ELSEVIER

Energy biotechnology: beyond the general lignocellulose-to-ethanol pathway

Editorial overview

Largus T Angenent

Current Opinion in Biotechnology 2007, **18**:191–192

Available online 25th May 2007

0958-1669/$ – see front matter

DOI 10.1016/j.copbio.2007.05.003

Largus T Angenent

Department of Energy, Environmental & Chemical Engineering, Washington University in St. Louis, St. Louis, MO 63130, USA
e-mail: angenent@seas.wustl.edu

Lars Angenent's research interest includes mixed culture bioprocessing of wastes to produce energy. Currently, the Angenent laboratory studies anaerobic digestion systems to produce methane and microbial fuel cells to generate electricity from wastewater. Molecular biology methods developed in the microbial ecology or environmental microbiology fields are used to ascertain the community composition and its function with the ultimate goal to engineer better bioprocesses.

Alternative energy generation through biotechnology is already carried out on a large scale. In the USA, for example, ~18 million m^3 of bioethanol were produced in 2006 from starch-rich corn kernels (mainly grown in the temperate climate of the Midwestern states) by using enzyme contact and/or steeping in dry- and wet-milling plants followed by yeast fermentation. This is similar to the volume that is produced by the Brazilian bioethanol industry from sugar cane. To really compete with nonrenewable energy and to make our society carbon neutral, however, energy biotechnology is now focusing on lignocellulosic feedstocks (e.g. corn stover, poplar wood, grasses) made of a tertiary structure of cellulose, hemicellulose, and lignin. In the USA, energy biotechnology has redirected its efforts mainly towards a general lignocellulose-to-ethanol pathway, which for the purpose of this editorial is summarized into a five-step pathway: (1) growing, harvesting, storing, and transporting agricultural crops; (2) pretreating lignocellulosic feedstock to open the cell-wall matrix and to remove lignin; (3) exposing the feedstock to a mixture of purified enzymes to hydrolyze hemicellulose and cellulose to five- and six-carbon sugars; (4) fermenting these sugars to ethanol; and (5) separating the produced ethanol. Nevertheless, improvements and innovations for all five steps must be made, as indicated by the fact that lignocellulosic ethanol production is still at the pilot stage. However, it is a mistake to focus all energy biotechnology efforts on just improving these five steps in the general lignocellulose-to-ethanol pathway. This issue of *Current Opinion in Biotechnology* reviews efforts outside the improvement of this conversion pathway. Other reviews published in 2007 are already available that specifically deal with the general conversion pathway [1,2].

Energy biotechnology uses carbon stored during photosynthesis from cyanobacteria, algae or plants. This carbon can then be converted to energy carriers, such as biodiesel or ethanol. For example, efforts are underway by microbiologists and bioprocess engineers to produce long-chain fatty acids in cyanobacteria with sunlight for further abiotic processing into biodiesel. Plant scientists are trying to improve lignocellulosic biomass yields, by manipulating plants via different breeding approaches or by genetic modification. The first review by Torney *et al.* focuses on the genetic modification of maize. Besides reviewing the literature in regard to improving yields, the authors also focus on other important concerns, such as increasing plant tolerance to stress (e.g. drought) and the alteration of stover composition (e.g. a lower lignin content). The latter is important to be able to reduce the rigorousness of the pretreatment step before lignocellulosic biofuel production.

An established alternative to the general lignocellulose-to-ethanol pathway is through biomass gasification to produce synthesis gas (syngas: H_2 and CO)

followed by, for example, the abiotic Fischer-Tropsch process to form organic compounds. This may be an economically more viable pathway for difficult-to-degrade lignocellulosic feedstock than the general pathway described above (circumventing steps 2, 3 and 4 in the general pathway). However, biotechnology could still play an important role, because bacterial processes have several advantages over the Fischer-Tropsch process to convert syngas into energy carriers. Thus, in their review, Henstra and Stams examine the use of thermophilic bacteria to produce pure hydrogen, acetate, butyrate, ethanol or butanol from hot syngas. Here, metabolic engineering might be required to produce other products. The advantage of thermophilic fermentation of syngas is the faster conversion rate compared with mesophilic fermentation (aside from not having to cool syngas as much).

