Green Energy and Technology

Faizal Bux
Yusuf Chisti *Editors*

# Algae Biotechnology

## Products and Processes

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# Contents

# Microalgae Cultivation Fundamentals

**Yuan Kun Lee**

**Abstract** Microalgal cultivation has attracted much attention in recent years, due to their applications in $CO_2$ sequestration, biofuels, food, feed and bio-molecules production. The general requirements for successful microalgal cultivation include light, carbon, macronutrients such as nitrogen, phosphorus, magnesium and silicates and several micronutrients. This chapter discusses the principles of microalgae cultivation with regards to essential requirements and growth kinetics.

**Keywords** Microalgae · Light · Nutrient supply · Culturing · Growth kinetics

## 1 Introduction

Microalgal cultivation has attracted much attention in recent years, due to their applications in $CO_2$ sequestration, biofuels, food, feed and bio-molecules production. Estimates of the number of algal range from 350,000 to 1,000,000 species, however only a limited number of approximately 30,000 have been studied and analysed (Richmond 2004). Microalgae are a diverse group of organisms that occur in various natural habitats. Many of the microalgae studied are photosynthetic, whilst only few of them are known to grow mixotrophically or heterotrophically (Lee 2004). The general requirements for successful microalgal cultivation include light (photosynthetic and mixotrophic), carbon, macronutrients such as nitrogen, phosphorus, magnesium and silicates and several micronutrients (species dependant) for their successful cultivation. This chapter will provide an overview of the fundamentals of microalgal cultivation.

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## 2 Illumination

Microalgal cultures receive light at their illuminated surface. The ratio between the illuminated surface area and volume of cultures (*s*/*v*) determine the light energy available to the cultures and the distribution of light to cells in the cultures. Generally, higher the *s*/*v* the higher the cell density and volumetric productivity could be achieved (Pirt et al. 1980). High cell density reduces the cost of harvesting, as well as cost of media. Thus high *s*/*v* photobioreactors (PBRs) are generally preferred. However it must be cautioned that high cell density may lead to shallow light-path. Thus turbulence in the systems must be sufficient to facilitate light supply to each of the cell in the culture system to sustain maximum photosynthetic activity and growth. This would lead to reduced volumetric productivity.

### *2.1 Light Absorption*

The quantity of light energy absorbed by a photosynthetic culture is mostly determined by cell concentration and not the photon flux density. That is, most photons of low flux density could pass through a culture of low cell concentration, but all photons of high flux density could be captured by a culture of high cell concentration. Thus, cell concentration of a photosynthetic culture will continue to increase exponentially until all photosynthetically available radiance (PAR) impinging on the culture surface are absorbed. For example, a *Chlorella* culture with an optical absorption cross-section of 60 $cm^2$ $mg^{-1}$ chlorophyll a, and chlorophyll a content of 30 mg Chl a/g-cell, will require 5.6 g-cell $m^{-2}$ or 0.56 g-cells $L^{-1}$ to absorb all available photons impinging on a culture of 1 m (wide) × 1 m (long) × 0.01 m (deep), irrespective of the photon flux density. Once this cell concentration is reached, biomass accumulates at a constant rate (linear growth phase, Pirt et al. 1980) until a substrate in the culture medium or inhibitors become the limiting factor.

### *2.2 Light Attenuation Through Mutual Shading*

Once all the photons are absorbed by cells nearer to the illuminated surface, the cells located below this "photic zone" do not receive enough light energy for photosynthesis. This leads to the phenomenon of mutual shading. Thus, cell growth is limited to the photic zone.

Let us consider a monochromatic light impinges on a microalgal culture, where $I_0$ = incident photon flux density, $I_t$ = transmitted photon flux density, $a$ = absorbance coefficient or extinction coefficient of the culture at the wavelength,

$L$ = light path, and $x$ = cell density. The relationship of absorbance and cell density can be described by the Beer-Lambert Law:

$$\log(I_o/I_t) = axL \tag{1}$$

For a *Chlorella pyrenoidosa* culture with absorbance coefficient of 0.11 $m^2\ g^{-1}$ cell at the wavelength of 680 nm, 99 % of the light could only penetrate 3.6 cm into a culture of 0.5 g $L^{-1}$, and 1.2 mm into a culture of 15 g $L^{-1}$ cell density. The final cell density in outdoor shallow algal pond cultures was about 0.5 g $L^{-1}$ (Richmond 2004), whereas the highest cell density achievable in a simple batch culture in a narrow light path PBR could be >10 g $L^{-1}$ (Cuaresma et al. 2009; Doucha and Livansky 1995; Pulz et al. 2013; Lee and Low 1991, 1992; Quinn et al. 2012; Ugwu et al. 2005). Hence in most algal cultures indoors and outdoors, a significant proportion of the algal cultures is kept in dark at any given time. As a consequence, cells circulating in the culture receive energy intermittently.

