MICROALGAE MASS PRODUCTION METHODS

Y. Shen, W. Yuan, Z. J. Pei, Q. Wu, E. Mao

ABSTRACT. *This article reviews the performance, special features, and technical and/or economic barriers of various microalgae mass production methods including open-pond, photobioreactor, and immobilized culture systems. Open ponds are the least expensive among the three systems; however, issues of vulnerable species contamination, low productivity, high harvesting cost, and large volume of water loss have to be addressed. High biomass productivity and cell density, reduced contamination, and better use of CO_2 are some advantages of photobioreactor systems, but the prohibitively high construction cost of photobioreactors still limits commercialization of such systems. Immobilized algae culture systems have great potential to obviate the harvesting problem of open ponds and photobioreactors and enhance biomass productivity; however, high material cost and limited choices of algae species require more investigation. Economics of algae biofuel manufacturing are also discussed. Algae biomass productivity, lipid content, and petroleum price are decisive factors in the economic viability of algae biofuels.*

Keywords. *Algae, Biodiesel, Immobilized algae culture, Open pond, Photobioreactor.*

U.S. and world economies depend on fossil fuels (coal, oil, and natural gas), which are finite and nonrenewable energy sources. For example, fossil fuels currently provide more than 85% of all energy consumed in the U.S., nearly two-thirds of the electricity, and virtually all of the transportation fuels (DOE, 2008). Although the exact time at which fossil fuels will run out is debated, it is probably inevitable that fossil fuel supplies will decline in the future and will become even more expensive. Furthermore, use of fossil fuels contributes to accumulation of CO_2 (a greenhouse gas) in the atmosphere. Therefore, there is an urgent need for alternatives to fossil fuels, such as biofuels, including biodiesel, ethanol, and other types of biomass-derived fuels. As one of the major biofuels, biodiesel can be directly used in diesel engines (Yuan et al., 2005, 2007; Hansen et al., 2006) and can play a significant role in diversifying transportation fuels in the U.S. Because biodiesel is renewable, cleaner, safer, and beneficial to the economy, it has been supported by the U.S. federal government and many state governments, and its consumption in the U.S. has increased exponentially in recent years.

The goal set by the U.S. government is to replace 20% of transportation fuels with biofuels by the year 2030 (English and Ewing, 2002). If biodiesel were the sole biofuel used to meet this goal, then 28 billion gallons of biodiesel would be needed each year at the current rate of consumption (Chisti, 2007). As illustrated in table 1, producing this amount of biodiesel would require an unsustainably large cropping area when using any other sources (corn, soybean, or oil palm) except algae. For example, 583 million acres of soybean (which is 130% of the total existing cropping area for all crops in the U.S.) would be needed to produce this amount of biodiesel. Even for oil palm, one of the best oil producers that can be grown on land, 10% of the total existing U.S. cropping area would be needed. This scenario can be changed, however, if algae are used to produce biodiesel. Given the demonstrated algae biomass productivity in photobioreactors (PBRs) and 30% oil content (Chisti, 2007), if 1% of the total existing U.S. cropping area, or 4 million acres, is used to grow algae, there will be sufficient algae biofuels to achieve the 20% replacement goal. Unlike other oil crops, algae can be grown in the desert or on marginal lands and, therefore, will not compete for arable lands currently used for human food and animal feed production. Algae can also grow in salty water, so competition for valuable fresh water can be avoided. Along with their CO_2 biofixation potential (Benemann, 2003) and wastewater treatment benefits (Hoffmann, 1998; Shen et al., 2008), algae have been regarded as the only potential source of biodiesel to completely replace fossil diesel (Chisti, 2007) and the most promising renewable energy source (Donohue and Cogdell, 2006; Chisti, 2007; Schenk et al., 2008; Rodolfi et al., 2009).

Although there are over 50,000 microalgal strains in habitats almost everywhere on earth (Chen, 1996), only a small fraction of them can be considered for biodiesel production because most of them either grow slowly or are low in lipid content. Tredici (2008) listed 31 algal strains as potential candidates for biodiesel production. The biomass productivity, lipid content, and lipid yield of these algal strains are shown in table 2 (Tredici, 2008). The marine algae *Nannochloropsis* were ranked the highest in lipid yield among the 31 strains. The freshwater strain *Scenedesmus* sp. DM also attracted attentions due its high biomass yield and acceptable lipid content.

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The authors are **Ying Shen, ASABE Member Engineer,** Doctoral Student, and **Wenqiao Yuan, ASABE Member Engineer,** Assistant Professor, Department of Biological and Agricultural Engineering, Kansas State University, Manhattan, Kansas; **Zhijian Pei,** Associate Professor, Department of Industrial and Manufacturing Systems Engineering, Kansas State University, Manhattan, Kansas; **Qingyu Wu,** Professor, Department of Biological Science and Biotechnology, Tsinghua University, Beijing, China; and **Enrong Mao,** Professor, College of Engineering, China Agricultural University, Beijing, China. **Corresponding author:** Wenqiao Yuan, Department of Biological and Agricultural Engineering, 129 Seaton Hall, Kansas State University, Manhattan, KS 66506; phone: 785-532-2745; fax: 785-532-5825; e-mail: wyuan@ksu.edu.

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Table 1. Comparison of biodiesel sources.

Source	Oil Productivity per Year (gal acre^{-1})[a]	Cropping Area Needed to Produce 20% of all U.S. Transportation Fuels (million acres)	Cropping Area Needed vs. Total Existing U.S. Cropping Area (%)
Corn	18	1,556	346
Soybean	48	583	130
Canola	127	220	49
Coconut	287	98	22
Oil palm	635	44	10
Algae	6,276	4	1

[a] From Chisti (2007).

Growth of microalgae is affected by many factors, such as abiotic factors (e.g., light, temperature, nutrients, dissolved oxygen content, CO_2 concentration, pH, salinity, and toxic chemicals in the growth media), biotic factors (e.g., presence of bacteria, fungi, viruses, and competition from other algae), and operational factors (e.g., shear forces generated by mixing, dilution rate, and harvest method and frequency) (Renaud and Parry, 1994; Fabregas et al., 2004; Moheimani, 2005; Hu and Gao, 2006; Chiu et al., 2009; Rodolfi et al., 2009; Shen et al., 2009a, 2009b, 2009c). However, it is difficult to determine which factor affects algae growth the most because all these factors may influence algae growth together (Abu-Rezq et al., 1999; Zittelli et al., 1999). An earlier thorough study was carried out from 1978 to 1996 through the U.S. DOE funded Aquatic Species Program to develop renewable transportation fuels from algae; the main focus was production of biodiesel from algae with high oil content. For about two decades, the program achieved tremendous advances in algal strain collection, screening, characterization, and improvement, as well as in the science of manipulating metabolism of algae and the engineering of algae production systems (Sheehan et al., 1998). However, funding for this program was eliminated in 1996 because of inexpensive crude oil prices at that time and DOE's strategic changes in biofuel research focus. Since then, no substantial government-supported research activities in algae biofuels have been reported. In recent years, because of increasing energy prices and the food vs. fuel debate, renewed interest in growing algae for fuels and other chemicals has arisen in the U.S. It is necessary to have an updated understanding of current microalgae mass production methods and be aware of the technical and economic challenges of each method. Through a literature review, this article summarizes the performance, special features, and technical and/or economic barriers of currently available algae mass production methods, including open ponds, closed PBRs, and immobilized culture systems. Heterotrophic culture of algae is not discussed because this article focuses only on photosynthetic algae production methods.

