Post-treatment of sanitary landfill leachate by coagulation–floculation using chitosan as primary coagulant

Inara Oliveira do Carmo Nascimento, Ana Rosa Pinto Guedes, Louisa Wessels Perelo and Luciano Matos Queiroz

ABSTRACT

Chitosan was chosen as an alternative primary coagulant in a complementary coagulation–floculation treatment of sanitary landfill leachate with the aim of removing recalcitrant organic matter. In order to optimize the process conditions, central composite design and response surface methodology were applied. To evaluate the performance of the process using chitosan, we also carried out tests with aluminium sulphate (Al2(SO4)3.14 H2O) as coagulant. In addition, acute toxicity tests were carried using the duckweed Lemna minor and the guppy fish Poecilia reticulata as test organisms. The analytic hierarchy process was employed for selecting the most appropriate coagulant. Mean values of true colour removal efficiency of 80% and turbidity removal efficiency of 91.4% were reached at chitosan dosages of 960 mg L−1 at pH 8.5. The acute toxicity tests showed that organisms were sensitive to all samples, mainly after coagulation–floculation using chitosan. CE50 for L. minor was not determined because there was no inhibition of the average growth rate and biomass production; LC50 for P. reticulata was 23% (v v−1). Multi-criteria analysis showed that alum was the most appropriate coagulant. Therefore, chitosan as primary coagulant was not considered to be a viable alternative in the post-treatment of landfill leachate.

Key words | chitosan, coagulation–floculation, landfill leachate, post-treatment, toxicity

INTRODUCTION

The process of coagulation–floculation can be used as a complementary post-treatment of sanitary landfill leachate for the removal of recalcitrant compounds that have not been removed by biological treatment (Renou et al. 2008; Ziyang et al. 2009). However, chemical coagulants may have adverse effects on the environment. Thus, the use of natural coagulants has been indicated as an interesting alternative to chemical coagulants by several authors (Bolto et al. 1999; Ødegård et al. 1999; Ahmad et al. 2006; Yang et al. 2007; Szygula et al. 2009; Rizzo et al. 2010; Al-Hamadani et al. 2011; Zoonoozi et al. 2011; Verma et al. 2012; Ramli & Aziz 2015).

Since most natural colloids are negatively charged, cationic polyelectrolytes are of particular interest as potential coagulants. The most promising cationic biopolymer for extensive application is chitosan, a linear cationic polymer of high molecular weight obtained by N-deacetylation of chitin, which is manufactured from the outer shell of crustaceans (Bratskaya et al. 2004; Kumar et al. 2004).

Chitosan is virtually insoluble in water under normal conditions, but in acid solutions (with the exception of sulphuric acid solutions), it behaves as a moderately basic cationic polyelectrolyte (pKa = 6.3–6.4) (Guibal & Roussy 2007). In addition, chitosan has advantages for the treatment of wastewater because of its ability to act as a coagulant/floculant, its non-toxic characteristics, biodegradability, and its function as a chelator of heavy metals, which helps to reduce conductivity and toxicity (Renault et al. 2009). Chitosan has been considered as an ‘ecologically friendly’ product. However, there are few studies on the use of this polymer as a coagulant in wastewater treatment.

This study aims to determine the optimum dosage and pH values for post-treatment of biologically-treated landfill leachate by coagulation–floculation using chitosan as the...
primary coagulant. The proposed conditions were tested and evaluated regarding their efficiency in removing recalcitrant organic matter, measured as the true colour, turbidity, and toxicity.

**METHODS**

Leachate samples treated in a pilot-scale sequence batch activated sludge reactor were collected in polyethylene containers (20 L) at 2-week intervals. The samples were immediately transported to the laboratory for characterization and performance of the coagulation-flocculation tests. The samples were characterized for pH, chemical oxygen demand (COD), total organic carbon (TOC), alkalinity, total nitrogen, ammonia nitrogen, nitrate, nitrite, total phosphorus, soluble phosphorus, true colour, apparent colour, turbidity, chloride, and solids in accordance with Standard Methods for the Examination of Water and Wastewater (2012). Carbohydrates and proteins were determined according to Dubois et al. (1956) and Lowry et al. (1951), respectively. Table 1 shows the characteristics of the samples.

As shown in Table 1, biological treatment was effective in removing ammoniacal nitrogen. Nevertheless, concentrations of COD (500–2,900 mg L\(^{-1}\) O\(_2\)), TOC (420.2–1,490 mg L\(^{-1}\)), carbohydrates (ND–321.8 mg L\(^{-1}\)), and proteins (431.1–891 mg L\(^{-1}\)) continued to be high. This indicates that the biologically-treated leachate contained compounds of low biodegradability. Therefore, additional physico-chemical treatment is required in order to meet environmental standards for discharge.