Turbulence facilitates cycling of cells between the photic and dark zones. It was indeed observed that the photosynthetic efficiency and biomass productivity of microalgal cultures (Hu and Richmond 1996; Vejrazka et al. 2012) of different cell densities were functions of the stirring speed or aeration rate. The mixing effect on the areal productivity of outdoor *Spirulina* cultures was also demonstrated (Richmond and Vonshak 1978). These studies suggest that long intermittent illumination (and/or dark phase) leads to lower photosynthetic efficiency and productivity. Improvement of both gas and nutrient mass transfer may also contribute to the enhanced biomass productivity.

## 3 Carbon Supply

In high *s*/*v* PBR where most of the algal cells receive sufficient light energy to sustain growth, $CO_2$ absorption, volumetric $O_2$ evolution, nutrient depletion and metabolite excretion proceed at high rates, which may determine the overall productivity of the culture (Pirt et al. 1980).

Inorganic carbon is usually supplied as $CO_2$ gas in a 1–5 % mixture with air. High $CO_2$ partial pressure is inhibitory to most algae (Lee and Tay 1991). Another mode of carbon supply is as bicarbonate. The balance between dissolved free carbon dioxide ($CO_{2(aq)}$), carbonic acid ($H_2CO_3$), bicarbonate ($HCO_3^-$) and carbonate ($CO_3^{2-}$) is pH and temperature dependent. Higher pH (alkaline) favours forward direction of the balance equation:

$$CO_{2(aq)} + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2H^+ \tag{2}$$

The $CO_2$ absorption rate, i.e. the rate of $CO_2$ transfer from the gas to the liquid phase ($R$) is expected to accord with the mass transfer equation (Lee and Tay 1991),

$$R = K_L a(c_s - c) \tag{3}$$

where $K_L$ is a constant, $a$ = interfacial area, $c_s$ = saturation concentration of $CO_2$, and $c$ = concentration of $CO_2$ in bulk liquid. The term $K_L a$ is known as the "volumetric gas transfer constant". In solution, the $CO_2$ hydrates to some extent to form $H_2CO_3$. The equilibrium constant of the reaction $CO_2 + H_2O \leftrightarrow H_2CO_3$ is given as $K = [H_2CO_3]/[CO_2]$. The amount of carbonic acid or dissolved $CO_2$ present at equilibrium will be directly proportional to the partial pressure of $CO_2$. The equilibrium between the $CO_2$, bicarbonate and carbonate ions will depend on the pH according to the Henderson-Hasselbach equations,

$$\log[HCO_3^-]/[CO_2] = \text{pH} - pK_1 \tag{4}$$

and

$$\log\left[CO_3^{2-}\right]/[HCO_3^-] = \text{pH} - pK_2 \tag{5}$$

where $pK_1$ and $pK_2$ are the negative logarithms of the first and second dissociation constants of carbonic acid respectively and the square brackets denote concentrations. The values of $pK_1$ and $pK_2$ are respectively 6.35 and 10.3 at 25 °C, hence, at equilibrium when the pH = 6.35, $[HCO_3^-] = [CO_2]$, and when the pH = 10.3, $[CO_3^{2-}] = [HCO_3^-]$. Thus at the neutral pH of 7.5 at which the level of the $[CO_3^{2-}]$ can be discounted and only the $[HCO_3^-]$ and $[CO_2]$ need be considered.

It should be noted that only the free $CO_2$ concentration ($c$) enters into Eq. (2) and apparently the bicarbonate concentration should not affect the $CO_2$ absorption rate.

In a homogeneous culture which derives its $CO_2$ from the gas phase, at a constant pH the net rate of $CO_2$ accumulation is given by

$$dc/dt = K_L a(c_s - c) - \mu x/Y \tag{6}$$

where $\mu$ = specific growth rate, $x$ = biomass concentration and $Y$ = growth yield from $CO_2$. The interconversion rates of free $CO_2$ and bicarbonate ions are assumed to be equal because they are in equilibrium.

