Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2014.903869

54 (2015) 3003–3010
June

Taylor & Francis
Taylor & Francis Group

Nonliving macrophyte *Salvinia* sp. application for nutrient removal in starchy wastewater treatment of cassava industry

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Received 23 September 2013; Accepted 10 March 2014

ABSTRACT

The removal of nutrients (nitrogen, phosphorus, and COD) of wastewater from a local cassava industry wastewater was investigated. Dried (nonliving) biomass of the aquatic macrophyte *Salvinia* sp. was used in a batch system and experiments were all bench-top laboratory-scale in Erlenmeyer flasks. The kinetic behavior was evaluated and a statistical central composite design (CCD) was used to verify the influence of the parameters pH (5.0–9.0), initial biomass concentration (0.0–0.0067 g mL^{-1}), and agitation (0–150 rpm). After 24 h of contact with the macrophyte, about 98% of nitrogen was removed, coinciding to pH and COD/N ratio elevations. After 96 h, according to CCD results, the dried biomass of *Salvinia* sp. removed significantly the nutrients from cassava wastewater, suggesting it was irrespective of all the evaluated parameters. The nonliving macrophyte *Salvinia* showed potential for application in the starchy wastewater treatment as an alternative way for pollutant removal, since this macrophyte is found in aquatics environments.

Keywords: *Salvinia;* Cassava industry; Starchy wastewater; Nutrient removal; Statistical design; Dried macrophyte

1. Introduction

Several aquatic ecosystems are commonly polluted with industrial effluents containing high concentration of substances, such as nitrogen, phosphorus, organic matter, and heavy metals. Many wastewater treatment methods are available as on date in order to ensure good-quality effluent before disposal into the municipal sewer systems [1]. The wastewaters vary in terms of the pollutant composition depending on the origin of the industry. The disposal of such effluents in the environment will lead to surface and groundwater contamination, increase in chemical oxygen demand, eutrophication, ecosystem imbalance, and human health risks [2,3]. The wastewater originating from agro-food industries primarily contain proteins, sugars, oils, and greases. These industries are predominantly loaded with organic wastes and are rich in organic content [4].

Most of the industrial units carry out pretreatments, namely grit removal, sieving, and degreasing before disposal into municipal wastewater treatment plants. The wastewater treatment encompasses physical–chemical and biological treatments. In a general

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way, biological processes (aerobic or anaerobic systems) are widely used for the treatment of agro-industry wastewaters, which contain high concentrations of biodegradable organic matter (in term of BOD) [5].

At the moment, in some countries, the use of advanced physical and chemical treatments to improve plant effluents is usually not feasible because of its capital and operational costs and also due to lack of specialized operators. Land is also relatively inexpensive. Therefore, natural treatment processes are appropriate alternatives. Natural treatment systems include wetlands, aquatic, and land treatment systems. Compared to advanced physical–chemical or biological treatment systems, these systems have less capital expenditures and lower operational costs; also they are easier to operate [6].

Cassava (*Manihot esculenta* Crantz) is a crop for food, animal feed, starch processing, and currently used as a main raw material for biofuel processing which has high comparative advantage of many countries in the world. According to FAO [7], the countries leading in cassava production in the world in 2011 are Nigeria 52.4 million tons, Brazil 25.44 million tons, Indonesia 24.00 million tons, and Thailand (21.91 million tons). In Brazil, cassava is a crop widely distributed throughout the national territory. It is an important crop for its hardiness and satisfactory performance under conditions of low soil fertility and in different climates of various regions of Brazil.

The cassava industrialization generates wastewater with a large possibility of causing pollution to the rivers and environment, in view of its high organic matter value. Developing countries, particularly, have been facing serious environmental problems due to the several cassava processing industries and the residues it generate.

