Alternative routes to biofuels: Light-driven biofuel formation from CO$_2$ and water based on the ‘photanol’ approach

K.J. Hellingwerf*, M.J. Teixeira de Mattos

Molecular Microbial Physiology Group, Swammerdam Institute for Life Sciences and Netherlands Institute for Systems Biology, University of Amsterdam, Nieuwe Achtergracht 166, NL-1018 Amsterdam, The Netherlands

1. Introduction

Photosynthesis, be it in natural or in artificial form, is a key element in a sustainable, global, supply of energy. In this process, a reaction center, often assisted by light-harvesting antennae, converts the free energy of visible irradiation emitted by the sun into the redox free energy of electrons (Fig. 1). In artificial photosynthesis (photovoltaics) the redox energy is directly converted into electricity and therefore artificial photosynthesis is optimally suited for electricity supply, particularly because of the minimal number of free energy conversion steps involved.

For fuel supply, however, the electrons with their more negative redox potential, have to be stored stably in chemical compounds (like H$_2$, CH$_3$OH, etc.) that can be transported at low cost to the required site of combustion. In spite of a considerable amount of work to achieve this, artificial photosynthesis has not yet provided stable catalysts to convert the electrons, derived from water, into stable fuel products. Rather than chemical catalysts, one can also set up an in vitro system, using the molecular components of natural photosynthesis, i.e. Photosystems I and II (Slater, 1976). However, particularly Photosystem II is very sensitive to auto-inactivation (Aro et al., 1993), and the in vitro systems lack auto-regenerative capacity. For these reasons we estimate that natural photosynthesis is much better suited for large-scale sustainable fuel production than artificial systems.

In natural photosynthesis, based on chlorophototrophy (Bryant and Frigaard, 2006), light emitted by the sun, is captured by the antennae of phototrophic organisms on the surface of the earth and transferred via resonance energy transfer to dedicated reaction centers, the Photosystems I and II. There, the free energy is stored by a series of electron transfer events, catalyzed by these two photosystems and a cytochrome b$_6$/f complex, that convert the free energy of light into the biochemical energy of NADPH. Simultaneously, the electron transfer steps generate a proton gradient that can lead to the formation of ATP, in an amount matching the requirements for conversion of both these nucleotides, together with CO$_2$, into phosphorylated carbohydrates, via the Calvin cycle (Calvin, 1989). The main catalytic components for natural photosynthesis are housed in the thylakoid membrane. Both its intrinsic electron-transfer pathway and its underlying structure are highly conserved in evolution and are used in micro-organisms and in the chloroplasts of plants and algae.

2. Current biofuel production strategies

Similar to CO$_2$, also protons can be used as the final electron acceptor in natural photosynthesis (Kruse et al., 2005; Prince and Kheshgi, 2005), thus producing overall (a mixture of) oxygen and hydrogen. In the following, however, we will concentrate on the former electron acceptor. Since the new millennium several alter-
native strategies have been developed for large-scale conversion of CO₂ into biofuels:

(i) The first of these implies a two-step procedure: First formation of (plant-derived) biomass, followed by a second processing step for conversion to products like methane and ethanol. Methane formation from biomass waste has been developed as a technology already several decades ago. The large-scale production of ethanol has been pioneered in Brazil, from sugarcane (Goldemberg, 2007), and more recently also in the US from various crops, in particular from corn (Hill et al., 2006). This latter approach, however, has become controversial for various reasons (Marris, 2006). First of all, the use of food crops for fuel production has led to sharp increases in food prices. Second, environmental concerns have been expressed regarding the environmental impact of the application of these processes at the very large scale, e.g. because of local nutrient imbalances and extent of net CO₂ sequestration (Searchinger et al., 2008).

