Bio-Based Adipic Acid for Renewable Nylon and Polyurethane Resins

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Sponsored by Verdezyne, Inc.

Contact:
Stephen Picataggio, Ph.D.
Chief Scientific Officer
Verdezyne, Inc.
2715 Loker Ave
Carlsbad, CA 92010

Phone: 760-707-5246
spicataggio@verdezyne.com

Project Title: Biobased adipic acid for renewable nylon and polyurethane resins

Milestone (November 2011): Verdezyne, Inc. has developed a yeast-based fermentation process to produce bio-based adipic acid, a key component of renewable nylon 6/6 and polyurethane resins, from natural oils and announced the opening of a pilot plant located in Carlsbad, CA to accelerate its commercialization.

Eligibility: This nominated technology is eligible for the small business award

Focus Area: The use of greener synthetic pathways

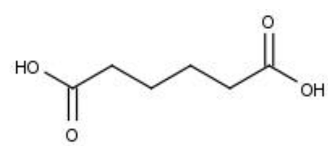
Eligibility: All elements of the nominated technology have been developed in the United States

Abstract: Adipic acid ($C_6H_{10}O_4$) is an important industrial dicarboxylic acid used to make Nylon 6/6 and polyurethane resins, with an estimated global market of $6.5 billion. It is currently produced from petrochemical sources by nitric acid-catalyzed oxidation of cyclohexane that generates, nitrous oxide, non-methane volatile organic compounds, carbon monoxide and nitrogen oxides in the waste gas stream. The production of adipic acid from renewable resources would allow the production of completely bio-based nylon and polyurethanes and result in substantial reduction of environmental pollutants. To this end, we have engineered an industrial strain of the yeast *Candida* to produce adipic acid from natural plant-based oils. This yeast normally grows on fatty acids as the sole carbon source via cyclic degradation through its β-oxidation pathway, and a strain in which this pathway has been completely blocked can convert these substrates to the corresponding dicarboxylic acids via selective oxidation of terminal methyl-groups through its ω-oxidation pathway, producing diacids with a chain-length distribution that precisely mimics the plant-based oil feedstock. We have engineered both the β-oxidation and ω-oxidation pathways to enable highly selective production of adipic acid from any natural plant-based oil, regardless of its fatty acid composition. In addition, Verdezyne has developed a fermentation and downstream purification processes to recover polymer-grade bio-adipic acid from the fermentation broth and has demonstrated its use for the synthesis of nylon 6/6 fibers and pellets. Verdezyne recently announced the opening of a pilot plant, located in Carlsbad, CA, to demonstrate the scalability of the process, validate our cost projections and generate sufficient quantities of bio-based adipic acid for commercial market development. Verdezyne's bio-based production technology offers compelling advantages over petroleum-based incumbent manufacturing processes including lower cost, sustainable feedstock supply and a smaller environmental footprint.

Technology Description

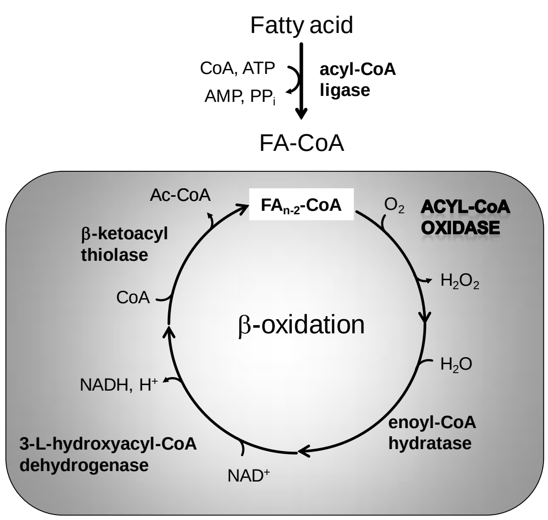
Verdezyne has developed a robust industrial yeast strain and fermentation process capable of producing bio-based adipic acid, a precursor for renewable nylon and thermoplastic polyurethane resins, at unsurpassed yield and selectivity from any plant-based oil, regardless of its fatty acid composition.

Recent advances in industrial biotechnology have made it possible to engineer microorganisms that can be deployed in fermentation-based processes to produce a variety of chemicals. Verdezyne is using proprietary methods in synthetic biology to develop and commercialize industrial yeast strains and fermentation processes for cost-effective production of renewable fuels and chemicals. We strongly favor yeast as the superior production host because it has a long history of industrial practice, is robust under industrial conditions; uses inexpensive feedstocks; produces multiple products; conducts fermentation at acidic-pH imparting resistance to bacterial contamination; and in contrast to bacterial platforms such as *E. coli*, is invulnerable to phage infection.



