Treatment of Simulated Wastewater Containing n-phenyl-n-isopropyl-p-phenylenediamine Using Electrolysis System with Ti/TiRuO$_2$ Electrodes

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ABSTRACT

This study investigated the effects of the electrolytic treatment in the simulated wastewater with aromatic amine n-phenyl-n-isopropyl-p-phenylenediamine (Flexzone 3P®) using Ti/TiRuO$_2$ electrodes under 0.025 A/cm^2 (DC) for different electrolysis durations (5; 15; 30; 45 and 60 min). Conductivity, pH, UV-visible spectra, gas chromatograms, toxicity and biodegradation tests were carried out. During the electrolytic treatment the pH decreased and conductivity increased slightly. After 60 min of electrolysis, the concentration of Flexzone 3P decreased by 65.1%. UV-vis spectra and chromatograms of simulated wastewater showed changes in the molecular structure of the aromatic amine. After 5 and 15 min of electrolysis, the simulated wastewater containing the Flexzone 3P showed detoxification by Saccharomyces cerevisiae *toxicity test. The electrolysis of 5 min improved the biodegradation of the simulated wastewater containing Flexzone 3P.*

Key words: Electrolytic treatment, wastewater, Ti/TiRuO$_2$ electrodes

INTRODUCTION

There are many industries, such as pulp and paper mills, pharmaceutical, and electroplating industries, which discharge wastewaters with certain pollutants which would cause a serious impact to the environment (Chiang et al., 1997). Many pollutants have certain substituints such as halogen, sulfo-, azo- or nitro-groups. Particularly the accumulation of such groups and specific substitution patterns confer xenobiotic character to a synthetic compound (Campbell, 1977; Knackmuss, 1996). As a consequence, many of these chemicals tend to persist in the environment. Persistent chemicals present the problem of accumulation in organisms near the top of food chains to a much higher level generally than in the environment (Alexander, 1977; Campbell, 1977; Melo and Azevedo, 1997; Tortora et al., 2000). Consequently, the treatment of the industrial wastewaters has always been conducted to minimize the threat to the environment. Biological processes are the treatment systems used most and they can usually remove readily biodegradable organics (Chiang et al., 1997). However, if the main part of the organic compounds present in a wastewater is persistent, microorganisms are not able to degrade it and the biological processes are not suitable. Depending on their quantity and their ecotoxicological behavior, the recalcitrant organics have to be removed by additional treatment (Gulyas, 1997; Chiang et al., 1997; Israilides et al.,

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1997; Panizza et al., 2000; Moraes et al., 2000; Janssen and Koene, 2002).

Electrochemical treatment is a powerful tool for the purification of this kind of wastewater. This technology has attracted a great deal of attention because of the versatility, which makes the treatment of liquids, gases and solids possible. Other characteristics include energy efficiency, amenability to automation and environmental compatibility. In fact, the main reagent is the electron, which is a "clean reagent" (Rajeshwar et al., 1994). The electrochemical methods have been successfully used for the treatment of wastewater from slaughterhouse (Marconato et al., 1998), textile wastewater (Naumczyk et al., 1996; Shen et al., 2001; Xiong et al., 2001), tannery wastewater (Vlyssides and Israilides, 1997; Szpyrkowics et al., 2001), domestic wastewater (Vlyssides et al., 2002), and biorefractory organics (Saracco et al., 2001). After the electrochemical treatment of wastewater containing persistent compound, the biodegradation and toxicity can be improved (Vlyssides and Israilides, 1997; Chiang et al., 1997; Israilides et al., 1997; Angelis et al., 1998).

The present study investigated the degradation of a simulated wastewater containing the compound n-phenyl – n – isopropyl – p – phenylenediamine (commercial name is Flexzone 3P), using Ti/TiRuO$_2$ electrodes. The Ti/TiRuO$_2$ electrodes are dimensionally stable anodes (DSA$^®$) and they are suitable for electro oxidation of organic compounds. They show high corrosion resistance and physical/chemistry stability under high positive potentials (Pelegrino et al., 2002). However, other kinds of electrodes have also been utilized for electro oxidation of organic compounds such as the synthetic boron-doped diamond (BDD) electrodes (Panizza et al., 2001). DSA$^®$ electrodes have been chosen for these experiments due their good features for treating wastewaters containing organic compounds (Régis and Bidoia, 2001a; Chung and Park, 2000).

