On-site enzyme production, as opposed to central enzyme manufacturing, can eliminate 30-50% of the total enzyme production costs

Next generation enzymes

The global population, which will surpass eight billion by 2030, is expected to consume over 30% more energy than today[1]. With oil reserves declining and greenhouse gas (GHG) emissions from fossil fuels increasing, the global community is looking for alternative energy sources such as biofuels, wind and solar.

First generation biofuels, derived from food sources like corn, starch, sugar, animal fats and vegetable oil, is already well-established and can create fuels that are readily used in today's petrol engines. There are mandates for blending biofuels in many countries, as the first generation biofuel processes are raising concerns about possible threat to the food supply, inefficiencies of farming food crops and cost competitiveness against fossil fuels.

Second generation biofuels offer great advantages over the current biofuel technology. Instead of competing with the food supply, it consumes agricultural waste or crops specifically grown for energy. These feedstocks are rich in the lignocellulose needed to make the cheap sugar used in the creation of cellulosic ethanol and bio-based chemicals.

With the building of three commercial plants in the US in 2014 by Agengoa, DuPont and Poet, and other plants under construction in Brazil and elsewhere, second generation biofuels have finally arrived after nearly a decade of development. Driven by second generation biofuels, the renewable fuels sector is expected to represent 5% of transportation energy consumption by 2030 (Figure 1).[2]

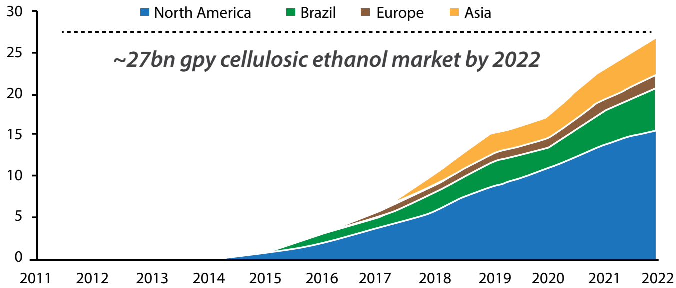

Figure 1: Global cellulosic ethanol market forecast[3]

Second generation biofuel production can be split into six different phases (Figure 2): feedstock collection, pretreatment, enzymatic hydrolysis, sugar fermentation, distillation and upgrading. The critical technology steps that differentiate various methods are generally considered to be the process that pretreats feedstock in preparation for enzymatic hydrolysis, the enzymatic hydrolysis that turns feedstock into fermentable sugars and the fermentation process that turns the sugars into biofuels and bio-based chemicals. While several companies such as DuPont and Abengoa are focused on the entire value chain, biotechnology companies like Dyadic, Amryis and Gevo are focused on the high-value steps in the process.

Initially, the cost of enzymes used in the hydrolysis step contributed nearly half the cost of second generation biofuels. However, in the past five years, enzyme producers such as Dyadic, Novozymes and DuPont have made progress to reduce the production cost of enzymes, reducing the enzyme cost per gallon from nearly \$2 (€1.50) in 2010 to as low as an estimated \$0.30 today.

