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REVIEW

Biodiesel production—current state of the art and challenges

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Abstract Biodiesel is a clean-burning fuel produced from grease, vegetable oils, or animal fats. Biodiesel is produced by transesterification of oils with short-chain alcohols or by the esterification of fatty acids. The transesterification reaction consists of transforming triglycerides into fatty acid alkyl esters, in the presence of an alcohol, such as methanol or ethanol, and a catalyst, such as an alkali or acid, with glycerol as a byproduct. Because of diminishing petroleum reserves and the deleterious environmental consequences of exhaust gases from petroleum diesel, biodiesel has attracted attention during the past few years as a renewable and environmentally friendly fuel. Since biodiesel is made entirely from vegetable oil or animal fats, it is renewable and biodegradable. The majority of biodiesel today is produced by alkali-catalyzed transesterification with methanol, which results in a relatively short reaction time. However, the vegetable oil and alcohol must be substantially anhydrous and have a low free fatty acid content, because the presence of water or free fatty acid or both promotes soap formation. In this article, we examine different biodiesel sources (edible and nonedible), virgin oil versus waste oil, algae-based biodiesel that is gaining increasing importance, role of different catalysts including enzyme catalysts, and the current state-of-the-art in biodiesel production.

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Introduction

Biodiesel is a clean-burning fuel currently being produced from grease, vegetable oils, or animal fats. Its chemical structure is that of fatty acid alkyl esters. Biodiesel is produced by transesterification of oils with short-chain alcohols or by the esterification of fatty acids. The transesterification reaction consists of transforming triglycerides into fatty acid alkyl ester, in the presence of an alcohol, such as methanol or ethanol, and a catalyst, such as an alkali or acid, with glycerol as a byproduct [20]. Chemical reaction at supercritical conditions without the use of a catalyst has also been proposed [36].

In the United States, oil is the fuel of transportation. Coal, nuclear, hydropower, and natural gas are primarily used for electric power generation. The United States with 5% of the world's population, consumes 25% of the world's petroleum, 43% of the gasoline, and 25% of the natural gas. According to Oil and Gas Journal (O&GJ) estimates, worldwide reserves at the beginning of 2004 were 1.27 trillion barrels of oil and 6,100 trillion cubic feet of natural gas. These are proven recoverable reserves. At today's consumption level of about 85 million barrels per day of oil and 260 billion cubic feet per day of natural gas, the reserves represent 40 years of oil and 64 years of natural gas.

Thus, because of diminishing petroleum reserves and the deleterious environmental consequences of exhaust gases from petroleum diesel, biodiesel has attracted attention during the past few years as a renewable and

environmentally friendly fuel. Since biodiesel is made entirely from vegetable oil or animal fats, it is renewable and biodegradable. Biodiesel also contains very little sulfur, polycyclic aromatic hydrocarbons, and metals. Petroleum-derived diesel fuels can contain up to 20% polycyclic aromatic hydrocarbons. For an equivalent number of carbon atoms, polycyclic aromatic hydrocarbons are up to three orders of magnitude more soluble in water than straight chain aliphatics. The fact that biodiesel does not contain polycyclic aromatic hydrocarbons makes it a safe alternative for storage and transportation.

Like petroleum diesel, biodiesel operates in compression-ignition engines. Biodiesel is most often blended with petroleum diesel in ratios of 2% (B2), 5% (B5), or 20% (B20). It can also be used as pure biodiesel (B100). Biodiesel fuels can be used in regular diesel vehicles without making any changes to the engines, although older vehicles may require replacement of fuel lines and other rubber components. (Biodiesel has similar materials compatibility to ultralow sulfur diesel (ULSD); so vehicles built to run on that should be compatible with pure biodiesel.) It can also be stored and transported using diesel tanks and equipment. Since biodiesel is oxygenated, it is a better lubricant than diesel fuel, increasing the life of engines, and is combusted more completely. Indeed, many countries are introducing biodiesel blends to enhance the lubricity of low-sulfur diesel fuels [1]. The higher flash point of biodiesel makes it a safer fuel to use, handle, and store. With its relatively low emission profile, it is an ideal fuel for use in sensitive environments, such as heavily polluted cities.

There are several technical challenges that need to be addressed to make biodiesel profitable. First, the high cost of virgin vegetable oil as the source of triglycerides plays a large role in process profitability. To reduce production costs and make it competitive with petroleum diesel, low cost feedstocks, such as nonedible oils, waste frying oils, and animal fats, could be used as raw materials. However, the relatively higher amounts of free fatty acids and water in this feedstock results in the production of soap in the presence of alkali catalyst. Thus, additional steps to remove any water and either the free fatty acids or soap from the reaction mixture are required. In fact, commercial processors often employ an acid-catalyzed esterification reactor to process excess free fatty acids prior to base-catalyzed transesterification.

Considerable research has been done on biodiesel made from virgin vegetable oils (e.g., soybean oil, sunflower oil, rapeseed oil) using alkali catalysts. The majority of biodiesel today is produced by alkali-catalyzed (e.g., NaOH, KOH) transesterification with methanol, which results in a relatively short reaction time [16]. However, the vegetable oil and alcohol must be substantially anhydrous and have a low free fatty acid content, because the presence of water or free fatty acid or both promotes soap formation. The soap formed lowers the yield of esters and renders the downstream separation of the products difficult [16], requiring additional processing.

In this review article, we examine different biodiesel sources (edible and nonedible), virgin oil versus waste oil, algae-based biodiesel that is gaining increasing importance, the role of different catalysts including enzyme catalysts, and the current state-of-the-art in biodiesel production.

