ABSTRACT

Cyanobacteria are photosynthetic prokaryotes organisms used on single-cell protein production and wastewater treatment for nitrogen and phosphorus removal. Moreover, some strains have the particular characteristic of growing in the dark on simple molecules such as acetate, glucose and organic acids, and consequently reduction of Chemical Oxygen Demand (COD) from nutrient medium. *Aphanthece microscopica Nägeli* is a cyanobacteria frequently found in south of Brazil, which has been studied with respect to residues valorization in the single-cell protein production. Wastewater from rice parboilization process presents characteristics that suggest the removal of nutrients and organic matter by incorporation into a biomass. The aim of this paper is to extend the findings of the previous studies about heterotrophic metabolism application of these cyanobacteria on wastewater treatment, considering influence of temperature and inoculum amount. Experiments indicated that *Aphanthece microscopica Nägeli* presented high potential of organic matter removal from parboilized rice effluent by 300 mgL$^{-1}$ inoculum at temperature range 25-35°C.

**Keywords:** Cyanobacteria, Aphanthece, Organic matter removal, Parboilized rice effluent.
RESUMO

Cianobactérias são microrganismos fotosintetizantes usados na produção de proteínas unicelulares e na remoção de nitrogênio e fósforo de águas residuárias. Algumas linhagens apresentam a característica particular de crescerem no escuro a partir de moléculas orgânicas simples como acetato, glicose e ácidos orgânicos, com consequente redução da DQO. Aphanothece microscopica Nägeli é uma cianobactéria de grande ocorrência no sul do Brasil, tendo sido estudada quanto a valoração de resíduos agroindustriais e na produção de proteínas unicelulares. O efluente da parboilização do arroz apresenta características que sugerem a remoção de nutrientes e matéria orgânica por incorporação em biomassa microbiana. Assim, o objetivo deste trabalho é prosseguir os estudos a respeito da aplicação do metabolismo heterotrófico desta cianobactéria no tratamento de águas residuárias, considerando a influência da temperatura e inoculo. Os resultados indicaram alto potencial de remoção de DQO do efluente da parboilização do arroz com 300mg/L de inoculo na faixa de 25 a 35ºC.

Palavras-chaves: Cianobactéria, Aphanothece, Remoção de matéria orgânica, Efluente da parboilização do arroz.

INTRODUCTION

The use of cyanobacteria cultures in wastewater treatment is suggested since many years for nitrogen and phosphorus removal from industrial and urban effluents (ARDELEAN & ZARNEA, 1998; ASLAN & KAPDAN, 2006; LINCOLN et al. 1996; NUÑEZ et al. 2001; QUEIROZ et al. 2007; TAM & WONG, 1996). Microalgae are prokaryotic, as green algae (Chlorophyta), or eukaryotic photosynthetic microorganisms, as cyanobacteria (Cyanophyceae) (MATA et al. 2010). Cyanobacteria or blue-green algae are distinguished from other prokaryotes by their generally low rates of endogenous respiration and by their limited ability to utilize organic substances as a source of carbon and energy (FAY, 1983). These microorganisms were always regarded to be typical photosynthetic in which the light-dependent fixation of CO₂ is the dominant mode of nutrition. This concept, however, was obscured by observation that in nature blue-greens are most abundant in habitats rich in organic matter. Thus, some strains of cyanobacterial are capable of using simple organic molecules (glucose and organic acid) as a source of carbon and energy for continuous slow growth in dark. Disaccharides could be converted readily to the component sugars in the heterocysts, degraded along the glycolytic pathway, and oxidized through the hexose monophosphate shunt (oxidative pentose phosphate pathway), providing energy and reductant for N₂ fixation. There is a great deal of evidence that the catabolic pathways remain operational after heterocyst differentiation (FAY, 1992). This heterotrophic potential could be used in biotreatment of agro-industrial wastewaters for simultaneous organic matter and nitrogen removal. BICH et al. (1999) indicated the possibility of using microalgae in a treatment system for the nitrogen removal from an agro-industrial wastewater with Chemical Oxygen Demand (COD) about 500 mg/L. BASHAN & BASHAN (2004) reported the application of Chlorella vulgaris in the biological treatment of recalcitrant anaerobic industrial effluent, mainly for phosphorus removal. Aphanothece microscopica Nägeli is a cyanobacterium that has been studied with respect to residues valorization in the single-cell protein production, due to high specific growth rate (0.24h⁻¹) on wastewater (QUEIROZ et al. 2002). QUEIROZ et al. (2007) evaluated nitrogen and organic matter consumption from parboiled rice effluent by with 100mg/L of Aphanothece inoculum and 20°C on stirred-batch reactor. Results indicated that maximum efficiency COD removal occurred at 15 hours of cultivation, approximately 83.4%, suggesting the consumption of organic matter components on dark.

