Sustainable production of industrial chemicals from sugars

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abstract

The use of sugar as a raw material for sustainable production of existing industrial chemicals offers great potential for cane- and beet-sugar producers to capture added value while helping to transform the chemical industry to a renewable feedstock base. Large-scale global manufacture of existing industrial chemicals requires approximately 3 billion barrels/year of oil and its equivalents as the primary source of raw materials, heat, and power. In addition to being energy intensive, the chemical industry contributed significantly to the nearly 30 billion tonnes of anthropogenic carbon dioxide emitted globally into the atmosphere in 2008.[1] This article highlights the important benefits of sustainable chemicals manufacturing and provides an overview of Genomatica's sucrose-based process for the manufacture of 1,4-butanediol, an existing 1.3 million tonne/year petrochemical. Commercial production of 1,4-butanediol from sugar using an engineered microorganism will require much less energy, will release significantly less carbon dioxide, and is expected to be substantially cost advantaged relative to current petro-chemical processes.

Keywords: **1,4-butanediol, bio-based chemicals, renewable feedstocks, sugar, sustainability**

Nachhaltige Produktion von Industriechemikalien aus Zucker

Die Nutzung von Zucker als Rohstoff für eine nachhaltige Produktion existierender Industriechemikalien bietet Rohr- und Rüberzuckerproduzenten gute Möglichkeiten zur zusätzlichen Wertschöpfung und liefert einen Beitrag dazu, die chemische Industrie in eine Basis von erneuerbaren Ausgangsstoffen zu verwandeln. Die globale Massenproduktion von existierenden Industriechemikalien erfordert jährlich zirka 3 Mrd. Barrel Öl und äquivalente Stoffe als Primärquelle von Rohmaterial, Wärme und Antriebskraft. Die chemische Industrie ist dadurch nicht nur energieintensiv, sondern hat 2008 weltweit auch nahezu 30 Tonnen anthropogenes Kohlendioxid in die Atmosphäre entlassen.[1] Dieser Artikel beleuchtet die wichtigen Vorteile der Herstellung von nachhaltigen Chemikalien und liefert einen Überblick über Genomaticas Saccharose-basiertes Verfahren zur Herstellung von 1,4-Butanediol, einer existierenden Petrochemikalie von 1,3 Mio. Tonnen/Jahr. Die kommerzielle Produktion von 1,4-Butanediol aus Zucker unter Einsatz eines genetisch veränderten Mikroorganismus wird viel weniger Energie erfordern, erheblich weniger Kohlendioxid freisetzen und voraussichtlich einen beträchtlichen Kostenvorteil gegenüber den derzeitigen petrochemischen Verfahren bieten.

Obtención sustentable de productos químicos industriales a partir de azúcares

El uso de azúcar como materia prima para la producción sustentable de productos químicos industriales existentes ofrece un gran potencial para los productores de azúcar de caña y de remolacha para obtener valor agregado en tanto se ayuda a la transformación de la industria química a una base de insumos renovables. La manufactura global en gran escala de las industrias químicas existentes requiere aproximadamente 3 mil millones de barriles/año de petróleo y sus equivalentes como fuente primaria de materias primas, calor y energía. Además de ser energía-intensiva, la industria química contribuye significativamente a las cerca de 30 mil millones de toneladas de dióxido de carbono antropogénico que se emitieron globalmente a la atmósfera en 2008.[1] Este artículo destaca los importantes beneficios de una producción sustentable de sustancias químicas y provee un panorama del proceso basado en sacarosa de Geomática para la manufactura de 1,4-butanodiol un petroquímico existente con 1,3 millones de toneladas/año. La producción comercial de 1,4-butanodiol a partir de azúcar, utilizando microorganismos transformados, requerirá mucha menos energía, liberará significativamente menos dióxido de carbono y se espera que tenga ventajas sustanciales de costos cuando se c ompara con los procesos petroquímicos.

