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Kinetic and Stoichiometric Relationships of the Energy and Carbon Metabolism in the Culture of Microalgae

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Abstract: Some microalgae can grow metabolizing inorganic and organic carbon sources, which might occur simultaneously and independently, while energy is supplied by light and/or an organic carbon source. In this context, the contribution of each metabolism to total growth can be determined by quantitative analysis. The illumination of microalgal cells growing in the presence of organic substances, might cause an effect which can drive the carbon metabolism in different ways. When analyzing the growth of different strains of microalgae, some differences could be distinguished, between additive or inhibitory effect of light on heterotrophic metabolism in mixotrophic or photoheterotrophic growth. This manuscript proposes, the integration of a kinetic and stoichiometric metabolic model which explains the differences of carbon and energy utilization modes between mixotrophic and photoheterotrophic growth in microalgae. This model presumably discloses relevant independent facts between the mechanisms of photosynthesis and the oxidative metabolism of organic compounds, such as glucose and the importance of these differences on the production of biomass and secondary metabolites.

Key words: Microalgae, photoautotrophy, heterotrophy, mixotrophy, photo-heterotrophy

INTRODUCTION

There are many aspects to be considered in order to achieve higher biomass concentration and productivities. Microalgae in general need an inorganic carbon source and luminous energy to carry out photosynthesis. However, many are capable to utilize organic compounds as carbon and energy source. Cyanobacteria, that are naturally photoautotrophic, through utilization of plant-like photosynthesis, nevertheless possess a respiratory pathway and all the enzymes necessary for sugar catabolism. But not all strains are capable to use this route and grow heterotrophically[1]. In some cases, cells are able to perform photosynthesis and simultaneously use organic carbon substrate in complete darkness (*Chlorella sp.*), but it happens that light is required for substrate assimilation (mainly for enzymes activation). In this case, the energy from light required is much lower than for photoautotrophic growth. The efficiency of organic and inorganic carbon incorporation to biomass changes during photosynthetic growth, depending on microalgal species, light intensity, energy and carbon source (inorganic or organic) and growth phase. *Chlorella vulgaris*[2,3], *Haematococcus* pluvialis[4] , *Arthrospira (Spirulina) platensis*[5] are strains found to grow under photoautotrophic, heterotrophic as well as mixotrophic conditions. All of them were found to perform photoautotrophic and heterotrophic growth as independent and simultaneous mechanisms[6]. However, there are other strains that grow preferably either photoautotrophically, such as *Selenastrum capricornutum*[7], *Scenedesmus acutus*[2], heterotrophically such as *Chlorella vulgaris*[8,9] or photoheterotrophically in the presence of light. In general, the energy and carbon sources for microalgae might be differentiated as in Table 1.

The term mixotrophic and photoheterotrophic metabolism are not well defined, in particular a fine difference of the energy source required between them to perform growth and specific metabolite production. Therefore it is important to define quantitatively the influence of light and the organic carbon source and their interrelation when considering microalgal use for production.

In other point of view the different metabolisms involved could be distinguished according to pH changes, which depend on the growth stoichiometry of microalgae as part of the metabolism involved (Table 2).

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Table 1: Summary of energy and carbon sources in different metabolisms found in microalge

Name	Energy source	Carbon source	Example
Photo autotroph	Light	Inorganic	Cyanobacteria
(Chemo)Heterotroph	Organic	Organic	*E. coli*
Photo heterotroph	Light	Organic	Purple and green photosynthetic bacteria i.e. *Rhodospirillum*
Mixotroph	Light and organic	Inorganic and organic	*Chlorella* (Photoautotrophic heterotroph)

Table 2: Stoichiometric equations considering pH changes in the culture of microalgae in various metabolisms

Metabolism	Stoichiometric equation
photoautotrophic	$H_2O + HCO_3^- \xrightarrow{hv} C(biomass) + 1/2O_2 + 3OH^- \Rightarrow$ pH increase
heterotrophic	$(1 + a)\,CH_2O + O_2 \longrightarrow C(biomass) + aCO_2 + (1+a)\,H_2O \Rightarrow$ pH decrease
mixotrophic	$bHCO_3 + cCH_2O \xrightarrow{hv} (b + (c-a))C(biomass) + 3OH- + aCO2 \Rightarrow$ pH changes are not significant

Symbols

A	-	illuminated area of photobioreactor (cm^2, m^2)
C	-	oxygen concentration (mg l^{-1}, ppm)
C_{CO2}	-	carbon dioxide concentration (mg l^{-1}, mmoles ml^{-1})
C_{Gl}	-	glucose concentration (g l^{-1})
I	-	light intensity (klux, Wm^{-2}, $kJcm^{-2}h^{-1}$, etc)
I_0	-	incident light intensity (klux, Wm^{-2}, $kJcm^{-2}h^{-1}$, etc)
I_{av}	-	average light intensity (klux, Wm^{-2}, $kJcm^{-2}h^{-1}$, etc)
K_i	-	inhibition constant (mg l^{-1})
K_I	-	Light saturation constant (klux, Wm^{-2}, $kJcm^{-2}h^{-1}$, etc)
K_s	-	Subtrate saturation constant (mg l^{-1}; mmoles l^{-1}, etc)
Q_P	-	photosynthetic rate (mol O_2 produced $l^{-1}h^{-1}$; mol O_2 g^{-1} biomass h^{-1}; mol O_2 mg^{-1} chlorophyll h^{-1})
Q_R	-	respiratory rate (mol O_2 consumed $l^{-1}h^{-1}$; mol O_2 consumed g^{-1} biomass h^{-1})
S	-	substrate concentration (mg l^{-1}; g l^{-1}; mol l^{-1})
S_0	-	initial substrate concentration (mg l^{-1}; g l^{-1}; mol l^{-1})
t	-	time (h)
V	-	culture volume (l)
X_A	-	biomass concentration (g l^{-1}), in photoautotrophically growing cells
X_H	-	biomass concentration (g l^{-1}), in heterotrophically growing cells
X_M	-	biomass concentration (g l^{-1}), in mixotrophically growing cells
Y_{kcal}	-	bioenergetic yield (g $kcal^{-1}$)
Y_{kJ}	-	bioenergetic yield (g kJ^{-1})
$Y_{X/S}$	-	biomass yield from substrate (g biomass produced g^{-1} substrate consumed)
α	-	heterotrophic fraction in the mixotrophic growth (dimensionless)
ΔH_s	-	enthalpy change for complete combustion of organic compound (kcal $mole^{-1}$)
μ	-	specific growth rate, d^{-1},
μ_m	-	maximum specific growth rate (d^{-1})

In the case of autotrophic metabolism alkalization proceeds due to gaseous CO_2 consumption, in the case of heterotrophy, growth medium becomes acidulated due to CO_2 produced from an organic carbon source and in mixotrophic culture pH value depends on the dominating constituent metabolism, but in the majority of cases remains approximately constant.

Kinetics and statics of microalgal growth: In the literature terms "mixotrophic" and "photoheterotrophic" are frequently mistaken. Therefore there is the need to define these terms precisely. Moreover, the complexity and inconsistence in trophic terminology of microalgae takes place due to overlapping of many types of metabolisms, since there is a possibility of metabolic shift as the respond to changes in environmental conditions[10]. It is of high importance to assess precisely what is meant by growth limiting substrate in each type of metabolism (autotrophic, heterotrophic, mixotrophic, photoheterotrophic).

Photoautotrophic cultures: Photoautotrophy involves utilization of light as a sole energy source which is converted into chemical energy through photosynthetic reactions[11]. Photosynthetic activity is closely related to the growth under this cultivation conditions. The most frequently growth limiting substrates studied are light and CO_2.

In photoautotrophic cultures, in which light could be considered as physical substrate, the influence of light intensity on specific growth rate is frequently described either with Monod (if no photoinhibition is observed, equation 1) or Haldane (when photoinhibition occurs, equation 2) equations.

$$\mu = \mu_m \left[\frac{I_0}{K_{I_0} + I_0} \right] \quad (1)$$

$$\mu = \mu_m \left[\frac{I_0}{K_{I_0} + I_0 + \frac{I_0^2}{K_i}} \right] \quad (2)$$

At low values of I_0 (incident light), the Monod equation approximates first-order equation in which the specific growth rate is linearly related to the limiting incident light intensity, then $\mu<\mu_m$, according to equation 3.

$$\mu = \mu_m \left[\frac{I_0}{K_{I_0}} \right] \quad (3)$$

Alternatively, if $I_0 = K_{I_0}$, then

$$\mu = \mu_m \left[\frac{I_0}{2I_0} \right] = \frac{1}{2}\mu_m \quad (4)$$

When $I_0 >> K_{I_0}$, a zero order relationship is established by equation 5.

$$\mu = \mu_m \left[\frac{I_0}{I_0} \right] = \mu_m \quad (5)$$

In which the specific growth rate is at maximum value and is no longer dependent on the incident light intensity represented by I_0, but is dependent on other environmental and physiological conditions, the latter duo to photoinhibition.