The research of new and cheaper treatment alternatives is a relevant subject of interest for this kind of industry. In recent researches, it has been tried to use the alternative systems that gather efficiency in the wastewater treatment, simplicity in the execution, besides the low cost of the material and operation. In this way, the use of aquatic macrophytes becomes an interesting alternative due to their low cost for the treatment of several industrial effluents. The aquatic macrophytes, such as, *Salvinia* sp. and *Eichhornia crassipes*, even nonliving, possess a high capacity to accumulate pollutants. However, the use of nonliving aquatic plants in the removal of pollutants is very recently studied and the main researches are related with the capacity of accumulation of heavy metals. Therefore, this research had the purpose of evaluating the capacity of the *Salvinia* sp. (dried) on the removal of nitrogen, phosphorus, and COD of cassava wastewater containing high load of biodegradable organic matter.

2. Materials and methods

2.1. Dried biomass

The biomass used in this work was the local aquatic macrophyte *Salvinia* sp. provided by the Center for Advanced Research in Aquaculture, in Toledo city, Paraná-Brazil. The plant tissues were washed in deionized water and dried at 40˚C. Only the leaf was used in experiments and the biomass, only for prevention, was conditioned in plastic sacks and preserved in room temperature for subsequent use.

2.2. Sampling and characterization of the wastewater

All the prepared solutions for the characterization of the wastewater were of analytical grade and deionized water was used in all experiments. Two sampling sites were selected for the collection of the wastewater in industry: the entrance of the treatment system and the exit of the last lagoon of stabilization. Twenty liters of wastewater were obtained from industry, and after bottled in smaller volumes, all material was kept frozen. In this research, the treated wastewater was used as substrate.

For the physicochemical characterization of the wastewater, the following parameters were analyzed: pH, total solids (TS), fixed solids (FS), volatile solids (VS), chemical oxygen demand (COD), nitrogen (N), and phosphorus (P). Analyses were performed following the standard methods [8]. All of the analyses were accomplished in duplicate. The removal of the substance was determined according to Eq. (1):

$$\text{Removal} = \frac{C_0 - C_f}{C_0} \times 100 \tag{1}$$

where C_0 and C_f are the initial and final concentration (mg.L^{-1}) of the analyzed component.

2.3. Kinetic behavior of nutrients removal

Batch experiments were accomplished under pre-established conditions for defining the best residence time. Erlenmeyer flasks containing 5.5 g of biomass (dry basis) and 150 mL of wastewater were incubated under constant agitation, with temperature controlled at 30˚C. The control experiment was accomplished with 150 mL wastewater and without biomass.

The pH was verified in the following intervals: 0, 5, 10, 20, 30, 40, 60 min, 2, 4, 12, 24, 48, 72, and 96 h. The first analysis of N, P, and COD were accomplished 8 h after the beginning of the experiment and then every 24 h until the end of the experiment. After each time of residence the solutions were filtered, diluted, and analyzed.

2.4. Tests for evaluating the capacity of removal of N, P, and COD using statistical experimental design

A statistical experimental design was elaborated with the purpose of verifying the influence of three independent variables on the removal of N, P, and COD: initial pH, initial concentration of biomass (C), and agitation (A). This study involved the application of a response surface methodology (RSM) with the use of a central composite design (CCD). Table 1 shows the range of the studied factors and the correspondent coded levels [9]. In this study, the levels for each variable were chosen according to the following criteria: pH from slightly acid to basic, the agitation (A) interval represents a range without agitation to a maximum agitation of 150 rpm, that would be the maximum limit to be used in operational conditions, and the levels of biomass concentration were defined according to data obtained in literature [10–12].

To evaluate the capacity of removal of N, P, and COD with the use of the dried *Salvinia* biomass, experiments were accomplished in batch systems, and in different pH conditions, initial concentration of biomass, and agitation, according to the conditions established in each run of the experimental design. Each flask was maintained in room temperature, under constant agitation in a mechanical agitator, and with pH adjustment, as defined for the experiment. For pH correction, 0.1 N NaOH or 0.1 N HCl were used. After 96 h, the treated wastewater samples were filtered and analyzed. The experiments were accomplished in duplicate.

