COMPARISON OF ADVANCED TECHNIQUES FOR THE TREATMENT OF AN INDIGO MODEL SOLUTION: ELECTRO INCINERATION, CHEMICAL COAGULATION AND ENZYMATIC DEGRADATION

COMPARACIÓN DE TÉCNICAS AVANZADAS PARA EL TRATAMIENTO DE UNA SOLUCIÓN MODELO DE ÍNDIGO: ELECTRO INCINERACIÓN, COAGULACIÓN QUÍMICA Y ENZIMÁTICO

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Abstract

Indigo blue is one of the most important textile dyes. The wastewaters for indigo blue used are thought to be contaminated because; indigo can be oxidized to isatin, which is hydrolyzed to antranilic acid, a compound that is toxic to aquatic life. Indigo oxidation was evaluated using a model solution that simulated a textile wastewater. Three oxidation systems: electro incineration, chemical coagulation using Al2(SO4)3, and enzymatic degradation with laccase. With electro incineration, the indigo solution was completely discolored; chemical oxidation demand diminution was 98 % in approximately 3 hours and indigo blue was mineralized with no sludge production. Chemical coagulation with 40 mg L−1 of sulfate aluminum removed some color though control of pH was necessary. At pH 5, the chemical oxygen demand was reduced by 76 %, with sludge formation. Laccase enzyme, processing required 72 hours for complete discoloration of the indigo model solution; chemical oxidation demand diminution was nearby 50 %, but laccase could not mineralize the dye. Toxicity assays indicated that the processes generated more toxic products than the control.

Keywords: electro incineration, indigo, oxidation, chemical coagulation, laccase, toxicity.

Resumen

El azul índigo es de los colorantes textiles más utilizados, las descargas que lo contienen son contaminantes porque, según algunos reportes, el indigo se oxida a isatin, compuesto fácilmente hidrolizable a ácido antranílico, sustancia tóxica para la vida acuática. Se evaluó la oxidación del indigo usando una solución modelo similar a las descargas textiles. La comparación se hizo con tres sistemas: electroincineración, coagulación y enzimático con lacasa. Con la electroincineración el indigo fue completamente decolorado y la demanda química de oxígeno disminuyó 98 % en aproximadamente 3 horas, el indigo se mineralizó sin formación de lodos. La coagulación química, usando 40 mg L−1 de sulfato de aluminio, removió parte del color; sin control de pH la reducción de la demanda química de oxígeno fue despreciable, mientras que controlando el pH a 5 durante la prueba de jarras, la demanda química de oxígeno disminuyó 76 %; hubo formación de lodos. Con la enzima lacasa, la decoloración fue en 72 h, la demanda química de oxígeno disminuyó en 50 %, la lacasa no mineralizó el colorante. La prueba de toxicidad mostró que los tres procesos formaron productos más tóxicos que el indigo.

Palabras clave: electroincineración, índigo, oxidación, coagulación química, lacasa, toxicidad.
1. Introduction

The textile industry is one of the top water polluting industries in terms of discharge volume, as well as color and chemical composition of residual wastewater. Textile effluents include dyes that have a complex chemical structure, which frequently are disposed untreated to municipal sewers or into surface waters (Nilson et al., 2006).

Azo dyes do not occur in nature and are produced only through chemical synthesis. Due to their chemical structure, these dyes are recalcitrant to conventional wastewater treatment processes (Pereira and Duran 2001; Abadulla et al., 2000). Azo dyes are often toxic or have toxic transformation products, when released into the environment. Therefore, their complete mineralization is desired (Kandelbauer et al., 2004). Almost one million tons of textile dyes are produced annually in the world, of which about seventy percent represent the azo dye group (Zille et al., 2004).

The release of azo dyes into the environment through the effluent of textile dyeing plants has become a major concern in wastewater treatment. Azo dyes can not be treated with conventional biological waste water systems. Under anaerobic conditions, azo reductases usually cleave azo dyes into their corresponding amines, many of which are mutagenic and/or carcinogenic (Camarero et al., 2005; Kim et al., 2002). Human exposure to azo dyes occurs through ingestion, inhalation, or skin contact. The human microflora plays roles in the degradation of azo dyes, with azo reduction being the most important reaction related to toxicity and mutagenicity. Ingested azo dyes are mainly metabolized by intestinal microflora to colorless aromatic amines (Xu et al., 2007).

