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Employment of factorial design to evaluate the organic loading and aeration of biological systems in the degradation of dairy wastewater

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ABSTRACT

Among industries producing foodstuffs, the dairy industry is notable for producing great amount of wastewater and high water consumption. Organic loading treatment based on biological degradation is a preferential treatment for dairy wastewater and effluents. However, it has some limiting factors for broader application due to the organic loading variation that results in an overload and wash-out of biological treatment systems. Considering these limitations, the organic loading and oxygen supply as factors in the rated efficiency of biological treatment systems with results expressed as removal efficiency of chemical oxygen demand ($\%COD_{removed}$) were evaluated. The variables investigated were: 1) initial concentration of organic load [raw, diluted 1:1 v/v (wastewater/distilled water) and diluted 1:3 v/v (wastewater/distilled water)] and 2) aeration (with or without). A Doehlert factorial design type (2x3) was employed for these studies. Aerobics systems with organic loading at a 1:3 ratio (v/v dairy effluent /distillated water) showed elevated efficiency in biodegradation (88.31 ± 2.16 $\%COD_{removed}$). The least efficient biodegradation was observed in anaerobic system for raw dairy effluent equal to 10.42 ± 3.97 $\%COD_{removed}$. This indicated that a dilution of effluent organic loading was necessary in obtaining greater efficiency from a biodegradation system and low hydraulic retention time.

Keywords: dairy; wastewater; effluent; biodegradation; design of experiment.

Emprego de planejamento fatorial para avaliação da carga orgânica e aeração de sistemas biológicos na degradação de efluente lácteo

RESUMO

As indústrias de alimentos do setor de laticínios estão entre as mais poluentes que há em termos de volume de resíduos líquido gerado e pelo alto volume de água consumido. Neste contexto, os processos de degradação de matéria orgânica baseados em sistemas biológicos continuam sendo uma das opções mais promissoras para a redução da carga orgânica dos efluentes lácteos. Entretanto alguns fatores inerentes ao efluente podem levar a sobrecarga dos sistemas biológicos de tratamento e consequente ineficiência de tratamento. Neste sentido, o objetivo dessa investigação foi avaliar a influência de diferentes cargas orgânicas [efluente bruto; diluído em 1:1 (efluente lácteo: água destilada); e diluído 1:3 (efluente lácteo: água destilada)] em sistemas de tratamento biológico aerado e não aerado. Para avaliação e otimização dos sistemas empregou-se um planejamento fatorial do tipo Doehlert (2x3). Os resultados foram apresentados em termos de eficiência de remoção da demanda química de oxigênio (%$DQO_{removido}$) e verificou-se que os sistemas aeróbios com diluição 1:3 v/v (efluente lácteo / água destilada) apresentaram maior eficiência de degradação (88,31 ± 2,16 %$DQO_{removido}$), enquanto que os sistemas anaeróbios com efluente bruto apresentou menor eficiência na redução da matéria orgânica, expressa em termos de %$DQO_{removido}$, igual a 10,42 ± 3,97%. Este fato evidenciou a necessidade de diluição com o intuito de elevar a eficiência do sistema de biodegradação e redução do tempo de retenção hidráulica.

Palavras-chave: laticínios; resíduos líquidos; efluente; biodegradação planejamento experimental.

1. INTRODUCTION

The primary constituents of dairy wastewater are variable amounts of diluted milk and floating solids (mainly lardy substances) originating from a variety of sources as detergents, lubricants, and domestic sewage (Braile and Cavalcanti, 1993). The quantity and pollutant-load of wastewater from dairy production varies widely depending on 1) the amount of water used during processing, and 2) the controls on various waste discharges (Braile and Cavalcanti, 1993; Faria et al., 2004; Alturkmani, 2007). The by-products resulting from the production process for milk and other dairy products are whey, buttermilk and sour milk (Gillies, 1974, Faria et al., 2004; Panesar et al., 2007).

Whey is the most important derivative of these by-products in relation to composition: 1) the volumes produced, 2) the characteristic of raw milk quality in dairy production, and 3) elevated pollutant factors (Faria et al., 2004; Panesar et al., 2007). The disposal of whey is one of the biggest problems facing by dairy industry in many parts of the world (Tawfik et al., 2006; Panesar et al., 2007; Pattnaik et al., 2008; Vourch et al., 2008).

