

The kinetics of the removal of nitrogen and organic matter from parboiled rice effluent by cyanobacteria in a stirred batch reactor

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Abstract

The aim of this research was to evaluate the kinetics of the removal of nitrogen and organic matter from parboiled rice effluent by the cyanobacteria *Aphanothece microscopica Nägeli*. From the results obtained, it was verified that maximum efficiency for the removal of organic matter expressed as COD and total nitrogen (N-TKN) occurred after 15 h of cultivation, being 83.44% and 72.74, respectively. The scale-up process indicated that the volume of the estimated reactor would be 100.2 m³, containing parboiled rice effluent and biomass.

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1. Introduction

The removal of carbon and nitrogen from industrial wastewater is usually accomplished using two different processes: anaerobic digestion followed by nitrification plus de-nitrification, involving at least three reactors. However, some studies have proposed the simultaneous removal of these components, since the separation of the carbon and nitrogen removal stages increases the costs of both the treatment and its technological control. In addition, the application of the conventional processes results in the waste of the nitrogen contained in the wastewater, not allowing for a possible valorization of this nutrient (Ortiz et al., 1997; Xing et al., 2000). Alternative technology, complementing the two operations and including nitrogen recovery from the wastewater should be examined. In this sense, cyanobacteria have been used in wastewater treatment, due to their great efficiency in the removal

of organic matter and inorganic nutrients, as well as the possibility of the valorization of the residues by incorporating the nutrients into biomass (Martínez et al., 2000).

Cyanobacteria are prokaryotic microorganisms that, like higher plants, carry out photosynthesis with the production of oxygen. Some strains have the ability for nitrogen fixation and to use organic compounds in heterotrophic metabolism. This particular metabolism provides the organisms with their simpler nutritional requirements (Fay, 1983).

Biological wastewater treatment by cyanobacteria was proposed, motivated by the heterotrophic metabolism of this microorganism, with the consumption of simple organic molecules and inorganic nutrients in the dark (Ardelean and Zarnea, 1998; Tam and Wong, 2000). Several authors have reported that the only objective of the respiration of cyanobacteria is to generate minimal energy for growth in the dark (Fay, 1983; Schmetterer, 1994; Anand, 1998). Hence, the bio-depuration of effluents using cyanobacteria has been studied in several countries such as the USA, Israel, Italy and Singapore, with subsequent use

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of the biomass for the production of single-cell protein (SCP) (Tam and Wong, 2000). Bich et al. (1999) indicated the possibility of using microalgae in a treatment system for the removal of nitrogen from an agro-industrial wastewater with about 500 mg COD L⁻¹. Bashan and Bashan (2004) reported the application of *Chlorella vulgaris* in the biological treatment of recalcitrant anaerobic industrial effluent. The microalgae reduced the ammonium ions (71.6%), phosphorus (28%) and COD (61%).

Aphanothece microscopica Nageli is a cyanobacterium that has been studied with a view to the valorization of agro-industrial residues in the production of SCP and treatment of parboiled rice effluent (Queiroz et al., 2001, 2003, 2002, 2004).

Currently 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. This process involves significant water consumption, on average equal to 4 m³/ton of processed grain, resulting in a considerable volume of effluent. The characteristics of this effluent with respect to its nitrogen and organic matter compositions, suggest the possibility of biological treatment for the incorporation of these nutrients into the cyanobacteria biomass (Queiroz and Koetz, 1998).

A study of the growth kinetics to be applied in wastewater treatment is an important parameter in process scale-up. The growth rates can be obtained numerically by adjustment of the cell concentration to the polynomial function according to the rate equation, and the kinetic data can be assessed by the differential and integral methods (Ahmad and Holland, 1995; Levenspiel, 2000). The progress of the reaction can be assured as a function of the time from the concentration of a given component (Bartholomew and Hecker, 1994; Roustrup-Nielsen, 2000). Studies accomplished in small-scale systems contribute significantly to the scale-up of processes (Katzer et al., 2001). Thus, the aim of this work was to evaluate the kinetics of the removal of nitrogen and organic matter from parboiled rice effluent by the cyanobacteria *A. microscopica Nageli*, as well as a scale-up study.