For *Arthrospira platensis*, the specific growth rate (μ) is independent on pH between 8.5 and 10.5, the optimum temperature lies between 35 and 37°C, with a thin cell suspension in a nutritionally sufficient medium, in which exponential growth proceeds, the relation between μ and the light intensity (I_0) is expressed with Monod equation in which K_{I_0} is the light saturation constant (klux), for μ_m=2.2 d^{-1} and K_{I_0}=10 klux were obtained at 35°C[12]. The biomass concentration X_A of photoautotrophically grown cells of *A. platensis* increased proportionally with the increase in light intensity, in both batch and continuous culture. Linear growth was a function of light intensity while all other nutrients remained in excess.

In the case of batch cultures using a photobioreactor, biomass increases produce a shading of the cells located behind the cells in the illuminated surface, then autotrophic growth can be assumed to occur mainly in the culture upper surface zone, since the light illumination from the illuminated surface of the fermentor was mostly hindered by the algae present in this zone. Hence, autotrophic growth of *A. platensis* may be expressed by:

$$\frac{dX_A}{dt} = Y_{kJ} I_0 A \quad (6)$$

in which biomass productivity may be defined with terms of $\Delta X_A/\Delta t$ for batch and DX_AV for continuous culture, indicating that productivity is proportional to I_0A, depending of the bioenergetic yield.

Eq. (6) may be simplified as:

$$\frac{dX_A}{dt} = KI_0 \quad (7)$$

where, $K = \frac{Y_{kJ}A}{V}$, constant. K can be used as a one design criteria for photobioreactor development[13].

Bioenergetic yield: The assessment of bioenergetic yield (Y_{kJ} or Y_{kcal} depending on the unit used) with respect to *A. platensis* was performed by an appropriate measurement of A(λ) in the λ=380-720 nm range of visible light, coupled with a separate evaluation of the wavelength distribution f(λ) of a light source. Because all the energy is gathered from light, $\Delta ATP_T = \Delta ATP_{hv}$, Y_{kJ} can be determined from the following equation (8):

$$Y_{kcal} = \frac{\Delta X_A}{IAt} \quad (8)$$

where I is the intensity of absorbed light (photosynthetically active radiation) (kJ $cm^{-2}h^{-1}$), A is illuminated surface area (m^2). The calculated values for Y_{kcal} were: 0.01-0.02 g cell $kcal^{-1}$ (*A. platensis*); 0.01 g cell $kcal^{-1}$ (*A. nidulans*) and 0.009 g cell $kcal^{-1}$ (*C. vulgaris*)[14,15]. Bioenergetic yields (Y_{kJ}) in both batch and continuous culture were obtained from the slope giving 5.0x10^{-3} g biomass kJ^{-1}, as compared with values of 2.4-4.8x10^{-3} g biomass kJ^{-1} [16] for *A. platensis*, as reported previously[17].

Heterotrophic cultures: It is well known that blue-green algae were generally considered as obligate photoautotrophs. On the other hand, some reports indicate that heterotrophic conditions could permit slow growth on certain carbon sources. Heterotrophy is defined as utilization of sole carbon and energy source from organic compounds. In this context, requirement for light is eliminated[18]. Heterotrophy gives the possibility to increase biomass concentration and thus productivity, when it function independent and simultaneously to autotrophy, which could be fatherly improved by the application of high-cell density techniques, frequently used for i.e. yeasts culture, such as fed-batch, chemostat culture and membrane cells recycle systems[19,20]. Heterotrophic aerobic growth was confirmed experimentally for the following strains of microalgae capable of photosynthesis: *Chlorella vulgaris*[2,3], *C. sorokiniana*, *C. regularis*[8] and *C. pyrenoidosa*[21], *Scenedesmus*[22], *Haematococus*[4], *Spirulina*[5,13,23], *Nitzschia laevis*[25], *Chlamydomonas reinhardtii*[24,1,26],

Table 3: Advantages and disadvantages of heterotrophic culture of microalgae (modified from Chen, 1996) (16)

Concept	Advantages	Disadvantages
Operation	Easy maintenance of optimal condition for growth and production	High costs of growth medium, axenic cultures required
Sterilization	Elimination of predatory organisms by sterilization	Potential contamination with bacteria
Yields	Higher biomass concentration, growth rate and productivity	Growth limitations when organic substrate concentration decreases. O_2 concentration should be controlled
Light supply	No need to supply light	In some cases inability to produce light induced metabolites or high valuable substances that can be solved by shifting to mixotrophic growth conditions

Scenedesmus obliquus[18,27,28], *Synechocystis, Plectonema boryanum* and *Nostoc*[29], where the organic compounds introduced were: glucose, peptone and acetate, though there might be a room for future investigations in searching for other effective organic compounds. The problem that might evolve is that, the quality of heterotrophically grown cells might be different than those grown photoautotrophically.

There are contradictory information in the literature whether lag-phase is extremely long in heterotrophic growth. *Arthrospira platensis* was grown heterotrophically on glucose as the sole carbon source under axenic conditions. Some studies show that growth could be detected only after several days[5], while in other reports on heterotrophic growth, 8 h long lag-phase was observed[30].

As observed previously, heterotrophic growth (dX_H/dt) occurred at the expense of glucose. The final biomass concentration attained was 4.14 g l^{-1} after 440 h of cultivation when an initial glucose concentration of 9.0 g l^{-1} was used[5]. These results contrasted with other reports in which heterotrophic growth of *Arthrospira* was not clearly found[14], suggesting that the cultivation time was not long enough to support growth in these studies. This could be also explained with the lack of bicarbonate in the growth medium, that shows the buffering capacity. When heterotrophic growth is carried out in the absence of bicarbonate, CO_2 is produced ($CO_2+H_2O \Leftrightarrow HCO_3^- + H^+$) and medium becomes strongly acidulated (pH drops to 3-4). Under these conditions growth is inhibited. In the presence of bicarbonate (as in typical Zarrouk growth medium) pH was only slightly acidulated (7.5) and the growth proceeded until glucose was exhausted[30].

It was confirmed that heterotrophic growth of *Arthrospira platensis* might contribute to at least in part of the biomass production. The specific growth rate of dX_H/dt was $8.3x10^{-3}$ h^{-1}, comparable to that obtained for *Haematococcus pluvialis* ($9.3x10^{-3}$ h^{-1})[4], but lower than that obtained for *Chlorella vulgaris* UAM 101[3]. The specific glucose consumption rate (0.018 h^{-1}) was almost constant using different initial glucose concentration between 0.5 and 9 g l^{-1} [5]. The overall growth yield (0.46 g biomass g $glucose^{-1}$) was comparable to the yield values of well-known heterotrophs such as yeasts and other aerobic-heterotrophs[4]. Another microalgae, *Chlorella regularis* could grow in dark-heterotrophic continuous culture using acetate.

Furthermore, it has also been reported by several nutritionists, that heterotrophically grown algal cells show high digestibility and biological value as feed for various animals and fish. Continuous cultures in acetate-limited chemostat and oxygen-limited turbidostat culture of *Chlorella regularis* S-50, using acetate as the sole carbon source were carried up to study the growth characteristics of this alga under dark-heterotrophic conditions. Due to the poor tolerance to acetate of the algal cells, turbidostat cultures based upon pH-stat were performed, under operating conditions almost identical to those of oxygen-limited chemostat cultures. In the oxygen-limited cultures, the sum of the amount of carbon in the cells grown and the carbon dioxide evolved was nearly equal to the quantity of carbon in the acetate consumed by the alga. Energetic analyses gave values of true growth yields, $Y_{X/S}$=25.6 (g cell mole $acetate^{-1}$), $Y_{X/O}$=23.8 (g cell mole O_2^{-1}) and Y_{kcal}=0.12 (g cell $kcal^{-1}$) and values of maintenance coefficients, m=0.2 (mmoles acetate g^{-1} cell h^{-1}), m_o=0.4 (mmoles O_2 $g^{-1}h^{-1}$) and m'=0.042 (kcal g^{-1} cell h^{-1}), respectively. It was confirmed that growth yields for acetate and oxygen-limited cultures were apparently higher than those in the acetate-limited. A cell productivity of 8.6 g cell $l^{-1}h^{-1}$ was obtained with oxygen consumption rate 0.36 mole O_2 $l^{-1}h^{-1}$, when the oxygen-limited turbidostat culture was carried out by feeding fresh medium containing 140 ml acetic acid l^{-1}. The content of cellular protein and chlorophyll was 40-65 and 1.0-2.5% per dry cell weight, respectively. These values tended to decrease with the increase of residual acetate in the culture broth[8].

