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Fish canning wastewater treatment by activated sludge: Application of factorial design optimization Biological treatment by activated sludge of fish canning wastewater

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ABSTRACT

The optimization of hydraulic retention time (HRT) and initial organic matter concentration for dissolved organic carbon (DOC) abatement of wastewater from a fish canning industry of northern Portugal by activated sludge was investigated using response surface methodology (RSM). The two parameters were chosen since it was found that the treatment efficiency is mainly influenced by them. The experimental data on DOC removal were fitted into a quadratic polynomial model using factorial design and RSM. The optimum process conditions were determined by analyzing the response surface of a three-dimensional plot and by solving the regression model equation. The obtained results showed a HRT of 6.4 h and an initial DOC of 406.2 mg/L as the best treatment conditions. Under these conditions, the maximum predicted DOC removal was 88.0%, confirming the feasibility and the reliability of fish canning wastewater treatment by activated sludge for organic content removal.

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1. Introduction

Portugal is a country with a long coastline (around 1800 km) and with great tradition and potential for fishing. According to data from the National Statistics Institute, in 2012, the Portuguese fleet captured more than 197,500 t of fish and nearly 44,000 tons of prepared and canned fish were manufactured, from which 27,500 t were exported. The commercial fish-processing industry generates large quantities of solid waste and wastewater [1]. The treatment of fish canning wastewater is particularly difficult due to the high content in organic matter and salt, and the characteristics of the companies, which are mainly small companies, widely dispersed and with high seasonal activity [2,3]. This sector is also known by the considerable variability in water consumption and effluent characteristics. In fact, the level of total soluble and suspended chemical oxygen demand (COD) varies largely among factories and fish type [4]. All these factors make it difficult to meet the requirements of the increasingly restrictive legislation and to deal with this problem in a sustainable way.

A treatment process suitable to treat or even valorize and recycle this wastewater must be found. Biological treatment is the most common process used to treat organics-containing wastewaters [5]. These processes are frequently used since they are more economic and environmentally friendly, using optimized natural pathways to actually destroy pollution, not only transform it into another form [6]. Artiga et al. [7] used a hybrid membrane bioreactor for the treatment of water generated during tuna cooking with brine, from a fish canning industry, with 7.8–11.7 g COD/L, 1.2–1.8 g N/L and up to 84 g/L of salt. After 73 operating days they achieved a COD removal efficiency of 92%. The effect of a lipase-rich enzyme preparation was evaluated in an up-flow anaerobic sludge blanket bioreactor (UASB) by Alexandre et al. [8] to treat fish-processing plant wastewater containing 1500 mg oil and grease (O&G)/L. They concluded that the enzymatic pre-hydrolysis step together with anaerobic treatment improved the quality of the treated effluent and reduced operational problems. Riano et al. [1] studied the treatment of fish processing wastewater with microalgae-containing microbiota at 23 and 31 °C, achieving approximately 70% of COD and phosphate removal regardless of temperature. However, further research is needed in order to optimize operational conditions considering the energy-efficiency of the system. Although biological treatment of carbonaceous, nitrogenous and phosphorous pollution has proved to be feasible at high salt concentrations [9], the performance achieved depends on proper reactor conditions and characteristics of the effluent.

Single-variable optimization methods are not only tedious but also can lead to misinterpretation of results, especially because the interaction between different factors is overlooked [10]. Therefore, at present, the multivariate optimization methods have been chosen for numerous research works. A factorial design of experiments has been extensively used to process development and optimization because it allows the simultaneous analysis of the effects of many process variables at different levels as well as their interactions. The experimental design and response surface methodology (RSM) are useful statistical techniques to identify and optimize factors that influence a particular process, with a reduced number of experiments to be performed. This multivariate technique fits the experimental data to a theoretical model through a response function, estimating this way the model coefficients [11,12]. RSM and factorial design have been extensively applied in many areas of wastewater treatment such as optimization of the treatment conditions of an oily wastewater by a nano-porous membrane process [13], optimization of an electrocoagulation process for the treatment of wastewater from biodiesel production [14], optimization of a textile dye wastewater degradation by enzymatic catalysis [15,16], minimization of organic content of simulated industrial wastewater by Fenton type processes [17], etc. To our knowledge, there are no reports in the literature on the optimization of fish canning wastewater treatment by activated sludge.