2. Methods

2.1. Inoculum

A. microscopica Nageli culture stocks were prepared and maintained in standard BGN medium (Rippka et al., 1979), with the following composition: K₂HPO₄ · 3H₂O (0.040 g L⁻¹), MgSO₄ · 7H₂O (0.075 g L⁻¹), EDTA (0.001 g L⁻¹), H₃BO₃ (2.860 g L⁻¹), MnCl₂ · 4H₂O (1.810 g L⁻¹), ZnSO₄ · 7H₂O (0.222 g L⁻¹), Na₂MoO₄ · 2H₂O (0.390 g L⁻¹), CuSO₄ · 5H₂O (0.079 g L⁻¹), CaCl₂ · 6H₂O (0.040 g L⁻¹), C₆H₈O₇ · H₂O (0.006 g L⁻¹), ferric Citrate and ammonium (0.006 g L⁻¹), pH 7.6. The conditions used were 30 °C, 2 Klux of light and constant agitation in the growth chamber for a 12 h dark/light photoperiod.

2.2. Characterization of the effluent

The effluent used was characterized according to the N-TKN, soluble COD, P-PO₄⁻³ and pH from eight samples collected on eight different days from the exit of the maceration tanks of a rice industry, according to Apha (1998). The carbon/nitrogen (C/N) and nitrogen/phosphorous (N/P) ratios were calculated from the COD, N-TKN and P-PO₄⁻³, and adjusted when necessary with sucrose, potassium dihydrogen phosphate and ammonium sulfate.

2.3. Obtaining of the kinetic data in an experimental bioreactor

The experiments were set up in a 4.5 L cylindrical batch bioreactor with an internal diameter of 10 cm and height of 100 cm. It was operated at 30 °C in the absence of light, with a C/N ratio of 50, N/P ratio of 1.98, pH adjusted to 8.0, constant aeration of 1 VVM and permitting saturation with oxygen. An inoculum of 100 mg L⁻¹ of *A. microscopica Nageli* in the exponential growth phase was added.

Total nitrogen (N-TKN) and organic matter expressed as the COD, were monitored for 24 h with sample collection every 3 h. The kinetic variables of specific growth rate, generation time and substrate yield coefficient were assessed as proposed by Ahmad and Holland (1995). The evaluation of the potential removal of nitrogen and organic matter was evaluated during the experiments. A differential method of kinetic data analysis was used to evaluate the substrate consumption kinetics according to Levenspiel (2000).

$$\int_{t_0}^t \mu dt = \int_{X_0}^X \frac{dX}{X} \quad (1)$$

where μ is the specific growth rate, dX is the variation in cell concentration and dt is the cultivation time

$$\text{tg} = \frac{\ln 2}{\mu_{\max}} \quad (2)$$

where tg is the generation time and μ_{\max} is the maximum specific growth rate

$$Y_{X/S} = - \frac{dX}{dS} \quad (3)$$

where $Y_{X/S}$ is the substrate yield coefficient and dS is the variation in substrate concentration

$$\frac{-dS}{dt} = q_s X \quad (4)$$

where q_s is the specific rate of substrate consumption

$$\log \left(\frac{dS}{dt} \right) = \log k + n \log S \quad (5)$$

where $\log(dS/dt)$ is the decimal logarithm of the variation in substrate, $\log_{10} k$ the logarithm of the reaction rate constant, n the reaction order and $\log S$ is the logarithm of the substrate concentration.

