



***ICEEM/03 – ENVIRONMENTAL ENGINEERING
SECTION
Environmental Pollution and Monitoring***

MICROALGAE AS SUSTAINABLE CELL FACTORIES

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Abstract

Microalgae and cyanobacteria are sunlight-driven cell factories that convert carbon dioxide to potential biofuels, foods, feeds and high-value bioactives. In addition, these microorganisms are useful in several bioremediation applications and as nitrogen fixing biofertilizers. This work discusses the industrial and environmental applications of microalgae and the production of algae in photobioreactors. Suitably designed photobioreactors are substantially more productive in comparison with open ponds and “raceways” that have been traditionally used for culturing microalgae. Furthermore, photobioreactors allow monoseptic culture of many more algae than can be grown in open systems. Design of photobioreactors requires an ability to accurately estimate the photosynthetic irradiance level; supply the necessary amount of carbon dioxide; remove the inhibitory oxygen produced by photosynthesis; ensure rapid light–dark cycling of cells for enhanced productivity; and limit the maximum level of turbulence that the fragile algal cells are exposed to. These and other aspects of photobioreactor engineering are discussed.

Keywords: microalgae; cyanobacteria; environmental sustainability; biofuels; biofertilizers; photobioreactors; carbon sequestration

1. Introduction

Microalgae and cyanobacteria are photosynthetic microorganisms that are responsible for at least 50% of the photosynthetic biomass production on Earth. Although these two groups of microorganisms are fundamentally different, for the purposes of this review they will be referred to as microalgae, in keeping with much of the literature in applied phycology. As sunlight-driven cell factories, microalgae are potential production vehicles for numerous possible products (Becker, 1994; Olaizola, 2003; Spolaore et al., 2006; Walker et al.,

2005). Microalgal products include biofuels, foods, feeds, high-value bioactives, and biomaterials. In addition, these microorganisms are useful as nitrogen-fixing biofertilizers and agents for bioremediation of the environment. This review discusses the applications of microalgae and their production in tubular photobioreactors. Design considerations for photobioreactors are discussed. With increasing emphasis on sustainability of production processes (Gavrilescu and Chisti, 2005), microalgae are bound to play an increasing role in production of goods and services.

2. Applications of microalgae

Microalgae and cyanobacteria have a long history of use as food (Kay, 1991). *Spirulina* products (Ciferri, 1983; Khan et al., 2005) are consumed all over the world. Algae such as *Chlorella* and *Nostoc* (Gao, 1998) are eaten particularly in Asia. About a dozen other microalgae are produced commercially in smaller quantities for use as feed in the aquaculture industry (Duerr et al., 1998). A majority of aquaculture-produced shellfish, shrimp and some fish are reared directly or indirectly on microalgae during one or more stages of their life (Duerr et al., 1998).

More recent is the interest in using microalgae, cyanobacteria and other photosynthetic bacteria as potential producers of biorenewable fuels, industrial chemicals, bioactive substances and specialty chemicals. Renewable fuels such as biodiesel, biohydrogen and biogas can be sourced from algae. Biodiesel production from microalgae is a relatively novel concept. Certain microalgae can accumulate 70% or more of their dry biomass as hydrocarbons and, therefore, are potential sources of biofuels (Banerjee et al., 2002; Sheehan et al., 1998; Tsukahara and Sawayamam, 2005). A New Zealand company, Aquaflow Bionomic, recently announced world's first commercial production of biodiesel from microalgae grown in sewage ponds (Kiong, 2006). Photobiological production of hydrogen as fuel is attracting much attention (Akkerman et al., 2002; Das and Veziroglu, 2001; Ghirardi et al., 2000; Kapdan and Kargi, 2006; Tsygankov et al., 2002) and may be commercially feasible in the distant future.

Certain cyanobacteria have been recognized as important biofertilizers and soil conditioners. In presence of sufficient water, crop productivity is limited primarily by the availability of assimilable nitrogen. Consequently, huge quantities of chemically produced nitrogenous fertilizers are consumed worldwide. Potentially, cyanobacteria can provide an alternative to chemical nitrogenous fertilizers in some applications. Many species of cyanobacteria such as *Anabaena* are diazotrophs, i.e. they can use atmospheric dinitrogen as the sole nitrogen source to produce proteins and other necessary biochemicals. Some cyanobacterial species have been used to provide up to 40 kg of nitrogen per hectare in rice cultivation in Asia's flooded paddy fields. Potentially, nitrogen fixation capabilities of cyanobacteria may exceed 300 kg nitrogen per hectare per year. Much interest exists in large-scale cultivation of various cyanobacteria for use as biofertilizers (Moreno et al., 2003; Vaishampayan et al., 2001).