To evaluate the performance of the process using chitosan, we also carried out tests with aluminium sulphate (Al\(_2\)(SO\(_4\))\(_3\).14 H\(_2\)O) as coagulant. The stock solution of chitosan at 1% (m/v) was prepared by dissolving chitosan (Polymar Ciência e Nutrição S/A) in 1% (v/v) hydrochloric acid solution. The stock solution of alum at 2% (m/v) was prepared by dissolving 20 g of aluminium sulphate (Cinética Ltda) in distilled water to make 1.0 L of solution. The tests were performed in a conventional jar-test apparatus (Jar Test Model Nova Ética) with three 2.0 L jars and three paddle rotors. Before rapid mixing, the pH was adjusted by the addition of H\(_2\)SO\(_4\) 1 N or NaOH 1 N, and different dosages of chitosan and alum (Table 2) were added to each jar filled with 1 L of treated leachate sample. After the reaction time, the sludge was then left to settle for 24 h. After the settling period, the supernatant liquid was withdrawn from the jar and the samples were analysed for true colour and turbidity.

The coagulant dosage (C) investigated ranged from 700 to 1,100 mg L\(^{-1}\) for chitosan and from 1,500 to 1,700 mg L\(^{-1}\) for alum; the pH value was varied from 6.0 to 9.0 for chitosan and from 8.0 to 10.0 for alum. The gradient for rapid mixing (G\(_{rm}\)), time for rapid mixing (T\(_{rm}\)), gradient for flocculation mixing (G\(_f\)), and flocculation time (T\(_f\)) were held constant at G\(_{rm}\) = 400 s\(^{-1}\), T\(_{rm}\) = 30 s, G\(_f\) = 30 s\(^{-1}\), and T\(_f\) = 10 min for chitosan and G\(_{rm}\) = 869 s\(^{-1}\), T\(_{rm}\) = 10 s, G\(_f\) = 30 s\(^{-1}\), and T\(_f\) = 10 min for alum.

In order to optimize the independent variables, central composite design (CCD) and response surface methodology were applied. The quadratic equation model for predicting the optimal conditions can be expressed according to

Table 1 | Characteristics of biologically-treated landfill leachate

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
<th>Median</th>
<th>Number of tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.5–8.7</td>
<td>7.9</td>
<td>13</td>
</tr>
<tr>
<td>COD (mg L(^{-1}) O(_2))</td>
<td>300–2,900</td>
<td>1,500</td>
<td>12</td>
</tr>
<tr>
<td>TOC (mg L(^{-1}))</td>
<td>420.2–1,490.0</td>
<td>910.1</td>
<td>7</td>
</tr>
<tr>
<td>Alkalinity (mg L(^{-1}) CaCO(_3))</td>
<td>175–2,000</td>
<td>500</td>
<td>13</td>
</tr>
<tr>
<td>Ammoniacal nitrogen (mg L(^{-1}) N-NH(_3))</td>
<td>ND–17.9</td>
<td>ND</td>
<td>13</td>
</tr>
<tr>
<td>TKN (mg L(^{-1}) N)</td>
<td>2.8–100.8</td>
<td>56</td>
<td>13</td>
</tr>
<tr>
<td>Nitrate (mg L(^{-1}) N-NO(_2))</td>
<td>ND–175.6</td>
<td>5.8</td>
<td>10</td>
</tr>
<tr>
<td>Nitrate (mg L(^{-1}) N-NO(_3))</td>
<td>7.6–107.9</td>
<td>20.1</td>
<td>10</td>
</tr>
<tr>
<td>Total phosphorus (mg L(^{-1}) P)</td>
<td>10.4–29.2</td>
<td>14.2</td>
<td>13</td>
</tr>
<tr>
<td>True colour (mg L(^{-1}) PtCo)</td>
<td>2478.4–9537.5</td>
<td>5385.3</td>
<td>13</td>
</tr>
<tr>
<td>Apparent colour (mg L(^{-1}) PtCo)</td>
<td>3,037–11,975</td>
<td>6983.9</td>
<td>13</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>42.0–192.5</td>
<td>112.5</td>
<td>13</td>
</tr>
<tr>
<td>Chloride (mg L(^{-1}) Cl(^-))</td>
<td>333.2–5 331.7</td>
<td>2665.8</td>
<td>5</td>
</tr>
<tr>
<td>Total solids (mg L(^{-1}))</td>
<td>8,635–30,298</td>
<td>11,770</td>
<td>11</td>
</tr>
<tr>
<td>Suspended solids (mg L(^{-1}))</td>
<td>125–3,700</td>
<td>477.5</td>
<td>12</td>
</tr>
<tr>
<td>Carbohydrates (mg L(^{-1}))</td>
<td>ND–321.8</td>
<td>153.1</td>
<td>7</td>
</tr>
<tr>
<td>Proteins (mg L(^{-1}))</td>
<td>431.1–891.0</td>
<td>770.1</td>
<td>7</td>
</tr>
</tbody>
</table>

TKN, total Kjeldahl nitrogen; ND, not detected.

