UnilaSalle Editora
REVISTA DE CIÊNCIAS AMBIENTAIS - RCA (ISSN 1981-8858)

http://revistas.unilasalle.edu.br/index.php/Rbca

Canoas, v. 11, n. 2, 2017

http://dx.doi.org/10.18316/rca.v11i2.2765

TREATMENT OF DAIRY EFFLUENTS IN WETLANDS SYSTEMS WITH FLOATING AQUATIC MACROPHYTES

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ABSTRACT

The aim of this study was to evaluate the *Eichhornia crassipes* and *Salvinia auriculata* efficiency as bioremediation alternatives for wastewater from a dairy company in Toledo, Paraná. The experiment was performed between June and August of 2014, for 36 days in the greenhouse at PUCPR *campus* Toledo. It was used 750 liters of effluent from a dairy plant in Toledo-PR, arranged in three polyethylene reactors, operating intermittently (batch). The repetitions were performed according to the hydraulic retention time (HRT), every 4 days. It was evaluated 7 physicochemical parameters of the effluent (temperature, pH, turbidity, total solids, COD, total nitrogen and total phosphorus). The results obtained during the experiment were satisfactory, it was observed the maximum efficiency of turbidity, total nitrogen, and COD of 94.4%, 90.26% and 82%, respectively, with *E. crassipes* and 91.7%, 75.65% and 82% respectively for *S. auriculata*. However, from the period between the day 16 and the day 20 of the experiment, it was observed increases in the level of the parameters because some plants got into the senescent process, returning to the environment half of all that were absorbed. This result suggests the necessity of removal plants in senescence, so the system can work properly. The aquatic macrophyte *E. crassipes* was better biorremediator for dairy effluents than *S. auriculata*, however, both of them can be widely used in the treatment of wastewater with high levels of organic matter and nutrients.

Keywords: Water Hyacinth; Salvinia; Phytoremediation; Dairy Effluents.

RESUMO

Tratamento de efluentes de laticínio em sistemas de zonas úmidas com macrófitas aquáticas flutuantes. O objetivo deste trabalho foi avaliar a eficiência de *Eichhornia crassipes* e *Salvinia auriculata* como alternativas de biorremediação para águas residuárias provenientes de uma indústria de laticínios em Toledo, Paraná. O experimento foi realizado entre os meses de junho e agosto de 2014, durante 36 dias, na casa de vegetação da PUCPR *campus* Toledo. Foram utilizados 750 litros de efluente de um laticínio em Toledo-PR, disposto em três reatores de polietileno, operando de forma intermitente (batelada). As repetições foram realizadas em função do tempo de detenção hidráulica (TDH), a cada 4 dias, foram avaliados

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7 parâmetros físico-químicos do efluente (temperatura, pH, turbidez, sólidos totais, DQO, nitrogênio total e fósforo total). Os resultados obtidos durante o experimento foram satisfatórios e, observou-se eficiência máxima de remoção de turbidez, nitrogênio total e DQO de 94,4%, 90,26% e 82%, respectivamente, com *E. crassipes* e, 91,7%, 75,65% e 82%, respectivamente para *S. auriculata*, entretanto, a partir do período entre o 16º e o 20º dia de experimento, foram observadas elevações nos teores dos parâmetros, devido a algumas plantas entrarem em processo senescente, devolvendo ao meio tudo que absorveram. Tal fato sugere que a retirada das plantas saturadas durante esse tempo, para que o sistema funcione adequadamente. A macrófita aquática *E. crassipes* foi a que melhor se comportou como biorremediadora de efluentes de laticínio na remoção de contaminantes em comparação com *S. auriculata*, porém, as duas podem ser amplamente utilizadas no tratamento de águas residuárias com altos níveis de matéria orgânica e nutrientes.

Palavras-chave: Aguapé; Salvínia; Fitorremediação; Efluentes de Laticínio.

INTRODUCTION

The production of milk *in natura* and its derivatives in processing industries requires large volumes of water to be used in the processing and cleaning activities. Therefore, it generates a great amount of wastewater with high pollution potential due to the presence of high levels of organic matter, solids, fats and nutrients, which represent the main environmental impact related to this sector (Andrade, 2011). Due to these characteristics, the treatment of dairy wastewater favors biological processes (Nirenberg and Ferreira, 2005), which have the main function of stabilizing organic content, remove nutrients and persistent toxic substances (Cavalcanti, 2012).

The reduction of wastewater treatment systems efficiency ends up reflecting in the aquatic environment, because of the release of improperly treated wastewater, which may cause adversity in the environment by reducing its quality, eutrophication, the formation of anaerobic conditions in the bottom of the water body and fish mortality (Von Sperling, 1996).