2.4. Estimation of the continuous processes

The volume of the continuous reactor (CSTR) was estimated from the mass balance of the COD, assuming constant volume, fluid density and flow-rate and steady state conditions (Eq. (6)).

$$V = \frac{F}{q_s} \left[\left(\frac{S}{S_0} \right) - 1 \right] \quad (6)$$

where V is the fluid volume, F is the fluid flow-rate, q_s is the specific rate substrate consumption, S is the final substrate concentration and S_0 is the initial substrate concentration.

3. Results and discussion

The parboiled rice effluent obtained directly from the maceration tanks was characterized according to the parameters N-TKN, COD, P- PO_4^{-3} , pH, C/N ratio and N/P ratio as shown in Table 1.

From the data analysis it was possible to verify the variability of the parameters that could be expressed by high coefficients of variation. The pH results indicated the need for a neutralization step in the process, since according to Valiente and Leganes (1989), cyanobacteria have a high optimum pH, between 7 and 10. The average pH registered (4.60) was similar to data found by Queiroz and Koetz (1998) (4.59) when monitoring the rice parboiling process in an industry for a period of 13 months.

The observed variations in COD and N-TKN can be attributed to variations in the rice parboiling process. Subramanian and Daksinamurthy (1977) reported that the length and temperature of the rice soaking operation allowed for different levels of protein and soluble carbohydrate losses to the wastewater, reflected in compositional variations of the effluent. This verification has consequences in the maximum and minimum results of total nitrogen (25.40–5.04 mg L⁻¹) and COD (2578–5022 mg L⁻¹). The average C/N ratio was 73.84, higher

than that required for development of the microorganisms (C/N = 20). So this effluent can be considered as a potential source of nitrogen and organic matter for the production of single-cell protein. Xing et al. (2000) found that variations in the C/N ratio in cultivation medium resulted in different rates of incorporation of the carbon and nitrogen in the cells. Thus Queiroz et al. (2003), assessing the influence of the C/N and N/P ratios on nutrient removal from rice parboiling effluent, verified that the highest removal of nitrogen and COD took place with a C/N ratio of 50 and N/P ratio of 1.98. So these parameters must be adjusted to maintain the efficiency of the process, using standardized samples with constant C/N and N/P ratios.

Fig. 1 describes the growth curve of *A. microscopica Nögeli* in the parboiled rice effluent, the results being obtained in two trials. Guerrero et al. (1999) reported that the absence of a lag phase is a characteristic of cyanobacteria growth curves when inoculated into food industry effluents, due to the high availability of carbonated compounds and nutrients such as organic acids and nitrogen. This fact was already verified by Queiroz et al. (2001), using an inoculum greater than 100 mg L⁻¹. In addition, Ahmad and Holland (1995) mentioned that an increase in inoculum resulted in a reduction in the lag phase.

In the present experiment the lag phase lasted 15 h, with a maximum cell concentration of 550 mg L⁻¹ during this period. These results show the possibility of applying the cyanobacteria *A. microscopica Nögeli* in the treatment of effluents in reactors with low hydraulic detention times, inferior to those applied in conventional activated sludge systems (Von Sperling, 1997).

Fig. 2 shows that the pH of the culture medium increased significantly throughout the cultivation period. Przytocka-Jusiak et al. (1977) reported that the heterotrophic growth of the cyanobacteria was usually accompanied by a change in pH. An increment change in pH occurred during the periods with high rates of substrate consumption.

Table 1
Characterization of the parboiled rice effluent

COD (mg L ⁻¹)	N-KTN (mg L ⁻¹)	P- PO_4^{-3}	pH	C/N	N/P
4090	75.60	22.01	4.54	56.03	3.43
6480	69.85	57.95	5.51	92.67	1.20
5022	72.10	75.92	4.31	69.75	0.94
4514	88.03	32.28	4.26	51.27	2.72
2821	48.43	52.82	4.51	58.24	0.91
3128	95.04	93.90	4.48	32.91	1.01
2578	80.14	84.91	4.22	32.16	0.94
5022	25.40	11.75	4.98	197.7	2.16
\bar{X}					
4206.8	69.32	53.94	4.60	73.84	1.66
CV					
31.56	32.44	55.71	9.34	72.73	59.03

\bar{X} , average, CV, coefficient of variation; data obtained from two repetitions.

