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Treatment of rice parboiling wastewater by cyanobacterium *Aphanothece microscopica* Nägeli with potential for biomass products

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ABSTRACT

Cyanobacterium *Aphanothece microscopica* Nägeli has been used in research for the removal of nitrogen and organic matter in order to associate single-cell protein production with wastewater treatment. These micro-organisms use photosynthesis as the main metabolic way, although some strains are able to grow in absence of light in heterotrophic cultures. Therefore, the main purpose of the work was to evaluate the growth kinetics of unicellular cyanobacterium *A. microscopica* in rice parboilization effluent without light source. Experimental conditions were 100 and 300 mg L^{-1} inoculum concentration at 25 and 35°C. Results showed that biomass production with maximum nitrogen and organic matter removal at 12 h of batch time was 300 mg L^{-1} inoculum at 35°C. Our results demonstrate that *A. microscopica* shows high yield of nitrogen and organic matter removal from rice parboilization effluent, promising of potential for biomass products and wastewater treatment.

Keywords: COD removal; Nitrogen removal; Rice parboilized effluent; Single-cell protein; Heterotrophic cultures

1. Introduction

Cyanobacteria have been used for many years for chemical oxygen demand (COD), nitrogen, and phosphorus removal from industrial and urban wastewater with cells in suspension [1–7] or immobilized [8] .These micro-organisms described as prokaryotes are able to perform photosynthesis with oxygen production, and found practically in all terrestrial niches with minimal availability of water, nutrients, and light intensity [9,10]. However, several studies mention that microalgae such as *Scenedesmus* sp., *Chlorella* sp., *Phormiduum* sp., and *Aphanothece* sp. can assimilate organic compounds under light period conditions, and are able to grow in heterotrophic metabolism in the dark using simple molecules such as acetate, glucose, and organic acids [4,11–16]. According to Perez-Garcia et al. [15], the heterotrophic growth approach eliminates the major deficiencies of illuminated autotrophic microalgae production, allowing the use of practically any bioreactor

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with a significant reduction in costs for most processes. Moreover, under some heterotrophic growth conditions, the microalgal biomass yields are consistent and reproducible, reaching cells densities of 50–100 g of dry biomass per liter, higher than densities obtained in photoautotrophic cultures.

Aphanothece microscopica Nägeli is a cyanobacterium that has been studied for wastewater treatment and valorization of residues, since these micro-organisms show digestibility and net protein utilization that can qualify them for single-cell protein production [17]. Single-cell proteins are obtained from micro-organism's cultures, such as yeast, algae, bacteria, and cyanobacteria [10,18]. On the other hand, biological wastewater treatment by cyanobacteria could be proposed, motivated by the heterotrophic metabolism of this micro-organism, with the consumption of organic substrate and inorganic nutrients in the dark. However, heterotrophic wastewater treatment by cyanobacteria is an approach that has be studied in laboratory scale regarding its potential application for the treatment of effluents. Queiroz et al. [4] evaluated the kinetics of the removal of nitrogen and organic matter from parboiled rice effluent by the cyanobacterium *A. microscopica* on 4.5 L cylindrical batch bioreactor operated at 30˚C in the absence of light, with a C/N ratio of 50 and N/P ratio of 1.98. Results indicated that the greatest removals occurred at 15 h, period corresponding to the exponential growth phase of the micro-organism, corresponding 83.44 and 72.74% for COD and N-TKN, respectively. Jacob-Lopes et al. [19] evaluated the protein fraction of *A. microscopica* cultivated in parboiled rice effluent submitted to different drying conditions. The electrophoresis profile of proteins from biomass presented bands with molecular weight between 62.5–15 kDa and amino acid profile was higher than the concentrations recommended by Food and Agriculture Organization, with the exception of lysine, methionine, and cysteine. Biochemical profile of cyanobacterium *A. microscopica* cultivated in parboiled rice effluent and submitted to different drying conditions were studied with the objective of evaluating its potential as a source of metabolites of commercial interest [18,19]. Parboiled rice effluent presents a range of nitrogen (25.40–5.04 mg L^{-1}) and COD (2,578–5,022 mg L^{-1}) leading to the average C/N ratio of 73.84, higher than that usually required for the development of the micro-organisms [4]. Thus, this effluent can be considered as a potential source of nitrogen and organic matter for the production of single-cell protein by this cyanobacterium. The drying conditions (tray dryer with parallel airflow 1.5 m s^{-1} at 40, 50, and 60˚C and tray thicknesses of 5 and 7 mm) were shown to significantly affect the macronutrient composition of the biomass with respect to protein, carbohydrate and lipid contents. Lipid profile showed a predominance of polyunsaturated fatty acids, especially gamma linolenic acid, but the drying conditions did not influence the polyunsaturated/saturated ratio of the biomass.