The commercial compound Flexzone 3P is an aromatic amine used by a chemical industry of rubber antioxidant and antiozonant (Régis and Bidoia, 2001a) located in Rio Claro, São Paulo State, Brazil. The compound Flexzone 3P has been chosen because it is one of the most important chemical compounds present in the effluent from that Brazilian industry. Many aromatic amines have been reported to be powerful carcinogens and mutagens, and/or hemotoxicants (Chung et al., 1997; Benigni and Passerini, 2002). Consequently

the treatment of the industrial wastewaters containing these kinds of chemicals is very important to minimize the threat to the environment.

We studied the electrochemical treatment in order to reduce the toxicity of the simulated wastewater containing Flexzone 3P. In particular, conductivity and pH were monitored with electrolysis duration. After electrolysis duration, samples of wastewater were submitted to UV-visible spectrophotometry analysis, gas chromatography, toxicity assay and biodegradation test using the respirometric Bartha flask.

MATERIAL AND METHODS

Simulated wastewater

The wastewater investigated in this study was prepared at laboratory using n-phenyl-n-isopropyl-p-phenylenediamine (commercial name is Flexzone 3P). This compound is an aromatic amine obtained from a chemical industry in Rio Claro, Brazil. The test compound has tablet form and for this reason it was macerated using a porcelain mortar and pestle. Thereafter 1g was dissolved in 5 mL of ethyl alcohol (Merck PA) and the volume was completed to 1000 mL in a volumetric flask with Na$_2$SO$_4$ 0.15M solution. A saturated solution was obtained and therefore it was filtered and stored in amber flask in cold room. The concentration of the saturated solution was 100.5 mg.L^{-1} and it was known by quantitative analysis using UV-visible spectrophotometer Shimadzu, 2401 PC.

Electrolytic system

The experiments were carried out in a 250 mL batch electrolytic cell (Fig. 1). A laboratory DC power supply (Dawer, FCC-3005D) with current-voltage monitor was employed to provide the electric power during electrolysis. The cell was equipped with a magnetic stirrer in order to keep the electrolyte well mixed. In system, Ti/TiRuO$_2$ electrodes were used and they were prepared according to an expired patent (De Nora, 1970), placed vertically and parallel to each other in the electrolytic cell. They were operated at 0.025 A.cm^{-2} with electrolysis duration of 5; 15; 30; 45 and 60 min.
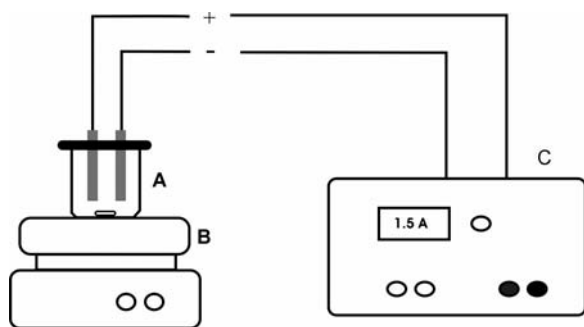
Figure 1 - Sketch of the electrolytic system used. A: Electrolytic cell 250 mL with Ti/TiRuO$_2$ electrodes. B: Magnetic stirrer. C: DC power supply.

Gas chromatography

HPI 5890, series 2, stationary phase 100% dymethylpolythilozane, volume sample: 0.5 to 1 microlitre, column length: 30 m, column internal diameter: 0.53 mm, film thickness: 0.88 micron, injector temperature: 250ºC, detector temperature: 350ºC, not polar column, detector flame ionization and flow of Helium gas.