Because all energy is gathered from glucose $\Delta ATP_T=\Delta ATP_{Glu}$, the growth yields for total available energy acetate, Y_{kJ} for heterotrophic culture can be calculated using the following equation:

$$Y_{kJ} = \frac{1}{\left(-\Delta H_a\right)+\left(-\Delta H_o\right)/Y_{X/O}} \quad (9)$$

Heterotrophic growth offers several advantages, in particular gives the possibility to culture photosynthetic

cells in the manner common for large-scale heterotrophic organisms (i.e. yeasts). But still many problems to be solved (Table 3).

Mixotrophic cultures: Mixotrophy (photolithotrophic heterotrophy) is defined as a metabolic process, in which photosynthesis is the main energy source, although both organic compounds and CO_2 are essential. Amphitrophy (subtype of mixotrophy) refers to organisms that are able to live either autotrophically or heterotrophically) depending on the ratio of organic substrate concentration to light intensity[10]. According to another definition, a mixotroph is an organism able to assimilate organic compounds as carbon sources while using inorganic compounds as electron donors (Table 1). Marquez[5] and Hata[11] suggest, that in mixotrophic culture a simultaneous uptake of organic compounds and CO_2 takes place as carbon sources for cell synthesis and it is then expected that CO_2 released via respiration, will be rapidly trapped and reused under sufficient light intensity. Thus, mixotrophic cells acquire the energy by catabolizing organic compounds via respiration and converting light energy into chemical energy via photosynthesis[11].

Stanier found, that the cellular growth rate of *Spirulina sp.* in the presence of glucose, even in the presence of light, respiration occurs. Photosynthetic (measured as O_2 production) and respiratory activity (measured as O_2 consumption) were studied in the medium supplemented with glucose in the presence and in the absence of photosynthesis inhibitor (DCMU, 5 mmol l^{-1}). Respiratory activity showed that light did not influence O_2 consumption rate, no matter whether DCMU was present or not[11] and with or without light[2-5] . This fact might serve as an indicator to differentiate mixotrophy from photoheterotrophy. Studies with radioactively labeled glucose ^{14}C for 80 h, revealed that 50% of isotope was incorporated into the biomass and the remaining 50% was excreted in the form of CO_2[10].

It has been proved experimentally that in mixotrophic culture, the addition of organic substrate resulted in the increase in the growth rate, as well as in the final biomass concentration. In mixotrophic culture no photoinhibition was observed, that typically occurred above 20 klux in photoautotrophic culture[31-33]. This could be explained with the protective role of glucose or with the shift in light intensity at which photoinhibition occurs. Photosynthetic growth produces O_2 which is accumulated along the cultivation time, by the photosynthetic activity depending of light intensity, in which high photosynthetic activity build up high dissolved oxygen concentration in close photobioreactors, fact than might accelerate oxidative reactions. Cells growing heterotrophically might use part of the O_2 produced by cells growing photoautotrophically, decreasing dissolved oxygen concentration, situation than can helps to reduce photoxidative damage.

Many attempts have been done to explain the mechanism of mixotrophic growth, in particular contribution of autotrophy and heterotrophy. The problem is difficult to clarify when uniquely distinguishing the constituent metabolisms, co-existing in a single cell, they must interact somehow. Therefore it is of high importance to establish whether light influences heterotrophic or heterotrophic light-drived metabolism.

Other effective carbon sources utilized in mixotrophic cultures in addition to glucose and peptone were arginine, aspartic acid, leucine, proline, TCA-cycle organic acids, acetic, butyric, tartaric and maleic acids. The specific growth rate (μ) and the growth rate (dX/dt) of mixotrophic cultures supplemented with 0.1% peptone increased 1.2–1.3 fold and 1.85–1.93 fold, respectively, as high as those observed in culture without peptone added. The effectiveness of peptone assimilation was remarkable in the light intensity range lower than the saturation intensity. The growth yields were much higher in mixotrophic cultures in the presence of glucose and/or peptone than in purely autotrophic cultures. It could be seen that mixotrophic growth yield could become almost twice as high as autotrophic growth yield[14].

Ogawa and Terui[12] reported glucose assimilation using different initial concentrations. In the logarithmic growth phase, the relation between the intensity of incident light (I_o) and the specific growth rate (μ) in mixotrophic cultures with glucose added could be analyzed with Lineweaver-Burk plots. The specific growth rate increased at low light intensity, but did not exceed the value of μ_m which was reached in autotrophic cultures growing under sufficiently strong illumination. Therefore, mixotrophic culture is effective, enabling to increase the biomass productivity at low light intensity or when the culture is in the linear growth phase. If cell density is sufficiently high, autotrophic growth is dependent of the intensity of incident light and the illuminated area.

In mixotrophic cultures of *A. platensis* grown at three different light intensities, the biomass concentration (X_M) increased with light intensity and increased with an increase in glucose consumption. The final biomass concentration in mixotrophic cultures at $5.07x10^{-3}$ kJ $cm^{-2}h^{-1}$ was 1.23 g biomass l^{-1}, 1.9 times greater than autotrophic cultures at the same light intensity. This indicates that mixotrophic growth of *A. platensis* might be useful for the biomass production from the practical viewpoint[13].

The carbon recovery for mixotrophic growth of *A. platensis* indicated that almost no extracellular

Table 4: How to distinguish the different metabolisms

situation 1	situation 2	situation 3	situation 4
no light no glucose	light no glucose	no light glucose	light glucose
only chemoautotrophic organisms	photoautotrophic and mixotrophic organisms	heterotrophic and mixotrophic organisms	photoautotrophic, heterotrophic, mixotrophic and photoheterotrophic organisms

products were produced. In fact, the bicarbonate consumption rates observed in autotrophic cultivation were 6.7, 9.8 and 15.5x10^{-3} g h^{-1}l^{-1} for light intensities of 5.07, 7.79 and 11.39x10^{-3} kJcm^{-2}h^{-1}, respectively which were larger than those observed in mixotrophic cultivation (1.1, 3.4 and 7.8x10^{-3}h^{-1}l^{-1}). This suggested that CO_2 produced from heterotrophic glucose metabolism might be used photosynthetically together with bicarbonate from the culture medium[13].

Since the energy comes from two sources, $\Delta ATP_T=\Delta ATP_{hv}+\Delta ATP_{Glu}$, the bioenergetic yields of mixotrophic cultures can be defined as follows:

$$Y_{kJ}=\frac{\Delta X_M}{IAT+V(\Delta H_S)+(S_o-S)} \quad (10)$$

The values of Y_{kJ} determined from equation 10 gave 8.1, 7.2 and 6.7x10^{-3} g kJ^{-1} for light intensities of 5.07, 7.79 and 11.39x10^{-3} kJ cm^{-2}h^{-1}, respectively were determined for *A. platensis*[16]. These values were smaller than those for *C. vulgaris* (17.9 to 23.9x10^{-3} g kJ^{-1})[2] because the contribution of the heterotrophic activity in mixotrophic cultures of *A. platensis* was smaller than in *C. vulgaris*.

Lee *et al.*[34] reported that the energetic yield for the algae in mixotrophic culture were larger than under photoautotrophic conditions. However, it resulted from the comparison of energies of such different nature. Generally bioenergetic yields from organic substrates are significantly higher than from light[30]. Similar results were obtained by Hata *et al.* (2000) who studied conversion efficiency and sharing of energies from organic compound (glucose) and light by measuring intracellular metabolic fluxes in mixotrophic batch cultures of *Marchantia polymorpha*. The authors found that the efficiency of energy conversion was larger in the case of respiration than through photosynthesis[11].