Table 2 | Criteria and values obtained for both coagulants (chitosan and alum)

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Chitosan</th>
<th>Alum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimum dosage (m L(^{-1}))</td>
<td>960</td>
<td>1,610</td>
</tr>
<tr>
<td>Efficiency removal of true colour (%)</td>
<td>80</td>
<td>87</td>
</tr>
<tr>
<td>Efficiency removal of turbidity (%)</td>
<td>91</td>
<td>81</td>
</tr>
<tr>
<td>Toxicity to fish (% LC(_{50}))</td>
<td>23</td>
<td>42</td>
</tr>
<tr>
<td>Cost of process ($ m(^{-3}))</td>
<td>34.47</td>
<td>26.19</td>
</tr>
</tbody>
</table>
Equation (1), proposed by Montgomery (2009):

\[ Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{i2} X_i^2 + \sum_{i<j}^{k} \beta_{ij} X_i X_j + \ldots + e \]

(1)

where \( i \) is the linear coefficient, \( j \) is the quadratic coefficient, \( \beta \) is the regression coefficient, \( k \) is the number of factors studied and optimized in the experiment, and \( e \) is the random error. Analysis of variance (ANOVA) was used for graphical analysis of the data to obtain the interaction between the independent variables and the dependent variables (responses). Model adequacy was investigated by the examination of residuals. The quality of the fitted polynomial model was expressed by the coefficient of determination \( R^2 \), and its statistical significance was checked by the Fisher’s F-test using Minitab® 16 software. Model terms were evaluated by the probability (P value) with a 95% confidence level. Three-dimensional plots and their respective contour plots were obtained based on the effects of the two factors (coagulant dosage and pH). Finally, the values of response variables were compared in an overlay plot to identify the optimum region of treatment, and confirmatory experiments were performed. Furthermore, the cost estimation was based on the amount of coagulant and \( \text{NAOH} \) used and it did not take into consideration the operational costs for the coagulation-flocculation process.

The experimental design, mathematical modelling, and optimization were performed using Minitab® Release 16 Statistical Software. The optimum values of the variables investigated were obtained by analysing the overlay plots and also by using the desirability function Response Optimizer available in Minitab® Release 16.

To assess the efficiency of post-treatment in the optimum region, characterization and toxicity tests were performed before and after treatment. Acute eco-toxicity of landfill leachate before and after physico-chemical treatment in the optimum region was determined using the \( \textit{Lemna minor} \) duckweed according to OECD Guideline no. 221 (2006) and \( \textit{Poecilia reticulata} \) fish according to OECD Guideline no. 205 (1992) as test organisms. All the samples were tested at concentrations of 50, 25, 12.5, 6.25, and 3.125% (v/v) and a control using dilution water. In order to evaluate the acute toxicity of the dissolved compounds contained in the samples and exclude the influence of pH values on the test organisms, all the samples were adjusted to a pH of 7.0.

Experiments with \( \textit{L. minor} \) were carried out in triplicate by exposing four colonies of two to four visible fronds (leaves), reaching a total of eleven fronds. The test was performed in a static system, that is, without renewal of the solution in a temperature-controlled chamber (24 °C) and under continuous cool-white fluorescent lighting. The percentage growth inhibition of plants and the inhibition of the biomass were determined at the end of the experiment, after 7 days of exposure. The mean effective concentration (EC\(_{50}\)) was determined using regression analysis.

For toxicity testing of the fish species \( \textit{P. reticulata} \), the exposure test was carried out in duplicate at a room temperature of 24 ± 1 °C for 96 h under 12:12 h light:dark conditions, exposing eight organisms at each concentration and in the control. Dechlorinated tap water was used as dilution water. The lethal concentration (LC\(_{50}\)) at 95% confidence limits was calculated by probit transformation of the mortality data using Minitab® 16 software.

To select the most appropriate coagulant for the coagulation-flocculation process, the analytic hierarchy process (AHP) (Saaty 2003) was employed for multi-criteria decision analysis (MCDA). The AHP has theoretical foundations which are accepted by the scientific community (Moura et al. 2007; Aragonés-Beltrán et al. 2009; Fazeli et al. 2014; Dragincic et al. 2015; Plakas et al. 2015). In addition to that, Aragonés-Beltrán et al. (2009) recommend its use for weighting calculations because it does not require complex information from the decision maker.