In Brazil, the marketing and reuse of this component are underdeveloped. In the first half of 2006, a total of 52,786 tons of dairy products were exported. From this quantity, only 5,149 tons corresponded to the sale of serum, representing less than 10% of total exports and less than 0.1% of that is generated and used to supply the internal market (Embrapa, 2008). Just an insignificant portion of the serum is employed as animal feed supplements. Serum is rarely recylcled and represents a problematic waste for treatment in dairy industries (Faria et al., 2004). Janczukowicz et al. (2008) evaluated the effectiveness of the dairy effluent biodegradability obtained from different sections of processing sequences. The results confirmed that all the wastes coming from the production process are collectively treatable in a single system of biological degradation, with the exception of the produced serum. The biodegradation of this complex waste can cause a diversity of overloads for any liquid-waste management technologies and, therefore, requires the monitoring and installed metering (Janczukowicz et al., 2008).

In an aerobic process the stabilization of wastewater is promoted by aerobic and facultative microorganisms, whereas in an anaerobic process, facultative and anaerobic microorganisms execute the degradation (Mendes, 2004). Aerobic reactors may be an activated sludge, biological filter or aerobic stabilization pond (Clark, 1962; Mendes, 2004; Pattnaik et al., 2008; Vourch et al. 2008). The successful implementation of each is related to prior knowledge about the physical and chemical characteristics of the effluent. As noted by Mendes (2004), "Anaerobic processes are conducted mainly by bacteria and *archaea* (a class of bacteria that survive in extreme environmental conditions)." Generally, the efficiency of organic matter conversion into stabilized and/or mineralized products will depend on the:

i. Efficiency of each reaction, and

ii. Equilibrium among different bacterium species in the anaerobic system´s microflora. In contrast with the aerobic processes, the anaerobic processes require no artificial aeration equipment and any amount of generated biogas (CH_4) can be used as fuel. The efficiency of the anaerobic system is, however, frequently below values observed in optimized aerobic systems.

Recent studies reported the use of hybrid systems for dairy wastewater treatment, where one of the modules is based on the biodegradation of organic matter (Lansing and Martin, 2006; Tawfik et al., 2006). Tawfik et al. (2006) proposed a hybrid system of treatment consisting of an upflow anaerobic sludge blanket (UASB) with a retention time of 24 h followed by an activated sludge system for the simultaneous treatment of domestic waste and milk. The initial loads ranged from 1.9 to 4.4 kg.COD/$m^{3.}$ The authors related a reduction of the COD_{total} and BOD of 69% and 79% respectively. Bae et al. (2003) proposed a hybrid system composed of a batch reactor, based on biological system coupled with a sequential reverse-osmosis membrane for the removal of nutrients. After this adaptation the aeration system was unable to maintain consistent operating conditions for more than 110 days when using a single membrane. The BOD removal was 97% to 99%. A COD removal-efficiency and process were around 80% when the system was optimized.

Ecological treatment systems (ETS) are composed of a series of anaerobic and aerobic reactors, clarifiers and wetlands, and have been used to remove nutrients from domestic and industrial effluents. Lansing and Martin (2006) noted this model system as capable of removing nutrients with positive performance via the incorporation of aerobic and anaerobic systems and different hydraulic retention times between media. Lansing and Martin (2006) obtained a system of continuous treatment with the capacity to treat effluent from milk at 1,310 L/day, but at a hydraulic retention time exceeding 20 days.

Despite the elevated efficiency of ETS in the removal of nutrients, the capacity of ETS to treat dairy wastewater containing different organic loads was not established prior to 2008. Morgan and Martin (2008) investigated the impact of dilution as a factor for ETS system efficiency and the organic loading as a factor in treating dairy wastewater. Three dilution volume ratios were used (dairy-wastewater/domestic-effluent): 1:3, 1:1 and 2:1. The efficiency was given in terms of reduction of inorganic nitrogenous and BOD values. Morgan and Martin (2008) observe that a medium with high organic load had a higher hydraulic retention time for aerobic module. The effects of seasonality on the ecological system in treatment have not been sufficiently investigated.

