

# A new dawn for industrial photosynthesis

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**Abstract** Several emerging technologies are aiming to meet renewable fuel standards, mitigate greenhouse gas emissions, and provide viable alternatives to fossil fuels. Direct conversion of solar energy into fungible liquid fuel is a particularly attractive option, though conversion of that energy on an industrial scale depends on the efficiency of its capture and conversion. Large-scale programs have been undertaken in the recent past that used solar energy to grow innately oil-producing algae for biomass processing to biodiesel fuel. These efforts were ultimately deemed to be uneconomical because the costs of culturing, harvesting, and processing of algal biomass were not balanced by the process efficiencies for solar photon capture and

conversion. This analysis addresses solar capture and conversion efficiencies and introduces a unique systems approach, enabled by advances in strain engineering, photobioreactor design, and a process that contradicts prejudicial opinions about the viability of industrial photosynthesis. We calculate efficiencies for this direct, continuous solar process based on common boundary conditions, empirical measurements and validated assumptions wherein genetically engineered cyanobacteria convert industrially sourced, high-concentration CO<sub>2</sub> into secreted, fungible hydrocarbon products in a continuous process. These innovations are projected to operate at areal productivities far exceeding those based on accumulation and refining of plant or algal biomass or on prior assumptions of photosynthetic productivity. This concept, currently enabled for production of ethanol and alkane diesel fuel molecules, and operating at pilot scale, establishes a new paradigm for high productivity manufacturing of nonfossil-derived fuels and chemicals.

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## Introduction

The capture of solar energy to power industrial processes has been an inviting prospect for decades. The energy density of solar radiation and its potential as a source for production of fuels, if efficiently captured and converted, could support the goals of national energy independence. Analyses of photosynthetic conversion have been driven by this promise (Goldman 1978; Pirt 1983; Bolton and Hall 1991; Zhu et al. 2008, 2010). The deployment of solar-

based industries for fuels has, however, been limited by the lack of efficient cost-effective technologies. Projects funded between 1976 and 1996 under the US Department of Energy (DOE) aquatic species program explored phototrophic organisms and process technologies for the production of algal oils and their refinement into biodiesel. The results of these efforts were summarized in a report that delineated the technological barriers to industrial development (Sheehan et al. 1998).

The traditional photosynthetic fuels process is one wherein triglyceride-producing algae are grown under illumination and stressed to induce the diversion of a fraction of carbon to oil production. The algal biomass is harvested, dewatered and lysed, and processed to yield a product that is chemically refined to an acyl ester biodiesel product. Many companies have been founded since the DOE final report that strive to make incremental improvements in this process to create viable solar energy-to-fuel technologies. However, many of the fundamental barriers to industrial photosynthetic efficiency remain and threaten to constrain this approach to one wherein only associated coproduct generation can salvage the process economics (Wijffels and Barbosa 2010).

Here, we reassess industrial photosynthesis in light of the development of powerful tools for systems biology, metabolic engineering, reactor and process design that have enabled a direct-to-product, continuous photosynthetic process (direct process). Many of these innovations were presaged by DOE as well as academic and industrial sources (Gordon and Polle 2007; Rosenberg et al. 2008) who suggested that these types of technological advances could enable the success of industrial photosynthesis (see Table 1 for a list of innovations and advances inherent in the direct process).

The direct process uses a cyanobacterial platform organism engineered to produce a diesel-like alkane mixture, to maximally divert fixed CO<sub>2</sub> to the engineered pathway, and to secrete the alkane product under conditions of limited growth but continuous production. This creates a process analogous to those of engineered fermentative systems that use heterotrophic organisms, e.g., yeast, *E coli*, etc., whose phases of growth and production are separated and whose carbon partitioning is controlled to achieve very high maximal productivities (for example, see Ohta et al. 1991; Stephanopoulos et al. 1998). Such processes, where cells partition carbon and free energy almost exclusively to produce and secrete a desired product while minimizing energy conversion losses due to growth-associated metabolism, have much longer process cycle times and higher system productivities than those requiring organism growth and downstream biomass harvesting and processing.