In parallel, several

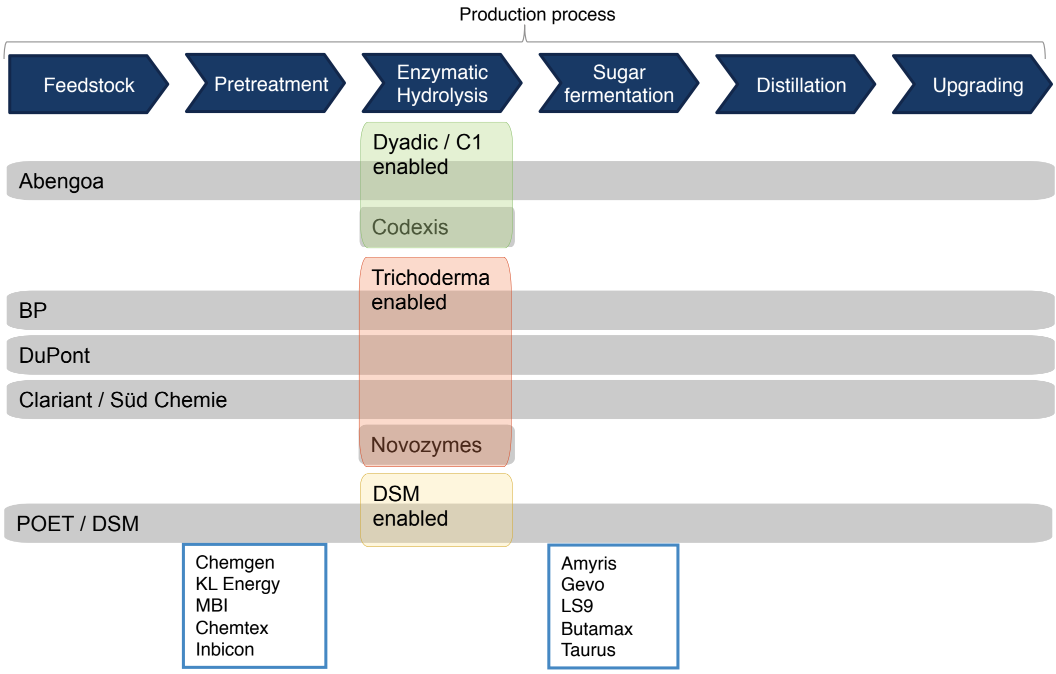

Figure 2: Second generation biofuel production process[4]

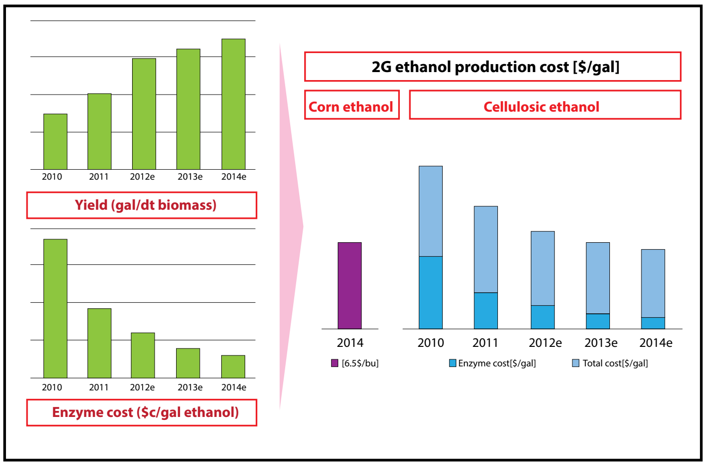

Figure 3: The reduction of second generation ethanol production over time[6]

ethanol producers such as Chemtex, Abengoa and Iogen have developed better, more cost effective pretreatment technologies for different feedstocks. As a result of those efforts, second generation cellulosic ethanol is on track to be cost competitive with corn-based ethanol by 2016[5]. The opportunity for technology companies at the forefront of this industry is enormous. If cost reductions and volume increases follow projections, the enzyme market alone could be $5 billion in a decade.

With respect to enzyme production, there are two approaches for second generation biofuel production: (1) a central enzyme manufacturing plant that distributes the enzymes to several ethanol production sites; and (2) an on-site manufacturing that couples the enzyme and the bioethanol production at one location.

While Novozymes remains committed to central enzyme manufacturing, other industry players such as Dyadic, DuPont and DSM have shifted towards the concept of on-site enzyme production. When produced on-site, biofuel producers can avoid downstream processing, adding stabilisation agents, warehousing and shipping of huge quantities of enzymes. A study conducted by Dyadic has shown that on-site production eliminates between 30 and 50% of the total enzyme production costs, significantly reduces inventory and streamlines the supply chain.