Edible sources—virgin oil

Biodiesel production from soybean oil is very popular. Researchers have focused on different catalyst systems, different solvents, and different acyl acceptors. Soybean oil has five fatty acids: approximately equal amounts of palmitic acid, oleic acid, and linolenic acid (about 13% each), linoleic acid (approximately 55%), and stearic acid (approxmately 4%). The average US production of soybean oil from 1993 to 1995 was 6.8 billion kg, and in 2002, soybeans were harvested from more than 30 million ha across the United States, which accounts for 40% of the total world soybean output [26]. This production capacity accounts for more than 50% of the total available biobased oil for industrial applications. A useful industrial application of soybean oil is in biodiesel blends. According to Kinney and Clemente [26], soybean oil-derived biodiesel possess enhanced biodegradation, increased flashpoint, reduced toxicity, lower emissions, and increased lubricity. However, oxidative instability and cold flow in northern climates limit the usefulness of a soybean oil-derived biodiesel as a fuel. The tools of biotechnology could be utilized to modify the fatty acid profile of soybean for performance enhancement, which may increase the attractiveness of biodiesel derived from this commodity crop [26]. There is still some disagreement in the literature over the oxidative stability of biodiesel, and in particular how well the "iodine value" characterizes its stability. The iodine value is a measure of the level of "unsaturation" of the fatty acids in the oil, with more saturated fatty acids being less susceptible to oxidation. However, other factors also significantly affect the stability, such as the level of natural antioxidants (such as vitamin E) in the fuel [15].

Soybean oil has a high iodine value compared to many other biodiesel feedstocks (indicating a relatively low level of saturation compared to other oils, such as rapeseed and canola), but Mushrush et al. [30] conducted storage stability tests and found soybean biodiesel (in concentrations up to 20%) to be stable in the "stable" fuel and to reduce the instability in the "unstable" fuel significantly. In

addition to fuel storage stability, fuel solubility, and oxidative stability, seawater stability should also be taken into consideration in water environments [31].

According to Mushrush et al. [31], US Navy shipboard fuel tanks compensate for diminishing fuel by the addition of seawater to the fuel tank. The authors found that this can lead to "fuel instability problems such as filter stoppage and other serious engine damage." Presence of trace fatty acids in the oil and seawater led to the formation of a soapy emulsion at the interphase. When using recycled oil, care should be taken to remove all acidic components or the biodiesel will not be stable [32].

Freedman et al. [16] have investigated the effect of the molar ratio of the alcohol to oil, type of catalyst (base vs acid), temperature and degree of refinement of the oil on the yield of biodiesel. They reported a 98% yield of biodiesel in 1 h using alkali catalysts such as sodium hydroxide or sodium methoxide with alcohols such as methanol, ethanol, and iso-butanol [17]. For the alkali-catalyzed reaction, the effect of alcohol to oil ratio was found to be the most important variable affecting the yield, while temperature had a significant effect on the initial reaction rate. Their study also shows that acid catalysts would be more effective when the degree of refinement of oil was low, and for oils that had a high free fatty acid content.

Enzyme catalysts

Biocatalysts are gaining more attention nowadays and have the potential to outperform chemical catalysts for biodiesel production in the future. New biochemical routes to biodiesel production, based on the use of enzymes, have become very interesting [6, 9, 18, 27, 33, 37, 39]. Most of the articles published have used a variety of substrates such as rice bran oil, canola, sunflower oil, soybean oil, olive oil, and castor oil. Several lipases from microbial strains, including *Pseudomonas fluorescens* [22, 40], *Pseudomonas cepacia* [10], *Rhizomucor miehei* [40], *Rhizopus oryzae* [28], *Candida rugosa* [7], *Thermomyces lanuginosus* [48], and *Candida antarctica* [27], have been reported to have transesterification activity.

Lipase has been shown to be effective in the transesterification of sunflower oil in a solvent-free medium [2]. One problem that arose was the inhibition of the enzyme due to glycerol formation. A number of different acyl acceptors have shown to be effective with lipase as the catalyst. Methanol and ethanol are the most commonly used alcohols. Longer chain alcohols have also been shown to be effective, but they provide lower yields than methanol. Recent studies using methyl acetate as the acyl acceptor and soybean oil show that the use of this acyl acceptor does not lead to inhibition of the enzyme [11]. Also, since no glycerol is produced in the process, this method is very convenient for recycling the catalyst, and byproduct triacetylglycerol shows no negative effect on the fuel property [47].

The results of biodiesel production by transesterification of olive oil using lipase as a catalyst were recently reported [37]. The final conversion and yield of biodiesel were unaffected by initial enzyme concentrations above 500 U/ml olive oil. The optimum reaction temperature was 60 °C.

The effect of different solvents and three different acyl acceptors on the transesterification of triolein (as a model compound) has been recently investigated [8]. The yield of biodiesel (methyl or ethyl ester) was monitored as a function of time. The yield of the product was also determined in a solvent-free system for two different modes of stirring. The results indicated that the highest yield was obtained in a solvent-free system with mechanical stirring. Methyl acetate was also effective as a solvent and acyl acceptor.

Other catalyst systems

In an attempt to reduce the problems with separation and soap formation, some nonenzymatic heterogeneous catalysts have been investigated. ZrO_2, ZnO, SO_4^{2-}/SnO_2, SO_4^{2-}/ZrO_2, KNO_3/KL zeolite, and KNO_3/ZrO_2 are some solid catalysts that were studied in the transesterification of palm and coconut oil [23]. The reaction was carried out at 200 °C, 50 bar, 3 wt% catalyst, and a 6:1 molar ratio of methanol to oil. All the solid catalysts exhibited some activity for both palm and coconut oil. The sulfonated metal catalysts gave the highest fatty acid methyl ester yields overall. ZrO_2 gave an 86.3% yield for coconut oil and 90.3% yield for palm oil. The study shows that SO_4^{2-}/ZrO_2 is deactivated quickly but can easily be regenerated.