For purposes of energy conversion analysis, we compare the direct process to a conventional algal pond biomass-based process producing biodiesel esters. A simple

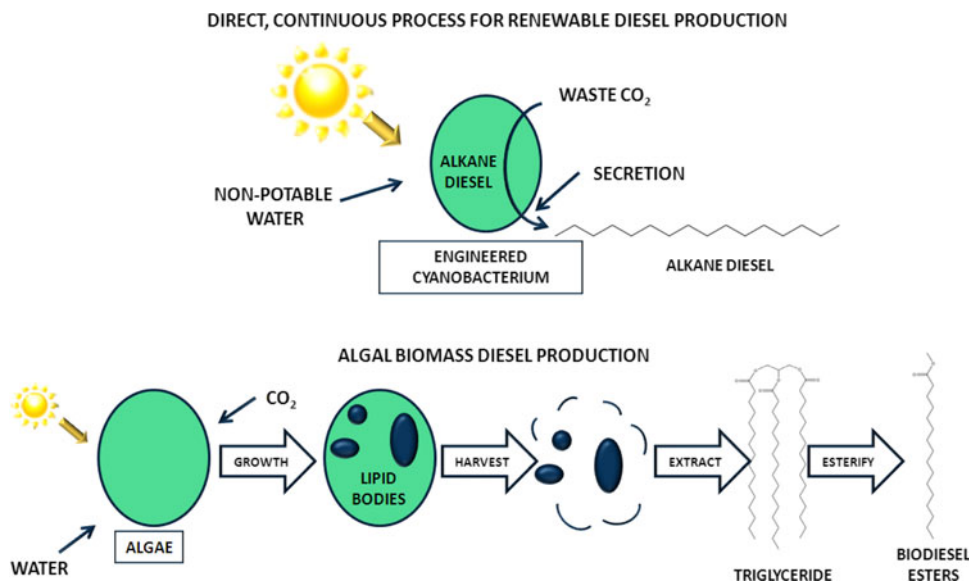
**Table 1** Technological innovations leading to high-energy capture and conversion characteristics of a direct, continuous process for photosynthetic fuel production

Process innovation	System design
Maximize energy capture and conversion by process organism	<ul style="list-style-type: none"> <li>• Metabolic engineering for recombinant pathway to directly synthesize final product</li> <li>• Gene regulation control to optimize carbon partitioning to product</li> <li>• Metabolic switching to control carbon flux during growth and production phases</li> </ul>
Minimize peripheral metabolism	<ul style="list-style-type: none"> <li>• Cyanobacterial system to obviate mitochondrial metabolism</li> <li>• Operation at high (&gt;1%) CO<sub>2</sub> to minimize photorespiration</li> </ul>
Maximize yield and productivity	<ul style="list-style-type: none"> <li>• Decoupling of biomass formation from product synthesis</li> <li>• Engineering continuous secretion of product</li> <li>• Optimization of process cycle time via continuous production</li> </ul>
Enable economic, efficient reactor and process	Photobioreactor that <ul style="list-style-type: none"> <li>• minimizes solar reflection</li> <li>• optimizes photon capture and gas mass transfer at high culture density</li> <li>• optimizes thermal control</li> </ul>

comparative illustration of the algal biomass process and the direct photosynthetic concept is shown in Fig. 1. Many analyses have been performed for the algal process (Benemann and Oswald 1994; Chisti 2007; Gordon and Polle 2007; Dismukes et al. 2008; Rosenberg et al. 2008; Schenk et al. 2008; Angermayr et al. 2009; Stephens et al. 2010; Weyer et al. 2009; Wijffels and Barbosa 2010; Zemke et al. 2010; Zijffers et al. 2010) and for photosynthetic efficiency associated with production of plant biomass (Zhu et al. 2008, 2010) and we have incorporated the relevant aspects of these published reports to bound the current analysis. Our analysis of the algal process closely follows the assumptions of Weyer et al. (2009) with the exception that we use the more common open-pond scenario. Note that we also make a clear distinction between biodiesel esters derived from algal biomass and fungible alkane diesel synthesized directly.

### Photosynthetic efficiency

The cumulative energy input and the derived energy output are critical factors in comparing processes for fuel production. In discussing energy input, photosynthesis has an