Another deviation from the general lignocellulose-to-ethanol pathway might be advisable unless the economics associated with using purified enzymes improve further (step 3 in the general pathway). Currently, a mixture of purified hydrolytic enzymes is still too expensive and not as potent with real pretreated lignocellulosic feedstock as under ideal laboratory conditions. Nature's most efficient systems to biodegrade lignocellulose are mixed cultures in insect and mammalian guts that have evolved with the host. Besides biomining these mixed cultures for novel hydrolytic bacteria or enzymes, they can also be used to convert pretreated lignocellulosic feedstock into biofuels. Bioprocess engineers have experience with using mixed cultures to produce energy from organic material by operating large-scale anaerobic digesters that produce bioenergy (biogas, i.e. CH_4 and CO_2). In these anaerobic digesters hundreds to thousands of bacterial species (some with cell-associated cellulose-degrading enzymes) degrade complex molecules, such as cellulose, in a food web. These bioprocesses are already used to convert complex waste streams from, for example, fermentation vessels (after ethanol distillation) into methane gas to partly power the biorefinery. To produce liquid biofuels, however, the mixed culture must produce other products, such as butyrate, lactate, ethanol or polyhydroxyalkanoates. Kleerebezem and van Loosdrecht examine the literature on mixed culture biotechnology in their review. They conclude that clear selection criteria must be present to establish a stable mixed culture for production of a specific product.

For a less-complex co-product stream, such as glycerol in biodiesel production, a single-culture bioprocessing step is feasible to add value. Yazdani and Gonzalez, for example, are using *Escherichia coli* to convert glycerol into succinic acid or other products. In their review, they highlight that an economical biotechnology process is only possible when all co-products are converted to their maximum value, similar to what is currently done in oil refineries. This would result in several steps added to the general bioconversion pathway and would lead to the development of a true biorefinery concept.

Ezeji *et al.* point out in their article that butanol is a better liquid biofuel than ethanol. Thus, rather than using genetically engineered yeast cultures or bacterial strains to convert five- and six-carbon sugars from hemicellulose and cellulose to ethanol, already existing solventogenic clostridia strains can convert both these sugars to butanol. This biotechnology process was popular in the first half of the twentieth century employing rather easily degradable sugars as feedstocks. The authors propose to integrate butanol fermentation in the general lignocellulosic pathway (replacing step 4). The purified enzyme exposure (step 3) could possibly be circumvented in such a proposed pathway, because clostridia have the machinery to produce hydrolytic enzymes. The authors also focus on recombinant DNA technology with the goal to create a hyper-butanol producing strain. Such a strain would produce butanol at high rates, generate a high butanol/acetone ratio stream, be resistant to product inhibition, and possibly be able to use cellulose as a feedstock. Finally, novel approaches for separating butanol (step 5) are discussed in detail.

Even after bioethanol has been produced, biotechnology still has a role to play in converting this ethanol to a useful energy carrier (electric current) for consumer electronics. In the final review, Minteer *et al.* discuss the latest findings with enzymatic biofuel cells. Workers have reported high power densities with membrane-less biofuel cells in which enzymes with high specificities employ direct electron transfer with the anodic and cathodic electrodes. Both these enzyme-coated electrodes are then placed in a single fuel solution, such as ethanol, to produce an electric current. Breakthroughs are reported on the lifetime and environmental stability of the enzymes by immobilizing them on the electrode surface. The authors suggest that work is necessary to completely oxidize the fuel by developing multienzyme cascade systems. The use of biobatteries made without any metal, which can be discarded in an environmentally friendly way after the oxidation of ethanol is completed, could therefore be on the horizon.

References

1. Stephanopoulos G: **Challenges in engineering microbes for biofuels production**. *Science* 2007, **315**:801-804.

2. Himmel ME, Ding SY, Johnson DK, Adney WS, Nimlos MR, Brady JW, Foust TD: **Biomass recalcitrance: engineering plants and enzymes for biofuels production**. *Science* 2007, **315**:804-807.