Table 1
Coded levels and real values for the variables of the experimental CCD

Variable	Level −1.68	−1	0	+1	+1.68
pH	5	5.8	7.0	8.2	9.0
A (rpm)	0	30	75	120	150
C (g mL^{-1})	0	0.0013	0.0033	0.0053	0.0067

Note: A = agitation; C = initial concentration of biomass.

3. Results and discussion

3.1. Wastewater characterization

Table 2 presents the results obtained for the partial characterization of the liquid residue at the entrance of the industrial wastewater treatment and at the exit of the last lagoon of stabilization. It was observed lower efficiency in industry in the removal of P and COD. The increase of the N concentration in elapsing of the treatment could be explained due to the low pH of the effluent and the complex metabolic pathways involved in the process.

The COD value was within the range established for this type of wastewater. Each factory produces a wastewater having its own characteristics with respect to organic content and volume, depending on the efficiency of the machines used. Wastewater characteristics were highly dependent on the level of technology of the plant, on the variety of cassava processed, and on the retention time of water in the sedimentation tanks [13]. It becomes necessary to use the of polishing lagoons, promoting additional removal of the nutrients as, for example, the use of aquatic macrophytes.

3.2. pH evaluation and COD removal

It is well known that pH of raw wastewater can either have a positive or negative influence on the treatment quality as it would affect the stability of various hydroxide species formed. Likewise, the change in pH can modify the surface charge of particles and greatly influence the removal of colloidal dispersed organics from solution [14]. Fig. 1 illustrates the time course of pH. It was verified similar behavior in the tests with and without macrophyte (control), where pH is practically constant until 12 h, later increasing value to 8.40. This increase happens due to the biodegradation of the organic matter and the probable oxygenation of the solution, due to agitation.

It was possible to realize that the effluent with an initial pH among 4.0 and 5.0 presented an odor similar to the sulfide and, after 48 h of agitation, a strong odor was observed, characteristic of whey, with the pH elevation (6.0–8.0) probably due to fermentation of the wastewater. After 72 h of agitation, when the pH reached superior values, the effluent did not present any unpleasant odor, probably indicating a degradation of the organic matter. The pH values obtained after 96 h (8.40 and 8.39) favored the removal of N by the volatilization of the ammonia and by the increment of the precipitation of the insoluble phosphate [10,15].

Table 2
Partial wastewater characterization

Parameter	System entrance (mg.L^{-1})	Final effluent (mg.L^{-1})	Removal (%)
COD	32,000.00	20,480.00	36.0
P	134.4	121.6	9.50
Total N	165.2	350.0	–
TS	6,000.00	7,000.00	–
FS	2,000.00	0	100.00
VS	4,000.00	5,000.00	–
pH	5.0	5.2	–

Notes: COD = chemical oxygen demand, P = phosphorus, Total N = total nitrogen, TS = total solids, FS = fixed solids, and VS = volatile solids.

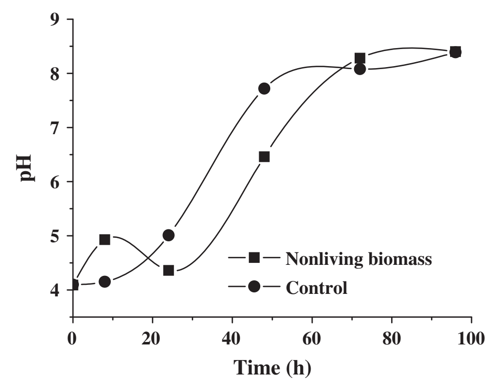

Fig. 1. Time course of pH in the experiments with macrophytes and control.

The evolution of COD in the experiments with and without macrophytes is observed in Fig. 2. The experiment with biomass that the reduction of COD was approximately 76.56% and the final effluent reached 4,800.00 mg.L^{-1} of COD. This reduction was superior to that obtained with the control, which reached 53.12%. Zimmels et al. [12] obtained 90.25% of COD removal with *E. crassipes* for sewage treatment. Maine et al. [16] had a similar behavior, with 86% of COD removal in a little scale of wetland system.