**Fig. 1** Schematic comparison between algal biomass and direct photosynthetic processes. The direct process, developed by Joule and called Helioculture™, combines an engineered cyanobacterial organism supplemented with a product pathway and secretion system to produce and secrete a fungible alkane diesel product continuously in a SolarConverter™ designed to efficiently and economically collect and convert photonic energy. The process is closed and uses industrial

waste CO<sub>2</sub> at concentrations 50–100× higher than atmospheric. The organism is further engineered to provide a switchable control between carbon partitioning for biomass or product. The algal process is based on growth of an oil-producing culture in an industrial pond on atmospheric CO<sub>2</sub>, biomass harvesting, oil extraction, and chemical esterification to produce a biodiesel ester

additional consideration. Unlike most chemical processes that scale three-dimensionally with volume, photosynthetic processes scale with the two-dimensional area of solar capture. Light energy scales with the number of photons striking an area per unit time, e.g.,  $\mu\text{E}/\text{m}^2/\text{s}$ , where E (Einstein) is equal to one mole of photons. In a photosynthetic industrial process, areal productivity is most sensitive to the amount of light energy captured over the area of insolation and its conversion to product. Typically, either open algal ponds or closed photobioreactors have been used. For efficient areal capture, a reactor design is required that optimizes solar insolation, culture density, gas mass transfer, mixing, and thermal management.

Different fields of photonic research use different boundary conditions when discussing cumulative energy demand and it is important to distinguish them: specifically, efficiencies may be stated based either on (1) total solar radiation directed to the earth, (2) total radiation penetrating the atmosphere and striking the earth, or (3) total useful radiation that drives a process or phenomenon, e.g., weather, solar PV generation, photosynthesis, etc. Misuse of percentages valid for one process leads to misconceptions of energy yields for another, so it is important to be specific when comparing sources and sinks for photons.

Each year, approximately 43,000 megajoules (MJ) of solar energy reach each square meter of space facing the sun just outside the earth's atmosphere (Frölich and Lean 1998). The amount of solar energy striking any point on the

earth's surface is considerably less than this value due to several factors, including the earth's rotation, the angle of the ground relative to the incoming radiation, and attenuation through the atmosphere by absorption and scattering. The solar radiation reaching the earth's surface in the continental USA is approximately 11–18% of the total extraterrestrial value, depending on location.

The National Renewable Energy Laboratory (NREL) has conducted long-term measurements of daily insolation rates at various locales in the United States (Marion and Wilcox 1994; Wilcox et al. 2007). Rates for a few locations are shown in Table 2. For example, measurements at Phoenix, AZ, between 1992 and 2003 yield an average annual insolation rate of 7,300 MJ/m<sup>2</sup>/year striking a flat horizontal stationary surface. Using these empirical results precludes the need to make assumptions about atmospheric attenuation of solar energy.

Photosynthetic systems utilize radiation of the visible portion of the solar spectrum, i.e., in the wavelength range from 400 to 700 nm. Other photosynthetic systems can function at longer wavelengths but we confine this analysis to the range utilized by algae and cyanobacteria. Photosynthetically active radiation (PAR), the integrated total photonic energy available for photosynthesis, is approximately 39% of the total solar energy directed earthwards. However, moisture in the atmosphere preferentially absorbs the infrared portion of the spectrum. As a result, the fraction of PAR in ground-incident radiation available

**Table 2** Average annual total and photosynthetically active (PAR) ground horizontal radiation (PAR) at various US locales

Locale	Historical average total ground radiation MJ/m <sup>2</sup> /year	Historical average PAR MJ/m <sup>2</sup> /year
El Paso, TX	7460	3460
Phoenix, AZ	7300	3400
Las Vegas, NV	7190	3320
Lanai, HI	7120	3530
Albuquerque, NM	6990	3240
Leander, TX	6050	3000
Cambridge, MA	4800	2380

PAR is computed using NREL models based on the ratio of the measured historical average total radiation reaching the ground (Gueymard 2005; Bird and Riordan 1984)

for photosynthesis is increased to a value of about 48% of the total. Higher energy ultraviolet photons and lower energy infrared photons sum to the remaining 52%. Average PAR values for any location, based on historical average solar insolation rates, can be calculated using NREL models (Gueymard 2005; Bird and Riordan 1984). Annual PAR insolation at Phoenix is ~3,400 MJ/m<sup>2</sup>/year (Table 2).

In photosynthesis, a number of molecules have been enlisted as sources of reducing power to be converted by photonic energy. During many millions of years, plants, algae, and cyanobacteria have evolved to oxidize water and generate oxygen. Oxygenic photosynthesis follows the well-established Z-scheme mechanism (Blankenship 2002) wherein photonic energy converts NADP<sup>+</sup> to the reductant NADPH and ADP to ATP. These molecules are generated stoichiometrically with photons and are the chemical currencies used to fix CO<sub>2</sub> and drive cell metabolism. Fixation of one CO<sub>2</sub> requires two NADPH and three ATP.

