

Assessment of toxicity in industrial wastewater treated by biological processes using luminescent bacteria

Evaluación de la toxicidad en aguas residuales industriales tratadas por procesos biológicos utilizando bacterias luminiscentes

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Abstract

The toxicity of wastewater from a meat by-products processing company was evaluated before and after treatment using the Sequencing Batch Reactor (SBR). Toxicity tests were carried out by analyzing the inhibitory effect of samples in relation to light emission from marine bacteria of the species *Vibrio fischeri*. The results found that the effluents prior to treatment were highly toxic ($EC_{50} < 60\%$) whereas post-treatment results showed low or no toxicity ($EC_{50} > 82\%$). In some operational stages of the SBR reactor, a high correlation between the ammonia nitrogen present in each sample and the toxicity of wastewater from both the influents and the effluents was found, with correlations (R^2) of 0.6141 and 0.8158, respectively. As a consequence of these results, the SBR system can be considered efficient at removing organic matter, and nitrogen, and thereby decreasing toxicity in treated water.

Key words: ammonia nitrogen, organic matter, Sequencing Batch Reactor, toxicity, *Vibrio fischeri*

Resumen

La toxicidad de las aguas residuales provenientes de empresas de procesamiento de productos cárnicos fue evaluada antes y después del tratamiento usando un reactor secuencial por lotes (SBR). Las pruebas de toxicidad fueron evaluadas mediante el análisis del efecto inhibitor de muestras en relación con la emisión de luz a partir de bacterias marinas de la especie *Vibrio fischeri*. Los resultados demostraron que los efluentes antes del tratamiento eran altamente tóxicos ($EC_{50} < 60\%$), mientras que los resultados después del tratamiento mostraron baja o nula toxicidad ($EC_{50} > 82\%$). En algunas etapas operativas del reactor SBR, se encontró correlación entre el nitrógeno amoniacal presente en cada muestra y la toxicidad de las aguas residuales tanto en el afluente y los efluentes, con correlaciones (R^2) de 0,6141 y 0,8158, respectivamente. Como consecuencia de estos resultados, el sistema SBR puede ser considerado eficiente en la eliminación de materia orgánica y nitrógeno, y de ese modo disminuir la toxicidad en el agua tratada.

Palabras claves: materia orgánica, nitrógeno amoniacal, reactor secuencial por lotes, toxicidad, *Vibrio fischeri*

INTRODUCTION

The treatment of industrial wastewater is a highly complex process that generally involves factors associated with load fluctuations and high concentrations of organic matter. These factors are often due to inhibitors in biological processes that have not been properly introduced in the environmental or contaminants that have not been treated before being discharged into water reservoirs. The Sequencing Batch

Reactor system (SBR) is a technology for wastewater treatment that combines different cycles and stages of operation depending on the quality required of the effluent water. The most common stages are filling, reaction (aeration and mixing), sedimentation, draw off, and purge, all carried out within the same tank (Rodríguez *et al.* 2011a, b).

In recent years, the need to implement effective systems for the treatment of industrial effluents has been established

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(Dalzell *et al.* 2002, Rigopoulos and Linke 2002) in order to reduce toxic waste (Araújo *et al.* 2005). Toxicity is usually determined by the capacity of a substance to have an adverse effect on an organism and depends on the chemical properties of the compound and its concentration. It is also determined by the duration and frequency of exposure to the toxin, and furthermore, by the relationship of the substance to the organism's life cycle (Morales 2004).

Toxicity was evaluated before and after treatment to determine the operational efficiency of the SBR system (Gutierrez *et al.* 2002). The organisms most frequently used for toxicity testing are bacteria, fish, algae, *Daphnia*, and Rotifera. For trials of this nature these types of organisms have the advantage of presenting biochemical pathways similar to those of higher organisms. Furthermore, they have short life cycles and respond quickly to changes in the environment (Jennings *et al.* 2001). The most common application of this form of toxicity testing is related to the determination of lethal concentration (LC_{50}) and effective concentration (EC_{50}), where LC_{50} is the concentration of a compound that causes death in 50% of the organisms tested, while EC_{50} is the concentration causing adverse effects in 50% of the population (Onorati and Mecozzi 2004). Since 1979, the luminous bacteria *Vibrio fischeri* have been used for toxicity testing. This species is a Gram negative, facultative anaerobic bacteria of the Vibrionaceae with bioluminescence being its most representative characteristic (Araújo *et al.* 2005).

