



Fish processing wastewater treatment by combined biological and chemical processes aiming at water reuse

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ABSTRACT

The objective of this work was to evaluate the potentiality of the reuse of fish processing wastewater by association between biological and chemical treatment. Heterotrophic microalgae systems were used in the biological treatment and coagulation–flocculation–sedimentation in the chemical step. Ammonia, chemical oxygen demand, total solids, suspended solids, turbidity, phosphorous, alkalinity, hardness, silica, sulfate, aluminium, calcium, magnesium, manganese, ferric and pH were determined in each stage of treatment. The results obtained showed the potentiality of the proposed process for reuse of fish processing wastewater. Among the water quality criteria evaluated, the treated wastewater complies with the Brazilian and US standards for water reuse in cooling systems.

Keywords: Microalgae/cyanobacteria; Heterotrophic cultivation; Coagulation–flocculation–sedimentation; Water reuse

1. Introduction

Fish processing is an important world economic activity. This process involves significant water consumption, on the average equal to 11 m³/ton of fish processed, resulting in a significant volume of wastewater [1]. This wastewater contains considerable quantities of carbon, nitrogen, phosphorus, metals and solids carried from the raw material, suitable for supporting cyanobacterial growth [2].

The use of cyanobacteria for wastewater treatment may offer an inexpensive alternative to conventional forms of secondary and tertiary wastewater treatment. In addition to usual photosynthetic ponds that operate in the presence of light energy, heterotrophic bioreactors

are a potential technology for treating wastewaters. The heterotrophic metabolism of these microorganisms is based on the consumption of simple exogenous organic molecules and inorganic nutrients in the dark, simultaneously removing organic matter, nitrogen, phosphorous and other pollutants in a single step, reducing capital and operational treating costs [3]. In addition, conversion partially occurs of the pollutants into biomass that has a potential for the production of single-cell protein and single-cell oil that can be used as feedstock in the animal feed and bioenergy industry [4,5].

A key issue in biological treatment systems with cyanobacteria is biomass separation. The size of the cyanobacterial cells is generally very small and the cellular concentration in the wastewater is diluted for separation by the simple sedimentation operation. In the majority of cases, biomass separation is the determining factor for the

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techno-economic viability of the process [6]. Combined operations of coagulation-flocculation-sedimentation have been proposed as an effective method for cyanobacterial cell separation. In the specific case of wastewater treatment, this operation causes amelioration in the final water quality [7].

Demands on water resources for household, commercial, agricultural, and industrial purposes are increasing greatly, and the situation is exacerbated by growing urbanization. Industrial activities in Brazil account for approximately 20% of water consumption; at least 10% of this water is discharged directly into water bodies and more than half is either treated inappropriately or not treated at all. The continuous demand for sustainability of industrial production calls for the development of new tools such as reuse and recycling in industry [8].

Water recycling within an industrial plant is usually an integral part of the industrial process and must be developed on a case-by-case basis. Industrial uses for reclaimed water include evaporative cooling water, boiler-feed water, process water and irrigation and maintenance of plant grounds. Of these uses, cooling water is currently the predominant industrial reuse application. The use of reclaimed water is an emergent area for industrial practice, with great promise for reduction of the industrial water footprint [9].

In this regard, the aim of this work was to evaluate the potentiality of the reuse of fish processing wastewater by association between biological and chemical treatment.

2. Materials and methods

2.1. Wastewater

Wastewater originating from the fish processing industry (Rio Grande, RS, Brazil) was investigated. Wastewater samples were collected, transported and stored at 4°C. The investigated parameters were analyzed on the basis of the parameters described for class 4 waters by the National Council for the Environment, Brazil (Conama) [10]. The investigated parameters included ammonium (NH_4^+), total solids (TS), total dissolved solids (TDS), suspended solids (SS), turbidity, chemical oxygen

demand (COD), pH, silica, hardness, alkalinity, sulfate, phosphorus (P-PO_4^{3-}), aluminum, calcium, magnesium, manganese, and iron, according to Standard Methods for the Examination of Water and Wastewater [11].