The requirement for CO<sub>2</sub> fixation under atmospheric conditions (where CO<sub>2</sub> concentration is ~0.04%) has been observed to be between 9 and 10 photons/CO<sub>2</sub> (Blankenship 2002). The extra photon requirement beyond eight is accounted for by the efficiency loss due to the process of photorespiration. Because of the low-O<sub>2</sub>/CO<sub>2</sub> selectivity of the first enzyme step of carbon fixation, at the ribulose-1,5-bisphosphate carboxylase, oxygenation competes with carboxylation and diverts some carbon to glycolate in systems operating under atmospheric CO<sub>2</sub> concentrations. Some phototrophs, particularly C-4 plants and the cyanobacteria, have evolved sophisticated CO<sub>2</sub> capture mechanisms to maintain high-CO<sub>2</sub> concentrations in physical contact with the carbon-fixing machinery. In photosynthetic processes utilizing CO<sub>2</sub> at 50–100 times atmospheric concentrations, it is reasonable to minimize the contribution of photorespiration and to assume photon/CO<sub>2</sub>

stoichiometries of eight (see Furbank and Hatch 1987; Zhu et al. 2008).

A photon/product ratio can be calculated for any metabolic intermediate or synthetic product from either a natural or engineered recombinantly expressed pathway. For example, though fixation of a mole of CO<sub>2</sub> into biomass with empirical formula CH<sub>2</sub>O requires eight photons, production of other metabolic intermediates requiring ATP and/or NADPH may require more photons per mole CO<sub>2</sub> fixed.

Processes relying on the refining of biomass must account for product yields in efficiency calculations. Algal processes for fuel production take advantage of intrinsically high triglyceride oil production, up to 30–50% dry cell weight (Zemke et al. 2010). Batch cultivation and processing of algae, either in open ponds or in closed photobioreactors, require subsequent harvesting, dewatering, oil processing, and transesterification to produce a biodiesel fuel product, e.g., a fatty acyl ester. The overall productivity of this process is affected by the amount of carbon fixed to triglyceride per unit time and the process efficiency over a given area. This analysis uses the higher yield value for algae.

Cyanobacteria, once classified as blue-green algae, are now taxonomically categorized as water-splitting bacteria. They differ from algae in that they lack a defined nucleus and other genome-containing organelle compartments, e.g., mitochondria and chloroplasts, and have bacterial-style inner and outer membranes. Some cyanobacterial genera are robust, unicellular, and readily transformable, and therefore amenable to genetic engineering and industrialization. In this analysis, the engineered cyanobacterial system is one engineered with a pathway for linear saturated alkane synthesis (Reppas and Ridley 2010) and an alkane secretion module, and with a mechanism to control carbon partitioning to either cell growth or alkane production.

### Comparison of efficiencies for an algal pond biomass-to-biodiesel and a cyanobacterial direct-to-fungible diesel process

For comparison, we present two process scenarios and a theoretical maximum and compute practical maximum efficiencies. To use the empirically determined surface insolation rates of NREL, each scenario assumes a common location, e.g., Phoenix, AZ, and the energy input begins with the boundary of photons incident on a horizontal surface at that locale, e.g., 7,300 MJ/m<sup>2</sup>/year. We compare the accumulation of energy losses at each process step and the resultant input for conversion by the organism. The factors that lead to photon loss are based on empirical measurements and on literature reports (see particularly Weyer et al. 2009; Zhu et al. 2008; also Benemann and Oswald 1994;

Chisti 2007; Gordon and Polle 2007; Dismukes et al. 2008; Rosenberg et al. 2008; Schenk et al. 2008; Angermayr et al. 2009; Stephens et al. 2010; Wijffels and Barbosa 2010; Zemke et al. 2010; Zijffers et al. 2010), and are described in photon utilization assumptions (below). Note that some loss categories are defined differently by different authors but we have attempted to account for all basic assumptions in our comparative analysis.

The direct scenario assumes conversion of fixed CO<sub>2</sub> directly to a hydrocarbon, while minimizing production of biomass, and further involves secretion and continuous capture of the hydrocarbon product from the culture medium during a defined process interval. This scenario is designed for efficient capture and conversion of solar radiation in a densely arrayed closed reactor format. The theoretical maximum scenario does not include the losses associated with culture growth, surface reflection, photon utilization, photorespiration, mitochondrial respiration, process cycling, and nonfuel production, (Table 3).

