

Cultivation of microalgae *Spirulina platensis* (*Arthrospira platensis*) from biological treatment of swine wastewater

Cultivo da microalga Spirulina platensis (Arthrospira platensis) a partir de tratamento biológico de efluente suíno

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Abstract

The microalgae biomass production from swine wastewater is a possible solution for the environmental impact generated by wastewater discharge into water sources. The biomass can be added to fish feed, which can be used in the formulation of meat products. This work addresses the adaptation of the microalgae *Spirulina platensis* (*Arthrospira platensis*) in swine wastewater and the study of the best dilution of the wastewater for maximum biomass production and for removal of Chemical Oxygen Demand (COD), ammonia and phosphorous to the microalgae. The cultivation of *Spirulina platensis*, strain Paracas presented maximum cellular concentrations and maximum specific growth rates in the wastewater concentration of 5.0 and 8.5%. The highest COD removals occurred with 26.5 and 30.0% of wastewater in the medium. The maximum removal of total phosphorous (41.6%), was with 8.5% of wastewater, which is related to the microalgae growth. The results of *Spirulina* culture in the swine wastewater demonstrated the possibility of using these microalgae for the COD and phosphorous removal and for biomass production.

Keywords: *Arthrospira*; biomass production; pollutant removal; *Spirulina*; swine wastewater.

Resumo

A produção de biomassa de microalgas a partir de efluente suíno consiste em uma possível solução para o impacto ambiental gerado pela descarga de efluentes em fontes naturais. A biomassa produzida pode ser adicionada a rações de peixes, os quais podem ser utilizados na formulação de produtos cárneos. O objetivo deste trabalho foi a adaptação da microalga *Spirulina platensis* (*Arthrospira platensis*) em efluente suíno e o estudo da diluição ideal de efluente para obter a máxima produção de biomassa e remoção de Demanda Química de Oxigênio (DQO), amônia e fósforo do efluente pela microalga. O cultivo da *Spirulina platensis*, cepa Paracas apresentou máxima concentração celular e máxima taxa específica de crescimento em concentrações de efluente de 5,0 e 8,5%. As maiores remoções de DQO ocorreram com 26,5 e 30,0% de efluente no meio. A máxima remoção de fósforo total (41,6%) foi obtida com 8,5% de efluente, sendo relacionado com o crescimento da microalga. Os resultados da cultura de *Spirulina* em efluente suíno demonstraram a possibilidade do uso desta microalga na remoção de DQO e fósforo, além da produção de biomassa.

Palavras-chave: *Arthrospira*; produção de biomassa; remoção de poluentes; *Spirulina*; efluente suíno.

1 Introduction

The swine culture is an important economical activity in rural methods. Brazil possesses the fourth largest worldwide swine flock, with the production of 2.87 million tons of meat. Swine dejects constitute one of the most important pollution sources when they are discharged into nature without previous treatment (SILVA et al., 2003). The residues are not adequately treated and their release into the environment cause serious pollution problems. The swine wastewater is rich in inorganic phosphorous and nitrogen, which usually accumulate in the water generating eutrophic systems (CAÑIZARES-VILLANUEVA et al., 1995).

The characteristics of treated swine wastewater are high values of Biochemistry Oxygen Demand (BOD), nitrogen

and phosphorous concentrations. The aerobic processes of wastewater treatment are the most appropriate ones for removing these compounds. Some examples are the stabilization ponds, which are favorable for cyanobacterium development, among them the *Spirulina platensis*.

According to Cañizares-Villanueva et al. (1995), the swine wastewater can be used as a medium for microalgae and cyanobacterium growth after stabilization through aeration or anaerobic digestion. Moreover, the nutrients found in the wastewater can be converted into biomass, removing the compounds that cause pollution, enabling the reuse of the water after the treatment (DOUMENGE et al., 1993). The *Spirulina platensis* culture in swine wastewater can be beneficial because

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the microalgae can use the nitrate as a source of nitrogen. Additionally, the oxygenation caused by photosynthesis reduces the total coliform count. The produced biomass can be used as animal feed, energy production, and fertilizers or to produce fine chemistry products such as pigments, polysaccharides, carotenes, sterols, vitamins, poly-unsaturated fatty acids and lipids (LODI et al., 2003).

This work addresses the adaptation of the microalgae *Spirulina platensis* in swine wastewater and studies the ideal wastewater dilution for maximum biomass production, in addition to Chemical Oxygen Demand (COD), and the microalgae capability to remove ammonia and phosphorous.