In many cases, cyanobacteria can use industrial residues as nutrients source substrate for energy [2,5,20–24]. Lincoln et al. [1] studied the growth of *Arthrospira platensis* on dairy wastewater of laboratory- and pilot-scale cultures. The results demonstrated rapid ammonia uptake and cyanobacterium growth, enhancing the prospects for the production of protein feed from dairy effluent. Also Dumas et al. [13] suggest that seems promising the biotreatment of fish farm effluent by cyanobacterium *Phormidium bohneri*.

Rice parboilization effluent shows characteristics with regard to its nitrogen and organic matter composition, proposing a biological treatment with incorporation of nutrients into a biomass [25]. Removal of carbon and nitrogen from industrial wastewater is usually accomplished using two different processes: anaerobic digestion followed by nitrification and denitrification, involving at least three reactors. In contrast, there are studies that suggest simultaneous removal of these compounds [4,17,26,27].

Temperature has a great influence in microbial metabolism, affecting the oxidation rates of carbonaceous and nitrogenous matter [4,7,28]. Martínez et al. [29] studied microalgae *Scenedesmus obliquus* cultivated in urban effluents, previously submitted to secondary sewage treatment on different conditions of temperature and stirring. The authors reported greater specific growth rates (μ_{max}) in stirred cultures, with the highest value of 0.0438 h^{-1} at 30˚C with increased biomass productivity in the linear growth phase after exponential growth, with the optimum appearing at 25˚C. Moreover, the highest maximum phosphorus removal was 98% at 94 h and ammonium depletion at 188 h in stirred cultures at 25˚C. The study showed higher growth rates at 30˚C. On the other hand, Renaud et al. [30] verified that *Nitzschia closterium* do not grow in temperatures higher than 30˚C or lower than 20˚C. Vasconcelos and Pereira [31] reported that due to high temperatures and luminosity, phytoplankton communities from tertiary lakes frequently show a high cyanobacterium concentration. The behavior of biomass concentration during cultivation is related to specific growth rate and inoculum concentration, which normally depend on initial concentration of limiting subtract, maximum growth rate, and a specific constant of each subtract [4,9,16].

In this context, the hypothesis of this study is that *A. microscopica* cyanobacterium is able to grow in

parboiled rice effluent in the range of 25–35˚C and starting 100 and 300 mg L^{-1} inocula, whereas temperature and inoculum are fundamental in the study of the scale-up process.

In this research, we investigated the growth kinetics of *A. microscopica* concerning temperature and inoculum concentration for single-cell protein production and extended findings about heterotrophic cultivation of this cyanobacterium in agro-industrial wastewater.

2. Materials and methods

2.1. Inoculum

Stock suspensions of cyanobacterium *A. microscopica* were maintained in complete BG11 medium (Braun–Grunow Medium), according to Rippka et al. [32], for at least 48 h with a light period of 12 h with photon flux density of 30 μmol ($m^{-2}s^{-1}$) and 30˚C. Inoculum in log phase was centrifuged at 3,000 × *g* for 15 min and cell concentration was measured by dry weight, through filtration of a given volume of culture medium in a 0.45 μm filter and drying at 105˚C for 24 h, as previously described [33].