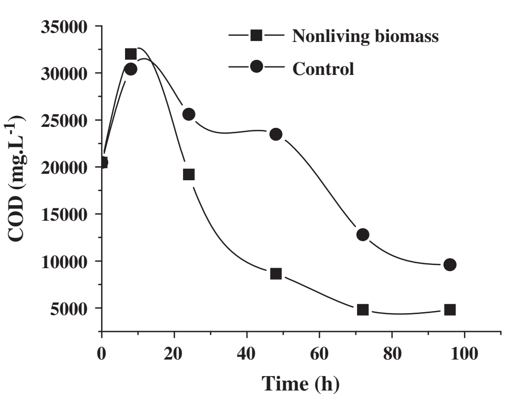

Fig. 2. Time course of COD for cassava wastewater treatment.

Table 3 shows the results of removal efficiencies of COD, N, and P obtained in the experimental design for each run. The values in bold and italic in the table represent the largest and smaller results obtained to each removal efficiency. The central composite design totalized 17 experiments including triplicate at the central point. All the runs were executed in random sequence.

Table 3
Removal efficiencies (%) of COD, N, and P obtained according to CCD design

	Factors			Removal efficiency (%)		
Run	pH	A	C	COD	N	P
1	–1	–1	–1	92.19	99.94	99.51
2	+1	–1	–1	*97.91*	*99.96*	99.42
3	–1	+1	–1	84.37	99.92	*99.67*
4	+1	+1	–1	76.56	99.85	99.26
5	–1	–1	+1	84.37	99.81	99.34
6	+1	–1	+1	85.94	99.80	*98.60*
7	–1	+1	+1	95.31	99.86	99.01
8	+1	+1	+1	84.37	99.95	99.51
9	–1.68	0	0	*47.91*	*99.53*	99.01
10	+1.68	0	0	87.50	99.91	99.09
11	0	–1.68	0	85.94	99.88	98.78
12	0	+1.68	0	94.79	99.93	99.42
13	0	0	–1.68	63.54	99.72	99.09
14	0	0	+1.68	68.75	99.94	99.34
15	0	0	0	58.33	99.68	98.93
16	0	0	0	68.75	99.69	99.18
17	0	0	0	68.75	99.70	98.89

Notes: A = agitation, C = initial concentration of biomass, COD = chemical oxygen demand, P = phosphorus, and N = nitrogen.

This kind of factorial design allows the obtainment of a mathematical model with linear and quadratic parameters (multiple regression). The main effects of factors, considering linear (L) and quadratic (Q) model parameters, and the two-way interactions between the factors were analyzed through the Pareto chart of effects. The best results were obtained in the experiment 2, for N and COD removals, and in run 3, for P removal. The center point (runs 15, 16, and 17) is a triplicate and gives the magnitude of the error associated to the experimental problem. The standard errors obtained for the coefficients of the models are about 0.035 for the response of nitrogen, about 0.09 for the response of phosphorus, and about 4.00 for the response of COD, for a 5% of significant level.

The COD removal (Table 3) ranged from 47.91 to 97.91%. The maximum removal (run 2) was observed at an initial pH 8.2, 30 rpm, and a biomass concentration of 0.0013 g mL^{-1}. The minimum removal (run 9) was observed with pH 5.0, 75 rpm, and biomass concentration of 0.0033 g mL^{-1}. The Pareto chart was used for identifying which effect estimates are significant. According to Fig. 3, only one parameter, the quadratic term of agitation, A(Q), was statistically significant, which is evidenced by the horizontal column going beyond the dot line. The positive value of the effect indicates that higher values of COD removal can be obtained for higher values of agitation.