Other sulfonated solid catalyst can be used to catalyze the transesterification reaction. Recently, one of the more interesting sulfonated solid catalysts was derived from amorphous carbon [42]. Carbon rings present in compounds such as starches and sugars provide a large number of sites available for sulfonation. Studies were performed using glucose and sucrose as carbon sources. The carbon source was pyrolyzed at low temperatures resulting in carbon rings. The sheets were then sulfonated by sulfuric acid. The result is an inexpensive solid catalyst that has properties similar to Nafion®. The authors show that it is an effective catalyst for the esterification of oleic and stearic acid. They claim an activity greater than half that of sulfuric acid and greater than regular solid catalysts at 80 °C. If true, this catalyst offers an inexpensive alternative to immobilized enzyme catalysts. However, studies carried

out in our laboratory both with virgin oil and waste oil showed substantially lower yields compared to enzyme catalysts. In these studies, the catalyst was made by a similar technique, which involved pyrolizing the sugar first and then sulfonating it. Sucrose was placed in test tubes in a tube furnace and was heated to 375 °C for a period of 15 h. The result was a black powder, which was ground using a mortar and pestle. The black powder was combined with 150 mL of 96 wt% sulfuric acid and was heated to 150 °C for 15 h. The solution was then vacuum-filtered using glass wool filters. The solid was washed with distilled/deionized water until the pH of the wash water was near neutral. Experiments were run with triolein, olive oil, and used olive oil as the source. The reactions were carried out at 85 °C with 0.05 g of the sugar catalyst. An 8:1 molar ratio of methanol to triolein was used. The yields in all cases were very small compared to Novozym 435. A high temperature was used, because runs at 40 °C showed an even smaller yield.

Other catalyst systems have also been investigated. Xie and Huang [46] have reported the synthesis of biodiesel from soybean oil using KF/ZnO catalyst. The catalyst with 15% KF loading and that calcined at 873 K showed the optimum activity. The results showed that the activity of the catalysts correlated well with their basicity. Wang and Yang [44] investigated the transesterification of soybean oil with nano-Mgo in supercritical and subcritical methanol. The authors report an increase in the transesterification rate when nano-MgO was added from 0.5 to 3 wt%.

Other recent advances

Recently, Fabbri et al. [14] reacted soybean oil with di-Me carbonate (DMC), which avoided the coproduction of glycerol. The main difference between the biodiesel like material, which the authors call DMC-BioD, and biodiesel produced from vegetable oil and methanol (MeOH-biodiesel) was the presence of fatty acid glycerol carbonate monoesters (FAGCs) in addition to FAMEs. The authors report that the presence of FAGCs influenced both fuel and flow properties, while the distribution of main pyrogenic compounds, including polycyclic aromatic hydrocarbons (PAHs), was not affected.

Dubé et al. [12] have developed a membrane reactor to produce biodiesel from canola oil and methanol via both acid- and base-catalysis. Several tests, using food-grade canola oil, were performed in the semibatch two-phase membrane reactor at various temperatures, catalyst concentrations, and initial feed loadings. The novel two-phase membrane reactor was particularly useful in removing unreacted canola oil from the fatty acid methyl ester product yielding a high purity biodiesel. In a recent article [5], canola oil was transesterified using methanol and caustic in a reactor with membranes of varying pore sizes. It was shown that all the membranes could retain the canola oil in the reactor, which indicated that the oil droplets present in the reactor were larger than all the pore sizes tested.

Other vegetable oils that have been used in biodiesel production include corn, sunflower, cottonseed, peanuts, canola, and rapeseed. However, expanding the use and production of a particular feedstock must be evaluated in terms of the environmental and economic impacts. According to a recent United Nations report, the global rush to switch from oil to energy derived from plants will drive deforestation, push small farmers off the land, and lead to serious food shortages and increased poverty unless carefully managed. The United Nations report points to crops like palm oil, maize, sugar cane, and soya and urges governments to beware of their human and environmental impacts, some of which could have irreversible and damaging consequences. Thus, it makes sense to examine biodiesel production from waste oil and other nonedible sources. This will be done in the following sections.

Waste oil

Several studies have been done on the production of biodiesel from waste oils or animal fats [45] describing the feasibility of making quality biodiesel from this feedstock while identifying the problems with the free fatty acids present in the raw materials. The presence of free fatty acids and water in this feedstock results in the production of soap in the presence of alkali catalyst. Thus, additional steps to remove any water and either the free fatty acids or soap from the reaction mixture are required. Despite the lower reaction rate associated with sulfuric acid-catalyzed transesterification processes, this approach has several advantages over the base-catalyzed method [4]: it employs a one-step process as opposed to a two-step process; it can handle feedstock with a high free fatty acid content; downstream separation of the biodiesel is straightforward; and a high quality glycerol byproduct is produced.

The acid-catalyzed process suffers from a number of drawbacks. In addition to the low reaction rate, a drawback of the acid-catalyzed process is the requirement for the reactor to withstand an acidic environment. Yet another drawback to the acid-catalyzed process is that high alcohol-to-oil ratios are necessary to promote the conversion of oil to fatty acid alkyl ester [17]. In their study on acid-catalyzed transesterification of soybean oil, Canakci and Van Gerpen [4] found that water strongly inhibits the ester-formation reaction. They recommended that the concentration of water in the reaction mixture should be

less than 0.5%. Therefore, water formed by the esterification of free fatty acid would limit the presence of free fatty acid in oil to 5%. However, this is highly dependent on the amount of alcohol present.

The use of insoluble solid catalysts (such as immobilized enzymes) facilitates its removal from the glycerol and fatty acid alkyl ester products and leads to a reduction in waste material requiring disposal. The biggest advantage of enzyme catalysts is the absence of soap formation. Aside from enzymes, several researchers have attempted to use acid or alkali solid catalysts (e.g., zinc and calcium oxides, calcium and barium acetates, hydrotalcite, NaX faujasites, titanosilicate structure-10, calcium carbonate rock, tungstated zirconia-alumina) [19]. Almost all the catalysts require temperatures in excess of 200 °C to achieve conversions greater than 90% within the time scale of the experiment. Recently, mesoporous silica multifunctionalized with both organosulfonic acid and hydrophobic organic groups such as allyl and phenyl was shown to be effective in esterifying free fatty acids while excluding water, a byproduct that inhibits the reaction, from the proximity of the active sites [29]. Such a catalyst seems promising because of its relatively high surface area, flexible pore size, and its potential for controlling catalytic functionalities at the molecular level.