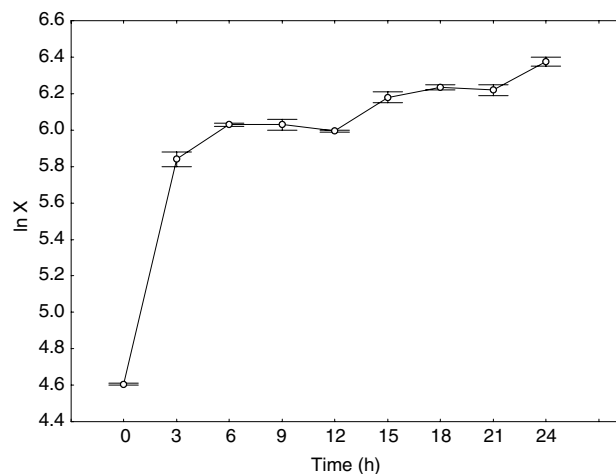


Fig. 1. Semi-logarithmic growth curve for *A. microscopica Nögeli* in the parboiled rice effluent.

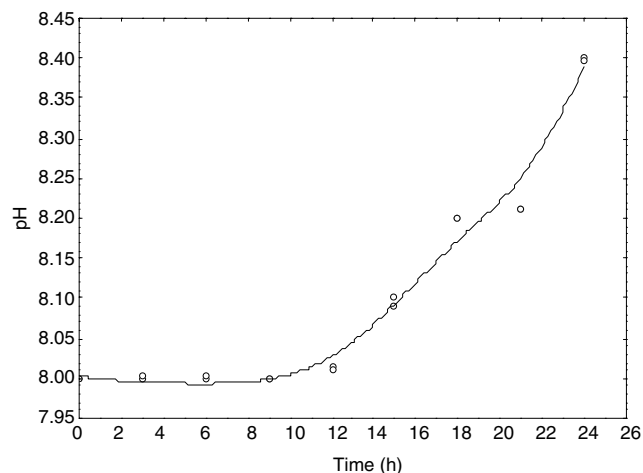


Fig. 2. Changes in the pH values of the parboiled rice effluent during the cultivation of *A. microscopica Nägeli*.

Fig. 3 shows the maximum efficiency of COD removal from parboiled rice effluent. The greatest removal was observed during 15 h of cultivation, a period corresponding to the log phase of growth, a removal efficiency of 83.44% being obtained. The high efficiency of COD removal can be explained by the ability of some cyanobacteria species to grow with a heterotrophic metabolism, assimilating organic compounds immediately when transferred to the dark (Fay, 1983; Ardelean and Zarnea, 1998; Tam and Wong, 2000; Queiroz et al., 2001). Thus, in this way the cyanobacteria *A. microscopica Nägeli* can be used in reactors with no light source for the removal of COD from parboiled rice effluent.

Fig. 4 presents the maximum efficiency for the removal of total nitrogen (N-TKN), during the experimental period. In the same way as for organic matter, maximum removal occurred in hydraulic detention times of 15 h, corresponding to the log phase, with a maximum efficiency of removal of 72.74%. These results are superior to those