An additional advantage of on-site production is that it facilitates can produce enzyme mixes that are tailored to hydrolyse the specific feedstock used in each ethanol plant. The combination of various feedstocks including corn stover, wheat straw, sorghum, wood pulp and other energy crops and often very different pretreatment processes can demand a variety of unique enzyme mixes to operate in optimal conditions. On-site production of enzymes allows custom cocktails, but requires a flexible, robust production organism that is capable of producing such enzyme mixes.

Currently, three separate enzyme platforms compete in the market. Several companies share the technology platform based on Trichoderma reesei. Since it is a proven organism for the production of industrial enzymes, it is a reliable starting point for the production of cellulases and hemi-cellulases needed in the enzymatic hydrolysis for cellulosic biofuels.

Poet-DSM has a proprietary enzyme platform that is expected to be used in its new Project Liberty facility coming online in 2014. Currently, only Dyadic and its licensees Abengoa and Shell through Codexis has access to the C1 platform.

Of the three platforms, Dyadic's C1 system could hold the most promise for the industry. C1 is an integrated enzyme production platform that is based on Dyadic's proprietary fungus strain Myceliophthora thermophile. C1 produces a large variety of enzymes economically at very high yields that can hydrolyse a wide range of feedstocks, perform at higher temperature and broader pH ranges, and is proven at industrial scale in fermenters upwards of 150,000 litres in size.

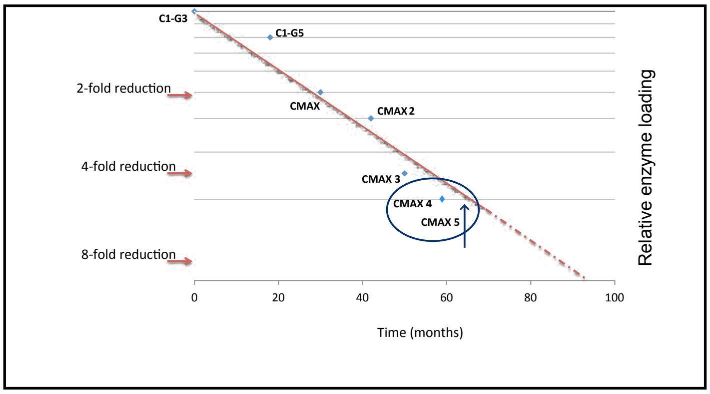

Figure 4: C1 enzyme mixtures have dropped in cost by over 5-fold in the past five years

The C1-based strains produce an enzyme mix in a single fermentation that can efficiently hydrolyse a wide variety of lignocellulosic feedstock. These strains are being continuously improved by overexpressing genes, increasing the level of key enzymes in the mix. As a result of these efforts, the efficiency of the enzyme mix has been improved and the amount of enzymes necessary to hydrolyse pretreated corn stover has reduced by a factor of more than five over the past five years (Figure 4).

Further, C1 is already proven to produce enzymes at large manufacturing scale, of up to 150,000 litres at relatively low cost. Abengoa, scheduled to open the first commercial second generation plant worldwide in Hugoton, Kansas this summer is using C1 technology for its process under a Dyadic licence. Abengoa has been able to decrease from $4.15 per gallon in 2010 to approximately $2 per gallon today, largely due to the effectiveness of its C1-based enzymes.

The next generation of enzymes developed at Dyadic have promise to decrease costs further. Focused on the on-site manufacturing approach, Dyadic's CMAX enzymes can be tailored towards different substrates and pretreatment technologies. C1's genome is over twice as rich as Trichoderma reesei, and can be genetically modified to produce specific homologous enzymes. Genes used within the genome, versus inserting genes from other organisms, are often more productive and meet non-GMO requirements in many countries.

Using C1-technology to turn biomass into cheap sugar, combined with an on-site manufacturing strategy, promises to make production of second generation biofuels and bio-based chemicals cost competitive in the near future. ●

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2 BP Energy Outlook 2030, page 31
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4 Source: Dyadic International, Inc.
5 Bloomberg New Energy Finance
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