2 Materials and methods

2.1 Swine wastewater characterization

The swine wastewater was collected in a property located in the state of Rio Grande do Sul, in the south of Brazil, at the end of the second facultative pond and stored under cooling to 4 °C. The parameters used for the wastewater characterization were: COD, ammonia and total phosphorous, determined according to APHA (2000).

2.2 Microorganism and culture medium

The microalgae *Spirulina platensis*, strain Paracas (MULLITERNO et al., 2005), was used in this study. The preparation and maintenance of the inoculum was accomplished using Zarrouk medium according to Raouf et al. (2006), standard for the cultivation of this microalgae.

The culture medium for the runs was the swine wastewater itself, collected after the second facultative pond of a treatment system that uses stabilization ponds, which was used after the dilution according to a Factorial Design.

2.3 Culture conditions and analytical determinations

The runs were carried out in 2 L Erlenmeyer flasks containing 1.8 L of medium and an initial biomass concentration of 5.000 cells.mL⁻¹, determined by microscopy cell count. Two glass tubes were passed through the Erlenmeyer's stopper, one for sample collection and the other for continuous aeration. The cultures were mixed and aerated using filtered and humidified air at a flux of 20 L.h⁻¹ provided by diaphragm pumps fitted with a system to remove oil-droplets. The cultures were maintained at 30 °C (RAOOF et al., 2006; ANDRADE; COSTA, 2007) in a greenhouse and illuminated with 20W fluorescent lamps (Philips, Brazil) at an illuminance of 2.000 lux and with a 12 hours light/dark photoperiod (TANTICHAROEN et al., 1994), for 30 d.

Samples were taken at 24 hours intervals and the biomass concentration determined by microscopy cell count, using a Sedgewick Rafter Cell F50 Chamber. The pH was determined potentiometrically each day (AOAC, 1995).

2.4 Factorial design

A 2² Central Composite Design (CCD) was used to study the influence of the wastewater concentration and sodium bicarbonate concentration on the *Spirulina* growth and the removal of wastewater pollutants. The maximum wastewater concentration for the Factorial Run Design was stipulated from the preliminary cultivation tests of the microalgae in the swine wastewater, using as standard? the tolerable ammonia concentration in the wastewater for the *Spirulina platensis* development (ANAGA; ABU, 1996). Table 1 shows the real and coded values of the variables used in the 2² Central Composite Design. Distilled water was used for the dilutions of swine wastewater. Table 1 shows the ammonia concentrations of the experiments after the wastewater dilution.

2.5 Analysis of the biomass growth

Data of biomass concentration versus culture times were plotted and submitted to polynomial adjusts. The polynomial equations obtained were used to calculate the maximum cellular concentration (X_{max}, cells.mL⁻¹), for each run of 2² Central Composite Design. The maximum specific growth rate (μ_{max}, d⁻¹) was obtained through exponential adjustment in the logarithmic phase of growth. The results of μ_{max} and X_{max} of the runs were analyzed statistically through analysis of variance and response surface methodology.

2.6 Analysis of pollutant removals

The determinations of COD and phosphorous were accomplished at the beginning and at the end of each run, in agreement with the methodology described by APHA (2000). The removal of COD and phosphorous were calculated by Equation (1):

$$\text{Removal (\%)} = \left(\frac{x_i - x_f}{x_i} \right) * 100 \quad (1)$$

being:

x_i = COD or phosphorous before the biomass growth; and

x_f = COD or phosphorous after the biomass growth.

The results of COD removal and phosphorous of the CCD runs were analyzed statistically through analysis of variance (Anova) at 95% confidence interval and response surface methodology.

3 Results and discussion

3.1 Swine wastewater characterization

The results of ammonia, COD and phosphorous (775 ± 6.9 mg.L⁻¹, 8,704 ± 362 mg.L⁻¹ and 2,964 ± 47 mg.L⁻¹, respectively) obtained for the effluent used in this experiment were similar to the values found in the literature for the swine wastewater of facultative ponds. Silva et al. (2003) found maximum COD values of 5.250 mg.L⁻¹, 701.6 mg.L⁻¹ for ammoniacal nitrogen and 2.800 mg.L⁻¹ for total phosphorous.

Table 1. Coded and real values of wastewater concentration (WC,%) and sodium bicarbonate concentration (SBC, g.L⁻¹) and results of maximum specific growth rate (μ_{\max} , d⁻¹), initial ammonia concentration (mg.L⁻¹), maximum cellular concentration (X_{\max} , cells.mL⁻¹) and removal of COD (%) and phosphorous (%) in the CCD runs.