Open Ponds

In open ponds, the oldest and simplest systems for algal culture, algae are cultivated under conditions identical to those in the external environment. Open-pond systems first appeared in the 1950s (Meier, 1955; Golueke et al., 1957; Golueke and Oswald, 1959) and are still widely used in large-scale outdoor microalgal cultivation. Many different designs have appeared for open-pond systems, but three major types succeeded and are still operated at commercial scales: raceway ponds, circular ponds, and unstirred ponds (fig. 1).

Table 2. Biomass and lipid productivities of 31 microalgal strains (Tredici, 2008).

Microalgae	Biomass Productivity (mg L^{-1} d^{-1})	Lipid Content (% of biomass)	Lipid Productivity (mg L^{-1} d^{-1})
Nannochloropsis sp. RM	278.2 ±0.0	31.0 ±0.5	86.3 ±0.0
Nannochloropsis sp. RP	232.7 ±25.7	37.0 ±0.5	86.1 ±9.5
Nannochloropsis sp. ZM	241.8 ±7.7	33.1 ±1.7	79.9 ±2.6
Pavlova lutheri	212.5 ±10.6	37.1 ±0.5	78.9 ±3.9
Scenedesmus sp. DM	348.2 ±2.6	21.8 ±0.6	75.8 ±0.6
Pavlova salina	240.0 ±7.1	31.1 ±1.4	74.6 ±2.2
Chlorococcum sp. UMACC 112	380.0 ±2.6	19.5 ±0.7	74.2 ±0.5
Nannochloropsis sp. CS 246	231.8 ±1.3	30.4 ±0.3	70.4 ±0.4
Nannochloropsis sp. MRS	270.0 ±2.6	24.9 ±0.7	67.2 ±0.6
Ellipsoidium sp. LW 70/01	235.5 ±1.3	28.4 ±0.4	67.0 ±0.4
Phaeodactylum tricornutum	335.0 ±31.1	19.2 ±0.4	64.3 ±6.0
Chlorella sorokiniana	315.5 ±10.3	19.8 ±0.7	62.3 ±2.0
Ellipsoidium sp. LW 277/01	275.5 ±21.9	22.5 ±0.8	62.1 ±4.9
Tetraselmis sp. LW	414.0 ±11.3	14.9 ±0.1	61.8 ±1.7
Chlorella sp. AMI2	307.3 ±7.7	19.2 ±0.4	59.0 ±1.5
Scenedesmus sp. cvc3	283.6 ±5.1	20.6 ±0.8	58.4 ±1.1
Porphyridium cruentum	613.3 ±77	8 9.4 ±0.2	57.5 ±7.3
Tetraselmis suecica CV	383.6 ±1.3	14.9 ±0.1	57.3 ±0.2
Isochrysis sp. MRS	194.0 ±5.7	28.7 ±0.5	55.6 ±1.6
Isochrysis (T-ISO) CS 177	252.5 ±1.8	22.0 ±1,6	55.4 ±0.4
Chlorella vulgaris UTEX 1200	274.5 ±21.9	19.4 ±0.9	53.2 ±4.2
Nannochloropsis sp. MI	237.3 ±1.3	22.3 ±0.5	52.8 ±0.3
Scenedesmus quadricauda	260.0 ±1.3	19.0 ±0.5	49.3 ±0.2
Chlorella vulgaris CCAP 211/11b	231.8 ±1.3	19.7 ±0.3	45.7 ±0.3
Skeletonema sp. CS 252	128.8 ±5.0	32.9 ±0.2	42.4 ±1.6
Monodus subterraneus UTEX 151	257.3 ±20.6	15.5 ±0.5	39.9 ±3.2
Tetraselmis suecica OR	448.0 ±0.0	8.4 ±0.3	37.5 ±0.0
Chaetoceros muelleri	92.0 ±4.2	34.7 ±0.2	32.0 ±1.5
Thalassiosira pseudonana	135.0 ±5.3	22.0 ±1.7	29.7 ±1.2
Skeletonema sp. CS 181	123.8 ±3.5	21.1 ±0.9	26.1 ±0.8
Chaetoceros calcitrans	62.0 ±1.4	40.9 ±0.1	25.3 ±0.6

Raceway Ponds

Raceway ponds (fig. 1a) are usually constructed either in singles or as groups of channels built by joining individual raceways together. Channels may be built in concrete or compacted earth or lined with plastics. Depths of raceway ponds are usually between 15 and 30 cm, and a paddlewheel is often used to drive water continuously around the circuit (Moheimani, 2005; Schenk et al., 2008). Other types of mixing systems, such as pumps and airlifts, can also be used, but these are less popular than paddlewheels. Mixing to expose algae cells to sunlight and CO_2 is one of the key factors in open-pond design and operation. The water-flow velocity required to prevent algae cells from deposition and setting depends on the sinking rate of the cells. A velocity of 10 to 20 cm s^{-1} was found effective, and higher velocities are preferred, but a velocity greater than 30 cm s^{-1} could consume too much energy to be viable (Sheehan et al., 1998). Raceway ponds are the most commonly used open systems for commercial algae culture because of their comparatively low construction and maintenance costs (Borowitzka, 2005). Companies such as Cyanotech (U.S.), Inner Mongolia Biological Engineering (China), Nature Beta Technologies (Israel), Tianjin Lantai

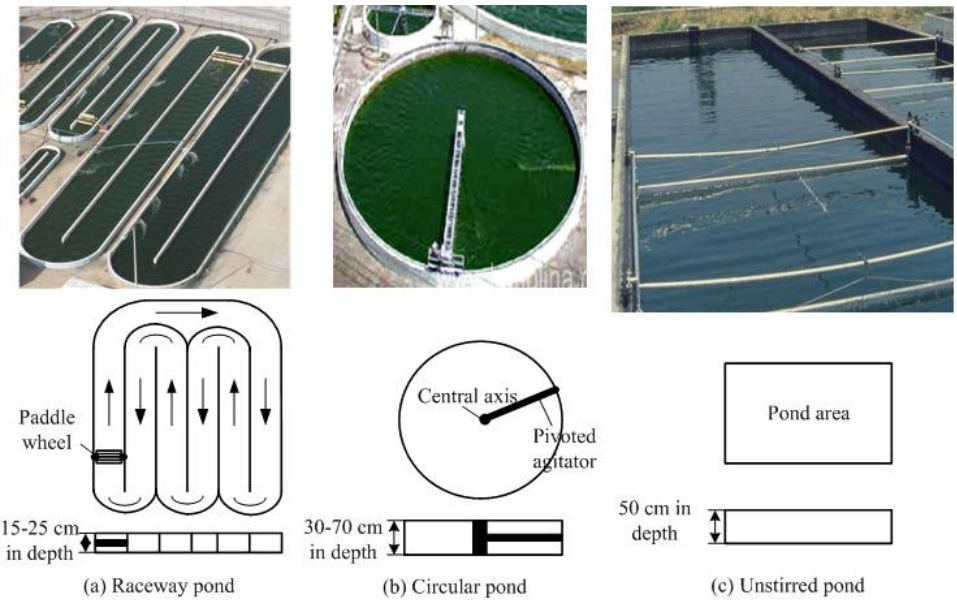

Figure 1. Three different designs of open-pond systems (a and b: courtesy of A. Ben-Amotz, National Institute of Oceanography, Israel; c: courtesy of M. R. Tredici, University of Florence, Italy).

