Producing Industrial Chemicals by Fermenting Renewable Feedstocks – and at a Lower Cost

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Milestone

In 2011, OPX Biotechnologies (OPXBIO) demonstrated the fermentation and primary purification of the renewable product 3-hydroxypropionate (3-HP) at scale of 3,000 liters via its engineered microbe and bioprocess. 3-HP can be readily converted by further processing to a number of other important industrial chemicals including acrylic acid, acrylamide, acrylonitrile, malonic acid and 1,3 propanediol. Also in 2011, OPXBIO converted 3-HP to bioacrylic at yields in excess of 90%. At pilot scale, OPXBIO demonstrated fermentation metrics that predict a modeled commercial cost of acrylic acid at approximately $0.75/lb., which is comparable to the average petro-based acrylic acid cost in 2011. OPXBIO's goal is to achieve costs that are 25 – 50% less than petro-based acrylic when it advances to commercial scale.

Nomination Qualifications

OPXBIO is a small 68-person, Boulder, Colo.-based corporation that was formed in 2007. The nominated technology is eligible for the small business award and focuses on award area (1) the use of greener synthetic pathways. The work on this nomination has been completed in Colorado and Michigan.

Abstract

The worldwide demand for fuels and chemicals shows no signs of slowing, nor does the appetite for creating these materials using environmentally sustainable methods. To meet these demands, chemical producers have been searching for alternative processes to produce many of today's industrial chemicals, including using renewable feedstocks. However, the transition to renewable feedstock has been slow due to the cost of these renewable chemical processes. To be successful, new processes must be both environmentally sustainable and cost competitive with traditional petro-based chemicals. OPXBIO's proprietary platform technology called Efficiency Directed Genome Engineering (EDGE™) allows it to develop and engineer microorganisms and bioprocesses faster and cheaper than traditional microbial engineering technologies, enabling it to develop multiple cost-effective chemicals from multiple renewable feedstocks. OPXBIO proved in 2011 that its patented technology can renewably and cost effectively produce chemicals by demonstrating a bioprocess for acrylic acid (bioacrylic).

OPXBIO's bioacrylic process significantly reduces greenhouse gas emissions and crude oil use when compared to traditional acrylic processing from propylene. An initial lifecycle analysis (LCA) indicates that OPXBIO's process for producing bioacrylic would reduce greenhouse gas emissions by more than 77% and crude oil use by 82%. If the entire 9-billion pound global market for acrylic was replaced with bioacrylic using OPXBIO technology, greenhouse gas reductions would be in excess of 5 million tons per year, and the industry would reduce the use of crude oil by approximately 2.5 million tons.

OPXBIO used its EDGE™ process to engineer both a microorganism to produce 3-hydroxypropionic acid (3-HP), and a process that renewably produces acrylic acid. A key focus was the strain development centered on increasing the cellular pools of malonyl-CoA, the first committed intermediate for the 3-HP production pathway. Many commercial products may be derived from the core metabolic precursor, malonyl-CoA, including fatty acids (and hence long chain alkanes), polyketides, and 3-HP.

Currently, 3-HP is being produced in pilot-scale with metrics that predict a commercial cost of bioacrylic at approximately $0.75/lb. (using dextrose as feed at $0.14/lb.). This cost is competitive with the average petro-based acrylic in 2011, making the process both more environmentally and economically sustainable.

Technology Overview

In 2011, OPX Biotechnologies, Inc. (OPXBIO) took major steps that proved its proprietary technology platform – Efficiency Directed Genome Engineering (EDGE™) – allows it to engineer and develop microorganisms faster *and* cheaper than other traditional microbial engineering tools and technologies. OPXBIO used its technology to develop a proprietary microorganism and bioprocess for the production of 3-hydroxypropionic acid (3-HP) that is further converted to acrylic acid (bioacrylic). OPXBIO demonstrated the fermentation of 3-HP at pilot scale, with performance that models to a projected commercial cost of approximately $0.75/lb. (using dextrose as feed at $0.14/lb.), which is competitive with the average petro-based acrylic in 2011. Also in 2011, OPXBIO scaled up the process and demonstrated the fermentation and primary purification of 3-HP at 3,000 liters. In addition to the process being cost competitive, it produces 77% fewer greenhouse gas emissions and will use 82% less crude oil than the traditional propylene-based acrylic acid processes.