(ii) Above discussions about the net energy yield and environmental impact of large-scale ethanol production has led to the development of several alternatives, often referred to as ‘second-generation biofuels’ (Chisti, 2008; Vasudevan and Briggs, 2008). For example, an economically viable alternative has been found in the harvesting of plants (or their seeds, e.g. rapeseed). As many seeds are rich in tri-glycerides, transesterification with methanol at high pH (i.e. alkali catalyzed) leads to the straightforward formation of methyl-esters of long-chain fatty acids, i.e. biodiesel, which can be directly used as a gasoline additive (Vasudevan and Briggs, 2008). Yet additional sources of triglycerides are available: Many algae form considerable amounts of these lipids, which upon harvesting and extraction can be processed in the same way as plant-derived oils (Chisti, 2007). For many species the neutral lipid content is in the order of ~30% by weight, but for selected species this content may go up to 80% (e.g. Schizochytrium sp., Botryococcus braunii, etc.). It should be noted, however, that for some of these organisms the neutral lipid fraction is dominated by polyisoprenoids (Ratledge, 2004) rather than triglycerides, such as the botryococcenes (Metzger and Largeau, 2005). Nevertheless, these latter may be converted into useful fuel through ‘cracking’.

Because of the inherent disadvantages of ‘first-generation’ biofuels, several proposals have been made for improvement. For the first stage of the process these include the improvement of the efficiency of photosynthesis (Mussgnug et al., 2007) and the use of phototrophic microorganisms because of their inherently higher biomass yield per unit surface area (Janssen et al., 2003; Dismukes et al., 2008), the use of more than only the polysaccharide fraction of the plant cells, including the lignin fraction (Weng et al., 2008), the application of genetic engineering to alter plant composition and to facilitate monomer release from the biopolymers (Bouton, 2007; Sticklen, 2008). For the second stage of the process it has been proposed to e.g. alter the solvent specificity of the solvent-producing organism, like to butanol (Durre, 2007) or a mixture of branched-chain alcohols (Atsumi et al., 2008), to increase its solvent tolerance (Stephanopoulos, 2007), or even to substitute it for an entirely chemical process (Agrawal et al., 2007).

Independent of these proposed improvements, suggestions for further altering and optimizing biofuel production systems have been made. An intriguing one of these is to use an engineered cyanobacterium (i.e. Synechococcus leopoldensi) equipped with the cloned bacterial cellulose synthase genes from Gluconobacter xylinus (Nobles and Brown, in press). This recombinant cyanobacterium produces extra-cellular deposits of non-crystalline cellulose; particularly its non-crystalline nature makes this polymer an ideal feedstock for biofuel production of various alcohols. Relevant in this respect is that cellulose production in this configuration is not limited by the intracellular storage capacity of the producer cell, a limitation that does apply to e.g. glycogen and poly-alkanoate formation. Significantly, homologous expression enzymes that catalyze the formation of extracellular cellulose has also been observed in natural isolates of cyanobacteria, like in the filamentous Crinum epipsammum (Dewinder et al., 1990).

3. The ‘photanol approach’: combining phototrophy and chemotrophy

Besides many attractive features, the first- and second-generation biofuel production systems described above do also have a number of less desirable aspects. The first-generation approach suffers from the disadvantage that in the first step a very complex product is formed that requires significant efforts before a major part of it can be converted to useful fuel. Second generation systems have the inherent limitation of the cellular lipid storage capacity (although botryococcenes are described to be ‘deposited’ outside of the producing cells) and require a complex biosynthetic machinery, with a limited capacity, and a nitrogen-limited growth. We therefore think that it is relevant to develop further alternatives.

In the realm of (micro)biology, molecular physiology has revealed two basic modes of life: The phototrophic and the chemotrophic mode (Madigan and Parker, 2003). A subclass of organisms that uses the former mode, the chlorophotoautotrophic organisms, convert CO₂ and minerals into new cells, using the energy of (sun)light. In their intermediary metabolism, the NADPH and ATP generated at the thylakoid membrane drive a cyclic metabolic pathway, called the Calvin cycle, in which CO₂ is converted into C₃ sugars like Glyceraldehyde-3-phosphate. These latter intermediates are key intermediates that feed most of the remaining anabolic pathways (Madigan and Parker, 2003).