Produção sustentável de produto químicos industriais a partir de açúcares

O uso de açúcar como matéria-prima para a produção sustentável de produtos químicos industriais existentes oferece aos produtores de açúcar de cana e de açúcar de beterraba enorme valor acrescentado potencial, ao mesmo tempo que ajuda a transformar a indústria química numa indústria à base de matérias-primas renováveis. A produção mundial a grande escala de produtos químicos existentes requer aproximadamente 3 mil milhões de barris/ano de petróleo e seus equivalentes como fonte principal de matérias-primas, calor e energia. A indústria química, além de consumir muita energia, contribuiu significativamente para a emissão global de cerca de 30 milhares de milhões de toneladas de dióxido de carbono antropogénico para a atmosfera em 2008.[1] Este artigo destaca as vantagens importantes da produção de produtos químicos sustentáveis e oferece uma visão geral do processo da Genomatica à base de sucrose para a produção de 1,4-butanodiol, um derivado químico do petróleo cuja produção anual é de 1,3 milhões de toneladas. A produção comercial de 1,4-butanodiol a partir de açúcar usando um microrganismo concebido para o efeito requer muito menos energia, liberta uma quantidade de dióxido de carbono significativamente menor e prevê-se ter uma vantagem económica substancial em relação aos processos petroquímicos actuais.

Introduction

Over 80 million tonnes of industrial chemicals are manufactured globally each year from petroleum-based feedstocks.[2] These petrochemicals, which encompass building blocks, intermediate chemicals, and derived polymers, are valued at over $2 trillion and provide the materials and products that impact and enable virtually every aspect of our daily existence. From fertilizers for food and engineered plastics that make cars lighter and safer to disposable diapers and treatments that make wood last longer, modern chemicals clearly improve our overall standard of living. However, these great benefits historically have come at great cost.

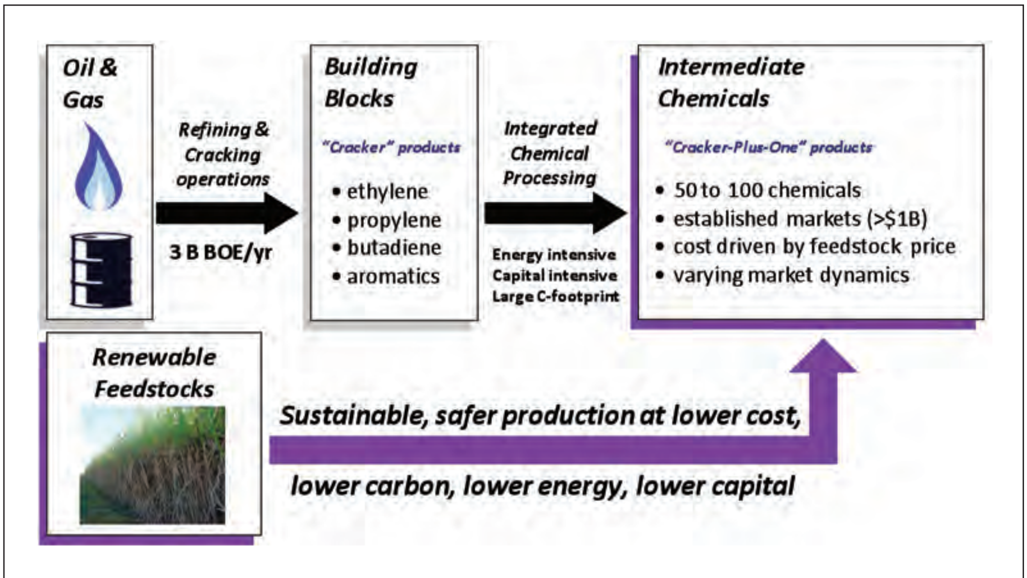
Petrochemicals are derived from crude oil, naphtha, and natural gas, which are refined into fuels and numerous base chemical building blocks, such as ethylene, propylene, butadiene, and aromatic compounds such as benzene, toluene, and xylenes. In addition to serving as the primary raw materials for major polymers such as polyethylene and polypropylene, these building blocks are converted into over 100 large volume intermediate chemicals that provide the basis for the entire chemical industry (Figure 1). While the chemicals themselves play a positive role in society, the petroleum-based processes used to manufacture chemicals engender challenges that can jeopardize the economy, the environment, and overall global security.