Organic load as a factor in treatment efficiency not based on biodegradation was investigated in published papers as per different technological systems in dairy waste treatment (Mendes, 2004; Pan et al., 2006; Villa et al., 2007; Banu et al., 2008; Salazar and Izário Filho, 2009). In 2007 Villa and co-workers could evaluate the efficiency of the process photo-Fenton/solar in reducing the organic load of dairy wastewater by total organic carbon (TOC) measurements. By analyzing the process efficiency for different organic loads (TOC 350, 2,500 and 5,000 mg/L) observed the reduction of TOC in 90.86 and 50%, respectively, for a radiation time of 3.5 hours.

The optimization and evaluation of parameters on these different systems in most investigation cited previously were used a univariate analysis. Sometimes statistical tools facilitating better comprehensions are required to show developmental and optimization of treatment systems. This is supported by chemometric concepts (area specifically intended for analysis of multivariate chemical data of nature). Using chemometric tools facilitates the identification of potential problems such as baseline or interfering in calibrations and predictions from new samples in statistical models (Brereton, 2000; Hopke, 2003). The development of analytical equipment and chemical processes have led to a need for advances in methods of design of experiments in order to rapidly obtain reliable data for calibration and instrumental analysis of process efficiency (Bruns et al., 2003; Hopke, 2003). Hence, there are few studies in the literature that report optimizing parameters in experiment design in different dairy wastewater treatment systems (Salazar and Izário Filho, 2009).

Experiment design is defined as a set of statistical techniques applied to planning, conducting, analyzing and interpreting controlled tests to assess and define factors influencing the values of a specific parameter or a group of parameters (Bruns et al., 2003; Peixoto et al., 2008). The basic principle uniformly alters all levels of all variables, discrete or continuous, in a planned and rational way, thus reducing the number of experiments without limiting the number of factors to be analyzed (Peixoto et al., 2008). The use of full factorial designs is needed when evaluating the influence of variables without running the risk of excluding factors or interactions that may be important (Bruns et al., 2003). The screening of variables in a process is customary in fractional factorial design applications and produces considerable cost-effectiveness (Bruns et al., 2003). This paper is a report on the evaluation of factorial design in organic loading and aeration efficiency for biological systems in the degradation of dairy wastewater.

2. MATERIAL AND METHODS

Extractions of 20.0 L of homogenous effluent from a dairy in the city of Lorena, São Paulo, were collected and stored under refrigeration at 4 °C in the Escola de Engenharia de Lorena (EEL / USP).

Experiments were conducted in order to evaluate the effect of different organic loads and the effect of aeration in the media employing a multivariate design (Doehlert: 2x3) (Figure 1). The objective was to optimize various conditions of the experimental design for aerobic pond system proposed (Table 1). The initial volume of sludge added to the systems was 3.0 mL (~ 3.0 mg of sludge). The initial concentration of sludge in each system was 6.0 mg/L. The efficiency of the process was also performed in terms of $\%COD_{removed}$. The system temperature was maintained at 30 ± 3 °C. Observations of the final degradation of the effluent were made after 72hours. After this period the concentration of organic load remained constant (Figure 2). Active sludge was collected previously from the treatment wastewater plant of EEL-USP for these observations.

Figure 1. Evaluation of different organic load and oxygen supply over organic matter biodegradation.

Figure 2. Microscopy of active sludge used in tests for organic loading and oxygen supply on the biologic treatment (20X of zoom).

The process of biological treatment with activated sludge was conducted in Erlenmeyer flasks with a nominal capacity of 0.5 L and an air diffuser in each reactor (Q_{ar} = 0.75 L/min). The dairy wastewater used was diluted with distilled water (1:1 v/v).

2.1. Statistical tolls for planning, conduct, analysis and interpretation of experiments

A Doehlert factorial design type (2x3) was used in the analysis of influences on concentration of organic loading and aeration in biological treatment. The variables investigated were: 1) initial concentration of organic load [raw, diluted 1:1 v/v (wastewater/distilled water) and diluted 1:3 v/v (wastewater/distilled water)] and 2) aeration (with or without) (Tables 1 and 2). The response variable was the $\%COD_{removed}$. The methodology *5220 D. Closed Reflux, Colorimetric Method for Chemical Oxygen Demand* of APHA-AWWA was adapted to COD determination in dairy wastewater and more details can be acquired at Salazar et al. (2010).

Table 1. Multivariable design of experiment (Doehlert: 2x3) employed to evaluate oxygen supply and organic load over biologic treatment.