AHP is based on the fact that the inherent complexity of a multiple criteria decision problem can be solved through the construction of hierarchic structures consisting of a goal, criteria (effective factors) and alternatives. Figure 1 shows a view of the hierarchy structure for selecting the most effective coagulant in this study.

At each hierarchical level, paired comparisons are made with judgments in relation to elements on a higher level using Saaty’s comparison scale. These comparisons lead to dominance matrices from which ratio scales are derived in the form of principal eigenvectors. These matrices are positive and reciprocal (\( a_{ij} = 1/a_{ji} \)). The synthesis of AHP combines multi-dimensional scales of measurement into a single one-dimensional scale of priorities.

In this study, the relative importance of criteria was determined based on the scientific literature and Brazilian legislation. It is worth mentioning that the toxicity to aquatic plants was not included among the criteria because it was not possible to quantify wastewater treated using chitosan-related effects which could distort the results analysis. And the weight of each alternative relative to each covering...
criterion was calculated based on the values obtained in the tests (Table 2). The results were analysed to identify the most appropriate coagulant by the ‘hierarchy analysis process’ in the Expert Choice© software.

RESULTS AND DISCUSSION

Optimization of coagulation–floculation post-treatment using chitosan and alum

Table 3 shows the CCD in the form of a $2^2$ full factorial design with five additional experimental trials (run numbers 5–9) as replicates of the central point and four axial points, as well as the experimental responses obtained from each assay. The experiments were run in random order to minimize the effects of unexpected variability in the observed responses.

The influence of dosage ($X_1$) and pH ($X_2$) on the true colour ($Y_1$) and turbidity ($Y_2$) of the treated leachate was determined using the sequential model of the sum of squares according to Equations (2) and (3) (using chitosan), Equations (4) and (5) (using alum).

$$Y_1(x) = 377.5030 - 0.2862X_1 - 56.417X_2 - 0.0001X_1^2 + 2.5365X_2^2 + 0.0148X_1X_2$$

$$Y_2(x) = -1636.300 + 0.3308X_1 + 422.2090X_2 - 34.3974X_2^2$$

$$Y_1(x) = 61.4125 + 0.3598X_1 - 1.0671X_2 - 0.0022X_2^2 + 0.0406X_1X_2$$

$$Y_2(x) = 630.658 - 4.712X_1 - 59.435X_2 + 0.507X_1X_2$$

Table 3 | CCD for the study of experimental variables for both coagulants (chitosan and alum) and obtained results

<table>
<thead>
<tr>
<th>Run no.</th>
<th>Blocks</th>
<th>Chitosan</th>
<th>Alum</th>
<th>Experimental design</th>
<th>Removal (%)</th>
<th>Experimental design</th>
<th>Removal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C (mg L$^{-1}$)</td>
<td>pH</td>
<td>True colour</td>
<td>Turbidity</td>
<td>C (mg L$^{-1}$)</td>
<td>pH</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>700</td>
<td>6.0</td>
<td>65.1</td>
<td>81.2</td>
<td>1,300</td>
<td>8.0</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1,100</td>
<td>6.0</td>
<td>84.5</td>
<td>-242.4</td>
<td>1,700</td>
<td>8.0</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1,100</td>
<td>9.0</td>
<td>78.0</td>
<td>-56.5</td>
<td>1,300</td>
<td>10.0</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>700</td>
<td>9.0</td>
<td>40.8</td>
<td>81.2</td>
<td>1,700</td>
<td>10.0</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>900</td>
<td>7.5</td>
<td>55.5</td>
<td>88.5</td>
<td>1,500</td>
<td>9.0</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>900</td>
<td>7.5</td>
<td>56.5</td>
<td>85.3</td>
<td>1,500</td>
<td>9.0</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>900</td>
<td>7.5</td>
<td>54.5</td>
<td>82.9</td>
<td>1,500</td>
<td>9.0</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>900</td>
<td>7.5</td>
<td>55.6</td>
<td>82.9</td>
<td>1,500</td>
<td>9.0</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>900</td>
<td>7.5</td>
<td>57.5</td>
<td>85.8</td>
<td>1,500</td>
<td>9.0</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>617</td>
<td>7.5</td>
<td>34.7</td>
<td>72.5</td>
<td>1,217</td>
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</tr>
<tr>
<td>11</td>
<td>2</td>
<td>1,183</td>
<td>7.5</td>
<td>73.3</td>
<td>-41.2</td>
<td>1,783</td>
<td>9.0</td>
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<tr>
<td>12</td>
<td>2</td>
<td>900</td>
<td>5.4</td>
<td>65.1</td>
<td>-183.5</td>
<td>1,500</td>
<td>7.6</td>
</tr>
<tr>
<td>13</td>
<td>2</td>
<td>900</td>
<td>9.6</td>
<td>44.0</td>
<td>72.5</td>
<td>1,500</td>
<td>10.4</td>
</tr>
</tbody>
</table>
The results obtained were analysed by ANOVA to assess the goodness of fit. The ANOVA revealed that the two independent variables, dosage and pH, were significant for the determination of true colour since the P-values were less than 0.05 (0.00) using chitosan and alum coagulants. The coefficient of determination ($R^2$) for the empirical Equation (2) was 99.6% and that for the empirical Equation (4) was 97.7%, which shows a good agreement between the experimental data and model-predicted values for true colour. This means that the models are statistically significant and only small variations are not explained by the models.