According to Sales (2011), to minimize the environmental impacts of effluent discharge, it is necessary viable alternatives to reduce concentrations of nutrients and organic matter in order to prevent the eutrophication of them. In this paradigm, the wetlands arise, which are reputable as artificial systems designed in a way to use water plants or put them on a substrate layer, where biofilms that join several communities of microorganisms that treat wastewater by biological, physical and chemical ways are spread (Zeb *et al*., 2013).

Over the last few years, the aquatic macrophytes have been widely used for the decontamination of polluted environments, because of the potential of their roots to absorb toxic substances and use the nutrients in the effluent as energy for their metabolism, with satisfactory results in terms of removing these contaminants (Leitao Jr. *et al*., 2005). The water hyacinth, *Eichhornia crassipes* (Mart) Solms, is the most frequent species used for aquatic environments phytoremediation.

The plants used for this purpose are called aquatic macrophytes, which are plants whose photosynthetic parts are in contact with water during all or part of the year, besides of being a fundamental community in aquatic ecosystems due to its wide availability, rapid growth and biological diversity (Andrade, 2007).

Given these contexts, the aim of this study is to evaluate the employability of wetlands systems using floating aquatic macrophytes as a remediation tool for wastewater from a dairy company in Toledo, in the state of Paraná, analyzing their effectiveness in the treatment of dairy effluent as to the removal of physical and chemical parameters.

MATERIALS AND METHODS

This research was conducted in the greenhouse at the Pontifical Catholic University of Parana - PUCPR experimental field, Toledo, Parana, Brazil. The laboratory activities were realized in the Environmental Analysis Laboratory of PUCPR and in the Environmental Chemistry and Instrumental Laboratory, from the Study Group on Soil and Environment - GESOMA, at the Sta+te University of West of Parana - UNIOESTE, campus of Marechal Cândido Rondon. The experiment was conducted for 36 days between June and August 2014.

The aquatic macrophytes were collected in natural systems unpolluted from the International Itaipu Lake in the city of Santa Helena, seven days before the beginning of the experiment, in order to acclimate them to the new environment. After being collected, the aquatic macrophytes used in this study were washed under running water to remove all impurities therein.

The effluent was obtained from a polishing pond of a small dairy production industry in Toledo. The industrial production of this company is exclusively sold at the group's store. Annually, the company produces an average of 350 pieces of mozzarella cheese, 70 pieces of provolone cheese and 170 pieces of ricotta.

After collection the source, the wastewater was transported in 50 liters plastic drums to the greenhouse at PUCPR, where they were arranged in three polyethylene reactors, with 310 liters capacity each one. The reactors were operating intermittently (batch), with repetitions by the hydraulic retention time, installed in a greenhouse with transparent polyethylene for insertion of natural light and avoiding the influence of rainfall on the systems.

For the effluent laboratory analysis, it was used 600 mL glass bottles previously sterilized in order to remove any contaminating source that may possible influence afterward results.

The experimental delineation was completely random (CRD) with repetitions performed by the retention time of the effluent in the reactor, each one containing one species of aquatic macrophytes, with 15 units each one: T1 (*Eichhornia crassipes*), and T2 (*Salvinia auriculata*).

It was collected the environmental samples (effluent and plant) in the reactors every four (4) days during the period of 36 days, totalizing 10 samples, all of the collections are due in the morning, between 09:00 and 10:00 o'clock, featuring samples in relation to detention time of the wastewater. It was collected the samples in 600 mL glass bottles, previously sterilized, and stored in refrigerators at 4° C. For the evaluation of plant tissue, the collection was done randomly.

The parameters analyzed in this study were the following, all in accordance with the methods described by the Standard Methods for the Examination of Water and Wastewater (APHA, 2005): Chemical

Oxygen Demand (COD; mgL^{-1}); Turbidity (NTU); Solid Series (Total, Fixed and Volatile; mgL^{-1}); Dissolved Oxygen (DO; mgL^{-1}); Total Kjeldahl Nitrogen (TN; mgL^{-1}); Total Phosphorus (TP; mgL^{-1}); pH and Temperature (ºC).

Statistical analysis consisted of average tests and standard deviation for all parameters analyzed. For regression analysis, quadratic models were used in turbidity parameters, TN, TP, total solids and COD. The physical-chemical results were compared with current environmental legislation related to discharge of effluents into water bodies, through the regulations of the Brazilian National Environmental Council (CONAMA) 357/2005 and 430/2011.

RESULTS AND DISCUSSION

The temperatures of the liquid and the air (maximum, minimum and average) recorded during the experiment-conducting period are illustrated on figure 1. The maximum and minimum temperatures recorded were 26.9 °C and 7.1 °C, respectively. The effluent low temperature can be attributed to the fact that it has a shade in the water surface caused by superficial biomass of floating aquatic plants. The superficial biomassprevents heat entering the aquatic environment, behavior which was also observed by Henry-Silva and Camargo (2008), as well as the experiment, was carried out in cold periods of the year, which also explains the low temperature of the effluent.