One of the authors, Vasudevan and his student Xiangping Shen, have recently investigated biodiesel production by transesterification of waste olive oil with methanol and Novozym®435. Experiments were carried out to investigate the influence of the molar ratio of methanol to triolein, mode of methanol addition, reaction temperature, and mixing speed on biodiesel yield.

For waste olive oil, the experiments results indicated that a molar ratio of 9:1 for methanol to triolein resulted in the highest biodiesel yield. This ratio is higher than the stoichiometric ratio of 3:1 probably due to the presence of other fatty acids in the feed and due to the fact that waste oil was used. At ratios higher than 9:1, the yield became lower due to enzyme deactivation by methanol.

Stepwise addition of methanol resulted in higher yields of biodiesel probably due to less inhibition of the enzyme by methanol. Higher yields of biodiesel were also obtained at a reaction temperature of 60 °C, which resulted in higher reaction rates and lower inhibition of the enzyme active sites by methanol. Mixing speed in the range 100–400 rpm had relatively little effect on the yield. The effect of different acyl acceptors or solvents or both on biodiesel yield was also evaluated. The highest yields were obtained when *tert*-butanol and methanol were both present as solvent/acyl acceptor perhaps due to the synergy that resulted as a result of better dispersion of the oil in the mixture.

The efficacy of Novozym®435 was also determined by reusing the enzyme after washing it with a solvent. The results showed that enzyme was very stable and still retained a high activity after several runs.

Wang et al. [43] investigated lipase-catalyzed alcoholysis of soybean oil deodorizer distillate (SODD) for biodiesel production. In this system, free fatty acids and glycerides were converted to biodiesel simultaneously. Butanol was adopted as the reaction medium in which the negative effects caused by excessive methanol and byproduct glycerol were eliminated. There was no obvious loss in lipase activity even after 120 cycles. Studies by Vasudevan and Shen have not demonstrated such high enzyme stability.

The addition of a cosolvent to generate a homogeneous reaction mixture has been discussed [3]. While this enhances reaction rate significantly, the cosolvent must eventually be separated from the biodiesel and this requires additional processing. Another issue that has an adverse effect on biodiesel production is the removal of residual triglycerides and glycerol from the biodiesel product. The employment of multiple water wash steps creates an environmental challenge due to the need to treat the wastewater. The presence of unreactable materials in waste oil leads to poor flow properties of the biodiesel in cold weather. The use of homogeneous base catalysts coupled with the presence of free fatty acids and the chemical nature of the reaction components serve to yield a low quality glycerol byproduct.

If the goal is to reduce or eliminate the formation of soap and/or to process more waste oil and produce high quality biodiesel and glycerol, then enzyme catalysis is very attractive. Unfortunately, the process is not economically viable. In 2005, Novozymes (Bagsværd, Denmark) in conjunction with National Renewable Energy Labs (NREL) announced a 30-fold enzyme cost reduction in the conversion of pretreated corn stover to ethanol. The cost of the enzyme was approximately $0.10/gal of ethanol. A similar reduction in the cost of lipase would make enzymatic transesterification/esterification process economically very viable. In fact, current research in our laboratory and other laboratories is focused on ways to minimize inactivation of the enzyme by methanol. This can be achieved by utilizing different acyl acceptors and solvents (such as *tert*-butanol or higher alcohols), which in turn will increase the number of times the enzyme can be regenerated and reused. Thus, if better solvents are developed that minimize enzyme deactivation and/or if better enzymes are made through directed evolution resulting in an increase in the number of regenerations, then the cost of the enzyme can be proportionally higher. Elimination of solvents and the use of a single acyl acceptor-solvent will also lead to a reduction in costs.

There is also renewed focus on finding alternate uses for the byproduct glycerol or to convert glycerol to

more useful products (including methanol or ethanol) via fermentation. Focus should also be on technologies to improve the conventional process for biodiesel production by perhaps utilizing membrane reactors to handle waste oil.

Nonedible sources

Nonedible oils, like Jatropha, Pongamia, Argemone, Castor, Sal, etc., can be used for the production of biodiesel. Jatropha curcas has tremendous potential for biodiesel production. A tropical plant that grows in low to high rainfall areas (rainfall as little as 25 cm per year) can be used to reclaim marginal soil.

Shah et al. [38] have investigated three different lipases (*Chromobacterium viscosum, Candida rugosa*, and *Porcine pancreas*) for transesterification of Jatropha oil in a solvent-free system to produce biodiesel; only lipase from *Chromobacterium viscosum* was found to give appreciable yield. Immobilization of lipase (*Chromobacterium viscosum*) on Celite-545 enhanced the biodiesel yield to 71% with a process time of 8 h at 40 °C.

Tiwari et al. [41] optimized the three important reaction variables in biodiesel production—methanol quantity, acid concentration, and reaction time for reduction of free fatty acid (FFA) content of Jatropha curcas oil. The optimum combination for reducing the FFA of Jatropha curcas oil from 14% to less than 1% was found to be 1.43% v/v H_2SO_4 acid catalyst, 0.28 v/v methanol-to-oil ratio, and 88-min reaction time at a reaction temperature of 60 °C. This process gave an average yield of biodiesel of more than 99%. The fuel properties of Jatropha biodiesel were found to be comparable to that of diesel.

Karmee and Chadha [24] have investigated biodiesel production from the nonedible oil of *Pongamia pinnata* by transesterification of the crude oil with methanol and KOH as catalyst. A maximum conversion of 92% (oil to ester) was achieved using a 1:10 molar ratio of oil to methanol at 60 °C. When tetrahydrofuran was used as cosolvent, the conversion increased to 95%. Important fuel properties of methyl esters of Pongamia oil biodiesel compared well with ASTM standards.

Algae-based biodiesel

There is growing interest in algae-based biodiesel especially as more states in the United States mandate blending biodiesel with petroleum diesel. In the following paragraphs, we examine the pros and cons of algae-based biodiesel. It is important to keep in mind that any biofuel is ultimately a means of collecting solar energy and storing it in an energy dense chemical. To make such a system as efficient as possible, it is beneficial to understand the entire process from beginning to end.

