GROWTH OF *Aphanothece microscopica* NÄGELI ON EXOGENOUS SUGARS

CULTIVO DE Aphanothece microscopica *NÄGELI A PARTIR DE AÇÚCARES*

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ABSTRACT: Biological processes for wastewater treatment generally produce biomass or active sludge without reuse. In this context, incorporation of organic matter and nutrients from agro industrial effluents into cell mass for single-cell protein allowed application of sustainable process. Cyanobacteria could be used due to its versatile metabolism. So, the aim of this paper was evaluate the growth of cyanobacteria *Aphanothece microscopica* Nägeli growth on heterotrophic medium with glucose, lactose and sucrose. Growth curves indicated that cultivation of cyanobacterial on the dark depend the type of carbon source and there are different mechanisms for glucose, fructose and sucrose consumption. Results suggest a useful application of cyanobacteria on organic matter removal from wastewater.

KEYWORDS: Cyanobacteria. *Aphanothece*. Wastewater treatment.

INTRODUCTION

Microalgae are eukaryotic, as the green algae (*Chlorophyta*), or prokaryotic photosynthetic microorganisms, as the cyanobacteria (*Cyanophyceae*) (MATA; MARTINS; CAETANO, 2010). Cyanobacteria are photosynthetic organisms with high protein content on biomass, called of single-cell protein (SCP) and capable of simple organic molecules consumption on heterotrophic in the dark (FAY, 1983; FAY, 1992). This particular metabolism provides the organisms with their simple nutritional requirements and the use of microalgae in biotechnology has been increased in food, cosmetic and pharmaceutical industries (MORENO-GARRIDO, 2008; HARUN et al., 2010). Nowadays, microalgae and cyanobacteria have been used also for biodiesel production (MATA; MARTINS; CAETANO, 2010) and carbon dioxide sequestration on photobioreactors (JACOB-LOPES et al., 2009). Furthermore, alternative technologies of bioremediation with simultaneous chemical engineering demand (COD) and nutrients (nitrogen and phosphorus) for incorporation into a microalgal biomass have been studied due to versatile metabolism of these microorganisms (TAM; WONG, 1996; LINCOLN; WILKIE; FRENCH, 1996; BICH; YAZIZ; KAKTI, 1999; MARTINEZ et al., 2000; TAM; WONG, 2000; XING et al., 2000; QUEIROZ; KOETZ; TREPTOW et al., 2001; BASHAN; BASHAN, 2004; ASLAN; KAPLAN, 2006).

Aphanothece microscopica Nägeli is a cyanobacteria that has been studied with a view to the valorization of agro-industrial wastewater and the production of SCP (QUEIROZ et al., 2002; BASTOS et al., 2004). Queiroz et al. (2007) reported COD and nitrogen removal from parboiled rice effluent by this microorganism of approximately 83% and 73%, respectively, on batch cultivation.

Despite of several papers about biological wastewater treatment by application of microalgae heterotrophic metabolism, literature is very poor concerning organic substrate consumption and oxygen demand for organic matter oxidation (OREN; SHILO, 1979). Literature report that dark endogenous metabolism in cyanobacteria serves mainly the adjustment of photosynthetic period (FAY, 1983). Glycogen or exogenous glucose supports a limited dark metabolism, it is being converted to glucose-6-phosphate and metabolized via the respiratory pathway (Figure 1). Cyanobacteria are distinguished from other prokaryotes by their generally low rates of endogenous respiration and by limited ability to utilized organic substances as a source of carbon and energy in the dark. Unlike aerobic heterotrophic bacteria and eukaryotic organisms, cyanobacteria present some of the essential TCA cycle enzymes in extremely low activities. Thus, the incomplete TCA doesn´t function in substrate oxidation, but performs a biosynthesis of various amino acids and lipids.

Respiration in cyanobacteria is considered a membrane-bound electron transport process leading to the formation of ATP in the dark. Oxygen is currently known as the terminal oxidant, but the existence of other terminal oxidants cannot be excluded. Amino acid oxidations reduce oxygen and there is no evidence that these reactions are couple

ATP formation. Reviews in the 90´s years established that the respiration has been found in all cyanobacteria tested for this activity and its probable function is the generation of a minimum amount of energy necessary for survival in the dark (SCHMETTERER, 1994). However, knowledge of cyanobacterial respiration is not yet satisfactory and their application depends of studies on several organic substrates and analysis of oxygen profiles.

