Mixotrophic cultivation of *Microchloropsis gaditana*

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ABSTRACT

The mixotrophic cultivation of *Microchloropsis gaditana* is studied. The microalgal species was cultivated in 10-liter cylindrical bioreactors filled up to 6 liters. All six cultivations were carried out in identical conditions in a glass greenhouse in the month of October. So, the temperature and the light intensity were allowed to vary naturally. Four cultivations were mixotrophic while, for comparison, one cultivation was autotrophic and one cultivation was totally heterotrophic as all light was excluded. In the four mixotrophic cultivations and in the heterotrophic cultivation the carbon (Co) was supplied from glycerol. In the autotrophic cultivation, the macro and micronutrients were supplied by inorganic salts. Air was supplied with a rate of 150 l/hr corresponding to 25 l/(l-hr). In the mixotrophic cultivations Co was equal to 2.87 g/l, 2.92 g/l, 3.96 g/l and 4.96 g/l and in the heterotrophic cultivation Co was equal to 2.24 g/l. The initial nitrogen concentration (No) was the same in all six cultivation and equal to 77.1 mg N/l as nitrate nitrogen. The Co/No ratio influenced both the time necessary for the organic carbon to be fully utilized and the rate of carbon uptake. For the heterotrophic cultivation, within 15 days, 92% of the carbon has been utilized, while for the mixotrophic cultivations after 32 days of cultivation 79%, 68% and 37% of the carbon has been utilized for Co equal to 2.92, 3.96 and 4.96 gr/l respectively. The organic carbon uptake rate, compared to the mixotrophic cultivations, is higher in the heterotrophic growth because during the day the microalgal cells via photosynthesis utilize CO_2 instead. The biomass yield, expressed per gr of organic carbon added to the growth medium, decreases for the high Co=4.96 gr/l and it is highest for the heterotrophic growth.

Keywords: *Microchloropsis gaditana*; mixotrophic; heterotrophic, autotrophic; biomass yield.

1. INTRODUCTION

Microalgae are unicellular photosynthetic organisms that use light and carbon dioxide, with higher photosynthetic efficiency than plants, for the production of biomass. Some microalgae species can also grow and multiply heterotrophically in the absence of light if an organic carbon source becomes available (Mata et. al., 2010). The main advantage of heterotrophic growth is higher biomass growth rates and biomass production because, unlike autotrophic growth, heterotrophic growth is not limited by light transmission through the growth medium (Liang Y., 2013). Another advantage of heterotrophic growth is the potential of achieving higher lipid content and, as a result, higher lipid productivities (Huang et. al., 2010). This is neededif microalgal cultivation is to be useful for biodiesel production. Disadvantages of heterotrophic growth arethe susceptibility to contamination which requires that all parts of the bioreactors as well as all growth mediamust be carefully sterilized and the cost of organic carbon which must be provided to the growth medium(Embury et. al., 2012). Mixotrophic cultivation is a combination of cultivation in autotrophic and heterotrophic modes. The major advantages of mixotrophic cultivation are the higher growth rate and biomass productivity, as well as the higher lipid and protein content of the biomass (Burkholder et. al., 2008). Additionally, it should be mentioned that the process can easily scaled-up and it can result in increased growth and resource utilization by the microalgae (Wan et. al., 2011). On the other hand, the major disadvantages of mixotrophic cultivation are the simultaneous need for light, CO_2, organic carbon, and O_2 and the reduced energy conversion efficiency in comparison to heterotrophic cultivation (Yang et. al., 2000). The biodiversity of microalgae is large; there are species that prosper in fresh water and others in saltwater. It should be mentioned that they are used in pharmaceutical industry, in biodiesel production, in wastewater management, as nutritional supplements for human nutrition and as feed for animals and fish (Pulz & Gross 2004). What is more, they contain a high percentage of proteins, (with a good amino acid profile) and lipids (with significant amounts of DHA and EPA that are essential for fish feeds), varying by algal species (Brown et. al., 1997). In addition, they have a high content of vitamins such as, A, B1, B2, B6, B12, C,