Some scientific researchers have studied techniques for azo dye treatment like ozonation, photocatalytic oxidation, electrochemical coagulation, chemical coagulation, biological treatment, adsorption with activated charcoal, ultra filtration and electro coagulation (Cañizares et al., 2006).

The degradation of azo dyes has been extensively studied using a wide range of fungi and bacteria. Fungi, with their ligninolytic enzyme systems are also used for the biological discoloration of textile dyestuff (Nyanhongo et al., 2002). Oxidative biodegradation takes place upon action of enzymes such as peroxidase and laccase (Camarero et al., 2005; Kim et al., 2002; Unyayar, 2005; Erkurt, 2007).

A new process for the treatment of recalcitrant compounds is electro incineration using Boron Doped Diamond (BDD) electrodes, to mineralize organic compounds. During the reaction some oxidant agents such as hydroxyl radicals OH' are formed in-situ on the BDD electrodes (Scialdone et al., 2008, Michaud et al., 2003, Zhi et al., 2003.). However, electro incineration has to be controlled for specific potential values (E) and current density (J) in order to favor hydroxyl radical (OH') production, and avoid H₂O₂ production. Hydrogen peroxide is a weak oxidant (Bedoui et al., 2009; Bahadrir et al., 2003). Hydroxyl radical is formed by water oxidation on the BDD surface, these radicals exhibit stronger oxidant properties than H₂O₂, so that more color molecules are degraded to CO₂ (Butrón et al., 2007).

An FM01-LC electrochemical reactor has been used in different areas including waste water treatment (Nava et al., 2007; Griffiths et al., 2005). Electrochemical incineration has been found to be adequate for the treatment of colored wastewaters. Several color mineralization studies mention systems with platinum electrodes and Dimensionally Stable Anodes (DSA) but these have shown low mineralization (Faouzi et al., 2006; Chen et al., 2005).

Chemical coagulation has been used for the waste water treatment. Coagulation agents produce charged particulates that combine with ions neutralizing the charges. The neutral particulates coagulate and subsequently sediment. Historically, dirty water is cleaned by treating with alum, Al₂(SO₄)₃·12 H₂O, and lime, Ca(OH)₂. However disposal of coagulation sludge is a concern (Cañizares et al., 2006; Sharp et al., 2006; Meric et al., 2005).

Analysis using a recognized set of water chemistry parameters is the most common method of assessing water quality by regulatory authorities. These parameters normally include chemical composition but not toxicity (Sharp et al., 2006; Meric et al., 2005). Determination of acute toxicity of chemical products is part of a general hazard assessment established by various environmental protection agencies. Regulatory agencies and research organizations commonly use conventional acute toxicity tests with fish or Daphnis in which immobility and mortality are the most frequently monitored end points. Daphnia magna has been evaluated as a useful organism to test effluent toxicity in textile wastewater, dye and dyes wastewater (Yogendra, 2008; Sponza, 2006).

The overall objective of this study was to compare and contrast three processes for the treatment of an indigo textile dye solution, which simulate industrial wastewater from a denim laundry: electro incineration with BDD electrodes, chemical coagulation using aluminum sulfate and enzymatic oxidation using laccase. Parameters determined in this study were COD, color removal, and the acute toxicity of the indigo oxidation product from the three processes compared with the indigo solution.
2. Methodology

2.1 Reagents

All reagents including indigo blue were analytical grade and purchased from Sigma Aldrich. All solutions were prepared with analytical grade reagents and deionized water having 18 mΩ−1 cm−1 resistivity from a Mill-Q™ system.

For the coagulation test, the reagent used was Al₄(SO₄)₆. The pH was adjusted with NaOH or CH₃CH₂OH.

Laccase was from a commercial product named Deni Lite II S, purchased from Novozymes México. This product contains laccase, fenotiazin-10 propionic acid as a mediator, and a non ionic surfactant in a phosphate buffer (Greca et al. 2001). Laccase was selected for this study because it is one of the enzymes most used for indigo discoloration, and its oxidation products are well known. All laccases oxidize indigo yielding isatin (indole-2,3-dione) (Campos, 2001).