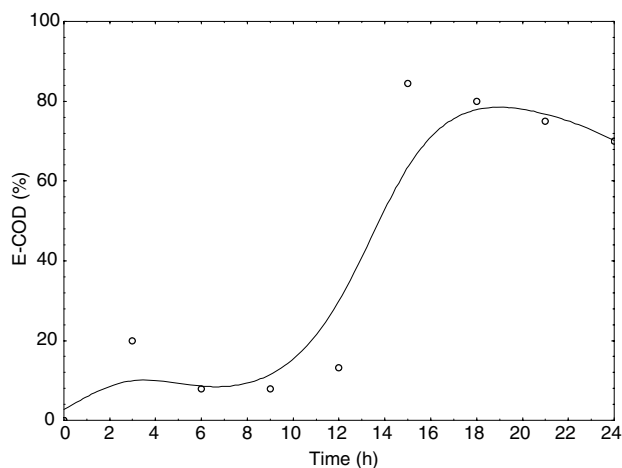


Fig. 3. Efficiencies of COD reduction by *A. microscopica Nägeli* in the parboiled rice effluent.

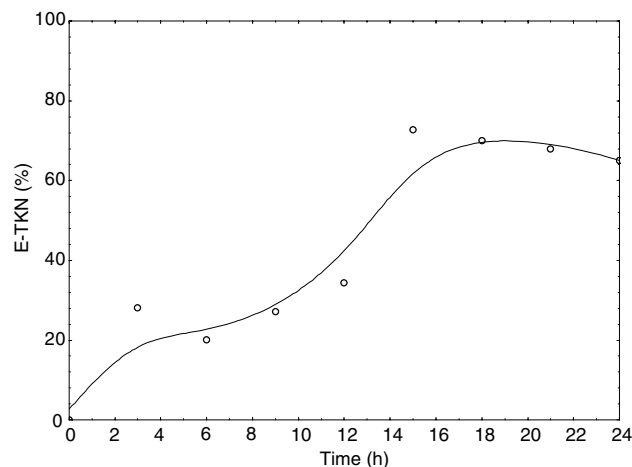


Fig. 4. Efficiency of N-TKN removal by *A. microscopica Nägeli* from the parboiled rice effluent.

obtained by Martínez et al. (2000) who verified a maximum removal of N-TKN in 188 h for *Scenedesmus obliquus* under conditions with light and a temperature of 20 °C. However, nitrogen removal by a microalgal culture in wastewater cannot be exclusively attributed to nitrogen conversion. Other mechanisms capable of eliminating nitrogen in intensively aerated microalgal systems are non-biological, such as air stripping, ammonia volatilization, absorption and sedimentation (Talbot and De La Noue, 1993; Bich et al., 1999).

The values for the kinetic variables of *A. microscopica Nägeli* cultivated in parboiled rice effluent are shown in Table 2. The substrate yield coefficient for the exponential phase of growth was 0.17 mg cel mg COD⁻¹. This value was inferior to those found for heterotrophic bacteria in activated sludge systems. This information suggests that *A. microscopica Nägeli* removes a great amount of organic matter with a low conversion to biomass. On the other hand, the substrate yield coefficient (nitrogen) for the exponential phase of growth $Y_{X/S}$ of 11.40 mg cel mg N-TKN⁻¹, was higher than that found in conventional nitrogen removal systems, where the growth of microorganisms was slow (Von Sperling, 1997).

Table 2
Growth kinetics under the experimental conditions

Kinetic variable	Values
Δt (h)	15
X (mg L ⁻¹)	520
X_0 (mg L ⁻¹)	100
t_g (h)	6.30
μ_{max} (h ⁻¹)	0.11
$Y_{X/S}$ (mg cel mg COD ⁻¹)	0.17
$Y_{X/S}$ (mg cel mg N-TKN ⁻¹)	11.40

Δt , log phase; X final cell concentration; X_0 , initial cell concentration; t_g , generation time; μ_{max} , maximum specific growth rate; $Y_{X/S}$, yield substrate coefficient.

