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Biological conversion of carbon dioxide and hydrogen into liquid fuels and industrial chemicals

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Non-photosynthetic routes for biological fixation of carbon dioxide into valuable industrial chemical precursors and fuels are moving from concept to reality. The development of 'electrofuel'-producing microorganisms leverages techniques in synthetic biology, genetic and metabolic engineering, as well as systems-level multi-omic analysis, directed evolution, and *in silico* modeling. Electrofuel processes are being developed for a range of microorganisms and energy sources (e.g. hydrogen, formate, electricity) to produce a variety of target molecules (e.g. alcohols, terpenes, alkenes). This review examines the current landscape of electrofuel projects with a focus on hydrogen-utilizing organisms covering the biochemistry of hydrogenases and carbonic anhydrases, kinetic and energetic analyses of the known carbon fixation pathways, and the state of genetic systems for current and prospective electrofuel-producing microorganisms.

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Introduction

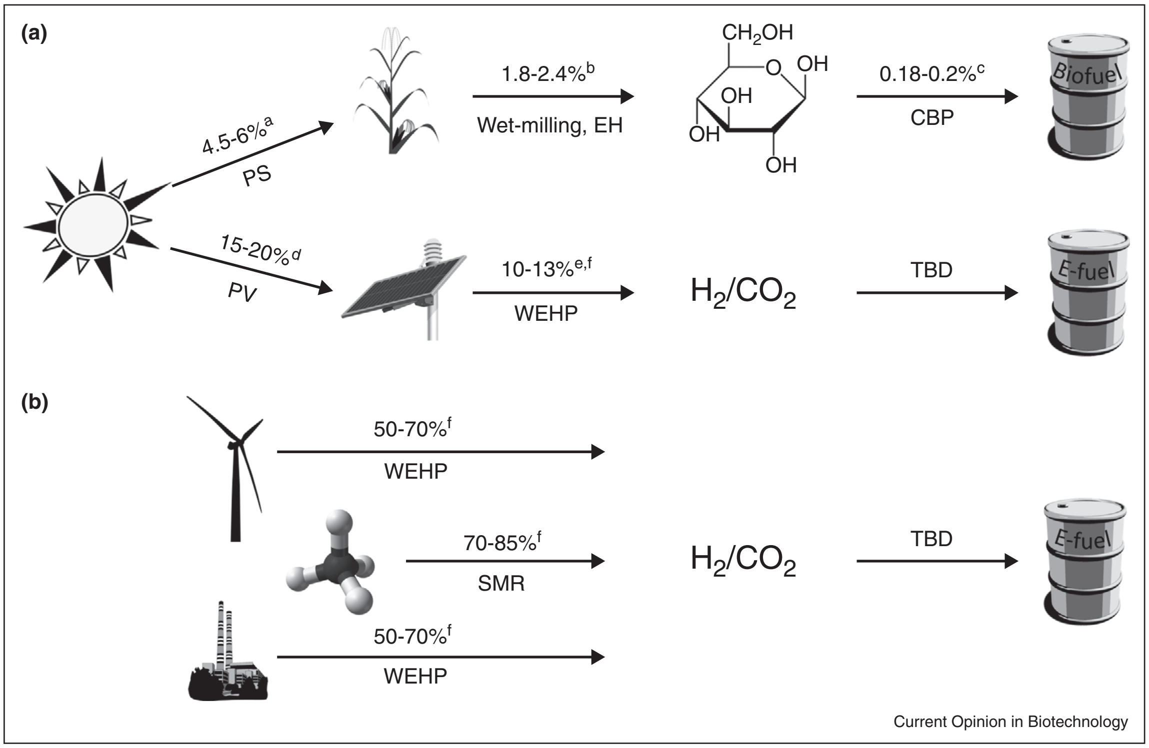
There are intrinsic limitations to the production of biofuels derived from photosynthetic organisms that impede the development of a renewable liquid fuel industry at large-scale. In particular, these include low efficiency of solar energy conversion (Figure 1) and competition for agricultural resources. Recent initiatives in the U.S. and elsewhere have the objective of harnessing the molecular mechanisms of non-photosynthetic organisms that can utilize CO_2 directly for the production of energy-dense liquid fuels, which are now referred to as 'electrofuels' [1••,2,3•]. Electrofuel-producing microorganisms are being developed that require the complementary expertise of synthetic biologists, metabolic engineers, and microbiologists to equip native CO_2-fixing species or autotrophs with pathways for targeted fuel production, or confer autotrophy on heterotrophic host organisms, or both. The range of possible sources currently being explored for low-potential, high-energy electrons to power an electrofuel process includes hydrogen gas, formate, carbon monoxide and electricity. This review will focus on electrofuel strategies that use hydrogen gas as source of reducing power for CO_2 fixation. Microorganisms that are able to use other sources of electrons including electricity directly are discussed in an accompanying review by Lovely [4]. A general scheme for electrofuel production from hydrogen and CO_2 is shown in Figure 2.