E and biotin, folic acid and inorganic salts (phosphate, zinc, iron, calcium, selenium, magnesium), antioxidants and pigments such as chlorophylls, carotenoids and phenolic compounds (Lubian et. al., 2000). Cultivation of microalgae can be carried out either in open or closed systems. Open-field cultivation is usually conducted in open raceway bioreactors that are exposed to the environment. Cultivation in closed systems can be carried out in photobioreactors of various configurations. However, their culture systems are quite complex and microalgal growth is influenced by different factors, such as light intensity, temperature, pH, carbon dioxide, mixing rate, salinity and nutrient composition of the culture medium (Dean et. al., 2010).

The aim of this study is to cultivate *Microchloropsis gaditana* in three different modes, i.e. autotrophically, mixotrophically and heterotrophically and compare the biomass yields and productivities. Also, for the heterotrophic and mixotrophic cultivations, the aim is to determine the effect of the initial carbon concentration on the carbon uptake rate and the biomass yield expressed in gr of biomass/(l-gr of organic carbon added).

2. MATERIALS AND METHODS

2.1 Bioreactors

The cultivations were carried out in cylindrical bioreactors each of 10 L capacity that were filled up to 6L. Air was continuously passed through the solution in each bioreactor at 150 L/hr through 2 mm glass tubing positioned at the tip of a magnetic bar and the air bubbles were dispersed with the magnetic bar at the bottom of the glass flasks at a rotational speed of 500 rpm. The carbon dioxide feed rate corresponds to 0.062 L CO_2 h^{-1} or 0.026 mole CO_2 h^{-1}. The experiments were performed in the greenhouse of the University of Thessaly under the same conditions of temperature, light intensity, orientation and aeration each time. The pH in all six bioreactors was fixed at 8.5 ±0.3 with the addition of a weak base or weak acid. This cultivation set up in a greenhouse environment where temperature and sun illumination vary naturally is useful since it can examine differences in biomass productivities for these different modes of cultivations under identical external conditions in a natural environment and could be useful in a potential application in open bioreactors.

2.2 Materials and Methods of Analyses

The microalgae *Michrochloropsis gaditana,* a species growing in brackish waters, SAG strain number 2.99, was obtained from the University of Göttingen and the inoculum was cultivated in a Brackish Water Medium (Sammlung von Algenkulturen der Universität Göttingen (SAG), Göttingen, Germany) (= 1/2 SWES) (SAG, 2007). Each liter of the culture medium contained: 0.2 g KNO_3/L, 0.02 g K_2HPO_4/L, 0.02 g $MgSO_4.7H_2O$/L, 30 mL of soil extract/L and 5 ml/L, of a solution containing the following micronutrients: (1 mg $ZnSO_4.7H_2O$, 2 mg $MnSO_4.4H_2O$, 10 mg H_3BO_3, 1 mg $Co(NO_3)_2.6H_2O$, 1 mg $MoO_4.2H_2O$, 0.005 mg $CuSO_4.5H_2O$, 700 mg $FeSO_4.7H_2O$ and 800 mg EDTA)/L. Also, synthetic salt was used to make the culture water brackish (455 mL filtered brackish water in 1 L water). Each bioreactor was inoculated with a standard quantity of 60 mL of *M. gaditana* inoculum of approximately 0.5 optical density.

Crude glycerol was obtained from a local biodiesel manufacturing plant. Its composition was approximately 86% glycerol, 0.5% methanol, 4% free fatty acids and 7.5% H2O. The carbon content was calculated so that the approximate Co/No ratios could be pre-estimated. Initial atomic nitrogen concentration was fixed in all six bioreactors at 77.1 mg/l and it was in the nitrate form ($NaNO_3$). Potassium was supplied from potassium chloride (KCl). The average daily temperature was 25.3 °C and the average sun illumination was 10.9 (MJ/m^2-d). Table 1 shows the initial parameters of each of the six experiments.