These data motivated new researches to extend the findings of the previous studies considering influence of temperature and inoculum amount, according to improve the wastewater treatment by cyanobacterial.
MATERIAL AND METHODS

Wastewater characterization

Some samples of effluent were collected directly from tanks of rice parboilization processing at six different days. Wastewater was characterized through pH, Kjeldahl Total Nitrogen (KTN), Chemical Oxygen Demand (COD) and Total Volatile Acids (TVA), according to APHA, (1998). Total Sugars (TS) were obtained for Felhing Method (AOAC, 1995). Data were analyzing by variation coefficient in percentage, i.e., average per standard deviation.

Experimental reactor and sampling

A stirred-batch reactor was filled with 4L of the effluent with 100 and 300mg/L of cyanobacterial concentration. Initial pH of effluent must be adjusted to 7.6 for Aphanothece growth (QUEIROZ & KOETZ, 1997). The cyanobacteria were cultivated without light source on 25 and 35°C, with constant aeration of 1VVM during all experimental period. Samples for cell concentration and wastewater assay were collected every 3 hour during 24 hours.

Inoculum

Stock suspensions of cyanobacterium Aphanothece microscopica Nägeli were maintained in complete BGN medium (Braun–Grunow Medium), according to RIPPKA et al. (1979), for at least 48 hours, light period of 12 hours at 30°C (QUEIROZ et al. 2002). Log phase inoculum was centrifuged at 3000xg for 15 minutes and cell concentration was measured by gravimetric method, through filtration of a given volume of culture medium in a 0.45 µm filter and drying at 105°C for 24 hours (APHA 1998).

Organic matter removal

Removal of matter organic was evaluated through soluble COD consumption, express in percentage (CONTRERAS et al. 2000). COD consumption rates were calculated through slope of COD profile by differential approach (LEVENSPIEL, 2000). Average specific rates were calculated by ratio of COD consumption rate and biomass concentration during the experiment, in terms of COD per cyanobacterial concentration per time (CONTRERAS et al. 2000).

RESULTS AND DISCUSSION

Table 1 presents characterization of wastewater from rice parboilization processing. All parameters showed the high variation coefficient (%), except pH (10.7%), due mainly to variations on the process and type of rice grains. Nowadays, rice is one of the more important cereals in the world, being the basic constituent of the Brazilian diet.

Brazil is among the 10 largest rice producers, and parboiling one of the most important improvement processes. As this process involves significant water amount (average 4m³ per ton of rice processed grain), resulting in a considerable wastewater amount, with characteristics that suggest the possibility of biotreatment for the incorporation of these nutrients into the biomass (QUEIROZ & KOETZ, 1997).

TABLE 1 - Characterization of wastewater from rice parboilation processing
(units in mg/L, except pH)

<table>
<thead>
<tr>
<th>pH</th>
<th>COD</th>
<th>KTN</th>
<th>TVA</th>
<th>TS</th>
</tr>
</thead>
<tbody>
<tr>
<td>4,5</td>
<td>2821</td>
<td>45</td>
<td>924</td>
<td>919</td>
</tr>
<tr>
<td>4,2</td>
<td>2578</td>
<td>49</td>
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<td>722</td>
</tr>
<tr>
<td>4,5</td>
<td>3286</td>
<td>93</td>
<td>624</td>
<td>1154</td>
</tr>
<tr>
<td>4,3</td>
<td>4514</td>
<td>88</td>
<td>1275</td>
<td>2085</td>
</tr>
<tr>
<td>4,3</td>
<td>6480</td>
<td>79</td>
<td>780</td>
<td>2554</td>
</tr>
<tr>
<td>5,5</td>
<td>5022</td>
<td>70</td>
<td>1290</td>
<td>2032</td>
</tr>
<tr>
<td>Average</td>
<td>4,6</td>
<td>4177</td>
<td>71</td>
<td>978</td>
</tr>
<tr>
<td>Variation (%)</td>
<td>10,7</td>
<td>33,3</td>
<td>28,6</td>
<td>24,8</td>
</tr>
</tbody>
</table>