A mediator 2,2 azino bis (3-ethylbenzotizolin 6 sulfonic) acid (ABTS) was used with laccase. The basis of the laccase-mediator concept is that low-molecular-weight compounds are oxidized by the enzyme to stable radicals, which then act as redox mediators, oxidizing compounds that are not normal substrates of laccase (Camarero, 2005). ABTS is oxidized by laccase to the cation radical (ABTS⁺), which oxidizes indigo by an indirect enzymatic oxidation without direct contact of the dye and enzyme (Solis-Oba et al. 2007).

2.2 Equipment

A potentiostat-galvanostat EG&G model PAR 273 and M270 software were used for all chronoamperometric experiments. Chemical oxygen demand (COD) analyses were performed using Hach equipment.

2.2.1 Electro incineration

The FM01-LC reactor was operated in an undivided mode with a single electrolyte compartment and electrolyte flowing circuit (Butrón et al. 2007). The turbulence promoter is a plastic routed mesh, promoter type D. In this work the spacer and promoter were 0.55 cm thick each. Working and counter electrodes were BDD and a platinum coated titanium flat sheet, respectively.

2.2.2 Chemical coagulation

The chemical coagulation was performed with the jar test, using a Kemira Kemwater Floculator 2000. The measured of residual Al was done by atomic absorption assay, using a GBC equipment model 932 AA.

2.2.3 Enzymatic oxidation

The color removal was measured in a Hewlett Packard spectrophotometer UV-VIS model 8453. Analysis was performed at 602 nm, the maximal absorption wavelength for indigo; It absorbs light in the yellow region of the spectrum (maximum at 602 nm), which gives it its intense blue color (Spellstone, 2004).

2.3. Indigo model solution

In order to prepare the indigo model solution there were analyzed some samples from a denim laundry, they contain principally: indigo, NaCl the neutral electrolyte most often used in dyeing (Barber, 1992), acetic acid and some additives like humectants and surfactants. The average parameters of these samples were: 536 mg/L of chemical oxygen demand (COD), with 18 mΩ−1 cm−1 resistivity, conductivity of 5.78 mS cm−1 and pH 6.34. The model solution was prepared with 1 M Indigo and 0.05 M NaCl to simulate laundry industrial wastewater.

2.4 Electro incineration methods

Electrochemical incineration of indigo was carried out in the FM01-LC cell using BDD electrodes at a current density of 15 mA cm−2; this optimized value was previously determined (Butron et al., 2007).

Electrolysis was performed using the indigo model solution, and incineration progress was estimated by COD analysis of samples taken at different times. The COD values were determined by the closed reflux dichromate titration method. Estimating residual organic matter by COD analysis eliminates interference from chloride species (Butron et al. 2007).

2.5 Chemical coagulation methods

Three experiments were conducted with the coagulation jar test: The first one using solutions with 40, 60, 80, 100, 120,140, 160, 180, 200, 220 and 240 mg L−1 (Al₂(SO₄)₃) added to the indigo model solution. A second experiment used 200 mg/L (Al₂(SO₄)₃) and the indigo model solution with different initial pH values: 2, 4, 6, 8 and 10. The third experiment used 40 mg L−1 (Al₂(SO₄)₃) as follows: one jar test without pH control and the other by controlling to pH 5 during the entire experiment. In each case, initial pH, final pH, final COD and the remaining Al were measured to compare the different experimental conditions.

The speciation diagram for Al was constructed with the constant of formation from the database HYDRA and with the software MEDUSA.
2.6 Laccase oxidation methods

Samples for enzymatic oxidation were prepared with 0.3 mL purified laccase in buffer acetate pH 5, and 0.1 mL of 5 mM ABTS. The electronic absorption spectrum was measured at 602 nm wavelength (indigo maximal absorption), during 72 h to record the color diminution. The final COD was determined after ultra-filtration with a 3 KDa membrane.