Acrylic acid is a major industrial commodity, with annual global demand exceeding 4 million tons (9 billion pounds in 2009) and with an estimated annual worldwide demand growth of 4%. Major consumer uses of acrylic acid include the production of acrylate esters with applications in paints and surface coatings, adhesives, sealants, and plastic additives, and the production of polyacrylic acid for uses in superabsorbents (for example, in disposable diapers), replacement of phosphates in detergents, and as flocculants and thickeners.

Traditionally, acrylic acid is produced from propylene, derived from petroleum, in a two-step chemical oxidation. The price volatility of these feedstocks, combined with their finite availability and their resulting greenhouse gas emissions, has prompted the search for processes to make acrylic acid from renewable resources, in particular by microbial fermentation from sugars. In addition, the producers of end-use products such as diapers, paints, coatings, detergents and adhesive have asked suppliers for more renewable and sustainable chemicals, thus increasing the demand for a bioacrylic. Producing bioacrylic through direct fermentation, however, has proven to be one of the most-challenging renewable processes due to its toxicity and instability. An alternative is the fermentative production of 3-HP, which is readily dehydrated to acrylic acid as shown in Figure 1. While 3-HP is not known to be the natural end-product of metabolism by any organism, it is present as an intermediate in a CO_2-fixation cycle first discovered in *Chloroflexus aurantiacus* [1].

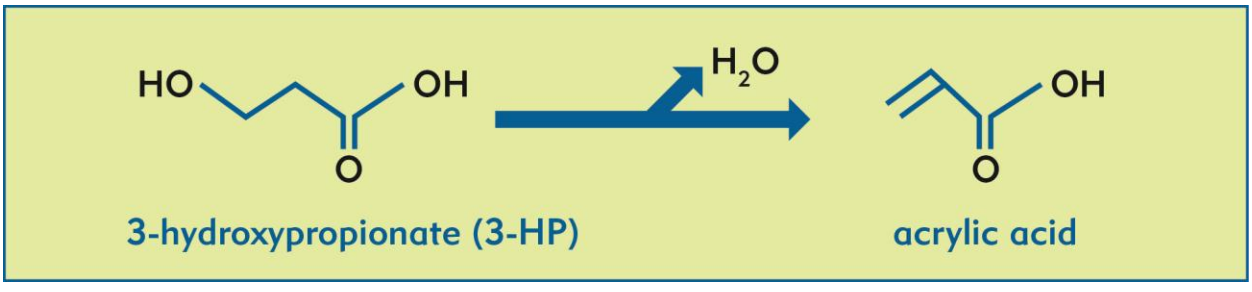

Figure 1: Dehydration of 3-HP to Acrylic Acid.

A metabolic pathway from glycerol to 3-HP is known [4], and significant production of 3-HP from this starting material by engineered biocatalysts has been demonstrated [5]. However, the supply and pricing of glycerol derived from the production of biodiesel from plant oils is subject to tenuous support by various governments, and thus production based on fermentation of sugars (corn-derived dextrose, cane sucrose, or cellulosic sugars) by metabolically-engineered biocatalysts is a more feasible long-term option.