Using photon utilization assumptions, we can illustrate the accumulating energy losses and the ultimate efficiencies for each of the scenarios. The individual losses, each accounting for a fraction of energy diverted away from conversion to the desired product, are summarized in Table 3. Figure 2 shows the stack-up of losses affecting the conversion efficiencies. The *large arrows* shown in the bottom of the plot indicate the overall conversion efficiency, i.e., the fraction of photons captured and converted to product. Because the losses combine multiplicatively, showing the loss axis in logarithmic terms allows a proper relative comparison. As shown in Fig. 2, various constraints result in nearly a 40% reduction in practical maximum conversion efficiency for the direct process relative to the theoretical maximum for this process. Even so, the conversion efficiency for the direct process is about seven times larger than that for an algal open pond. Note that these calculations do not account for downstream-processing efficiency. Also note that the results presented in

Fig. 2 show the potential for converting photons to product, but do not indicate the cost for building and operating facilities for implementing these processes.

Figure 3 shows the relationship between the calculated energy conversions expressed for any liquid fuel in per barrel energy equivalents (bble). By using the photosynthetic efficiency calculated above, the extrapolated metric of barrel energy equivalents (bble is equal to  $6.1 \times 10^9$  joule) and any product density expressed in kg/m<sup>3</sup> and energy content, e.g., heating value in MJ/kg, the output of this analysis can be converted to areal productivity for any molecule produced from either an endogenous or an engineered pathway. For example, the direct process, operating at the calculated 7.2% efficiency would yield 350 bble/acre/year. This equates to 15,000 gal alkane/acre/year where a C17 alkane has a heating value of 47.2 MJ/kg and density of 777 kg/m<sup>3</sup>. Given the flexibility of genome engineering to construct production organisms that make and secrete various fuel products, a similar calculation can be applied for any product synthesized via a recombinant enzymatic pathway and a productivity value extrapolated. By comparison on an energy basis, the practical efficiency of the algal biomass process would equal about 3,500 gal/acre/year of the target triglyceride (71 bble; heating value 41 MJ/kg; density 890 kg/m<sup>3</sup>). Note that 1 gal/acre/year is equivalent to 9.4 l/hectare/year.

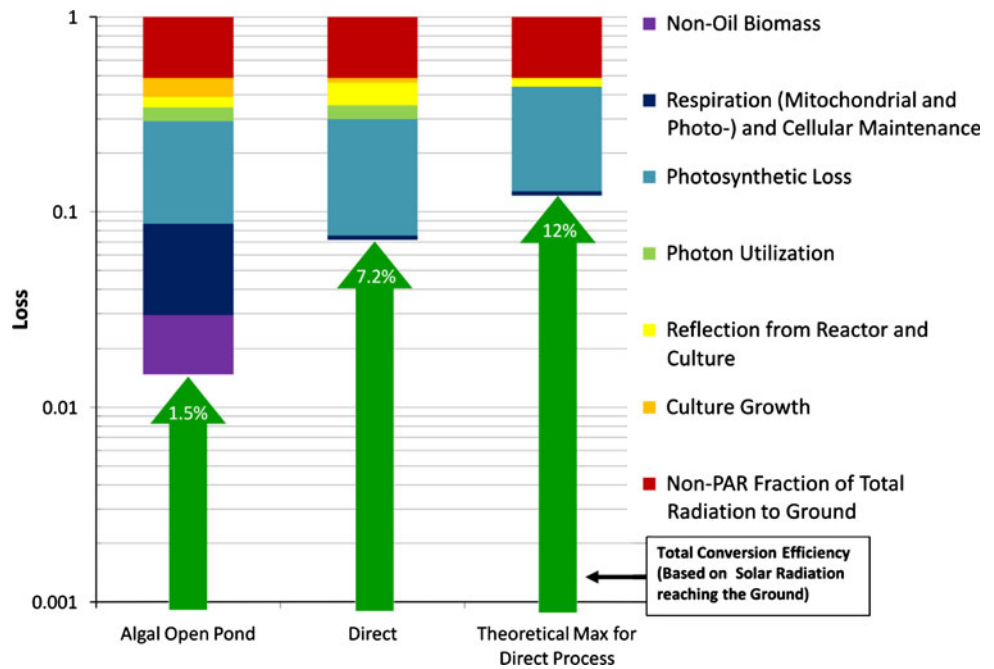
The areal productivity estimate for the direct process surpasses the best estimates for fuel productivity potential by any biomass-derived fuel process, e.g., for grain or cellulosic ethanol, for algal or vegetable oils for biodiesel, or biomass gasification and Fischer–Tropsch reforming for hydrocarbons. The photon energy densities and process productivities, plus the advantage of no arable land or freshwater displacement, create a scenario in which a minimal dedication of marginal land can serve to meet US renewable fuel standards.