Petrochemical production requires about 3 billion barrel-of-oil-equivalents (BOE) per year of non-renewable fossil fuels, which are being depleted and are subject to substantial price volatility and supply vulnerability. In addition, petrochemical production processes generally are capital- and energy-intensive, produce chemical waste and pollution, and emit substantial amounts of greenhouse gases that are considered a major contributor to global climate change. To address this foreboding situation, new technologies based upon renewable feedstocks are being developed and represent a major paradigm shift toward more sustainable manufacturing with dramatically reduced cost, energy, and carbon footprint.

Sustainability, sustainable chemicals, and sugar

Sustainable is a term that is heard more frequently to describe new processes and products, but this term is rarely defined and it is often misused. On a macroscopic scale, sustainability is the capacity to endure and involves maximizing the benefits and minimizing the detriments of human activity.[3] Sustainable development and advancement of human societies will depend upon provision of low cost and readily available energy, nutritious food, clean water, and useful materials, while maintaining a healthy, natural environment. In general, sustainability requires both social and environmental responsibility combined with economic robustness integrated over time. The element of time is implicit in the term sustainable and efforts to develop sustainable new processes and products are intended to introduce lasting transformational changes. A list of more specific attributes associated with sustainable manufacturing processes and products is shown in Figure 2.

Figure 1. Production of industrial chemicals from fossil fuels versus renewable feedstocks



Decades of chemical research and development have resulted in refined consumer products and highly optimized chemical manufacturing processes. However, three recent factors have put the traditional chemical industry under unique pressure:

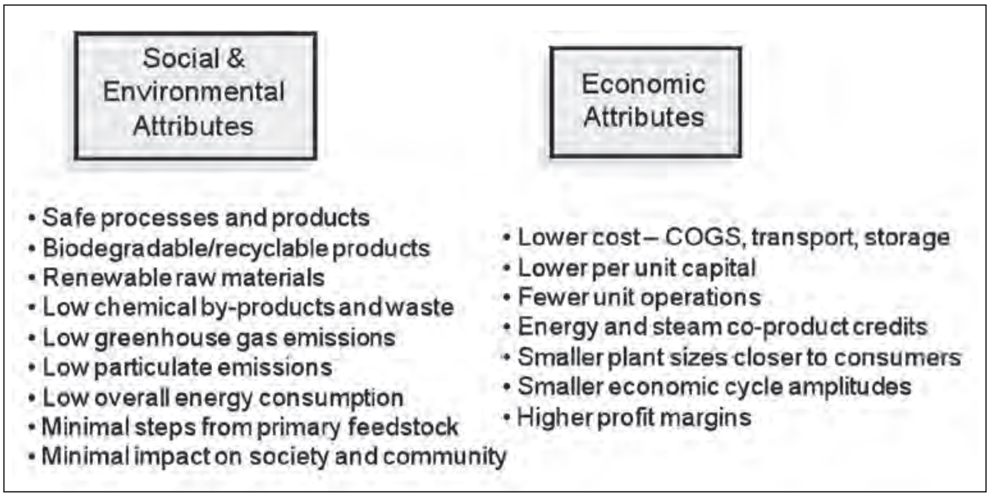
1. dropping consumer demand,

2. high and fluctuating oil and gas prices; and

3. environmental concerns associated with energy use, waste, and greenhouse gas emissions.

According to a recent survey conducted by Genomatica and ICIS, 57% of chemical industry respondents believe their companies should reduce exposure

Figure 2. Key attributes for sustainable manufacturing processes and products



to the petroleum-based commodity markets.[4] Of interest for sugar growers and refiners, 46% believe there is an economic advantage to switching chemical manufacturing process inputs to renewable feedstocks, like sugars, starches or biomass, and 24% already incorporate sugar or other carbohydrates into at least some of their processes. Chemical manufacturing based upon sugar would allow producers to lock in their supply costs on a more stable global market - the sugar market - which would ease pressure to raise chemical prices. Not surprisingly, however, the pricing and availability of sugar (or other renewable feedstock) are the two dominant concerns held by chemical executives about sustainable chemicals.