Toxicity assay with *Saccharomyces cerevisiae*

The toxicity of simulated wastewater containing Flexzone 3P treated during different electrolysis durations was evaluated by survival data analysis for *Saccharomyces cerevisiae*, according to Régis and Bidoia (2001b). A cell suspension was obtained with 14.0 g of Fleishmann Royal® yeasts. Yeast cells were washed in distilled water by centrifugation for three times. The sediment was resuspended in 100 mL saline solution (0.85%) and later it was diluted (10^{-1}). An aliquot of 1 mL of this dilution was added to 9 mL of simulated wastewater treated during electrolysis in test tubes (6 repetitions each treatment) at 28ºC. After seven days exposure, the cells colored with erythrosine (Sharf, 1972) were counted in Neubauer Counting Chamber. The expression of results was obtained considering the total cellular viability [(living cells/ living and dead cells) x 100].

Bartha respirometric test (Cetesb, 1990)

The soil was classified as sandy, sandy fine sub-class by Instituto Campineiro de Análise de Solo e Adubo, Brazil. After the collection the soil was dried at ambient temperature, sifted in sieve of mesh 0.2 mm and stored in polyethylene plastic. During the respirometric test, carbon dioxide evolution rates were monitored using Bartha respirometric flask (Fig. 2). Carbon dioxide, which was evolved during the respiration process, was trapped in 10 mL of aqueous solution of KOH (0.2 N) placed in the side arm attached to the respirometric flask. The amount of carbon dioxide absorbed was then measured by titrating the residual KOH with a standard solution of HCl (0.1 N) after 1 mL of BaCl$_2$ 0.1 N was added to precipitate the carbonate ions (Balba et al., 1998). The calculations of CO$_2$ evolution, carbon biodegraded and biodegradation efficiency were carried out as described in Technical Norm L6.350 (Cetesb, 1990). Since respiration rate was closely correlated with the level moisture content of the soil (Campbell, 1977; Mitchel, 1992; Balba et al., 1998), soil moisture was corrected at 60% of the soil's moisture-holding capacity.

The microorganisms used as inoculum in respirometric test were collected from the soil of the chemical industry and from the wastewater treatment plant. In laboratory, the inoculum was exposed to the test compound Flexzone 3P to obtain an adaptation using test medium in which Flexzone 3P was the sole source of carbon and energy. This test medium was described by Aaronson (1971) and some alterations were made: 1.0 g of K$_2$HPO$_4$; 0.2 g of MgSO$_4$.7H$_2$O; 0.1 g of NaCl; 0.1 g of CaCl$_2$; 0.02 g of FeCl$_2$; 1.0 g of (NH$_4$)$_2$SO$_4$; 1000 mL of H$_2$O; 0.6 g of Flexzone 3P; pH = 7.2 to 7.5. Thereafter, nutrient agar plate-counting technique was used for obtaining the microorganisms using Plate Count Agar® medium (Difco Laboratories).

Each respirometric flask contained 50 g of soil, 1.5 mL of inoculum (suspension with absorbance about 0.4, at λ = 540 nm) with bacteria previously adapted to the Flexzone 3P and 5 mL of simulated wastewater containing Flexzone 3P treated during 5, 30, and 60 min by electrolysis system. Two blank controls were maintained: (a) only soil and (b) inoculum added to the soil. All the respirometric flasks were incubated (in duplicate) at 28ºC for 44 days. During each sampling, air without CO$_2$ was supplied.
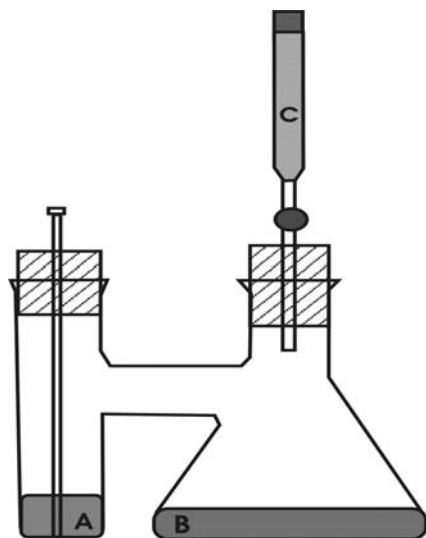
Figure 2 - Sketch of Bartha respirometric flask.
A: KOH solution. B: Soil. C: Filter of soda lime.