Equation (3) for the percentage turbidity removal was modified by eliminating terms that were found to be statistically insignificant in the first ANOVA analysis. The second ANOVA analysis showed that the model was significant for the determination of turbidity (0.01 < 0.05) using chitosan as well as having a low coefficient of determination ($R^2 = 67.03$%). On the other hand, considering the terms of the significant variables, a mathematical model of the first order polynomial for turbidity removal efficiency using alum was determined in order to promote high true colour removal efficiency, since the change of independent variables would influence the good results obtained for true colour.

Based on Equation (2), true colour removal was optimal at a higher dosage of chitosan of 1,100 mg L$^{-1}$ at a pH of 5.4. On the other hand, the highest turbidity removal (90%) was obtained at a lower dosage, less than 900 mg L$^{-1}$, with a pH between 6.5 and 9.5. Graphical optimization was used to determine the process parameters for maximum removal of turbidity and true colour from wastewater. The contour lines for response surfaces were superimposed on an overlaid contour plot (Figures 2 and 3).

The white area of the overlay plot as shown in Figures 2 and 3 defines the optimum region of coagulant dosage and pH for the highest true colour and turbidity removal efficiencies. Figure 2 shows that chitosan dosages lower than 700 mg L$^{-1}$ and pH values between 6.0 and 6.5 or chitosan dosages around 900 mg L$^{-1}$ and pH values between 8.0 and 8.5 lead to greater removal efficiencies of recalcitrant organic matter. Meanwhile, Figure 3 clearly shows that an alum dosage between 1,542 and 1,762 mg L$^{-1}$ and pH between 8.5 and 10.0 lead to greater removal efficiencies.

The pH is a critical parameter in the efficiency of the coagulation–floculation process. This parameter influences the wastewater properties and the behaviour of coagulants in the solution. However, the results have shown that chitosan, when compared to alum, is more sensitive to pH. With a decrease of the pH value, part of the humic substances in the leachate becomes insoluble, which reduces the amount of remaining organic matter and, consequently, the dosage of chitosan required for the destabilization of the colloidal system. In addition to that, at a pH of 6.0 or less, more than 90% of the amine groups are protonated. This means that at lower pH values, the amount of chitosan required for efficient coagulation–floculation will be lower than that needed at higher pH values. This can be explained by the acid–base properties of chitosan and the degree of dissociation of the polyelectrolyte as stated by Guibal & Roussy (2007). The pKa of amine groups is close to 6.3–6.4 for fully dissociated chitosan (with a deacetylation degree of close to 90%). Therefore, less coagulant would be required to destabilize colloidal particles.

However, the addition of chitosan changes the initial pH values of the wastewater because of the acidity of the chitosan solution (pH value near to 3.0). As the chitosan dosage increased, the pH of wastewater decreased. The pH decrease shows that coagulation–floculation with
chitosan as coagulant can be used to treat wastewaters that may have high pH values.

In order to investigate the predicted optimum combination of the process variables of each of the coagulants, confirmatory experiments were carried out, applying the process conditions determined by the Response Optimizer function using Minitab® 16 software (960 mg L$^{-1}$ of chitosan at pH 8.5 and 1,610 mg L$^{-1}$ of alum at pH 9.5). Under these conditions, the maximum efficiency removal of true colour was 80% and the turbidity removal reached 91% using chitosan; using alum, the true colour removal efficiency was 87% and the turbidity removal reached 81% at an optimum dosage of 60 mg L$^{-1}$. Ramli & Aziz (2015) obtained a colour removal efficiency of only 14.67% at a pH 4 and turbidity removal efficiency of 23.52% at pH 9 using chitosan as primary coagulant in the treatment of landfill leachate. Rizzo et al. (2010) obtained a turbidity removal efficiency of 94% (600 UNT) when they added 400 mg L$^{-1}$ of chitosan coagulant to the wastewater of olive oil without adjusting the pH of the liquid medium (4.4). In the treatment of wine wastewater, the same authors achieved 80% (36 NTU) turbidity removal efficiency with 20 mg L$^{-1}$ of chitosan.