Then, the major goal of the experimental work being reported here was to create a methodology for optimization and control of a process that is still poorly controlled and that could be applied in similar factories. Thus, the aim was to characterize the effluent of one unit of canned fish production, over time, regardless of the type of fish, in order to assess the effluent seasonal variability. Based on this characterization, the conditions of treatment by activated sludge were optimized. Response surface methodology and a polynomial function were applied to set the optimum operating conditions for maximum reduction of dissolved organic carbon (DOC).

2. Material and methods

2.1. Wastewater characterization

During the period of the biological tests, 110 L of wastewater from a fish canning industry of northern Portugal were collected at each time and left to settle for 1.5 h. DOC and total soluble nitrogen ($N_{total\ soluble}$) of each effluent sample were determined following the Standard Methods for the Examination of Water and Wastewater [18]. DOC was determined by using Total Organic Carbon analyzer (5000 A analyzer from Shimadzu, Japan). The values reported represent the average of at least two measurements; in most cases each sample was injected three times, being the validation performed by the apparatus only when coefficient of variation (CV) was smaller than 2%.

2.2. Biological treatment

The biological treatment was applied to previously settled fish canning wastewater in order to evaluate the organic matter removal efficiency by the activated sludge process. A sample of suspended biomass from a municipal wastewater treatment plant (Freixo WWTP, Porto, Portugal) was used as inoculum.

The experimental home-made set-up used for this study consists of a 110 L feed tank containing the wastewater to be treated, a biological reactor or aeration tank (internal diameter (ID)=19 cm, height (H)=33 cm, working volume (V)=6 L) equipped with air diffusers at the bottom to ensure the oxygen supply and the mixing of the whole liquid volume (the air flow rate was about 6 L min^{-1}), a secondary sedimentation tank (ID=19 cm, $H_{cylinder}$=31 cm, H_{conic}=15 cm, V=6 L) equipped with a sludge recirculation system, and a storage tank to receive the final effluent (treated wastewater). There are also two peristaltic pumps operating at adjustable flow rate: one for reactor feeding and another for sludge recirculation (Fig. 1).

Before conducting the biological treatment studies themselves, an acclimatization and growing period of the activated sludge used as inoculum was carried out in batch mode during 20 days in order to obtain a biomass concentration in the reactor greater than 1500 mg VSS/L. Once reached this value, the operation was shifted to continuous mode. Throughout the experiments, temperature, pH and DO were approximately $T=22\pm2$ °C, pH=7.5 ± 0.5, DO=2.0 ± 0.5 mg/L, respectively.

2.3. Factorial design

A 3^2 factorial design was carried out in order to analyze the influence of the hydraulic retention time (HRT) and the feeding stream dissolved organic carbon (DOC_i) on the fish canning wastewater DOC reduction by activated sludge treatment. The operating ranges selected for each factor were established according to the values normally used in this type of biological treatment and considering the wastewater characterization initially made. The wastewater DOC value is highly variable as can be seen in Table 3, with a minimum value of 153 mg/L and a maximum of 984 mg/L. Since the average value is approximately 500 mg/L, upper and lower limits of 800 and 200 mg/L, respectively, were chosen in order to encompass the majority (74%) of the original values recorded. This way, the initial DOC range was established as 200–800 mg/L and the HRT between 4.2 and 8.1 h. Each independent variable was coded at three levels between −1 (low level), 0 (middle point) and +1 (high level). The coding of the variables was done by the following [19]:

$$x_i = (X_i - X_z)/\Delta X_i \quad , \quad i = 1, 2, 3, \ldots, k \tag{1}$$

where x_i, is the dimensionless value of an independent variable, X_i is the real value of an independent variable, X_z is the real value of an independent variable at the center point and ΔX_i is the step change of the real value of the variable i corresponding to a variation of a unit for the dimensionless value of the variable i. The levels of each factor are listed in Table 1. Table 2 presents the experimental design matrix.