Photosynthesis begins with a photon being captured by a 2p electron in a ring of conjugate double bonds within a pigment molecule (with the 2p electron being part of a conjugate pi bond), causing a π–π^* excitation (where the energy level of this excitation determines the wavelength of light that can be "harvested," with the pigments in photosynthetic organisms allowing the capture of photons with wavelengths from 400 to 700 nm). Recently published research [13] appears to finally explain the near 100% efficiency with which this captured energy is transmitted to the reaction center of a chloroplast. Their observation of coherent electronic oscillations between donor and acceptor pigment molecules (classically viewed as exchanging energy through virtual photon emission and absorption) demonstrates the wavelike behavior of the excitation energy transfer through the chromophore, accounting for almost loss-less energy transmission.

At the reaction center, the excitation energy is used to split CO_2 and H_2O molecules, ultimately producing carbohydrates (through the many steps of the Calvin cycle), with an overall process that can be summarized as

$$6CO_2 + 12H_2O + \text{photons} \rightarrow C_6H_{12}O_6 + 6O_2 + 6H_2$$

A crude analysis of the quantum efficiency of photosynthesis can be done without getting into the details of the Calvin cycle; rather simply by looking at the photon energy required to carry out the overall reaction and the energy of the products. The Z-scheme, well-established in photosynthesis research, indicates that eight photons must be absorbed to split one CO_2 and two H_2O molecules, yielding one base carbohydrate (CH_2O), one O_2 molecule, and one H_2O (which, interestingly, is not made of the same atoms as either of the two input H_2O molecules).

With the average energy of "photosynthetically available radiation (PAR)" photons being roughly 217 kJ, and a single carbohydrate (CH_2O) having an energy content taken to be one-sixth that of glucose [$(CH_2O)_6$], or 467 kJ/mole, we can make a rough maximum efficiency of 26.9% for converting captured solar energy into stored chemical energy. With PAR accounting for 43% of incident sunlight on earth's surface, the quantum limit (based on eight photons captured per CH_2O produced) on photosynthetic efficiency works out to roughly 11.6%.

In reality, most plants fall well below this theoretical limit, with global averages estimated typically between 1 and 2%. The reasons for such a difference generally revolve around rate limitations due to factors other than light (H_2O and nutrient availability, for example), photosaturation (some plants, or portions of plants receive more sunlight than they can process while others receive less

than they could process), and Rubisco (the protein that serves ultimately as a catalyst for photosynthesis) also accepting atmospheric O_2 (rather than CO_2), resulting in photorespiration, releasing some of the already captured carbon.

In the United States, the average daily incident solar energy (across the entire spectrum) reaching the earth's surface ranges from 12,000 to 22,000 kJ/m^2 (varying primarily with latitude). If the maximum photosynthetic efficiency is 11.6%, then the maximum conversion to chemical energy is around 1,400–2,550 kJ/m^2/day, or 3.8×10^{12} J/acre-year in the sunniest parts of the country. Assuming the heating value of biodiesel to be 0.137 GJ/gal, the maximum possible biodiesel production in the sunniest part of the United States works out to be approximately 28,000 gal/acre-year, assuming 100% conversion of algae biomass to biodiesel, which is infeasible.

It is important to keep in mind that this is strictly a theoretical "upper limit" based on the quantum limits to photosynthetic efficiency, and does not account for factors that decrease efficiency and conversion, or the efficiency with which algae convert carbohydrates into triglycerides (which is not well quantified at this point, and is dependent on many environmental factors). Based on this simple analysis though, it is clear that claims of algal biodiesel production yields in excess of 40,000 gal/acre-year or higher should be viewed with considerable skepticism. While such yields may be possible with artificial lighting, this approach would be ill-advised, as at best only about 1% of the energy used to power the lights would ultimately be turned into a liquid fuel (clearly, one needs to look at the overall efficiency).

This upper limit also allows us to assess how truly inefficient many crops are when viewed strictly as biofuel producers. With soybeans yielding on average 60 gal of oil (and hence biodiesel) per acre-year, the actual fuel production is staggeringly small in comparison to the amount of solar energy available. This should further make it clear that using typical biofuels for the purpose of electricity generation (as opposed to the transportation sector) is an inefficient means of harnessing solar energy. Considering that photovoltaic panels currently on the market achieve net efficiencies (for solar energy to electrical energy) of the order of 15–20%, with multilayer photovoltaics and solar thermal-electric systems achieving efficiencies twice that in trial runs, biomass to electricity production falls far behind (considering typical plant photosynthetic efficiencies of 1–2%), with conversion of that biomass energy to electrical energy dropping the net efficiency to well under 1%.

Viewed in this light, it becomes clear that biofuels must offer some other benefits in addition to fuel production, to be energetically (or economically) appealing, in terms of how efficiently we can harness an energy source (solar energy) and turn it into a higher value form. Corn and soy, which dominate US agriculture, have long been grown for producing animal feed. The emerging ethanol and biodiesel industries, which have primarily relied on these crops, are ultimately a coproduct from crops grown as a food source for humans and animals. But, the relatively low net photosynthetic efficiency of the crops, and low total fuel yields, means that neither is a desirable approach if our goal is producing more fuel than that could be produced from those crops as a coproduct of animal feed production.

As the search for other feedstocks continues, it would be desirable to look for crops that can give a high net conversion of solar energy to energy in the form of fuel, while providing additional side benefits (coproducts, for example), since the net efficiency for harnessing solar energy through photosynthesis into liquid fuels is rather low.

Aquatic species such as microalgae have become appealing because of the potential for significantly higher average photosynthetic efficiency than with typical land crops, due to their aquatic environment providing them with better access to water, CO_2, and nutrients (depending on the system they are grown in). Additionally, while land crops may require substantial energy inputs for irrigation, planting, fertilization, and harvesting, these can be greatly minimized with an aquatic crop, with a well-designed system. Unfortunately, there are significant challenges to making this an economically viable energy crop.