2.7 Toxicity assay methods

The toxicity assay for the indigo solution and for the oxidation products, formed after the treatment with each one of the three process were done following the standard method indicated in the NMX-AA-087-1995-SCFI. The assay evaluates the effect of each sample in *Daphnia magna straus* and reporting the LD50, which is the concentration causing lethality of 50 % of the organisms.

3. Results and discussion

3.1 Electro incineration

Fig. 1 shows the indigo COD variation measured at current density of 15 mA cm\(^{-2}\) as a function of time and considering different flow rates. Indigo COD values decreased as the electrolysis process continued, decreasing was as a function of electrolysis time. However, incineration of organic matter did not depend on the flow rate, mineralization was the same at the different flows tested.

At the end of the process, after 3 hours of electro incineration, COD decrease to near zero indigo mineralization was almost 100 %. Dogan et al (2005) reported only 60 % indigo mineralization using platinum anodes, comparing with this information, for indigo mineralization using BDD electrodes have shown better results than platinum ones. The same result has been reported for mineralization of other compounds such as chlorobenzene (Lei, 2009). During the electro incineration there was no sludge formation and indigo solution was completely discolored.

Ammar et al. (2006) reported that anodic oxidation of indigo carmine with BDD electrodes produced isatin 5-sulfonic acid as the main aromatic product formed, and oxalic and oxamic acids are generated as ultimate carboxylic acids. Mineralization continues until CO\(_2\) and H\(_2\)O production; and the nitrogen of the dye is converted into NH\(_4^+\) and NO\(_3^-\). Bechtold et al. (2006a, 2006b) reported the production of hypochlorite for indigo oxidation using electrolysis or BDD electrodes, both in presence of NaCl.

Fig. 1. COD changes during the indigo model solution oxidation with electro incineration at 15 mA cm\(^{-2}\) at different flow rates.

3.2 Chemical coagulation

3.2.1. Using different aluminum sulfate concentrations

Fig. 2 shows the indigo solution COD diminution by adding different quantities of aluminum sulfate. Under the conditions tested, the coagulation process required more than 120 mg L\(^{-1}\) of Al\(_2\)(SO\(_4\))\(_3\) to diminish the COD; at lower concentrations of aluminum salt the COD removal was negligible. An increase in coagulation salt concentration from 150 to 240 mg L\(^{-1}\) (Al\(_2\)(SO\(_4\))\(_3\)) did not have a substantial effect on the coagulation efficiency, some of the aluminum remains in solution.

With all the evaluated (Al\(_2\)(SO\(_4\))\(_3\)) concentrations, the initial pH was 6.3, and the final pH value was less than 3.5. This change was due to the acid chemical species (protons) formed during the coagulation process.

Fig. 2. COD changes after the indigo model solution coagulation using the jar test, with different concentrations of aluminum sulfate as a coagulant agent.

3.2.2. Using different initial pH

Table 1 shows final values of pH, COD and Al remained, from the jar test with 200 mg L\(^{-1}\) aluminum salt and varied initial pH. As in the experiment above, final pH for all cases was lower than 3.5. The maximal COD diminution was obtained with an initial pH of 12, however COD decreased only to 35 % of the initial value and the Al
Table 1. Results for indigo coagulation with 200 mg L\(^{-1}\) aluminum sulfate at different initial pH

<table>
<thead>
<tr>
<th>Initial pH</th>
<th>Final pH</th>
<th>Final COD</th>
<th>Al Residual</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 2</td>
<td>2.05</td>
<td>570</td>
<td>165</td>
</tr>
<tr>
<td>pH 4</td>
<td>2.8</td>
<td>574</td>
<td>171</td>
</tr>
<tr>
<td>pH 6</td>
<td>2.98</td>
<td>507</td>
<td>165</td>
</tr>
<tr>
<td>pH 8</td>
<td>3.06</td>
<td>529</td>
<td>162</td>
</tr>
<tr>
<td>pH 12</td>
<td>3.41</td>
<td>360</td>
<td>64</td>
</tr>
</tbody>
</table>

remained was high. Initial pH did not have a marked effect on the coagulation efficiency, because the solution acidifies during the coagulation process. The subsequent experiment was performed adjusting the pH at 5 during the total coagulation process.