Algal density obtained mixotrophically at 2.2 g l^{-1} in *Arthrospira* cultures was comparable to the density obtained in outdoor mass culture (1.0 g l^{-1}) using PVC-constructed pond with 10 cm depth and intensities of 3.5 to 6.3 g l^{-1} obtained in a tubular photobioreactor (2.6 cm diameter)[35]. In both cases, solar radiation was about 10 times greater (121.42 and 104.75x10^{-3} kJ cm^{-2} h^{-1}, respectively) than the light illumination used for *Arthrospira* in mixotrophic conditions (11.39x10^{-3} kJ cm^{-2} h^{-1}). Therefore, axenic mixotrophic cultures of *Spirulina platensis* in a closed photobioreactor might be useful to produce biomass containing high amounts of pigment such as phycocyanin, chlorophyll and carotenoids.

Photoheterotrophic culture: Photoheterotrophy (photoorganotrophy, photoassimilation, photometabolism) describes metabolism in which light is required to use organic compounds as carbon source[10]. Different reports state that photoheterotroph is an organism able to use light as a source of energy and organic materials as carbon source (Table 1). Zhang defined photoheterotrophy as the use of sugar exclusively as carbon source, ATP provided by electron transfer via Photosystem I or respiration[36]. Inhibition of photosynthesis by DCMU, causes inhibition of heterotrophy in any case with or without light supply. According to Orus *et al.*[37] photoheterotrophism occurs under light natural condition of dim light, that does not support photoautotrophic growth that was stimulated by inhibiting photosystem II with DCMU. In this category *Synechocystis sp.* that uses glucose with light supply, but does not grow without both light or glucose, should be included.

The inability of all cyanobacterial strains to grow heterotrophically and mixotrophically might result, as Zhang suggests, from a lack of an efficient uptake of organic substrates, a fact demonstrated for *Nostoc sp.* PCC7118, *Synechocystis* PCC6308 and PCC7008 strains[38-40]. The presence of an active glucose uptake system was described for facultative phototrophs, all capable of photoheterotrophy and chemoheterotrophy (sugar is the source of both: carbon and energy), for example *Plectonema boryanum*, *Synechocystis* PCC 6714, *Synechocystis* PCC 6803, *Nostoc* MAC PCC8009. The first two strains transported glucose via proton/glucose symport, the remaining through facilitated diffusion by the glucose transporter. The lack of sugar transport system could explain why some cyanobacteria are obligate photoautotrophs[36].

This could be discussed by considering the following four growth cases (Table 4):

1. No organic carbon source (glucose) nor light is available
2. Only light is available
3. Only organic compounds are available
4. Both light and organic compounds are available

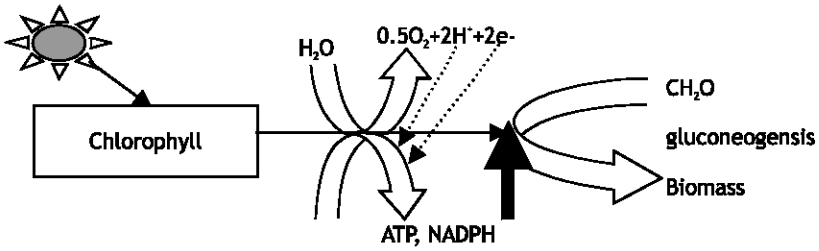

Fig. 1: Photoheterotrophic metabolic pathway. Light is the sole source of energy

Photoheterotrophic organisms cannot grow solely on glucose nor solely on light, but can grow only when light and glucose are present at same time (situation 4). This means that glucose is used only as a building material, but not as energy source, seems to be, there is not Krebs cycle actively working. Microalgae which can perform mixotrophic growth can easily shift between autotrophic and heterotrophic nutrition mode, driving situations 2, 3 and 4 depending on environmental condition specially light and organic compound availability.

In photoheterotrophic growth, energy can be taken from light and transformed into chemical energy as ATP and NADPH in the light phase of photosynthesis. In dark phase, in which is Calvin cycle is not active, CO_2 can not be assimilated for glucose synthesis. Therefore, probably the following situation occurs (Fig. 1): energy from light is converted into chemical energy of ATP/NADPH, that are used in metabolic transformations of glucose into biomass, therefore CO_2 is not or minimal produced. Oxygen is produced due to water photolysis. According to these, two clear differences between photoheterotrophy and mixotrophic metabolisms can observed. CO_2 is not produced by oxidative metabolism in photoheterotrophy as in mixotrophy and a part of the O_2 is produced by glucose photolysis in photoheterotrophy a difference of mixotrophy in which O_2 is produced by photosynthesis.

Therefore, if the energy is gathered only from light, $\Delta ATP_T = \Delta ATP_{h\nu}$, bioenergetic equation will be the same as for photoautotrophic growth[8].

Light-activated heterotrophic cultures: Some species require light to grow heterotrophically, i.e. *Synechocystis* that does not grow on glucose in complete darkness unless receiving some minimal light[41], glucose can stimulate its growth rate in light. Light-activated heterotrophic growth (LAHG) is heterotrophic growth on organic compounds (such as glucose) under conditions without continuous illumination, when short period light pulses are supplied i.e. every day to the cells culture. An example could be *Synechocystis* 6803 – chemoheterotroph that is incapable to grow when light is not supplied periodically, although light is not directly used as the energy source. LAHG includes at least two basic biological processes: light-sensing/signal transduction and heterotrophic growth under very weak light[42]. Then, quantitative analysis of mixotrophic growth has not been clear. *Synechocystis sp.* PCC6803 was grown in a 2.5 l enclosed photobioreactor on medium with or without glucose. The incident light intensities ranged from 1.5 to 7 klux. The highest average specific growth rates of mixotrophic cultures and photoautotrophic cultures were, respectively, 1.3 h^{-1} at a light intensity of 7 klux on 3.2 g l^{-1} glucose and 0.3 h^{-1} at both light intensities of 5 and 7 klux. The highest cell density 2.5 g l^{-1} was obtained at both light intensities 5 and 7 klux on 3.2 g glucose l^{-1}. Glucose consumption decreased with decreasing light intensity. The energy yields of mixotrophic cultures were 4 and 6 times higher than in autotrophic cultures. Light favored mixotrophic growth of *Synechocystis sp.* PCC 6803, particularly at higher intensities (5-7 klux)[43].

Contribution of heterotrophy in mixotrophy: In the case of mixotrophy, the contribution of heterotrophically growing cells to total biomass cultured in mixotrophic conditions, might be generalized using the mathematical description obtained for *Arthrospira platensis*[16]. The sum of biomass produced under autotrophic and heterotrophic growth coincided with the biomass produced mixotrophically X_M, i.e.,

$$X_M = X_A + X_H \quad (11)$$

Assuming that α is the heterotrophic fraction in the mixotrophic growth, X_H in the mixotrophic culture can be expressed as follows:

$$X_H = \alpha X_M, \qquad 0 < \alpha < 1 \quad (12)$$

from equations 11 and 12, X_M is expressed as:

$$X_M = \frac{X_A}{1-\alpha} \quad (13)$$

In dense cultures, the light is utilized only by the algae in the illuminated surface zone of the reactor, i.e., the

value of α at the surface zone is low, while cells located under this zone are less able to utilize the light energy due to the dense layer of algae in the surface, i.e., the value of α in this zone (light limited zone) becomes larger.

Hence, the apparent (average) value of α in mixotrophic culture would increase with an increase in biomass density during the cultivation. In order to estimate the apparent value of α and α_i during the culture, the value of α_i is constant during a short period of time, Δt_i.

Then equation 12 can be expressed as follows:

$$\frac{\Delta X_M}{\Delta t_i} = \frac{1}{1-\alpha_t}\frac{\Delta X_A}{\Delta t_i} \tag{14}$$

by substituting equation 12 into equation 13

$$\frac{\Delta X_M}{\Delta t_i} = \frac{1}{1-\alpha_t} KI_0 \tag{15}$$

then, integration of equation 14 yields

$$\int_{X_{Mi}}^{X_{Mi+1}} \Delta X_M = \frac{KI_o}{1-\alpha_t}\int_i^{i+1}\Delta t_i \tag{16}$$

i.e.,

$$\alpha_i = 1 - \frac{KI_o}{X_{Mi+1}-X_{Mi}}(t_{i+1}-t_i) \tag{17}$$

Basing on equation 17, the values of α_i for the mixotrophic cultures were calculated using K=0.53, I_o= 5.1, 7.8,11.4x10^{-3} kJ $cm^{-2}h^{-1}$, Δt_i = 24 h and the values of X_{Mi+I} and X_{Mi}, for final and initial biomass concentration, respectively, during Δt_i. The data indicate that α_i increased with the cultivation time, ranging from 0.02 to approximately 0.61 after 240 h of cultivation. The values of α_i depends directly of the biomass density.