Sustainable chemicals must be designed from the outset to employ efficient, effective, safe and more environmentally responsible raw materials and processes. In addition, sustainable chemicals must minimize energy consumption, emissions and overall economics. Finally, sustainable manufacturing processes typically will require the fewest number of conversion steps from a primary feedstock, which ideally is renewable. Sustainable chemicals clearly represent a major opportunity for sugar growers and marketers, who can play a vital role in transforming the chemical industry feedstock base from petroleum to renewable carbohydrates.

Role of biotechnology in sustainable chemicals

The chemical industry is increasingly promoting sustainable practices such as lowering energy usage and recycling of raw materials and by-products. However, the chemical industry is still dependent upon oil, naphtha, and natural gas as their primary feedstocks. Moreover, most petrochemical processes have been optimized as much as possible, and a major shift in feedstock utilization and manufacturing designs will be required to lower energy, emissions, and cost below current levels. Biotechnology offers a new approach for the production of industrial chemicals from renewable carbohydrates such as glucose, sucrose, and xylose derived from plants. Microorganisms are well known to naturally produce a range of commercially important chemicals such as alcohols (ethanol, butanol), organic acids (acetate, butyrate, citrate, lactate), amino acids (lysine, phenylalanine, tyrosine), drugs (penicillin, tetracycline) and even polymers (polyhydroxy-butyrates).[5] Unfortunately, many existing large volume industrial chemicals are produced only at very low levels or are not produced at all naturally by micro-organisms. However, recent advances in biotechnology now are paving the way to a new sustainable chemicals industry.

Modern biotechnology involves the integrated use of many different biological methods to manipulate living cells. A microorganism consists of basic building blocks such as nucleotides that make up DNA and RNA, fatty acids that make up lipids, and amino acids that are the main constituents of proteins. Important chemical reactions take place inside of cells to interconvert materials and provide the cell with energy (e.g., ATP), redox co-factors (e.g., NADH or NADPH) and metabolic intermediates required to build the cell components. Each of these metabolic reactions inside the cell is catalyzed by an enzyme, and the cell produces these enzymes by linking together amino acids in a sequence dictated by a short segment of DNA referred to as a gene. Biotechnology tools have been developed to rapidly determine the DNA sequence of individual genes as well as entire genomes of microorganisms. Using this genetic blueprint and other biochemical information, models of metabolism for any organism can be created and these models can help guide efforts to engineer the organism for production of many products of interest, such as chemicals. This is referred to as the discipline of metabolic engineering.[6]

Metabolic engineering is now being used to design and improve the performance of microorganisms for the fermentative production of an increasing number of high value chemical products. For example, DuPont has engineered the bacterium *Escherichia coli* to produce the valuable intermediate, 1,3-propanediol (PDO), which is a building block for their branded fiber Serona™.[7] DuPont teamed up with corn glucose provider Tate & Lyle to build a 45,000 tonne/year capacity facility that has been in operation since 2006. Other notable developments in this area include Telles, a joint venture formed between Metabolix and ADM, which is constructing a plant for the annual production of 50,000 tonnes of biodegradable polyhydroxybutyrate polymers from glucose using *E. coli.*[8] Similarly, Cargill is now producing polylactic acid, another biodegradable polymer, from glucose at their 135,000 tonne capacity facility in Blair, Nebraska.[9] Recently, Genencor announced that they were commercializing a process with Goodyear for the production of isoprene from glucose, again using *E. coli*. It is interesting to note that, to date, none of these commercial fermentation processes employ sucrose as the feedstock. Clearly the use of readily available sucrose for the production of high value chemicals has exciting prospects for the future.