Comparisons are often made between the energy efficiencies of photosynthesis and those for solar electricity

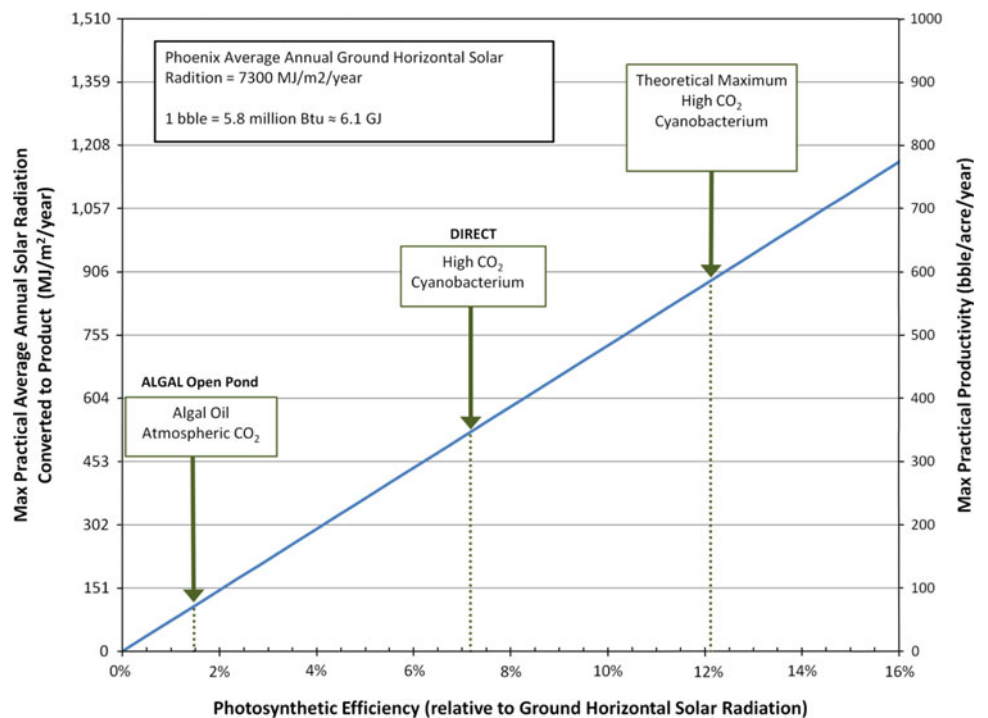
**Table 3** Individual contributions to photon energy losses in algal open pond and direct process scenarios (see photon utilization assumptions for a description). Cumulative contributions are illustrated in Fig. 2

Energy loss factor	Algal open pond (%)	Direct, continuous (%)	Direct theoretical maximum (%)
Unusable radiation (non-PAR fraction)	51.3	51.3	51.3
Culture growth loss	20	5.4	0
Reactor surface reflection loss	2	15	0
Culture reflection loss	10	10	10
Photon utilization loss	15	15	0
Photosynthetic metabolic loss	70.2	74.8	70.9
Cellular maintenance loss	5	5	5
Mitochondrial respiration loss	30	0	0
Photorespiration loss	49	0	0
Nonfuel production loss	50	0	0

**Fig. 2** Sum of individual contributions and accumulated photon losses for two fuel processes and a theoretical maximum for energy conversion. The losses are represented on a logarithmic scale and accumulated serially for the processes beginning with the percent of PAR in empirically measured solar ground insolation. Total practical conversion efficiency after accounting for losses is indicated by the *green arrows*



**Fig. 3** Relationship between practical photon capture efficiency and productivity calculated on a barrel equivalent energy basis



generation. It is important to make these comparisons in the proper context. Solar thermal or photovoltaic systems generate power requiring economical and efficient storage and transmission into the electrical grid, whereas the systems described here generate easily stored energy in liquid form. Moreover, values quoted for solar power systems are peak efficiencies that fall off precipitously under even momentary shading (Curtright and Apt 2008). Solar electricity efficiencies are also compounded by battery

efficiencies and impedance losses that introduce system-specific variability. Manufacturing fuels to direct them into an existing refining, distribution, and transportation infrastructure would be more fairly compared to other existing and developing technologies for energy conversion to reasonably storable forms and not to electricity.