2.2. Experimental reactor and sampling

A stirred batch reactor-type column bubbles with 10 cm internal diameter and 100 cm height, without light source (heterotrophic condition) was filled with 4 L of effluent, which was collected directly from the output of rice parboilization tanks. Effluent samples were characterized by pH, COD, and Total Kjeldahl Nitrogen (KTN), following methodology proposed by APHA [33], and the initial pH was adjusted to 7.6 for cyanobacterial cultivation [24]. Cyanobacteria were cultivated without light source in isothermical batch reactor at 25 and 35˚C, with initial inoculum concentrations of 100 and 300 mg L^{-1} and constant air flow of 1VVM (volume of air per volume of effluent per minute) during all experimental periods. Biomass concentration was determined every 3 h as the dry cell mass remaining after 24 h at 105˚C, following filtration with a 0.45 μm membrane filter [33]. All experiments were performed in duplicate

2.3. Kinetic analysis

Growth curves were plotted in experimental conditions and the kinetic constants were calculated according to Ahmad and Holland [34]. Maximum specific growth rates were calculated by linear regression of the semi log curve biomass concentration vs. time and by calculating of the slope for each point [12].

2.4. Wastewater parameters

Nitrogen and organic matter removal efficiency by cyanobacteria was calculated by initial and final conditions. Protein content in cyanobacterial biomass was calculated by multiplication of KTN obtained of filter with biomass recovered by 6.25 factor, as previously described by Voltolina et al. [35].

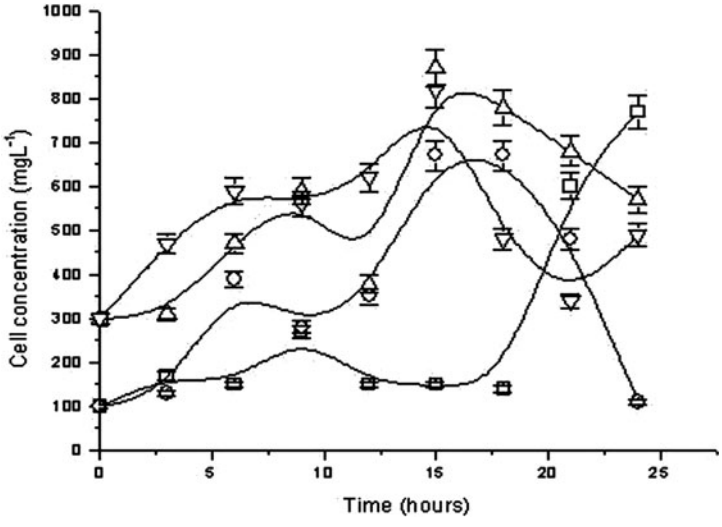

Fig. 1. Growth curves for AmN at different conditions of inoculum concentration and temperature, respectively: 100 mg L^{-1} – 25˚C (□); 100 mg L^{-1} – 35˚C (○); 300 mg L^{-1} – 25˚C (△); 300 mg L^{-1} – 35˚C (▽).

3. Results and discussion

Maximum cell concentrations were observed before 24 h of cultivation, independent of the considered experimental condition (approximately 800 mg L^{-1}). There was no lag phase when the inoculum was provided at 300 mg L^{-1}, comparing the curves, which can be explained by the use of a high concentrated inoculum that reached log phase in shorter period of time [31,36]. Fig. 1 shows the growth curves for AmN in experimental conditions at 25 and 35 ℃.

These results agree with Queiroz et al. [4], which found maximum biomass concentration before 48 h at 30 ℃ and 12 h of light period using 300 mg L^{-1} of inoculum. According to Lincoln et al. [1], the extent of biotreatment by algae and cyanobacterium is proportional to the biomass amount. These authors found 900 mg L^{-1} of cyanobacterium *A. platensis* in dairy effluent, with specific growth rates lower than our results in rice wastewater.

Results indicate that *A. microscopica* could be used for the treatment of this effluent in stirred bioreactors with lower periods of cell detention, shorter than periods applied for conventional systems of active sludge [36].

A low value of duplication time was observed in the experimental conditions, which suggests the predominance of respiratory metabolism. Table 1 shows the specific growth rates (μ_{max}) and duplication time (t_d). Furthermore, it should be noted that there were no significant differences at the 5% level of significance between the maximum specific growth rates. These results are corroborated by Queiroz et al. [4] that found low duplication times for *A. microscopica* cultivated on rice parboilization effluent in absence of light in comparison with cyanobacteria cultivated in presence of light. Maximum growth rates were higher than the ones observed by other authors for species of *S. obliquus* and *Chlorella vulgaris* cultivated on synthetic effluents and mediums [12,29]. On other hand, values are slightly lower than those obtained by Sansawa and Endo [37] with *Chlorella regularis* in heterotrophic cultures for the production of intracellular phytochemicals.