Bio-based chemicals versus biofuels

Currently, the two main uses of sucrose are as a food product (refined sugar) and for ethanol production. In Brazil, approximately 55% of sucrose derived from sugarcane is converted by fermentation to ethanol, which is used as a renewable transportation fuel. Biofuels, such as sucrose-derived ethanol, are of great interest to countries seeking to reduce dependence on imported oil, attenuate fuel price fluctuations, and minimize greenhouse gas emissions. However, fuels are very large-volume, low value, single-use commodity products. Once fuels are combusted, they are converted to products (e.g., CO, CO_2, and H_2O) that are emitted into the atmosphere. Accordingly, fuels typically are sold at low prices and typically garner low unit profits for producers. A biofuel like ethanol has a low energy density, so the selling price is even lower on an energy equivalent basis relative to an equal volume of gasoline. Advanced biofuels that look more like the hydrocarbons in gasoline and diesel are being developed and have higher energy density, although substantially more sugar is required to manufacture these products fermentatively. For example, 1 tonne of sugar can provide a maximum yield of 680 litres ethanol, but yields at most 547 litres butanol, and as little as 450 litres of longer chain alcohols or hydrocarbons.[10] Although fuel volumes are large, product value per kg sugar will remain invariably low when biofuels are being produced, and more advanced biofuels will not address this challenge. In addition, biofuel manufacturers are likely to face constant price pressures from competing petroleum producers, and so sugar producers serving the biofuel sector may see profits pushed even lower.

Finally, ethanol is one of the few fermentatively-derived biofuels that have been ASTM approved for blending with gasoline for use as a transportation fuel. It is unclear what other biofuels will gain regulatory approval, making biofuels a speculative enterprise. Fortunately, there is a lower risk, higher value opportunity for sugar that is emerging - **sustainable production of existing industrial chemicals.**

Industrial chemicals are the source of many different materials that are not transiently used like fuels, but rather provide substantial value over time. Hence, chemicals are sold in large established markets at higher prices and higher profits relative to fuels. For example, a typical profit for US ethanol is in the range of $0.05-0.10 per liter, while many chemicals can achieve over 5 times that profit for the same amount of material produced. The projected cost advantage and higher profits for chemicals production from sugar are based upon the ability to convert the primary feedstock (sucrose) in a single step (fermentation) directly into the chemical of interest with significantly lower capital and lower raw material costs relative to petrochemicals. It is estimated that over 30 different intermediate chemicals could be manufactured sustainably and economically from inexpensive sugar in the future. For example, Genomatica recently has developed a new bio-process for the manufacture of the intermediate chemical 1,4-butanediol (BDO). Below we describe this program and provide a vision indicating that sugar will play a major role in redefining the chemical industry while creating new revenue streams and helping to improve the overall competitiveness of the sugar industry.

Genomatica's 1,4-butanediol process

Genomatica's overall mission is to sustainably manufacture industrial chemicals from low-cost renewable feedstocks using engineered microorganisms that serve as miniaturized chemical factories. Genomatica's strategy is built upon an integrated technology platform that combines computational modelling and design with lab-based experimental engineering.[11] Proprietary metabolic models and simulation algorithms are used to test all possible ways that a targeted chemical could be produced inside the cell of a microorganism. The optimal paths to create the chemical inside the cell from sugar are then selected on the basis of criteria such as yield and the number of known enzymatic steps. These pathways and strain designs are then implemented in the lab to afford a microorganism and fermentation process for the target chemical production. The close working relationship between the lab and the computational modelling operation is key to refining the process as experimentation takes place. Downstream process development then leads to optimum separation and purification designs for a fully integrated cost-advantaged, energy-efficient, clean, safe, renewable bio-manufacturing process for sustainable chemicals.