RESULTS AND DISCUSSION

According to Régis and Bidoia (2001a) the molecular structure of Flexzone 3P is like substituted benzene, which consists of aromatic amine. The peaks between 230-270 nm are typical of benzene UV-Vis spectrum and other aromatic hydrocarbons. When aromatic rings are bonded to the $-NH_2$ group, generally the peaks are shifted to larger wavelengths and there is an increase in the intensity absorption (Dyer, 1965). This UV spectrum region is related with $\pi - \pi^*$ transition. The simulated wastewater untreated by electrolysis (i.e., control sample) showed this behavior described by Dyer (1965). Fig. 3 shows UV-visible spectra of the simulated wastewater treated by electrolysis system and the untreated sample (time zero in the Fig. 3). In the spectra of 5 and 15 min of electrolysis times high molecular alterations for Flexzone 3P (presence of a new peak at 244.5 nm) was observed. In the spectra of 45 and 60 min this peak disappeared.

The peak intensity of Flexzone 3P decreased after electrolysis as electrolysis time increased (Fig. 3). The concentration was reduced during the experiment from 100.5 mgL^{-1} to 35.1 mgL^{-1} (Fig. 4). Results showed that the electrochemical process destroyed Flexzone 3P. The presence of a new peak at 244.5 nm suggested that there were new substances formed during the electrolysis.

According Rajeshwar et al. (1994), pollutants capable of undergoing electrochemical oxidation or reduction at an electrode could be transformed and/or removed from water streams or reservoirs by the application of appropriate potentials in electrochemical reactors. However, rather than the removal of unwanted material being process, side reactions, particularly solvent breakdown almost always occur, for example,

$$2H_2O \rightarrow O_2 + 4H^+ + 4e^- \qquad (1)$$
$$2H_2O + 2e^- \rightarrow H_2 + 2OH^- \qquad (2)$$
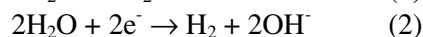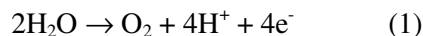
Although these reactions have a deleterious effect on the overall process efficiency, schemes have been devised to exploit the pH changes associated with Reactions 1 and 2 to bring about useful reaction chemistry (Rajeshwar et al., 1994).

In this work, the pH gradually decreased from 6.9 to 3.9 as shown in Fig. 5. It was evident that H$^+$ production (Reaction 1) was the dominant reaction resulting in a change of pH to the acid region. Furthermore, H$^+$ ions could be produced during the degradation of Flexzone 3P. The conductivity increased slightly with electrolysis time evidencing that there was small increment in total ionic concentration during the treatments.

Fig. 6 shows the gas chromatogram of the untreated simulated wastewater containing Flexzone 3P (control sample). There were 3 peaks with the following retention times: 10.316; 10.851 and 18.793 min. Flexzone 3P probably corresponded to the peak with the largest area (46.2%) (retention time 10.851 min). The other peaks in the chromatogram corresponded to other substances contained in the tablet of the test compound. Figs. 7 to 9 show the chromatograms of simulated wastewater samples treated during 15; 30 and 45 min by electrolysis.