Microalgae and cyanobacteria are potentially useful in bioremediation of organic and inorganic compounds. For example, microalgae can hyperaccumulate heavy metals from wastewater (Mallick, 2002; Suresh and Ravishankar, 2004) and some are capable of degrading polyaromatic hydrocarbons and other organics (Juhasz and Naidu, 2001). Microalgae appear to be potentially useful in removing uranium from mining wastewater (Kalin et al., 2005). Microalgae contain about 50% carbon in their biomass. In most cases, all of this carbon can be obtained from atmospheric carbon dioxide. Consequently, algae are attracting interest as vehicles for sequestering carbon dioxide produced in various industrial operations (Doucha et al., 2005; Ono and Cuello, 2004). Use of algae for carbon dioxide absorption may have advantages over higher plants, as microalgae generally grow faster than plants. Microalgae can potentially be used in life-support systems for absorbing carbon dioxide and replacing it with oxygen in confined atmospheres such as those found in space stations (Gòdia et al., 2002).

Nitrate and phosphate pollution of water bodies because of runoffs from land fertilized with inorganic fertilizers is an important environmental issue. Microalgae have the potential to remove nitrate and phosphate pollutants from runoffs and wastewaters (Drenner et al., 1997; Kent et al., 2005). Algae play an important role in wastewater treatment in high-rate algae ponds and other systems.

Microalgae are recognized sources of numerous bioactives (Borowitzka, 1999; Dittmann and Wiegand, 2006; Lebeau and Robert, 2003a, b; Proksch et al., 2002; Walker et al., 2005). Polysaccharides with antiviral and other activities have been widely reported in microalgae (Yim et al., 2003). Microalgae are being investigated for commercial production of omega-3 polyunsaturated fatty acids such as eicosapentaenoic acid (Belarbi et al., 2000; Molina Grima et al., 2003; Wen and Chen, 2003). Production of astaxanthin (Guerin et al., 2003; Lorenz and Cysewski, 2000) and β -carotene (Jin and Melis, 2003) from microalgae *Haematococcus* and *Dunaliella*, respectively, are now well-established commercial processes.

Some algae can grow in the dark, i.e. they are heterotrophic. Heterotrophic growth requires a supply of organic carbon in the culture medium but eliminates the need for light. The spectrum of products formed in heterotrophic growth can be quite different from those produced in photosynthetic culture. Use of heterotrophic algae has been suggested for producing eicosapentaenoic acid (Wen and Chen, 2003). In view of their capabilities as cell factories, genetic engineering of microalgae is attracting much attention (León-Bañares et al., 2004). For example, in 2001, Martek Biosciences (www.martekbio.com) was successful in genetically modifying the marine microalga *Phaeodactylum tricorutum* so that it could live in the dark on glucose (Anon., 2001). This and other genetic modifications of microalgae are likely to greatly improve their potential for inexpensively providing various useful products (León-Bañares et al., 2004).

3. Large-scale microalgal culture systems

Large-scale outdoor culture of microalgae and cyanobacteria in open ponds, raceways and lagoons is well-established (Becker, 1994). Open culture is used commercially in the United States, Japan, Australia, India, Thailand, China, Israel and elsewhere, to produce algae for food, feed, and extraction of metabolites. Open culture systems allow relatively inexpensive production, but are subject to contamination. Consequently, only a few algal species can be cultured in open outdoor systems. Species that grow successfully in the open include rapid growers such as *Chlorella* and species that require highly selective extremophilic environment that does not favor growth of most potential contaminants. For example, species such as *Spirulina* and *Dunaliella* thrive in highly alkaline and saline selective environments, respectively. Algae produced in quantities in open systems include *Spirulina*, *Chlorella*, *Dunaliella*, *Haematococcus*, *Anabaena* and *Nostoc*.

Contamination-free monoseptic culture of most microalgae requires a fully closed culture system, or a photobioreactor. Photobioreactor culture is expensive relative to open culture, but for many cases it may be the only feasible option. Photobioreactors enable a highly controlled culture and are specially useful for culturing high-value algae that may be needed in tonnage quantities.