Biotechnology (China), Parry Agro Industries (India), and Earthrise Farms (U.S.) reported having large-scale raceway ponds for β-carotene or food supplement production (Walker et al., 2005). By using raceway ponds, a cell concentration of up to 1 g L^{-1} can be achieved, and productivities of about 10 to 25 g m^{-2} d^{-1} have been reported (Lee, 2001; Moheimani and Borowitzka, 2006). However, because of seasonal light and temperature changes, such productivities are difficult to maintain on an annual basis.

Circular Ponds

Circular ponds, which have a design similar to raceway ponds, are normally up to 45 m in diameter and 30 to 70 cm in depth with a centrally pivoted agitator (fig. 1b) (Moheimani, 2005). Circular ponds are commonly used in Southeast Asia for health food, such as β-carotene production. For example, Taiwan and Japan produce thousands of tons of algal biomass annually by using large-scale circular ponds for production of β-carotene with *Chlorella* sp. (Lee, 2001). Algal circular ponds can also be combined with wastewater treatment (Garcia et al., 2000). *Oscillatoria* was cultured in circular ponds using diluted wastewater, and the biomass productivity achieved was around 15 g m^{-2} d^{-1} along with reductions of more than 80% of ammonia and 50% of total organic carbon in wastewater (Sheehan et al., 1998). Size is a limiting factor for circular ponds because of poor mixing efficiency when the rotating arm gets too long (e.g. >50 m in diameter).

Unstirred Ponds

The other commonly used open-pond system is unstirred ponds (fig. 1c), the most economical and least technical of all commercial culture methods. Very large unstirred open ponds are simply natural lakes or constructed from natural water ponds with uncovered beds and are usually less than 50 cm deep (Borowitzka and Borowitzka, 1990). These types of ponds have been used for culturing *Dunaliella salina* for β-carotene production in Western Australia and South Australia by Betatene Ltd (Borowitzka 1988a, 1988b, 1997). The company produces 7 to 10 tons $year^{-1}$ of β-carotene in 460 ha shallow unstirred ponds in Whyalla, South Australia, and produces 6 tons $year^{-1}$ of β-carotene in 250 ha shallow unstirred ponds in Hutt Lagoon, Western Australia. Lee (1997) reported harvesting more than 30 tons $year^{-1}$ of microalgal biomass from natural lakes in Southeast Asia. Unstirred open ponds are, however, limited to microalgae that are capable of growing in poor conditions or have a competitive advantage that allows them to outgrow contaminants such as protozoa, other microalgae, viruses, and bacteria (Chaumont, 1993).

Features and Barriers of Open Ponds

Open-pond systems offer several advantages, including:

1. Relatively low construction and maintenance costs. For example, reported raceway pond construction cost was about \$25 m^{-2}, including \$15 m^{-2} ground work and \$10 m^{-2} infrastructure costs for air, pumps, pipes, sensors, control, containers, paddle wheels, power, and computer rooms (Ben-Amotz, 2008a).
2. Easy to scale up. Because each pond can be operated independently, scaling up can be easily achieved by increasing the number of ponds.
3. The possibility of integration with wastewater treatment processes.

However, open ponds also face some technical barriers that prevent them from being commercialized for biofuel manufacturing:

1. Species contamination. Because they are open to the environment, open ponds are easily contaminated by fast-growing wild algae or microorganisms that feed on algae. Single-species cultivation can be maintained only for a short period of time (e.g., a few months). Over 50 years of repeated attempts, very few species have proven amenable to large-scale cultivation in open ponds. For example, the high-salinity species *Dunaliella salina* for β-carotene production (Boro-

witzka et al., 1984; Gonzalez et al., 2003) and the highly alkaline species *Spirulina platensis* (pH > 9.2) and fast-growing species *Chlorella* sp. for protein production (Belay 1997; Chen and Zhang, 1997; Miron et al., 1999; Tredici, 2008; Hu and Sommerfeld, 2008) have been cultivated for purposes other than biofuel manufacturing.

2. Low productivity. Theoretically, a productivity of 50 to 60 g m^{-2} d^{-1} of dry algae biomass is possible with open ponds. However, currently even 10 to 20 g m^{-2} d^{-1} is difficult to achieve in large-scale open ponds on an annual basis. This is largely due to poor mixing and water-gas transfer in open ponds that limit photosynthetic efficiency. Seasonal temperature and sunlight intensity changes also negatively affect biomass productivity.
3. High harvesting cost. Harvesting is another problem associated with open-pond systems. It is costly to separate algae from water because algae concentration in open ponds is usually very low, e.g., 0.6 to 1 g L^{-1}, which is less than 0.1% by weight (Becker, 1994; Pulz, 2001; Tredici, 2004), and algae sizes are very small, e.g., 5 to 10 μm in diameter. Many harvesting methods, e.g., filtration, flotation, flocculation, sedimentation, and centrifugation, have been investigated, but none have proven to be simple, inexpensive, and suitable to large-scale algae production (Hoffmann, 1998).

Large amount of water evaporation is also a challenging problem in the use of open ponds, especially in tropical or desert areas. All these issues have led to the appearance of other types of algae production systems.

Closed PBR Systems

Photobioreactors are not directly exposed to the atmosphere; instead, they are covered with a transparent material or contained within transparent tubing. Of the different designs of closed systems, most consist of tubes of various shapes, sizes, and lengths constructed of various transparent materials such as glass and plastic. Photobioreactors are also widely designed as plate (flat panel) types to maximize light exposure.

Tubular PBRs

Tubular PBRs are most commonly used in commercial algae cultivations because of their ease of construction, improved control of gas transfer, large surface area to volume ratio, and fairly good biomass productivities (Moster, 1991; Pulz, 2001, Ugwu et al., 2008). The tubes can be arranged in various configurations: straight vertical, horizontal, inclined, or helical. Figure 2 shows five tubular PBR designs. One of the world's largest PBRs is designed as a vertically aligned fence-like straight tubular system, which is arrayed in a greenhouse in Klotze, Germany. The system occupies an area of 10,000 m^2 with 700 m^3 culture volume. Annual production achieved in this system is 130 to 150 dry ton biomass, which is about 35 to 41 g m^{-2} d^{-1} (Pulz, 2001; Schenk et al., 2008). Carlozzi (2003) reported a maximum productivity of 47 g m^{-2} d^{-1} in a tubular PBR with south-north orientation. Other types of tubular PBRs were also widely reported. A conical helical tubular PBR with a cone angle of 60° and an installation area of 0.5 m^2 was reported by Morita et al. (2001). *Chlorella sorokiniana* was cultured in this PBR, and maximum biomass productivity reached was 33.2 g m^{-2} d^{-1} on a sunny day in August, when solar energy input was around 11.5 MJ m^{-2} d^{-1}. Average biomass productivity was 25.2 g m^{-2} d^{-1} in two culture periods in August. A 0.2 m^2 horizontal airlift tubular PBR was tested by Grima et al. (2001) for culturing *Phaeodactylum tricornutum* outdoors from March to August. Because of variations in solar irradiation and temperature, the biomass dry weight (DW) varied from 19.1 g m^{-2} d^{-1} (in April) to 31.7 g m^{-2} d^{-1} (at the end of July).