Adipic acid

We have engineered an industrial strain of the yeast *Candida* to produce adipic acid from natural plant-based oils. The parental strain has proven to be robust in commercial fermentations for production of long chain dicarboxylic acids and has many of the essential traits needed for effective and reliable performance at industrial scale - traits that would otherwise be difficult to engineer into the strain by any rational means. The strain tolerates saturating concentrations of adipic acid in the fermentation broth, growing at the same rate and to the same final cell density in its absence. The strain is diploid with two copies of every chromosome providing the genetic redundancy that underlies its stability and robustness at commercial scale. Verdezyne has sequenced its genome and established a versatile genetic toolbox that facilitates its rapid development for a commercial bioprocess.

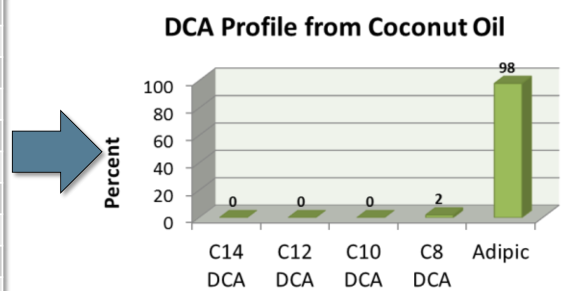


The parental strain was originally isolated from petroleum contaminated soil and can normally utilize alkanes and fatty acids as the sole carbon source for growth via cyclic degradation through its β-oxidation pathway. Each round of β-oxidation releases a molecule acetyl-CoA for respiration through the tricarboxylic acid cycle and shortens the fatty acyl-CoA substrate by two carbon atoms, concomitantly generating one molecule of $FADH_2$, NADH with each cycle. This process normally continues until the entire fatty acid chain is cleaved into acetyl-CoA units.

The first and rate-limiting step in the β-oxidation pathway is catalyzed by the enzyme, acyl-CoA oxidase. *Candida* has two acyl-CoA oxidase isozymes with different substrate specificities. We have engineered the selectivity of entry into the β-oxidation pathway so that fatty acids are degraded sequentially only to adipic acid, which is then excreted directly into the fermentation broth. The cells cannot reutilize the adipic acid produced as a substrate for growth. This novel process allows the use of inexpensive mixed chain-length natural oil feedstocks, including the soapstock and fatty acid distillate waste streams from vegetable oil processing.

The fermentation is a two-stage fed-batch process. In the first stage, the biomass is grown to high cell density on renewable sugars, after which the natural oil is continually fed into the fermentor for selective conversion to adipic acid, which is subsequently recovered and purified from the fermentation broth by proprietary methods. Verdezyne's pathway has a maximum theoretical yield which is unsurpassed among other considered bio-based adipic acid pathways. The maximum theoretical yield of the conversion is dependent upon the fatty acid chain length distribution of the natural oil feedstock. The example presented here illustrates the conversion of the mixture of fatty acids in coconut oil exclusively to adipic acid, with a maximum theoretical yield of 0.60 g/g (or 0.69 g/g for coconut oil soapstock).

Coconut Oil	
Formula	Percent
C6:0	0.5
C8:0	7.8
C10:0	6.7
C12:0	47.5
C14:0	18.1
C16:0	8.8
C18:0	2.6
C20:0	0.1
C18:1	6.2
C18:2	1.6



Adipic acid represents over 98% of the dicarboxylic acids produced and further purification through downstream processing produces polymer-grade bio-adipic acid. We have demonstrated the synthesis of nylon 6/6 fibers and pellets from bio-based adipic acid.

Further modifications to the omega-oxidation pathway have improved the yield, titer and productivity of the process so that it is now cost competitive, and with some feedstocks even cost-advantaged, compared to the incumbent petrochemical process. Verdezyne has applied for patent protection of the genes, yeast strains and fermentation process to produce adipic acid (see PCT US2010040837).

Verdezyne recently announced the opening and operation of its pilot plant, located in Carlsbad, CA, to demonstrate the scalability of the process, validate our cost projections and generate sufficient quantities of bio-based adipic acid for commercial market development.

Verdezyne's Pilot Plant in Carlsbad, California, where bio-based adipic acid is manufactured.

Market Opportunity

The global chemicals industry has over $375 billion in sales. Most of these are derived from petroleum-based feedstocks and manufactured using harsh chemical processes. The volatility of oil prices have eroded profit margins and along with environmental concerns have compelled chemical manufacturers to seek alternative sustainable ways to make these chemicals.