Genomatica has applied this approach to develop a breakthrough bio-manufacturing process that will enable production of hundreds of thousands of tons of the industrial chemical 1,4-butanediol (BDO) solely from renewable materials - sugar and water.[12] Approximately 1.3 million tons of BDO is manufactured globally each year for use in the production of solvents, fine chemicals and high-performance polymers such as spandex fibers for textiles and polybutylene terephthalate (PBT) for engineered plastics used in the

Figure 3. A BDO pathway constructed and engineered into *E. coli*. Each arrow represents enzymatic conversions. Glycolysis and the TCA cycle are multiple-enzyme metabolic processes

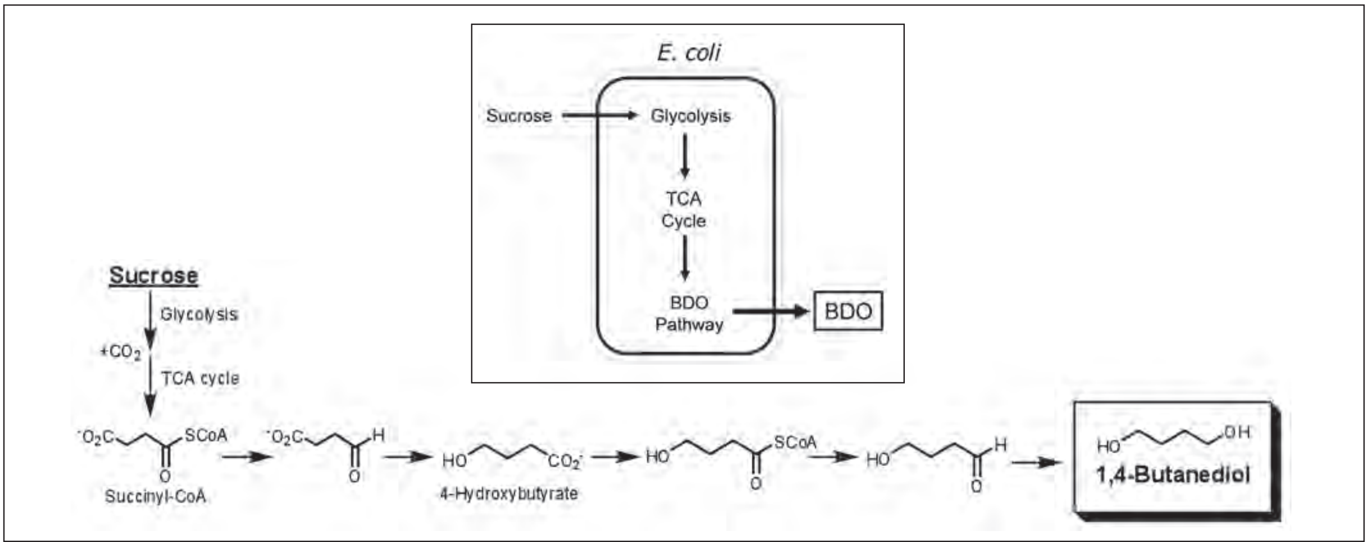
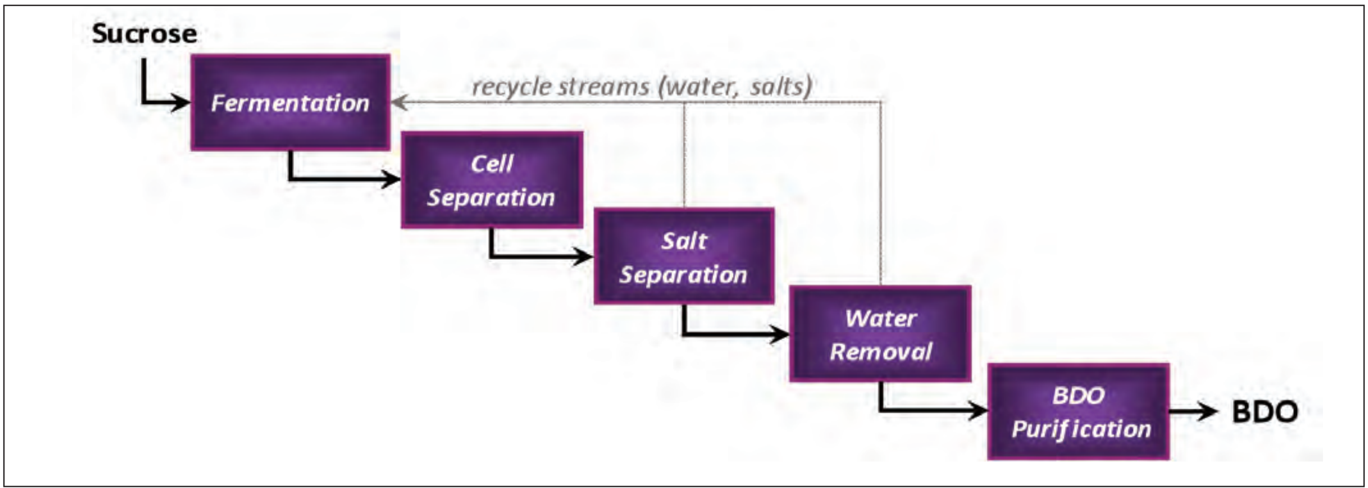