2.2. Microorganism and culture conditions

A monoculture of *Aphanothece microscopica* Nágeli (RSMAn92), originally isolated from the Patos Lagoon estuary, located in the state of Rio Grande do Sul, Brazil ($32^\circ 01' \text{S} - 52^\circ 05' \text{W}$) was used. Stock cultures were propagated and maintained in a synthetic BGN medium [12] with the following composition (g/L): $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ (0.040), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.075), EDTA (0.001), H_3BO_3 (2.860), $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (1.810), $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (0.222), $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (0.390), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.079), $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ (0.040), NaNO_3 (15), $\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$ (0.006), ammonium iron citrate (0.006), pH 7.6. The incubation conditions used were 25°C, photon flux density of $15 \text{ mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ and a photoperiod of 12 h.

2.3. Experimental set-up

Fig. 1 shows the schematic diagram of the experimental set-up. The system consists of an equalization tank, a bubble column bioreactor and a sedimentation.

2.4. Biological wastewater treatment

The experiments were carried out in bioreactors operating in an intermittent regime, fed with 4.5 L of fish processing wastewater. The experimental conditions were as follows: initial cell concentration of 0.2 g/L, isothermal reactor operating at a temperature of 30°C, C/N ratio of 20, (adjusted when necessary with sucrose and ammonium sulfate), pH being adjusted to 7.6, constant aeration of 1 VVM (volume of air per volume of wastewater per min) and absence of light. The tests were carried out in triplicate and the kinetic data referred to the mean of six repetitions.

2.5. Biomass harvesting and chemical wastewater treatment

Biomass harvesting experiments were performed using a Jar Test apparatus. A rapid agitation of 110 rpm for

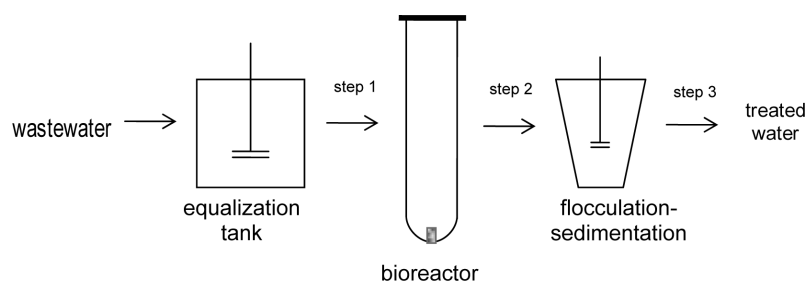


Fig. 1. Schematic representation of the treatment steps aimed at water reuse. Step 1 (effluent from the equalization tank), Step 2 (biological treatment), Step 3 (chemical treatment).

Table 1
Values of the independent variables for the different levels of the experimental design

Independent variable	Symbol	Level		
		-1	0	+1
Type of coagulant	X_1	FeCl ₃	—	Al ₂ (SO ₄) ₃
Coagulant concentration	X_2	50	300	550
pH	X_3	6.0	7.0	8.0

30 s was used followed by a slow stir of 50 rpm for 10 s and 15 min of sedimentation time. The best condition was obtained by response surface methodology, aiming to determine the optimal conditions for turbidity, COD and solids removal, as a function of three experimental factors (type of coagulant, coagulant concentration and pH). A complete $3^2 \times 2$ factorial design was used to evaluate the relationship between the chemical treatment (independent variables) and the removal efficiency (dependent variable). The experimental design and the statistical analyses were carried out using the Statistica 7.0 software (Statsoft, USA). Table 1 shows the levels of the experimental variables used. For a three-factor system, the statistical model is defined by Eq. (1):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (1)$$

where Y is the variable response, β are the parameters of the statistical model and X_1, X_2, X_3 are the independent variables of the statistical model

2.6. Analytical procedures

In every step the wastewater generated in the process was characterized in accordance with the parameters described in resolution 357/2005 of the National Council on the Environment, Brazil [10] for classes of water intended for reuse in cooling systems. All results are reported as concentration units and removal efficiency (%).

