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Effect of Glucose on the Productivity and Biochemical Constituents of a Few Microalgae

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Abstract: ***In the present investigation effect of glucose uptake was studied on the micro algae Chlorococcum humicola and Chlorella vulgaris obtained from Algal culture collection of Vivekananda Institute of Algal Technology (VIAT), cultivated in a designed bioreactor was investigated. Experiments were conducted to study the growth rate of micro alga and biochemical analysis was also done. Analytical data revealed that Chlorococcum sp. was able to grow well in heterotrophic. Data on the biochemical characteristics of biomass was generated and presented.***

Keywords: Microalga, Growth rate, Biochemical analysis Heterotrophic and Autotrophic

1. Introduction

Some microalgae assimilate and thus utilize organic carbon as an energy source for growth in the dark (heterotrophy) or combined with CO_2 uptake under light (mixotrophy), offering the possibility of increasing cell concentration (up to 40 g/l) and productivity Lee YK (2004). It is widely observed that the maximum specific growth rate of algae increases in the following order: heterotrophy, autotrophy and mixotrophy. Mixotrophic cultures exhibit high cell densities and specific growth rates and thus, higher biomass output rates Lee YK (2001). Heterotrophic cultures are denser (up to 100 g/l) and, according to Lee YK(2004) , projected costs of producing algae in industrial fermenters seems to be approximately ten times lower when compared to the conventional autotrophic cultivation mode. On the other hand, dense heterotrophic cultures are often oxygen-limited. The aeration conditions are of crucial importance for cell growth as the specific growth rate decreases when the cells grow under restricted supply oxygen conditions. Moreover, oxygen transfer is likely to be a limiting factor during a commercial-scale high-cell density cultivation of heterotrophic microalgae, leading to a decrease in process productivity. In such conditions, to maintain aerobic conditions, a very high stirrer speed has to be maintained during much of the process, resulting in power input increase and increased costs. Cell proliferation of microalgae can be negatively affected by mechanical agitation due to severe shear stress, which is the way generally used to improve mass transfer in submerged fermentations Gudin C, Chaumont D (1991)

Another solution was suggested by Pack M Y (1991), who claimed a method for producing economically biomass of microalgae and fish, by establishing a symbiotic relation between the above two organisms in a culture pond exposed to sunlight or artificial illumination.

This symbiotic approach could be extended to any microalgae capable of growth under hetero and autotrophic nutritional modes, particularly, microalgae belonging to *Chlorella* Genus, such as *Chlorella protothecoides*, which have been recognized as a good lipid producer for biodiesel Lopes da Silva T, Santos CA, Reis A (2009) and Miao X, Wu Q (2006).

According to the present work, the microalga was grown in designed bioreactor for autotrophic, Phototrophic and Heterotrophic mode of nutrition with and without glucose treatment. The biomass obtained was analysed for lipid, protein and carbohydrate and fatty acid, amino acid profile.

2. Materials and Methods

Cultivation of Microalgae:

Chlorococcum humicola and *Chlorella vulgaris* obtained from Algal culture collection of Vivekananda Institute of Algal Technology (VIAT), were cultivated in a suitable medium in a thermostatically controlled room at 24± 1ºC and illuminated with cool white fluorescent lamps at an intensity of 2000 lux in a 12:12 light and dark regime

The microalgae were grown in **Bold Basal Medium** (*Nichols and Bold* 1965) and **CFTRI –Medium** Light intensity during the trials was measured using lux meter (Lutron LX – 101A).The micro algal cultures were microscopically examined using Olympus (HB) microscope and photomicrographed using Nikon digital camera (Coolpix E8400)

Growth Measurement

Growth was measured by counting cells using a haemocytometer (Neubauer, improved) and the results were plotted in a semi-logarithmic graph. Growth rate (divisions/day) was arrived at using the formula.

$$\frac{\log N - \log N_0}{\log 2 \times t}$$

where,
N - No. of cells per ml at the end of log phase or mg weight/L
N_o - Initial count of cells per ml or mg weight/L
t - Days of log phase

For dry weight method, the algal cultures were pelleted by centrifugation at 7500 rpm (Remi cooling microfuge) for 15 minutes. Cells were washed with glass distilled water, again centrifuged and dried in an oven for 24 hours or until constant weight.

pH AND CONDUCTIVITY MEASUREMENT

For all the trials, pH was measured using digital pH meter (Elico LI 120) and conductivity using digital Conductivity meter (Equiptronics EQ - 660A) respectively.

BIOMASS HARVESTING

When the microalgae are well grown a known volume of culture was harvested and added with diluted flocculent PHYCOFLOC and was evenly mixed for 20 minutes and left for overnight to settle in the bottom of the container. The next day the pellet was collected after centrifuging at 2000 rpm for 15 minutes. The pellet was dried and the algal biomass was collected.