The Microtox toxicity test is based on the relationship between the reduction of light produced by these bacteria (Bennett and Cabbage 1992, Jennings *et al.* 2001) and the toxicity of the sample. The bioluminescence reaction of these bacteria is linked to the electron transport system in their cellular respiration and is indicative of the metabolic status of the cell. Therefore, a decrease in bioluminescence indicates a decrease in cellular respiration. Physicochemical pollutants all affect cell respiration by altering the rate of synthesis of proteins, and lipids and thus changing the level of luminescence emission (Onorati and Mecozzi 2004).

The results reported by the Microtox tests are presented as effective concentration (EC_{50}). However, in order to standardize the results and the correlation with physicochemical parameters from the Pearson index (Zar 1996), toxicity units (TU_{50}) were calculated using following formula (Araújo *et al.* 2005):

$$TU = \frac{100}{EC_{50} (15 \text{ min})}$$

In this way, toxicity can be classified as: *highly toxic* if $EC_{50} < 60\%$; *moderately toxic* if $60\% < EC_{50} < 82\%$; and *not toxic* if $EC_{50} > 82\%$ or when a decrease in luminance is not exhibited (Araújo *et al.* 2005, Lanciotti *et al.* 2004, Movahedian *et al.* 2005).

In this study, toxicity tests were conducted using the luminescent bacteria *V. fischeri*, industrial wastewater generated in a meat by-products processing company and the effluents of this water that were treated using an SBR system. The SBR system operated for 252 days, in eight different stages, in order to remove organic matter and ammonia nitrogen. The results are of great importance due to their contribution to the field of wastewater treatment at industrial level.

MATERIALS AND METHODS

Study area. A pilot-scale SBR system with a volume of 2.96 m³ (figure 1) was installed in the Agropecuaria San Fernando S. A. (AGROSAN) meat products processing company for the removal of both organic matter and nitrogen. The company is located in the southwest of the department (administrative region) of Antioquia, Colombia. It is engaged in the production of meat and bone meal and fat for meat by-product based animal feed (using viscera, feathers, blood, bone, and fat), from slaughterhouses, farms involved in the slaughter of poultry, and swine, and from other actors in the meat industry.

Wastewater. Two types of wastewater were studied in this work. The first was water from the washing of vehicles, equipment, barrels, and packages containing meat products. The second was from the condensate water generated when transforming raw materials into meal, and fat for animal feed through cooking. The average COD_T , COD_F , BOD_5 , TSS, VSS, $N-NH_4^+$, pH values for the washing water were $8308 \pm 1823 \text{ mg l}^{-1}$, $3922 \pm 1540 \text{ mg l}^{-1}$, $2684 \pm 1686 \text{ mg l}^{-1}$, $1711 \pm 974 \text{ mg l}^{-1}$, $1242 \pm 817 \text{ mg l}^{-1}$, $365 \pm 14 \text{ mg l}^{-1}$, 6.11 ± 0.40 , respectively, while for the condensate water they were $1381 \pm 484 \text{ mg l}^{-1}$, $822 \pm 215 \text{ mg l}^{-1}$, $563 \pm 219 \text{ mg l}^{-1}$, $7 \pm 3 \text{ mg l}^{-1}$, $6 \pm 3 \text{ mg l}^{-1}$, $616 \pm 129 \text{ mg l}^{-1}$, 9.64 ± 0.47 , respectively.

SBR operation. The steps undertaken by the SBR in each cycle were filling, reaction, sedimentation and emptying. Each full 8 hour cycle was divided into 6 hours for the reaction, which included, intermittent aeration (for a duration of 8 minutes), anoxia-mixture, (for a duration of 15 minutes), and 2 hours for the sedimentation phase. The

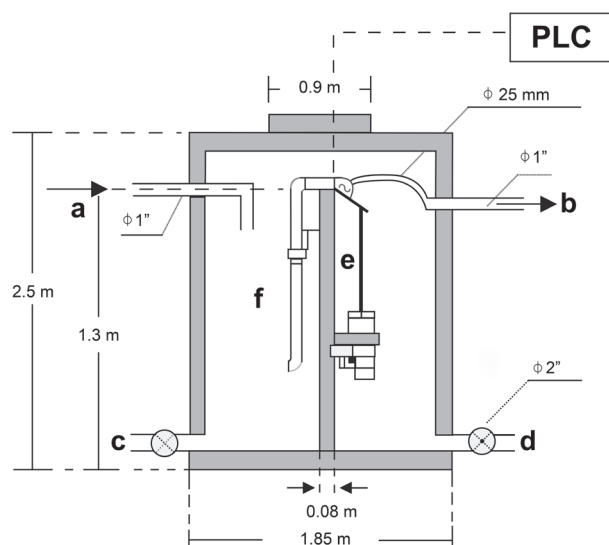


Figure 1. Sequencing batch reactor (SBR) with programmable logic controller (PLC) system for cycles and dimensions to: A. Tributary; B. Effluent; C. and D. Purge valves; E. SBR with aspiration tube for filling of the SBR-through Communicating Vessels; F. Homogenizer or coarse particle separator