Factors	**Level**		
	- 1	**0**	**+ 1**
Aeration (0.75 L/min)	with	--	without
Organic load concentration (v/v)	raw effluent	1:1	1:3

Table 2. Experiments conditions employed in the multivariable design of experiment (Doehlert: 2x3) – Evaluation of organic load and oxygen supply over biologic treatment efficiency.

Experiment	Factors	
	Aeration	Organic load
1	-1	-1
2	-1	0
3	-1	+1
4	+ 1	-1
5	+ 1	0
6	+ 1	+1

Statistical calculations were done using an Excel 2003 spreadsheet for ease of organization, simplified algebraic calculations and data export. The statistical software MINITAB ® Release 14.12.0 - Statistical Software, 2004, was used for the proposition factorial design in assessing the significance of factors studied.

3. RESULTS AND DISCUSSION

Before discussing the results, it is important to emphasize that we sought the repeatability of results while maintaining the minimum physical and chemical variation of the effluent in question (Villa et al., 2007). Paired batches of experiments were conducted over brief periods with a total of 12 tests under multivariate experimental design (Doehlert: 2x3). Duplicated efforts made estimates and calculations for Variance error of each individual observation possible (Bruns et al., 2003). This facilitated the evaluation of statistical significance on the effects and estimated influences of factors and interactions on the efficiency of degradation of biological treatment system. The results (Table 3) are reported as a percentage of COD removed.

Table 3. Results, in (%) $COD_{removed}$, of dairy effluent biodegradation, employing multivariable design of experiment (Doehlert: 2x3) (n = 2).

Experiments	**Factors**		**(%)$COD_{removed}$**				
	Aeration	**Dilution**	**1ª batch**	**2ª batch**	**Average**	**deviation**	**Rep.[1] (%)**
1	With	1:1(v/v)	69,78	67,21	68,50	1,82	2,65
2	Without	1:1(v/v)	18,80	13,85	16,33	3,50	21,44
3	With	*In Natura*	54,91	54,19	54,55	0,51	0,93
4	Without	*In Natura*	13,23	7,61	10,42	3,97	38,14
5	With	1:3(v/v)	89,84	86,78	88,31	2,16	2,45
6	Without	1:3(v/v)	28,76	21,89	25,33	4,86	19,18

[1]repeatibility

The data in Table 4 shows low deviation values between batches. Aerobic conditions showed a higher repeatability than anaerobic conditions, i.e. the values obtained in aerobic conditions showed lower values of relative standard deviation than those under anaerobic conditions. Thus, results for microflora in aerated and diluted activated sludge were similar assuming a control of the process variables as offsets values. It was observed that the aerobic system presented more percentage reduction of organic matter in dairy wastewater degradation than in the anaerobic one in relation to COD data obtained. From these experimental results, as per Doehlert guidelines, with significant values of statistical evaluation of whole process, first-order effects on the response investigated (%$COD_{removed}$) were evaluated (Figure 3).

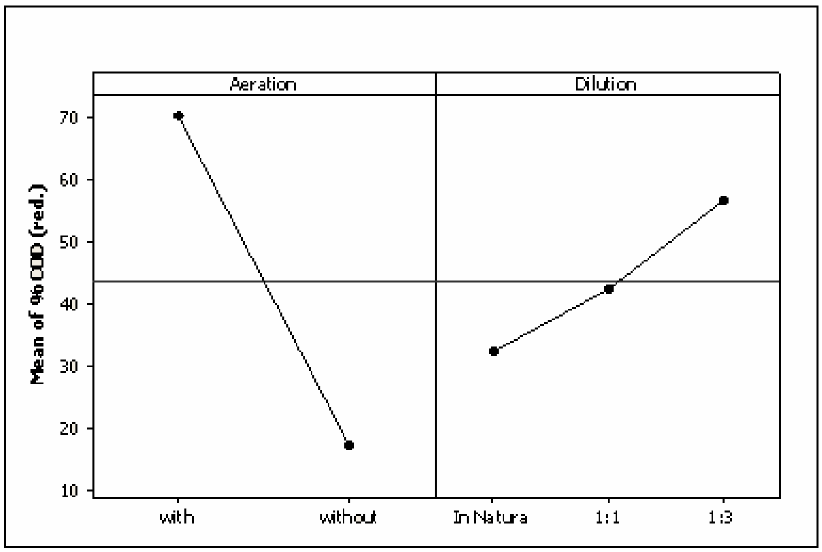

Figure 3. Effects of 1st order over %$COD_{removed}$ obtained from dairy effluent biodegradation employing multivariable design of experiment (Doehlert: 2x3).