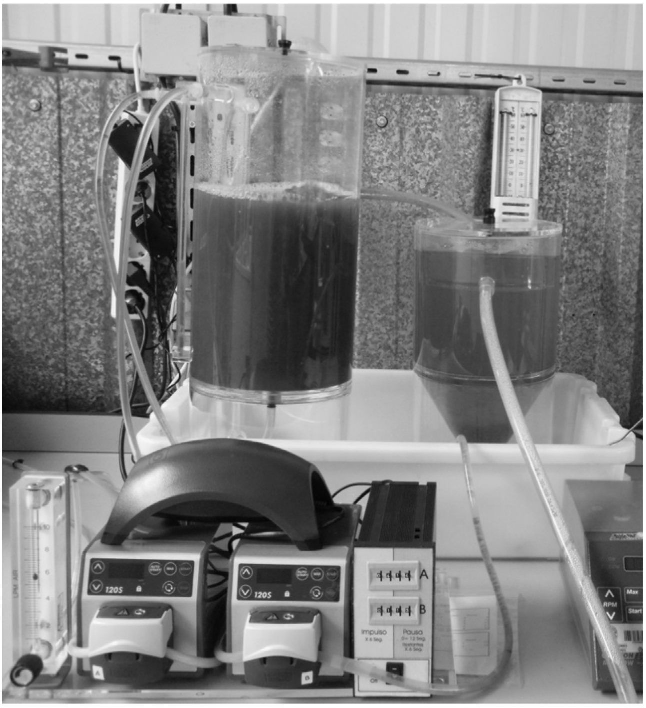

Fig. 1. Equipment of biological treatment with activated sludge.

The experimental design, analysis of variance (ANOVA) and 3D response surface were carried out using the software Statistica v7.0 (Statsoft Inc.). Eq. (2) describes the regression model of the present system, which includes the interaction terms

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 + \beta_{11} x_1^2 + b_{22} x_2^2 \tag{2}$$

where Y is the predicted response, i.e. the DOC removal; x_1 and x_2 are the coded levels of the independent factors, hydraulic retention time and initial wastewater DOC. The regression coefficients are: β_0 the intercept term; β_1 and β_2 the linear coefficients; β_{12} the interaction term and β_{11} and β_{22} the quadratic coefficients. The model evaluates the effect of each independent factor on the response.

3. Results and discussion

The fish canning industry is known to have wastewaters with different characteristics according to the type of fish being processed and to the individual streams produced: cooking effluents, effluents generated during the sterilization, washing effluents, etc. Wastewater characterization is a critical factor in establishing a corresponding effective management strategy or treatment process. Thus, in order to obtain a representative set of information of fish canning industrial wastewaters, several samples were collected and characterized during almost one year. Table 3 presents the seasonal variability of a fish canning wastewater regarding the initial organic matter content (DOC_i) and total soluble nitrogen ($N_{total\ soluble}$). The performance of the biological treatment of this kind of wastewater by activated sludge was optimized as regards the initial DOC and hydraulic retention time by applying a factorial design with two factors and three levels (Table 1).

Before conducting the biological treatment studies themselves, as referred above, an acclimatization and growing period of the activated sludge used as inoculum was carried out in batch mode during 20 days, in order to obtain a biomass concentration in the reactor greater than 1500 mg VSS/L.

Table 1
Factor levels for a 3^2 factorial design.

Factors	Parameters	Coded level		
		1	**0**	**−1**
X_1	HRT (h)	8.10	6.15	4.20
X_2	DOC_i (mg/L)	800	500	200

Table 2
Factorial design matrix with experimental results and predicted values for fish canning wastewater DOC removal by activated sludge.

Run	Factors		DOC removal (%)	
	X_1 (h)	X_2 (mg/L)	Actual value	Predicted value
1	4.20	200	39.7	37.3
2	4.20	500	38.7	42.6
3	4.20	800	31.5	30.0
4	6.15	200	82.2	82.9
5	6.15	500	86.2	86.5
6	6.15	800	73.3	72.3
7	8.10	200	57.2	58.8
8	8.10	500	64.9	60.7
9	8.10	800	42.3	44.8

Table 3
Seasonal variability of initial DOC and $N_{total\ soluble}$ parameters of a fish canning wastewater.