Plate (Flat Panel) PBRs

Plate PBRs consist of a transparent rectangular container with the light path usually between 1 and 30 cm (Hu et al., 1996; Moheimani, 2005; Hu and Sommerfeld, 2008). This type of PBR is usually inclined or vertically aligned. Figure 3 shows two commonly used plate PBR designs. Zhang et al. (2001) successfully cultivated a thermophillic cyanobacterium (*Synechocystis aquatilis*) in outdoor 192 L vertical plate reactors for three seasons (winter, spring, and summer). An eight-plate reactor was installed in parallel over an area of 3.5 m^2 inside a glass greenhouse. The light path of each plate was 1.5 cm. Under a mean irradiation of 11.2 ±2.1 MJ m^{-2} d^{-1}, the reactor achieved an average biomass productivity of more than 30 g m^{-2} d^{-1} and a cell concentration of 1 to 2 g L^{-1}. Using the vertical plate PBRs as shown in figure 3b, Hu and Sommerfeld (2008) achieved 12.5 g m^{-2} d^{-1} and a high cell density of about 7 g L^{-1} in Arizona. Another example is the 110 L Green Wall Panel PBRs located at Livorno, Italy. The system uses 0.3 mm thick flexible low-density polyethylene films instead of high-cost transparent tubes. *Nannochloropsis* sp. was cultured in this system by two phases: nutrient sufficient and nitrogen (N) starving. With about 30% lipid content, biomass productivity was around 30 g m^{-2} d^{-1}. The system was estimated to have a lipid yield of 20 tons ha^{-1} $year^{-1}$ in the Mediterranean climate and about 30 tons ha^{-1} $year^{-1}$ in sunny tropical areas (Rodolfi et al., 2009). Productivities of plate and tubular PBRs are generally comparable.

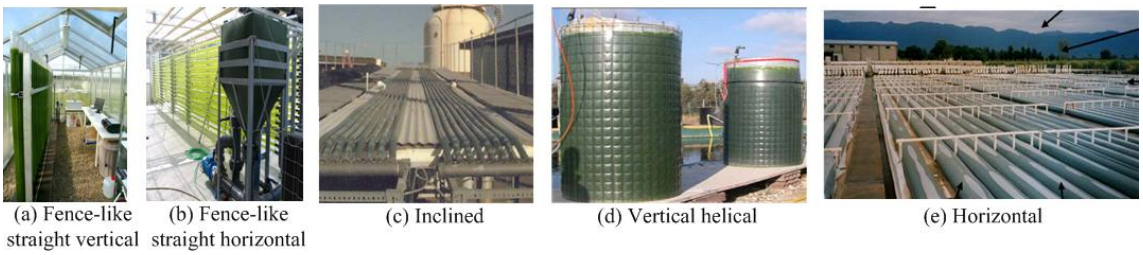

Figure 2. Different designs of tubular PBRs (a and b: courtesy of L. Thomsen, Jacobs University, Germany; c, d, e: courtesy of M. R. Tredici, University of Florence, Italy).

(a) Inclined plate PBR (b) Vertical plate PBR

Figure 3. Two different designs of plate PBRs (a: courtesy of A. Richmond, Ben-Gurion University, Israel; b: courtesy of Q. Hu and M. Sommerfeld, Arizona State University).

Features and Barriers of PBRs

PBRs have a number of advantages over open ponds:

1. Higher biomass productivity and cell density. This is probably the most important reason for using these systems. Because of better mixing and maximized sunlight capture, biomass productivity per unit area of PBR can be as twice that of open ponds, whereas cell density can be 30 times higher (Chisti, 2007). Higher cell density can significantly reduce algae harvesting (dewatering) and drying costs (Hu and Sommerfeld, 2008).
2. Reduced contamination risks because of less exposure to the environment. Although single-species cultivation is technically possible with PBRs, it is not economically practical to keep the system sterilized. Therefore, contamination from fungi, bacteria, or even other algae may still be possible.
3. Better control of culture conditions such as temperature, light, pH, and nutrients for prolonged durations. PBRs usually have a heat exchanger or are placed inside a greenhouse to maintain optimum temperatures during daytime and overnight throughout various seasons. Light control can be achieved by adjusting the orientation of PBRs so light saturation and shading can be reduced. Because of higher cell concentration, pH and nutrient adjustment can be easier in PBRs than in open ponds.
4. Reduced CO_2 losses, which is attractive to recycling flue gas from power plants.

However, even with all these advantages, it is still difficult to justify the use of algae PBRs for biofuel manufacturing largely because of the prohibitively high construction cost at large scales. An estimated low-boundary PBR field capital investment was \$180 m^{-2} (Dimitrov, 2007), almost seven times that required for open ponds per unit area.

Comparison of Open-Pond and PBR Systems

Grima (2009) compared the performance of the three most popular algae culture systems: the open raceway pond, closed vertical plate PBRs, and horizontal tubular PBRs, as shown in figure 4. This is one of the best comparisons of various culture systems because the same algae strain (*Scenedesmus almeriensis*) was tested at the same location back to back. Some major design and operating parameters along with biomass productivities are shown in table 3. It is apparent that open ponds have the highest volume to surface ratio (VSR), while tubular PBRs have the lowest. A typical biomass productivity of 15, 35, and 50 g m^{-2} d^{-1} (on the basis of surface area of the pond or PBR) was achieved for the raceway pond, plate PBRs, and tubular PBRs, respectively. When calculated by actual land area on an annual basis (about 300-day operation per year), the productivities were 45, 80, and 100 tons ha^{-1} $year^{-1}$, respectively. The biomass productivity seems to be positively proportional to VSR, probably due to better cell exposure to sunlight at lower VSRs. Although PBRs achieved 2 to 3 times higher biomass yield per surface area, volume-based construction costs of the PBRs were 10 to 25 times higher (or 3.5 to 10 times higher per surface area) than for the raceway pond, which makes it difficult to justify the use of PBRs vs. open ponds for algae production. A more detailed cost analysis is presented in a later section.

As mentioned previously, harvesting is a big problem associated with suspended algal culture systems including open ponds and PBRs. As shown in table 4, the typical biomass concentration is about 0.25 g L^{-1} in open ponds and 1 to 1.5 g L^{-1} in PBRs. Separating such low concentrations of biomass from water remains a challenge. Among the various methods, centrifuging is the most effective and reliable; however, a cost of \$20 to \$50 per gallon of water prevents it from being used in biofuel production (Massingill et al., 2008). It is mostly used in high-value product development or following other methods, such as sedimentation or flocculation in the second step after algae are initially concentrated (Ben-Amotz,

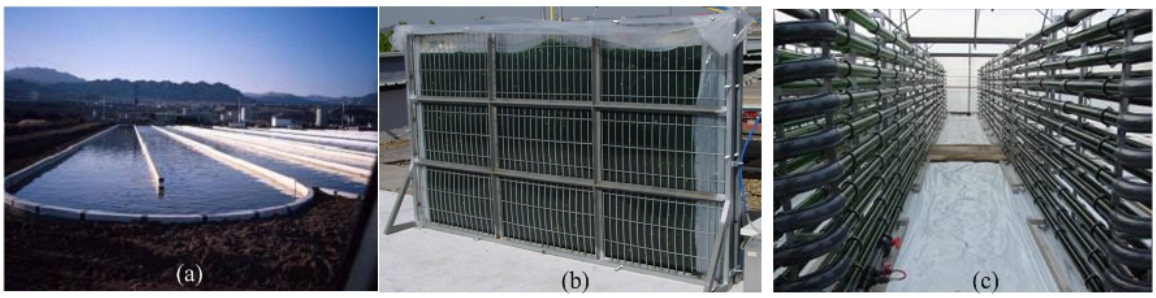

Figure 4. (a) Raceway pond, (b) plate PBR, (c) tubular PBR (all pictures courtesy of E. M. Grima, University of Almeria, Spain).