A correlation was found between initial organic load and hydraulic retention time (HRTs) in relation to the aeration and dilution parameters per each condition from the design of experiments proposed. The highest initial organic load (*in natura*) required long reaction time to reach the same biodegradation efficiency at 50% of efficiency treatment. These effects were also relevant due to the effluent recalcitrance (different dilutions promoted different initial organic load for biodegradation) and the aeration system employed and as per the biodegradation mechanism used (aerobic and anaerobic). When Driessen and Yspeert (1999) evaluated the effect of organic loading on an anaerobic system over hydraulic retention time, they noted results similar to those found in this paper (the highest organic load system presented the highest hydraulic retention time). Such results were also reported by Morgan and Martin (2008), when employing ETS for the degradation of dairy wastewater with high phosphorus and nitrogen content. As a result, estimation of 2nd order interactions for the factors studied was possible (Figure 4).

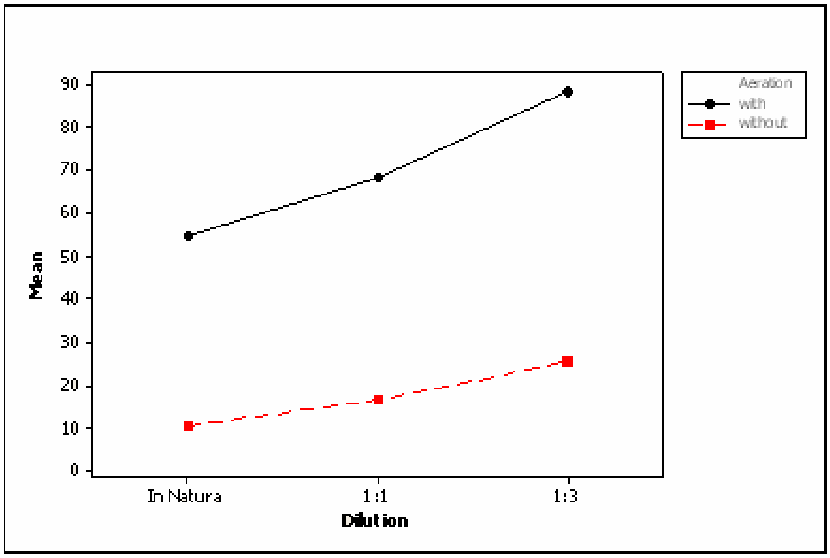

Figure 4. Effects of 2nd order over %$COD_{removed}$ obtained from dairy effluent biodegradation experiments employing multivariable design (Doehlert: 2x3).

The aerobic and more diluted system showed a significant effect on the rate of COD reduction in relation to the anaerobic system as per previous observations. This fact agrees with prior results. In order to evaluate the proposed biological system, a statistical approach of individual factors and their synergism based on analysis of variance (Table 4) was employed to determine the degree of confidence factors and respective interactions.

Table 4. Analyses of Variance for results of %$COD_{removed}$ obtained from dairy effluent biodegradation employing multivariable design of experiment (Doehlert: 2x3).

Source	DF[1]	QS[2]	MS[3]	F[4]	p[5]
Blocking or Batch	1	47.2	47.2	18.54	0.008
Aeration	1	8457.2	8457.2	3323.95	0.000
Dilution	2	1197.5	598.8	235.33	0.000
Aeration/Dilution	2	179.0	89.5	35.18	0.001
Error	5	12.7	2.5		
Total	11	9893.7			

[1]degree of freedom; [2]quadratic sum; [3]mean square; [4]Factor F; [5]p-value (degree of confidence).

Initially, significant statistical interactions between aeration and dilution were observed, as were factors of the first-order discussed previously. From this analysis, experimental deviations were attributed to the analytical sequence used (blocking or batch) with 99.2% confidence. The overall standard deviation was set at 1.595 and the adjusted square at 99.72%. These tests have strengthened the reliability of data obtained experimentally.

