Effect of pH and potassium concentration on the autotrophic cultivation of *Microchloropsis gaditana*

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Abstract

The influence of the pH and of the potassium concentration of the growth medium during the autotrophic cultivation of the microalgae *Microchloropsis gaditana* on its growth rate and biomass productivity was studied. The experiments were carried out in 10 L cylindrical glass bioreactors in a glass greenhouse under the same environmental conditions namely, temperature and light intensity. Growth rates were determined from the specific growth rate coefficient in the exponential phase of the cultivation. The basic growth medium in all cultivations was the Brackish Water Medium. In the first series of experiments the pH of the growth medium was varied and was set equal to 5, 6.5, 7.5, 8.5 and 10 while the concentration of macro and micronutrients was kept the same in all bioreactors. In the second series of experiments, where the effect of potassium concentration was studied, the pH in all bioreactors was fixed at 8.5 and the potassium concentration in each bioreactor was varied and was equal to 9 mg/l, 86,3 mg/l, 300 mg/l, 700 mg/l and 2000 mg/l. The concentration of potassium affects the growth rate of the microalgae *M. gaditana*. A very low potassium concentration seems to have a negative effect on the growth rate of *M. gaditana*and biomass productivity. Optimal concentrations for growth were around 300 mg/l. The biomass productivity varied between 0.10 g/(l-d) and 0,12 g/(l-d) for potassium concentrations of 86.3 mg/l and up to 2000 mg/l, being very lowest and equal 0.06 g/(l-d) for a potassium concentration equal to 9 mg/l. The pH of the growth medium also affects the growth rate of the microalgae *M. gaditana*. Very low pH values seem to significantly reduce the growth rate of microalgae. Optimal pH, in terms of specific growth rate is about 8.5. The specific growth rate coefficients were equal to 0.11, 0.11, 0.15, 0.18 and 0.13 d^{-1} at pH values equal to 5, 6.5, 7.5, 8.5 and 10 respectively. However, this is not in line with the biomass productivity as a very high biomass productivity was measured at pH=10. While biomass productivities ranged between 0.10 and 0.15 g/(l-d) for pH values between 5 and 8.5 the biomass productivityat pH 10 was equal to 0.28 g/(l-d).

Keywords: *Michrochloropsis gaditana; autotrophic; pH; potassium*

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1. INTRODUCTION

The global aquaculture industry has grown in recent years, reaching a peak in 2010. According to the FAO, world production in fisheries and aquaculture will reach 172 million tonnes in 2021, mainly due to the increased demand for fish. This implies the need to increase fish feed production in order to meet the requirements of aquaculture.Fish meal and fish oils are currently the main source of protein and fat respectively used as food in fish.Vegetable proteins, such as soy flour, oilseed rape flour, corn gluten meal or wheat gluten meal, have been used successfully as ingredients in fish feed in many studies [1-5]. However, plant protein sources contain a wide range of non-nutrient factors, and therefore, a high percentage of these components can adversely affect fish growth [2]. Thus, many studies have focused on the search for alternative sources of protein and fats suitable for fish feed. The main sources of EPA and DHA in farmed fish diet are fish oils from various species of fish. However, the over-exploitation of fishery resources combined with the growing demand for fish oil has resulted in stagnation in its production and the consequent increase in its price. In addition, the presence of chemical compounds such as PCBs and dioxins can have adverse health effects [6].

It is well accepted that microalgae have excellent nutritional properties, as they contain high amounts of proteins, lipids, antioxidants, vitamins(such as A, B1, B2, B6, B12, C, E, biotin, folic acid), minerals (phosphorus, zinc, iron, calcium, selenium, magnesium) and are rich in pigments such as chlorophylls, carotenoids and phenols [7]. Some species of microalgae are rich sources of lipids with significant amounts of DHA, EPA and arachidonic acid (AA) that are essential in the diet of fish. Protein content in microalgae varies depending on the species, but also on the growing conditions. The same applies to the content in lipids. Significant amounts of polyunsaturated fatty acids (HUFAs), which are essential in the diet of fish, are also found in the biomass of microalgae[8].It is worth mentioning that the growth of microalgae and biomass production depend to a large extent on various parameters, such as temperature, light intensity, pH, the degree of mixing of the crop and the concentration of macronutrients and micronutrients in the culture medium. Even the composition of the biomass produced is significantly affected by changes in environmental and agricultural parameters. In particular, the content of proteins, fats and other biochemical compounds, such as antioxidants, dyes, chlorophylls, phenols and carotenoids, is affected by changes in the aforementioned parameters.