High alterations were observed both in the retention times and the quantity the peaks (Figs. 7 to 9). After electrolysis duration of 45 min (Fig. 9) there was only one peak, which showed retention time of 18.858 min. It suggested that Flexzone 3P was completely altered and degraded by electrolytic treatment. This result could be related to the detoxification of the simulated wastewater. The toxic effects of the wastewater before and after electrolysis were determined by toxicity test with *S. cerevisiae* in this study.
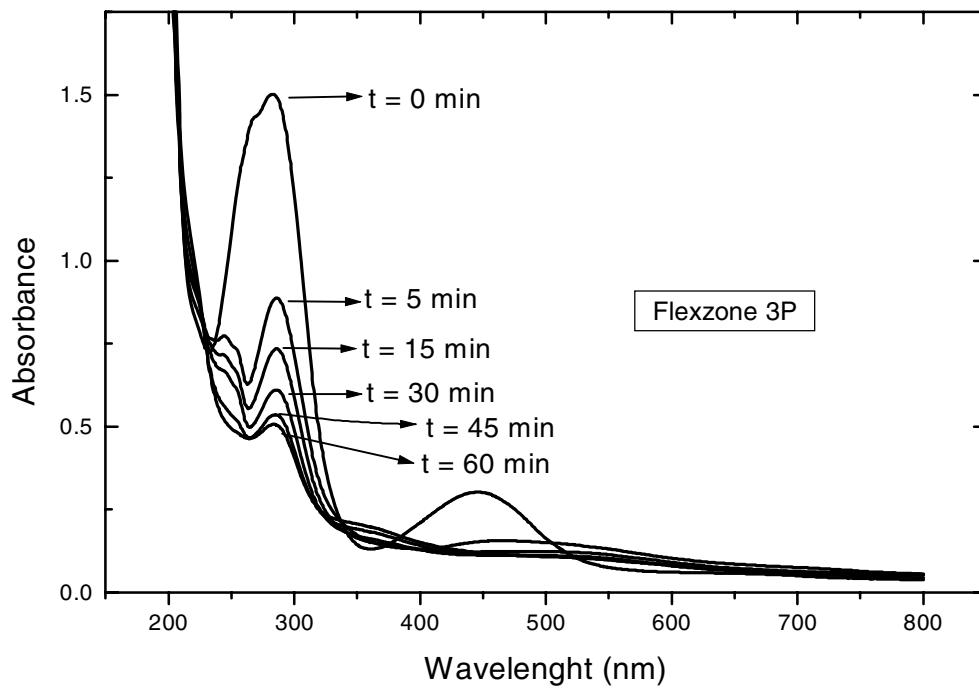
Figure 3 - UV-visible spectra of the simulated wastewater containing Flexzone 3P treated by electrochemical system with different electrolysis duration using Ti/TiRuO$_2$ electrodes. Time zero is the control spectrum, i.e., untreated simulated wastewater.
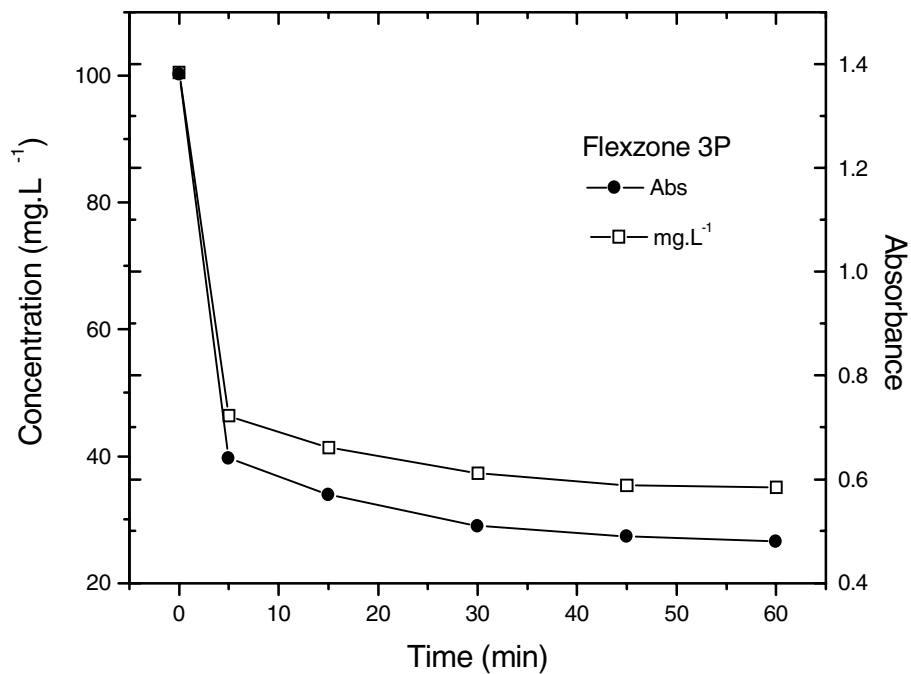