Dual substrate limitation approach to mixotrophy and photoheterotrophy : There is the scarcity of information on the influence of light intensity on the growth rate in mixotrophic culture[18,32,44-52] and photoheterotrophic culture. Vonshak (2000), showed that μ_M was greater than μ_A (20-40%) at each light intensity.

Vonshak disagrees that autotrophy and heterotrophy function independently in *Spirulina sp.* cells. The arguement used by the author was that autotrophy and heterotrophy possess a common link, which are the components of electrons transport system, therefore mixotrophy is lower than the simple superposition of autotrophy and heterotrophy. Vonshak also suggests (after Stanier) that cells require less light for growth than autotrophic cells[32].

Mixotrophic culture could be discussed as dual limited process: i) with light as physical substrate and ii) glucose as chemical substrate. The influence of these two substrates could be described either with the additive

$$\mu = \sum_{i=1}^{n}\left(\frac{\mu_{m_i} S_i}{K_{Si}+S_i}\right) \tag{18}$$

or multiplicative

$$\mu = \prod_{i=1}^{n}\left(\frac{\mu_{m_i} S_i}{K_{Si}+S_i}\right) \tag{19}$$

growth kinetics[10,44].

Since mixotrophic metabolism is the sum of two distinctive metabolisms: autotrophic and heterotrophic, a mixotrophic cell utilizes simultaneously two energy sources (light and glucose) and two carbon sources (CO_2 and glucose). Photohoterotrophic organism, though uses the energy of light and carbon of glucose for cellular growth and maintenance. Therefore in the case of mixotrophic growth, light and glucose could be considered as growth enhancing substrates (S_{enh}) and in the case of photoheterotrophic growth, both: light and glucose are thought to be growth essential substrates (S_{ess}), since they are sole energy and carbon source, respectively. In these situations, of multiple substrate limitation for heterotrophic growth, the following growth kinetic model can be modified from that proposed[44]:

$$\mu = \left(\sum_{i=1}^{n}\frac{\mu_{mi} S_{enh,i}}{K_{enh,1}+S_{enh,i}}\right)\left(\prod_{i=1}^{m}\frac{\mu_{mj} S_{ess,j}}{K_{ess,j}+S_{ess,j}}\right) \tag{20}$$

that distinguishes between growth enhancing and essential substrates.

The first term in the above equation shows the stimulatory effect of growth enhancing substrates (glucose and light in mixotrophic metabolism) and $\mu_{m,i}$ represents the maximum specific growth rate in the presence of growth enhancing substrates. The second term represents photoheterotrophic growth, involving essential substrates.

According to definition of volumetric growth rate, the following rate equations could be proposed for mixotrophic (r_M) and photoheterotrophic (r_{PH}) growth mode:

$$r_M = \frac{dX_M}{dt} = r_A^M + r_H^M = \frac{dX_A^M}{dt} + \frac{dX_H^M}{dt} \tag{21}$$

$$r_M = \mu_M X = \mu_A^M(I)X_A^M + \mu_H^M(C_{Gl})X_H^M \tag{22}$$

$$r_{PH} = \mu_{PH}(I, C_{Gl}) X_{PH} \neq (r_H^M + r_H^M) \quad (23)$$

where μ(S) could be i.e. Monod-type equation or inhibition equation.

Zhang described the growth in mixotrophic culture with the multiplicative approach[16]. He used Haldane equation to describe the influence of light intensity and glucose concentration on specific growth rate. The model parameters were determined for light: μ_m=0.0919 h^{-1}, K_S=3.02 klux, K_i=2.34 klux and for glucose: μ_m=0.14 h^{-1}, K_S=3.68 g l^{-1}, K_i=1.24 g l^{-1}.

Contribution of autotrophic and heterotrophic in mixotrophic metabolism could be also discussed by studying the influence of light intensity and glucose concentration on specific growth rate (Fig. 2 and 3). *Spirulina sp.* was cultured with the use of different metabolisms, at various light intensities and initial glucose concentrations (Table 5) in low-light path photobioreactor under sterile conditions. It was found that growth parameters have the substantial influence on static as well as kinetic growth pattern and under conditions of high prosperity (under high light intensity and/or glucose concentration) cells metabolize less economically, that was reflected by the lower values of Y_{kJ} and $Y_{X/Gl}$. Also, in the presence of higher glucose concentrations, $Y_{X/N}$ decreased. This means that intense denitrification occurs. Therefore it is not advantageous to culture cells at high glucose concentration. The optimal growth parameters determined were C_{Gl}=2.5 g l^{-1}, I_{av}=10.5 klux (Table 5)[30].

Photoinhibition was observed only in autotrophic culture, but not in mixotrophic (Fig. 3). It was found, that μ_A is always greater than μ_M but μ_M at lower light intensities was lower than μ_H. If mixotrophy was a simple superposition of autotrophy and heterotrophy (after assumption that light does not influence heterotrophic constituent), the dependence of μ_M=f(I) should have the same character as μ_A=f(I). But the function μ_M=f(I) seems to be rather Monod-type, than of inhibitory character[30].

In the mathematical model it might be assumed that the influence of growth parameters could be described with multiplicative approach (equation 20), in which the primary metabolism is autotrophy. Therefore, photoheterotrophy depends of the influence of light to glucose uptake as energy and carbons source respectively, the following growth equation can be proposed,

$$\mu_M(I, C_{Gl}) = \mu_A(I) \cdot f(C_{Gl}) \quad (24)$$

where $f(C_{Gl})$ is Monod-type function describing stimulatory effect of glucose presence[30].

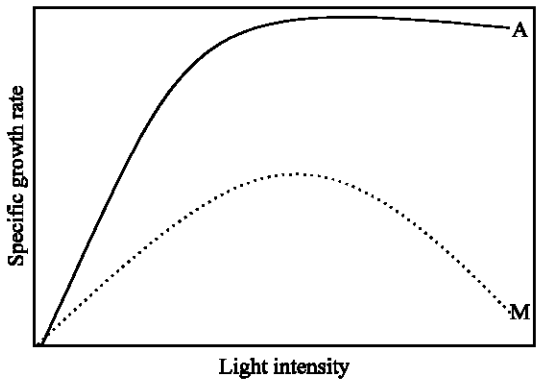

Fig. 2: The influence of light intensity on specific growth rate in photoautotrophic (A) and mixotrophic (M) culture (31)

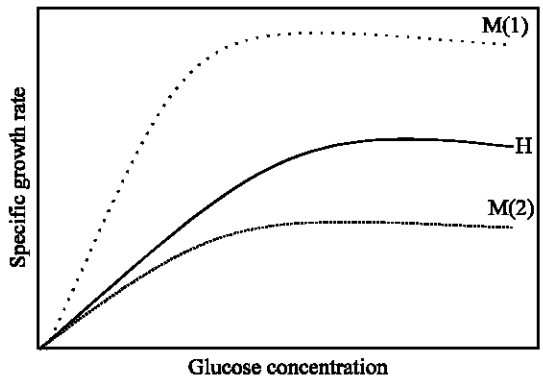

Fig. 3: The influence of glucose concentration on specific growth rate in heterotrophic (H) and mixotrophic (M) culture at different light intensities I1 and I2 (I1>I2)[30]

Zhang studied phycocyanin production in fed-batch high cell mixotrophic culture of *Spirulina platensis* with stepwise increase in light intensity. This mode of culture was carried out in order to avoid substrate inhibition. The author found that the cell density reached was 10.2 g l^{-1}, 4.3-fold higher than in batch culture (2.4 g l^{-1}). He also investigated the influence of initial glucose concentration and light intensity in mixotrophic batch culture and proposed a number of mathematical models to describe the optimal glucose concentration (also as 2.5 g l^{-1}) and light intensity (3.86 klux)[26]. The optimal light intensity differs from determined by Chojnacka[30]. This difference could be due to different method of light intensity measurement and distribution inside the culture vessel. Zhang also did not observe photoinhibition in mixotrophic culture, but observed inhibitory influence of glucose at higher concentrations. This could be explained with nitrogen limitation[30].