The most common species of microalgae used in the field of aquaculture are: *Nannochloropsis sp*., *Chaetoceros sp*., *Skeletonema sp.*, *Isochrysis sp*., *Chlorella sp*., *Pavlova sp*., *Thalassiosira sp.*, *Pseudoisochrysis sp*., *Spirulina sp*., *Tetraselmis sp*. and *Dunaliella sp*. [9]. Some species of microalgae are the primary producers of EPA and DHA in the marine food chain and are emerging as a promising alternative to fish oil. Species of microalgae with high EPA content are *Phaeodactylum tricornutum* and *Nannochloropsis sp*., while *Thraustochytrium sp*. and *Schizochytrium limacinum* are good sources of DHA [10]. *Michrochloropsis sp.* (formerly *Nannochloropsis sp*.) contain relatively large amounts of valuable fats and are used in aquaculture. Sukenik et al *investigated* the effect of environmental factors (such as light intensity, nitrogen availability and temperature) on cell chemical composition, with particular emphasis on the lipid content [11]. Seasonal

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fluctuations were further evaluated using large external shallow lakes, with the aim of mass production of *Microchloropsis sp*. for application in aquaculture. The percentage of EPA in the biomass of microalgae ranged between 1.6 and 3.8% (w/w). Despite the significant variations in the protein content of the cells, the amino acid composition in the biomass of *Microchloropsis sp.* remained relatively stable during the various production seasons. Changes in the composition of fatty acids were attributed to differences in the climatic conditions of each season and in the daily cycles of light intensity and temperature.

The aim of this study is to study the influence of the pH and separately of the potassium of the culture mediumon the growth kinetics and on the biomass productivity of the microalgae *Michrochloropsis gaditana* (SAG, strain number:2.99) in an environment of greenhouse where the light intensity and temperature are allowed to vary naturally.

2. MATERIALS AND METHODS

2.1 Bioreactors

The cultivations were carried out in five cylindrical bioreactors each of 10L capacity that were filled up to 6L. Air was continuously passedthrough the solution in each bioreactor at 300 L/hr through 2 mm glass tubing positioned at the tip of a magnetic bar and the air bubbles were dispersed withthe magnetic bar at the bottom of the glass flasks at a rotational speed of 500 rpm. The carbon dioxide feed rate corresponds to 0.10 L CO_2 h^{-1} or 0.042 mole CO_2 h^{-1}. All five bioreactors were set up in a glass greenhouse in the University of Thessaly, Gaiopolis Campus. They were positioned in such a way so that the cultivations would be under identical environmental conditions in a greenhouse environment such as as temperature, irradiation and orientation.

2.2 Materials

The microalgae species *M. gaditana* (SAG strain number 2.99) was obtained from the University of Göttingen and 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: 0.2g 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 1L water)[12]. Each bioreactor was inoculated with a standard quantity of 60 mL of *M. gaditana* inoculum of approximately 0.5 optical density.

2.3 Methods

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. Table 2.1 shows the parameters of the two experiments described above.

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Table 2.1 Initial parameters of the variable pH and variable potassium concentration experiments. The concentration of phosphorus and micronutrients is the same in all cultivations and equal to that of the standard solution.

BR*	K=138,7 mg/l N=77,1 mg/l	pH=8.5 N=77,1 mg/l	Iav (MJ/m^2-d)	Tav °C
	pH (±0,3)	K (mg/l)		
1	5	9	15,4	28,3
2	6,5	86,3	15,4	28,3
3	7,5	300	15,4	28,3
4	8,5	700	15,4	28,3
5	10	1600	15,4	28,3