Indeed, 3-HP is a molecule uniquely suited as a target for metabolic engineering tools [6]. Its stoichiometry (the molecular formula for 3-HP is $C_3O_3H_6$) predicts 100% mass recovery from glucose ($C_6O_6H_{12}$), unlike more reduced compounds with lower stoichiometric yields such as ethanol (50%) or isoprenoid hydrocarbons (37%). A number of potential pathways to 3-HP from sugar have been proposed [ref BNICE, Cargill patents, Genomatica patent application, OPXBIO OAD patent application], largely branching from microbial central metabolism. Most are thermodynamic feasible [7] and use the combination of heterologous existing enzymatic activities in a pliable host organism or require the engineering of, at most, one non-naturally occurring enzyme. The implementation of several of these pathways has been documented in the patent literature [5].

The OPXBIO team discovered a way to engineer microorganisms to produce 3-HP via the pathway from malonyl-CoA (Figure 2) using fewer steps from a readily available metabolic intermediate to 3-HP, and the enzyme(s) and genes involved have been characterized [1]. Metabolic supply of malonyl-CoA has been studied intensely because it is the precursor to fatty acids, and thus manipulating its biosynthesis is key not only to the efficient production of 3-HP, but numerous other products including fatty acid-derived chemicals and biofuels.

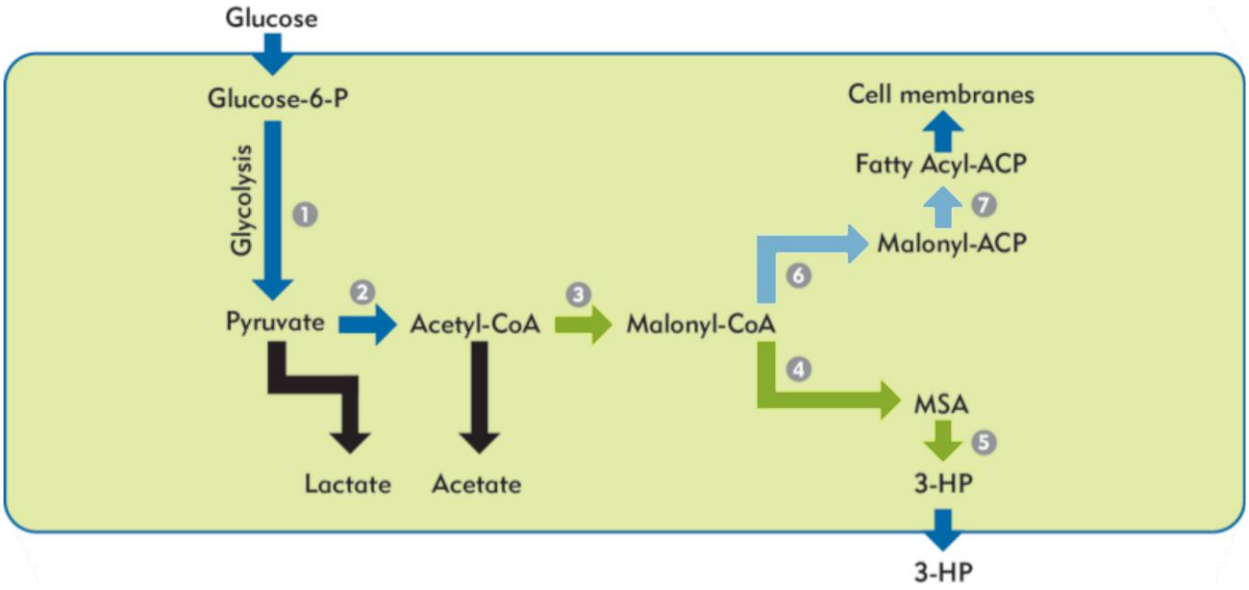

Figure 2: Simplified Biocatalyst Design for the Production of 3-HP through malonyl-coA *Sugar is taken into the cell and metabolized through the ubiquitous glycolysis pathway (1) to make pyruvate, which is then converted to the intracellular intermediates acetyl-coA and then malonyl-coA through the actions of pyruvate dehydrogenase and acetyl-coA carboxylase (2) and (3) respectively. Normally malonyl-coA levels are regulated by intermediates of fatty acid biosynthesis (steps 6 and 7) such as fatty acyl-ACPs. We have decreased activity through fatty acid biosynthesis (shown in white) to reroute malonyl-coA to 3-HP through the actions of malonyl-coA reductase and 3-HP dehydrogenase (steps 4 and 5 respectively). Green arrows have been increased in strains, whereas white have been decreased. In addition, the production of the key byproducts, lactate and acetate, has been eliminated (shown as black arrows).*

