

# Opportunities for Renewable Bioenergy Using Microorganisms

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**ABSTRACT:** Global warming can be slowed, and perhaps reversed, only when society replaces fossil fuels with renewable, carbon-neutral alternatives. The best option is bioenergy: the sun's energy is captured in biomass and converted to energy forms useful to modern society. To make a dent in global warming, bioenergy must be generated at a very high rate, since the world today uses  $\sim 10$  TW of fossil-fuel energy. And, it must do so without inflicting serious damage on the environment or disrupting our food supply. While most bioenergy options fail on both counts, several microorganism-based options have the potential to produce large amounts of renewable energy without disruptions. In one approach, microbial communities convert the energy value of various biomass residuals to socially useful energy. Biomass residuals come from agricultural, animal, and a variety of industrial operations, as well as from human wastes. Microorganisms can convert almost all of the energy in these wastes to methane, hydrogen, and electricity. In a second approach, photosynthetic microorganisms convert sunlight into biodiesel. Certain algae (eukaryotes) or cyanobacteria (prokaryotes) have high lipid contents. Under proper conditions, these photosynthetic microorganisms can produce lipids for biodiesel with yields per unit area 100 times or more than possible with any plant system. In addition, the non-lipid biomass can be converted to methane, hydrogen, or electricity. Photosynthetic microorganisms do not require arable land, an advantage because our arable land must be used to produce food. Algae or cyanobacteria may be the best option to produce bioenergy at rates high enough to replace a substantial fraction of our society's use of fossil fuels.

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## Introduction—The Challenge

Human society seems to have an insatiable appetite for fossil fuels. We now consume about 13 terrawatts ( $1 \text{ TW} = 10^{12} \text{ W} = 3.2 \text{ EJ/year}$ ) of energy worldwide, and approximately 80% of that comes from burning fossil fuels (Goldemberg and Johansson, 2004). Our dependence on fossil fuels poses three giant risks for the survival of human society as we now know it. The first risk is that we will deplete fossil-fuel reserves, leaving human society metaphorically and perhaps literally “cold, hungry, and in the dark.” The second risk is that geopolitical strife from competition for dwindling resources will lead to economic and energy disruptions, political turmoil, and war. The third risk is from global climate change caused by the net increase in atmospheric  $\text{CO}_2$  due to combustion of the fossil fuels. Although the portent of catastrophe may seem more immediate from the first two risks, it is the third risk that will have the most long-lasting and profound impact. Fortunately, we have the potential to reduce, or perhaps even eliminate, the third risk by converting to renewable, carbon-neutral energy sources that provide the energy services now obtained from fossil fuels.<sup>1</sup> Eliminating the third risk by finding substitutes for fossil fuels should have the happy consequence of minimizing risks one and two, as well.

Except for the problems they cause, fossil fuels are an ideal energy source. They provide high-density, transportable

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<sup>1</sup>Renewable and carbon neutral are related, but distinct features. Renewable means that the energy service comes from an energy source that was produced recently, not mined from hundreds of millions of years of accumulation, and that can continue to be produced. Carbon neutral means that any  $\text{CO}_2$  released was taken from the atmosphere recently, creating a short-term C cycle, not a net addition of  $\text{CO}_2$ .

energy that can be converted to what society needs: heat, electricity, and motive force. We have come to expect the benefits of fossil fuels; renewable substitutes must match them in convenience and utility. Furthermore, fossil fuels can provide energy services on a massive scale: currently over 10 TW worldwide. Substitutes must match that large scale, too, but without adding to the accumulation of CO<sub>2</sub> in the atmosphere.

Combustion of fossil fuels is adding about 6 gigatons (Gton = 10<sup>9</sup> tons) per year of C (in the form of CO<sub>2</sub>) to the atmosphere each year (IPCC, 2007). Ophuls (1977) insightfully described extracting and burning fossil fuels as harvesting “phantom acres,” or the concentrated biomass accumulated over hundreds of millions of years. In just a few hundred years, humans have released the organic carbon accumulated over hundreds of millions of years. Thus, the atmospheric CO<sub>2</sub> level has been increasing at an accelerating rate since the start of the Industrial Revolution. Table I summarizes the increases in atmospheric CO<sub>2</sub> and the concomitant rise in the Earth’s temperature. The parallel increases are no accident, and the projected trends are alarming (IPCC, 2007), particularly if the atmospheric CO<sub>2</sub> level does not stabilize at the hoped for 550 ppmv (parts per million by volume). Should the CO<sub>2</sub> concentration continue to rise through 2100, average temperatures could rise by more than 3°C, ocean levels could rise up to 80 cm (nearly 3 feet), most lake and reservoir levels will decline, extreme weather events (e.g., hurricanes, drought, and flooding) will occur more frequently, and disease transmission may become more prevalent (IPCC, 2007). The impacts will not be distributed equally, and those already clinging to a most tenuous existence surely will suffer the greatest from flooding, drought, starvation, disease, and war (UNDP, 2007).