* BR=bioreactor, Iav =mean intensity of radiation Tav = mean temperature

Each time all the components of the medium were the same and only the value of the parameter studied was changed. In order to study the influence of pH, its value was varied in the five bioreactors and was equal to 5, 6.5, 7.5, 8.5 and 10. These values were monitored three times daily and were adjusted with dilute HCl or NaOH. The initial nitrogen concentration was fixed at 77.1 mg/l. In the experiments were the potassium concentration was varied the pH was fixed at 8.5±0.3 and the concentration of potassium in the five bioreactors was equal to 9.0, 86.3, 300, 700 and 1600 mg/l.The microalgae growth progression was monitored daily using optical density measurements at 655 nm with the use of a spectroscopy UV/Vis instrument. Three samples were collected daily from each culture and all measurements were carried out in triplicate. At the end of each cultivation period, the total production of each culture was measured (in g L^{-1}) after harvesting the biomass using centrifugation at 4000 rpm for 10 min and drying of the biomass at 40 °C in an air circulating oven until a constant weight was attained.

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$$\mu_{exp} = \frac{In(\frac{\alpha_2}{\alpha_1}}{(t_2 - t_1)} \quad (2.1)$$

where, α_1 and α_2 are the optical density readings at the beginning and the end of exponential growthphase, at time $t_1$and t_2, respectively.

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3. RESULTS AND DISCUSSION

Figures3.1a and 3.1b show the optical density as a function of culture time for pH values equal to 5, 6.5, 7.5, 8.5 and 10 and the specific growth rate, μ_{exp}, in the exponential growth phasevs pH respectively.

Optimum pH for the cultivation of *Microchloropsis gaditana*, as determined by the optical density, is 8.5 which, is the pH closest to the water encountered in the sea. Acidic cultivation media appear to significantly slow down the growth of *Microchloropsis gaditana*. It is worth mentioning that even in a highly the highly pH of 10 growth, as measured by optical density readings, is faster than those in acidic environment. It is also worth mentioning that carbon dioxide solubility increases substantially with increasing pH.

Figures3.2a and 3.2b show the optical density as a function of culture time for the potassium concentrations equal to 9, 86.3, 300, 700 and 1600 mg/l and the specific growth rate, μ_{exp}, in the exponential growth phase versus the potassium concentration respectively. The concentration of potassium varies from 9 mg/l to 1600 mg/l, ie more than 2 orders of magnitude. The potassium concentration used in the standard solution ("Brackish Water Medium") is 86.3 mg / l.

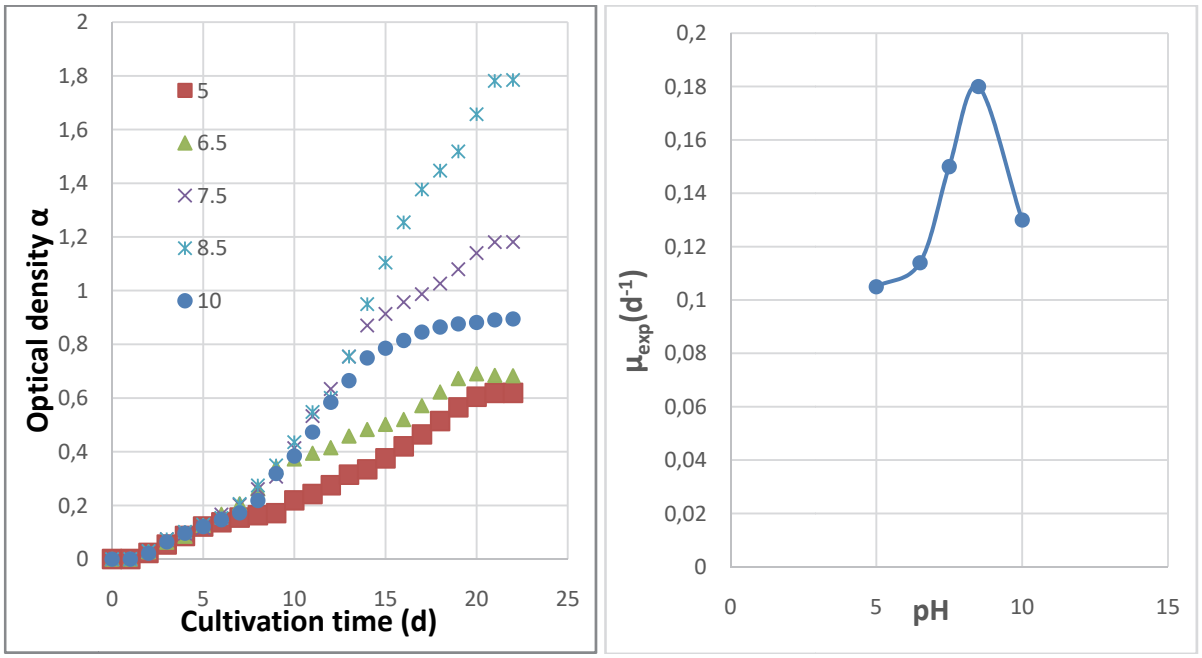

Figure 3.1. *a) The optical density versus the cultivation time for the pH values shown and b) the specific growth coefficient in the exponential growth phase versus the pH for the cultivation of Microchloropsis gaditana.*