To accomplish these goals, OPXBIO leveraged its flexible and efficient technology platform to rapidly develop commercial microbes for producing chemicals and fuels. This platform leverages a strong core competency in metabolic engineering and microbial physiology. OPXBIO developed a proprietary strain engineering technology, EDGE (Efficiency Directed Genome Engineering) which allows for the rapid, robust and comprehensive understanding and manipulation of multiple complex phenotypes. The core understanding gained through the deployment of these technologies allows for both a strong intellectual property position and the efficient direct engineering and optimization of organisms for industrial bioprocesses (11-22).

Using EDGE, the team made numerous genetic modifications to manage and increase metabolic flux though malonyl-coA by controlled inhibition of fatty acid synthesis as shown in Figure 2 above. Figure 3, below, demonstrates the increases in specific productivity of 3-HP of several engineered strains. Specific productivity (grams of product per gram of biocatalyst per unit time) is a measure of how much product each cell can make within a given time period, and is an important metric to measure biocatalyst performance.

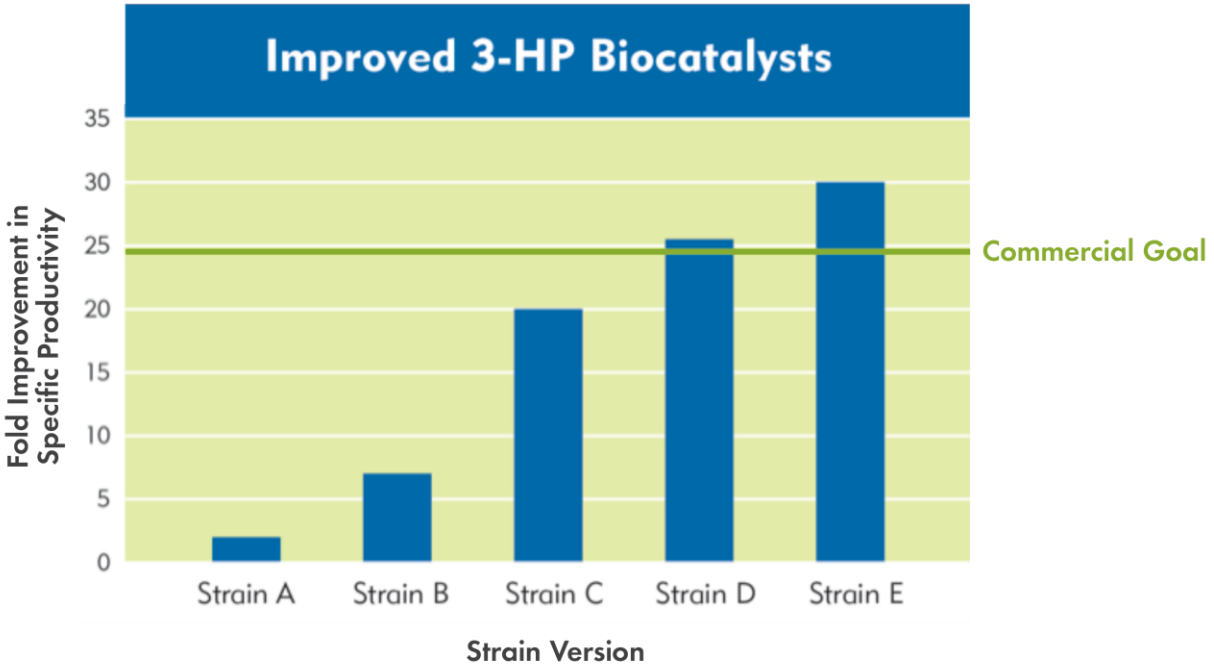

Figure 3: Improved Biocatalysts for the Production of 3-HP through malonyl-coA. *Fold improvement in specific productivity is shown for several strains. Strain A lacks manipulation of fatty acid synthesis, whereas Strain E has multiple genetic modifications to modify fatty acid biosynthesis, achieving specific productivities above those needed for commercial-level production.*