3. Results and Discussion

Analytical data revealed that the effect of Glucose on the growth rate of *Chlorococcum* humicola and *Chlorella vulgaris* were studied under varied growth conditions. The growth rate was calculated and it was found to be 0.1868 in *Chlorella vulgaris* and 0.1955 in *Chlorococcum humicola* (Table 1 and 2) when grown under heterotrophic condition followed by the phototrophic and autotrophic conditions .But Lee (2004) stated that the maximum specific growth rate of algae, cultured heterotrophically on simple sugars, is lower than that of photosynthetic cultures. No contamination was detected during the growth. The result has showed that under heterotrophic condition the growth was appreciably higher when compared to autotrophic and phototrophic condition.

Jiří Doucha and K. Lívanský (2011) reported that pH of the culture decreased during cultivation as a result of increased production of carbon dioxide in the culture. After the end of growth caused by glucose consumption, the cells stopped CO_2 production, resulting in a pH increase.

The glucose uptake system of *Chlorella vulgaris* has been studied intensively. It is induced within 60 to 90 min following the supply of glucose in darkness Komor E, Tanner W (1974), Tanner W (1969) Kamiya and Kowallik (1987) have reported that the glucose uptake mechanism was light sensitive and, furthermore, that the uptake was inhibited in the light. However, Flor Martinez* and Maria Isabel Orus (1990) reported that the presence of glucose stimulated growth even in the presence of 2% CO_2 indicated that the cells were using glucose as a carbon source in the light. They concluded that the rate of glucose utilization in the light and dark was similar was further supported by the observation that the rate of respiration in the presence of glucose was similar in the light and dark.

Table 1: Effect of Glucose on the Growth rate and pH of *Chlorella vulgaris* under varying Growth Conditions

S. No	Growth Division Per Day			pH		
	Autotrophic	**Phototrophic**	**Heterotrophic**	**Autotrophic**	**Phototrophic**	**Heterotrophic**
1	-	-	-	7.3	7.1	7
2	0.2456	0.3333	0.3689	7.4	7.2	7.3
3	0.3473	0,2643	0.4213	7.4	7	7.4
4	0.2614	0.2146	0.3152	7.5	7.3	7.6
5	0.2222	0.2008	0.2555	8.1	7.4	7.61
6	0.1854	0.202	0.217	8.2	7.5	7.8
7	0.1598	0.1786	0.1868	8.2	7.6	8.3

Table 2: Effect of Glucose on the Growth rate and pH of *Chlorococcum humicola* under varying Growth Conditions

S. No	Growth Division Per Day			pH		
	Autotrophic	Phototrophic	Heterotrophic	Autotrophic	Phototrophic	Heterotrophic
1	-	-	0	7.1	7.01	7.1
2	0.5849	0.447	0.3608	7.3	7..03	7.2
3	0.3715	0.3137	0.3112	7.4	7.54	7.3
4	0.2724	0.2497	0.2699	8	7.6	8.1
5	0.2196	0.2274	0.2222	8.6	8.2	8.4
6	0.1935	0.2093	0.2185	8.8	8.5	8.5
7	0.1669	0.18	0.1955	9	9.2	9.3

Biochemical analysis

The biomass obtained was analysed for carbohydrate, protein, lipid, fatty acid and amino acid profile. From the data it is inferred that the level of carbohydrate (31.56 g) and protein content (23.23g) drastically increased in heterotrophic condition of ***Chlorella vulgaris.*** But the Lipid content (0.0971 g) was increased in phototrophic condition. The level of **Palmitic acid (0 .0034 mg)**, vitamin content and amino acid content was increased in heterotrophic condition of ***Chlorella vulgaris.*** The level of protein, fatty acid, vitamins and amino acid content was increased in heterotrophic condition of ***Chlorococcum humicola.*** **(Refer Fig 1 to 8)**

4. Conclusion

In the present study, it was concluded that ***Chlorococcum humicola*** was able to grow well in heterotrophic condition with glucose when compared to *Chlorella vulgaris* in both phototrophic and heterotrophic conditions. The growth was much more than the autotrophic condition. Due to glucose utilization the production of biomass of the microalgae can

be increased. Biochemical analysis revealed that Protein and fat content was raised in heterotrophic condition in the case of *Chlorella vulgaris.*The level of carbohydrate content, protein content, fatty acid content and Amino acid compound content is drastically increased in heterotrophic condition of *Chlorococcum humicola.*