3. Results and discussion

The results of the turbidity, chemical oxygen demand and suspended solids of fish industry wastewater before and after biological treatment are presented in Table 2. In the raw wastewater turbidity, COD and SS values of 206 NTU, 1809 mg/L and 178 mg/L, respectively were observed. A COD removal efficiency of 87.95% was evidenced after biological treatment, in parallel to the increase in the turbidity (155.8%) and suspended solids (187.6%), characteristic of microbial growth. The partial composition of the raw effluent is in accordance with the results observed by Hornes and Queiroz [13] when monitoring fish effluent in a fish industry for a period of

Table 2
Partial characterization of the raw wastewater and after the biological treatment

Parameters	Step 1	Step 2
Turbidity, NTU	206 ± 83.3	321 ± 5.1
COD, mg L ⁻¹	1809 ± 1063.6	218 ± 13.1
SS, mg L ⁻¹	178 ± 81.3	334 ± 9.3

$n = 6$

12 months. The authors found maximum and minimum values of chemical oxygen demand (1900–594 mgL⁻¹), total solids (1552–7220 mgL⁻¹), fixed solids (393–5355 mgL⁻¹) and volatile solids (775–2422 mgL⁻¹). The composition of this wastewater is generally variable, in function of the fish species, seasonal variability of the particular species and the processing type [14]. The high COD removal is typical of the heterotrophic cyanobacterial systems that use exogenous organic compounds in the dark [15], in a metabolism potential to wastewater treatment. However, in this process, a significative biomass growth is normally associated with organic matter removal, and the harvesting of the biomass is a necessary step. Generally, the harvesting of biomass is a significant problem because of the small size (3–30 µm diameters) of the microalgal cells. In addition, cell concentration is generally relatively dilute and hence large volumes need to be handled to recover the biomass [6,7]. The size of the cells of the cyanobacteria used in this study is 9.0–9.5 µm in diameter [16].

Various methods of coagulation-flocculation have been used to aggregate the microalgae cells in order to increase the effective particle size and hence ease sedimentation. Microalgal cells carry a negative charge that prevents the aggregation of cells in suspension. The surface charge can be neutralized or reduced by adding flocculants such as multivalent cations and cationic polymers to the wastewater. Several authors have reported the use of multivalent metal salts as effective flocculants. The commonly used salts include ferric chloride, aluminum sulfate [17–19].

According to the proposed experimental design, 18 experiments were carried out, evaluating different combinations of type of coagulant, coagulant concentration and pH in the removal of turbidity, COD and SS. The contour curves (Fig. 2) show the removal efficiencies of the parameters turbidity, COD and SS, considering the wastewater after the biological treatment, as a function of the factors studied. The results indicated that the best removal occurred at conditions of 300 mg/L of ferric chloride and pH 7.0. The maximum removal efficiencies for COD (85%), suspended solids (93%) and turbidity (97%) were observed in this condition.

In fact, coagulation-flocculation operations in wastewater treatment are mainly used for the removal of colloidal material which causes coloring and turbidity