For the determination of organic carbon, the method of Ciavatta *et al.* was used (1991). The samples were first centrifuged and then filtered. According to this method, organic carbon was oxidized by a mixture 5 ml of 2N $K_2Cr_2O_7$ and 20 ml of concentrated H_2SO_4 at 160 ± 2 °C. The excess dichromate was titrated with iron (II) sulphate. The ammonium nitrogen content was measured by distillation in the presence of MgO and collection of the product in a solution 2% H_3BO_3 in presence of indicator methyl red, and subsequently titration of the product with 0.1 N HCl. The biomass, at the end of the cultivation period, was collected via centrifugation at 4000 rpm for 5 minutes and was dried in an air circulation oven at 45 °C.

Table 1 *The basic experimental set up for the six cultivations of M. gaditana in a glass greenhouse.*

BR	No (mg/l)	K (mg/l)	Trace Elements & P	Co (gr/l)	Co/No	Mode of Cultivation	pH (±0.3)
1	77.1	138.7	SAG, 1/2 SWES	0	----	Autotrophic	8.5
2	77.1	138.7	SAG, 1/2 SWES	2.24	29.0	Heterotrophic	8.5
3	77.1	138.7	SAG, 1/2 SWES	2.87	37.2	Mixotrophic	8.5
4	77.1	138.7	SAG, 1/2 SWES	2.92	37.8	Mixotrophic	8.5
5	77.1	138.7	SAG, 1/2 SWES	3.69	47.9	Mixotrophic	8.5
6	77.1	138.7	SAG, 1/2 SWES	4.96	64.3	Mixotrophic	8.5

3. RESULTS AND DISCUSSION

Figure 1 shows the carbon reduction versus the cultivation time for the heterotrophic and the four mixotrophic cultivations. The two curves having Co= 2.87 and 2.92 g/L with Co/No equal to 37.2 and 37.8 respectively and are independent cultivations are basically identical.