3.2.3. Controlled pH

Table 2 shows the results of the jar test using 40 mg L\(^{-1}\) aluminum sulfate and controlling the pH at 5.0 in contrast to uncontrolled pH. The maximal COD removal reached was 76 % when pH was controlled during the complete jar test. This reaction was done using a few quantity of coagulating salt (40 mg L\(^{-1}\)). Without pH control, indigo removal was negligible. Efficiency attained by controlling pH at 5.0 during the complete jar test was higher than using elevated concentration of aluminum sulfate or by using the initial alkaline pH values. The pH has an important effect on coagulation efficiency but pH must be controlled during the entire coagulation process to be effective.

Table 2. Coagulation of Indigo model solution using 40 mg L\(^{-1}\) Al\(_2\)(SO\(_4\))\(_3\) and initial pH 5.

<table>
<thead>
<tr>
<th>Controlled pH at 5.0</th>
<th>Without pH control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final COD mg L(^{-1})</td>
<td>128</td>
</tr>
<tr>
<td>COD Removal</td>
<td>76 %</td>
</tr>
</tbody>
</table>

These results can be explained by evaluating the chemical coagulation with aluminum sulfate: when Al\(^{3+}\) is added to water, the aluminum ions enter into a series of complicated reactions, initially forming a mononuclear complex (Holt \textit{et al.}, 2002):

\[
\text{Al}^{3+} + \text{H}_2\text{O} \rightarrow \text{Al(OH)}^{2+} + \text{H}^+ \quad (1)
\]

\[
\text{Al(OH)}^{2+} + \text{H}_2\text{O} \rightarrow \text{Al(OH)}^+_2 + \text{H}^+ \quad (2)
\]

\[
\text{Al(OH)}^+_2 + \text{H}_2\text{O} \rightarrow \text{Al(OH)}^0 + \text{H}^+ \quad (3)
\]

\[
\text{Al(OH)}^0 + \text{H}_2\text{O} \rightarrow \text{Al(OH)}^- + \text{H}^+ \quad (4)
\]

In concentrated solutions having a low pH, the aluminium sulphate can be found under the form of ions: Al\(_2\)(SO\(_4\))\(_3\) \rightarrow 2\text{Al}^{3+} + 3\text{SO}_4^{2-}\), but if the same reagent is placed in natural water (which is a diluted solution and pH near to neutral), the Al\(^{3+}\) ion hydrolyzes under the form of polynuclear ionic groups. These groups contain di- and trivalent ions (other than OH\(^-\)) and up to 8 Al atoms. The forming of such species depends on the H\(^+\) and OH\(^-\) ions concentration (the water pH) and the raw water chemical composition (Blăgoi, 2002); aluminium begins to polymerize when the pH of an acid solution increases significantly beyond 4.5:

\[
2\text{Al(OH)}\left(\text{H}_2\text{O}\right)^{2+} \rightarrow \text{Al}_2\left(\text{OH}\right)\left(\text{H}_2\text{O}\right)^{2+} + 2\text{H}_2\text{O}
\]

The polymerization products grow, and eventually lead to the formation of polycation Al\(_2\)(OH)\(_{24}\) (H\(_2\)O)\(_{12}\)\(^{2+}\) called Al\(_{13}\) (Meghzili, 2008). Some authors indicate that the higher quantity of OH relative to H\(_2\)O makes the aluminum polycation a better coagulant substance than the aluminum mononuclear species; because polynuclear species absorbs onto specific sites of contaminant particles by desestabilization of them and causes the aggregation of the particle to the polycation (Snoeyink and Jenkins, 1987).

3.2.4. Speciation diagram

Fig. 3 shows the speciation diagram constructed with the software MEDUSA. It corresponds to the Al\(_2\)(SO\(_4\))\(_3\) – NaCl system molar fractions diagram. In textile laundries NaCl is commonly used. Therefore we studied the Al\(_2\)(SO\(_4\))\(_3\) coagulation process in the presence of NaCl, used at the same concentration as the real wastewater. How the coagulation process works by formation of ions, it is very important to study the effect of salt in the indigo coagulation with aluminum sulfate.