Table 3. Comparison of three culture systems (Grima, 2009).

Parameter	Raceway	Flat Panel PBR	Tubular PBR
Volume (m^3)	1,000	5	5
Volume to surface ratio (m^3 m^{-2})	0.2	0.07-0.1	0.04-0.08
Gas holdup	0.01	0.05	0.01
Mass transfer coefficient (m s^{-1})	0.0005	0.005	0.003
Dispersion coefficient (m^2 s^{-1})	0.0001	0.03	0.04
Typical biomass productivity			
(g m^{-2} d^{-1})	15	35	50
(tons ha^{-1} $year^{-1}$)	~45	~80	~100
Typical biomass conc. (g L^{-1})	0.25	1	1.5
Construction cost ($ m^{-3})	270	2,700	6,750

2008b; Massingill et al., 2008). Filtration and screening processes both separate solids from liquids by passing the suspension through a permeable medium that retains the solids, with the aid of pressure or vacuum. They are less costly, but they are limited in handling fast filtering speeds on a large scale. They also lead to eventual clogging of the filter by the packed cells when vacuum or pressure is applied. Flocculation is triggered either by adding chemical flocculants or microorganisms (called biofloculation) to the algal broth to modify the culture medium to aggregate algal cells and increase particle sizes to recover biomass. Flocculation and biofloculation are popular in large-scale algae harvest and relatively inexpensive; however, the methods are algae-strain specific, and recovery or recycle of flocculants is difficult. Settling/sedimentation are processes of solid-liquid separation that separates a feed suspension into slurry of higher concentration and an effluent of substantially clear liquid. Examples include gravity sedimentation, baffled sedimentation, and lamella plate pack sedimentation. Sedimentation is least expensive, but final solid concentration and cell recovery rate are low. Apparently, there is no single method that is effective, reliable, and reasonably cost effective. Details of the harvesting methods can be found in related references and are beyond the scope of this review.

Immobilized Culture Systems

A much less studied algal mass production method is the non-suspended, or immobilized, system, in which unialgal cultures are immobilized in a polymeric matrix or attached algal communities grow in shallow, artificial streams or on surfaces of rotating biological contactors (Hoffmann, 1998). Non-suspended algae cultivation includes enclosure and non-enclosure methods.

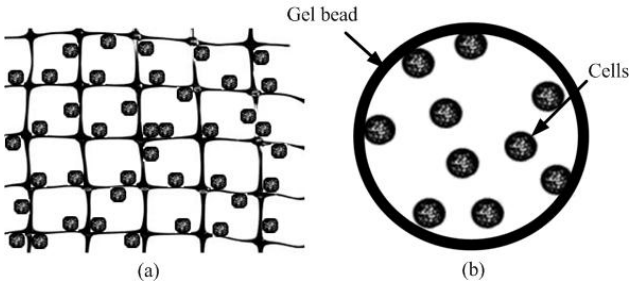

Figure 5. Enclosure methods: (a) cells in a polymer matrix sheet, and (b) cells in a gel bead.

Enclosure Methods

Enclosure methods involve use of a polymeric matrix to confine algae cells in a particular region of space or use of encapsulation to prevent algae cells from being washed away. Figure 5a shows cells enclosed in a polymer matrix sheet under a microscope, and figure 5b illustrates encapsulated cells in a gel bead.

The concept of growing cells on solid carriers resulted from the development of enzyme technology (Chibata and Tosa, 1977; Klibanov, 1983; Alexandria, 1985). The technology has been widely used in industry for enzyme, yeast, and bacterial cultures (Durand and Navarro, 1978; Kolot, 1981; Kennedy and Cabral, 1983), but its applications for algae production are limited and have been tested at the laboratory scale only (Hoffmann, 1998). Most efforts reported to date of growing entrapped or enclosed algae have focused on wastewater treatment because of better species control, faster removal rates of pollutants, avoidance of washout, etc. (Robinson et al., 1986; Huntley et al., 1989). A few algae species (e.g., *Anabaena doliolum* and *Chlorella vulgaris*) and various polymers (e.g., chitosan, alginate, and carrageenan) have been successfully applied to wastewater treatment (Da Costa and Ferreira Leite, 1991; Mallick and Rai, 1994). For example, Kaya et al. (1995) investigated four systems for tertiary wastewater treatment by *Scenedesmus bicellularis*: (1) non-immobilized cells with air bubbling (NCA), (2) cells immobilized in alginate beads (CBW), (3) cells immobilized on alginate screens (CSW), and (4) cells immobilized on alginate screens but conditioned in air (CSA). The CSW and CSA systems achieved higher efficiency in removal of N and phosphorous (P) as well as biomass productivity. In a 2 h cultivation after nutrient starvation, more than 800 μmol L^{-1} N and P in wastewater medium were completely removed in the CSA and CSW systems with biomass DW concentrations of 1.79 and 1.86 g L^{-1}, respectively. The CBW system achieved

Table 4. Comparison of commonly used harvest methods of microalgae.

Harvest Methods	Suspended Solids Concentration (%)	Operating Cost	Cell Harvesting Efficiency	Algal Species	References
Centrifuging	High (<22%)	Very high ($20 to $50 gal^{-1})	>90%	Almost all algae species except those very fragile	Mohn, 1980; Massingill et al., 2008; Green, 2008
Filtration/ screening	Medium to high (5% to 18%)	Medium to high ($10 to $20 gal^{-1})	20% to 90%	Algae with large cells	Mohn, 1980; Rossignol et al., 2000; Green, 2008
Flocculation	Low to medium (3% to 6%)	Low to medium ($3 to $10 gal^{-1})	50% to 90%	Algae with low cell density	Oh et al., 2001; Divakaran and Pillai, 2002; Brune et al., 2008; Green, 2008; Massingill et al., 2008
Bioflocculation	Low to medium (2% to 5%)	Low ($0.2 to $0.5 gal^{-1})	~90%		
Sedimentation/ settling	Low (0.5% to 3%)	Low to medium ($0.5 to $1.5 gal^{-1})	10% to 90%	Algae with high cell density	Brune et al., 2008; Green, 2008; Massingill et al., 2008

about 75% N and P removal with 1.57 g L^{-1} biomass DW concentration, and the NCA system achieved about 50% N and P removal with 0.19 g L^{-1} biomass DW concentration. Other research also showed that immobilized systems could achieve more biomass DW than free cell systems. For example, Leon and Galvan (1995) studied the production of glycerol in *Chlamydomonas reinhardtii* (~20% lipid content) cells immobilized in Ca-alginate. The immobilized cells showed a production rate of 7 g L^{-1}, which is higher than the 4 g L^{-1} achieved by their free-cell counterparts. A major problem with these enclosure systems for non-suspended algae production is the prohibitive cost of scaling up the polymeric matrix (García et al., 2001). This is probably why there have been no reports on use of this technology for large-scale algae production to manufacture biofuels.