COD: chemical demand; KTN: Kjeldahl total nitrogen; TVA: total volatile acid; TS: total sugar
COD average for wastewaters samples was 4117 mg/L, with 33.3% of variation, occurred due to variations on maceration time, temperature and type of grain (QUEIROZ & KOETZ, 1997).

According to CONTRERAS et al. (2000), the effluents generated for food industries present variable discharges concentrations of COD, solids and nitrogen on different forms. Volatile acids and total sugars are represented 62% of COD, calculated with parameters express in Table. 1. It is very important because some strains of cyanobacterial and microalgae could be used as simple molecules as glucose and acids organic or acetate for growth heterotrophic on dark cultivation (FAY, 1983; TAM & WONG, 1996).

Figure 1 presents soluble COD profile in different experimental conditions of inoculum and temperature. It can be observed in high consumption with 300mg/L inoculum for 25 and 35°C, which suggests that the extent of biotreatment of wastewaters are proportional to the amount of microorganism (LINCOLN et al. 1996).

**FIGURE 1 -** COD soluble consumption from rice parboilized effluent by *Aphanotche microscopica Nägeli* at different temperatures 25°C ( ) and 35°C (o) with inoculums 100mg/L (a) and 300mg/L (b)

COD consumption rate and efficiency of removal are express in Table 2. High specific COD consumption rates (0.25mg/mg.h) suggest high organic matter consumption with 300mg/L of inoculum, reflecting the profiles presented in Fig. 1, with practically COD depletion at 15 hours for 25 and 35°C. The results are important for possible application this cyanobacterium on rice wastewater with stirred tank in the temperature range, typical of subtropical climate. Specific rates found in this paper was higher than obtained by QUEIROZ et al. (2007) working *Aphanothece* in rice parboiled rice effluent at 20°C and inoculum of 100mg/L, about 0.08h^{-1}. Moreover, the rates are similar to obtained by BELTRAN-HEREDIA et al. (2000) in your study of oxidative degradation of the organic matter present in the washing waters from the black table olive industry by activated sludge, i.e., conventional processes of wastewater treatment. Thus, *Aphanotche microscopica Nägeli* presented heterotrophic metabolism and it could be used in stirred tank-reactors on dark to COD removal in wastewater from rice parboilization processing in the experimental conditions tested on this paper.
TABLE 2 - Comparison of average specific consumption rates and COD removal for inoculum and temperature experimental conditions

<table>
<thead>
<tr>
<th>Temperature [°C]</th>
<th>Inoculum [mg/l]</th>
<th>Specific COD consumption rate [mg COD/mg biomass.h]</th>
<th>Maximum COD removal and time [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>100</td>
<td>-0.046</td>
<td>66.7 at 24 hours</td>
</tr>
<tr>
<td>25</td>
<td>300</td>
<td>-0.250</td>
<td>97.7 at 15 hours</td>
</tr>
<tr>
<td>35</td>
<td>100</td>
<td>-1.160</td>
<td>84.8 at 12 hours</td>
</tr>
<tr>
<td>35</td>
<td>300</td>
<td>-0.250</td>
<td>97.7 at 15 hours</td>
</tr>
</tbody>
</table>

CONCLUSIONS

Based on the experimental data, maximum COD removal by *Aphanotoche microscopica Nägeli* suggest the application of this cyanobacterium on biotreatment of wastewater from rice parboilization processing for inoculus of 300 mgL⁻¹ at temperature range 25-35°C.

ACKNOWLEDGMENTS

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REFERENCES