Figure 4 - Concentration (mg.L^{-1}) and intensity absorbance (reading at 266.0 nm) reduction during the electrolysis treatment.
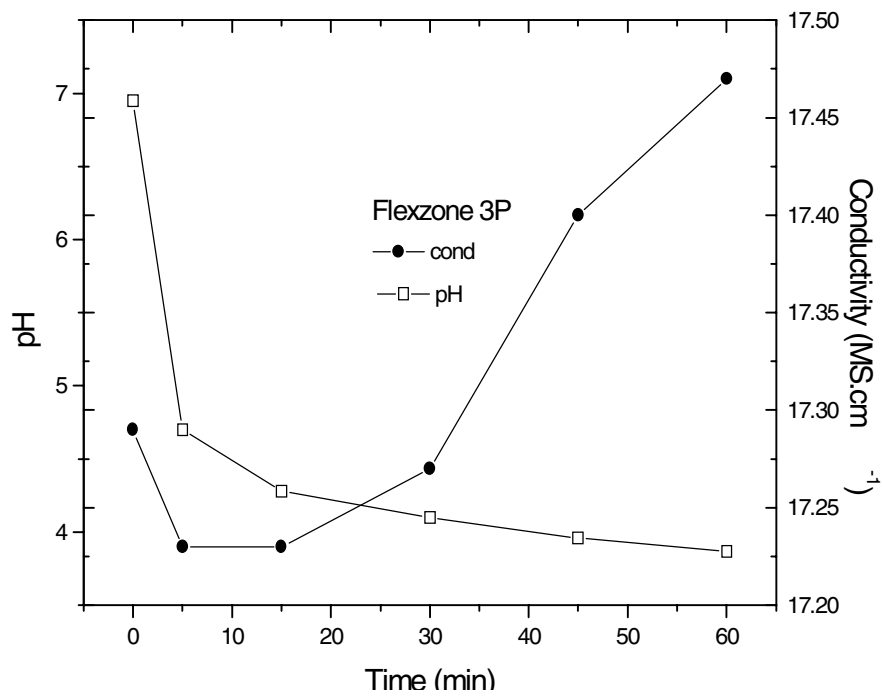
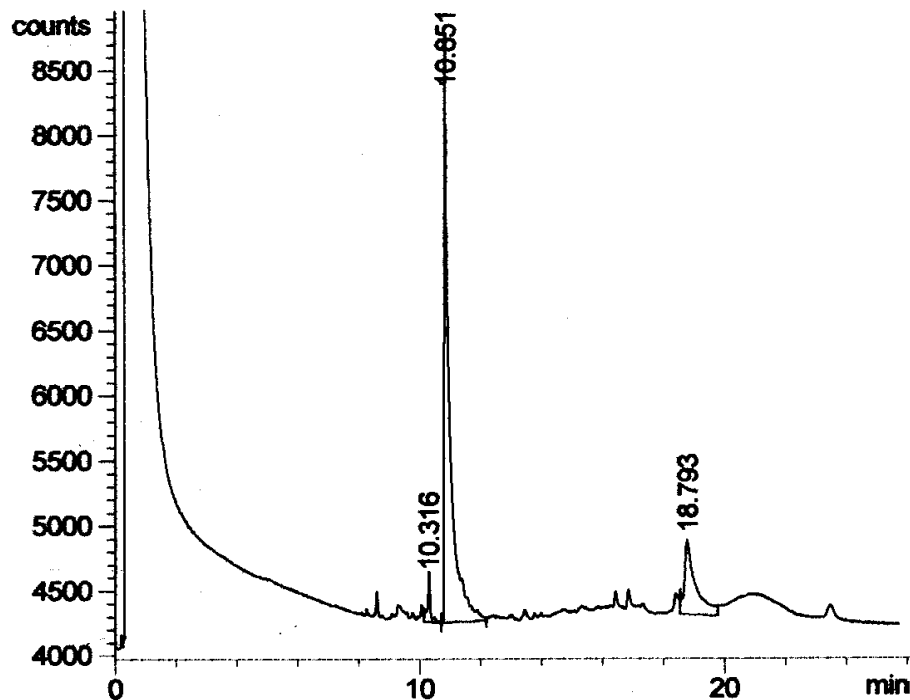**Figure 5 -** pH and conductivity (MS.cm^{-1}) changes during the electrolysis treatment.