Our greatest challenge—and the most essential—is to find means to reduce the extraction and combustion of fossil fuels. The ~10 TW of fossil fuels we use today is divided approximately 43% from petroleum, 40% from coal, and 17% from natural gas. While the world’s petroleum reserves will decline gradually over the next 20–40 years, coal has very large reserves and is being extracted more and more rapidly by the developing countries, such as China and India

(Masters and Ela, 2008). Even though petroleum use eventually must decline, consumption of all fossil fuels will produce higher CO<sub>2</sub> concentrations unless renewable sources of energy replace fossil fuels. In fact, the shift from petroleum to coal will only worsen the CO<sub>2</sub> balance, since coal releases almost two times more CO<sub>2</sub> than does petroleum for the same energy output (Masters and Ela, 2008).

The fossil fuel we consume today is a very large amount, and our consumption will rise by 60% or so in the next 25 years unless we can replace it on a very large scale (Energy Information Administration, 2005). Conservation and efficiency will be needed to keep the total energy demand from growing to totally unmanageable levels. With conservation and efficiency in effect, renewable substitutes for fossil fuels will have a chance to slow or reverse global warming, but only if they can be implemented on a very large scale. Thus, scale must be considered when evaluating the value of any renewable-energy scheme.

Two options for renewable energy have the potential to provide renewable, C-neutral energy in large amounts. Both capture solar energy, either through photovoltaics or in biomass. This Perspectives article focuses on biomass energy, which presents many options. I describe the major options, but devote most of my attention on the most promising options, which involve using microorganisms.

## Options and Principles of Biomass Energy

Table II lists leading options for biomass energy, which can be differentiated by the form of the biomass that is used (e.g., a food crop, cellulosic plant material, complex biomass, or microorganisms) and the final energy output (e.g., ethanol, butanol, methane, hydrogen, electricity, or biodiesel). Table II provides succinct statements of problems and advantages of each option, and I will elaborate on them after describing two principles that are common to all bioenergy approaches.

The first principle is that the energy is in the electrons (Rittmann, 2006). All of the bioenergy outputs listed in Table II consist of high-density electron carriers. Some are organic (e.g., ethanol, methane, and biodiesel), while others

**Table I.** Trends in atmospheric CO<sub>2</sub> and average air temperature.

Year	Atmospheric CO <sub>2</sub> (ppmv)	Average temperature (°C)	Comment
1800	280	15	Pre-industrial revolution
1870	280	15	Early industrial revolution
1950	305	15.2	Target for 2006 CO <sub>2</sub> levels <sup>b</sup>
1970	325	15.2	Major increases observed
1988	350	15.5	Increases accelerate
2000	360	15.8	Increases accelerate
2006	375	16.0	Increases accelerate
2050 est.	~550	Up to 17.2	Hoped for stabilization <sup>a</sup>
2100 est.	Up to ~800	Up to ~19.2	Stabilization does not occur

Source: IPCC (2007).

<sup>a</sup>Stabilization requires that CO<sub>2</sub> emissions are lower in 2050 than today and that they continue to decline.

<sup>b</sup>The CO<sub>2</sub> emission rate of 1950 would hold the 2006 CO<sub>2</sub> concentration.

**Table II.** Leading options for biomass energy.

Biomass-energy option	Problems	Advantages
Food crop (e.g., corn or sugar cane) to ethanol ( $C_2H_5OH$ )	Very low net energy yield, competition with food crops, water pollution, inherently low yield per unit area	Strong political lobbies; can be used with gasoline
Food crop (e.g., corn or sugar cane) to butanol ( $C_4H_9OH$ )	Low net energy yield, competition with food crops, water pollution, inherently low yield per unit area	Better net energy yield than ethanol
Cellulosics (e.g., switchgrass or <i>Miscanthus</i> ) to ethanol or butanol	Unproven at large scale; low net energy yield	Higher yield per unit area, less severe competition with food crops, and less water pollution than with food crops
Complex biomass (e.g., animal waste) to methane ( $CH_4$ )	Conversion efficiency is not yet high enough; unit cost is higher than from natural-gas deposits today	Mature technology; can use residues and wastes, turning a pollution problem into an energy resource; $CH_4$ infrastructure is in place
Complex biomass (e.g., animal waste) to hydrogen ( $H_2$ )	Technology is immature; conversion efficiency today is very low	Can use residues and wastes, turning a pollution problem into an energy resource; $H_2$ can be used in fuel cells
Complex biomass (e.g., animal waste) to electricity ( $e^-$ ) via the microbial fuel cell (MFC)	Technology is nascent; conversion efficiency is not established	Electricity infrastructure is in place; an MFC is a combustionless, pollution free fuel-cell technology that uses renewable organic fuel directly
Plants (e.g., <i>Jatropha</i> , soy beans, or sunflowers) to biodiesel (mainly C-16 and C-18 aliphatics)	Technology is immature; yield per unit area is inherently low; competes with food crops	Biodiesel is a high-density fuel that is an ideal substitute for petroleum
Phototrophic microorganisms (algae or cyanobacteria) to biodiesel	Technology is at an early stage; may require a significant capital investment	Biodiesel is a high-density fuel that substitutes ideally for petroleum; possible to have very high yield per unit area, allowing TW output; does not compete with food crops

are inorganic (hydrogen gas and electricity). All outputs are produced totally or in part by microorganisms.