Considering the influence of temperature, the growth activation energy (Ea) for cyanobacteria in parboilized rice effluent was estimated and it fitted the Arrhenius Model [38]. The activation energy was 17.36 ± 0.52 kJ mol^{-1} K^{-1} for the temperature range of 25 and 35 ℃, value lower than obtained to Xin et al. [37] with *Scenedesmus* sp. LX1 grown in BG11 medium.

Table 1
Growth rates and duplication time for experimental conditions

	μ_{max} (h^{-1}) *	R^2	t_d (h)
1	0.085a	0.9682	8.14
2	0.053a	0.9147	13.06
3	0.106a	0.8543	6.56
4	0.067a	0.9166	10.34

Notes: Experiments: (1) 25 ℃, 100 mg L^{-1}; (2) 25 ℃, 300 mg L^{-1}; (3) 35 ℃, 100 mg L^{-1}; (4) 35 ℃, 300 mg L^{-1}.
μ_{max}: maximum specific growth rate; R: coefficient of determination; and t_d: duplication time.
*Same letters indicate no significant difference at the 5% significance.

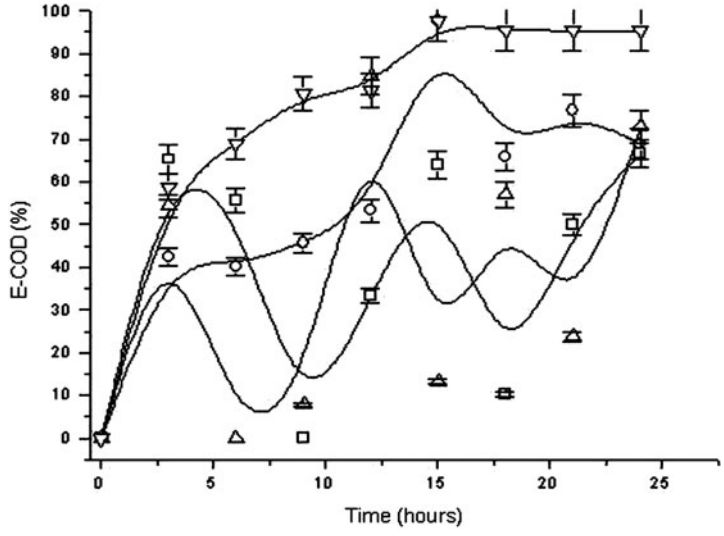

Fig. 2. COD removal from rice parboilization effluent by *A. microscopica* at different conditions of inoculum and temperature, respectively: 100 mg L^{-1} – 25 ℃ (□); 100 mg L^{-1} – 35 ℃ (○); 300 mg L^{-1} – 25 ℃ (△); 300 mg L^{-1} – 35 ℃ (▽).

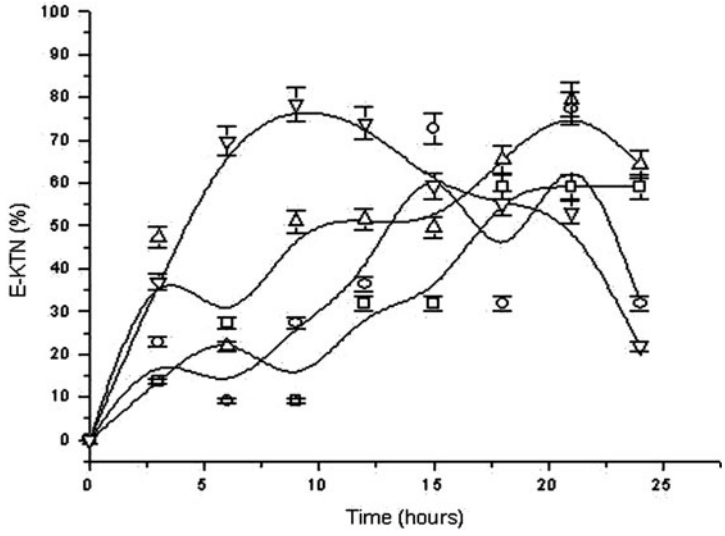

Fig. 3. KTN removal from rice parboilization effluent by *A. microscopica* at different conditions of inoculum and temperature, respectively: 100 mg L^{-1} – 25°C (□); 100 mg L^{-1} – 35°C (○); 300 mg L^{-1} – 25°C (△); 300 mg L^{-1} – 35°C (▽).