Various kinds of photobioreactors have been developed (Acién Fernández et al., 2001, 2003; Molina Grima et al., 1999; Muller-Feuga et al., 2003; Pulz, 2001; Sánchez Mirón et al., 2000; Tredici, 1999;). Of the available photobioreactors, tubular devices have proved most successful for large-scale use. Tubular photobioreactors consist of one or more relatively small diameter transparent tubes that may be arranged in various ways: a single continuous run loop (Fig. 1a); multiple parallel tubes originating and ending in common headers (Fig. 1b); and a tube coiled helically around a cylindrical or conical supporting frame (Fig. 1c). Sometimes the tubes may have static mixers or other internals to improve radial mixing. The array of tubes constitutes the solar collector of the photobioreactor, i.e. the region where most of the sunlight is captured. The solar collector may be mounted parallel to the ground, or vertically like a fence (Molina Grima et al., 1999). The photobioreactor may be placed outdoors to make use of inexpensive but cyclic sunlight, or it may be illuminated artificially (Muller-Feuga et al., 2003; Pulz, 2001). Artificial illumination is generally only feasible for relatively small-capacity photobioreactors that are being used to produce extremely high-value products.

Airlift-driven continuous run tubular photobioreactors have been shown to be able to reliably culture a wide range of microalgae over long periods. Design aspects of this type of photobioreactor are discussed here.

4. Airlift-driven tubular photobioreactors

Airlift-driven tubular photobioreactors consist of a transparent continuous run tubular solar receiver (Fig. 2) connected to an airlift pump that is

used to circulate the culture fluid through the solar tube. Air injected at the base of the upflow section (i.e. the riser zone) of the airlift device provides the energy necessary to cause flow of the culture broth.

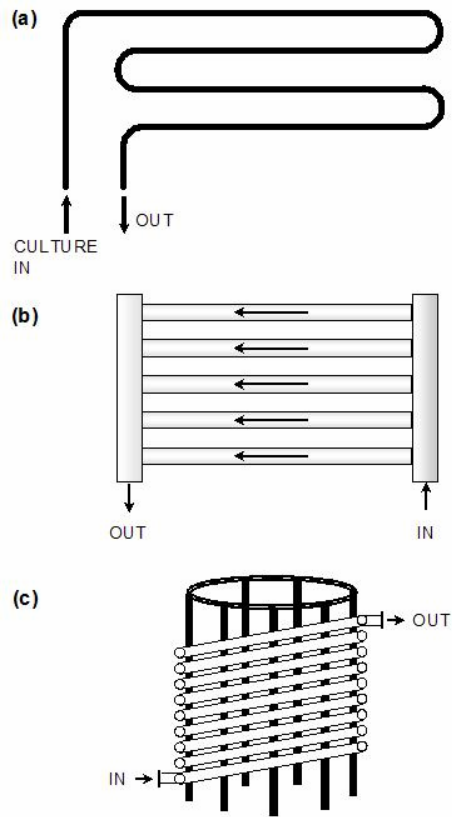


Fig. 1. Common arrangements for the solar collector a tubular loop photobioreactor: (a) a single continuous run loop; (b) multiple parallel tubes originating and ending in common headers; (c) a tube coiled helically around a cylindrical supporting frame

The air injected in the riser zone all disengages from the broth in the degassing zone (Fig. 2) so that essentially bubble-free broth returns to the solar receiver tubes. Degasser design for ensuring complete disengagement of bubbles, is discussed by Chisti and Moo-Young (1993) and Chisti (1998). Because the gas-liquid separator is generally optically deep compared to the solar tubes, it tends to be poorly illuminated and, therefore, its volume should be kept small (e.g. less than 15% of the total bioreactor volume) compared to the volume of the solar loop. Multiple solar loops may be linked to the same degasser.

In addition to providing the motive force for recirculating the broth, the airlift zone of the photobioreactor must remove the photosynthetically generated oxygen from the broth into the exhaust gas, before the broth returns to the solar loop. Carbon dioxide is injected at the entrance to the solar loop, in response to pH measurements. Fresh

medium is fed to the bioreactor and an equal amount of broth is harvested continuously.