The second principle is that the ultimate source of energy is sunlight; photosynthesis captures the sun's energy and invests it in organic molecules, which are the carriers of the electrons and their energy. In some cases, the organic molecules are quite homogeneous, such as the simple carbohydrates found in corn and sugar cane. In other cases, the organic molecules come in complex, heterogeneous forms, such as cellulose, plant residues, and animal wastes (de facto processed plant residues). Biomass energy is renewable, because its ultimate source is sunlight. It is C-neutral, because the time from photosynthesis to humans' energy use is short—days to years—not hundreds of millions of years, as with fossil fuels.

While biomass is a convenient electron carrier from the point of view of the photosynthetic plants and microorganisms, as well as the animals that eat them, biomass is quite an inconvenient electron carrier for fueling the activities of our industrial society. Biomass is not dense enough; society prefers concentrated energy forms, such as the fossil fuels. Furthermore, biomass tends to be wet, often stinky, and sometimes hazardous.

Can biomass capture enough of the sun's energy in a convenient form to meet the human demand of ~10 TW now derived from fossil fuels? On the one hand, the total incident solar energy reaching the earth's surface is plentiful: ~173,000 TW (Goldemberg and Johansson, 2004), which is 17,000 times what humans consume in fossil fuels. On the other hand, all photosynthesis on earth today captures about 140 TW of energy as biomass (Hall and Rosillo-Calle, 1998); this is only about 10 times today's energy demand of human society. Much of the natural energy flow captured from sunlight must go to run the earth's ecosystems, including a modest flow for feeding humans. To fuel its civilization, humans cannot simply divert one-tenth of the natural flow of high-energy electrons through biomass. This suggests that for biomass to be a major renewable, C-neutral source, photosynthetic energy capture must be expanded to produce some "new biomass." Furthermore, existing and new biomass must be used in ways that do not disrupt normal ecosystem function. Which of the options listed in Table II have the potential to meet these criteria?

## Biomass Options That Cannot Attain Large Enough Scale

All approaches that rely on diverting food crops to biofuels will fail to meet the criteria because they compete with food production for high-grade arable land and because their bioenergy output is limited. The ramp-up in corn-to-ethanol production in the United States already have led to an increase in the cost of corn by over 50% in the past year (Carroll and Parker, 2007; UNDP, 2007), and demonstrations have taken place due to rising prices of tortillas in Mexico, pasta in Italy, and bread in Pakistan (Clayton,

2008). Furthermore, ethanol is very water soluble, which means that the energy cost of separating it from water consumes a significant fraction of the energy value of the ethanol product. When all energy costs to produce the corn and convert it to ethanol fuel are considered, experts estimate that ~90% of the energy output in bioethanol from corn is lost in the processes required to make it (Giampietro and Mayumi, 2007; Shapouri et al., 2001). Due to this low net energy output, diverting large portions of the United States arable land to producing corn to make bioethanol would satisfy less than on a few percent of the country's demand for gasoline alone. It would, however, substantially increase water demand, water pollution, and soil erosion (NRC, 2007). The impact on food prices could be serious (Clayton, 2008). The negative impacts of the crop-based approach are becoming evident (Clayton, 2008; Rosenthal, 2008; Searchinger et al., 2008).

A simple computation illustrates the scale problem using crop-based ethanol. Let's set as a long-term goal replacing the ~4.3 TW of energy currently supplied by petroleum. This converts to  $1.4 \times 10^{17}$  kJ/year of energy. Since ethanol has an energy content of  $8.9 \times 10^4$  kJ/gallon ( $2.4 \times 10^4$  kJ/L) and can be produced from corn at a gross yield of ~350 gal/acre (230 L/ha), the area required to meet the petroleum demand would be  $\sim 4.4 \times 10^9$  acres ( $1.8 \times 10^9$  ha). This is more than 10% of the entire terrestrial area of Earth,  $\sim 4 \times 10^{10}$  acres ( $1.5 \times 10^{10}$  ha). However, the situation is not so favorable, because the net energy output of corn-to-ethanol is only about 10%, which says that the entire land surface area of the planet would be needed to supplant our current use of petroleum. Clearly, energy yield must increase by orders of magnitude over corn-to-ethanol if we are to meet the goal of replacing petroleum use; a factor of 3 or 10 is simply too little.

Switching the source of biomass away from food crops to cellulotics, like switchgrass, could improve the energy output and lower the negative environmental costs. However, the technology is not close to being ready. Even if the technological barriers to cellulosic ethanol or bioethanol are overcome, they still will have only a small impact on reducing fossil-fuel consumption.

Another crop-based approach is to convert high-lipid plants—such as soybeans, sunflowers, and *Jatropha*—to biodiesel, which is a high-quality transportation fuel. The net energy yield is higher this way than when converting crops to ethanol or butanol, since the long-chain aliphatics that comprise biodiesel are naturally immiscible in water. Sunflowers can produce up to about 1 m<sup>3</sup> of lipids per hectare-year (Huber et al., 2006), but land cultivable in high-lipid plants is limited.