Figure 6 - Gas chromatogram of untreated simulated wastewater containing Flexzone 3P (control sample).
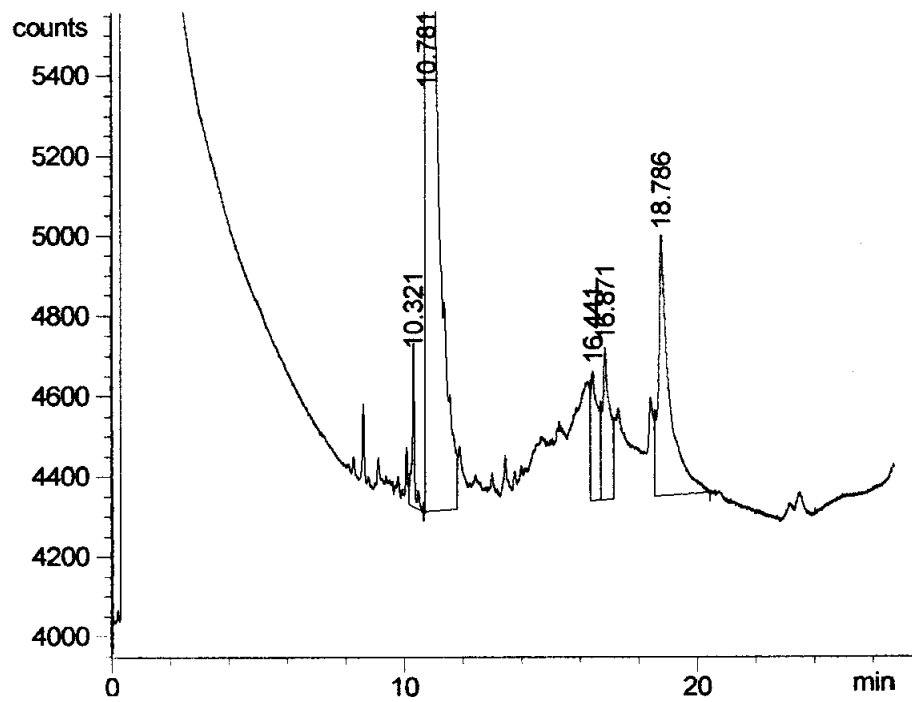
Figure 7 - Gas chromatogram of simulated wastewater containing Flexzone 3P treated during 15 min using Ti/TiRuO$_2$ electrodes.
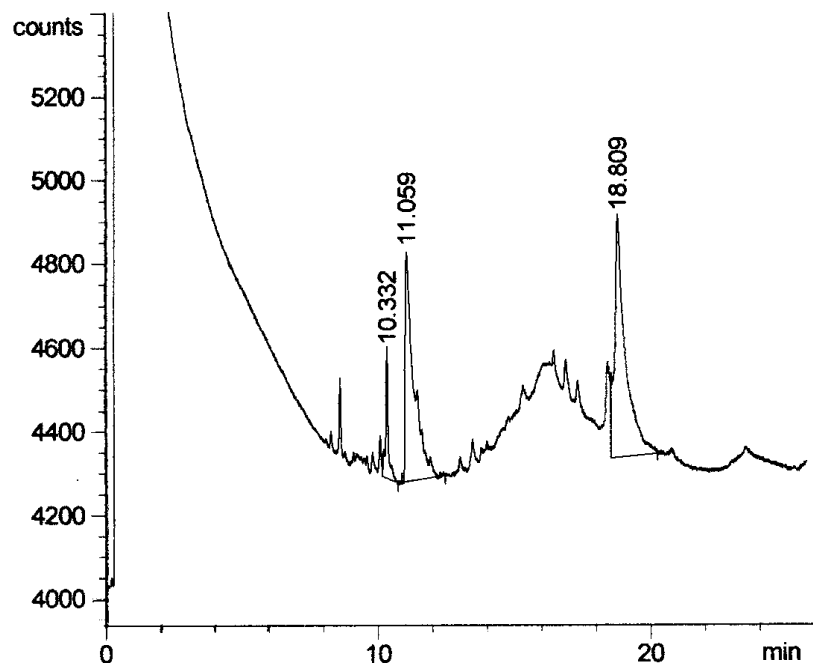