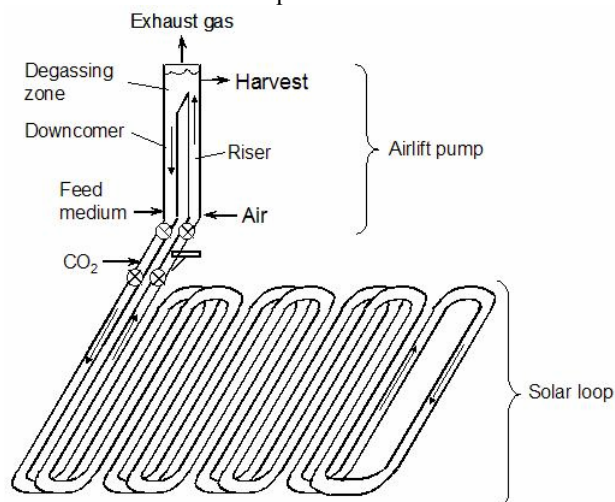


Fig. 2. An airlift-driven continuous run tubular photobioreactor (Molina Grima et al., 2001)

Algal growth medium must be suitably formulated to provide the elements necessary for growth. As an initial guide, the required levels of nitrogen, phosphorus and carbon can be estimated from the approximate molecular formula of the algal biomass, i.e. $\text{CO}_{0.48}\text{H}_{1.83}\text{N}_{0.11}\text{P}_{0.01}$. This is based on data presented by Grobbelaar (2004). Nutrients such as phosphorus must be supplied in significant excess because phosphates complex with metal ions and therefore not all the added P is bioavailable. Other essential nutrients include iron and in some cases silicon. Carbon is provided in the form of carbon dioxide that is fed continually, usually in response to signals from pH sensors. For marine microalgae, seawater supplemented with commercial nitrate and phosphate fertilizers and a few other micronutrients is commonly satisfactory. Nutrient requirements for phototrophic cultivation have been discussed by Grobbelaar (2004). Culture temperature needs to be maintained within 20–30 °C for most algae. Temperature control in large tubular photobioreactors would typically rely on passing the broth through a heat exchanger. Environmental factors and their control in culturing of microalgae are further discussed by Molina Grima (1999), Tredici (1999) and Lebeau and Robert (2003a).

Photobioreactor tubes may be made of any material that is strong and stable and transmits light in the photosynthetically active wavelength range. Suitable materials include glass and clear plastics such as poly(vinyl chloride) or PVC, poly(methyl methacrylate) or Plexiglas[®], polycarbonate, Teflon[®], and polyethylene. Silicone rubber may be used also. Glass is expensive for large scale construction and plastics are generally preferred. All plastics tend to lose clarity on extended exposure to outdoor ultraviolet radiation. Tredici (1999) provides other useful information for selecting tube materials.

Some algae will preferentially grow attached to the internal wall of the

photobioreactor tube, thus preventing light penetration into the tube and reducing bioreactor productivity. Wall growth is controlled by some of the following methods: 1) use of large slugs of air to intermittently scour the internal surface of the tube; 2) circulation of close fitting balls in continuous run tubes to clean the internal surface; 3) highly turbulent flow; and 4) suspended sand or grit particles to abrade any biomass adhering to the internal surface. Potentially, enzymes that digest the polymer glue that binds algal cells to the tube walls may be used for controlling wall growth.

In a properly designed photobioreactor culture, light should be the only factor limiting growth. About 40% of the direct solar radiation received at the Earth's surface is photosynthetically active radiation, or PAR, with a wavelength between 400 and 750 nm. Peak summer value of PAR in southern Mediterranean region is about 2,000 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at solar noon. Irradiance varies with season (i.e. day of the year), geographic location (i.e. the latitude), the solar hour, and the angle of inclination of the photobioreactor tubes with respect to the horizon. Methods for estimating the incident irradiance on photobioreactor tubes rely on well-known principles of solar power engineering, as discussed in the literature (Ación Fernández et al., 1997; Duffie and Beckman, 1980; Liu and Jordan, 1960; Molina Grima et al., 1999).