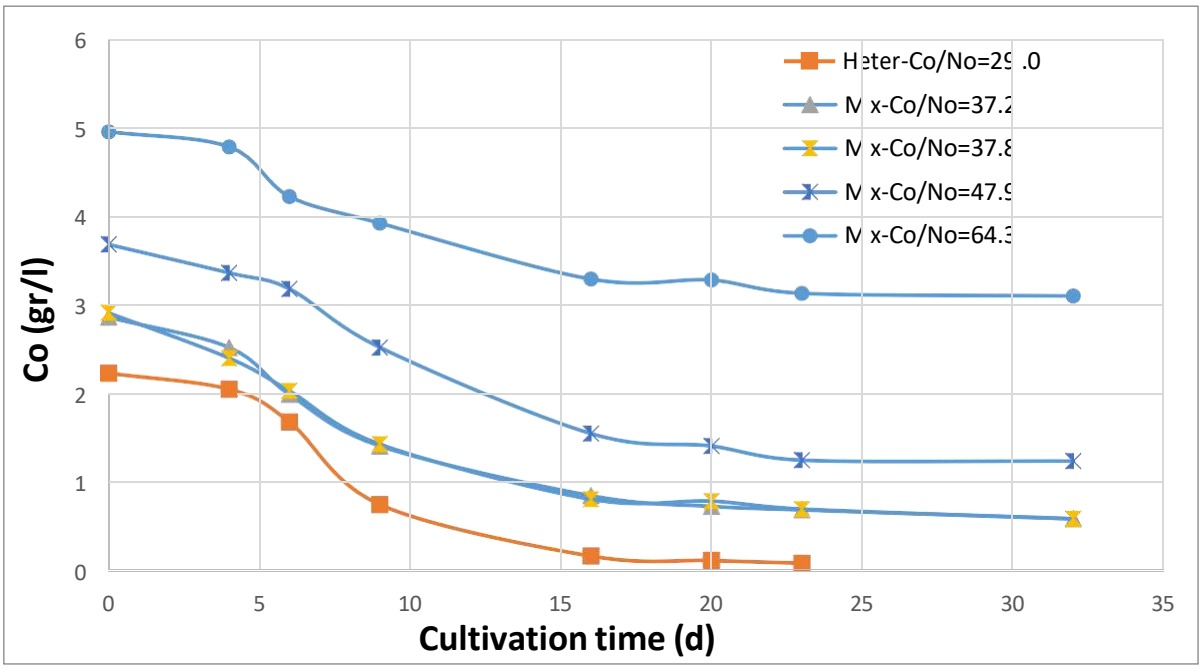

Figure 1. *The carbon reduction versus the cultivation time for the heterotrophic and the four mixotrophic cultivations for the initial carbon concentrations equal to 2.24, 2.87, 2.92, 3.69 and 4.96 gr/l and for Co/No ratios shown in the figure. Initial atomic nitrogen concentration is equal to 77.1 mg/l. Curves were drawn between data points for clarity.*

It is evident from figure 1 that the cultivation time is strongly dependent on the initial carbon concentration and as Co increases so does the cultivation time. For the heterotrophic cultivation, within 15 days, 92% of the carbon has been utilized. For the mixotrophic cultivations after 32 days of cultivation 79%, 68% and 37% of the carbon has been utilized for Co equal to 2.92, 3.96 and 4.96 gr/l (Co/No=37.8, 47.9 and 64.3) respectively. This substantially increased cultivation time required for the reduction of organic dissolved carbon (compared to the corresponding one for the heterotrophic cultivation) is probably due to the decreased carbon uptake rate because during the daylight hours in the mixotrophic cultivations the microalgal cells work via photosynthesis and therefore do not absorb dissolved organic carbon as efficiently.

Figure 2 shows the carbon uptake rate versus the Co/No ratio for the four mixotrophic cultivations of table 1.

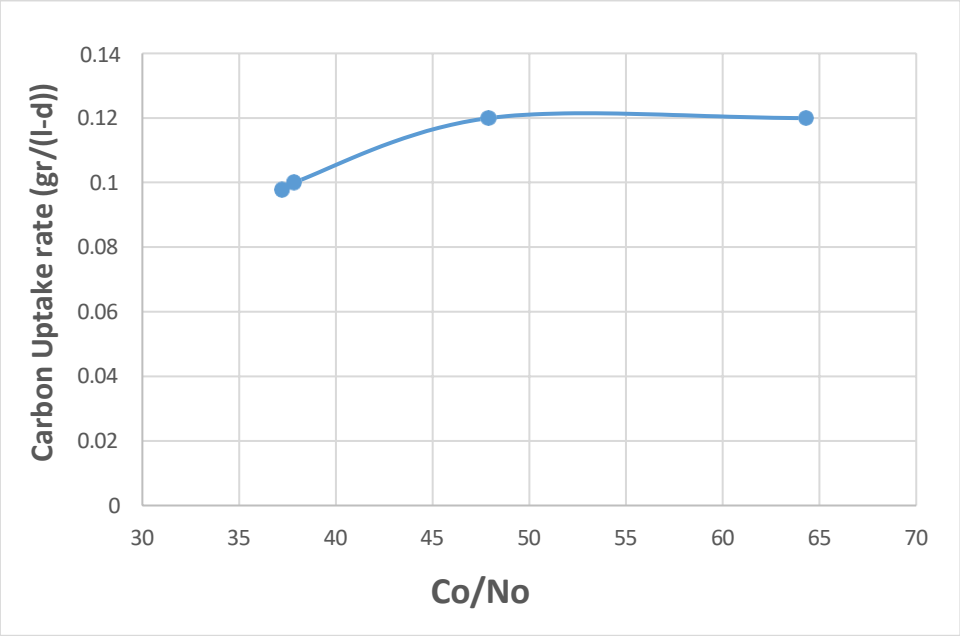

Figure 2. The carbon uptake rate versus the Co/No ratio for the four mixotrophic cultivations of table 1. Curves were drawn through the data points for clarity. For comparison the carbon uptake rate for the purely heterotrophic cultivation with Co/No=29.0 is 0.14 gr/(l-*d).*