While any form of biomass can be processed into a liquid fuel through various thermochemical processes (such as pyrolysis or gasification and Fischer-Tropsch synthesis), the energy and economic requirements of such processes are substantially greater than is required for transesterifying plant oils into biodiesel. Therefore, it is desirable to have a higher oil content to minimize processing costs (energetic and economic).

The storage of energy as oil rather than as carbohydrates slows the reproduction rate of any algae; so, higher oil strains generally grow slower than low oil strains. The result is that an open system (such as open raceway ponds) is readily taken over by lower oil strains, despite efforts to maintain a culture of higher oil algae. Attempts to grow higher oil extremophiles, which can survive in extreme conditions (such as high salinity or alkalinity) that most other strains cannot tolerate, have yielded poor results, in terms of the net productivity of the system. While an extremophile may be able to survive in an extreme condition, that does not mean it can thrive in such conditions.

Many research groups have therefore turned to using enclosed photobioreactors of various designs as a means of preventing culture collapse or takeover by low oil strains, as well as decreasing the vulnerability to temperature fluctuations. The significant downside is the much higher

capital cost of current photobioreactor designs. While such high costs are not prohibitive when growing algae for producing high value products (specialty food supplements, colorants, pharmaceutical products, etc.), it is a significant challenge when attempting to produce a low value product such as fuel. Therefore, substantial focus must be placed on designing much lower cost photobioreactors and tying algae oil production to other products (animal feed or fertilizer from the protein) and services (growing the algae on waste stream effluent to remove eutrophying nutrients, or growing nitrogen fixing algae on power plant emissions to remove NO_x emissions).

An additional challenge, when trying to maximize oil production with algae, is the unfortunate fact that higher oil concentrations are achieved only when the algae are stressed—in particular due to nutrient restrictions. Those nutrient restrictions also limit growth (thus limiting net photosynthetic efficiency, where maximizing that is a prime reason for using algae as a fuel feedstock). How to balance the desire for high growth and high oil production to maximize the total amount of oil produced is no small task. One of the goals of DOE's well-known Aquatic Species Program was to maximize oil production through nutrient restriction; however, their study showed that while the oil concentration went up, there was a proportionally greater drop in reproduction rate, resulting in a lower overall oil yield.

One approach to balancing these issues has been successfully tested on a small commercial scale (2 ha) by Huntley and Redalje [21], using a combination of photobioreactors and open ponds. The general approach involves using large photobioreactors for a "growth stage," in which an algal strain capable of high oil content (when nutrient restricted) is grown in an environment that promotes cell division (plentiful nutrients, etc.)—but which is enclosed to keep out other strains. After the growth stage, the algae enter an open raceway pond with nutrient limitations and other stressors, aimed at promoting biosynthesis of oil. The nutrient limitations discourage other strains from moving in and taking over (since they also require nutrients for cell division).

The economic picture Huntley and Redalje [21] presented is perhaps rosy due to the inclusion of substantial revenue from selling a high-value carotenoid coproduct, astaxanthin. Producing coproducts is perfectly fine and desirable; unfortunately, the potential market for a carotenoid is far smaller than the potential market for biodiesel—so, it could only help out with the economics of fuel production until that market is saturated. Since carotenoid synthesis increases with oil synthesis, the same conditions can be employed though to maximize total yield of each (resulting in an average oil yield of 25% of dry weight, using *Haematococcus pluvialis*).

The average biomass energy production reported by Huntley and Redalje [21] of 763 GJ/ha/year at their site in Hawaii works out to a net photosynthetic efficiency of just over 1%, based on an assumed average daily solar radiation of 19,300 kJ/m^2 (or 70,445 GJ/ha/year, calculated by NREL based on the National Solar Radiation Database). Unfortunately, this is not substantially different from what is routinely achieved with typical land crops. However, the average oil yield reported was 422 GJ/ha/year, roughly 0.6% of incident solar energy, equating to over 1,200 gal of biodiesel per acre-year—far better than conventional oil bearing crops.

While their trials can be counted a success by many measures, it is worth pointing out how low the yield is in terms of comparison to the potential yield based on the quantum limits of photosynthetic efficiency, as well as compared to other means for harnessing solar energy. It should be no surprise though that their yield achieved came well short of the potential yield, since nutrient depletion in the open pound phase greatly limits cell division and hence biomass production (ultimately limiting photosynthetic efficiency for converting sunlight to chemical energy). An open pond system probably could be useful in cultivating high oil algaes either through the approach taken by Huntley and Redalje [21], in which nutrient restriction in the pond prevents any form of algae from growing well (thus preventing takeover) and forcing oil concentration in the algae cultivated in a nutrient-rich photobioreactor stage, or through the use of an oil-rich "extremophile" algae that can survive in an extreme environment (such as very high salinity) that other strains cannot tolerate. One form of this approach would be engineering algae to be resistant to an inhibitor that would be dumped into an open pond to keep other strains out, but this is likely to be controversial.

It may also be possible to increase algal biosynthesis of oil by identifying the enzyme that regulates lipid production and attempting to increase its activity through genetic engineering. Acetyl-CoA carboxylase (ACCase) catalyzes the carboxylation of acetyl-CoA to maloynl-CoA, believed to be the rate-limiting step in fatty acid synthesis in plants and animals [25]. While efforts focused on genetic manipulation to increase the activity of ACCase have been going on for at least 15 years, and certainly much has been learned in that time, the research has not yet reached the stage of actually being able to substantially increase the net oil yield from algae (and thus increase the commercial viability). Most of the research has focused on developing a detailed knowledge of the enzymatic pathways for lipid biosynthesis, before beginning to pursue genetic modification. NREL has identified a gene that plays a large role in controlling ACCase activity, and has studied naturally occurring genetic mutations in algae strains that affect oil synthesis [35].