Verdezyne has developed yeast that use natural oil feedstocks for the production of a variety dicarboxylic acids that are the precursors for different kinds of nylon and identical drop-in replacements to their legacy counterparts, including:

- Adipic acid ($C_6H_{10}O_4$) for nylon 6/6, with a market value of $6.5 billion
- Dodecanedioic acid ($C_{12}H_{14}O_4$) for nylon 6/12, with a market value of $250 million
- Sebacic acid ($C10H_{12}O_4$) for nylon 6/10 with value of approximately $600 million

In 2008, the global adipic acid market was estimated at 5.6 billion pounds per year and is projected to reach 6.3 billion pounds by 2012. The US is the world's largest consumer at 2.4 billion pounds in 2008. Adipic acid's largest end-use segment, Nylon 6/6 is expected to consume nearly 4.3 billion pounds with a market value of approximately $6.5 billion, growing annually at 4% to 6%. Polyurethane resins offer the highest growth opportunity, with a CAGR of more than 4.5%. Global demand forecasts show growth of 6% per year to 2010. Increasing prices of energy and raw materials are hampering profits despite the growing demand in the adipic acid industry. Interviews with numerous end users and producers indicate high market awareness for bio-based adipic acid and strong market pull providing the product becomes available at equivalent cost.

Potential Benefits

Adipic acid is a high-volume chemical building block used to make many common polymers, including nylon 6/6 and thermoplastic polyurethane resins with a global market over $6 billion. Using sophisticated genetic engineering techniques, Verdezyne has developed a robust industrial yeast strain and fermentation process that produces bio-based adipic acid at high yield and selectivity from renewable fats and oils, regardless of their fatty acid composition. This feedstock flexibility enables production anywhere in the world. When produced at commercial scale, Verdezyne's bio-based adipic acid will be less expensive than the petrochemical incumbent and is expected to have a reduced environmental footprint than the current process.

The incumbent petrochemical process for commercial production of adipic acid involves hydrogenation of benzene to cyclohexane which is then oxidized with air to a mixture of cyclohexanol and cyclohexanone. This mixture is then oxidized with nitric acid to adipic acid, generating nitrous oxide, and other non-methane volatile organic compounds, carbon monoxide and nitrogen oxides in the waste gas stream that do not have a direct global warming effect but indirectly affect terrestrial and/or solar radiation absorption by influencing the formation or destruction of greenhouse gases, including tropospheric and stratospheric ozone. Despite significant abatement and controls, adipic acid manufacture in the US still produces approximately 1.9 $TgCO_2$ Eq (EPA 430-S-11-001, April 2011) with a Greenhouse Warming Potential approximately 300 times greater than CO_2. The BREW report estimates a potential cradle-to-gate NREU savings of 30%-60% from a successful bioprocess based on corn starch. Although we have yet to conduct a detailed life cycle analysis from natural plant-based oils, our preliminary estimates (based on The BREW report and adjusted for Verdezyne fermentation assumptions) suggest our bioprocess will reduce GHG emissions by approximately 2.2 tons of CO_2 equivalents per ton bio-adipic acid produced from corn starch.

Verdezyne's pathway has a maximum theoretical yield ranging from 0.47-0.69 g adipic acid/g consumed fatty acid, depending on the fatty acid content and composition of the natural oil feedstock, and is unsurpassed among other considered bio-based adipic acid pathways. A bioprocess developed by Draths Corporation based on conversion of glucose to *cis-cis* muconic acid by recombinant *E. coli*, followed by catalytic hydrogenation to adipic acid has a maximum theoretical yield of 0.41 g *cis-cis* muconic acid /g consumed glucose, with the with the best published results corresponding to a yield of 0.17 g *cis-cis* muconic acid /g consumed glucose. Rennovia Corporation is developing a high-temperature/high-pressure thermo-chemical route for conversion of glucose to adipic acid, but conversion yields have yet to be published.

Whereas other bio-based chemical processes are based on conversion of renewable biomass sugars, Verdezyne's bio-adipic acid process in uniquely based on renewable natural oils. World production of natural oils is projected to reach 132MM tons by 2012, approximately 14% of which has been used historically for chemicals production.

Verdezyne estimates that its production costs will be 30% to 35% lower than the incumbent process. The Company is moving the first of these chemicals, bio-based adipic acid, into pilot-scale production and is negotiating both manufacturing and off-take agreements with chemical and companies and end-users to accelerate its commercialization.

Company Description

Verdezyne is an industrial biotechnology company located in Carlsbad, CA, developing commercial yeast strains and fermentation processes for cost-effective production of renewable fuels and chemicals. The Company's bio-based production technology offers compelling advantages over incumbent manufacturing processes including lower cost, more reliable feedstock supply and a smaller environmental footprint. Verdezyne's initial chemical targets are a trio of diacids that are the precursors for different kinds of nylon, addressing a combined market of over $7 billion. The Company is beginning pilot-scale production of these chemicals which it will commercialize through partnerships with leading chemical manufacturers. Verdezyne has also engineered yeast for both first and second-generation cellulosic ethanol that are being commercialized by established players in the value chain. The Company has a management team with a successful track record in high growth companies and strong protection for its intellectual property portfolio through patents and trade secrets.

Verdezyne currently employs 46 people and had revenues in 2011 of $3 million. Current investors in Verdezyne include BP Alternative Energy Ventures, DSM Venturing B.V., OVP Venture Partners and Monitor Ventures.