## Conversion of Residual Biomass to Useful Energy

A potentially large and untapped source of biomass for making useful energy is residual biomass from normal

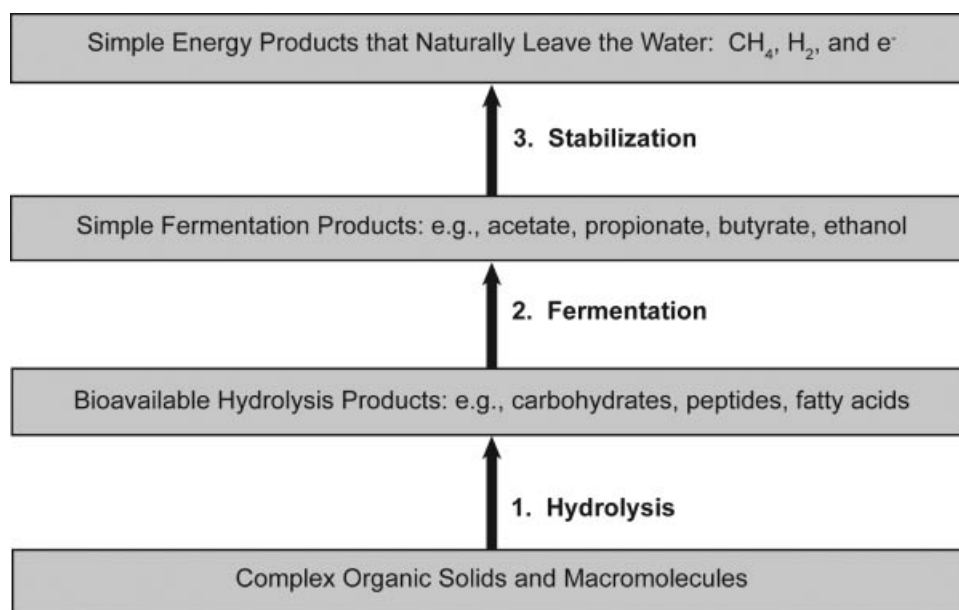
human activities. Agriculture, the food-producing industry, and municipal and industrial wastewaters contain enough energy to meet a significant fraction of the world's entire energy demand, if they could be collected and converted efficiently to useful energy forms. The U.S. Department of Energy (Chynoweth et al., 2001; Energy Information Administration, 2005) has estimated that the energy value of all residual biomass in the United States is 0.2–0.3 TW. This includes agricultural, animal, industrial, and human wastes and residues. Conversion of these biomass-energy sources to useful forms would meet approximately 7% of the United States' total annual energy use (~3.3 TW) (Energy Information Administration, 2005). The fraction is much higher worldwide, perhaps 25% or more (Goldemberg and Johansson, 2004). Furthermore, the wastes often cause serious environmental harm, and their collection and conversion to energy would provide a giant benefit to environmental quality.

Conversion of residual biomass to useful energy is different from production of ethanol from a food crop, because the starting material usually is much more chemically complex. Being the residue from living organisms, it normally contains a mixture of carbohydrates, proteins, lipids, and nucleic acids. Plant-derived materials also contain cellulose and lignin. Almost all of the biomass is in macromolecules, and much is solid.

Figure 1 defines the three steps needed to convert complex organic matter to useful bioenergy outlets: break down of the complex solids and macromolecules to simple chemical forms that can be taken up by the microorganisms, fermentation to simpler products, and final stabilization by specialized microorganisms to an electron- (and energy-) rich form that naturally leaves the water. The last step—generation of an energy-rich form that naturally leaves the water—is the inherent advantage of microbial energy-conversion systems, because it avoids the large energy cost for extracting the fuel from water.

In the first step, these complex materials need to be broken into constituent parts by a combination of chemical, mechanical, and enzymatic attack. This step is called pre-treatment, and many techniques are being tested to make complex biomass more bioavailable for ultimate energy conversion: high temperature, high or low pH, hydrolytic enzymes, microwaves, ultrasound, radiation, and pulsed electric fields. All of these methods are able to solubilize and make the residual organic material more bioavailable. At the same time, most of the pre-treatment techniques have drawbacks that include high energy demand, high chemical cost, production of odors, production of inhibitory products, and a large increase in salt content. Hence, an ideal technology for pre-treatment is not yet at hand, and this places limits on the what we can obtain from energy-conversion schemes. A conversion process today may capture 50% of the energy value from most residues. Clearly, the missing 50% is a large potential source of bioenergy, if we can figure out how to make that energy bioavailable.



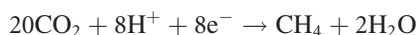


**Figure 1.** The three steps for converting complex organic material in biomass to useful energy outputs. The stabilization step is what determines the form of bioenergy that is produced: methane, biohydrogen, or bioelectricity (in a microbial fuel cell).

Once the organic matter is bioavailable, a good conversion process captures almost 100% of the electrons in a useful energy output. It is essential to avoid products that have the inevitable large energy cost associated with separating ethanol from water. Fortunately, different groups of microorganisms naturally do exactly what we desire: that is, produce an electron sink that naturally leaves the water and can be captured for human use. The three best electron and energy outputs (Fig. 1) are methane gas ( $\text{CH}_4$ ), hydrogen gas ( $\text{H}_2$ ), and the electrons themselves (i.e., electricity).

### Methanogenesis

The generation of  $\text{CH}_4$  by anaerobic microbial communities is called methanogenesis (Rittmann and McCarty, 2001; Speece, 1996). Eight electrons taken from biomass are ultimately routed to form 1 mol of  $\text{CH}_4$ , as shown in the following half reaction:



$\text{CH}_4$ , which is the same as natural gas, is a low-solubility gas that rapidly bubbles out of the water and can be captured, cleaned, and used for its energy value.  $\text{CH}_4$  has a high energy value ( $\Delta H^\circ = 816 \text{ kJ/mol}$  or  $102 \text{ kJ/e}^- \text{ eq}$ ) that can be captured through combustion. The heat released in combustion can be used for process or space heating, or it can be converted to electricity by conventional steam-turbine systems.