Growth rate dependence on average irradiance inside a bioreactor tube may follow Monod kinetics, or some other growth pattern (Molina Grima et al., 1999). For Monod kinetics (Eq. 1),

$$\mu = \frac{\mu_{\max} I_{av}}{K_I + I_{av}} \quad (1)$$

where μ is the specific growth rate, μ_{\max} is the maximum specific growth rate, I_{av} is the average irradiance inside the photobioreactor tube, and K_I is the light saturation constant (i.e. the irradiance value at half the maximum growth rate). Theoretical maximum specific photosynthetic growth rate of microalgae is $\sim 0.2 \text{ h}^{-1}$ (Masojídek et al., 2004). K_I depends on the alga and the culture conditions. For example, the reported saturation constant value for the cyanobacterium *Rhodospseudomonas capsulate* is between 25 and 103 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Lee and Shen, 2004). For the algae *P. tricornutum* and *Porphyridium cruentum* the light saturation constants are 185 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Mann and Myers, 1968) and $\sim 200 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Molina Grima et al., 2000), respectively. Methods of estimating the average irradiance I_{av} from the incident irradiance on the surface of the photobioreactor tube have been discussed extensively in the literature (Ación Fernández et al., 1997; Molina Grima et al., 1999).

Photobioreactor tubes of any realistic diameter operated with high-density culture for achieving a high productivity will inevitably contain a light limited central dark zone and a relatively well lit peripheral zone. Turbulence in the tube causes rapid cycling of the fluid between the light and dark zones. The frequency of light-dark cycling depends on several factors, including the level

of turbulence, the density and optical properties of the culture, the diameter of the tube, and the external irradiance level (Molina Grima et al., 2000, 2001). Under conditions of sufficient and excess external irradiance, light–dark cycling of above a certain frequency can increase biomass productivity relative to the case when the same quantity of light is supplied continuously over the same total exposure time (Camacho Rubio et al., 2003; Grobbelaar, 1994; Grobbelaar et al., 1996; Nedbal et al., 1996; Philliphs and Myers, 1953; Terry, 1986). Various attempts have been made to estimate the frequency of light–dark cycling caused by turbulence in the optically dense culture of a photobioreactor tube (Janssen et al., 2003; Molina Grima et al., 1999, 2000; Richmond, 2004; Sánchez Mirón et al., 1999), but this problem remains to be resolved satisfactorily.

Photobioreactor tubes of 0.01 to 0.1 m in diameter have been used commonly, but biomass productivity declines as tube diameter increases. This is because algal cells absorb light and deeper regions of bioreactor may be essentially dark. For example, the decline in light intensity with culture depth for a 0.05 m deep culture of the diatom *P. tricornutum* is shown in Fig. 3 for a high incident irradiance level of $2,000 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in a culture broth having a suspended biomass concentration of $2 \text{ kg}\cdot\text{m}^{-3}$. In such a culture device, ~90% of the culture volume constitutes a dark zone. Only 2% of the culture volume is light saturated and about 3% of the volume is photoinhibited (Fig. 3). In view of these results, a photobioreactor tube of a diameter bigger than 0.05 m is not recommended, unless it is internally illuminated in addition to external illumination.

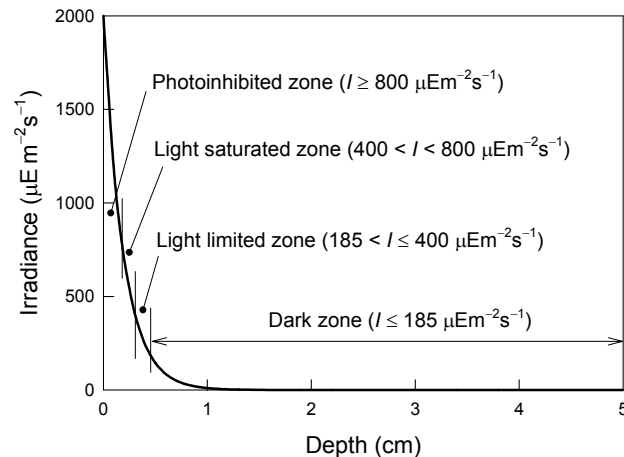


Fig. 3. Computed irradiance (I) profile inside a 5 cm deep pool of the diatom *P. tricornutum* ($2 \text{ kg}\cdot\text{m}^{-3}$ dry biomass concentration) for an external irradiance level of $2,000 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ incident perpendicular to the surface of the pool

(The light limited zone is defined as that corresponding to an irradiance level of less than or equal to the Monod light saturation constant ($\sim 185 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Approximate depths of the dark zone, light limited zone, light saturated zone and photoinhibited zone are shown. Nearly 90% of the culture volume is in the dark. About 80% of the culture volume is below the light compensation

point estimated at $\sim 4 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)