Figure 4. Basic block flow diagram for bio-based BDO process



automotive and electronics industries. All current BDO production involves the use of fossil fuel feedstocks such as acetylene, butane and propylene and requires three to four additional energy-consuming transformations to convert these materials into BDO. The ability to manufacture BDO from sugar will contribute to the creation of a more sustainable and environmentally responsible chemicals industry.

Constructing the BDO pathway

Genomatica has engineered a microorganism successfully for the first time to produce BDO directly from glucose or sucrose in a single step. Genomatica's process takes advantage of the selectivity and efficiency of biological systems. When sugar is fed to organisms such as the commonly used and safe bacterium, *E. coli*, it is naturally transformed to a series of metabolites through glycolysis and the tricarboxylic acid cycle (TCA cycle). Since no organism previously has been found or engineered to produce BDO from sugars, enzymes had to be found and introduced using biotechnology tools to create a pathway that converts natural metabolites, such as succinyl-CoA, into BDO.

Preferred pathways were defined using our computational platform and candidate enzymes for each individual step were identified by screening numerous variants for the desired activity. One BDO pathway is shown in Figure 3. Each of the required enzymes was introduced into *E. coli* in the proper order on plasmids to provide the activities needed to convert glucose and sucrose into 4-hydroxybutyrate (4-HB) and subsequently to BDO. Note that in addition to carbon from sugar, one carbon of BDO derives directly from CO_2. The first BDO pathways were thus constructed, although the amount of BDO produced was still rather low. Strain engineering was required to coax the organism into producing more BDO.

Optimizing BDO production

The strain had to be engineered to optimize the yield and rate of BDO production, while minimizing by-products. Here again, we relied on our computational platform, which allowed us to determine which enzymes should be added or deleted from *E. coli* to increase BDO and eliminate the formation of undesirable products such as acetate, pyruvate, and ethanol. This process involves an iterative approach that uses the models to guide our experiments and interpret data, followed by using the experimental results to improve the model and provide further experimental guidance. Through this unique strategy, we were able to increase BDO production over 20,000 fold in just 18 months.

Developing the bio-based BDO process

Development and optimization of the sugar-to-BDO manufacturing process is ongoing. One important factor that can impact the overall process viability is product tolerance. Our economic models indicated that cost-advantaged commercial production of BDO will require *E. coli* to produce and tolerate BDO concentrations of 100 g/L (10 % BDO in water) or higher. Since *E. coli* naturally can tolerate only 5- to 6-% BDO concentrations, Genomatica scientists leveraged adaptive evolution technology that allows organisms to gradually acclimatize and adapt to different environments over a period of months. In order to carry this out effectively, Genomatica developed proprietary machines that automate the ability to maintain organisms in changing conditions (e.g., increasing BDO concentrations) over extended periods. Using these machines, the tolerance of *E. coli* was increased to BDO concentrations above 10 percent, thus overcoming a potential major hurdle to commercialization.