Run	WC	SBC	Initial ammonia concentration	μ_{\max}	C_{\max}	COD	Phosphorous
1	8.5 (-1)	6.5 (-1)	69.6	0.415	204,68	43.9	21.2
2	26.5 (+1)	6.5 (-1)	217.0	-	5,143	88.5	-*
3	8.5 (-1)	13.5 (+1)	69.6	0.379	150,001	61.0	41.6
4	26.5 (+1)	13.5 (+1)	217.0	-	6,501	84.3	-*
5	5.0 (- α)	10 (0)	40.9	0.460	130,529	33.7	6.0
6	30.0 (+ α)	10 (0)	245.7	-	7,339	79.3	-*
7	17.5 (0)	5 (- α)	143.3	-	6,513	58.7	-*
8	17.5 (0)	15 (+ α)	143.3	0.237	118,095	53.7	-*
9	17.5 (0)	10 (0)	143.3	0.194	120,562	57.3	-*
10	17.5 (0)	10 (0)	143.3	0.224	105,664	60.9	0

$\alpha = 1.414$; *no phosphorous removal.

According to Silva et al. (2003) different factors can contribute to the variations in the physico-chemical composition of the wastewater, such as the animals' nutrition and handling factors.

The nitrate and ammonia assimilation is related to the pH of the medium, the absorption of nitrogen varying with the pH. The assimilation of nitrate tends to increase the pH and when ammonia is used as the only source of nitrogen, the pH of the medium can decrease quickly at levels smaller than 3.0. Some microalgae are sensitive to high concentrations of ammonia and its growth can be inhibited by concentrations of 1 mM (ABALDE et al., 1995).

3.2 Cultivation of the microalgae *Spirulina platensis* and pollutant removal

The pH of the CCD runs for the cultivation of *S. platensis* remained within the optimal pH of growth for this microalga, between 8.5 and 10.5, which is in agreement with Richmond (1990). The minimum growth temperature *Spirulina* was approximately 8 °C and the maximum 46 °C (ANDRADE; COSTA, 2007).

Figures 1 to 3 show the growth curves of *S. platensis* obtained for each treatment of factorial design.

Table 1 presents the maximum specific growth rate of the runs 1 to 10 of the Factorial Design for evaluating the influence of wastewater concentration and sodium bicarbonate concentration in the growth of the microalgae *S. platensis*, as well as the results of maximum specific growth rate, maximum cellular concentration and the COD and phosphorous reduction.

The largest SBC in run 3 (13.5%), compared to run 1 (6.5%), caused the decreasing of the values of μ_{\max} and X_{\max} . Runs 1 and 3 presented a 2 day lag phase and exponential phases of 16 and 15 days, respectively.

Run 5, which was accomplished with the smallest WC (5.0%), consequently the smallest initial ammonia concentration

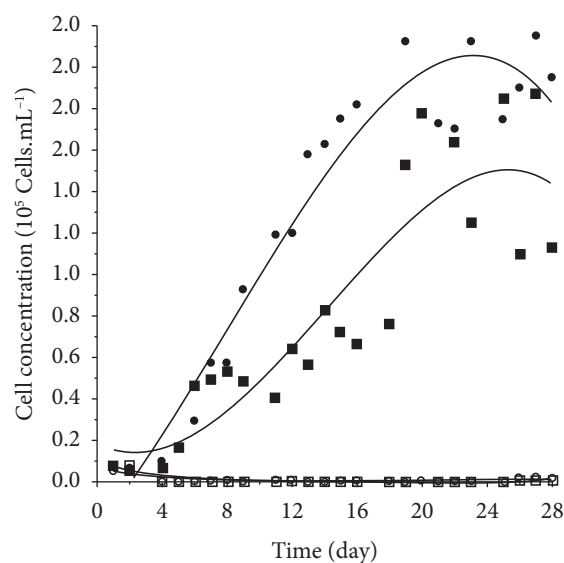


Figure 1. Time course of *S. platensis* cell concentration (cells.mL⁻¹) for the runs 1 to 4 of the CCD. (●) Run 1 (WC = 8.5%; SBC = 6.5 g.L⁻¹); (○) Run 2 (WC = 26.5%; SBC = 6.5 g.L⁻¹); (■) Run 3 (WC = 8.5%; SBC = 13.5 g.L⁻¹) and (□) Run 4 (WC = 26.5%; SBC = 13.5 g.L⁻¹). WC: wastewater concentration; SBC: sodium bicarbonate concentration.