The system agitation facilitates the atmospheric oxygen transfer to the wastewater, where it is consumed for oxidation of organic matter. In this sense, the agitation is important to maintain the necessary aerobic conditions for the organic matter oxidation. As the experiments were accomplished in Erlenmeyer's under agitation, an aerobic system was probably constituted, which results in a fundamental condition for organic matter removal. In other way, the dried macrophyte may be working as a medium of support for micro-organisms' growth, which are responsible for the oxidation of the organic matter. According to Bindu et al. [9], the plant root system also serves as an excellent substratum for the attachment and development of microbial. It is also reported in the literature that the oxygen available in such treatment systems would be first used by the micro-organisms for the oxidation of organic matter and, only after considerable removal of organic matter, the oxygen would be spared for the nitrification process [17,18].

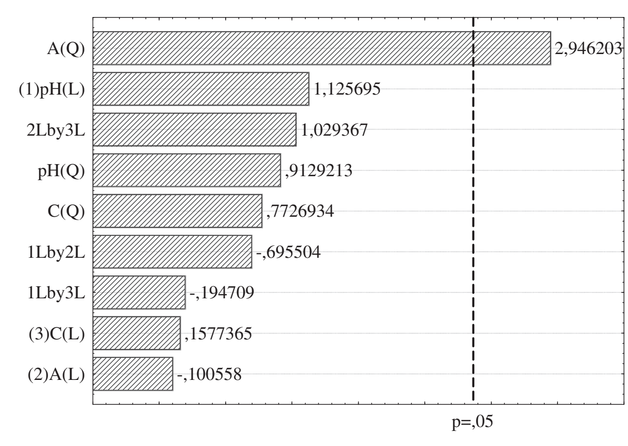

Fig. 3. Pareto chart of effects for COD removal (5% of significance level). Linear (L) and quadratic (Q) parameters in model for pH (factor 1), A (factor 2), and C (factor 3), and the two-way interactions between the linear factors.

3.3. Nitrogen removal

It was verified that a reduction of 98.88% of nitrogen was observed (Fig. 4) with the use of dried biomass, meanwhile 79.20% of reduction was observed for the control experiment. It was observed a reduction of 98.88% of nitrogen (Fig. 4) with the use of dried biomass, meanwhile 79.20% of reduction was observed for the control experiment. This concentration continues declining until the interval of 48 h, remaining constant to the end of the experiment.

The N removal reached almost 100% in all runs (Table 3). The agitation was again the most important variable in the removal of N (Fig. 5). In this case, only the quadratic term of the agitation, A(Q), was important, in a significance level of 5%. The positive effect of this variable indicates that the removal efficiency of N should increase if the agitation is increased.

There are three major mechanisms often referred in the literature regarding the nitrogen removal: (i) bacterial nitrification and denitrification, (ii) uptake by plants, (iii) volatilization of ammonia [19].

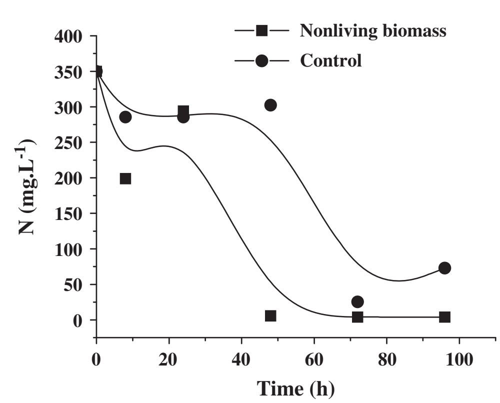

Fig. 4. Time course of nitrogen for cassava wastewater treatment.