Several authors have shown evidence of a great reduction in COD with low conversion to biomass when the consumption of simple molecules such as acetate, glucose and organic acids occurred in the absence of light (Fay, 1983; Ardelean and Zarnea, 1998; Tam and Wong, 2000). This suggests the existence of a cyanobacterial metabolism capable of assuring slow growth in the dark, explaining the high COD removal, however with low conversion to biomass. Nevertheless, the high nitrogen yield coefficient qualifies this residual water as a source of nitrogen for SCP production. According to Faintuch et al. (1992), one of the main difficulties in SCP production is the cost of the nitrogen-containing compounds. In this sense, the incorporation of the nitrogen contained in the parboiled rice effluent into biomass could contribute to solving related pollution problems and generate an alternative protein source. The maximum specific growth rate registered was of 0.11 h^{-1} , higher than that obtained by other authors for *Scenedesmus* and *Chlorella* when cultivated in effluents and synthetic media (Tam and Wong, 2000; Martínez et al., 2000). The lowest generation time of 6.3 h was registered at the hydraulic detention time (HDT), corresponding to the exponential growth phase of 15 h. This information is fundamental for the efficiency of processes in stirred tank-reactors without the re-circulation of solids (Von Sperling, 1997).

The kinetic constants for COD in the exponential growth phase period of the microorganism, obtained using the differential method of analysis, are shown in Table 3. Levenspiel (2000) reported that zero order reactions only occurred in intervals where the concentration of a certain reagent was high. On the other hand, if the concentration is lowered sufficiently, the reaction becomes dependent on the concentration and the order increases. This can be verified in Table 3, which presents the variation in reaction order with HDT, confirming the dependence of reaction order on the substrate concentration, since at minimum COD values, the highest reaction orders are obtained. In the log phase, the reaction order for COD occurred when $n = 0.73$ (concentration interval from 2821 to 430 mg L^{-1}).

Fig. 5 presents the variation in the rate of COD and it can be seen that the maximum rate of COD occurred after 15 h of cultivation, a period corresponding to the exponential growth phase. The data for the kinetic constants for

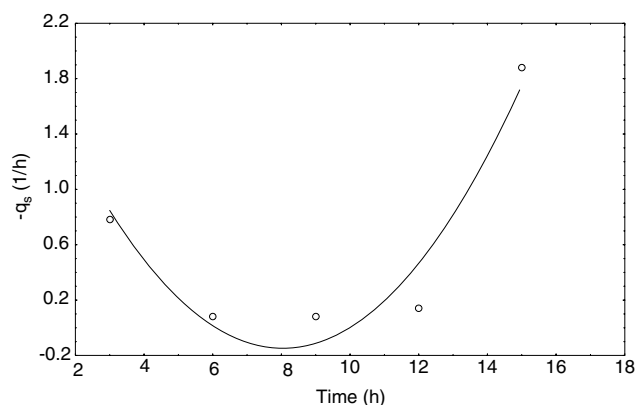


Fig. 5. COD rate under the experimental conditions.

N-TKN consumption in the exponential growth phase are shown in Table 4. The variation in reaction order with HDT confirmed that the N-TKN consumption rate depended on the substrate concentration. In the log growth phase, the reaction order for N-TKN consumption occurs when $n = 0.82$ (concentration interval from 48.43 to 13.20 mg L^{-1}), indicating that the reaction rate did not depend on the substrate concentration. Fig. 6 shows that for the N-TKN consumption rate, the maximum rate also occurred in the period corresponding to the exponential growth phase. Using the kinetic variables (Table 2) it was possible to carry out a scale-up study and estimate the CSTR volume, assuming a cylindrical geometry, industrial wastewater feed, COD and hydraulic detention time. The effluent flow-rate was assumed to be $18 \text{ m}^3 \text{ h}^{-1}$ for a medium sized industry. The hydraulic detention time was 15 h, determined from the maximum COD removal. Table 5 presents this estimation, calculated on the basis of the COD balance. The estimated bioreactor volume would contain 100.2 m^3 of fluid containing parboiled rice effluent and biomass.