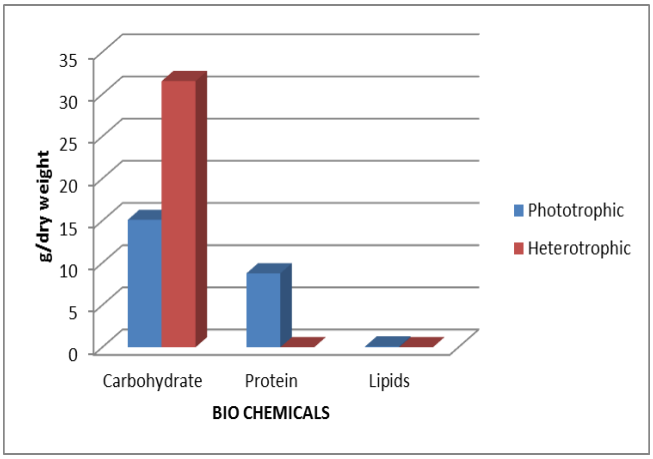

Figure 1: Effect of Glucose on the Composition of Biochemical Components in *Chlorella vulgaris*

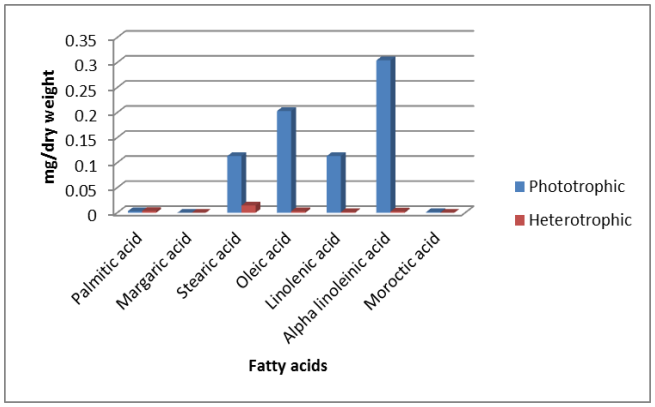

Figure 2: Effect of Glucose on the Composition of Fatty Acids in *Chlorella vulgaris*

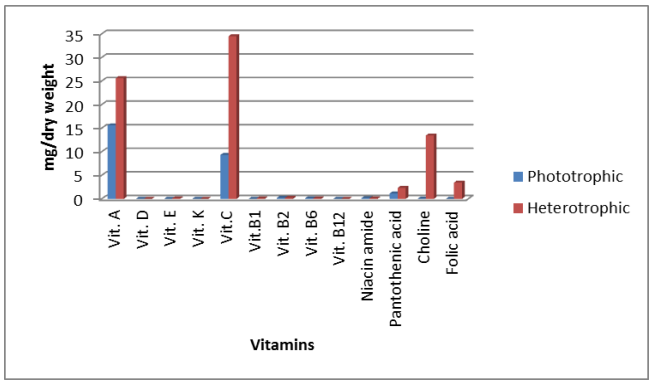

Figure 3: Effect of Glucose on the Composition of Vitamins in *Chlorella vulgaris*

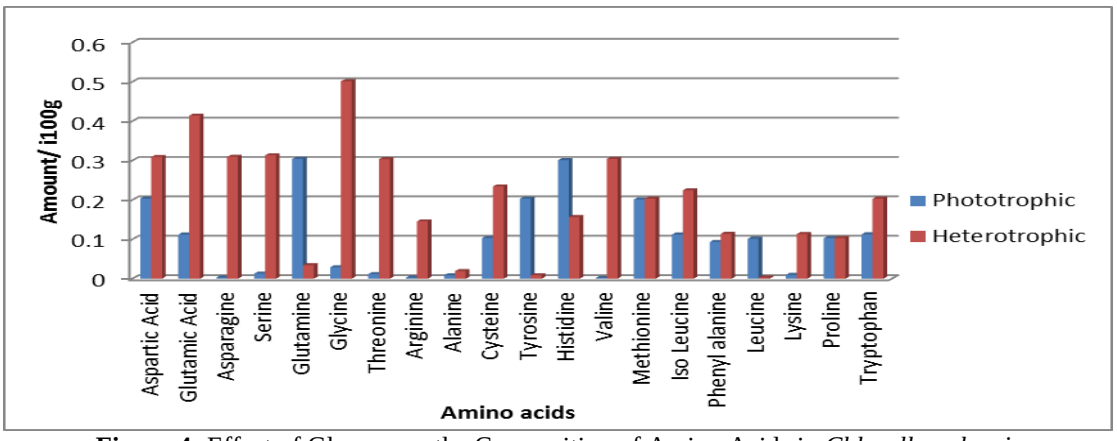

Figure 4: Effect of Glucose on the Composition of Amino Acids in *Chlorella vulgaris*