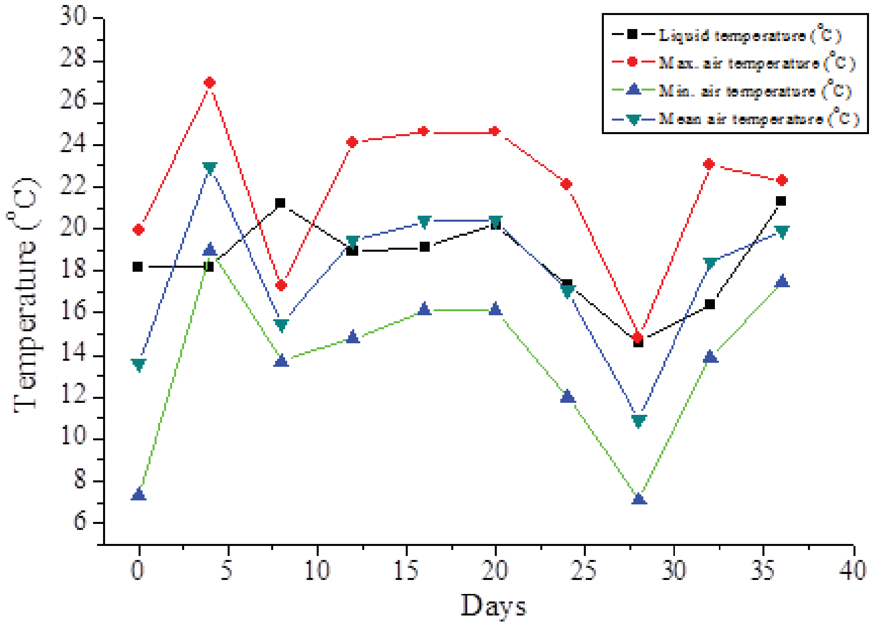

Figure 1. The effluent temperature variation during the days of the experiment.

Table 1 shows the pH increased according to the time when the effluent was retained in the reactor. During all 36 days of the experiment, the effluent pH remained within the limits set by CONAMA Resolution 430/2011, which determines pH limits among 6.00 to 9.00, proving the efficiency of the treatment.

The increased of pH in the wastewater can be attributed to the intense phytoplankton activity in the environment (Henares, 2014). As the samples were taken between 09:00 and 10:00 o'clock, phytoplankton had its ideal conditions to initiate the process of photosynthesis, because the time of solar light incidence is at least two hours. The choice of this time range for collecting plants and effluent is justified because at this way it has enough time, to perform all the laboratory tests on the same day of the collection The rise in pH found throughout the experiment is probably from the process of removing CO_2 from the water during phytoplankton photosynthesis. According to Cavalcanti and Sá (2010), this release is caused by the increase in the proportion of carbon inorganic forms (carbonate and bicarbonate). The chemical reaction between the carbonate ion (CO_3^{2-}) and the water molecule, releases bicarbonate ions (HCO_3^{2-}) and hydroxyl radical (OH^-) to the environment, thereby increasing the pH of the water. The averages found in Table 2 were above the ideal pH range for most of the plants, as according to Braccini, Braccini and Martinez (1999), it is between 5.00 and 6.50. Rates above this limit, elements such as calcium, iron, and phosphorus would precipitate, and became unusable for the plant.

Table 1. The effluent pH values for both treatments during the days of the experiment. Legend: SD (Standard deviation) and CV (coefficient of variance).

Day	**pH**	
	E. crassipes	***S. auriculata***
0	6.24	6.24
4	6.85	6.88
8	6.73	6.72
12	6.95	7.21
16	7.16	7.29
20	7.13	7.17
24	7.24	7.29
28	7.22	7.20
32	7.31	7.39
36	7.28	7.11
Average	7.05	7.01
SD	0.35	0.33
CV%	4.94	4.75

For the turbidity parameters, the initial concentration of the effluent was 128.0 NTU, with significant reductions over the effluent retention time in the reactor (Figure 2). In the treatment with *S. auriculata*, the final turbidity was 10.6 NTU with removal efficiency around 91.7 for *E. crassipes*, the turbidity level reduced to final 7.2 NTU, with the total efficiency of 94.4%. Given these results, the *E. crassipes* was the species that best behaved about the turbidity removal. This reduction can be justified by such plants have more developed root system when compared to *S. auriculata* and by the reactors having reduced depth, plants can absorb and precipitate the suspended particulate material, thus reducing the turbidity concentrations in the environment (Sales, 2011).