Methanogenesis is carried out by a complex community of microorganisms that work in an exquisite form of syntrophy. The last step—generation of  $\text{CH}_4$ —is performed

by two unique groups of strictly anaerobic *Archaea* (Parkin and Owen, 1986; Rittmann and McCarty, 2001; Speece, 1996). One group oxidizes  $\text{H}_2$  and respire  $\text{CO}_2$ :  $\text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$ . The second group ferments acetic acid to  $\text{CH}_4$  and  $\text{CO}_2$ :  $\text{CH}_3\text{COOH} \rightarrow \text{CH}_4 + \text{CO}_2$ . Both groups of methanogens are slow growers, require strictly anaerobic conditions, and have almost no other metabolic options besides the reactions shown here.  $\text{H}_2$  and acetic acid are produced from more complex organic molecules by a wide range of fermenting bacteria. In some cases, rapid consumption of  $\text{H}_2$  by the  $\text{H}_2$ -oxidizing methanogens is necessary to make the fermentation reactions to  $\text{H}_2$  thermodynamically possible. The fermenting bacteria produce extracellular hydrolytic enzymes to cut macromolecules into small, simple molecules that they can take up and ferment.

Methanogenesis has been employed for over 150 years (McCarty and Smith, 1986; Tchobanoglous and Burton, 1991) for the digestion and stabilization of sludge (also called biosolids) generated in wastewater treatment. It constitutes a mature technology that can be adapted to most forms of residual biomass. The key is making the input biomass more bioavailable. The cost of methane production is still relatively high compared to the cost of natural gas from fossil-fuel deposits, but this situation should change as natural-gas costs rise and methanogenesis and pre-treatment technologies improve.

### Biohydrogen Generation

$\text{H}_2$  is another low-solubility gas that can be a naturally separating electron and energy output from anaerobic

microbial systems (Fig. 1).  $H_2$  has approximately the same heat of combustion as  $CH_4$  ( $\Delta H^\circ = 237$  kJ/mol or 119 kJ/ $e^-$  eq), and it can be combusted to gain the energy value similarly to  $CH_4$ . However,  $H_2$  has the very large added benefit that it can be used as the fuel for a conventional fuel cell, where electricity can be produced without combustion, producing pollution-free electrical energy with an efficiency at least 50% higher than with a combustion-steam-turbine approach. Today,  $H_2$  gas is a large and mature industry (about  $10^8$  m<sup>3</sup>/year in the United States), but virtually all of our hydrogen comes from reforming fossil fuels. Having a biomass source for  $H_2$  would be a giant boon for renewable, C-neutral energy.

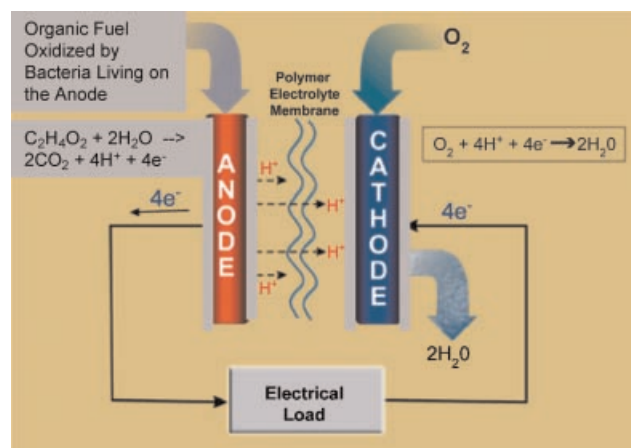
The most common approach for converting biomass to  $H_2$  is a bacterial fermentation process that is essentially a truncated version of methanogenesis. A combination of moderately acidic pH and short solids retention time suppresses methanogens and accentuates  $H_2$  production. Although fermentation to  $H_2$  is straightforward, the main drawback today is that only a small fraction of the electrons in the starting organic material ends up in  $H_2$ . This is true even when the starting material is pure glucose. The problem is that fermenting bacteria channel most of their electron flow to organic products, not to  $H_2$ . In the  $H_2$ -fermentation literature, this is recognized by the definition of the “maximum  $H_2$  yield” as 4 mol  $H_2$ /mol glucose, which comes from the fermentation of glucose to only  $H_2$  and acetic acid:



In this “maximum” reaction, the 24  $e^-$  eq of  $C_6H_{12}O_6$  are routed to 8  $e^-$  eq in  $H_2$  and 16  $e^-$  eq in  $CH_3COOH$ . Thus, two-thirds of the electrons and energy are “misdirected” to acetic acid. In practice, the situation is worse. The practical  $H_2$  yield is around 2 mol  $H_2$ /mol glucose, due to the formation of butyrate, ethanol, propionate, and a few other organic fermentation products (reviewed and explained in Lee et al., 2008b). Due to this yield problem, traditional  $H_2$  fermentation seems to be “stuck” even before it is applied in a serious way to real residual biomass. At the end of the next section, I describe a way to get biohydrogen “unstuck.”