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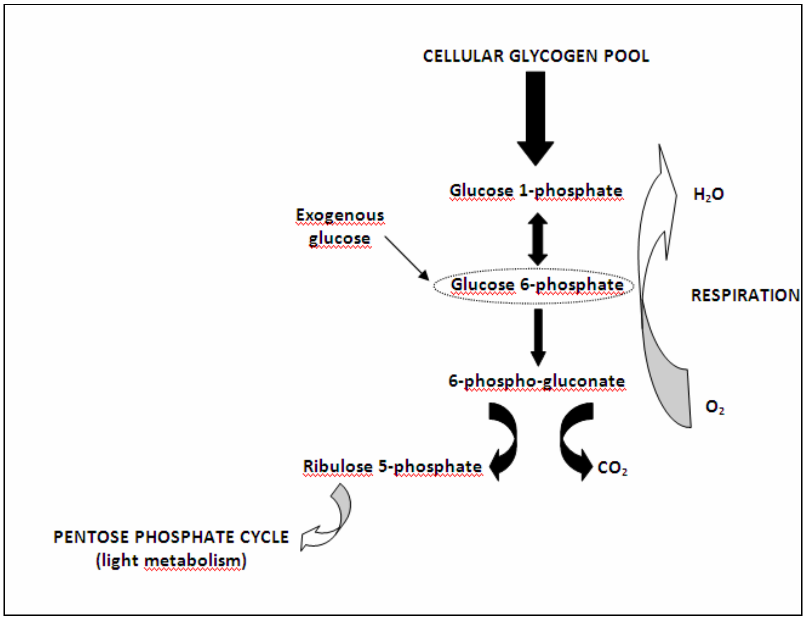

Figure 1. Schematic pathways of light and dark carbon metabolism in cyanobacteria (adapted by FAY, 1983)

In the 1970s, Sansawa and Endo (2004) found a strain of *Chlorella regularis* that has a high grow heterotrophically in the dark using organic carbon sources as well as autotrophically in the light, with glucose depletion in 6h cultivation at a constant oxygen consumption rate. Queiroz et al. (2007) showed COD removal from parboiled rice effluent with low biomass yield by *Aphanothece microscopica* Nägeli. It suggests the existence of metabolism capable that assuring slow growth in the dark.

In this context, the aim of this paper is to perform studies on cyanobacteria respiration and biomass production using an organic medium in the dark. These tests will evaluate the utilization of glucose, lactose and sucrose as exogenous carbon for the cultivation of *Aphanothece microscopica* Nägeli.

MATERIAL AND METHODS

Inoculum of *Aphanothece microscopica* Nägeli RSMan 92 was gently given from the Biotechnological Laboratory of FURG, Rio Grande, RS, Brazil. Cyanobacterial culture was grown on BG11 medium (RIPKA et al., 1979) for two weeks at 12h:12h (light:darkness) photoperiod, with forced aeration.

Experiments were set up with a 5% cyanobacterium inoculum in 200mL Erlenmeyer´s flasks containing BG11 medium and 2.5, 5, 7.5, 10, 15 and 20% of glucose, lactose and sucrose at 1VVM aeration; 30^{o}C; dark.

Samples were quantified for biomass concentration through a standard absorbance curve at 556nm (QUEIROZ et al., 2007). The specific growth rates and productivity were calculated from the growth curve. Oxygen and sugar consumption were obtained for maximums productivity. Dissolved oxygen concentration was measured using oxygen analyzer Digimed® DM-4P and glucose concentration was evaluated by glucose-oxidase method after acid treatment of sample (BASTOS, 2006).

RESULTS AND DISCUSSION

Figures 1a, b and c present biomass profile for *Aphanothece*'s cultivation on glucose, lactose and sucrose, respectively. Profiles represent higher growth on glucose and sucrose, lower on lactose only 10 and 15%. Biomass concentration maximum was obtained for glucose (400mg/L at 10 and 2.5%), sucrose (200mg/L at 5%) and lactose (500mg/L at 10 and 15%). Maximums biomass productivity was 5,5mg/L.h for 10% glucose, 6,6mg/L.h for 10%

lactose and 2,9mg/L.h for 5% sucrose. High biomass productivity for lactose suggest rapid assimilation this sugar and enzymatic mechanism suitable for this exogenous sugar. However, the lactose profile presented different pattern, suggesting a different incorporation mechanism with a long lasting lag phase, specific growth rate and biomass productivity (Table 1).