(40.9 mg.L⁻¹) and SBC of 10.0 g.L⁻¹, obtained the largest μ_{\max} of 0.4 d⁻¹ and X_{\max} of 130,5 cells.mL⁻¹, with the smallest lag phase and a 7 day exponential growth phase.

Runs 2 (WC = 26.5%; SBC = 6.5 g.L⁻¹), 4 (WC = 26.5%; SBC = 13.5 g.L⁻¹) and 6 (WC = 30.0%; SBC = 10.0 g.L⁻¹) presented cellular death within the first days of culture and consequently the smallest values of X_{\max} , which can be attributed to their largest initial ammonia concentration.

The comparison of Runs 7 (WC = 17.5%; SBC = 5.0 g.L⁻¹) and 8 (WC = 17.5%; SBC = 15.0 g.L⁻¹), demonstrate that the addition of sodium bicarbonate in the superior levels resulted

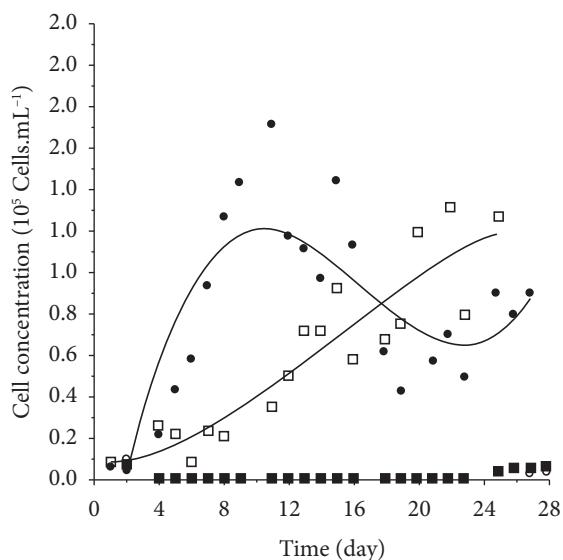


Figure 2. Time course of *S. platensis* cell concentration (cells.mL⁻¹) for the runs 5 to 8 of the CCD. (●) Run 5 (WC = 5.0%; SBC = 10.0 g.L⁻¹); (○) Run 6 (WC = 30.0%; SBC = 10.0 g.L⁻¹); (■) Run 7 (WC = 17.5%; SBC = 5.0 g.L⁻¹) and (□) Run 8 (WC = 17.5%; SBC = 15.0 g.L⁻¹). WC: wastewater concentration; SBC: sodium bicarbonate concentration.

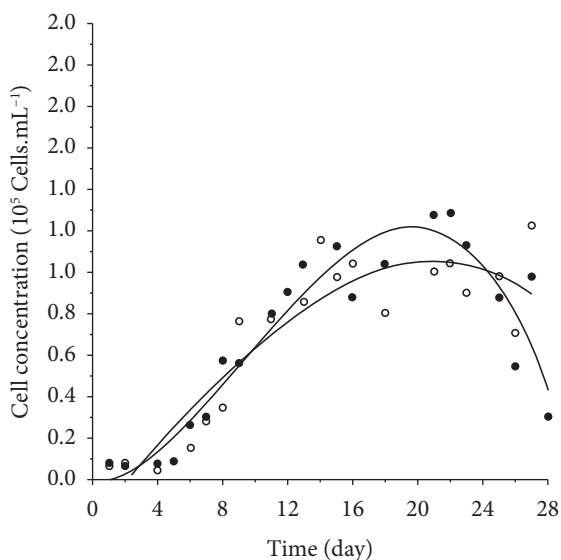


Figure 3. Time course of *S. platensis* cell concentration (cells.mL⁻¹) for runs 9 to 10 of the CCD. (●) Run 9 (WC = 17.5%; SBC = 10.0 g.L⁻¹); (○) Run 10 (WC = 17.5%; SBC = 10.0 g.L⁻¹). WC: wastewater concentration; SBC: sodium bicarbonate concentration.

in obtaining higher values of μ_{\max} and of X_{\max} , of 0.2 d⁻¹ and 118.0 cells.mL⁻¹ for run 8, in comparison to 0 d⁻¹ and 6.5 cells.mL⁻¹ for run 7. This behavior was contradictory to runs 1 and 3, in which the largest μ_{\max} and X_{\max} were obtained in the smallest sodium bicarbonate concentration. It can be supposed that the low wastewater concentration does not cause pH variations that limit the growth of the microalgae, thus the buffer effect of the bicarbonate is not as necessary and, therefore,

the concentrations of bicarbonate used did not have influence on the variables μ_{\max} and X_{\max} . However, in larger wastewater concentrations, observed in runs 7 and 8, the control of the pH through the sodium bicarbonate buffer is necessary due to the fact that the wastewater causes a decrease of the pH in the medium, making the microalgae growth unfeasible.