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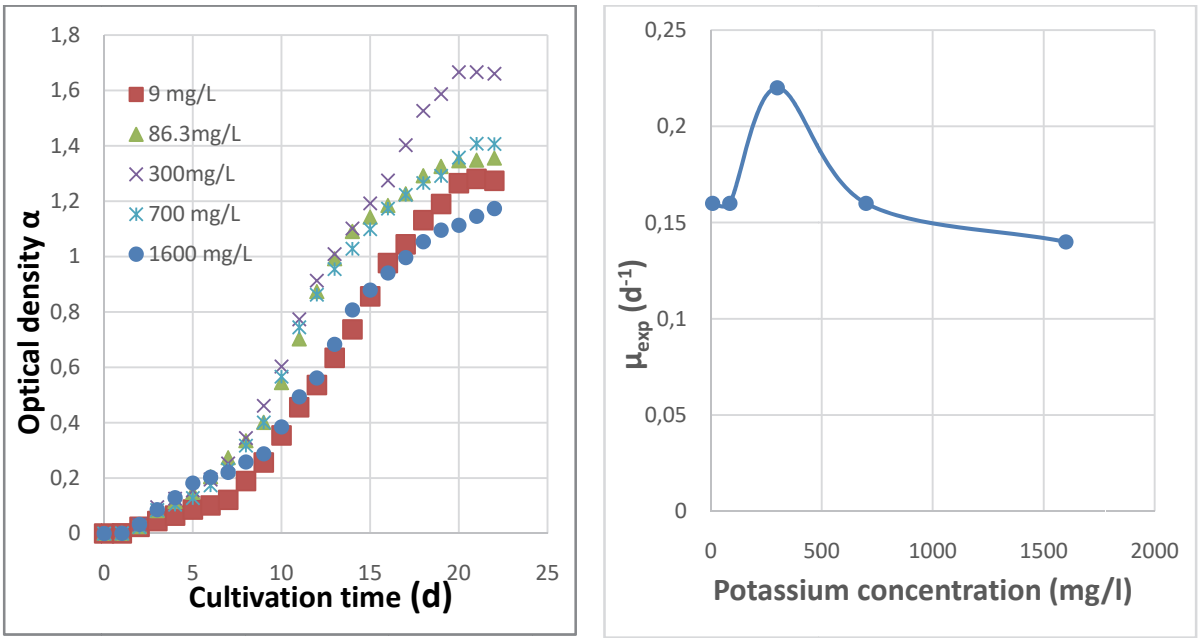
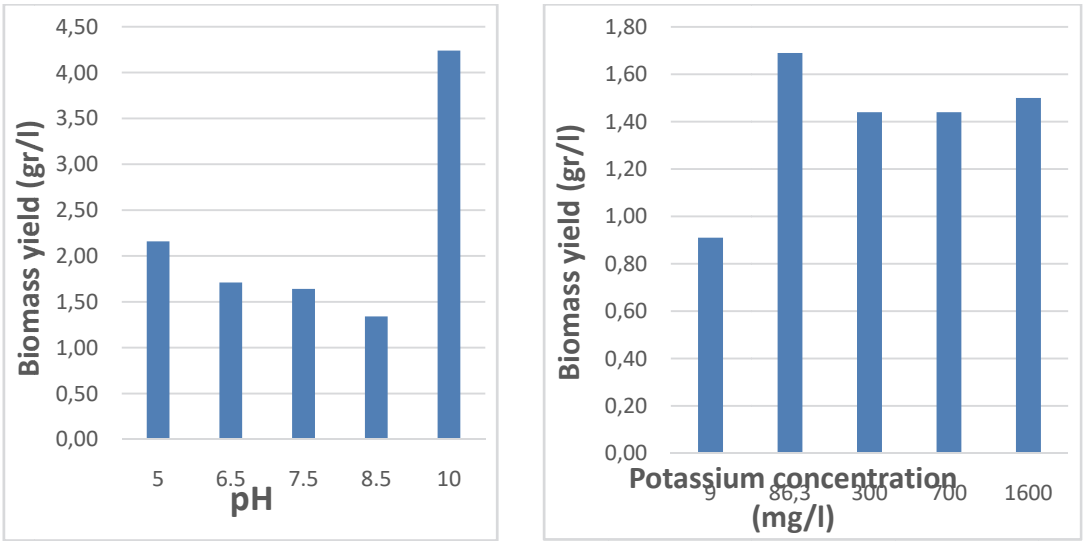