According to the results, there was a slow growth in the dark, with consumption of sugars, featuring the respiratory metabolism. This results showed growth higher than heterotrophic experiments on BG 11 medium with *Aphanothece microscopica* Nägeli RSMan92 (BASTOS et al., 2004). Others authors have reported that the only objective of the respiration of cyanobacteria is to generate minimal energy for growth in the dark (FAY, 1992; ANAND, 1998). Indeed, minimal energy represents a slow growth on heterothophic metabolism comparing the growth profile with previous papers about *Aphanothece sp*. on photosynthetic medium. Pelroy and Basshan (1973a) reported that dark growth of blue-green algae *Aphanocapsa* 6714 is slow in comparison to photosynthetic growth, decreasing by almost an order of magnitude.

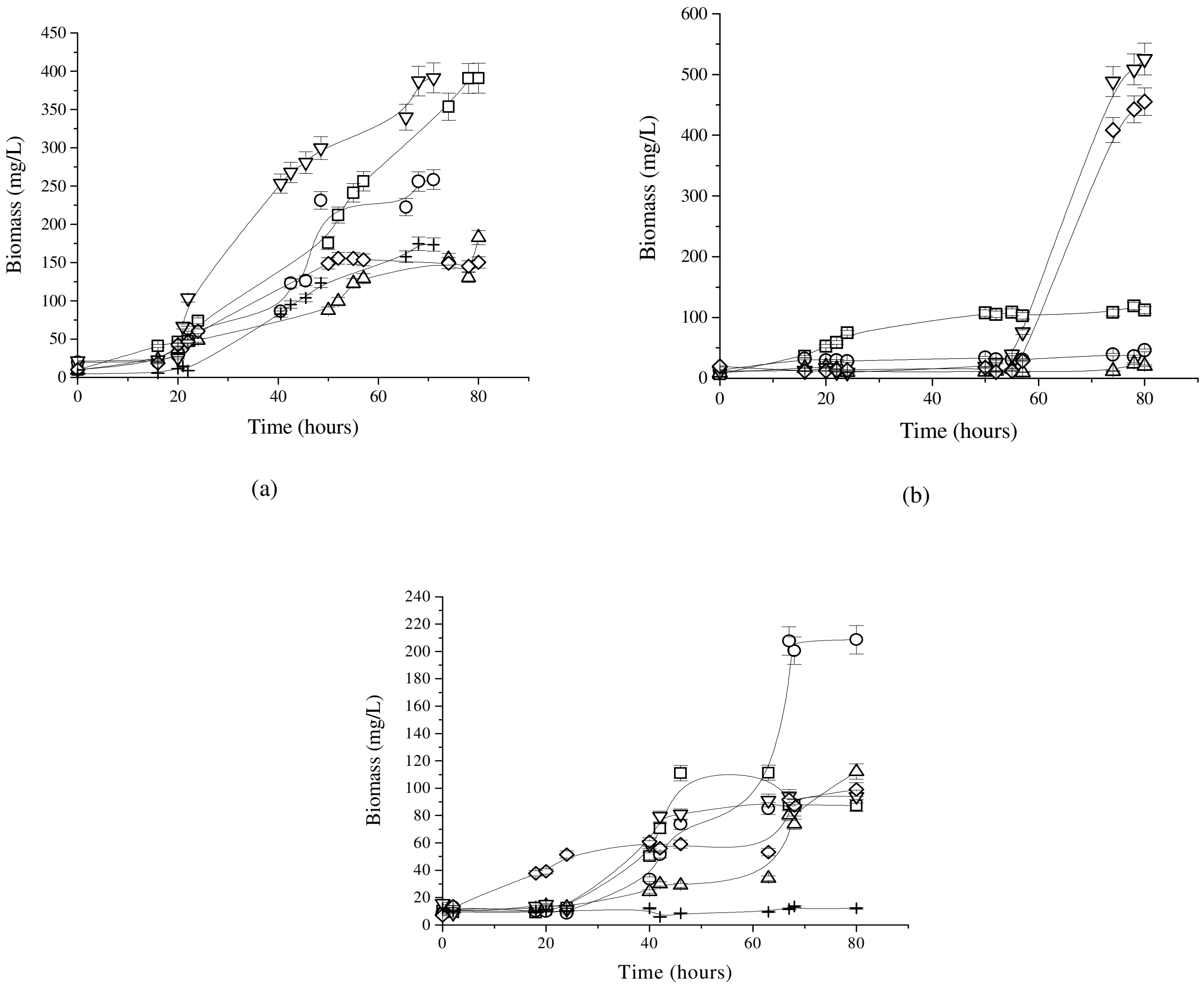

Figure 1. Biomass profile for heterotrophic *Aphanothece*'s growth on 2.5% ( ), 5% (○), 7,5% (Δ), 10% (∇), 15% (◊) e 20% (†) of glucose (a), lactose (b) and sucrose (c).

The assay on sucrose present slight growth for concentrations higher than 2.5%, in contrast to other sugars. This observation in concern of the profiles presented suggests different mechanisms of sugars assimilation, which support dark growth. Studies on the enzymatic mechanism of disaccharides and monosaccharides by cyanobacteria must be further developed to the better knowledge of this bioreaction. Analysis of enzymes in cell-free extracts revealed that ribulose-1,5 biphosphate was a strong inhibitor of glucose-6-phosphate dehydrogenase, the first enzyme of the oxidative pathway (PELROY; BASSHAN, 1973b). Moreover, dark incubation of cells led to the immediate disappearance of this metabolite, at the same time as the oxidative pathway was being activated. Besides, these authors suggests that the potential for heterotrophic growth by microalgae is probably due to the permeability of the cell membrane for organic molecules rather than a fundamental biochemical difference between *Aphanothece* or *Aphanocapsa* and strictly photoautotrophic others microalgae. Moreover, transport across the cell membrane may depend on the presence of specific carriers, which mediate the uptake of a particular substance (FAY, 1983). This information supports our data growth by *Aphanothece* on glucose and lactose, higher than on sucrose. Another consideration is a low oxygen demand by *Aphanothece* in Table 1, indicating the oxidative pathway different the aerobic microorganisms. It is an important engineering parameter for design of heterotrophic bioreactors using these microalgae.