The diagram shows that at pH values higher than 4.5 to higher, aluminum hydroxide is the sole species formed. Aluminum hydroxide is the principal species needed for the aluminum polymerization, and the polymer is the molecular species which coagulates with the indigo. At pH lower than 3.5, the quantity of Al(OH)\(_3\) formed is negligible, this explains the minimal efficiency of indigo coagulation in jar test without pH control, because the formation of Al mononuclear particles is favored, these particles can not coagulate the dye. In summary aluminum sulfate as a coagulant can be effective at the pH range of 4.5 or higher.

Fig. 3. Speciation diagram for Al\(^{3+}\) (7mM) in presence of chloride (84 mM) and sulfates (1.7 mM).
Below pH 4.5, alkalinity in the water supply is generally insufficient and the aluminum ions become soluble rather than insoluble and do not participate in the hydration and reactions necessary to make the aluminum sulfate effective as a coagulant in the case here considered.

3.3 Oxidation with laccase

Fig. 4 shows the electronic absorption spectrum of indigo model solution incubated with laccase and ABTS. It illustrates the indigo discoloration process (absorbance measured at 602 nm, maximal for indigo); because isatin, the indigo oxidation product using laccase (Campos et al, 2001) does not absorb between 450 and 750 nm, the absorbance spectra would not show accumulation of isatin. Campos et al. (2001) found that enzymatic oxidation of indigo with laccase from Trametes hirsuta and Sclerotium rolfsii produces isatin. Gursharan et al. (2007) reported the same chemical mechanism using laccase from the bacteria γ-Proteobacterium JB. Discoloration by laccase (about 72 h) was slower than by electroincineration (about 3 h). The maximal indigo COD diminution by laccase was only 53 %, showing that laccase could not mineralize the dye as effectively as electro incineration. Because laccase processing forms isatin, an organic compound, COD diminution was slow; this result is in accordance with Nyahongo et al. (2002) and Rodriguez (2007).

Fig. 4. UV-Visible electronic absorption spectra of Indigo model solution during the reaction with laccase and ABTS. Indigo concentration was measured by absorbance at 602 nm.

3.4 Toxicity

Table 3 presents a comparison of the three processes tested to oxidize the indigo model solution contrasted to control (indigo dye model solution).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% COD removal</th>
<th>LD50 ± Standard deviation at 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indigo model solution</td>
<td>--------</td>
<td>82.5 ± 9</td>
</tr>
<tr>
<td>Electro incineration</td>
<td>98 %</td>
<td>55 ± 6</td>
</tr>
<tr>
<td>Chemical coagulación using Al₂(SO₄)₃</td>
<td>76 %</td>
<td>19 ± 6</td>
</tr>
<tr>
<td>Laccase + ABTS</td>
<td>53 %</td>
<td>20 ± 4</td>
</tr>
</tbody>
</table>

The LD50 values reflect the required dilution of the process solution to yield 50 % lethality.

Conclusions

Three potentially useful procedures for treating indigo dye in textile laundry wastewater were compared and contrasted. The information that was obtained may be helpful in determining whether these technologies can be applied to large scale wastewater treatment. In particular, the duration required for either COD or decolorization, and requirements for pH control, provide information on likely large scale process requirements.

Some specific guidance that may be derived from these studies include 1) electro incineration with BDD electrodes seems particularly effective. Perhaps hypochlorite, if it presents a significant ecotoxicity problem, could be neutralized with sodium sulfite, bisulfate, or thiosulfite. 2) Control of pH (4.5 to neutral pH) during chemical coagulation would diminish requirement for aluminum sulfate and reduce effluent acidity, 3) a combination of treatments may be most practical. For example, an initial decolorizing treatment with laccase followed by electroincineration (to mineralize isatin and...
antranilic acid) might be cost effective as BDD electrodes are expensive.

The toxicity evaluation provided a preliminary indication that chemical coagulation and laccase ABTS treatment may accentuate acute toxicity. The small difference in toxicity of the electro incineration product solution would not likely be considered biologically significant. Product solutions from chemical coagulation and laccase / ABTS treatment appeared somewhat more toxic than control. For any of these indigo dye treatment processes, large scale use would require more extensive testing (including residual chemicals and ecotoxicity) with careful evaluation of the findings.

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