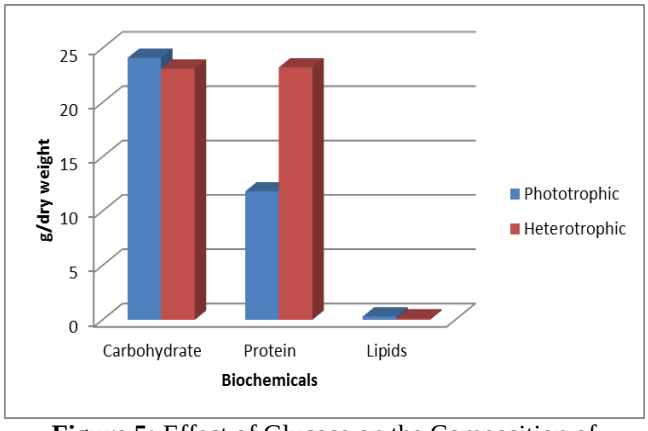

Figure 5: Effect of Glucose on the Composition of Biochemical Components in *Chlorococcum humicola*

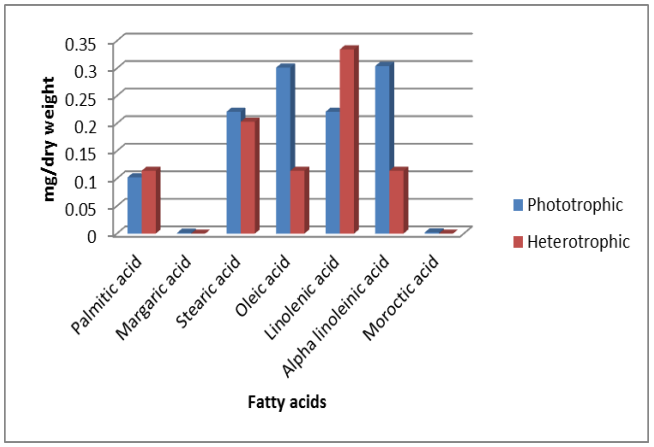

Figure 6: Effect of Glucose on the Composition of Fatty Acids in *Chlorococcum humicola*

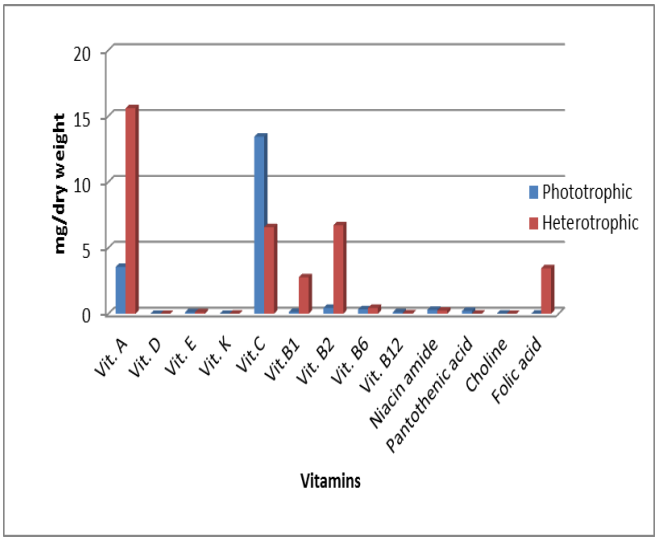

Figure 7: Effect of Glucose on the Composition of Vitamins in *Chlorococcum humicola*

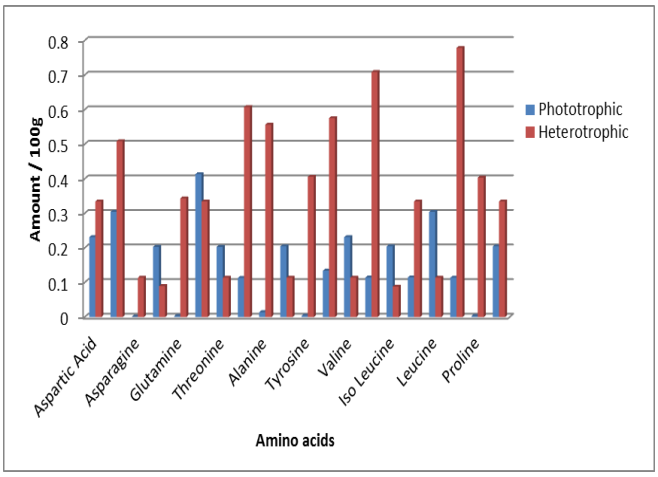

Figure 8: Effect of Glucose on the Composition of Amino Acids in *Chlorococcum humicola*

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