The maximum $CO_2$ absorption rate is given by

$$R_{max} = K_L a\, c_s = \mu x Y \tag{7}$$

And the volumetric $CO_2$ absorption coefficient is given by

$$K_L a = \mu x/c_s Y \tag{8}$$

The terms used in Eqs. (7) and (8) are similar to those used in Eqs. (3)–(6).

In a $CO_2$ limited culture with a $P_{CO_2}$ of 0.05 atm (5 % $CO_2$ in air), it is assumed that Eq. (8) would apply since $c_s$ would be about $10^{-3}$ M and generally for carbon substrate-limited growth c is of the order $10^{-5}$ M.

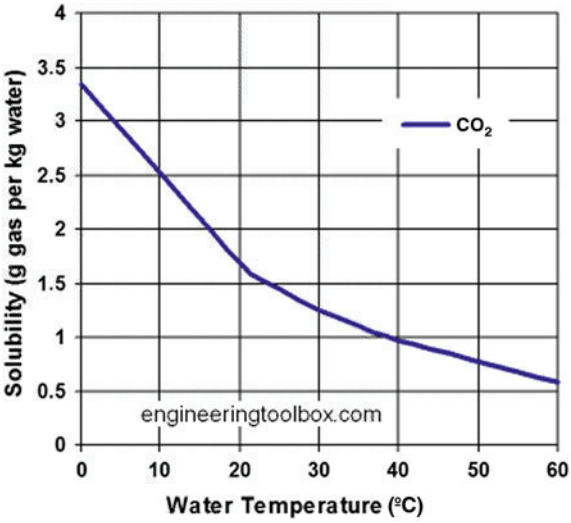


**Fig. 1** The effect of water temperature on the solubility of carbon dioxide (www.engineeringtoolbox.com)

According to the theory outlined, although change in pH will affect the degree of conversion of $CO_2$ to $HCO_3^-$, this should have no effect on the $CO_2$ absorption rate. It is not to be excluded, however, that a pH change could affect the diffusivity of $CO_2$ and the reaction kinetics of $CO_2$ at the interface, both of which could affect $K_L$. Also the interfacial area (*a*) could be affected by the pH.

The effect of temperature on the overall solubility of $CO_2$ is depicted in Fig. 1: The higher the temperature the lower the $CO_2$ in the culture (www.engineeringtoolbox.com).

## 4 Oxygen Accumulation

Accumulation of photosynthetically generated $O_2$ is one of the main factors that limit the scale-up of enclosed PBR. Oxygen production is directly correlated with volumetric productivity, and dissolved $O_2$ concentrations equivalent to 4–5 times that of air-saturations are toxic to many algae (Richmond 1986). These dissolved $O_2$ concentrations could be easily reached in outdoor cultures, especially in tubes of small diameter (high *s*/*v*). At maximal rates of photosynthesis, a 1 cm diameter reactor accumulates about 8–10 mg $O_2$/L/min. In order to keep the $O_2$ level below the toxic concentration requires frequent degassing and thus one may have to resort to short loops or high flow rates (Pirt et al. 1983). Manifold systems and vertical reactors offer a significant advantage in this respect.

## 5 Nutrient Supply

Main considerations in developing nutrient recipes for algal cultivation and maintenance are as follows.

- Nitrogen source: Nitrate, ammonia and urea are widely used as the nitrogen source, depending on the ability of the alga to use the nitrogen substrate and culture pH. pH of a culture medium containing nitrate tends to increase due to the removal of proton ($H^+$), whereas medium containing ammonium tends to decrease due to the accumulation of $H^+$. The pH of a urea-containing culture maintains constant, in this case the algae must be able to produce urease to utilize urea. Algae contain 7–9 % nitrogen per dry weight. Thus to produce 1 g of cells in 1 L of culture, a minimum of 500–600 mg/L of $KNO_3$ is required.
- Minerals: These include potassium, magnesium, sodium, calcium, sulfate and phosphate. Sea water is often phosphate deficient.
- Trace elements: These include aluminum, boron, manganese, zinc, copper, iron, cobalt and molybdenum. For solubility of the mixture of trace elements, chelating agents such as citrate and EDTA are included.
- Vitamins: Some algae (e.g. *Euglena* and *Ochromonas*) require vitamins such as thiamin and cobalamin.
- Total salt concentration: Depending on the ecological origin of the alga, for example the green alga *Dunaliella* can only survive in a medium containing 0.5 M NaCl and the optimal salinity for growth is 2 M NaCl.
- pH: Most media are neutral or slightly acidic to prevent precipitation of calcium, magnesium and trace elements.