## Bioelectricity

A third electron and energy outlet that removes itself from the water naturally (Fig. 1) is electricity produced from a microbial fuel cell (MFC), or bioelectricity. As illustrated in Figure 2, an MFC is a special form of a fuel cell in which bacteria grow as a biofilm on the anode. The bacteria catalyze the oxidation of organic compounds either in or derived from biomass (after it has gone through fermentation), and the bacteria then transfer the electrons to the anode. The electrons move through an electrical circuit to the cathode. At the cathode, the electrons are transferred to an electron acceptor, normally oxygen ( $O_2$ ). The energy value of the electrons is harvested in the circuit as electrical power, again an electron sink that naturally leaves the water (Fig. 1).



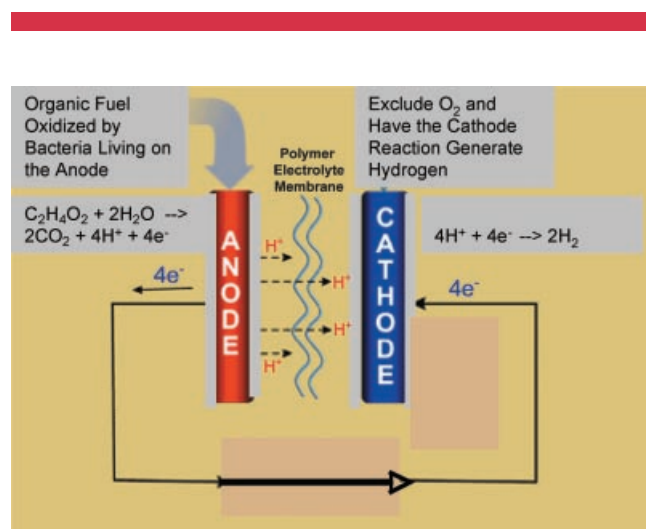
**Figure 2.** Schematic of a microbial fuel cell (MFC). Bacteria attached to the anode remove electrons from an organic electron donor (or fuel) and transfer them to the anode. The electrons move through the electrical circuit to the cathode, where they reduce  $O_2$  to  $H_2O$ . To maintain electroneutrality, protons ( $H^+$ ) or other ions move between the anode and cathode via the aqueous solution. Bioelectrical energy is harvested from the flow of electrons from the anode to cathode.

The biggest advantage of an MFC is that it can generate combustion-less, pollution-free bioelectricity directly from the organic matter in biomass. The intermediate step of generating  $H_2$  for a conventional fuel cell is eliminated. The most unique aspect of an MFC is that a biofilm of anode-respiring bacteria (ARB) transfers electrons to the surface of the anode. The mechanisms by which ARB transfer electrons are hotly researched and debated today. While it is possible that direct contact of the ARB with the anode can play a role, large-scale electron transfer uses soluble electron shuttles, conduction through the biofilm matrix, or a combination (reviewed in Kato Marcus et al., 2007; Rabaey et al., 2005; Rittmann, 2006; Rittmann et al., 2008). The recent discovery of conductive nanowires (Gorby et al., 2006; Reguera et al., 2006) in MFC biofilms suggests that ARB can create their own “electricity grid.” It is also possible that the extracellular polymeric substances (EPS) that hold the biofilm together and to the surface are sufficiently conductive, with or without nanowires, to support significant current fluxes (Kato Marcus et al., 2007).

Researchers from around the world have documented bioelectricity generation from a range of fermentation products and waste organics (reviewed in Logan, 2004; Logan and Regan, 2006; Logan et al., 2006; Rittmann et al., 2008). Thus, the concept of the MFC is proven, and making bioelectricity from organic fuel is not difficult. The challenge is to make bioelectricity at a fast enough rate that the capital costs of the MFC are offset by the value of the energy output. Today’s MFCs are far from fast enough to be commercially viable. However, current and power densities have increased steadily as researchers understand where the losses of electrons and energy occur and solve problems with kinetic bottlenecks (Cheng and Logan, 2007; Rittmann et al., 2008). For example, the highest current density to date,  $\sim 12$  A/m<sup>2</sup> of anode area

(Fan et al., 2007; Ringeisen et al., 2006; Rozendal et al., 2007; Torres et al., 2007) is more than 10 times larger than typical rates only 5 years ago (Lee et al., 2008a; Rittmann et al., 2008). Likewise, power densities have increased similarly, up to  $\sim 2.4 \text{ W/m}^2$ , in the same period (Cheng and Logan, 2007). Comprehensive research that treats the MFC as a system should continue to improve performance. No one part of an MFC is solely controlling. Integrated improvements need to be made in many areas, including the ARB, physical structure and conductivity of the anode biofilm, the anode materials and surface properties, the solution conductivity and buffering, the membrane or other means to separate the anode from the cathode, the cathode catalyst and electron acceptor, and the physical architecture of the system. While the complexity of the MFC systems makes research challenging, it also offers many opportunities to improve performance.