experiments were divided into eight different stages that varied the mix of washing water, and condensate ratios (figure 2). The sludge retention time (θ_c) was 30 days, in order to enhance oxidizing bacterial growth.

Inocula. The reactor was inoculated with 1.0 g l^{-1} of VSS from a UASB reactor located in the same company, which was acclimated to high organic loads. The VSS had a Sludge Volume Index (SVI) of 16.8 ml g^{-1} indicating that it was a sludge with good sedimentability. It was also inoculated with 1.075 g l^{-1} of mixed liquor from the VSS of an activated sludge system from a municipal wastewater treatment plant, with a SVI of 142.97 ml g^{-1} , therefore being sludge with acceptable sedimentability (Rodríguez *et al.* 2011a).

Chemical analysis. In the experiments the following physicochemical parameters were determined: chemical oxygen demand (COD), complying with the established protocols of Standard Methods (APHA 2005); ammonium, (N-NH_4^+) using a Kjeldahl instrument (Büchi), and pH, employing a Shott handylab pH 11/SET.

Toxicity test. The toxicity was determined with a BioFix Lumi-10 (Macherey-Nagel) apparatus, using the marine bacteria *V. fischeri*. The procedure was performed in

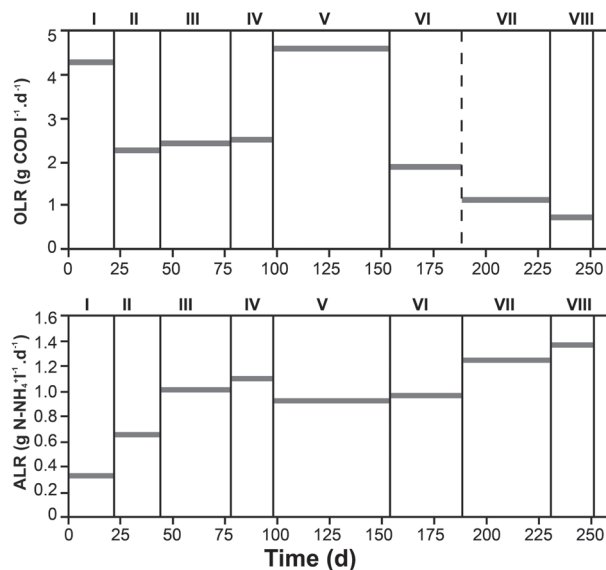


Figure 2. Organic loading rate (OLR) and ammoniacal loading rate (ALR) applied to the SBR system

accordance with that established by DIN ISO 11348-3 (Araújo *et al.* 2005). Readings were taken by measuring the emission of light for 15 minutes with different dilutions of wastewater and a suspension of luminescent bacteria prepared from a strain of lyophilized *V. fischeri*. The tests were carried out at 15°C using a thermostated Lumis therm block. For controlling bacteria *V. fischeri*, luminescence was measured in NaCl solution. Samples were taken from the influent and effluent of the SBR system in each of the eight stages, and were transferred to the laboratory at 4°C . The pH was maintained within the range of 6.0-7.0 and samples high in turbidity were centrifuged for 10 min at 5000 rpm.

Statistical analysis. Multidimensional analysis was performed to find correlations between the different parameters analyzed. Using the Statgraphics plus 5.1 software, and ANOVA analysis, the Pearson product moment correlations were obtained.

RESULTS AND DISCUSSION

The washing water showed an EC_{50} of 18.1% while the condensate water revealed an EC_{50} of 5.9%. These results indicated that both the washing water and the condensate are highly toxic ($\text{EC}_{50} < 60\%$), with the condensate being more so. These toxicity results are consistent with the characterization carried out on the two types of water,

which revealed that they had an average concentration of 365.14, and 615.54 mg l⁻¹ of N-NH₄⁺ and a pH of 6.11, and 9.64, respectively. According to the pKa, the ratio of NH₃ to NH₄⁺ depends mainly on pH, meaning that for washing water and condensate water the N-NH₃ concentrations were 0.324, and 532.67 mg l⁻¹. When free ammonia is greater than 0.2 mg l⁻¹ it causes mortality in several fish species (Anthonisen 1976), due to physiological damage associated with high concentrations of ammonium. It poses an environmental risk and because of this, the National Academy of Sciences (NAS) in the United States does not allow values higher than 0.02 mg l⁻¹. Therefore, the toxicity percentages found for the two types of water studied can be considered extremely high.