Non-Enclosure Methods

There are reports on methods of growing algae on solid carriers without enclosure. Most of these methods were designed for wastewater treatment (Przytocka-Jusiak et al., 1984; Adey et al., 1993; Mulbry and Wilkie, 2001; Kebede-Westhead et al., 2006). One such method was known as algal turf scrubber (ATS; Kebede-Westhead et al., 2006). Figure 6a shows a schematic diagram of the ATS system used for wastewater treatment at laboratory scale. Essential elements are a solid support for growth and harvesting of algae as well as wave surge for agitation. Success of this method has been reported for wastewater treatment and algae biomass production. Mulbry and Wilkie (2001) cultured benthic freshwater algae in an ATS system with a 1 m^2 growing area and 200 L of continuously circulated medium. The system achieved approximately 5 g m^{-2} d^{-1} biomass DW by using dairy manure. Daily ammonium–N and total P removal reached 42% to 100% and 58% to 100%, respectively. Kebede-Westhead et al. (2006) investigated the effect of wastewater loading rates on N and P removal efficiency and algae biomass productivity. Mean biomass productivities were between 7.1 and 9.4 g m^{-2} d^{-1} with 90% N and 68% to 76% P removal at the best loading rate. Figure 6b shows a large-scale ATS system in Florida (HydroMentia, 2005). The system consumed 926.1 lb total P $acre^{-1}$ $year^{-1}$ in 2005. According to P balance analyses, 6.69% total P was translated into algal P and harvested as animal feed. Adey et al. (1993) reported that annual biomass productivity of an ATS system in Florida ranged from 15 (winter) to 27 (spring) g m^{-2} d^{-1}. By applying gravity sieving in the ATS system, mean biomass productivity from March to May was improved to 33 to 39 g m^{-2} d^{-1}. Another method is rotating biological contactors (RBCs), illustrated in figure 6c. This method involves using a rotating disk or drum approximately 40% submersed in wastewater tanks, with algae growing on the disk or drum. For example, Suzuki and Yamaya (2005) investigated a novel rotating biological contactor with biodrum to remove hydrocarbons in wastewater from industrial discharges. Algal strain *P. zopfii* (ATCC 30253) was immobilized by physical entrapment within the open-pore network of 10 mm cubes. Maximum biomass in the polyurethane cubes reached about 3×10^6 cells mL^{-1} (60 g L^{-1}). With such biomass, the system removed about 95% of hydrocarbons in 2 days. Successful applications of this method were also reported by other researchers (Torpey et al., 1971; Przytocka-Jusiak et al., 1984).

Features and Barriers of Immobilized Systems

Immobilized algae culture systems are unique in that cells are attached to carriers instead of suspended in culture media. Because of that, several advantages are apparent:

1. Easy harvesting of algae biomass. Harvesting is a challenging issue for suspended algae production (using either open-pond or closed PBR systems), as explained previously. Immobilized algae production has great potential to obviate the harvesting problem because algal cells are enclosed in small spaces (e.g., beads) or attached to solid carriers, so water separation can be simple. For example, by using the ATS system, 65% to 85% of the algae biomass might be collected from the turf scrubber (HydroMentia, 2005).
2. Improved productivity. Immobilized systems, especially the non-enclosure systems, can improve nutrient/gas transfer and prevent light shielding. For example, in the ATS system, algae cells on the solid carrier are directly exposed to air and light, not through a water layer like in the suspended system. This prevents development of boundary layers that limit nutrient and metabolite exchange and light transfer, which could, consequently, lead to enhanced productivity. However, it should be noted that almost all immobilized algae systems in use today are not designed to produce biomass; instead, the main purpose is to remove nutrients. There will be plenty of room for improving algae biomass and oil production through optimizing growth conditions and selecting the right algae species, solid carrier materials, and designs.

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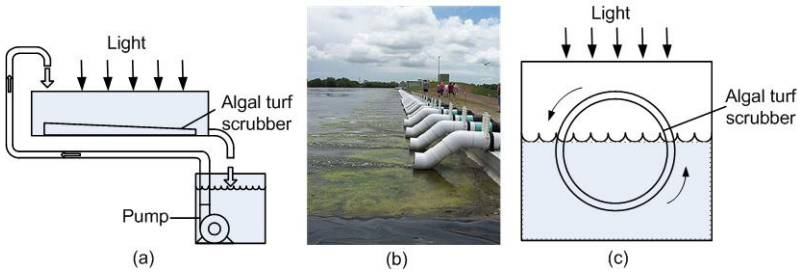

Figure 6. Algal turf scrubber systems (b: courtesy of HydroMentia, Inc.)

Some barriers of immobilized algal culture systems are as follows:

1. High material costs for the solid carriers. Polymeric matrix materials that are porous and allow water/air/nutrient exchange, such as chitosan, alginate, and carrageenan, are expensive. For the non-enclosure methods, although some inexpensive materials such as thin plastics and fabrics have been used, the lifespans of these materials are relatively short due to constant handling, and maintenance costs would be high in large-scale systems because of frequent replacement. Proper materials that are both inexpensive and durable have yet to be found for algae to attach to and grow on in large-scale systems.
2. Not all algae species can attach to and grow on solid carriers, especially non-enclosure carriers. Although a few species have been found applicable, most of them are used for wastewater treatment. It is not surprising that these species are low in lipid content. More investigations to identify some high lipid content species for immobilized culture systems are needed.
3. Scaling up outdoor immobilized systems may be difficult. More investigation is needed in this area.

The Economics of Algae Biofuel

Economic viability of algae mass production for biofuel manufacturing depends on at least three factors: (1) production cost, or how much lipid per acre can be produced; (2) the price of petroleum, and (3) future research and development innovations in algae cultures.

Maximum Biomass and Lipid Yield of Photosynthetic Algae

Photosynthetic organisms use at least eight photons to capture one molecule of CO_2 into carbohydrate $(CH_2O)_n$; thus, the maximum theoretical conversion efficiency of photosynthetically active radiation (PAR) energy into carbohydrate (η_{theo}) can be estimated as (Dimitrov, 2007):

$$\eta_{theo} = \frac{HV_{carbohydrate}}{8 \times E_{photon}} \quad (1)$$

where $HV_{carbodrate}$ is the heating value of CH_2O (~468 kJ mol^{-1}), and E_{photon} is the mean energy of a mole of PAR photons (~217.4 kJ). This gives a maximum theoretical efficiency of approximately 27%.

Actual solar conversion efficiencies, however, are much lower than this number because: (1) plants cannot absorb every PAR photon that falls on the surface of the earth because of photosaturation, photoinhibition, and light reflection; (2) plants also spend some energy on life-support functions besides building carbohydrates; and (3) there are transmission losses from sunlight to algae cells through the PBR wall or water/gas, and energy losses due to shading effects and light reflection from the PBR wall or water surface. By combining all these losses, it was estimated that only about 37% PAR energy can actually be used (Dimitrov, 2007), which leads to a final PAR conversion efficiency of about 10% (27% × 37%). However, it must be noted that the 10% PAR conversion efficiency is almost the theoretical maximum, and most algae mass production systems cannot reach this efficiency. Benemann (2008) suggested that 3.7% was the maximum in algae open ponds.