One key hurdle encountered was the fact that 3-HP produced from engineered biocatalysts is somewhat toxic. The inhibitory effect of 3-HP on *E. coli* growth has been examined [10] and has been shown to be significant at levels above 30 g/L. By combining multiple genetic modifications effecting tolerance, OPXBIO overcame this challenge by developing microorganisms that can tolerate levels of 3-HP at industrially relevant titer levels (8-10, 21, 22).Using the OPXBIO EDGE toolkit, numerous metabolic modules have been optimized and integrated to increase strain performance, including those discussed above such as

malonyl-coA flux and tolerance. This, in combination with bioprocess design and optimization, has led to a very rapid decrease in the predicted commercial production cost of 3-HP, as well as bioacrylic acid. Figure 4 shows the rate of cost reduction from the beginning of 1 liter fermentation runs to produce 3-HP until achieving commercially competitive cost performance.

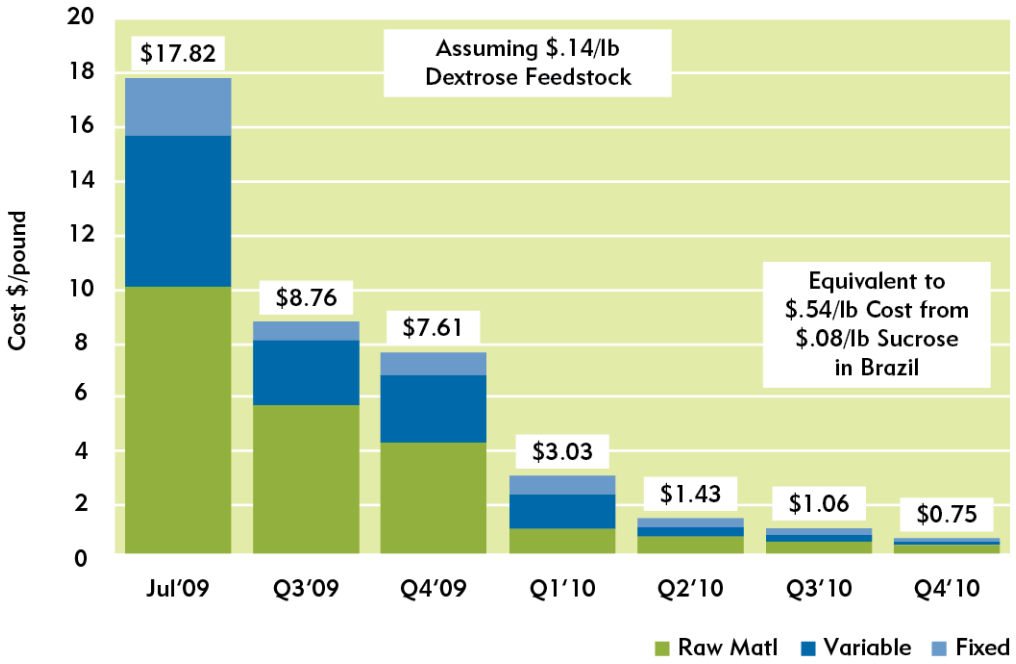

Figure 4: Cost-Reduction Curve.