The present results showed that specific growth rates were higher than the growth rates of cyanobacterium *P. bohneri* cultivated on fish farm effluents [13] and also higher than those observed by AmN in fish processing wastewater [39]. Thus, the results demonstrated that *A. microscopica* can be used for the production of single-cell protein on rice parboilization effluent.

The effluent used in experiments presents pH, COD, and KTN of 4.55 ± 0.5, 4,117 ± 1,372, and 71 ± 20.3 mg L^{-1}, respectively. After 24 h of cultivation, maximum values for COD and KTN removal efficiency by cyanobacterium were 97 and 78%, respectively, observed by 300 mg L^{-1} inoculum at 35°C (Figs. 2 and 3). These results were higher than maximum values found by Queiroz et al. [4] with *A. microscopica* cultivated at 20°C on rice effluent with similar physical-chemistry characteristics of this study. These results indicated the importance of biomass concentration and temperature on removal of COD and KTN from wastewaters by cyanobacteria.

Fig. 4 shows the protein biomass profile produced at log phase on optimal conditions of temperature and inoculum concentration, with an average value of 35.5 ± 2%. It is noteworthy that at 12 h the protein content was 37.7%, similar to values observed by Queiroz et al. [39] for *A. microscopica* collected directly from the environment. This value is equivalent to the average values observed for microalgae ***S. obliquus*** used for urban wastewater treatments [3], and also similar to average values observed by Markou et al. [40] in cultures of *A. platensis* in olive-oil mill wastewater. However, there is still scarce research concerning the protein content of cyanobacterial biomass cultivated on wastewaters.

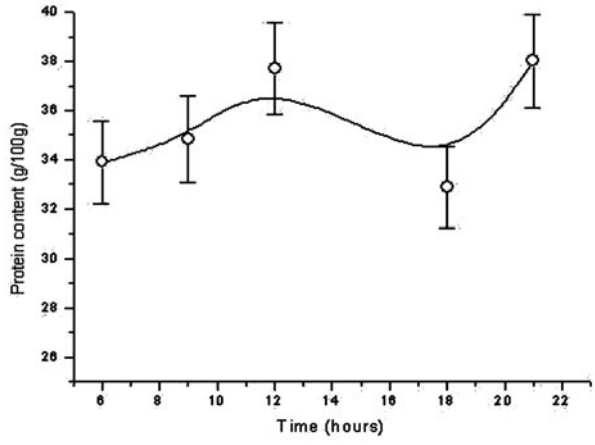

Fig. 4. Biomass protein content of AmN at log phase for optimum condition (300 mg L^{-1} inoculum at 35°C).

These results demonstrate that *A. microscopica* shows great efficiency for nitrogen and organic matter removal from rice parboilization effluent. Therefore, this cyanobacterium may be a potential source of single-cell protein.

4. Conclusions

In *A. microscopica* cultures, optimal production for biomass occurred between 9 and 12 h. The highest

nitrogen and organic matter removal occurred at 12 h with 300 mg L^{-1} inoculum at 35°C and protein-biomass content of 37.7%. Thus, biomass products by AmN could be associated with COD and TNK removal from rice parboilization effluent.

Notation

μ	—	specific growth rate (h^{-1})
μ_{max}	—	maximum specific growth rate (h^{-1})
COD	—	chemical oxygen demand (mg L^{-1})
E-COD	—	COD removal efficiency (%)
E-KTN	—	NTK removal efficiency (%)
KTN	—	Kjeldahl total nitrogen (mg L^{-1})
R^2	—	coefficient of determination
t_d	—	duplication time (h)
X	—	biomass concentration (mg L^{-1})
X_o	—	biomass concentration at begin log phase (mg L^{-1})

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