Runs 9 and 10, which are replicates (WC = 17.5%; SBC = 10.0 g.L⁻¹), demonstrated that the best result of μ_{\max} was found in run 10 (0.3 d⁻¹) and of X_{\max} in run 9 (120.5 cells.mL⁻¹). The exponential phases of these runs began in the 4th day. The growth curves of runs 9 and 10 presented a similar behavior and log phase.

The cellular deaths observed in runs 2, 4 and 6 can be associated to the high wastewater concentration and, therefore, of high initial ammonia in the culture medium (about 217.0 mg.L⁻¹ for runs 2, 4 and 245.7 mg.L⁻¹ for run 6). Olguín (2003), studying the cultivation of *Spirulina* from swine wastewater in tropical conditions, obtained high productivities in biomass in constant concentrations of total nitrogen from 30.0 to 50.0 mg.L⁻¹, and most of that is reduced to ammoniac nitrogen. In our experiment, for runs 2, 4 and 6, the initial ammonia concentration was about four times larger than the concentrations mentioned in the study of Olguín (2003), which justifies the cellular death of these runs. Studies performed by Ayala and Vargas (1987) on the use of swine wastewater for *Spirulina* cultivation concluded that the swine wastewater was toxic for the microalgae when used without dilution, because the coloration of the wastewater causes sunlight penetration problems in the culture medium, decreasing the cyanobacterium growth.

Canizares-Villanueva et al. (1995) reported that the wastewater dilution is necessary to reduce the toxic effect of the ammonia in the growth of *Phormidium* sp. That result is similar to that obtained in this work, once in high WC, the ammonia was toxic for the microalgae growth. In larger dilutions, the toxic effect of the ammonia was not limitand.

The final COD concentrations for all runs were smaller than the initial ones, evidencing the COD removal during the time of this research study. However, it was observed that the COD removal occurred in runs, in addition to those that presented cellular death. This can be explained by the fact that COD removal might not have been accomplished exclusively by the microalgae *Spirulina*, but also by other microorganisms in the wastewater. The chemical oxidation caused by the aeration of the system, according to Metcalf and Eddy (1993) and Ramalho (1980), can also promote the COD reduction of the medium. Therefore, according to Queiroz et al. (2004), some cyanobacterium can develop heterotrophic metabolism, however this hypothesis is the least probable, once the final COD and cell concentrations did not have a direct relationship.

In runs 1, 3 and 5, the phosphorous removals were of 21.2, 41.6 and 6.0%, respectively, which presented the lowest WC (8.5%), while the maximum cellular concentrations were of 204.6, 150.0 and 130.5 cells.mL⁻¹, demonstrating that the phosphorous removal was for biological assimilation. In the runs that presented larger WC, there was not phosphorous removal.