Among the most interesting opportunities is to change the electron acceptor at the cathode from  $\text{O}_2$  to  $\text{H}^+$ . As is illustrated in Figure 3, this change allows  $\text{H}_2$  to be generated at the cathode (Cheng and Logan, 2007; Lee et al., 2008a; Torres et al., 2007), and this form of an MFC is called a microbial electrolytic cell, or MEC. In an MEC, the energy output is  $\text{H}_2$ , not electricity. In fact, a small amount of electrical energy is consumed to boost the potential high enough to produce  $\text{H}_2$  at the cathode (Cheng and Logan, 2007). In the end, the net energy conversion to  $\text{H}_2$  is around 80% (Cheng and Logan, 2007). Using an MEC overcomes several problems associated with MFCs: for example, leakage of  $\text{O}_2$  into the anode compartment and the high potential loss for reducing  $\text{O}_2$  at the cathode. In addition, the MEC seems to be the ideal way to utilize the organic fermentation products that plague traditional fermentative  $\text{H}_2$  processes. In principle, almost all of the electrons and energy in biomass can be converted to  $\text{H}_2$  by a combination of fermentation and an MEC.



**Figure 3.** Schematic of a microbial electrolytic cell (MEC). An MEC is similar to an MFC (Fig. 2), except that  $\text{O}_2$  is excluded from the cathode compartment so that the electrons reduce  $\text{H}^+$  to form  $\text{H}_2$  as the energy output. A small amount of electrical energy is consumed to boost the potential high enough to produce  $\text{H}_2$  at the anode (not shown).

## Microbial Biomass as the Fuel

The previous section described how microbial systems convert energy from diffuse and inconvenient forms of biomass to useful forms that naturally separate themselves from the water:  $\text{CH}_4$ ,  $\text{H}_2$ , and electricity. In those cases, the microorganisms are not the energy output, but the means to channel electrons to a desired end product. This section focuses on microorganisms being the high-value energy output themselves. In particular, they are photosynthetic microorganisms that capture sunlight energy and concentrate it in lipids that can be used to produce biodiesel. Biodiesel directly replaces petroleum-based diesel fuel for transportation and other uses that demand a very high-density fuel.

Biodiesel can be produced from three sources (Gilman, 2007). The first is residual fats from food preparation. Waste fat from deep-fry cooking is a popular example today; Willie Nelson drives a car that runs on grease-trap biodiesel. The second source of biodiesel comes from lipids extracted from high-oil plants, such as soybeans, sunflowers, and *Jatropha*. The third source is lipids extracted from photosynthetic microorganisms, which include algae and cyanobacteria.

The most common source of biodiesel today is from waste cooking fats. This is an excellent way to capture the resource value of an otherwise waste material, but the quantity is miniscule compared to society's needs to replace fossil fuel used in transportation. Oils extracted from plants and photosynthetic microorganisms have the potential to produce much more significant quantities of biodiesel and are where the serious attention must be directed. How do plants and microorganisms compare?

Table III compares plants and photosynthetic microorganisms for features important to their ability to displace petroleum as a source of high-density, liquid fuel. While high-oil plants are a good source of lipids for conversion to biodiesel, photosynthetic microorganisms (algae or cyanobacteria) offer many significant advantages. The most important one is the last row in the table: a roughly 100-fold higher yield of lipids per hectare. This remarkably higher lipid yield comes from the other features of photosynthetic microorganisms: a much shorter doubling time, continuous harvesting, and a more homogeneous physical structure. When combined with the fact that photosynthetic microorganisms do not require arable land and need not compete with food production, the factor of  $\sim 100$ -fold higher yield means that microbial biodiesel has the potential to displace large amounts of fossil fuel for powering automobiles, trucks, airplanes, and power plants.

Microbial biodiesel is beginning to attract attention and investment today. At this point, two important questions confront scientists, engineers, and investors in microbial biodiesel. The first question is about which photosynthetic microorganism to use. Most efforts today focus on photosynthetic algae, such as *Chlorella* and *Spirulina*. Probably this attention on algae occurs because algae are well known as

**Table III.** A comparison of plants and photosynthetic microorganisms as a source of high-lipid material to make biodiesel.

Feature	High-lipid plants <sup>a</sup>	Photosynthetic microorganisms <sup>b</sup>
Doubling time	Relatively long, weeks	Relatively short, ~1 day
Needs arable land?	Yes	No
Harvesting	Seasonal, 1 or 2 crops per year	Continuous
Biomass quality—homogeneity	Heterogeneous, with leaves, stems, seeds, roots, etc.	Homogeneous
Biomass quality—lignocellulose	Yes	No
Water use	High due to evapotranspiration	Low to moderate if controlled
Fertilizer use	High use rate and subject to runoff	Amenable to nutrient capture and recycle
Areal yield of lipids <sup>c</sup>	Low <1,000 L ha <sup>-1</sup> year <sup>-1</sup>	High ~100,000 L ha <sup>-1</sup> year <sup>-1</sup>

<sup>a</sup>High-lipid plants considered here are soybeans and sunflowers.

<sup>b</sup>Photosynthetic microorganisms include algae and cyanobacteria.

<sup>c</sup>Yields per unit area are based on Huber et al. (2006).

photosynthetic microorganisms. The alternative is photosynthetic bacteria, such as the cyanobacterium *Synechocystis*. Both types of photosynthetic microorganisms can accumulate high levels of lipids, for example, ~25% in wild-type strains (Ahlgren et al., 1992). However, they differ in other ways that may be important to biodiesel production.

