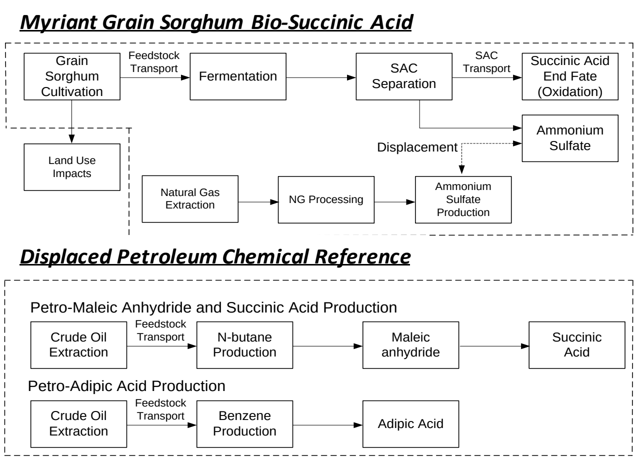


Table 2 LCA result for Myriant bio-succinic acid process

Scenario:	Demonstration	Commercial Plant
Feedstock:	Grain Sorghum	Grain Sorghum
Units:	Btu/lb, g/lb succinic acid	
Farming Equipment	71.3	71.3
Soil Carbon Credit	0.0	0.0
Ag. Chemical Use	40.6	40.6
Feedstock Transport	12.6	12.6
SAC Production Thermal Input	1,097.5	1,097.5
SAC Production Electricity Input	160.6	160.6
SAC Production Chemical Inputs	422.7	422.7
Fermentation Non-Combust. Emissions	0.0	0.0
Co-Product Ammonium Sulfate	-330.3	-330.3
SAC T&D	5.4	5.4
Chemical Fate (Oxidation)	676.0	676.0
Biogenic Chemical Carbon Credit	-676.0	-676.0
Total GHG (g/lb)	**1,480.3**	**1,480.3**
Total GHG (g/Kg)	3,263.6	3,263.6
Reduction from petroleum-derived SAC	62.1%	62.1%
Reduction from petroleum-derived MA	52.0%	52.0%

Chemical inputs for production are the second highest life cycle emission category. The ammonium sulfate co-product credit is significant, and offsets the succinic acid production and agricultural chemical total emissions. This study showed a significant reduction in green house gases from common petroleum routes to produce succinic acid (SAC) and maleic anhydride (MA). The total reduction in green house gases for the production of bio-succinic acid by Myriant's pathway as compared to petroleum derived succinic acid is 62% and from petroleum derived maleic anhydride is 52%.

Applicability and Impact

Organic chemicals are a class of chemicals based on or containing carbon atoms. Petrochemicals, which are organic chemicals, have traditionally been derived from fossil fuels such as petroleum, natural gas or coal. Organic chemicals can also be produced from renewable carbon sources such as plant-derived matter, including sugars, oils and cellulosic materials. These renewable plant sources are the basis of the biochemicals industry.

Organic chemical building blocks are used by the petrochemical industry to produce, through different chemical reactions, downstream chemicals used in thousands of industrial and consumer applications. These building blocks are classified according to the number of carbon atoms per molecule. For example, ethylene, the building block for such materials as polyethylene and ethylene glycol, has two carbon atoms per molecule (C2), is primarily derived from natural gas found in the U.S. and the Middle East, and is considered a light feedstock (i.e., its molecules contain few carbon atoms). Building blocks with three (C3), four (C4) and six (C6) carbon atoms are most commonly derived from a heavier petroleum feedstock. Supplies of these building blocks are influenced in part by the broader supply and demand dynamics of fossil-based feedstocks. For example, in the U.S., due to the widening pricing spread between natural gas and oil, many cracker operators are using lighter natural gas feedstocks to earn higher margins, thus limiting the production of heavier C3, C4 and C6 building block chemicals, which are derived from higher-priced petroleum feedstocks. We are targeting high-value chemicals traditionally derived from these heavier feedstocks. Given their supply and demand dynamics, proprietary nature and barriers to entry, these target chemicals generally command higher prices than traditional commodity chemicals.

Myriant's bio-succinic acid, a four carbon molecule, and bio-lactic acid, a three carbon molecule, will be used as drop-in and replacement chemicals in their current petroleum based markets. Succinic acid can be used to produce butanediol, a high-value chemical intermediate with end markets in polymer resins, fibers, coatings and other downstream chemical products. Also succinic acid can be used in the production of pyrrolidones, a chemical widely used as solvents in electronic process, polyurethane processing, coating, or as a replacement for methylene chloride in paint strippers. Acrylic acid, produced using lactic acid as precursor chemical, is used to impart hardness, tackiness and durability to thousands of polymer formulations. Its main derivatives, acrylates, are used in super absorbents, coatings, adhesives, sealants, textiles, paper chemicals and plastic additives.

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