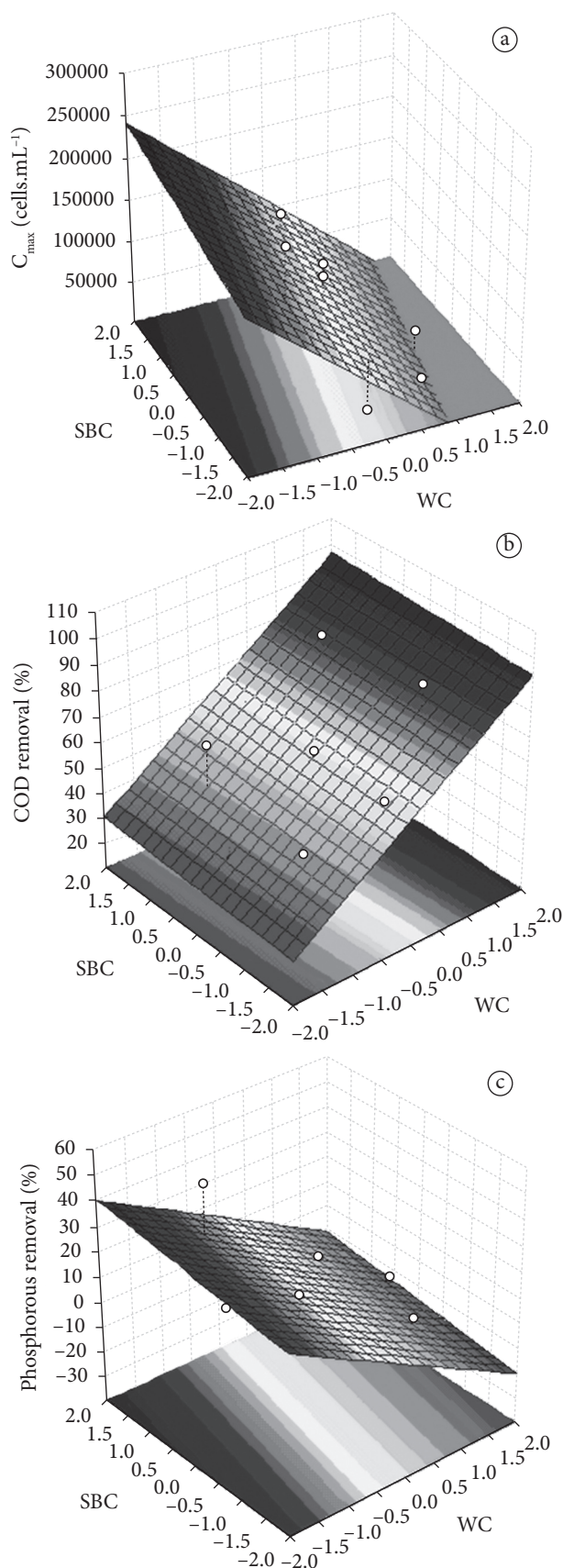


Figure 4. Surface response for the influence of wastewater concentration (WC) and sodium bicarbonate concentration (SBC) on a) X_{max} ; b) COD removal; and c) phosphorous removal.

Laliberté et al. (1997) studied the *Spirulina* cultivation for wastewater treatment and reported that in an aerobic treatment system, the phosphorous removal could have started by two different mechanisms: by the biological assimilation during the biomass growth and by the chemical precipitation that occurred predominantly when the biomass concentration decreases, in other words, in the decline phase or cellular death.

The initial inoculum concentrations used in this work ($5.000 \text{ cells.mL}^{-1}$) correspond to concentrations of around 0.1 g.L^{-1} . Lodi et al. (2003) studied the removal of nitrate and phosphorous from wastewater with *S. platensis* cultivation and concluded that biomass concentrations between 0.25 and 0.86 g.L^{-1} result in larger removals of these pollutants. The results obtained in this study suggest that the use of larger initial inoculum concentrations can reduce the toxic effect of the ammonia found in the swine wastewater, reducing the competition of the microalgae with the microorganisms in the wastewater and, consequently, the adaptation phases of the microalgae to the medium.

Figure 4a, b and c show the surface responses of μ_{max} , X_{max} , COD and phosphorous removal due to WC and SBC.

The linear effects of wastewater concentration evidenced significant influences in the answers of μ_{max} ($p = 0.026$), X_{max} ($p = 0.036$), COD removal ($p = 0.034$) and phosphorous removal ($p = 0.037$) to a confidence level of 95%. Other factors did not evidence significant influences.

Figure 4 indicates that sodium bicarbonate concentration did not influence significantly the responses of μ_{max} , X_{max} , COD and phosphorous removal. Largest μ_{max} , X_{max} and phosphorous removal were obtained in the smallest wastewater concentration added, in the order of 5.0 and 8.5%, respectively, while higher COD removals were obtained in superior levels of WC (26.5 and 30.0%).

4 Conclusion

The cultivation of the microalgae *Spirulina platensis*, strain Paracas showed maximum cellular concentration ($204,6 \text{ cells.mL}^{-1}$), maximum specific growth rate (0.4 d^{-1}) and maximum phosphorous removal (41.6%) in the smallest wastewater concentration added to the cultivation medium (5.0 and 8.5%), while larger COD removals (84.3 and 79.3%), were obtained in the largest wastewater concentration in the cultivation medium (26.5 and 30.0%). The cultivation of *Spirulina platensis* in swine wastewater demonstrated the capability of biomass production, COD and total phosphorous removal, therefore this microalgae can be an alternative to assist in the swinculture wastewater treatment, reducing the environmental impact caused by their pollutants and also to use the biomass produced in animal feed and fertilizers, in the production of pigments, vitamins, polysaccharides, including others.

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