Another area where genetic engineering of microalgae could prove useful would be reducing the size of pigment antenna. Algae tend to have long pigment antennas for absorbing incident sunlight, to allow individual cells to thrive in low-light conditions. This also results in individual algae "harvesting" more energy (photons) in individual photosystems than the metabolic processes can handle, with excess energy being radiated as heat or fluorescence. In high light conditions, without good agitation to rotate the algae nearest the surface, up to 80% of incident sunlight can be wasted through this photosaturation [34]. Maximizing efficiency in high sunlight would require either physical agitation or other means to rotate algae to the solar exposure region, or shortening (through selective breeding or genetic manipulation) of the pigment antennae to reduce the amount of light harvested by each algae.

Overall, while there is significant interest in algal biodiesel; it is important to keep in mind that this is still years away from being ready for actual commercial implementation. If we want to grow high oil algaes, two approaches appear possible—the use of an "extremophile" that can tolerate extreme conditions, and therefore be grown in an open pond under those conditions (which other strains cannot tolerate), or the use of photobioreactors for keeping invasive strains out, and optimizing the growth environment. The biggest challenge with the latter approach is the capital cost of current photobioreactors. Unfortunately, the focus does not appear to be in developing lower cost photobioreactors to bring down the capital cost for building a "photobioreactor farm," which ultimately will present a barrier to commercialization. Many current designs use vertical tube systems, which require expensive metal support structures. Economic viability will likely require much simpler, less expensive systems that can be placed on the ground—such as simple troughs covered with plastic film.

Conclusions

Biodiesel is a clean-burning fuel that is renewable and biodegradable. A recent United Nations report urges governments to beware of the human and environmental impacts of switching to energy derived from plants. There should a healthy debate about turning food crops or animal feed into fuel and the consequences of the switch to biofuels needs to be carefully thought out. The focus of biodiesel production needs to be on sources like waste oil and grease, animal fats, and nonedible sources. It is important to ascertain a priori what quantities of these materials may be annually collectible, and what proportion of transportation-fuel needs could these sources supply. Current research has focused on these areas as well as on algae-based biofuels. Many technical challenges remain and these include development of better and cheaper catalysts, improvements in current technology for producing high quality biodiesel, use of solvents that are nonfossil-based, conversion of the byproducts such as glycerol to useful products such as methanol and ethanol, and development of low cost photobioreactors.

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References

1. Anastopoulos G, Lois E, Serdari A, Zanikos F, Stournas S, Kalligeros S (2001) Lubrication properties of low-sulfur diesel fuels in the presence of specific types of fatty acid derivatives. Energy Fuels 15:106–112
2. Belafi-Bako K, Kovacs F, Gubicza L, Hancsok J (2002) Enzymatic biodiesel production from sunflower oil by *Candida antarctica* lipase in a solvent-free system. Biocatal Biotransformation 20(6):437–439
3. Boocock DGB, Konar SK, Mao V, Lee C, Buligan S (1998) Fast formation of high-purity methyl esters from vegetable oils. J Am Oil Chem Soc 75(9):1167–1172
4. Canakci M, Van Gerpen J (1999) Biodiesel production via acid catalysis. Trans Am Soc Agric Eng 42:1203–1210
5. Cao P, Tremblay AY, Dubé MA, Morse K (2007) Effect of membrane pore size on the performance of a membrane reactor for biodiesel production. Ind Eng Chem Res 46:52–58
6. Chang HM, Liao HF, Lee CC, Shieh CJ (2005) Optimized synthesis of lipase-catalyzed biodiesel by Novozym 435. J Chem Technol Biotechnol 80(3):307–312
7. Chen JW, Wu WT (2003) Regeneration of immobilized *Candida Antarctica* lipase for transesterification. J Biosci Bioeng 92(2):231–237
8. Coggon R, Vasudevan PT, Sanchez F (2007) Enzyme transesterification of olive oil and its precursors. Biocatal Biotransformation 25(2–4):135–143
9. De Oliveira D, Di Luccio M, Faccio C, Dalla Rosa C, Bender JP, Lipke N, Menoncin S, Amroginski C, De Oliveira JV (2004) Optimization of enzymatic production of biodiesel from castor oil in organic solvent medium. Appl Biochem Biotechnol 113–116:771–780
10. Deng L, Xu XB, Haraldsson GG, Tan TW, Wang F (2005) Enzymatic production of alkyl esters through alcoholysis: A critical evaluation of lipases and alcohols. J Am Chem Soc 82(5):341–347
11. Du W, Yuanyuan X, Dehua L, Jing Z (2004) Comparative study on lipase-catalyzed transformation of soybean oil for biodiesel production with different acyl acceptors. J Mol Catal B Enzym 30(3/4):125–129
12. Dubé MA, Tremblay AY, Liu J (2007) Biodiesel production using a membrane reactor. Bioresour Technol 98:639–647
13. Engel GS, Calhoun TR, Read EL, Ahn T, Mancal T, Cheng Y, Blankenship RE (2007) Fleming G.R. Evidence for wavelike energy transfer through quantum coherence in photosynthetic systems. Nature 446:782–786
14. Fabbri D, Bevoni V, Notari M, Rivetti F (2007) Properties of a potential biofuel obtained from soybean oil by transmethylation with dimethyl carbonate. Fuel 86(5/6):690–697