Feedstock Flexibility

The EDGE technology has proven it can allow OPXBIO to engineer microorganisms to produce a variety of chemicals using a variety of feedstocks. This addresses a key challenge of renewable chemicals – the inability of many processes to use more than one limited feedstock. The initial feedstock for the fermentation of 3-HP is either glucose from corn starch or sucrose from cane sugar, both of which have been used successfully at laboratory scale. It should be noted that for its first commercial plant with a capacity of 100 million pounds per year, OPXBIO will only require approximately 0.04% of the U.S. corn crop. If the entire 9-billion pound global market for acrylic acid was converted to bioacrylic using corn sugar as a feedstock, it would only require approximately 2.9% of the U.S. corn crop.

The power of the EDGE technology is that OPXBIO can switch to different feedstocks as they become economically viable. If cellulosic sugars are available at an economically viable price from non-food sourced biomass (municipal solid waste, woodchips, etc.), OPXBIO could engineer the microorganism to use this sugar. In addition, if syngas (hydrogen and carbon dioxide) from the gasification of non-food biomass becomes economically viable, OPXBIO could use it as a feedstock for its fermentation of 3-HP. OPXBIO has proven it can use syngas through a microorganism it has engineered that utilizes hydrogen and carbon dioxide as a feedstock and produces fatty acids through fermentation as part of a federal grant.

Environmental Benefits

A 2008 lifecycle analysis (LCA) of OPXBIO's bioprocess was compared to traditional petrochemical processes to produce acrylic, specifically propylene two-stage oxidation utilizing propylene from steam cracking of naphtha. The bioprocess compared glucose from corn starch and syngas from the gasification

of woodchips. Table 1 below compares both the amount of greenhouse emissions produced per kilogram of acrylic and the amount of crude oil used to produce a kilogram of acrylic. Table 2 shows the percent reductions by using the OPXBIO process, and Table 3 shows the tons of greenhouse gas reductions for a standard 100-million pound acrylic plant versus traditional technology and the tons of oil saved.

Table 1: Lifecycle greenhouse gas emissions and crude oil consumption for conventional acrylic acid production and bio-acrylic acid production from glucose and syngas feedstocks.

Parameter	Conventional	Glucose	Syngas
Greenhouse gas emissions (kg CO_2e/kg acrylic acid)	2.3	0.53	0.73
Crude oil consumption (kg oil/kg acrylic acid)	0.82	0.14	0.046

* All values rounded to two significant figures

Table 2: Percent reductions from conventional acrylic acid production for bio-acrylic acid produced from glucose and syngas feedstocks.

Parameter	Glucose	Syngas
Greenhouse gas emissions	-77%	-68%
Crude oil consumption	-82%	-94%

* All values rounded to two significant figures
* Negative values indicate percentage reduction in impacts from the conventional acrylic acid base case

Table 3: Reductions compared to conventional acrylic acid production for a 100-million pound per year bio-acrylic acid production facility.

Parameter	Glucose	Syngas
Greenhouse gas emissions(CO_2e)	80,000 tons	71,000 tons
Crude oil consumption	31,000 tons	35,000 tons

If the world's entire 9-billion pound acrylic market used OPXBIO's process, it would reduce greenhouse gas emissions by more than 5 million tons per year, save more than 2.5 million tons of oil per year. As mentioned, 3-HP can be used to produce other industrial chemicals such as acrylamide (a 2.5 billion pound market) and while a specific LCA has not been completed, OPXBIO expects similar percent reductions in greenhouse gas emissions and oil use. Aside from the reductions in greenhouse gas emissions, OPXBIO's process produces the targeted chemicals directly and will produce very minimal hazardous by-products. Additionally, the water and ammonia produced during the process will be recycled.

As the worldwide demand for fuels and chemicals increases, producing them from renewable feedstocks by fermentation becomes a more critical means of supplementing or even replacing traditionally petroleum-based products – but the production must make sense financially for this trend to truly take hold. OPXBIO's process, and the major milestones it met in 2011, is a major step in that direction.

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