Table 5: Static and kinetic growth parameters of *Spirulina sp.* culture under autotrophic, heterotrophic and mixotrophic growth conditions at different light intensities (0-23 klux) and initial glucose concentration (0-10 g l^{-1}) (31)

	$Y_{X/Gl}$	$Y_{X/N}$	Y_{kJ} [kJ g^{-1}]	C_{chl} [mg g^{-1}]	μ_m [h^{-1}]
Autotrophic					
I↑	-	1.3-2.1	$8x10^{-3}$-$1x10^{-3}$	4.4-3.7	0.024
Heterotrophic					
C_{Gl} ↑	0.90-0.10	0.8-2.0-0.8	$14x10^{-3}$-$2x10^{-3}$	4.5-4.0	0.045
Mixotrophic					
I ↑ (C_{Gl}=2.5 g l^{-1})	0.13-0.23	3.0-1.2	$10x10^{-3}$-$5x10^{-3}$	2.5	0.055
C_{Gl} ↑ (I=10.5 klux)	0.80-0.13	2.4-1.2	$20x10^{-3}$-$5x10^{-}$	3.7-2.5	0.055

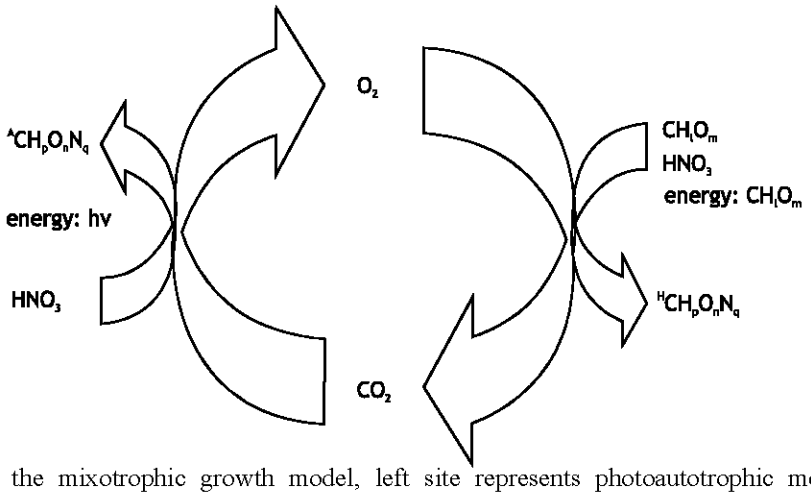

Fig. 4: Diagram of the mixotrophic growth model, left site represents photoautotrophic metabolism, right site heterotrophic metabolism. O_2 is produced by photosynthesis and CO_2 - by oxidative decomposition of organic compounds

Stoichiometric equations in microalgal metabolism

Mass balance: In mixotrophic growth (Fig. 4), low concentration of oxygen might influence heterotrophic metabolism. However, autotrophic constituent produces oxygen in the process of photosynthesis. Heterotrophic metabolism produces CO_2 to be used by autotrophic growth. If the rate of photosynthesis (Q_P) equals the rate of respiration (Q_R), no net production nor consumption of O_2 and CO_2 is observed, thus organic carbon is incorporated to biomass utilizing light energy and CO_2 (Fig. 4). But this ideal situation of balanced self-production and consumption is not observed in real systems, due to substrate limitations.

In photoheterotrophic growth (Fig. 5) only a single route proceeds: O_2 is produced by water photolysis with the simultaneous consumption of glucose and nitrogen source and with the utilization of luminous energy.

Thus, each metabolism could be described with the following growth equations and elemental balance:

Photoautotrophic

$$aHCO_3^- + bHNO_3 + cH_2O \xrightarrow{kcal_{hv},\ A} CH_pO_nN_q + \alpha O_2 + \beta OH^-$$

C: a = 1
N: b = q
H: a+b+2c = p+β
O: 3a+3b+c = n+2α+β
*: a=β

Heterotrophic

$$dCH_lO_m^- + b'HNO_3 + eO_2 \xrightarrow{kcal_{CHO},\ H} CH_pO_nN_q + \varepsilon H_2O + \Phi HCO_3^- + \chi H^+$$

C: d = 1+ϕ
N: b' = q
H: d *l*+b'= p+2ϵ+ϕ+χ
O: md+3b'+2e = n+ϵ+3ϕ
*: ϕ=χ

Photoheterotrophic

$$dCH_lO_m^- + b'HNO_3 \xrightarrow{kcal_{hv},\ PH} CH_pO_nN_q + \varepsilon H_2O + \Phi HCO_3^- + \chi H^+ + \alpha O_2$$

C: d = 1+ϕ
N: b' = q
H: d *l*+*b'* = p+2ϵ+ϕ+χ
O: md+3b' = n+ϵ+3ϕ+2α
*: ϕ=χ

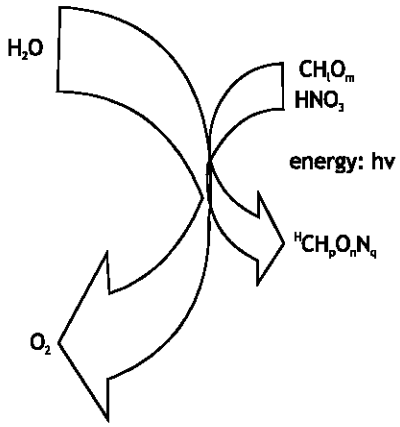

Fig. 5: Diagram of the photoheterotrophic growth model. This mode of growth consists of only one part, in which energy is obtained from light and carbon from organic carbon source

Mixotrophic: When assuming that the composition of autotrophically, heterotrophically and mixotrophically grown cells is the same ($CH_pO_nN_q$) and there is no product formation nor external O_2 supply, then O_2 used for heterotrophy originates from photoautotrophy. Equations can be arranged in two steps:

$$aHCO_3^- + b'HNO_3 + cH_2O \xrightarrow{i)kcal_{hv}} {}^{A}CH_pO_nN_q + \alpha O_2 + \beta OH^-$$

$$+ b'HNO_3 \xleftarrow{ii)\ F:\ +dCH_2O \rightarrow\ R:\ \leftarrow kcal_{hv}} {}^{H}CH_pO_nN_q$$
$$+ \varepsilon H_2O + \Phi HCO_3^- + \beta HNO_3$$

in ii) F means forward and R the reverse reactions

In the initial balance, αO_2 is the amount of oxygen produced by photosynthesis, $b'NO_3$ is the amount of nitrate source available for growth;

^{A}C: a = 1 ^{A}C : biomass produced autotrophically
N: b = q+b'
H: 2c+a+b = p+b'+β
O: 3a+3b+c = n+3b'+2α+β

The term ϕHCO_3^- is the amount of carbon dioxide produced by oxidative metabolism of the organic carbon source consumption, it is suggested to be an amount available for photosynthesis and $b''HNO_3$ is the amount of nitrate source that is still available for growth. CO_2 produced heterotrophically and O_2 produced photoautotrophically are used into two steps, forward in which biomass is produced heterotrophically and reverse phase, in which biomass is produced photoautotrophically to expenses of CO_2 coming from oxidative metabolism of glucose.

Forward
^{H}C: d = 1+ϕ ^{H}C : biomass produced heterotrophically
N: b' = q+b''
H: *l*d+β+b' = p+2ϵ+ϕ+b''
O: 3b'+2α+β+md = n+ϵ+3ϕ_+_3b''
Reverse
^{A}C: ϕ = 1
N: b'' = q+b'
H: 2ϵ = p+β
O: ϵ+3ϕ+3b'' = n+3b'+2α+β

Under these cultivation conditions, oxidative metabolism depends on the oxygen amount produced by photosynthesis. Heterotrophic growth does not proceed, if there is oxygen limitation occurring at low Q_P (photosynthetic rate), in this case $dX_M/dt = dX_A/dt$, which might happen under microaerophilic conditions. In other words: $Q_P = Q_R$. When no oxygen limitation is present, which could happen when $Q_P > Q_R$, then $dX_M/dt > dX_A/dt$. In terms of carbon and energy flux, growth rate and biomass concentration under mixotrophic growth should be = than growth rate and biomass obtained in autotrophic growth.

Yields: In photoautotrophic growth, algae utilize CO_2 as their sole carbon source. They depend on light as the source of all available energy for growth and maintenance. Under these conditions cells produce O_2. In heterotrophic growth organic compounds (i.e. glucose) are the sole energy source. The cells produce CO_2 and consume O_2. The energy is taken from glucose. In mixotrophic growth the net production/consumption of CO_2 and O_2 depends on the dominating metabolism (autotrophy or mixotrophy), which is depending on growth conditions (light intensity and glucose concentration). In mixotrophic growth light is utilized and organic substrate is consumed. In photoheterotrophic growth light is the sole energy source (with adventitious production of oxygen) and glucose is the sole source of carbon. Basing on these assumptions and definitions the biomass yields from carbon (mass $Y_{X/C}$ and molar $y_{X/C}$), from oxygen ($y_{X/O}$ or $y_{O/X}$) and bioenergetic yields ($Y_{X/kcal}$) are proposed (Table 6).