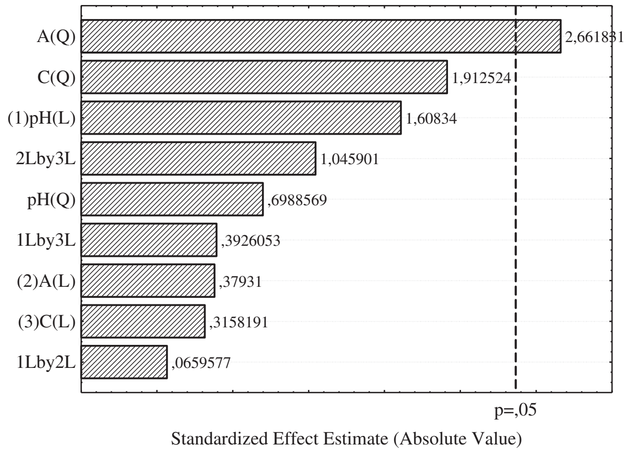

Fig. 5. Pareto chart of effects for nitrogen removal (5% of significance level). Linear (L) and quadratic (Q) parameters in model for pH (factor 1), A (factor 2), and C (factor 3), and the two-way interactions between the linear factors.

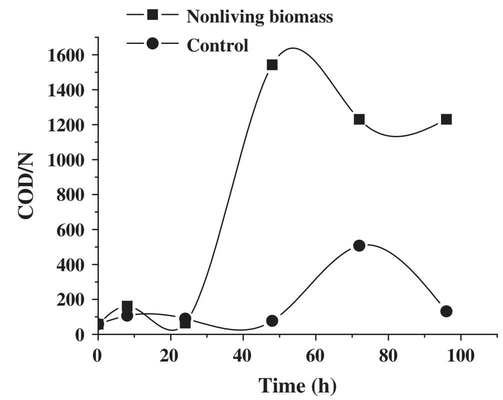

Fig. 6. Time course of ratio COD/N for cassava wastewater treatment.

In spite of the responsible biological action for the N removal, the dried biomass of the macrophyte influences the process of reduction of this parameter, because it acts as a support for the formation of a microbial film, responsible for the stabilization. In an industrial scale, this biomass makes possible that different populations of micro-organisms (bacteria, mushrooms, protozoa, and yeasts among others) grow adhered in the middle of supports, providing high biomass concentrations activates (micro-organisms) in his interior.

Besides, the dried macrophyte, when used as bioadsorbent, enhances the biological activity due to the adsorption of toxic compounds that would inhibit the action of micro-organisms that are responsible for the nitrification and denitrification process [20].

The COD/N ratio of influent is one of the most critical parameters for wastewater nitrogen removal process, because it directly effects functional micro-organism populations. In a nitrogen removal system, different micro-organism populations compete for substrate which caused fluctuation in effectiveness of organic and nitrogen removal. Carrera et al. [21] quantified the influence of influent COD/N ratio on a biological nitrogen removal process. They observed that nitrification rate decreased when the influent COD/N ratio increased from 0.71 to 3.4, and the relationship between nitrification rate and COD/N ratio could be defined by an exponential function. In this work, parallel to macrophyte activity, micro-organisms present in the wastewater may have contributed to nutrient removal through a denitrification process. Fig. 6 shows the evolution of the ratio of COD/N during the operation. At 24 h, according to Figs. 4 and 6, an exponential function was observed to COD/N ratio indicating a high denitrification process due to the presence of the macrophyte, which ensured efficiency to the removal of nitrogen due to the attachment of micro-organisms. Otherwise, removal efficiencies of N at 96 h were above 99.0% (Table 3) suggesting that it was irrespective of operational parameters like pH, initial biomass concentration and COD/N ratios.

3.4. Phosphorus removal

Fig. 7 shows the phosphorus concentration during the experiments with the macrophyte and control. At 24 h, there was almost a complete removal of P, coinciding to COD/N ratio elevation, after this period. It was observed that at 96 h the values of the P concentration reduced 85.20 and 90.13%, in the case of the experiments with biomass and control, respectively.