The aquatic species program report of 1998 (Sheehan et al. 1998) and the recently published National Algal Biofuels Technology Roadmap (2009) each conclude that

photosynthesis could support viable fuel processes given advances in organism and process productivities. Organism engineering, direct production, product secretion, and process optimization are areas for improvement to achieve viability. The direct photosynthetic platform is an alternative approach that addresses many of these ideas and offers efficiencies nearest to a thermodynamic maximum with more advantageous process economics.

Further application of systems and synthetic biology approaches could extend the range of efficiency for photosynthetic processes. For example, some photosynthetic microorganisms, particularly the nonoxygenic bacteria, have light capture systems allowing them to extend the PAR range into the near infrared (up to  $\sim 1,100$  nm; Kiang et al. 2007). Incorporating these alternate photon-capturing and reaction center complexes into oxygenic production organisms to supplement endogenous systems and broaden the spectrum of light harvesting could further optimize efficiency relative to PAR. Other innovations that reduce culture reflection, enhance photon capture, and broaden temperature optima can also be envisioned using advanced organism-engineering tools.

Because policy, grant funding, and private investment in transformative industrial processes are founded on their economics, any statements of efficiencies without the benefit of substantive analysis can be prejudicial to the development and implementation of technologies that may, in fact, be revolutionary. At this time of global need for sustainable fuels, the deployment of game-changing technologies is critical to economies and environments on a global scale. It is clear from this and other recent analyses focused on life cycles and energy balances (Stephens et al. 2010) that a very compelling case can be made for photosynthesis as a platform technology for renewable production of fuels. More specifically, an engineered cyanobacterial organism for direct continuous conversion of  $\text{CO}_2$  into infrastructure-compatible, secreted fuel molecules surpasses the productivities of alternatives that rely on the growth of biomass for downstream conversion into product.

### Photon utilization assumptions

The assumptions inherent in a calculation of overall efficiency of a photosynthetic process are based on areal insolation, capture, and conversion, and are analyzed relative to a sequentially accumulating loss of photons that are not gainfully utilized for the production of product. When accounting for the ultimate contingent of photons that are converted, the loss at each process step is a percentage fraction of the total available from the previous step. The descriptions below follow the sequence of process

conversion steps and reflect the accumulating losses and resultant efficiencies illustrated in Fig. 2. Values described below are summarized in Table 3.

### PAR radiation fraction

The analysis assumes that only the solar radiation reaching the ground is available for conversion and the cumulative loss is computed with respect to this boundary value. Although the average total solar radiation reaching the ground varies throughout the world, we assume that the relative efficiency of each subsequent step in the conversion process is location-independent to a first-order approximation. The energy fraction of solar radiation reaching the ground that lies in the PAR range does vary with location and time of day. Results obtained from NREL models (Gueymard 2005; Bird and Riordan 1984) indicate that the PAR radiation fraction ranges from about 47–50% in the southwest USA. For the calculations performed in this article, we use a value of 48.7% for PAR radiation fraction to remain consistent with Zhu et al. (2008), resulting in a loss of 51.3%.

### Culture growth

In the direct process, once reactors are inoculated, cells must be grown up to high density before the production phase. Thereafter, the process is continuous for an extended period. Based on pilot experience, we assume an 8-week process time, 3 days of growth at doubling times  $\sim 3$  h followed by 53 days of production with no biomass accumulation, before the reactors must be emptied and reinoculated. Direct production of a fungible product minimizes downstream processing. This results in a reactor availability loss of about 5%.

In the case of an algal biomass process, energy and carbon are dedicated to batch growth and stress-induced triglyceride accumulation, followed by harvesting and downstream processing. The DOE Algal Biomass report process summary indicates that the algal growth phase is followed by an equal triglyceride accumulation phase, which would indicate a cycling efficiency loss of 50%. Coupled growth and triglyceride process would result in an approximate 20% loss (see Fig. 3; Sheehan et al. 1998) which we take here.