Collection date	$DOC_{initial}$ (mg/L)	$N_{total\ soluble\ initial}$ (mg/L)
29/02/2012	734.8	ND[a]
13/03/2012	620.1	312.4
20/03/2012	659.9	269.4
23/04/2012	968.4	470.8
08/05/2012	317.1	156.0
17/05/2012	434.2	210.7
22/05/2012	984.3	400.4
04/06/2012	265.5	281.6
12/06/2012	272.0	240.0
19/06/2012	165.1	125.5
10/07/2012	716.7	343.1
17/07/2012	569.7	248.9
31/07/2012	739.5	338.6
07/08/2012	363.4	147.9
13/08/2012	363.0	257.0
24/08/2012	753.0	259.3
10/09/2012	166.0	96.6
18/09/2012	181.2	21.2
25/09/2012	768.2	268.7
07/11/2012	407.3	145.9
21/11/2012	672.0	261.6
10/12/2012	153.0	37.0
23/12/2012	547.0	131.0

[a] ND: Not detected

Once reached this value, the operation was shifted to continuous mode. For the further studies, it was considered that the biomass concentration in the biological reactor was kept constant and also that the reactor was perfectly mixed, i.e., the substrate and biomass concentrations were constant over the entire reactor. The condition of perfect mixing was supported with dissolved oxygen measurements in different points inside the reactor. The excess of biological sludge produced was removed from the secondary settler and the remaining sludge was recirculated into the reactor to maintain approximately constant the biomass concentration in the reactor ($\approx$ 3000 mg VSS/L).

The results from the 9 experiments are presented in Table 2. There was no test replication due to the nature of the real industrial wastewater with high temporal variability: samples taken at different times may have very different compositions. Using the experimental data, the second order polynomial model (Eq. (2)) was fitted to these results and the following equation was obtained in terms of coded factors:

$$Y = -313.4 + 118.8x_1 + 0.099x_2 - 9.17x_1^2 - 0.003x_1x_2 \tag{3}$$

The DOC removal results predicted by the model presented above, at each experimental point, are presented in Table 2. The quadratic model, including linear interactions, fitted adequately to the experimental data giving a coefficient of determination, R^2, of 0.9846. The model shows to be adequate for the prediction of DOC removal within the range of factors chosen. The value of adjusted R^2 for Eq. (3) is 0.9590 and suggests that 95.9% of the COD removal total variation can be attributed to the independent variables and only about 4.1% of the total variation cannot be explained by the model. The closer the values of adjusted R^2 to 1 are, the better is the correlation between the experimental and predicted values [20,21].

The significance of each coefficient was determined through a *p value* test, considering 95% of confidence, in which low *p values* ($p < 0.05$) indicate a high significance of the corresponding coefficient. The Pareto chart displays the statistically relevant effect of each factor on the response and it is a practical mode to view the results. These are sorted from the largest to the smallest, and the effects to the right of the divisor line (that corresponds to a *p value* of 0.05) are considered statistically significant. From Fig. 2, it is possible to observe that the significant factors are only the quadratic and the linear terms of HRT parameter and all the other terms are to the left of the divisor line, having, this way, a much smaller influence.

The statistical significance of the estimated effects was evaluated by ANOVA. The ANOVA results (Table 4) show that the model adequately describes the DOC removal of a fish canning industry wastewater by activated sludge and it is possible to observe that the factors that most influenced the DOC removal are the quadratic and the linear terms of HRT, as already was verified by the Pareto chart, being the quadratic term of HRT the most significant, since it has the lowest *p-value*. The linear and the quadratic terms of initial concentration of DOC parameter seem to slightly affect the DOC oxidation too, but the interaction between both factors was shown to be almost statistically insignificant. These verified influences are in accordance with the expected. Taking into account the substrate (DOC) balance to the biological reactor, it is possible to verify that the substrate removal efficiency increases with HRT (and then with the square of HRT). On the other hand, the food to microorganisms ratio (F/M) is defined as following:

$$F/M = (Q.\text{DOC}_i)/(V.\text{VSS}) \tag{4}$$

where F/M is food to microorganisms ratio (g DOC/ (g VSS.d)), Q is the wastewater flow rate (L/h), V is the reactor volume (L), VSS represents the biomass concentration in the reactor (mg VSS/L) and DOC_i is the concentration of the wastewater soluble substrate (mg DOC/L). The substrate removal efficiency decreases when the ratio (F/M)/DOC_i increases, which means that the increase of HRT (and thus of F/M) can originate a negative effect on the efficiency, depending on the substrate concentration (DOC_i) of the wastewater to be treated. The combined effect of the substrate removal kinetics and ratio (F/M)/DOC_i can explain the existence of a maximum in the variation of the efficiency with HRT. Since the value of DOC_i also affects the F/M ratio the variation of the substrate removal efficiency with DOC_i is lower. Additionally, better reactor performances are achieved for F/M values within a given range, which explains the increase of the efficiency up to a maximum and a decrease for higher DOC_i values.

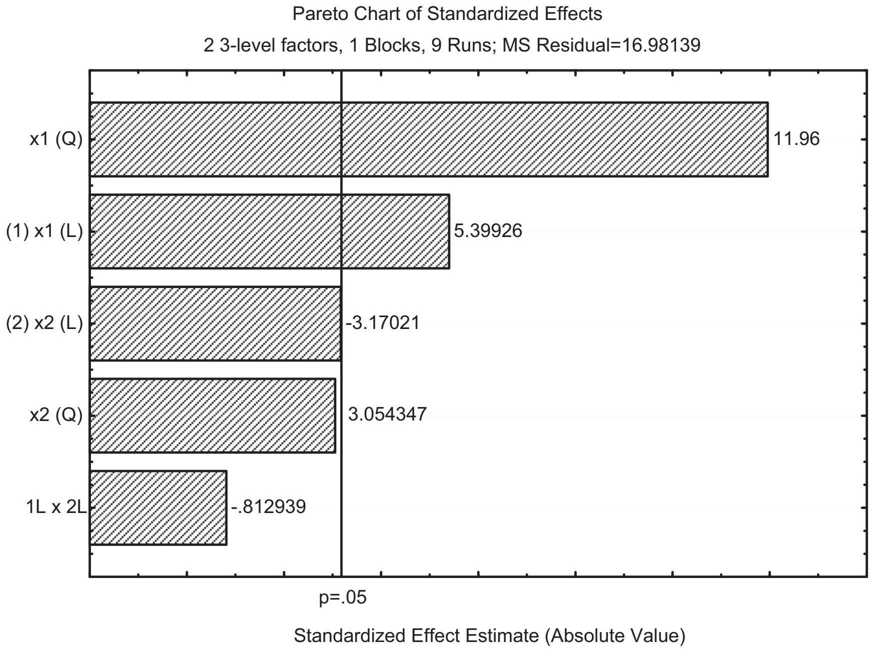

Fig. 2. Pareto chart of standardized effects for the 3^2 factorial design for DOC removal of a fish canning wastewater by activated sludge. (1) hydraulic retention time; (2) initial DOC.

Table 4
Analysis of variance (ANOVA) for the fitted quadratic polynomial model for optimization of DOC removal by activated sludge of a fish canning wastewater.

	Sum of squares (SS)	df	Mean square (MS)	*F*-value	*p*-Value
(1) x_1 (L)	495.0	1	495.0	29.2	0.012
x_1 (Q)	2429.0	1	2429.0	143.0	0.001
(2) x_2 (L)	170.7	1	170.7	10.1	0.05
x_2 (Q)	158.4	1	158.4	9.3	0.055
1 L by 2 L	11.2	1	11.2	0.7	0.476
Error	50.9	3	17.0		
Total SS	3315.3	8			

R^2=0.98463; adj R^2=0.95902.
df: degrees of freedom.
L: linear.
Q: quadratic.