From figure 2 it is seen that carbon uptake initially increases and then above Co=3.96 gr/l (Co/No=47.9) it remains constant. For comparison the carbon uptake rate of the heterotrophic cultivation, although it had a lower Co and Co/No equal to 2.24 gr/l and 29.0 respectively, is equal to 0.14 gr/(l-d) higher by 40% than the 0.1 gr/(l-d) for the mixotrophic cultivations with Co=2.87 and 2.92 gr/l and Co/No= 37.2 and 37.8. As discussed previously, this is most likely due to the decreased carbon uptake rate for the mixotrophic cultivations because during the day they photosynthesize and therefore do not utilize dissolved organic carbon as efficiently. Therefore, both the mode of cultivation and the initial carbon to nitrogen ratio influenced both the time necessary for the organic carbon to be utilized and the rate of carbon uptake.

Table 2 shows the biomass yield and the biomass productivities of the six cultivations namely, the autotrophic, the heterotrophic and the four mixotrophic cultivations of *Microchloropsis gaditana*. As noted in table 2 the biomass yield differed in these cultivations. In the mixotrophic cultivations as the Co/No ratio increased the biomass yield as well as the biomass productivity increase as the carbon added to the growth medium increases. However, when the biomass yield is expressed per gr of organic carbon added to the growth medium, it is equal to 1.1, 1.1 and 0.96 gr/(l-gr of C) for Co/No=37.2, 37.8 and 47.9 respectively, declining to 0.85 for Co/No=64.3. For the heterotrophic growth for Co/No=29 the biomass yield as well as the biomass productivity are higher and equal to 1.3 gr/(l-gr of C) and 145 mg/(l-d) respectively. The autotrophic cultivation gave a biomass yield of 2.8 g/l. The productivity is higher for the heterotrophic growth because of the shorter cultivation period required. For the autotrophic growth the biomass productivity was 0.086 g/(l-d).

Table 2. *The biomass yield (Mb), the biomass productivity (Pb) and the biomass yield per gr of organic carbon added in the growth medium (M'b) for the autotrophic, the heterotrophic and the four mixotrophic cultivations of Microchloropsis gaditana.*

	Mode of Cultivation					
	Autotrophic	**Heterotrophic**	**Mixotrophic**	**Mixotrophic**	**Mixotrophic**	**Mixotrophic**
Co → Quantity ↓	-----	2.24 gr/l	2.87 gr/l	2.92 gr/l	3.96 gr/l	4.96 gr/l
M_b (gr/l)	2.8	2.9	3.2	3.2	3.8	4.2

P_b (mg/(l-d)	86	145	107	107	125	140
M'_b (gr/(grC-l)	-----	1.3	1.1	1.1	0.96	0.85

4. CONCLUSIONS

The species *Microchloropsis gaditana* can grow in a variety of modes since it absorbs and utilizes glycerol efficiently. The mode of cultivation of the microalgal species *Microchloropsis gaditana* affects the kinetics of carbon reduction as well as the carbon uptake rate. The biomass productivity for the autotrophic growth is 0.086 g/(l-d). Heterotrophic growth appears to be superior if the biomass productivity is expressed in gr/(l-d-gr of carbon supplied). Excess carbon, 4.96 gr/l, in the mixotrophic growth leads to a decrease in biomass productivity per gr of carbon and does not appear to increase the kinetics of carbon reduction because, after 30 days of cultivation only about 37% of the carbon supplied is utilized. This is to compare with a 79% and a 68% reduction for 2.92 gr/l and 3.69 gr/l carbon added respectively. For the heterotrophic growth at the end of the cultivation with 2.24 gr/l carbon added 96% of the carbon had been utilized. Therefore, other modes of heterotrophic or mixotrophic cultivation such as heterotrophic semi-batch or mixotrophic semi-batch, in which carbon is added in small amounts, may lead to higher productivities.

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