The cost estimates ($ m^{-3}$) were determined by determining the cost for each item per kg multiplied by the amount of each item used in the process. According to Table 4, it is clear that the kind and dosage of coagulant not only improve the wastewater characteristics but greatly influence the cost of the final coagulation–floculation process. Despite the lower dosage, the use of chitosan has a high effect on the cost of the process compared to alum.

**Assessment of coagulation–floculation process using chitosan and alum as coagulant**

The samples from the confirmatory experiments were characterized and toxicity was investigated. Table 5 shows the characteristics of the biologically-treated leachate (T1) and leachate post-treated by coagulation–floculation using chitosan (T2) and alum (T3).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>Limit values of Brazilian legislation</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>8.0</td>
<td>4.3</td>
<td>4.4</td>
<td>5-9</td>
</tr>
<tr>
<td>COD (mg L$^{-1}$ O$_2$)</td>
<td>1500.0</td>
<td>1200.0</td>
<td>900.0</td>
<td>–</td>
</tr>
<tr>
<td>TOC (mg L$^{-1}$)</td>
<td>763.4</td>
<td>521.7</td>
<td>291.7</td>
<td>–</td>
</tr>
<tr>
<td>Alkalinity (mg L$^{-1}$ CaCO$_3$)</td>
<td>350.0</td>
<td>ND</td>
<td>ND</td>
<td>–</td>
</tr>
<tr>
<td>Ammoniacal nitrogen (mg L$^{-1}$ N-NH$_3$)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>20.0</td>
</tr>
<tr>
<td>TKN (mg L$^{-1}$ N)</td>
<td>2.8</td>
<td>5.6</td>
<td>2.8</td>
<td>–</td>
</tr>
<tr>
<td>Nitrate (mg L$^{-1}$ N-NO$_3$)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.0</td>
</tr>
<tr>
<td>Nitrate (mg L$^{-1}$ N-NO$_2$)</td>
<td>69.9</td>
<td>88.4</td>
<td>76.4</td>
<td>10.0</td>
</tr>
<tr>
<td>Total phosphorus (mg L$^{-1}$ P)</td>
<td>14.0</td>
<td>14.5</td>
<td>ND</td>
<td>0.030/0.050</td>
</tr>
<tr>
<td>True colour (mg L$^{-1}$ PtCo)</td>
<td>5001.9</td>
<td>955.9</td>
<td>374.8</td>
<td>75</td>
</tr>
<tr>
<td>Apparent colour (mg L$^{-1}$ PtCo)</td>
<td>6983.9</td>
<td>1671.2</td>
<td>442.0</td>
<td>–</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>169</td>
<td>14.5</td>
<td>3.8</td>
<td>100</td>
</tr>
<tr>
<td>Chloride (mg L$^{-1}$ Cl$^-$)</td>
<td>2999.9</td>
<td>2332.6</td>
<td>2665.8</td>
<td>250</td>
</tr>
<tr>
<td>Total solids (mg L$^{-1}$)</td>
<td>11110.0</td>
<td>10488.0</td>
<td>10314.0</td>
<td>–</td>
</tr>
<tr>
<td>Suspended solids (mg L$^{-1}$)</td>
<td>725.0</td>
<td>260.0</td>
<td>150</td>
<td>–</td>
</tr>
<tr>
<td>Carbohydrates (mg L$^{-1}$)</td>
<td>321.8</td>
<td>419.5</td>
<td>378.0</td>
<td>–</td>
</tr>
<tr>
<td>Proteins (mg L$^{-1}$)</td>
<td>750.2</td>
<td>271.7</td>
<td>87.5</td>
<td>–</td>
</tr>
</tbody>
</table>
and leachate post-treated by coagulation–floculation using chitosan (T2) and alum (T3).

Treatment efficiency was also assessed by toxicity monitoring using L. minor and P. reticulata as test organisms. Based on the inhibition of average growth rate ($\mu$) and biomass production, tests with L. minor showed that the plants were sensitive to wastewater samples, with a higher inhibition being caused mainly by post-treated leachate after coagulation–floculation using chitosan. The inhibition of biomass production (Figure 4) was more pronounced than the inhibition of frond multiplication. Mackenzie et al. (2003) evaluated the toxicity of untreated landfill leachate and the reduction of its toxicity after aeration and passage through a constructed wetland using four strains of L. minor. Toxicity was determined by the difference and the amount of chlorophyll fluorescence of leaves, the number of fronds, and total leaves. At the end of the test, the results showed an increase in toxicity with increasing untreated leachate concentrations that were exposed to macrophytes. Moreover, the authors report that the inhibition concentration values of the bodies (IC$_{50}$) progressively decreased at each stage of treatment, reaching an IC$_{50}$ value of 65.2% at the output of the constructed wetland.