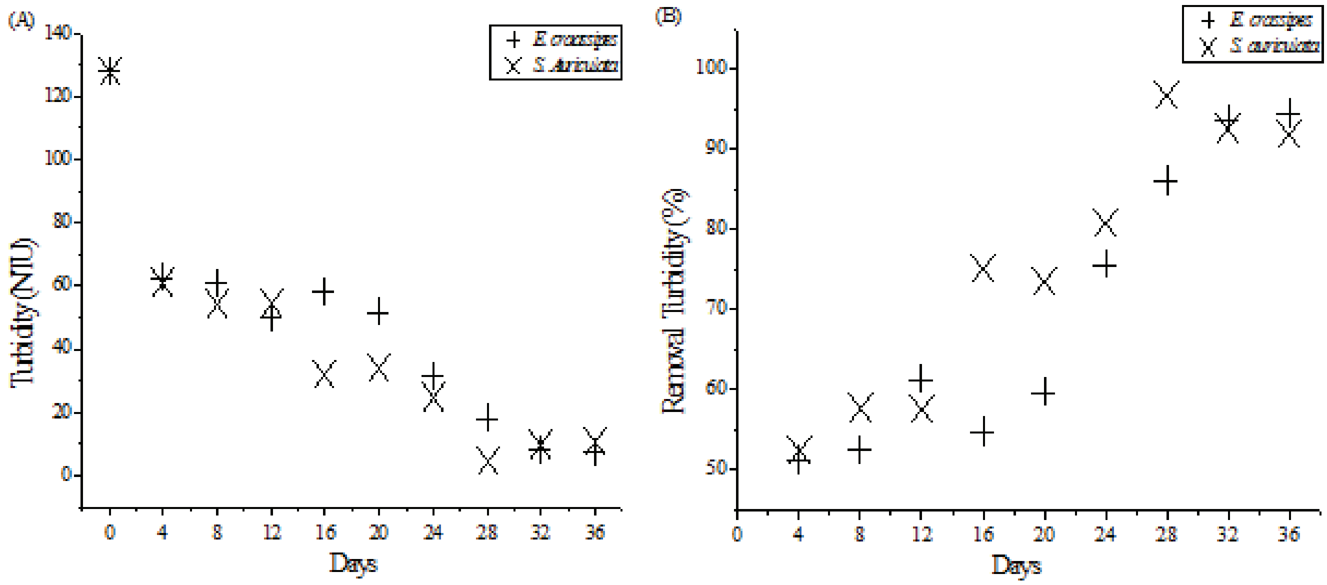

Figure 2. (A) Turbidity removal efficiency of the effluent for both treatments during the days of the experiment. (B) Levels of turbidity in the effluent during the experiment.

Henry-Silva and Camargo (2008) using *E. crassipes* and *Pistia stratiotes* in the treatment of shrimp effluent found 80.2% and 75.2% of efficiencies, respectively, after hydraulic retention time – HRT of 6 weeks. Freitas (2010) using *E. crassipes* for bioremediation purposes in fish farming effluents obtained an average efficiency of 96% in turbidity after 8 days, with total HRT of 12 days. Hussar and Bastos (2008) applying water hyacinth for fish effluent obtained average removal rate of 92% for turbidity, similar to what was found in this study.

Figure 2 shows that the high in the retention time of the effluent in the reactor, is the turbidity removal ratios, and this is mainly due to the removal of suspended solids by the root system of *E. crassipes* and precipitation of compounds in the effluent.

For total solids, figure 3 shows that *S. auriculata* showed a median reduction in the concentration of that parameter, even with a slight increase from the day 16 of the experiment, reducing from 1,210.0 mgL^{-1} initially to 856.0 mgL^{-1} in day 34, with a reduction of 29.26% at the end of the study. So far, *E. crassipes* behaved better compared to the previous species, but also with low ST removal values, thereby reducing the concentration from 1,210.0 mgL^{-1} on the first day to 790.0 mgL^{-1} at the end, resulting in 34.55% of removal. Based on these values, it was concluded that both plants were efficient in the total solids removal for effluents, also they are in accordance with the Resolution CONAMA 430/2011, which defines maximum removal efficiency of 20% of ST.