CO_2 fixation

The reduction of CO_2, the most oxidized form of carbon, into technologically useful organic compounds remains a daunting task for abiological chemical catalysis. There are, currently, six naturally occurring biological pathways for carbon fixation, and these have been reviewed extensively in recent years [5••,6,7,8]. Each pathway has unique features arising from the ecological and molecular context in which it evolved. Although there are many examples of CO_2-fixing carboxylases that are utilized for metabolic purposes other than carbon assimilation, such as energy conservation, anaplerosis, and redox-balancing [1••,2,3•,9••,10], this review will focus on the autotrophic CO_2 fixation pathways that are relevant for electrofuel production in addition to the primary host microorganisms that are currently being considered that use hydrogen (Table 1).

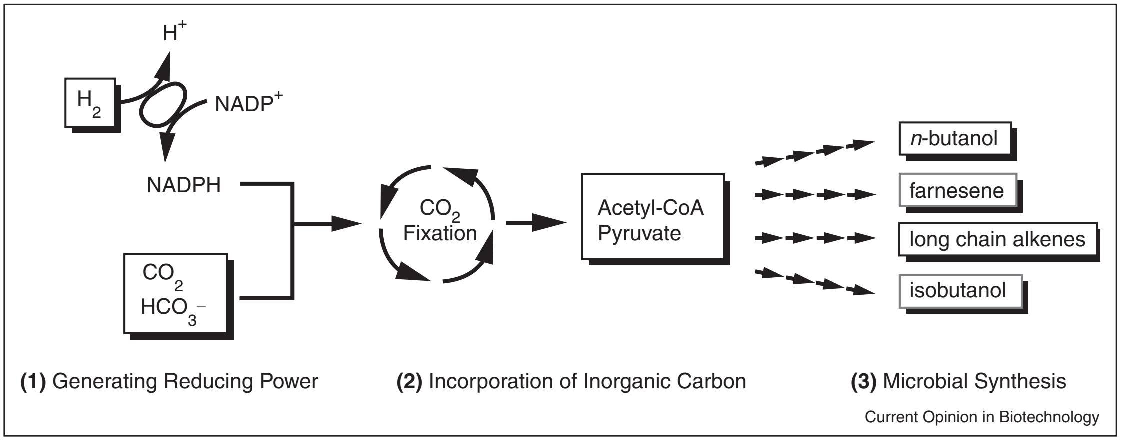
The most ubiquitous CO_2-fixation pathway is the Calvin–Benson–Bassham (CBB) cycle found in plants, algae, cyanobacteria, purple bacteria, and also in some proteobacteria, such as *Ralstonia eutropha*. *R. eutropha* is a metabolically diverse, facultatively autotrophic bacterium that can grow on sugars, fatty acids, amino acids, triacylglycerides as well as on H_2/CO_2 [5••,6,7,8,11•]. Previous work on *R. eutropha* has focused on its ability to store excess carbon as polyhydroxyalkanoates [12,13] (PHAs) and now efforts seek to divert carbon flux away from PHA storage and into other molecular targets. For example, *R. eutropha* is a proposed host for isobutanol production via the 2-ketoisovalerate pathway for branched chain amino acid synthesis [11•] from H_2 and CO_2. This strategy has already been successfully used in *E. coli* to produce

Figure 1



Electrofuel production from H_2 and CO_2. **(a)** Comparison of overall photon-to-fuel efficiency of biofuels versus electrofuels. Percentages represent the cumulative efficiency of solar energy conversion at different stages. Although the conversion efficiency of an electrofuel process is currently unknown, the improved efficiency of solar hydrogen compared to photosynthetic sugars indicates that a solar electrofuel process would require less land area than current biofuels. **(b)** Hydrogen inputs for electrofuels are highly flexible and generation strategies can include electrolysis of water by renewable wind power, electrolysis of water by conventional electricity (e.g. coal), or by steam reformation of methane, shown with conversion efficiencies for each process. Abbreviations: PS – photosynthesis, EH – enzymatic hydrolysis, PV – photovoltaic, WEHP – Water Electrolysis Hydrogen Production, SMR – steam methane reformation, TBD – To be determined. [a]Zhu *et al.* [76], [b]Assuming grain starch represents 40% of total corn biomass, [c]Conrado *et al.* [1••], [d]Parida *et al.* [77], [e]Assuming 65% overall electrolysis efficiency, [f]Holladay *et al.* [78].

Figure 2



Schematic drawing of the primary biochemical modules involved in electrofuel formation. Intracellular reducing power is generated from hydrogen gas via hydrogenase enzymes. Carbon fixation cycle incorporates inorganic carbon into central metabolism via key intermediates. Microbial synthesis of the target fuel molecule proceeds via endogenous and/or engineered metabolic pathways.