15. Ferrari R, da Silva Oliveira V, Scabio A (2005) Oxidative stability of biodiesel from soybean oil fatty acid ethyl esters. Sci Agric 62(3):291–295
16. Freedman B, Pryde EH, Mounts TL (1984) Variables affecting the yields of fatty esters from transesterified vegetable oils. J Am Oil Chem Soc 61:1638–1643
17. Freedman B, Butterfield RO, Pryde EH (1985) Transesterification kinetics of soybean oil. J Am Oil Chem Soc 63:1375–1380
18. Fukuda H, Kondo A, Noda H (2001) Biodiesel fuel production by transesterification of oils. J Biosci Bioeng 92(5):405–416
19. Haas MJ, Foglia A (2004) Alternate feedstocks and technologies for biodiesel production. In: Knothe G, Krahl J, Van Gerpen J (eds) The biodiesel handbook. AOCS Press, Illinois, USA
20. Hoydoncx HE, De Vos DE, Chavan SA, Jacobs PA (2004) Esterification and transesterification of renewable chemicals. Topic Catal 27(1–4):83–96
21. Huntley M, Redalje D (2007) CO_2 mitigation and renewable oil from photosynthetic microbes: a new appraisal. Mitigat Adapt Strateg Glob Change 12(4):573–608
22. Iso M, Chen BX, Eguchi M, Kudo T, Shrestha S (2001) Production of biodiesel fuel from triglycerides and alcohol using immobilized lipase. J Mol Catal B Enzym 16(1):53–58
23. Jitputti J, Kitiyanan B, Rangsunvigit P, Bunyakiat K, Attanatho L, Jenvanitpanjakul P (2006) Transesterification of crude palm kernel oil and crude coconut by different solid catalysts. Chem Eng J 116:61–66
24. Karmee SK, Chadha A (2005) Preparation of biodiesel from crude oil of *Pongamia pinnata*. Bioresour Technol 96(13):1425–1429
25. Kim KH, Lopéz-Casillas F, Bai DH (1989) Role of reversible phosphorylation of acetyl-CoA carboxylase in long-chain fatty acid synthesis. FASEB J 3:2250–2256
26. Kinney AJ, Clemente TE (2005) Modifying soybean oil for enhanced performance in biodiesel blends. Fuel Process Technol 86(10):1137–1147
27. Lai CC, Zullaikah S, Vali SR, Ju YH (2005) Lipase-catalyzed production of biodiesel from rice bran oil. J Chem Technol Biotechnol 80(3):331–337
28. Matsumoto T, Takahashi S, Kaieda M, Ueda M, Tanaka A, Fukuda H, Kondo A (2001) Yeast whole-cell biocatalysts constructed by intracellular overproduction of *Rhizopus oryzae* lipase is applicable to biodiesel fuel production. Appl Microbiol Biotechnol 57(4):515–520
29. Mbaraka I, Shanks BH (2005) Design of multifunctionalized mesoporous silicas for esterification of fatty acid. J Catal 229:365–373
30. Mushrush GW, Beal EJ, Hughes, JM, Wynne, JH, Sakran, JV, Hardy, DR (2007) Biodiesel fuels: the use of soy oil as a blending stock for middle distillate petroleum fuels. http://www.biodiesel.org/resources/reportsdatabase/reports/mar/20000701_mar-021.pdf
31. Mushrush GW, Wynne JH, Lloyd CT, Willauer HD, Beal EJ (2007) Soybean biodiesel: instability reactions. Pet Sci Technol 25(9):1163–1171
32. Mushrush GW, Wynne JH, Lloyd CT, Willauer HD, Hughes JM (2007) Instability reactions and recycled soybean-derived biodiesel fuel liquids. Energy Sources A Recovery Util Environ Eff 29(5):491–497
33. Noureddini H, Gao X, Philkana RS (2005) Immobilized *Pseudomonas cepacia* lipase for biodiesel fuel production from soybean oil. Bioresour Technol 96(7):769–777
34. Polle JEW, Kanakagiri S, Jin E, Masuda T, Melis A (2002) Truncated chlorophyll antenna size of the photosystems—a practical method to improve microalgal productivity and hydrogen production in mass culture. Int J Hydrogen Energy 27(11):1257–1264
35. Roessler PG, Brown LM, Dunahay TG, Heacox DA, Jarvis EE, Schneider JC, Talbot SG, Zeiler KG (1994) Genetic engineering approaches for enhanced production of biodiesel fuel from microalgae. ACS Symp Ser 566:255–270
36. Saka S, Sukdiana D (2001) Biodiesel fuel from rapeseed oil as prepared in supercritical methanol. Fuel 80(2):225–231
37. Sanchez F, Vasudevan PT (2006) Enzyme catalyzed production of biodiesel from olive oil. Appl Biochem Biotechnol 135(1):1–14
38. Shah S, Sharma S, Gupta MN (2004) Biodiesel preparation by lipase-catalyzed transesterification of jatropha oil. Energy Fuels 18(1):154–159
39. Soumanou MM, Bornscheuer UT (2003) Lipase-catalyzed alcoholysis of vegetable oils. Eur J Lipid Sci Technol 105(11):656–660
40. Soumanou MM, Bornscheuer UT (2003) Improvement in lipase catalyzed synthesis of fatty acid methyl esters from sunflower oil. Enzyme Microb Technol 33(1):97–103
41. Tiwari AK, Kumar A, Raheman H (2007) Biodiesel production from jatropha oil (Jatropha curcas) with high free fatty acids: an optimized process. Biomass Bioenergy 31(8):569–575
42. Toda M, Takagaki A, Okamura M, Kondo J, Hayashi S, Domen K, Hara M (2005) Green chemistry: biodiesel made with sugar catalyst. Nature 438(7065):178
43. Wang L, Du W, Liu D, Li L, Dai N (2006) Lipase-catalyzed biodiesel production from soybean oil deodorizer distillate with absorbent present in tert-butanol system. J Mol Catal B Enzym 43(1–4):29–32
44. Wang L, Yang J (2007) Transesterification of soybean oil with nano MgO in supercritical and subcritical methanol. Fuel 86(3):328–333
45. Watanabe Y, Shimada Y, Sugihara A, Tominaga Y (2001) Enzymatic conversion of waste edible oil to biodiesel fuel in a fixed-bed bioreactor. J Am Oil Chem Soc 78:703–707
46. Xie W, Huang X (2006) Synthesis of biodiesel from soybean oil using heterogeneous KF/ZnO catalyst. Catal Lett 107(1/2):53–59
47. Xu Y, Du W, Liu D, Zeng J (2003) A novel enzymatic route for biodiesel production from renewable oils in a solvent-free medium. Biotechnol Lett 25(15):1239–1241
48. Xu YY, Du W, Zeng J, Liu DH (2004) Conversion of soybean oil to biodiesel fuel using lipozyme TL 1M in a solvent-free medium. Biocatal Biotransformation 22(1):45–48