Figure 5 shows that the value of the effective concentration at 50% of the organisms (EC$_{50}$) of the effluent treated by coagulation–floculation with alum could be located between the concentration values of 6.25 and 12.5%. Through linear interpolation, it was determined that this value would be equal to 10.36%. Additionally, at that concentration, 100% of the average growth rate ($\mu$) was inhibited. However, when the plants were exposed to different concentrations of biologically-treated leachate before (T1) and after coagulation–floculation using chitosan (T2) and alum (T3), all inhibition values were negative (Figure 5), indicating that, compared to the control, the growth rate increased, which made it impossible to determine the EC$_{50}$. In all samples, the growth rate increase was significant for lower concentrations (3.125 and 6.25%).

At low concentrations, the growth of the plants may have been stimulated by nutrients, mainly nitrate and phosphorus, as well as other compounds present in the samples. On the other hand, the higher concentrations may have contained enough toxic compounds to cause an antagonistic effect on growth enhancement and, therefore, to decrease the growth rate when compared to low concentrations.

The relation between the decrease of the contaminant and the development of the plant has also been confirmed and reported by Clément & Bouvet (1995). According to the authors, the results showed that all samples were significantly toxic to the plants and most of them caused 100% inhibition of growth rate at a concentration of 100%. On the other hand, inhibition of biomass production was significantly higher than the growth inhibition rate, because of the amount of plant fronds with a reduced size. The authors also reported that the organisms developed well when exposed to lower concentrations due to the nutrients still present in the effluent.

Regarding the inhibition of biomass production (Figure 5), biologically-treated leachate showed the same
effects as on growth rate. For the leachate after coagulation–floculation treatment, the inverse relationship could be observed. Higher chitosan concentrations in the test solution increased the biomass production of aquatic plants. This was due to the development of larger frond surfaces, which could be observed in the plants exposed to solutions containing post-treated leachate with chitosan.

Figure 5 shows that there was no gradual increase in the values of inhibition of the production of biomass with increasing concentration of the effluent post-treated with alum and, thus, the relationship between the percentage inhibition of the growth rate and the effluent had low significance.

The acute toxicity of the effluents for the fish *P. reticulata* was determined by the number of dead fish present at the end of 96 h. LC50 values were determined, generating a probability graph that considered a confidence interval of 95% using the Minitab® 16 software. The probability graphs of Figure 6 show that the lines have different slopes for the wastewaters before and after coagulation–floculation treatment, revealing that their toxic effects on fish are significantly different. A line with a lower slope indicates the presence of substances with lower toxicity, which may be due to their poor absorption or rapid detoxification, while the line with the greater slope indicates higher toxicity of the tested solution.

The LC50 value of biologically-treated wastewater was determined to be 47% (v v−1), while it was 42% (v v−1) after physico-chemical treatment using alum and decreased to 23% (v v−1) after physico-chemical treatment using chitosan, representing an increase in the toxicity of the wastewater for *P. reticulata*.

Bullock et al. (2000) carried out toxicity tests with fish of the species *Oncorhynchus mykiss* (rainbow trout) exposed to different chitosan concentrations (0.75, 0.075, 0.038 and 0.019 ppm). Concentration tests were obtained by diluting 1% solution of chitosan prepared in 1% acetic acid. The authors concluded that the toxicity to rainbow trout was due to the acidified chitosan and not the acetic acid solvent. Although the chitosan had been dissolved in hydrochloric acid solution in that study, the data showed that acidified chitosan was also toxic to fish of different species.

On the other hand, Dautremepuits et al. (2004) investigated the potential toxicity of chitosan to fish of the species *Cyprinus carpio Linnaeus* exposed to different concentrations (37.5 to 375 mg L−1). In the experiments, water-soluble chitosan was added to the dilution spring water in tanks to obtain test concentrations. The stress induced in the fish by chitosan was demonstrated by the increased activity of antioxidant enzymes after 4 days of exposure and could be related to chitosan concentration. In concentrations lower than 300 mg L−1, the production of these enzymes would be linked to the organism’s defence mechanism. But at higher concentrations, the stress induced by chitosan cannot be controlled, increasing the mortality of the fish. This phenomenon indicated a non-negligible toxicity of chitosan in fish physiology, even using water-soluble chitosan.