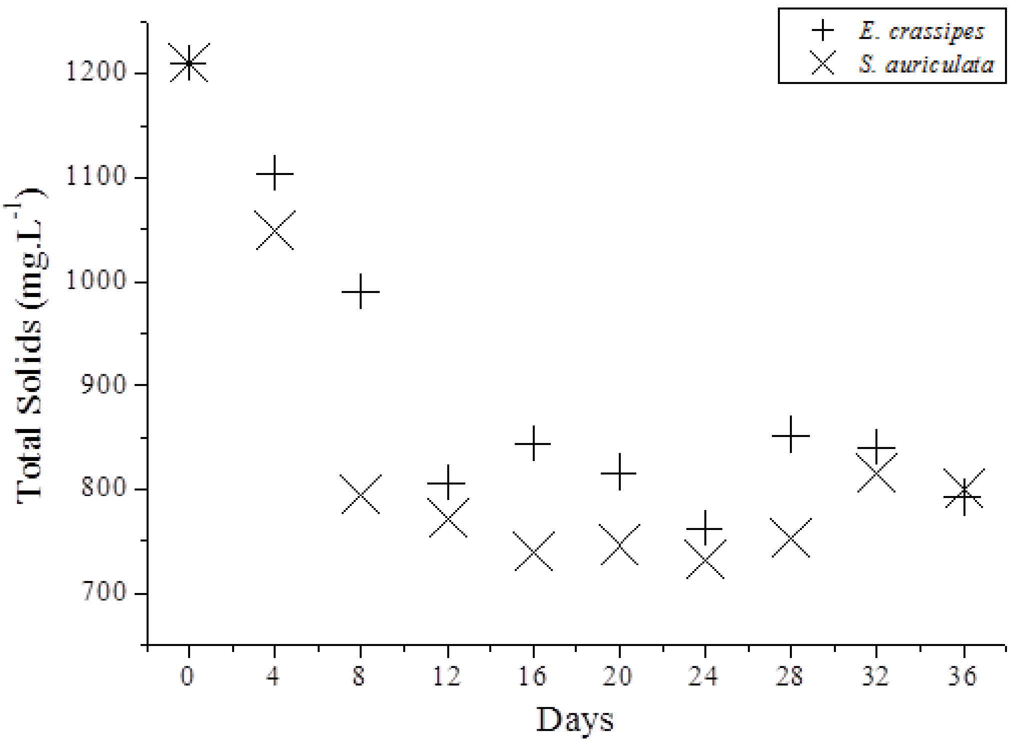

Figure 3. The effluent total solids variation for both treatments during the days of the experiment.

It was observed elevated concentrations of total solids between the day 12 and the day 20 of the experiment, in both treatments. Salati (2009) argues that the removal of suspended solids from effluents with floating aquatic macrophytes, mainly from water hyacinth, were related to their sedimentation or adsorption in plants root system. In experimental cases in the greenhouse, as there is no wind, there is no mixing of the solids and the surface coverage of the plants minimizes thermal mixtures.

The total nitrogen values (TN) for *E. crassipes* showed significant reductions in concentrations over the course of the experiment, ranging from 24.39 mgL^{-1} initially to 2.01 mgL^{-1} after day 36, with a small rise after 24 days, with the efficiency of 90.26% at the end of the experiment (Figure 4). While for *S. auriculata*, the results were not so satisfactory when compared to water hyacinth. It was observed a significant increase in TN concentration in the effluent after day 8, from 2.58 mgL^{-1} to 16.42 mgL^{-1} in day 16, and then it reduced again after this period, which made it statistically discrepant. Even so, at the end of the 36 days of the experiment, the TN concentration in *Salvinia auriculata* was 5.64 mgL^{-1}, totaling 75.65% of efficiency in this parameter removal.