For the effluent in phases I, II, V, VII, and VIII (table 1), EC₅₀ values were of high toxicity, whereas phases IV and VI were not found to be toxic. In contrast, all influents showed high toxicity (stages I to VIII), with EC₅₀ percentages below 60% for all cases. The increase in EC₅₀ in the effluent at each stage indicates that the treatment system used is capable of removing substances regarded as being toxic to the environment, such as ammonium (Campos *et al.* 2008, Carrera *et al.* 2004, Pynaert *et al.* 2003).

The removal results are shown in figure 3, where the maximum removal of COD and N-NH₄⁺ occurred during phases IV and VI, with 98.7 and 97.6% for COD, and 70.5 and 60.9% for N-NH₄⁺, respectively. Furthermore, when the removal of toxicity was correlated with the results of the removal of COD and N-NH₄⁺, toxicity was reduced by as much as 88.7% in stage IV and in by 91.2% in stage I. Therefore, it is evident that the SBR system is efficient at treating industrial wastewater from the processes involved in the transformation of meat by-products, operating under different organic (OLR) and ammonia (ALR) loading rates.

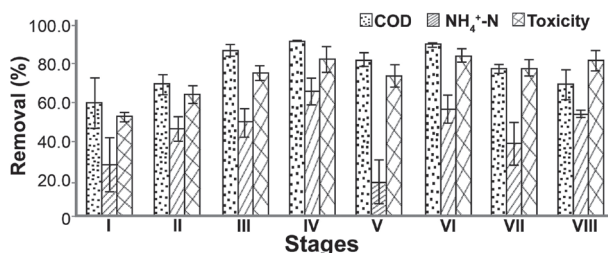


Figure 3. Removals of chemical oxygen dissolved (COD), ammonium (NH₄⁺-N) and toxicity in each of the eight stages of operation of the SBR (the bars indicate the standard deviations of each parameter)

Table 1. Effective concentration (EC₅₀) for influent and effluent at each stage of operation of the Sequencing Batch Reactor (SBR) (the samples where luminescence did not decrease were reported as > 82%; time = # of days)

Stage	Time	Effective concentration (EC ₅₀)	
		Influent (%)	Effluent (%)
I	7	17.0	34.1
	14	19.0	48.4
	21	18.2	44.2
II	28	15.0	42.0
	35	14.3	47.5
	43	14.0	51.6
III	51	13.0	71.0
	58	13.8	63.9
	65	13.5	78.6
	70	12.9	79.4
IV	84	10.8	> 82.0
	86	11.7	> 82.0
	93	11.4	> 82.0
V	100	10.2	50.0
	107	10.0	55.2
	114	9.7	35.4
	128	10.4	38.2
	133	9.3	65.0
	148	9.5	55.7
	149	9.8	46.9
VI	161	8.4	> 82.0
	163	8.2	> 82.0
	170	9.1	> 82.0
	177	8.3	> 82.0
	184	9.7	> 82.0
VII	189	9.2	> 82.0
	203	6.7	39.7
	210	6.9	40.6
VIII	231	6.4	42.6
	238	5.8	45.9
	245	5.6	57.6
	252	6.2	50.0

Toxicity unit (TU₅₀) values were correlated with the concentration of COD and N-NH₄⁺ for both influents and effluents. In the case of COD (figure 4), ANOVA analysis

shows an R^2 of 0.4995 with a P-value of 0.0507 and a confidence level of 90% for the influent of each stage. As for the effluents, the correlation was also low, with an R^2 of 0.4085, a P-value of 0.079, and a confidence level of 90% (figure 5).