Figure 3.2. *a) The optical density as a function of culture time for the potassium concentrations equal to 9, 86.3, 300, 700 and 1600 mg/l and b) the specific growth rate, μ_{exp}, in the exponential growth phase versus the potassium concentration for the cultivation of Microchloropsis gaditana.*

It is seen that the optimum concentration of potassium, as determined by the optical density readings, is 300 mg/l at which value the highest specific growth rate coefficient is obtained. Potassium plays an important role by activating dozens of important enzymes, involved in protein synthesis, sugar transport, nitrogen and carbon metabolism, and the process of photosynthesis [13]. Potassium is also very important for cell growth, which is an important process for plant function and growth.

Figures 3.3a and 3.3b show the biomass yield for the various pH and potassium concentration values respectively.



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It is seen that in both figures the trend in biomass yield does not match the trend observed with the optical. For the variable pH experiments, we observe that while the optimal growth rate is at pH 8.5, biomass production is maximum, with a significant difference, at pH 10. In terms of absorption measurements this can be explained by the fact that at high pH, above 9, strong cell aggregation and precipitation occurs which intensifies over time. In bioreactors, such precipitation is prevented by strong agitation. However, during the measurement, which is done by transferring a sample from the greenhouse to the laboratory, time favors strong agglomeration, thus reducing the absorption of the sample. As for the high biomass production at pH 10, this can be explained by the increase in the solubility of carbon dioxide at pH=10. Carbon dioxide is essential for photosynthesis. In general, however, the absorption kinetics data of Figures 3.1a and 3.1b do not coincide with the measured values of biomass collected at the end of the cultivations. This difference is under investigation. It is possible that the pH of the solution affects the absorption of the various dyes absorbing at 665 nm which, is the wavelength where the optical density was measured. Two such substancesare chlorophyll αand β and differences in the relative chlorophyll content in cultivations of different pH may lead to differences in absorption and biomass productivity. The cultivation time, if the latency phase is removed, is about 18 days. Thus, the maximum biomass productivity observed in a fairly alkaline environment, at pH 10, is 0.24 g/(l-d), higher than any other productivity in all experiments of this Deliverable.For the variable potassium experiments we observe that the maximum biomass production occurs at a concentration of potassium equal to 86.3 mg /l, although the optimal concentration of potassium for the growth of microalgae is at 300 mg /l. However, the two values differ only by 14.7% and biomass yield values for the 86.3, 700 and 1600 mg/l potassium concentrations are similar while,for potassium deficiency (9 mg/l) the difference is significant and equal to 46.2%. From the culture time of 22 days if the latency phase time which is about 3 days is subtracted it results that the culture time is about 19 days. Therefore, biomass productivity ranges from a low of 0.048 g/(l-d) for potassium concentrations of 9 mg/l to 0.089 g /(l-d) for potassium concentrations of 86.3 mg/l.

CONCLUSIONS

Both the pH and the potassium concentration affect the growth rate and biomass productivity of *M. gaditana* which was cultivated in an autotrophic mode. The effect of the pH was much stronger. High pH values lead to strong growth but, biomass productivity was significantly higher at PH=10 apparently because of the high solubility of carbon dioxide. Optical density measurements are apparently affected by the pH and this may be due to biomass agglomeration and changes in the absorptivity of chlorophyll. Very low concentrations of potassium lead to low biomass growth rates and low biomass productivities. However, intermediate and high potassium concentrations do not affect substantially the biomass growth rates and productivities.

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