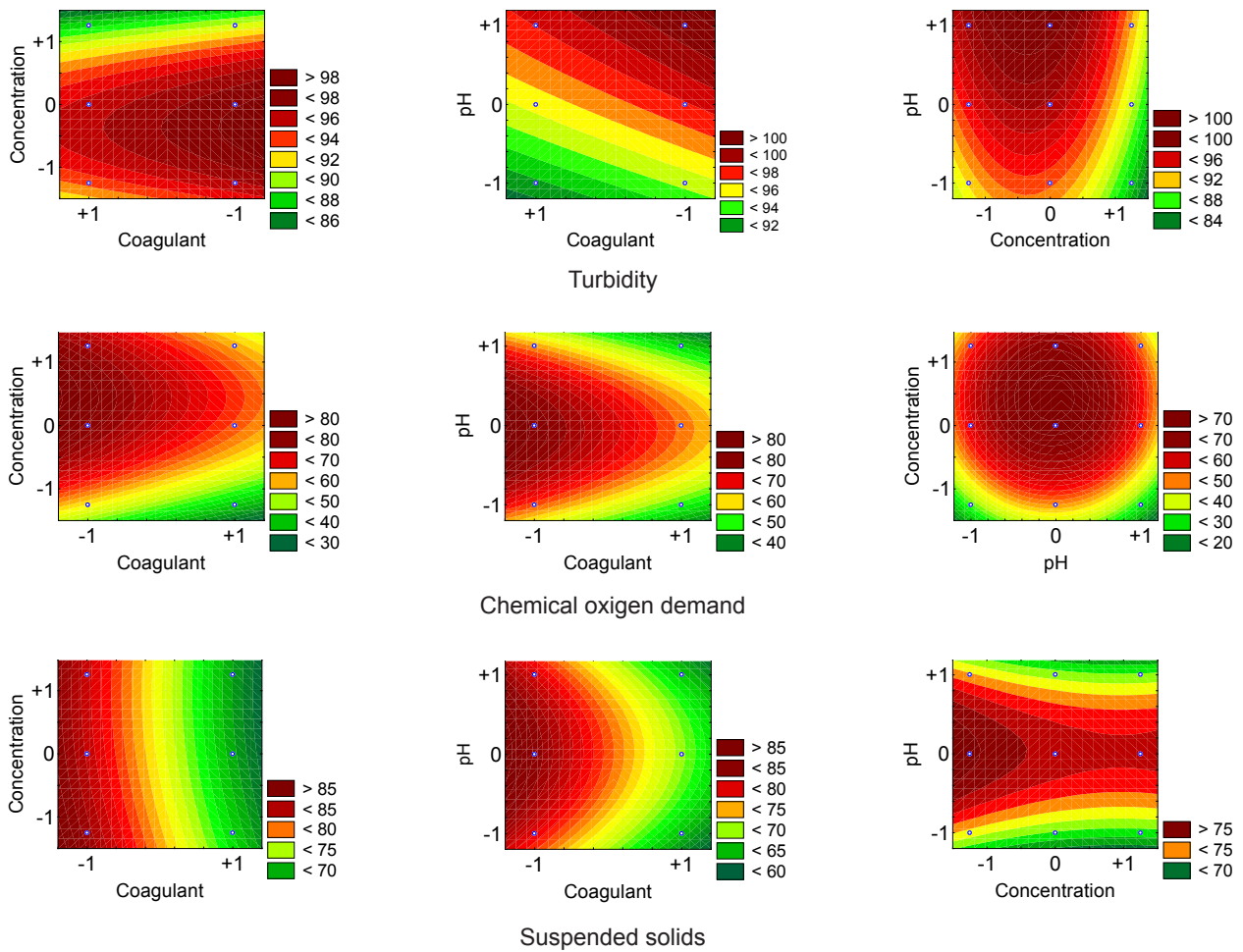


Fig. 2. Contours curves for the variables COD, turbidity and SS removal.

characterized by the elimination of suspended solids as well as the removal of as much of the organic material as possible [18]. Effective coagulation-flocculation associated with biological treatment has been reported for recovery from biomass microalgae with a COD removal range of 32–90% [20]. In this work main removal efficiencies of 85 and 77% were obtained at concentrations of 300 mg/L at pH 7.0 of ferric chloride and aluminum sulfate respectively.

The results of the effects and interactions between the type of coagulant, coagulant concentration and pH and also the coefficients of the models, suggested the following statistical models for the variables removal efficiencies of COD, turbidity and SS:

$$Y_{\text{COD}} = 51.4 - 7.2X_1 + 9.6X_2 + 6.9X_2^2 - 1.5X_3 + 1.5X_1X_3 - 2.3X_2X_3 \quad (2)$$

$$Y_{\text{Turbidity}} = 93.5 - 0.9X_1 - 3.2X_2 + 2.1X_2^2 - 3.0X_3 + 0.7X_3^2 + 3.1X_1X_2 + 0.4X_1X_3 + 0.4X_2X_3 \quad (3)$$

$$Y_{\text{SS}} = 74.1 - 7.2X_1 - 1.1X_2 \quad (4)$$

The goodness of fit of the models was evaluated by the *F*-test, which determines whether the regression equation is statistically significant by the values of *F* calculated and *F* critical. According to Box et al. [21], in order that a regression should be not only statistically significant but also useful for predictive purposes, the ratio between the mean square for regression and the mean square for residual (MS regression/MS residual, which corresponds to *F* calculated), must be at least three times greater than the value higher than the critical *F* value for COD removal ($F = 23 > F = 3$), turbidity removal ($F = 168 > F = 3$) and suspended solid removal ($F = 26 > F = 3$). Thus, the models proposed can be considered statistically sufficient and robust to describe the COD, turbidity and SS removal in these conditions.