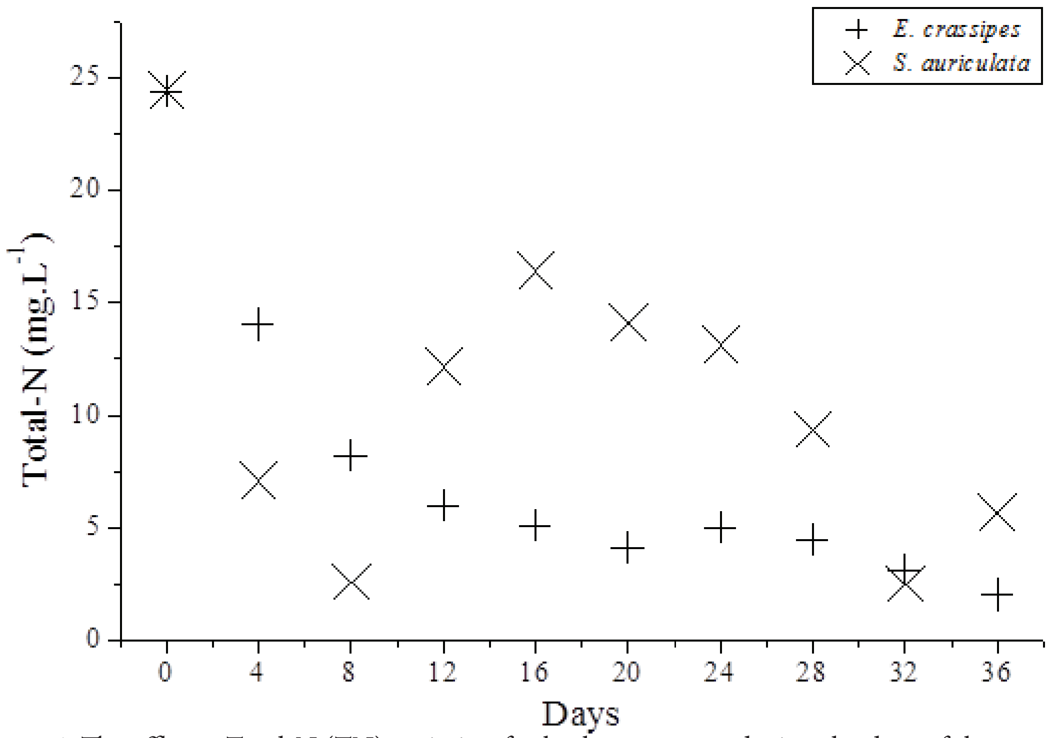

Figure 4. The effluent Total-N (TN) variation for both treatments during the days of the experiment.

The TN removal efficiency indices for *E. crassipes* exceeded those found by Weirich (2009) who evaluated swine manure effluents in summer and winter and found TN removal rates of 71.29% for *E. crassipes* in the coldest season with HRT of 30 days. Henry-Silva and Camargo (2006a), in the treatment of fish farming effluents, got 41.6% of TN removal with HRT of 33 hours. Petruchio and Esteves (2000), evaluating uptake rates of N in *S. auriculata* found 40% removal of total-N concentration under controlled conditions for 24 hours of HRT. Pistori *et al.* (2010) observing the *Salvinia molesta* behavior and two more aquatic macrophytes in nutrient solutions at high and low concentrations, observed the best results of TN absorption between 10 and 15 days of the experiment.

About *S. auriculata*, the increase in TN concentration it was possibly caused by the saturation of the plants absorbing the nutrient, since the plants were collected at different development stages, which is absorbed only the necessary for its development. Other authors attribute to HRT the responsibility of the reduction of TN absorption in *wetlands*, as Kawai and Grieco (1983), Lin *et al.* (2002) and Henares (2014).

For comparative purposes between the final values found and the current legislation in Brazil can be considered that only treatment with *E. crassipes* fits within the bounds set by CONAMA Resolution No. 357/2005 for total-N, which is 2.18 mgL^{-1}. Whereas treatment with *S. auriculata* needs a complementary treatment to reduce total-N levels to the allowed ones as previously mentioned resolution.

Figure 5 shows the behavior of the total phosphorus (TP) removal during the day of the experiment, both treatments. The initial concentration in the effluent was 9.03 mgL^{-1}. In treatment with *E. crassipes*, it has been found a satisfactory reduction in TP levels until day 8, with 5.70 mgL^{-1}, and between this period and the day 16 there was a slight attenuation in concentration, decreasing to 4. 96 mgL^{-1}, with 45% efficiency, the maximum efficiency found. However, after such time was noticed an increase in the concentration of TP where, at the end of the experiment, the concentration was 7.45 mgL^{-1}, making low removal percentage, of 19.26%. These low removals and efficiency levels found can be justified by the non-removal of plants already developed that, in their process of decomposition return to the environment half the nutrients previously absorbed and also by the increase of hydraulic retention time – HRT. Reidel (2005) observed the same behavior in the treatment of slaughterhouse effluents, where the author got an average efficiency of 21%.

The percentage removal in the present study was lower than those found by Henares (2014), that using water hyacinth for nutrient removal in aquaculture effluents has found TP removal of 43.61%, and Sales (2011), that using *E. crassipes* in treating brewery effluents in hot periods of the year achieved a reduction of 66.45% in the concentration of TP.