There are differences between mixotrophy and photoheterotrophy in terms of flux of carbon as well as the source of energy. In the case of photoheterotrophy, energy depends only on light and should be used to drive dX_{PH}/dt, some amount of energy could be used for maintenance and some is dissipated. If this assumption was withdrawn, photoheterotrophy could be distinguished from mixotrophy by a deficiency in electron mass transfer or energy limitation to drive heterotrophy

Table 6: Summary of biomass, energetic and oxygen yields equation, for different metabolic growth modes found in microalgae

Growth mode	Biomass yield from carbon source $Y_{X/C}$	Biomass yield from carbon source $y_{X/C}$	Energetic Yield $Y_{X/kcal}$	Oxygen yield y	Oxygen yield
Photoautotrophic	$\frac{\Delta X_A}{\Delta C_{CO_2}}$	$\frac{1}{a}$	$\frac{\Delta X_A}{kcal_{hv}}$	$Y_{O/X} = \frac{\alpha}{1}$	$Y_{O/X} = Q_P X_A$
Heterotrophic	$\frac{\Delta X_H}{\Delta C_{Glu}}$	$\frac{1}{d}$	$\frac{\Delta X_H}{kcal_{Glu}}$	$Y_{X/O} = \frac{1}{e}$	$Y_{X/O} = Q_R X_H$
Mixotrophic	$\frac{\Delta X_M}{\Delta C_{CO_2}\ \Delta C_{Glu}}$	$\frac{X_A + X_H}{(a+\phi)+d}$	$\frac{\Delta X_M}{kcal_{hv} + kcal_{Glu}}$	$Y_{X/O} = \frac{X_H}{e} - \frac{X_A}{\alpha}$	$C = Q_P X_A - Q_R X_H$ $C \geq 0$
Photoheterotrophic	$\frac{\Delta X_{PH}}{\Delta C_{Glu}}$	$\frac{1}{d}$	$\frac{\Delta X_{PH}}{kcal_{hv}}$	$Y_{O/X} = \frac{\alpha}{1}$	$Y_{O/X} = Q_P X_A$

and autotrophy simultaneously, since light is the only source of energy.

Microalgae, naturally photoautotrophic (performing photosynthesis) sometimes posses a respiratory activity and all the enzymes necessary for sugar catabolism, but only almost half of the species tested are capable to live heterotrophically and mixotrophically. Obligate photoautotrophy was found to be not only the lack of sugar transport, but also disability to sustain metabolic equilibrium, that disables survival. Usually photoautotrophic growth yields cells of the highest quality, but the lowest biomass concentration. Heterotrophic and mixotrophic mode gives higher cellular concentration, but biomass quality (i.e. photosynthetic pigments content) could be lower, accessory pigments content such as phycobiliproteins could be higher.

The terms mixotrophy and photoheterotrophy are frequently used interchangeable in the literature. Although these two metabolisms operate on both light and organic carbon source, reactions involved differ substantially. This should be considered when proposing a mathematical model (kinetic or static). Although the models used to describe microbial growth are based on Monod or Haldane equations, it is of high importance to precisely identify growth limiting substrates and the type of interaction in the case of multiple substrate kinetics. This is also significant when defining biomass growth yields and energy requirments.

Mixotrophs are capable to shift between different nutrition modes depending on growth conditions. Mixotrophy carried out two constitute metabolic pathways: autotrophy and heterotrophy, which probably slightly differ in efficiency from the situation when they do not coexist in a single cell. In mixotrophy, organic compounds and carbon dioxide might be employed simultaneously as carbon source and organic compounds and light used simultaneously as energy source. Therefore, growth kinetics should be described in terms of the differences stated among photoautotrophy, mixotrophy and photoheterotrophy, specially when the process is influenced by the presence of growth enhancing substrates.

Photoheterotrophy perform a single metabolism in which light is the sole source of energy and organic compounds are sole source of carbon. It involves the use of sugar exclusively as carbon source, ATP is provided through cyclic electron transfer via Photosystem I. Therefore these two substrates are growth essential.

Another difference between mixotrophy and photoheterotrophy is oxygen production and consumption. In mixotrophic metabolism, the net oxygen production/consumption could equal one when the component metabolisms equal. In the case of photoheterotrophic metabolism though, oxygen is produced via water photolysis, in the reaction in which light energy is stored in the form of organic compounds such as ATP and NADPH, are furtherly used in carbon assimilation from organic compounds (not CO_2).

Another way to distinguish these two metabolisms is that mixotrophs are able to employ single constituent metabolisms: can grow solely on light (in the absence of organic compounds) or solely on organic compounds (in complete darkness). Photoheterotrophs do not grow under these conditions, since they require the simultaneous presence of these two substrates.

A better knowledge of metabolic differences and requirements of energy and carbon for microalgae cultivation, is necessary to produce high valuable products, such as pigments, antioxidants, tocopherols, enzymes, etc., that can be used in the biomedical, food and chemica industries.

REFERENCES

1. Zhang, X.W., F. Chen and M.R. Johns, 1999. Kinetic models for heterotrophic growth of *Chlamydomonas reinhardtii* in batch and fed-batch cultures. Process Biochem., 35: 385-389.
2. Ogawa, T. and S. Aiba, 1981. Bioenergetic analysis of mixotrophic growth in *Chlorella vulgaris* and *Scenedesmus acutus*. Biotechnology and Bioengineering, 23: 1121-113.
3. Martinez, F. and M.I. Orus, 1990. Interactions between glucose and inorganic carbon metabolism in *Chlorella vulgaris* strain UAM 1011. Plant Physiol., 95: 1150-1155.
4. Kobayashi, M., T. Kakizono, K. Yamaguchi, N. Nishio and S. Nagai, 1992. Growth and astaxanthin formation of *Haematococcus pluvialis* in heterotrophic and mixotrophic conditions. J. Fermentation and Bioengin., 74: 17-20.
5. Marquez, F.J., K. Sasaki, T. Kakizono, N. Nishio and S. Nagai, 1993. Growth characteristics of *Spirulina platensis* in mixotrophic and heterotrophic conditions. J. Fermentation and Bioengine., 76: 408-410.
6. Wood, B.J.B., P.H.K. Grimson, J.B. German and M. Turner, 1999. Photoheterotrophy in the production of phytoplankton organisms. J. Biotechnol., 70: 175-183.
7. Goldman, J.C., W.J. Oswald and D. Jenkins, 1974. The kinetics of inorganic carbon limited algal growth. J. Water Pollution Control Federation, 46: 554-574.
8. Endo, H., H. Hosoya and T. Koibuchi, 1977. Growth yields of *Chorella regularis* in dark-heterotrophic continuous culture using acetate. J. Fermentation Technol., 55: 369-379.
9. Martinez, M.A., F. Camacho, J.M. Jimenez and J.B. Espinola, 1997. Influence of light intensity on the kinetic and yield parameters of *Chlorella pyrenoidosa* mixotrophic growth. Process Biochem., 32: 93-98.
10. Kaplan, D., A.E. Richmond, Z. Dubinsky and S. Aaronson, 1986. Algal Nutrition in: Richmond A. (Ed) CRC Handbook of Microalgal Mass Culture CRC Press Boca Raton FL.
11. Hata, J., Q. Hua, C. Yang, K.M. Shimizu and M. Taya, 2000. Characterization of energy conversion based on metabolic flux analysis in mixotrophic liverwort cells *Marchantia polymorpha*. Biochem. Engin. J., 6: 65-74.
12. Ogawa, T. and G. Terui, 1972. Growth kinetics of *Spirulina platensis* in autotrophic and mixotrophic cultures Proc IV IFS: Fermentation Technol. Today, pp: 543-549.
13. Marquez, F.J., K. Sasaki, N. Nishio and S. Nagai, 1995. Inhibitory effect of oxygen accumulation on the growth of *Spirulina platensis*. Biotechnol. Letters, 17: 225-228.
14. Ogawa, T. and G. Terui, 1970. Studies on the growth of *Spirulina platensis* (I) On the pure culture of *Spirulina platensis*. J. Fermentation Technol., 48: 361-367.
15. Ogawa, T., H. Kozasa and G. Terui, 1970. Studies on the growth of *Spirulina platensis* (II) growth kinetics of the autotrophic culture. Journal Fermentation Technol., 50: 143-149.
16. Marquez, F.J., N. Nishio, S. Nagai and K. Sasaki, 1995. Enhancement of biomass and pigment production during growth of *Spirulina platensis* in mixotrophic culture. J. Chem. Technol. Biotechnol., 62: 159-164.
17. Ogawa, T., T. Fujii and S. Aiba, 1978. Growth yield of microalgae: Reassessment of Ykcal. Biotechnol. Bioengin., 20: 1493-1500.
18. Chen, F., 1996. High cell density culture of microalgae in heterotrophic growth. Tibtech, 14: 421-426.
19. Chen, F., Y. Zhang and S. Guo, 1996. Growth and phycocyanin formation of *Spirulina platensis* in photoheterotrophic culture. Biotechnol. Letters, 18: 603-608.
20. Chen, F. and Y. Zhang, 1997. High cell density mixotrophic culture of *Spirulina platensis* on glucose for phycocyanin production using a fed-batch system. Enzyme and Microbial Technol., 20: 221-224.
21. Lin, L.P. and T. Chen, 1994. Factors affecting the mixotrophic growth of *Chlorella pyrenoidosa*. J. the Chinses Chemical Soc., pp: 91-102.
22. Fargašovα, A., 1997. The Effects of Organotin Compounds on Growth Respiration Rate and Chlorophyll a Content of *Scenedesmus quadricauda*. Ecotoxicol. Environ. Safe, 37: 193-198.
23. Marquez, F.J., 1999. Reassessment of the bioenergetic yield of *Arthrospira platensis* using continuous cullture. World J. Microbiol. Biotechnol., 15: 209-211.
24. Chen, F. and M.R. Johns, 1996. Heterotrophic Growth of *Chlamydomonas reinhardtii* on Acetate in Chemostat Culture. Process Biochem., 31: 601-604.
25. Wen, Z.Y. and F. Chen, 2001. Optimization of nitrogen sources for heterotrophic production of eicosapentaenoic acid by the diatom *Nitzschia laevis*. Enzyme Microbiol. Technol., 29: 341-347.
26. Zhang, X.W., Y.M. Zhang and F. Chen, 1999. Application of mathematical models to the determination optimal glucose concentration and light intensity for mixotrophic culture of *Spirulina platensis*. Process Biochem., 34: 477-481.
27. Neilson, A.H., W.F. Blankley and R.A Lewin, 1973. Growth with organic carbon and energy sources in: Stein JRD (ED) Handbook of Phycological Methods Cambridge University Press Cambridge, pp: 275-285.
28. Camacho-Rubio, F., M.E. Martinez-Sancho, S. Sαnchez-Villasclaras and A. Delgado-Perez, Influence of pH on the kinetic and yield parameters of *Scenedesmus obliquus* heterotrophic growth. Process Biochem., 8: 133-136.