Excepting a batch phase during startup, photobioreactors are almost always operated as pseudo steady state continuous cultures. As in any continuous culture, the dilution rate cannot exceed the maximum specific growth rate, or the culture will be washed out. Growth ceases after sunset, therefore, feeding and dilution are confined to daylight hours. For any dilution rate D and the steady state biomass concentration X_b , the biomass productivity P of a bioreactor is calculated using Eq. 2.

$$P = DX_b \quad (2)$$

Optimal dilution rate depends on the prevailing irradiance level (Ación Fernández et al., 1998, 2001). Volumetric biomass productivity does not usually exceed $2 \text{ kg}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$. As much as 25% of the biomass produced during daylight, may be lost during the night because of respiration. The extent of this loss depends on the irradiance level under which the biomass was grown, the growth temperature, and the temperature at night.

Although the Monod growth relationship (Eq. 1) does not reflect this, in most cases irradiance levels only slightly higher than the saturation light intensity inhibit algal growth. This phenomenon is known as photoinhibition and it is well documented (Camacho Rubio et al., 2003). Photoinhibition results from generally reversible damage to the photosynthetic apparatus, as a consequence of excessive irradiance (Camacho Rubio et al., 2003). Outdoor photobioreactors operating under intense midday irradiance levels in the summer are almost always photoinhibited. This phenomenon needs to be considered in properly estimating the average annual biomass productivity from a photobioreactor installation.

Photosynthesis generates oxygen. The maximum rate of oxygen generation in a typical tubular photobioreactor may be as high as $10 \text{ g O}_2\cdot\text{m}^{-3}\cdot\text{min}^{-1}$ under conditions of high irradiance. Dissolved oxygen levels much greater than the air saturation values inhibit photosynthesis. Furthermore, a high concentration of dissolved oxygen in combination with intense sunlight produces photooxidative damage to algal cells. To prevent inhibition and damage, the maximum tolerable dissolved oxygen level should not generally exceed about 400% of air saturation value. Oxygen cannot be removed within a tube; therefore, the maximum continuous run length of a tube must remain below a certain value, or oxygen inhibition will occur. Typically, the maximum continuous run length would not exceed 80 m. Oxygen is removed in the airlift zone of the reactor (Fig. 2), before the culture broth reenters the solar loop. The maximum length of the solar tube before oxygen removal becomes necessary depends on the linear culture velocity in the tube, the average rate of oxygen generation, the oxygen concentration at the inlet of the tube and the acceptable maximum concentration of oxygen at the outlet of the solar tube (Ación Fernández et al., 2001; Camacho Rubio et al., 1999). An airlift driven photobioreactor's ability to remove oxygen is governed by the gas-liquid mass

transfer characteristics of the airlift zone as discussed elsewhere (Acién Fernández et al., 2001; Chisti, 1989, 1999a; Gavrilescu and Tudose, 1998).

Many microalgae are susceptible to damage by intense shear fields, but a good level of turbulence is necessary in the photobioreactor tubes to ensure that cells do not settle. High levels of turbulence in a dense culture reduce photoinhibition and photolimitation by producing rapid light–dark cycling of the flow. The ideally high frequency of light–dark cycling appears to be impossible to achieve in tubes unless some form of static mixing elements are used. In principle, algal culture can be circulated at high flow rates using mechanical pumps instead of the airlift circulator. Passage through mechanical pumps and narrow orifices of valves can damage algal cells. Damaging effects of excessive agitation on microalgae and methods of protecting the cells against damage have been discussed by Chisti (1999b), García Camacho et al. (2001), Sánchez Mirón et al. (2003), and Mazzuca Sobczuk et al. (2006).

5. Concluding remarks

Microalgae and other phototrophic microorganisms are already used commercially in a number of important applications. In view of their potential as rapid-growing photosynthetic cell factories, algae are attracting increasing attention as vehicles for sustainable production of biofuels, foods, bioactives and industrial materials. Two significant factors that are expected to contribute to increased future use of the algae in commercial operations are advances in genetic engineering to enhance their capabilities and developments in engineering of large-scale photobioreactors to enable inexpensive contained culture. For many microalgae, tubular photobioreactors appear to hold the greatest promise for monoseptic culture for producing tonnage quantities of the biomass.

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