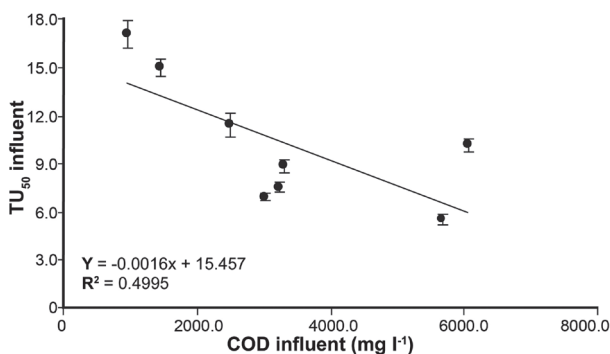


Figure 4. Correlation between toxicity units (TU_{50}) and chemical oxygen dissolved (COD) in the influent (results from ANOVA analysis)

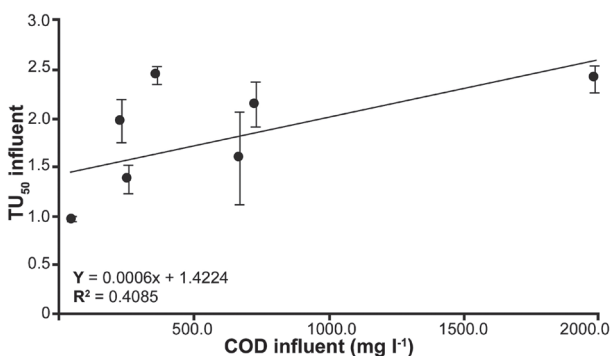


Figure 5. Correlation between toxicity units (TU_{50}) and chemical oxygen dissolved (COD) in the effluent (results from ANOVA analysis)

According to Araújo *et al.* (2005), wastewater generally has a broad mix of different components, which implies that the resulting toxic properties may vary depending on the interaction between each of them. This high variability may be responsible for the zero or poor correlation of COD and toxicity values. Furthermore, Boluda *et al.* (2002) note that in complex samples such as in the case of wastewater, COD cannot provide a good correlation with toxicity.

On the other hand, looking at the $N-NH_4^+$ in the influent (stage I to VIII), an R^2 of 0.6141 can be seen with a P-value of 0.0215 and a confidence level of 95% (figure 6). In the effluents (figure 7) the relationship was directly proportional ($R^2 = 0.8158$ and P-value = 0.0019 with

a confidence level of 99%). Thus, high concentrations of $N-NH_4^+$ caused increases in toxicity, while for low concentrations toxicities were low. Although the result is expressed as $N-NH_4^+$, in reality it is the ammonia that produces the toxicity. At $pH > pK_a$, ammonia strongly influences the toxicity of the water. As the pH decreases below the pK_a (9.1 at 28 °C), ammonia no longer influences toxicity. While the effect of temperature was not evaluated, it is possible to infer that an increase in this variable would have a positive effect on microorganisms as these better metabolize compounds as temperature increases.

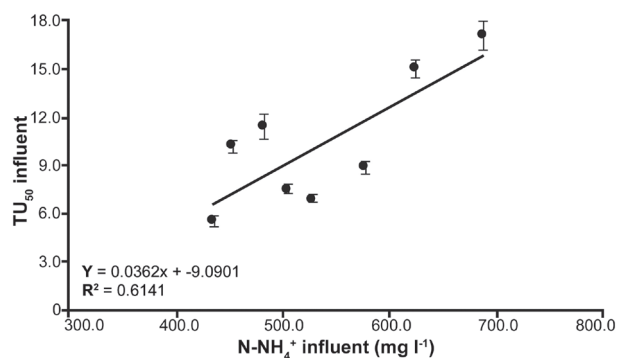


Figure 6. Correlation between toxicity units (TU_{50}) and ammonium (NH_4^+-N) in the influent (results from ANOVA analysis)

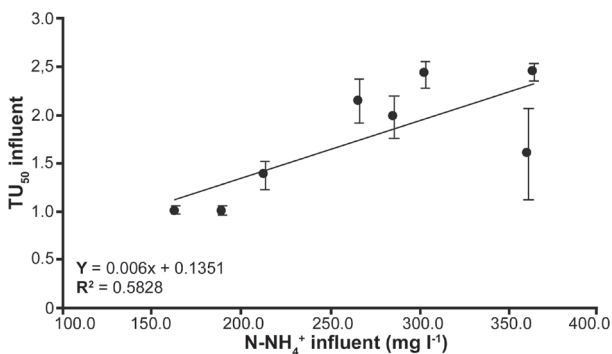


Figure 7. Correlation between toxicity units (TU_{50}) and ammonium (NH_4^+-N) in the effluent (results from ANOVA analysis)

CONCLUSIONS

The results indicated that both the washing water, and the condensate were highly toxic $EC_{50} < 60\%$, with the condensate being more so. However, in stages IV and VI where greatest ammonium removal occurred (70.5% and 60.9%, respectively), the EC_{50} in the effluents reached values greater than 82%, indicating effluents that were non-toxic.

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