The characterization of the fish processing wastewater is expressed in Table 3, based on the parameters indicated by the National Council on the Environment, Brazil (Conama) [10] for water reuse in cooling systems as

Table 3
Legislation limits, concentration values and removal efficiencies

Parameters	Conama [10]	EPA [27]	Step 1	Step 2	E_1 (%)	Step 3	E_2 (%)	E_T (%)
ST, mg/L	1000	ns	1436 ^a	1253.3 ^b	12.7	950.0 ^c	24.2	33.8
SS, mg/L	ns	ns	89.3 ^a	168.0 ^b	—	19.3 ^c	88.5	78.3
TDS, mg/L	ns	500	1346.7 ^a	1085.3 ^b	19.4	930.7 ^c	30.8	44.5
Turbidity, NTU	50	50	117 ^a	165.0 ^b	—	4.0 ^c	97.6	96.6
COD, mg/L	75	75	1824.6 ^a	195.3 ^b	89.3	0.0 ^c	100.0	100.0
Ammonia, mg/L	1	1.0	49.4 ^a	3.4 ^b	93	0.9 ^c	72.2	98.0
Phosphorus, mg/L	4	4	8.8 ^a	0.09 ^b	98.9	0.07 ^c	22.2	99.2
Hardness, mg/L	650	50–180	156.7 ^a	141.3 ^b	9.8	139.7 ^b	1.2	10.8
Alkalinity, mg/L	350	350	6.04 ^a	4.6 ^b	23.3	1.6 ^c	64.6	72.8
Sulfate, mg/L	200	200	0.07 ^a	0.05 ^b	28.5	0.05 ^b	0.0	28.5
Silica, mg/L	50	50	2.1 ^a	2.1 ^a	1.8	0.6 ^b	68.7	69.3
Aluminium, mg/L	0.1	0.1	0.16 ^a	0.11 ^b	26.6	0.16 ^b	—	26.6
Calcium, mg/L	50	50	44.2 ^a	43.1 ^b	8.3	42.0 ^b	2.6	5.0
Magnesium, mg/L	0.5	0.5	23.3 ^a	20.3 ^b	12.7	18.5 ^b	9.0	20.6
Manganese, mg/L	0.5	0.5	0.08 ^a	0.04 ^a	50.0	0.1 ^b	—	—
Ferric, mg/L	0.5	0.5	0.27 ^a	0.09 ^b	64.8	14.6 ^b	—	—
pH	6.9–9	6.9–9	9.2	7.7	—	7.0	—	—

Within the same line, means having different superscripts are significantly different ($p < 0.05$) by Tukey test E_1 : removal efficiency in the steps 1 to 2; E_2 : removal efficiency in the steps 2 to 3; E_T : total removal efficiency ns: not specified; —: not determined

well as the limits suggested and the removal efficiencies of each process step.

Analyzing the characterization data obtained (EE), mainly for the levels of COD, N-NH₄⁺, P-PO₄³⁻ and solids, it can be observed that the effluent used in the experiments is within the typical profile of the effluent from the fish industry [13]. It is important to note that when comparing the results for characterization of the effluent from the equalization tank, with the limits indicated by Conama [10], it is observed that the parameters involved in metals, with the exception of magnesium and the parameters SO₄²⁻, alkalinity and hardness are below the required limits by law. This makes this wastewater a potential for reuse in cooling systems.

Cyanobacteria act as agents of highly efficient purifying not only by oxygenation of the water but also by their efficiency in the removal of nutrients and metals [22]. In experimental conditions, the greatest efficiencies were recorded for ferric (64.81%), for removal coagulant effect highlighting the removal of silicon (silica) with a percentage of 68.72%, as we assess in Table 3, which expresses the efficiency of *Aphanothece* regarding overall efficiency of the process for the most significant parameters.

Analyzing comparisons of the data recorded after treatment with cyanobacteria *Aphanothece microscopica Nægeli* (Step 2) with the results of the effluent from the fish industry equalization tank (Step 1), there are significant removals of the wastewater components, stressing mainly

COD and P-PO₄³⁻ with removal efficiencies of 89% and 99% respectively, reaching phosphorus concentrations lower than indicated for reuse in cooling systems in accordance with Conama [10]. Queiroz et al. [3] obtained organic matter removals expressed as COD in the order of 83% for *Aphanothece microscopica Nægeli*, cultivated in parboiled rice effluent, equivalent to the value recorded in this work for the fish industry effluent. The high rate of phosphorus removal (99%) found is in agreement with that reported by Hornes and Queiroz [13], who obtained 100% of removal of P-PO₄³⁻ using the microorganism in studying the treatment of the effluent from the fish industry. However, some authors suggest the importance of other factors, such as precipitation and adsorption in phosphorus removal [23,24].