Assuming that algae only accumulate carbohydrates and lipids, theoretical biomass yield (*BY*, g m^{-2} d^{-1}) can be expressed by the following equation:

$$BY = \frac{QT\varsigma}{E_c(1-L)+E_l L} \quad (2)$$

where Q is the annual average PAR energy (W m^{-2}), T is time (86,400 s d^{-1}), η is the theoretical final PAR conversion efficiency (10%), E_c is the energy necessary for building 1 g of carbohydrate (17 KJ g^{-1}), E_l is the energy necessary for synthesizing 1 g of lipid (38 KJ g^{-1}) (Bender and Bender, 1999), and L is lipid content. Lipid yield is simply the multiplication of biomass productivity and lipid content. Using the U.S. Southwest as an example, we can estimate how much biomass and lipid can be produced. Annual PAR energy (Q) in the Southwest is about 105 W m^{-2} (Dimitrov, 2007). If we assume that all PAR energy is used to build carbohydrate (lipid content is 0), then the theoretical maximum biomass productivity on an annual basis is 54 g m^{-2} d^{-1} (86 mt $acre^{-1}$ $year^{-1}$) with the 10% PAR conversion efficiency discussed previously. However, most algae build proteins and lipids more than carbohydrates, and the energy requirements of proteins and lipids are greater than those of carbohydrates. Because lipids are the major target products, we assume that algae build only lipids and carbohydrates. The biomass and lipid yields of algae at various levels of lipid contents based on equation 2 are shown in figure 7. It is evident that at higher lipid content, the lipid yield will be higher although biomass yield will be lower. With 60% lipid content, the theoretical maximum lipid yield is below 8,000 gal $acre^{-1}$ $year^{-1}$ from photosynthetic algae cultivation. However, at this level of lipid content, biomass yield is only 31 g m^{-2} d^{-1}, which means the target algae species may not be able to compete with other lower lipid content but faster growing algae species. This explains why most fast-growing algae in natural environments are low in lipid content.

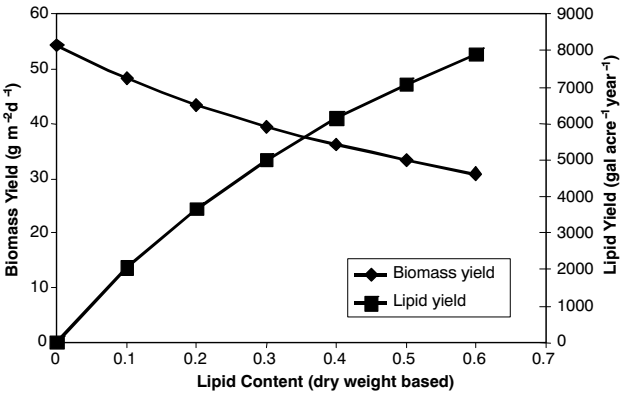

Figure 7. Theoretical algae biomass and lipid yield.

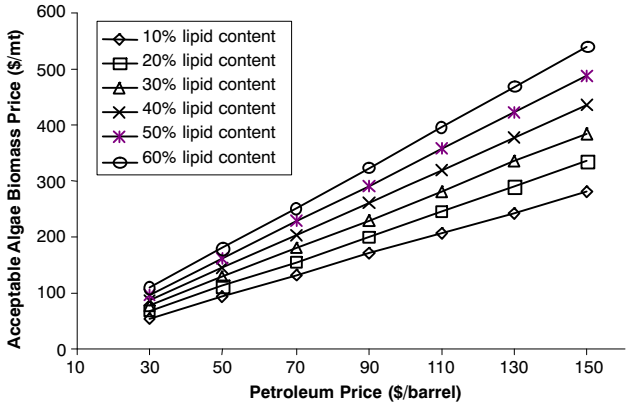

Figure 8. Competitiveness of microalgal biomass depends on its lipid content and the price of petroleum.

The Economics of Algae Mass Production

Assuming that the lipids in algae biomass are extracted for biodiesel production and the leftover is converted into biogas through anaerobic digestion, an economic model based on the energy value of algae-derived products was given by Chisti (2008), as shown in equation 3:

$$Z = \frac{X}{E_{petroleum}} \times \left[q(1-w)E_{biogas} + ywE_{biodiesel} \right] \qquad (3)$$

where Z is the acceptable price of algae biomass ($ ton^{-1}), X is the price of a barrel of petroleum, $E_{petroleum}$ is the energy contained in a barrel of crude petroleum (~6100 MJ), q is the biogas volume produced by anaerobic digestion of residual algal biomass (expected to be ~400 m^3 ton^{-1}), w is the lipid content of the biomass in percent by dry weight (~10% to 60% in commercial species), E_{biogas} is the energy content of biogas (expected to be ~23.4 MJ m^{-3}), y is the yield of biodiesel from algal oil (~80% by weight; Chisti, 2007), and $E_{biodiesel}$ is the average energy content of biodiesel (~37,800 MJ ton^{-1}; Chisti, 2008).

Acceptable algae biomass prices at various lipid content levels based on this model are shown in figure 8. For example, the current price of crude oil is about $50 per barrel. At this price, microalgal biomass with an oil content of 60% will need to be produced at about $180 ton^{-1} to be competitive with petroleum diesel. At 30% lipid content, the acceptable price has to be as low as $130 ton^{-1}. The current microalgal biomass price was estimated at around $3000 ton^{-1} and $3800 ton^{-1} for open ponds and PBRs, respectively (Chisti, 2007). Actual production costs of $8000 to $15,000 ton^{-1} (James and Boriah, 2008) and $4500 to $45,000 ton^{-1} (Tredici, 2008) were reported for open ponds and PBRs, respectively, which are far beyond the acceptable prices at the current petroleum price.

Figure 9 shows the prices of lipids from algae biomass produced at the acceptable prices determined by equation 2. At more than 30% lipid content, algae lipids can be cost-competitive with soybean oil, even without revenue from by-products. For example, if algae biomass can be produced at $130 ton^{-1} at 30% lipid content, then algae lipid cost will be about 24 cents lb^{-1}, which is below the current soybean oil price of about 35 cents lb^{-1}. However, at less than 20% lipid content, it is difficult for algae lipids to be cost-competitive with soybean oil, even if algae biomass can be produced at the acceptable prices determined by equation 2. It has to be noted that the current algae biomass production cost was estimated at around $3000 ton^{-1}, which is far beyond the acceptable price. At such a high biomass price, lipid cost would be $17.05 lb^{-1} and $2.84 lb^{-1} at 10% and 60% lipid content, respectively. Therefore, the key is to significantly reduce algae biomass production cost while keeping lipid contents high, which requires innovations in algae mass production.

Algae Production Costs

Detailed cost analyses of large-scale algae production are rarely available in the literature. Table 5 summarizes two up-to-date examples that include the details of capital investment and operating costs. The raceway pond (Ben-Amotz, 2008a, 2008b) is located in Israel with 10 ha area and a productivity of 7 tons ha^{-1} year^{-1}. Since the target product was β-carotene by growing *Dunaliella*, the system had very low biomass yield (2 g m^{-2} d^{-1}). The final production cost was found to be 26 $ kg^{-1}. The same author (Ben-Amotz, 2008b) projected that by growing some other algae species for biofuel production, the production cost could come down to 1.3 $ kg^{-1} assuming the same capital investment. However, the author assumed a biomass yield of 20 g m^{-2} d^{-1}, which is difficult to achieve. In addition, the author assumed much lower operating costs, which was not justified. A more conservative estimation will assume a biomass yield of 15 g m^{-2} d^{-1} and the same capital investment and operating costs. That would lead to a final production cost of about $3.5 kg^{-1}. Grima (2009) studied a 30 m^3 horizontal tubular PBR system. Although 100 t ha^{-1} year^{-1} biomass productivity was

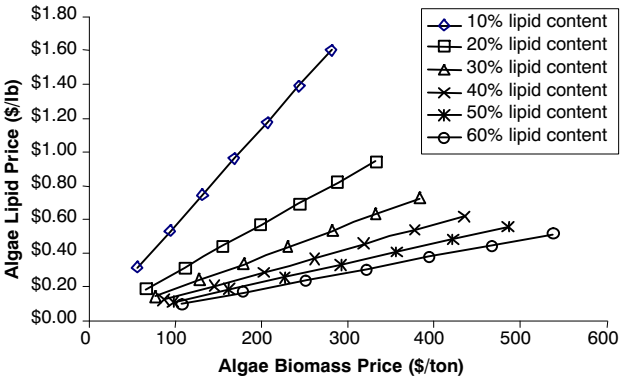

Figure 9. Algae lipid price vs. biomass price without revenue from by-products.