### Reactor surface reflection

Any process using an enclosed reactor must account for reflective and refractive losses as light passes through the outward facing surface. A 15% loss is estimated for the direct process to account for light reflected away from the reactor. The reactor is assumed to have two layers of

plastic containing the organisms (an outer protective layer and an inner container), resulting in three air/plastic interfaces that light must pass through before reaching the culture. Each of these interfaces will result in about a 5% reflective Fresnel loss, assuming no antireflective coating is used. For the algal open pond, a single air/water interface results in about a 2% reflective Fresnel loss.

#### Culture reflection

According to Zhu et al. (2008), about 10% of the incoming PAR radiation is reflected away by a plant or culture, with most of this reflection occurring at the green wavelengths. This loss is applied to all cases, including the theoretical maximum.

#### Photon utilization

Not all photons that enter a reactor are available for conversion. For instance, it may be too costly to maintain the reactor in a condition in which it can convert every photon, such as early in the morning and late in the day when solar radiation is very diffuse. Likewise, depending on how the reactor temperature is maintained, the organisms may not be at optimal production temperature early in the morning. In addition, at very high intensity levels, the organisms may not be able to convert all of the photons. Based on models that integrate solar and meteorological data with a thermal and production model, we estimate that about 15% of the incoming photons will not be available for conversion for the direct case. We assign a comparable loss to the algal open pond.

#### Photosynthetic loss

The main fractional loss in photosynthetic conversion results from energy-driven metabolism. Because the photosynthetic process is ultimately exothermic, the available energy contained in the product formed by metabolism is a fraction of that contained in the incoming photons. The remaining energy is dissipated as heat into the culture. For the production of alkane, we calculated that ~12 photons are required to reduce each molecule of CO<sub>2</sub>. Assuming an average PAR photon energy of 226 kJ/mol and a heating value of 47.2 MJ/kg for alkane, the photosynthetic conversion efficiency is about 25% (equivalent to a loss of 74.8%). For the simpler triglyceride, we assume only eight photons are required to reduce each molecule of CO<sub>2</sub>, but that the product consists of half triglyceride (heating value ≈ 37 kJ/kg) and half simple biomass (heating value ≈ 15.6 kJ/kg), resulting in a photosynthetic conversion efficiency of about 29.8%. This value for algal open ponds is considered to be very conservative, with the actual value

likely a few percent lower. Finally, for the theoretical maximum, we use the value computed in Zhu et al. (2008) for a maximum photosynthetic efficiency of 29.1% (obtained by combining the loss for photochemical inefficiency and carbohydrate synthesis).

#### Cellular maintenance

Maintenance energy is a variable that may affect photoefficiency by drawing away energetic currencies of ATP and NADPH for cell division, repair, and other functions not directly associated with product formation. The maintenance energy in any given process situation depends on rates of metabolism, cell division, etc., as shown in differences in measured values in dividing versus resting cells (Pirt 1965; Pirt 1975). A batch bioprocess, therefore, wherein cell division and product formation are proceeding simultaneously versus a continuous process where growth is minimized and carbon is partitioned to a secreted product may differ considerably in maintenance energy. However, because the concept and measurement are controversial, we have attributed a 5% loss to the analyses of all three scenarios.

#### Mitochondrial respiration

Under illumination, eukaryotic photosynthetic organisms, e.g., plants and algae, lose efficiency because of respiratory metabolism in the mitochondria. Because cyanobacteria have no subcellular organelles and the engineered organisms are partitioning nearly all fixed carbon to product, we have assumed negligible respiration loss in the direct process and have also zeroed out this loss in the theoretical practical maximum scenario. The algal open-pond analysis includes a 30% loss for mitochondrial respiration. This value is based on the plant value used by Zhu et al. (2008).

#### Photorespiration

According to Zhu et al. (2008), processes at atmospheric CO<sub>2</sub> concentrations, such as an open algal pond, will have a substantial loss (≈49%) due to photorespiration. This loss is minimized at high-CO<sub>2</sub> levels (>1%) maintained in the enclosed direct process (see text for explanation).

#### Biomass versus fuel production

In the direct process, most fixed-carbon output is in the form of a chemical product from a cloned heterologous pathway. For the algal process, we assume a generous value for oil yield of 50% by weight and thus apply a 50% loss to productivity.

The losses discussed above are summarized in Table 3. We define conversion factor as (1 – loss factor) for each of



the above losses. For instance, the conversion factor for cellular maintenance (loss = 5%) is 95%. Total conversion efficiency, as shown in Fig. 2, is computed by taking the product of each of the conversion factors computed from the values in Table 3.

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