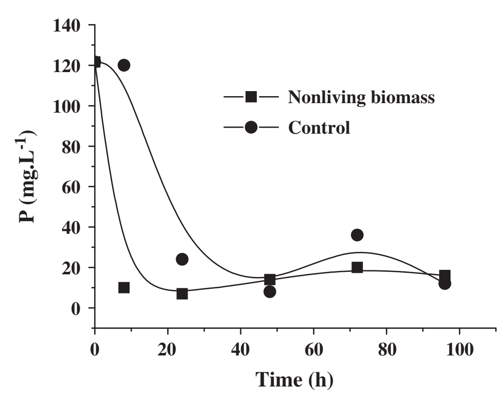

Fig. 7. Time course of phosphorus for cassava wastewater treatment.

Comparing both results of phosphorus, for the experiments with the macrophyte and the experiments without the macrophyte (control), it was difficult to conclude which is the experiment with higher phosphorus removal.

Different kinetic behaviors were observed for COD, N, and P removals due to the different decreasing ratios indicating the complex metabolic pathways involved in the system. As the better result of COD removal was observed after 96 h, this residence time was used in subsequent experiments.

According to the experimental design (all runs interrupted at 96 h), the P removal ranged from 98.60 to 99.67% (Table 3) suggesting that it was irrespective of all operational parameters like pH, agitation, initial biomass concentration, and COD/N ratios. The maximum removal was observed in experiment 3 (pH 5.8, 120 rpm and biomass concentration of 0.0013 g mL^{-1}) and the minimum removal was in experiment 6 (pH 8.2, 30 rpm and biomass concentration of 0.0053 g mL^{-1}). Fig. 8 shows that no variable was significant for the P removal which reached almost 100% of phosphorus removal for all runs.

An increase in pH values was verified along the experiments. This factor did not show significant influence on P removal results since the increase in pH occurred in all the experiments. When pH values are higher than 7.8, the P removal occurs predominantly by precipitation as an insoluble phosphate. pH increases progressively due to the biodegradation of the organic matter, contributing to the P removal through the precipitation of the phosphate in conditions of higher pH values [15].

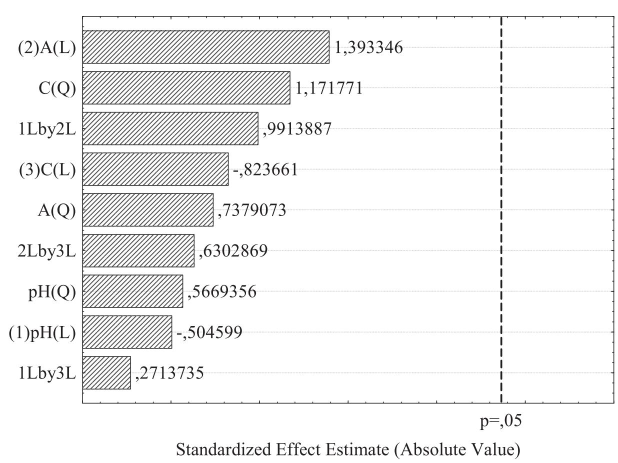

Fig. 8. Pareto chart of effects for phosphorus removal (5% of significance level). Linear (L) and quadratic (Q) parameters in model for pH (factor 1), A (factor 2), and C (factor 3), and the two-way interactions between the linear factors.

Another form of P removal is through the incorporation of micro-organisms known as accumulative organisms of polyphosphate, which biologically removes P [22]. The sedimentation process of the total solids, with subsequent filtration of the solution, collaborates significantly for the P reduction of the wastewater [23].

4. Conclusions

It was verified in this work that the nonliving biomass of the aquatic macrophyte *Salvinia* sp. has potential for the industrial application in the treatment of wastewater of cassava industrialization due to the observed removal of nutrients (up to 97% of COD, nitrogen, and phosphorus). The use of the dried macrophyte *Salvinia* sp. has the advantage of being a biomass found abundantly in several aquatics environments and it contributes significantly in the nutrients removal efficiency in wastewater treatment systems. However, larger scale studies should be conducted to certify the applicability of the macrophyte in the industry in terms of efficiency, and relating in economic aspects and environmental impact.

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