Although chitosan has been considered as a non-toxic polyelectrolyte (Guibal & Roussy 2007; Renault et al. 2009; Ramli & Aziz 2015), the results obtained in the toxicity tests suggest better investigation about the use of chitosan as coagulant.

### Selecting the most appropriate coagulant for coagulation–floculation post-treatment

Bearing in mind such considerations, the selection of the most appropriate coagulant must necessarily be based on a multi-criteria objective, with coagulant dosage, true colour removal efficiency, turbidity removal efficiency, toxicity to fish and costs of the coagulation–floculation process using chitosan and alum being considered the main effective factors. It is worth mentioning that it is not possible to select and determine a coagulant as the best for coagulation–floculation in wastewater treatment, and so the coagulant should be selected based on the quality conditions of the wastewater. Moreover, in this study, this MCDA technique is considered as a complement to the jar-tests and toxicity tests. It has the advantage of bringing more information to the decision process and justifies the selection of the coagulant.
Table 6 | Weights of the criteria according to the tests

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Weights</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxicity to fish (% LC50)</td>
<td>0.483</td>
</tr>
<tr>
<td>Cost of process ($ m⁻³)</td>
<td>0.184</td>
</tr>
<tr>
<td>Efficiency removal of turbidity (%)</td>
<td>0.158</td>
</tr>
<tr>
<td>Efficiency removal of true colour (%)</td>
<td>0.106</td>
</tr>
<tr>
<td>Optimum dosage (m L⁻¹)</td>
<td>0.069</td>
</tr>
</tbody>
</table>

After determining the factors in decision making for the coagulant, the next step was to make a pairwise comparison of criteria in AHP decision matrices. After that, the weights were calculated with the aid of the Expert Choice software, which allows both individual and combined results. The weights obtained for the criteria are shown in Table 6. The toxicity to fish was the most important factor to be considered (48.3%), followed by the cost of the process (18.4%). The toxicity tests are a measure of the environmental impacts of wastewater post-treated in the aquatic environment and, according to Brazilian legislation, the final effluent must not cause or have the potential to cause toxic effects to aquatic organisms, which explains the result obtained for the criteria. It is important to note that the order of priorities of the criteria can change if new factors are included in decision making.

After assessing the relative importance of the criteria, the decision score was found for each alternative. The best alternative is the one with the highest score. According to the results of AHP analysis, the most appropriate coagulant is alum at a concentration of 1,610 mg L⁻¹ with a relative importance of 67.5%, and the score of chitosan at a concentration of 960 mg L⁻¹ was 32.5%.

CONCLUSION

This study examined the post-treatment of biologically-treated landfill leachate by coagulation–flocculation using chitosan as the primary coagulant and alum as a comparative coagulant. CCD was employed to study and optimize the process variables such as pH and coagulant dosage for the removal efficiency of true colour and turbidity from landfill leachate that was treated biologically. The optimum process variables were found to be 960 mg L⁻¹ of chitosan at pH 8.5 and 1,610 mg L⁻¹ of alum at pH 9.5. Under these conditions, the maximum efficiency removal of true colour was 80% and the turbidity removal reached 91% using chitosan. Alternatively, alum coagulant reached 87% true colour removal efficiency and 81% turbidity removal, indicating that chitosan as a primary coagulant performed as well as alum in the removal of recalcitrant organic matter. In addition to the chemical effectiveness, the cost of the process should be considered when selecting a coagulant. In this study, the cost of the process using chitosan (34.47 $ m⁻³) was higher than cost using alum (26.19 $ m⁻³).

In addition to that, the results of the acute toxicity tests showed that organisms were sensitive to all samples, mainly to biologically-treated leachate after coagulation–flocculation using chitosan: CE₅₀ for L. minor was not determined because there was no inhibition of the average growth rate and biomass production; LC₅₀ for P. reticulata was 23% (v v⁻¹). These results suggest that chitosan has a toxicity level which is not negligible.

The use of multi-criteria analysis allowed us to aggregate the whole volume of information generated with jar-tests, toxicity tests and to select the most suitable coagulant for the specific problem to be solved. Therefore, the application of AHP, which took into account mainly an environmental cost-benefit approach, showed that the most appropriate coagulant in this study was alum.

In the future, in order to extend the knowledge and improve the coagulation–flocculation process, it would be useful to examine the use of chitosan as a supplementary coagulant. Although its non-negligible toxicity and high cost, in comparison with alum, constrain the application of chitosan as a primary coagulant, the high sensitivity of the performance of chitosan in removing true colour and turbidity concentration opens up possibilities for its use as a coagulant to aid in the removal of recalcitrant contaminants in landfill leachate.

REFERENCES


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