A process for recovery of BDO from the fermentation broth has been developed and involves the four basic steps of cell separation, salt separation, water removal, and distillation (Figure 4). Each of these unit operations has been demonstrated at lab scale and BDO of >99% purity has been produced by processing 30 L fermentation broths.

Genomatica currently is designing a demonstration plant that will allow validation of the fully integrated bio-based BDO process at larger scale. These demonstration studies will lead to the complete engineering package that will serve as a basis for the construction of commercial scale bio-BDO plants. Achieving our production targets using sugar is expected to provide a significant cost advantage relative to petroleum-based BDO. In addition, preliminary life cycle analysis indicates that producing BDO from sugarcane-derived sucrose is expected to provide substantial sustainability benefits by lowering fossil energy usage by at least 86% and reducing carbon dioxide emissions by at least 117%, assuming that bagasse is burned to power the facility.[13] So overall, this process will not just lower carbon dioxide emissions relative to petrochemical processes, but it will actually remove CO_2 from the atmosphere.

It is important to note that the bio-based BDO process is able to switch between glucose and sucrose, depending on relative prices and availability. We have engineered *E. coli* to use both glucose and sucrose equally well, which will allow chemical manufacturers to control their costs more effectively. Different feedstocks are also preferred in different geographies, based on local growing conditions and markets. For example, while glucose from corn may be favoured in the US, low-cost sugarcane-derived sucrose will be the preferred feedstock for BDO production in Brazil, and sucrose from sugar beets is available and would be preferred in the UK and other parts of Europe. Such feedstock flexibility should facilitate wide adoption of these new bio-BDO manufacturing processes.

Sugar as a sustainable primary feedstock

Our manufacturing processes use sugar as a renewable feedstock that we believe is both environmentally and economically sustainable. Sugar cane is easier and less costly to grow and yields significantly more carbohydrate per hectare when compared with corn. Importantly, sugar producers in locations like Brazil have ample capacity to increase production (less than 15% of arable land is currently farmed) without impacting environmentally sensitive areas like the Amazon region. This ability to expand sugarcane production should sustainably maintain low and stable sugar prices as demand increases.

The amount of sugar needed for chemicals production is small relative to the fuels market. For example, four world-scale BDO plants (45,000 tonnes/year BDO) representing 15% of the total BDO market will require only approximately 0.25% percent of currently available world sugar supply.[14] By contrast, almost 50% of global sugar is used for ethanol production. Because of these factors, we believe the robust sugar market can easily grow to include chemicals production without facing supply and demand disruption.

As indicated above, significantly higher profits can be expected for sugar-based production of chemicals, relative to biofuels like ethanol. Of the 420 mills currently operating in Brazil, approximately one-third of these are dedicated to the production of ethanol, while two-thirds produce both sugar and ethanol. Dedicated ethanol mills are particularly vulnerable to low and fluctuating ethanol and gasoline prices, as well as the credit markets and the overall global economy. Production of BDO and other high value existing chemicals with stable markets represents a robust opportunity for mills to diversify their product portfolio while reducing risk and increasing revenue and profits generated from sugar.

Conclusion

The sustainable production of industrial chemicals from sugar using fermentation is an emerging area of development that represents a lucrative new direction for sugarcane and sugar beet growers. Relative to fossil fuels, which are finite and experience price volatility, sugar is a low cost and abundant feedstock that should offer desirable price stability to the chemical industry. In addition, sugar is a renewable raw material derived from atmospheric carbon dioxide and hence its use greatly reduces greenhouse gas emissions and fossil energy usage. For example, Genomatica has developed a new sustainable process for the efficient production of 1,4-butanediol from sucrose. This process will enable low cost manufacture of a high value chemical with substantially reduced energy consumption and carbon dioxide emissions relative to petroleum-based routes. We believe that we are witnessing the beginning of a transformation in the chemical industry that will engender a plethora of similar new chemical production processes based upon sugar as the primary feedstock of choice in the future.

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