Thus, microalgae wastewater treatment systems present a good option for the biological treatment of agro-industrial wastewater. The design, construction and operation of the microalgal wastewater treatment system were influenced by two major factors: first, the need for adequate mixing to maintain efficient treatment, and second, the

Table 3
Efficiency of COD reduction and data for the COD rate as a function of the hydraulic detention time

Time (h)	COD (mg L^{-1})	E-COD (%)	dS/dt	Log (dS/dt)	Log S	q_s (h^{-1})	n
0	2821				3.45		
3	1289	54	-510.66	2.70	3.11	-0.783	0.91
6	2600	7.83	-73.66	1.86	3.41	-0.081	0.94
9	2597	7.95	-74.66	1.87	3.38	-0.082	0.86
12	2448	13.23	-124.33	2.09	3.41	-0.141	0.87
15	430	84.44	-797.0	2.90	2.63	-1.881	1

E-COD, efficiency of COD reduction.

Table 4
Efficiencies of N-TKN removal and data for the rate of N-TKN consumption as a function of the hydraulic detention time

Time (h)	N-TKN (mg L^{-1})	E-TKN (%)	dS/dt	Log (dS/dt)	Log S	q_s (h^{-1})	n
0	48.43				1.68		
3	37.42	28.18	-3.67	0.56	1.57	-0.257	0.73
6	44.03	9.08	-2.20	0.34	1.64	-0.095	0.83
9	35.20	27.27	-2.93	0.46	1.54	-0.318	0.62
12	30.83	36.34	-1.46	0.16	1.48	0.471	0.32
15	13.20	72.74	-5.87	0.76	1.12	-1.299	1.46

q_s , rates of substrate consumption; E-TKN, efficiency of N-TKN removal; n , reaction order.

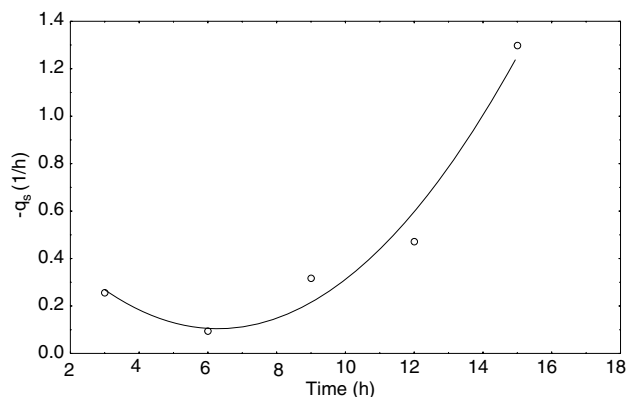


Fig. 6. N-TKN consumption rate under the experimental conditions.

Table 5
Estimation of CSTR volume for COD reduction

Kinetic variable	Value
F ($\text{m}^3 \text{h}^{-1}$)	18
q_{DQO} (h^{-1})	-0.14
S_0 (mg L^{-1})	2821.89
S (mg L^{-1})	467.20
V (m^3)	100.2

F , feed flow-rate; q_{COD} , COD rates; S_0 , initial COD; S , COD; V , volume of the reactor.

difficulty to separate the biomass separating from the treated effluent efficiently and economically in order to complete the process (Oswald, 1988; De La Noue and De Pauw, 1988; Molina Grima et al., 2002).

4. Conclusions

Under the experimental conditions used, we concluded that the greatest removals occurred at a HDT of 15 h, a period corresponding to the exponential growth phase of the microorganism, being respectively, 83.44% and 72.74% for COD and N-TKN. The reductions in COD and N-TKN occurred according to kinetics to the order of 0.73 and 0.82, respectively, for the period corresponding to the exponential growth phase. The volume of the bioreactor estimated for the simultaneous removal of nitrogen and organic matter and for SCP production by the industry under analysis, should be 100.2 m^3 . Therefore, the application of the cyanobacteria *A. microscopica Nageli* in the treatment of parboiled rice effluent is viable.

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