In chemotrophic organisms C₃ sugars play a key role in catabolism: A wide range of sugars is first degraded to the C₃ level, after which they can be converted to CO₂, provided that sufficient oxygen is available, or to a range of more reduced products. The latter is particularly important when anaerobic conditions prevail (for instance in the ABE fermentation in clostridia (Awang et al., 1988; Ezeji et al., 2007), the basis of the classical ‘solvatogenesis process’), but takes place also under (micro)aerobic conditions, e.g. as a form of ‘overflow metabolism’ (Deboer et al., 1990). A well-known example of the latter is the ethanol formation in Crabtree-positive yeasts like Saccharomyces cerevisiae (Vandijeken et al., 1993).

The key role of C₃ sugars in both modes of metabolism brings up the question whether or not it would be possible to combine them in one organism (see also Fig. 2). Using the methods of synthetic biology
biology (Hasty et al., 2002), this should lead to a type of metabolism that one might classify as ‘photofermentative’. It would circumvent the need to first convert CO₂ into the complex mixture of biopolymers of which the average cell is composed (protein, nucleic acids, cell walls, neutral and phospholipids, etc.) and to apply a series of subsequent processing steps to convert this complex mixture into a specific biofuel. Accordingly, the overall efficiency of the biofuel production process may be significantly increased. In the theoretical limit the number of incident photons per area could be quantitatively converted into e.g. ethanol, which implies a theoretical limit of $\sim 10^3$ l/ha/yr.

For the production of ethanol from CO₂ in the cyanobacterium Synechococcus sp. strain PCC 7942, Deng and Coleman (1999) (see also Fu, 2009) that this approach is feasible by heterologous expression of two enzymes only. The former investigators selected the coding sequences of pyruvate decarboxylase (pdc) and alcohol dehydrogenase II (adh) from the bacterium Zymomonas mobilis and showed that in the resulting recombinant strain ethanol accumulates up to 5 mM in a period of four weeks.

Extending on this idea, through application of molecular genetic engineering, a set of strains can be constructed that will each lead to any of a series of desired products, including various alcohols and organic acids, well-know from microbial fermentation processes. We refer to this approach of biofuel production as the ‘Photanol approach’. Furthermore, by a properly timed blocking of anabolism of the host organism, e.g. through a lack of minerals in the growth medium, it should be possible to redirect all carbon derived from CO₂ to a pre-selected fermentative pathway. The genetic engineering and synthetic biology required for this approach is most straightforwardly done in the cyanobacterium Synechocystis PCC6803 because of the natural transformability (Sheshakov and Khyen, 1970; Dzelzkalns and Bogorad, 1986) of this species and the many genomics analyses to which it has been subjected (e.g. Hihara et al., 2001; Shastri and Morgan, 2005).

4. Challenges

To make the Photanol approach an economically viable strategy, several scientific challenges still lie ahead. One of these is the downstream processing of the products formed. Nevertheless, for many aspects of this process use can be made of the knowledge base derived from the ABE fermentation (Awang et al., 1988). From such considerations it is clear already that ideally the growth temperature of the production organism should be as close as possible to the boiling point of the product formed; furthermore, the product should have very low solubility in water, or both. Nevertheless, this may lead to problems of product toxicity, a phenomenon that possibly can be counteracted through the (engineered) involvement of broad-specificity efflux pumps. In the light of these considerations it is relevant to note that genes encoding an ethylene synthase have been cloned successfully in the cyanobacterium Synechococcus elongatus PCC 7942 (Takahama et al., 2003). Significant rates of ethylene production were reported.

Because of the oxygenic character of photosynthesis in cyanobacteria, one of the important criteria in the selection of all coding sequences to be heterologously expressed will be the oxygen sensitivity of the encoded enzymes.

References