29. Zhang, Ch-C., R. Jeanjean and F. Joset, 1998. Obligate phototrophy in cyanobacteria: more than a lack of sugar transport. FEMS Microbiol. Reviews, 161: 285-292.
30. Chojnacka, K., 2003. Heavy metal ions removal by microalgae *Spirulina sp* in the processes of biosorption and bioaccumulation. Ph.D. Thesis, Wroclaw.
31. Becker, E.W., 1994. Microalgae Biotechnology and Microbiology. *Cambridge University Press* Cambridge, 13.
32. Vonshak, A., 2000. Mixotrophic growth modifies response of *Spirulina (Arthrospira) platensis* (cyanobacteria) cells to light. J. Phycol., 36: 675-679.
33. Chojnacka, K. and A. Noworyta, 2003. Mechanisms of Self-Control of Cells Concentration In Microalgal Culture Used In Microbiological Wastewater Treatment: H. Górecki and Z, Dobrzañski (Eds) Czech-Pol Trade Prague-Brussels-Stockholm Czech (in press).
34. Lee, H.Y., L.E. Erickson and S.S. Yang, 1984. The Estimation of Growth Yield and maintenance Parameters for Photoautotrophic Growth of *Spirulina platensis*. Biotechnol. Bioengin., 26: 926-935.
35. Torzillo, G., P. Carlozzi and B.A. Pushparaj, 1993. Two-Plane Tubular Photobioreactor for Outdoor Culture of *Spirulina*. Biotechnol. Bioengin., 42: 891-898.
36. Zhang, X.W., Y.M. Zhang and F. Chen, 1998. Kinetic models for phycocyanin production by high cell density mixotrophic culture of the microalga *Spirulina* platensis. J. Industrial Microbiol., 21: 283-288.
37. Orus, M.I., E. Marco and F. Martinez, 1991. Suitability of *Chlorella vulgaris* UAM 101 for heterotrophic biomass production. Bioresource Technol., 38: 179-184.
38. Austin, P.A., I.S. Ross and J.D. Mills, 1996. Regulation of pigment content and enzyme activity in the cyanobacterium *Nostoc sp.* Mac grown in continuous light a light-dark photoperiod or darkness. Biochimical and Biphysics Acta, 1277: 141-149.
39. Canizares-Villaneuva R.O., A.R. Dominguez, M.S. Cruz and E. Rios-Leal, 1995. Chemical Composition of cyanobacteria grown in diluted aerated swine wastewater. Bioresource Technol., 51: 111-116.
40. Radmer, R.J., 1996. Algal diversity and commercial algal products. Bio Sci., 46: 263-270.
41. Smart, L.B., S.L. Anderson and L. McIntosh, 1991. Targeted genetic inactivation of the photosystem I reaction center in the cyanobacterium *Synechocystis sp* PCC 6803. EMBO J., 10: 3289-3296.
42. Kong, R., X. Xu and Z.A. Hu, 2003. TPR-family membrane protein gene is required for light-activated heterotrophic growth of the cyanobacterium *Synechocystis sp* PCC 6803. FEMS Microbiology Reviews, 219: 75-79.
43. Wang, Y., Y. Li, D. Shi, G. Shen, B. Ru and S. Zhang, 2002. Characteristics of mixotrophic growth of Synechocystis sp. in an enclosed photobioreactor. Biotechnol. Letters, 24: 1593-1597.
44. Blanch, H.W. and D.S. Clark, 1996. Biochemical Engineering Marcel Dekker New York, pp: 195-197.
45. Chauhan, V.S., S. Gurbaksh and V. Ramamurthy, 1995. Eucalyptus Kraft Black Liquor Enhances Growth and Productivity of *Spirulina* in Outdoor Cultures. Biotechnol. Progress, 11:457-460.
46. Ogbonna, J.C., E. Ichige and H. Tanaka, 2002. Interactions between photoautotrophic and heterotrophic metabolism in photoheterotrophic cultures of *Euglena gracilis*. Applied Microbiol. Biotechnol., 58: 532-538.
47. Ogbonna, J.C. and H. Tanaka, 2000. Light requirement and photosynthetic cell cultivation-Development of processes for efficient light utilization in photobioreactors. J. Applied Phycol., 12: 207-218.
48. Olguin, E.J., S. Galicia, O. Angulo-Guerrero and E. Fernandez, 2001. The effect of low light flux and nitrogen deficiency on the chemical composition of *Spirulina sp* (*Arthrospira*) grown on digested pig waste. Bioresource Technol., 77: 19-24.
49. Olguin, E.J., S. Galicia, R. Camacho, G. Mercado and T.J. Perez, 1997. Production of *Spirulina sp* in sea water supplemented with anaerobic effluents in outdoor raceways under temperature climatic conditions. Applied Microbiol. Biotechnol., 48: 242-247.
50. Richmond, A.E., 2000. Microalgal Biotechnology at the turn of the millennium: a personal view. J. Applied Phycol., 12: 441-451.
51. Singh, R.J., R.M. Kothari, R.K. Sharma and V. Ramamurthy, 1995. Enhancement of *Spirulina* Biomass Productivity by a Protein Hydrolysate. Applied Biochem. Biotechnol., 50: 285-290.
52. Takeyama, H., A. Kanamaru, Y. Yoshino, H. Kakuta, Y. Kawamura and T. Matsunaga, 1997. Production of antioxidant vitamins β-carotene vitamin C and vitamin E by two-step culture of *Euglena gracilis*. Biotechnol. Bioengin., 53: 185-190.