System degradation was efficient in anaerobic degradation of organic matter around 10-25% dependent upon oxygen supply methodology. Systems under aerobic conditions showed levels of COD reduction of around 70% and 90%, dependent on the dilution factor. This characteristic behavior of a biological treatment may have occurred because of the nature of the effluent used for tests where aeration and dilution factors improve the degradation by the microbial flora. Perle et al. (2000) analyzed the biochemistry aspects of anaerobic degradation of a dairy wastewater and reported inhibitory effects in biodegradation in relation to the presence of casein and especially regarding the levels of milk lipids. Much has been researched for the development of systems for treatment of dairy wastewater using UASB reactors; however, the effects of inhibition caused by levels of lipids and casein (low efficiency) are often discussed in the literature (Perle et al., 2000; Demirel et al., 2005; Umaña et al., 2008).

The dilution parameter was also significant for the reduction of COD values in the experiments under anaerobic conditions, but with much lower values when compared with those experiments in aerobic conditions. The effluent diluted in a ratio of 1:3 v / v under anaerobic conditions was observed the formation of filaments, featuring a rich flora of bacteria and fungi (Figure 5). Filamentous fungi structure may hinder the settling of the sludge, reducing the efficiency of the biodegradation process. The incidence and prevalence of this type of flora are favored under anaerobic conditions, as microorganisms and facultative anaerobes metabolize carbohydrates present in the environment, converting them into products such as organic acids, carbon dioxide and water (Campbell, 2000; Voet et al., 2002). These products have led to reduction of pH in anaerobic conditions, where the final pH was 5.16 ± 0.02. Similar behaviors were observed, but formation of filaments was not observed, corresponding to anaerobic systems employing dilutions of 1:1 v / v (dairy wastewater/distilled water) and raw effluent, where the final pH values were 4.93 ± 0.02 and 5 05 ± 0.01, respectively. The initial pH measured in each system and condition – aerobic and anaerobic – comprised between 10.25 ± 0.51.

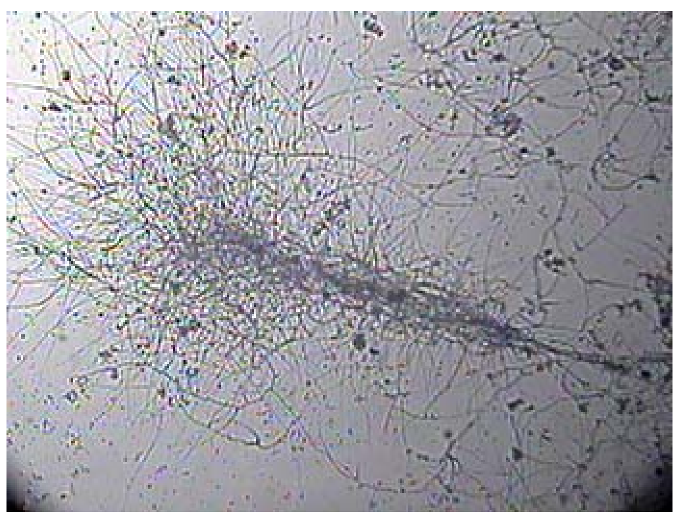

Figure 5. Microscopy of microbial flora under anaerobic condition and diluted effluent diluted to 1:3 v/v (zoom of 10 times).

It was observed that microbial flora was less dispersed under aerobic conditions than the anaerobic microbial flora was. The presence of microorganisms and rotifers (classified as protozoan) served as an indicator of sludge quality, explaining the elevated efficiency of degradation of organic matter in the effluent (Figure 2). Protozoan flora showed higher degradation performance than bacterial flora observed in anaerobic conditions.

4. CONCLUSION

Greater efficiency of biodegradation was observed for the aerated treatment systems. There was less hydraulic retention time for the aerobic systems with dilution 1:3 v / v (dairy wastewater/distilled water), resulting in a $\%COD_{reduced}$ of 88.31% ± 2.16%, representing a reduction of COD of 3342. 2 ± 184.2 $mg.L^{-1}$ to 390.6 ± 32.2 $mg.L^{-1}$. The evaluated factors were significant for adjusting the efficiency of degradation of organic matter present in the samples.

Chemometric tools and statistical software facilitated planning, experimental processes, analysis and interpretations of the biological assays. With multivariate planning (Doehlert: 2x3) was possible to evaluate and optimize the effect of organic loading and aeration on the efficiency of biological degradation. Factorial design allowed for estimates of the importance and value of each of the factors investigated in efficiency degradation and interactions under multivariate analysis with greater repeatability than those obtained using univariate analysis.

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