Algae accumulate large quantities of lipid as storage materials, but they do this when under stress and growing slowly. By contrast, cyanobacteria accumulate lipids in thylakoid membranes, which are associated with high levels of photosynthesis and a rapid growth rate. Thus, photosynthetic bacteria have a natural advantage for producing lipids at a high rate. Furthermore, cyanobacteria, being prokaryotes, can be improved by genetic manipulations much more readily than can eukaryotic algae (Vermaas, 1998). For example, Vermaas and colleagues (W. Vermaas, personal communication) have produced a single-gene mutant of *Synechocystis* that accumulates up to 50% of its dry weight in lipids.

The second question concerns the type of reactor system to employ. The trade off is between capital costs versus the rate and reliability of biomass production. The simplest systems for growing photosynthetic microorganisms are large, open ponds or raceways (e.g., Borowitzka, 1999; Christi, 2007; Pulz, 2001). They keep down the capital costs, but offer limited control over the growth conditions, evaporative water loss, and invasion of non-desired species. Enclosed photobioreactor systems (e.g., Borowitzka, 1999; Christi, 2007; Hai et al., 2000; Morita et al., 2001; Pulz, 2001) can overcome the limitations of the open ponds, but incur increased capital costs. Furthermore, enclosed systems vary widely, ranging from transparent bags exposed to the sun to highly sophisticated photobioreactor systems that employ the latest technology for monitoring, control, light collection, water management, and biomass harvesting.

It is far too early to judge which systems are going to prevail—algae versus cyanobacteria, open ponds versus sophisticated photobioreactor systems. Perhaps a number of systems will succeed, providing good options for producing renewable biodiesel at rates large enough to displace petroleum. Because of the advantages stated above, my team is focusing on biodiesel from *Synechocystis*. My team

also is devoting its efforts to sophisticated, closed photobioreactors, since they have the greatest potential to exploit the advantages of *Synechocystis* that has very high lipid content.

For any of the approaches to microbial biodiesel, several important challenges must be met successfully if the benefits are to be obtained without introducing environmental or economic disbenefits. One challenge is locating the production facilities in areas with suitably high sunlight and warm temperatures to spur maximum production. Arid and semi-arid regions seem to be the most favorable, and they can have the added benefit of not taking out of service high-grade arable land. A second issue is water management, particularly in arid regions where water is scarce. Clearly, uncontrolled evaporative losses are not allowable, and this leads to the use of closed photobioreactor systems. A third issue is the source of CO<sub>2</sub>. While it is possible to obtain CO<sub>2</sub> from the atmosphere, growth rates will be higher if a concentrated stream of CO<sub>2</sub> is available. Therefore, co-location of the photobioreactors near a power plant is desirable. Finally, the cost of the biodiesel product must be competitive. While the meaning of “competitive” is going change with time, research and development work must focus on keeping the unit costs down, since liquid fuels present a large commodity market.

A final issue about microbial biodiesel concerns the biomass that is not lipids. Even if 50% of the biomass is lipids that are captured for biodiesel, the other 50% of the biomass should be converted to useful products. The most obvious use for the carbohydrate and protein fractions of the microbial biomass is conversion to CH<sub>4</sub>, H<sub>2</sub>, or electricity by the microbial conversion processes described earlier. On top of producing biodiesel, photosynthetic microorganisms produce a lot more energy value when they become a major feedstock for the other microbial energy-conversion processes.

## Conclusion

Global warming is upon us today and will accelerate unless we take action to reduce the net addition of CO<sub>2</sub> to the



atmosphere. The only hope for achieving a major slowing and ultimately a reversal in net CO<sub>2</sub> accumulation is greatly reducing the combustion of fossil fuels. Fossil-fuel use will decline only when society comes up with renewable, C-neutral alternatives in very large quantity. One of the best options in the long term is bioenergy, in which the sun's energy is captured as biomass and converted to useful energy forms.

Successful bioenergy faces two serious challenges. The first is producing enough biomass-derived fuel to replace a significant fraction of the ~10 TW of energy generated today from fossil fuels. The second challenge is producing the bioenergy without incurring serious damage to the environment and to the food-supply system. Of the many bioenergy options on the table today, most fail on both counts. However, several microorganism-based bioenergy options have the potential to produce renewable energy on a large scale, without disrupting the environment or human activities.

Different microbial communities can convert the energy value of various biomass residuals to socially useful energy forms that naturally come out of water: methane, hydrogen, and electricity. Society generates biomass residuals from agricultural, animal, and a variety of industrial operations, as well as from human wastes. These residuals have large energy value and can replace a significant portion of our use of fossil fuel. Microbial methane production is well established, but can be improved. The other energy outlets are very promising, but still at the research stage.

Perhaps the largest potential for C-neutral energy comes from the conversion of sunlight into high-value photosynthetic microorganisms. Certain algae (eukaryotes) and cyanobacteria (prokaryotes) have high lipid contents that can be extracted and converted to biodiesel. Under proper conditions, systems employing photosynthetic microorganisms can produce lipids for biodiesel at rates 100 times or more greater than is possible with any plant system. In addition, the non-lipid biomass can be converted to methane, hydrogen, or electricity by the microbial conversion processes. The high per-area yields of lipids and other biomass by photosynthetic microorganisms, coupled with the fact that they do not require arable land, make it feasible to produce bioenergy at rates high enough to replace a substantial fraction of our society's use of fossil fuels.

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