### *5.1 Common Culture Media*

The following are common culture media used for the maintenance and cultivation of algae in laboratories (www.utex.org/prodmedia).

#### 5.1.1 Bold Basal Medium

A medium commonly used for fresh water algae. It may be supplemented with soil extract for growing algae isolated from soil.

| | | |
|---|---|---|
| Base media | (i) $NaNO_3$ | 10 g/400 mL |
| | (ii) $MgSO_4 \cdot 7H_2O$ | 3 g/400 mL |
| | (iii) $K_2HPO_4$ | 3 g/400 mL |
| | (iv) $KH_2PO_4$ | 7 g/400 mL |
| | (v) $CaCl_2 \cdot 2H_2O$ | 1 g/400 mL |
| | (vi) NaCl | 1 g/400 mL |
| Trace element solutions | (i) EDTA | 50 g/L |
| | KOH | 31 g/L |
| | (ii) $FeSO_4 \cdot 7H_2O$ | 4.98 g/L |
| | Water acidified with 1 mL $H_2SO_4$ | |
| | (iii) $H_3BO_3$ | 11.42 g/L |
| | (iv) $ZnSO_4 \cdot 7H_2O$ | 8.82 g/L |
| | $MnCl_2 \cdot 4H_2O$ | 1.44 g/L |
| | $MoO_3$ | 0.71 g/L |
| | $CuSO_4 \cdot 5H_2O$ | 1.57 g/L |
| | $Co(NO_3)_2$ | 0.49 g/L |

10 mL of each of the base medium (i)–(vi) and I mL of each of the trace element solution (i)–(iv) are made up to a final volume of 1 L. The medium may be solidified in 15 g/L agar.

### 5.1.2 N8 Medium for *Chlorella* and Other Green Algae

| | mg/L |
|---|---|
| $Na_2HPO_4 \cdot 2H_2O$ | 260 |
| $KH_2PO_4$ | 740 |
| $CaCl_2$ | 10 |
| Fe EDTA | 10 |
| $MgSO_4 \cdot 7H_2O$ | 50 |
| Trace elements | 1 mL |
| *Trace element stock* | |
| | g/L |
| $Al_2(SO_4)_3 \cdot 18H_2O$ | 3.58 |
| $MnCl_2 \cdot 4H_2O$ | 12.98 |
| $CuSO_4 \cdot 5H_2O$ | 1.83 |
| $ZnSO_4 \cdot 7H2O$ | 3.2 |

### 5.1.3 Bristol Medium

For 1 L medium, to approximately 900 mL of $dH_2O$ add each of the components in the order specified while stirring continuously. Bring total volume to 1 L with $dH_2O$.

| | mL | Stock solution (mL) | Final concentration (mM) |
|---|---|---|---|
| $NaNO_3$ | 10 | 10 g/400 mL $dH_2O$ | 2.94 |
| $CaCl_2 \cdot 2H_2O$ | 10 | 1 g/400 mL $dH_2O$ | 0.17 |
| $MgSO_4 \cdot 7H_2O$ | 10 | 3 g/400 mL $dH_2O$ | 0.3 |
| $K_2HPO_4$ | 10 | 3 g/400 mL $dH_2O$ | 0.43 |
| $KH_2PO_4$ | 10 | 7 g/400 mL $dH_2O$ | 1.29 |
| NaCl | 10 | 1 g/400 mL $dH_2O$ | 0.43 |

## *5.2 Sterilization*

Sterilization of culture media is mandatory, even if the algal cultures are not bacteria-free, to avoid additional contamination. All glassware, pipettes and media should be sterilized before use by autoclaving for 15 min at 15 pounds pressure. Seawater could not be autoclaved, for this would cause precipitation of salt from the medium, sterilization could be achieved through pasteurization and filtration sterilization (Little et al. 1987).

# 6 Culture Methods

## *6.1 Cultivation on Solid Media*

Streaking method is commonly applied. This could be done on agar plate in petri dish (Fig. 2), or on agar slant in test tube (Fig. 3). The slant in test tube has a smaller surface area compared to the petri dish, thus allowing less growth. However it has the advantage of preventing drying thus the culture could be stored for relatively long term (6 months to a year).

To prepare an agar slant, the molten agar medium, after autoclaving, is introduced into a test tube. The test tube is tilted, the agar medium is allowed to cool and harden in a slant fashion.