Table 5. Algae production costs of raceway and tubular PBR systems.

	Raceway[a]	Tubular PBR[b]
Algae strain	*Dunaliella*	*S. almeriensis*
Final product	β-carotene	biofuel
Scale	10 ha	650 m^2, 30 m^3
Biomass yield		
g m^{-2} d^{-1}	2	50
tons ha^{-1} $year^{-1}$	7	100
Capital cost ($)		
Major purchased equipment	4,300,000	290,720
Installation	--	29,070
Building	1,000,000	29,070
Infrastructure	1,000,000	264,140
Other	300,000	--
Total capital costs	6,600,000	613,000
Depreciation (10 years, $ $year^{-1}$)	660,000	61,300
Operating cost ($ $year^{-1}$)		
Fertilizers	36,000	4,720
Labor	500,000	127,930
Electricity	180,000	18,130
Water	220,000	--
CO_2	150,000	8,810
Other	800,00	--
Total operating costs	1,166,000	159,590
Total production cost ($ $year^{-1}$)	1,826,000	220,890
Algae biomass production cost ($ kg^{-1})	26	34

[a] Ben-Amotz (2008a, 2008b).
[b] Grima (2009).

achieved, the final biomass production cost was still \$34 kg^{-1}.

A cost distribution analysis of the two systems is shown in figure 10. As can be seen from figure 10a, depreciation was the largest portion (36%) of the total production cost of the open pond due to the large initial capital investment. For the tubular PBR system, labor accounted for the major part (58%) of the total cost, mainly due to the small scale of the system. When converted to unit biomass produced, the depreciation and raw material costs of the two systems were similar. The biggest difference was in labor because of the effect of the scale. Utility cost per unit biomass of the PBR system was lower than that of the open pond due to higher biomass productivity. However, it must be noted that the open pond example presented here was not for biofuel production. As explained previously, if another algae species, (e.g., *S. almeriensis*) was grown, much higher biomass yield could be achieved, which should be able to reduce all costs significantly (e.g., 80% to 90%).

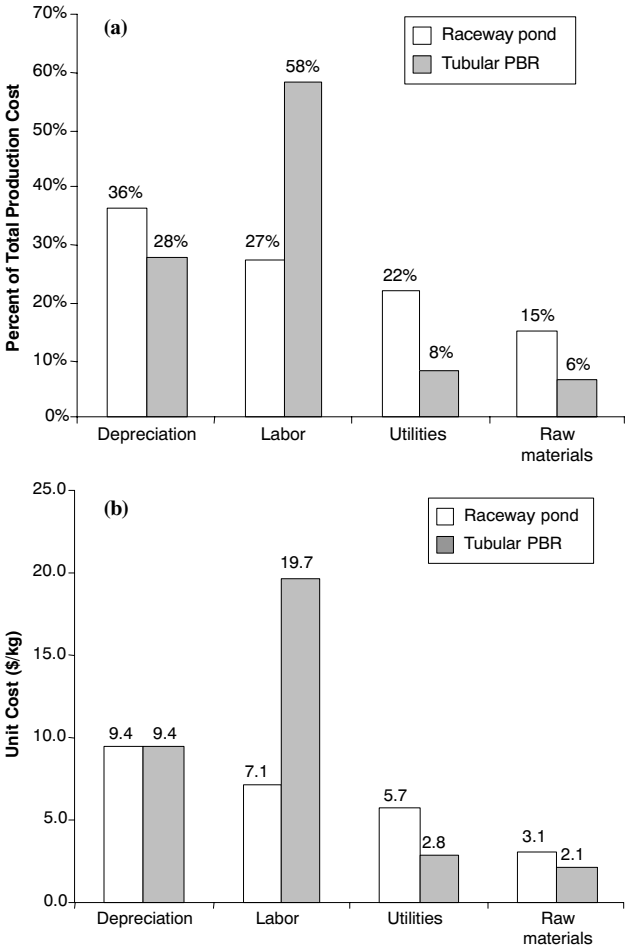

Figure 10. (a) Percentage and (b) unit biomass cost of each cost category of the raceway pond and tubular PBR system.

Future Innovations in Algae Mass Production

Producing biodiesel from microalgae is technically feasible, but because of high production costs, it is still not economically viable. Future research will have to significantly reduce production costs through innovations in several areas:

1. Metabolic and genetic engineering. Molecular-level engineering can potentially increase biomass productivity and oil content and reduce the sensitivity of algae to culture conditions, such as light, temperature, oxygen level, etc. (Roessler et al., 1994; Dunahay et al., 1996). The key is to push algae photosynthesis to the limit while keeping lipid content high.
2. Algae biorefinery. Efficient use of algae residues may offset biofuel cost to some extent. In addition to oil, most algae also contain a large quantity of proteins and carbohydrates and other nutrients in cells (Pyle et al., 2008). If economically viable technologies are available, these proteins and carbohydrates can be converted to value-added products such as feed and ethanol, respectively. Ben-Amotz (2008a) estimated that the potential commodity market value of microalgae can be \$1 kg^{-1}, which includes \$0.3 feed from proteins, \$0.4 biodiesel from lipids, and \$0.3 bio-ethanol from carbohydrates.
3. Photobioreactor/open-pond engineering. The high construction cost of PBRs (largely due to high material costs) is one of the major factors limiting use of PBRs. Materials with low cost, low light-dilution, and high thermal insulation should be developed and used. For open ponds, engineering efforts should focus on improving the efficiency of mixing, reducing mixing cost, and optimizing culture conditions to improve biomass yield and reduce contamination risks.
4. Downstream processing. High costs for harvesting, drying, and oil extraction are still limiting the commercialization of algae biofuel. It is estimated that 14%, 10%, and 16% of total production costs come from harvesting, drying, and oil extraction, respectively (Hu and Summerfeld, 2008). Downstream processing

accounts for 40% of the total cost, which is about the same as algae culture cost.

Summary and Conclusions

Producing microalgae biodiesel is technically feasible. It is regarded as one of the major renewable sources of biodiesel that can completely displace liquid fuels derived from petroleum. Among various algal culture systems, open ponds are the oldest and simplest. Open ponds are relatively inexpensive to build and maintain, easy to scale up, and it is possible to integrate them with wastewater treatment processes. However, they are low in productivity and easily contaminated by other microorganisms. Photobioreactors are the other commonly used algal culture systems. With complex designs of light dilution, gas transfer, and thermal insulation, PBRs can achieve double (or more) the biomass productivity of open ponds and have better control of contamination. However, high construction and maintenance costs are the main concerns. Immobilized systems have great potential to solve problems of suspended algae culture systems; however, proper algal species, effective and low-cost attachable solid carrier materials, and optimized designs all need further study. Research and development innovations that significantly reduce algae mass production costs are required to achieve economically viable algae biofuel manufacturing.

Acknowledgements

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