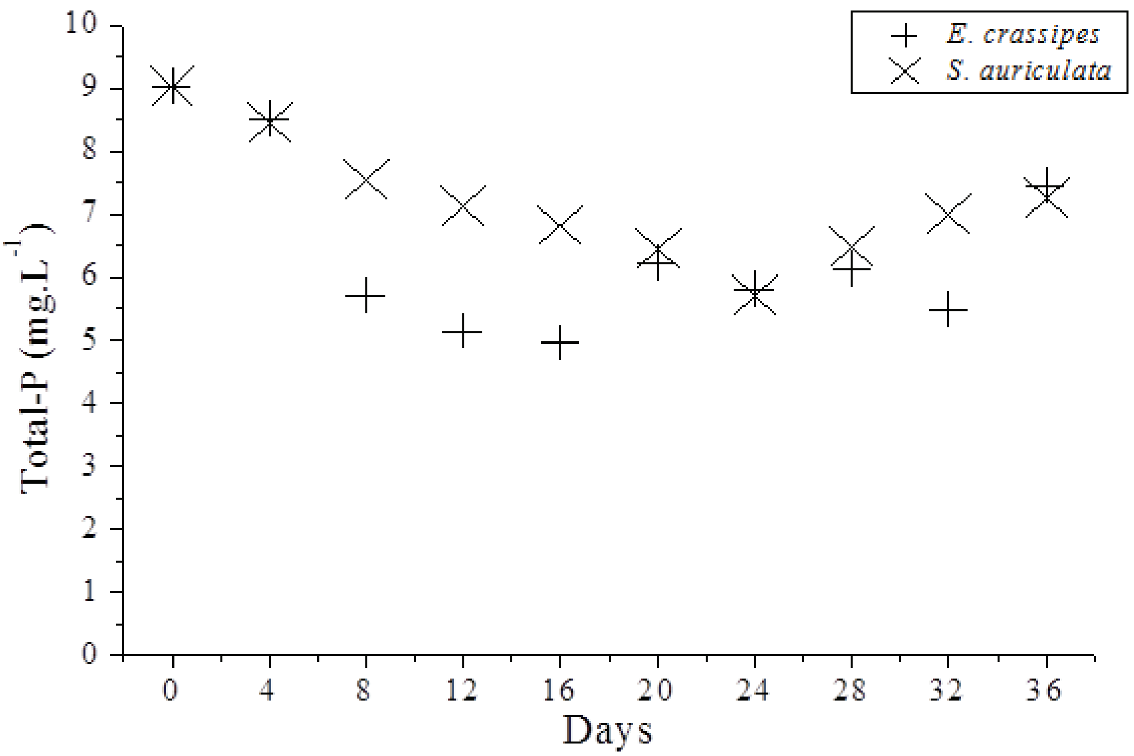

Figure 5. The effluent Total-P (TP) variation for both treatments during the days of the experiment.

When treating with *S. auriculata*, was noticed a reduction of TP concentrations until day 24, starting from 9.03 mgL^{-1} to 5.70 mgL^{-1} at the end of this period. However, in the following days until the conclusion of the experiment, TP rates gradually increased, ending with 10.68 mgL^{-1}. The efficiency at the end of the experiment was 25.36%, but higher than the levels found in the present study of *E. crassipes*. These rates were lower than the ones found by Henry-Silva and Camargo (2006b) that, by observing *Salvinia molesta* behavior in the treatment of Nile tilapia, found 72% removal of TP.

However, the values found do not meet the 0.15 mgL^{-1} as required as maximum allowed value - VMP determined by CONAMA Resolution 357/2005, requiring a complementary treatment for this reduction to acceptable levels for the Brazilian legislation.

Figure 6 shows the Chemical Oxygen Demand – COD values found during the experiment. Initially, the concentration of COD was 730 mgL^{-1}, where treatment with *E. crassipes* drastically reduced levels starting on the day 4 and stabilized after the day 8, finishing with 131.67 mgL^{-1}, a total efficiency of 82%.

However, with *S. auriculata*, the result was satisfactory at the end of the experiment, with final concentration of 118.33 mgL^{-1}, however, an abnormal increase in COD concentrations were observed in the period between the day 16 and 24, showing an adverse effect to that expected for this parameter, but showed 82% efficiency. Anomalies like that occurred probably because of the death of some plants, since the degradation of their biomass increases the organic matter in the effluent, as biologically, the plant behaves in decreasing order in the removal of organic matter. These results show that, from 12 days of the experiment, it should start a management of plants by removing the dead and old ones, so that young plants can develop faster and absorb more MO.