Table 1. Biomass productivity and specific growth rate at the best conditions at different sugars

Experiment	Biomass productivity (mg/L.h)	Specific growth rate (h^{-1})	Oxygen consumption rate (mmol/L.min)
Glucose 10%	5.5	0.013	0.025
Lactose 10%	6.6	0.113	0.023
Sucrose 5%	2.9	0.054	0.053

Heterotrophic growth of *Aphanothece microscopica* Nägeli on sugar medium explains the occurrence of cyanobacterial blooms in niches with high organic load (QUEIROZ; KOETZ; TREPTOW, 2001). So, the growth data from these sources of organic carbon is a reasonable prospect in the possible use of these microorganisms in the treatment of agro-industrial effluents, which are rich in soluble organic matter.

CONCLUSION

Cyanobaterial growth on darkness depends of carbon source; there are different mechanisms of glucose, fructose and sucrose consumption. The result of this research suggests a possible application of cyanobacteria to the removal of organic matter from wastewater.

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RESUMO: Os processos biológicos de tratamento de águas residuárias produzem grandes quantidades de biomassa geralmente sem utilização posterior. Neste contexto, a incorporação de matéria orgânica e nutrientes de efluentes agroindustriais em células microbianas visando a produção de proteínas unicelulares corresponderia a um processo sustentável. Nesse sentido, as cianobactérias poderiam ser aplicadas devido ao seu metabolismo versátil. Sendo assim, o trabalho teve como objetivo avaliar o cultivo heterotrófico da cianobactéria *Aphanothece microscopica Nägeli* em meios contendo glicose, lactose e sacarose. As curvas de crescimento indicaram que o cultivo heterotrófico depende do tipo de fonte de carbono, sugerindo diferentes mecanismos de incorporação e consumo da glicose, lactose sacarose. Os resultados indicam uma possível aplicação desta cianobactéria na remoção destas moléculas orgânicas em águas residuárias.

PALAVRAS-CHAVE: Cianobactéria. *Aphanothece*. Tratamento de águas residuárias.

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