In regard to ammonia, a high removal efficiency (98.06%) was verified. This contaminant causes problems of severe corrosion in heat exchangers when in concentrations above those permitted in the legislation [10]. Cyanobacteria have been listed as important agents of removal of ammoniacal nitrogen [24], with removal efficiencies of up to 100% by these microorganisms, including the cyanobacterium *Aphanothece microscopica Nægeli*, being recorded [13].

Negative values of removal efficiencies from SS and turbidity are attributed to the growth of biomass, because the cells of the cyanobacteria present a negative charge when they are suspended. In addition, cultures gener-

ally are made fairly diluted, with a concentration below 0.5 kg/m³ [6].

The wastewater treated by *Aphanothece* and subject to flocculant action (FeCl₃) showed 99% of phosphorus removal, satisfying the limits specified in the legislation [10]. The efficiency of COD removal is in agreement with a series of works that relate to high efficiency of organic matter and solid removal when ferric chloride is used to treat wastewater [23,25]. According to Semmens and Field [18], this effect is attributed to the property of rapid kinetic association between organic compounds and ferric ions. This reflects the recorded data, which show the effect of the combination of biological treatment with coagulating treatment, resulting in wastewater suitable for reuse in refrigeration systems.

Reclaimed water in cooling systems must be treated for hardness and alkalinity, to avoid the production of insoluble salts of calcium and magnesium and corrosion. Analyzing the data, it may be noted that fish processing wastewater has a great potential for reuse in this type of system, since it presents a concentration of hardness and alkalinity below the limit recommended in the legislation for this kind of reuse; concentrations of 156.67 and 6.04 mg/L CaCO₃, respectively are recorded. According to Hilal et al. [26] the hardness of water is proportional to the content of calcium and magnesium salts. The amount of calcium is usually two times higher than that of magnesium; the results obtained in this work are in agreement with the literature, since the concentration of calcium was 47 and magnesium 24 mg/L.

The choice of one or a combination of two or more techniques of treatment will depend on the potential of each technique, the mechanisms involved in reducing the contaminant of interest and the quality of water reuse that is needed. This shows that the use of cyanobacteria *Aphanothece microscopica* Nägeli as biological treatment of effluent from the fish industry, seeking its reuse in cooling systems is very effective because it is a secondary treatment, since almost all parameters were studied within the limit established in the legislation.

While evaluating the data given in Table 3 the significant effect of the action of coagulant FeCl₃ on removal efficiencies for the parameters considered should be noted. Treatment with the coagulant resulted in an increase of 13.84% in the efficiency of COD removal obtained when the effluent was treated with *Aphanothece*, resulting in total removal (ET) of 100% of COD. Values no less important were recorded for solids. These findings are reflected in the final value of turbidity efficiency (93.05%). Results for the parameters assessed are very close to those indicated by these authors, using experimental conditions applied in this work.

In the analysis of Table 3, after the Tukey test it was found that for most of the parameters under study there was a significant difference ($p < 0.05$) between the various treatments offered, and this difference is evident when the

effluent of fish industry was treated by *Aphanothece* and coagulant. Regarding requirements for microbiological parameters to Conama [10], indicates 2.2 NMP/100 mL for use of Class 4 water, which can be used in refrigeration systems, lower indices than 2 NMP/100 mL were recorded. The results of this work meet the requirements of legislation. Comparatively with the US Environmental Protection Agency standard [27], the system proposed also complies with the requirements for water reuse in cooling systems.

4. Conclusion

The association between biological and chemical treatment of the fish processing wastewater is a suitable strategy to reuse water in cooling systems, aiming to reduce the water footprint of the industrial activities. Biological treatment with cyanobacterial was effective in the removal of organic matter and nutrients in a single step. In addition, chemical treatment, based on coagulation/sedimentation complemented the removal of other contaminants required for water reuse.

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