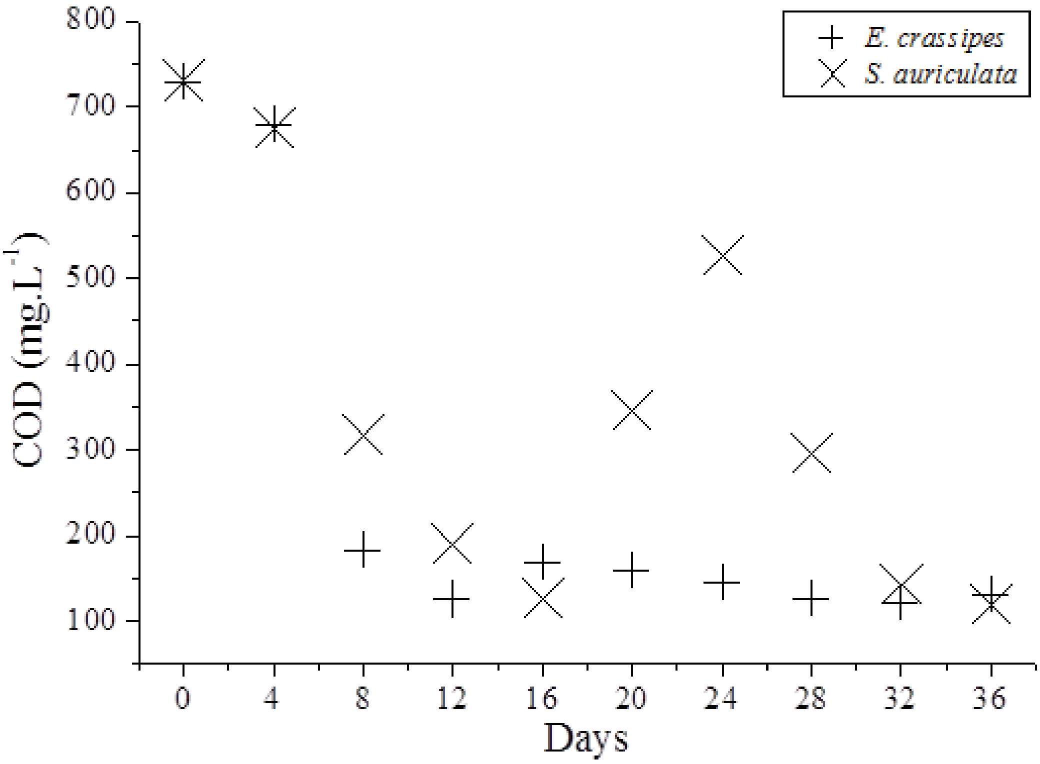

Figure 6. The effluent COD variation for both treatments during the days of the experiment.

The efficiency of COD removal found in *E. crassipes* was higher than those obtained by Jonas and Hussar (2010) that got an average removal of 61.9% using water hyacinth in the treatment of UASB reactor effluent with 8 days of HRT. Sezerino *et al.* (2012) found removal efficiency of 82% after treatment of anaerobic effluent using constructed wetlands systems. As for *S. auriculata*, Silva *et al.* (2006) found 77% removal of COD in cassava manufacturer effluents using such species.

COD levels found in this study are in accordance with the Resolution of the State Council for the Environment of Paraná State (CEMA-PR) Nº 070/2009, in its Annex 7, provides 200 mgL^{-1} as maximum value permitted for COD for dairy effluent releasing.

Regarding DO, the initial concentration was at 11.54 mgL^{-1}. In the two treatments, statistically, the results showed a significant difference, however, with concentrations presenting variation coefficient of 16% (between 11.54 to 21.00 mgL^{-1}) during the experiment. The results with *E. crassipes* showed better DO levels when compared to treatment with *S. auriculata,* with the same variation of the previous one, however, statistically differing from each other (between 11.54 to 20.07 mgL^{-1}) (Table 2).

Table 2. The effluent DO values for both treatments during the days of the experiment. Legend: SD (Standard deviation) and CV (coefficient of variance).

Day	DO (mgL^{-1})	
	E. crassipes	*S. auriculata*
0	11.54	11.54
4	15.69	17.89
8	12.41	16.33
12	12.77	13.61
16	16.06	15.56
20	16.42	16.33
24	16.42	15.56
28	16.79	16.72
32	20.07	21.00
36	15.33	14.00
Average	15.35	15.86
SD	2.52	2.56
CV%	16.41	16.16

Based on table 2, the values stand above the values exposed by CONANA 430/2011 which determines DO levels for the discharge of effluents above 5 mgL^{-1}, showing that the experiment was constantly aerated, with no anaerobic conditions formation in the environment. Situations with the lack of oxygen can contribute to the formation of undesirable organic compounds such as methane and carbon dioxide, reducing the activity of microorganisms dependent of oxygen to perform their functions (Von Sperling, 1996).

FINAL CONSIDERATIONS

The floating aquatic macrophytes *Eichhornia crassipes* and *Salvinia auriculata* were efficient in removing turbidity, COD, and nutrients (total-N and total-P) of the effluent; therefore, they are an effective tool of remediation of contaminated aquatic environments.

From the period between the day 16 and the day 20, there were increases in concentrations of some parameters, because some plants got into the senescent process, returning to the environment everything they have absorbed. This observation shows that from day 16 to day 20 the withdrawal of older and saturated plants is necessary, so that the younger plants can effectively exercise their role to remove contaminants.

According to the results presented, the management of these plants should be made between 15 to 20 days after their insertion in the environment because, after that period, they begin to proliferate, because they find favorable conditions for their growth, as organic matter and nutrients, besides forming chains between the roots, hindering their removal.

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