Using RSM, the effects of the independent factors (HRT and initial DOC) and their interaction on the DOC removal are known, the response can be predicted and the optimum values of DOC oxidation can be determined. The response surface plot (Fig. 3), generated from factorial design, shows the DOC removal of a fish canning wastewater as function of the two factors in study. By this plot is then possible to observe that, in addition to the HRT factor has shown to be the most important factor affecting the DOC removal, the initial DOC parameter also has a little influence on the response. As it can be seen, the optimum conditions for obtaining the maximum degradation are within the experimental ranges tested: the DOC removal increases with the increase of both HRT and initial DOC parameters up to, approximately, 6.4 h and 400 mg/L, respectively. From these values the removal begins to decrease with the increase of both parameters. Maximum DOC removal was, then, observed at a HRT of 6.42 h and an initial DOC of 406.2 mg/L, predicting a DOC removal of 88.0%. Considering

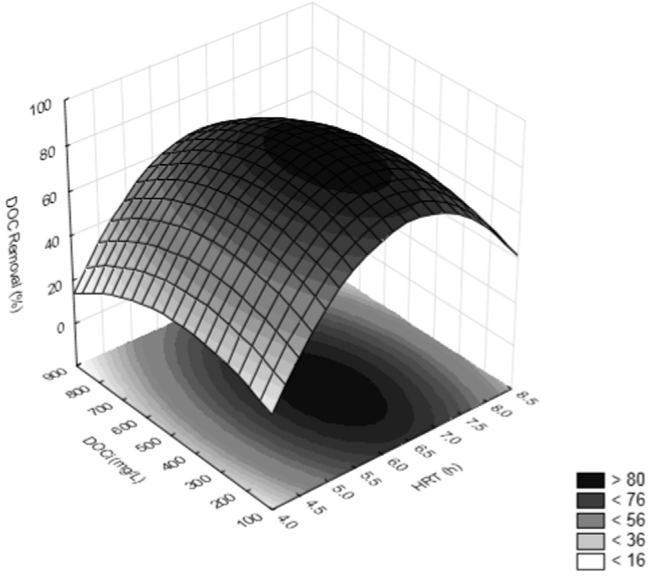

Fig. 3. Response surface plot for DOC removal of a fish canning wastewater by activated sludge as a function of hydraulic retention time and initial DOC.

that the biomass concentration in the reactor is about 3000 mg VSS/L, F/M is approximately 0.5 g DOC/(g VSS.d). As observed, the optimum HRT is within the upper values, which is consistent with results already observed in other studies [22,23] that show that the best removals of organic matter are obtained for longer hydraulic retention times, since the activated sludge is in contact with the wastewater for a longer time. Regarding the optimal value of initial effluent DOC, since these types of industrial effluents have a high variability, this optimal value may help in the decision of sending the effluent directly to the biological treatment or if it will be better to concentrate or dilute it with other effluents or even washing waters.

Other biological treatment processes for fish processing wastewaters were already studied by other researchers. Najafpour et al. [24] studied the treatment of a fish canning industry wastewater by a rotational biological contactor reaching between 85 and 98% of COD removal. Mendez et al. [25] studied the organic content removal of a tuna cooking wastewater by anaerobic digestion achieving a removal of COD of 80%. An integrated bioprocess for the treatment of tuna processing liquid effluents was also designed by Achour et al. [26], which included a physical pre-treatment unit used to remove the fats and the suspended solids through a decanter, an anaerobic digester and an activated sludge aerated bio-reactor. This integrated system, combining the three steps, allowed the removal of up to 95% of the COD with minimal energy consumption and minimal sludge production. Lim et al. [27] also studied the treatment of a fish processing wastewater but with a co-culture of *Candida rugopelliculosa* and *Brachionus plicatilis*. However, the maximum reduction of influent soluble chemical oxygen demand concentration was 70%. The comparison of these studies results with the optimal removal obtained in this work, allows confirming the feasibility and the reliability of fish canning wastewater treatment by activated sludge for organic content removal.

4. Conclusions

Activated sludge was tested for biological treatment of fish canning industrial wastewaters. The statistical experimental design and response surface methodology were found to be efficient tools to optimize some parameters. The optimum conditions in the ranges studied were found to be a

HRT of 6.42 h and an initial DOC of 406.2 mg/L, being the HRT the factor that most affected the DOC removal of the effluent.

This work showed the feasibility of using an activated sludge biological treatment to reduce significantly (88%) the organic content present in fish canning industrial wastewaters. Depending on the purpose for the treated water, it might or might not be necessary further polish treatment.

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