Figure 8 - Gas chromatogram of simulated wastewater containing Flexzone 3P treated during 30 min using Ti/TiRuO$_2$ electrodes.
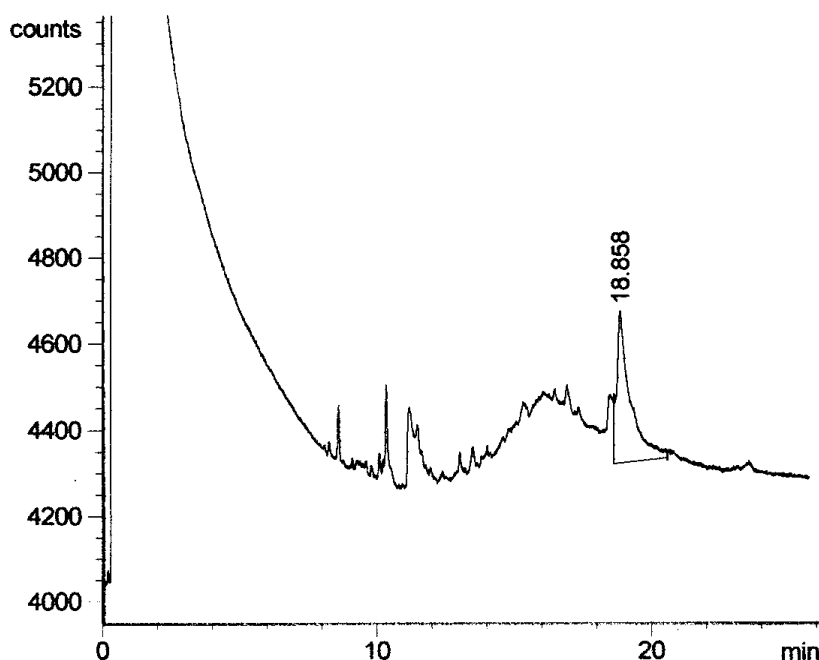
Figure 9 - Gas chromatogram of simulated wastewater containing Flexzone 3P treated during 45 min using Ti/TiRuO$_2$ electrodes.

Results (Fig. 10) showed that before electrolysis wastewater containing Flexzone 3P exhibited toxic effect, i.e., low cellular viability. After 5 min of electrolysis was obtained an increasing of cellular viability for the yeasts when the concentration reduction of Flexzone 3P was 53.8%.

The Bartha respirometric test showed that an aerobic mixed culture previously adapted to Flexzone 3P was capable of degrading the simulated wastewater treated and untreated by electrolysis system (Fig. 11).

Fig. 12 shows accumulated CO_2 evolution and CO_2 evolution per day. During seven initial days of experiment the CO_2 evolution rates for the treated simulated wastewater were: 1.37 mg CO_2 day^{-1} (soil), 2.51 mg CO_2 day^{-1} (soil and inoculum), 4.96 mg CO_2 day^{-1} (soil, inoculum and untreated wastewater), 4.96 mg CO_2 day^{-1} (soil, inoculum and wastewater treated during 5 min), 5.04 mg CO_2 day^{-1} (soil, inoculum and wastewater treated during 30 min) and 4.74 mg CO_2 day^{-1} (soil, inoculum, and wastewater treated during 60 min).

After this, the CO_2 evolution rates for the samples of treated wastewater were: 0.55 mg CO_2 day^{-1} (soil), 0.56 mg CO_2 day^{-1} (soil and inoculum), 1.02 mg CO_2 day^{-1} (soil, inoculum and untreated wastewater), 0.97 mg CO_2 day^{-1} (soil, inoculum and wastewater treated during 5 min), 0.95 mg CO_2 day^{-1} (soil, inoculum and wastewater treated during 30 min) and 0.86 mg CO_2 day^{-1} (soil, inoculum and wastewater treated during 60 min).

No initial lag phase was observed by mixed culture because the microorganisms were previously adapted to Flexzone 3P. All samples showed higher CO_2 evolution rates on 7th day of the experiment following a decreasing CO_2 evolution. It suggested that the microorganisms degraded more biodegradable substances and then persistent compounds remained. These persistent compounds were degraded slowly till the end of experiment. The untreated wastewater also showed degradation by mixed culture because the inoculum was previously adapted to the test compound. The sample of simulated wastewater treated during 5 min of electrolysis showed better biodegradation than other treated samples.
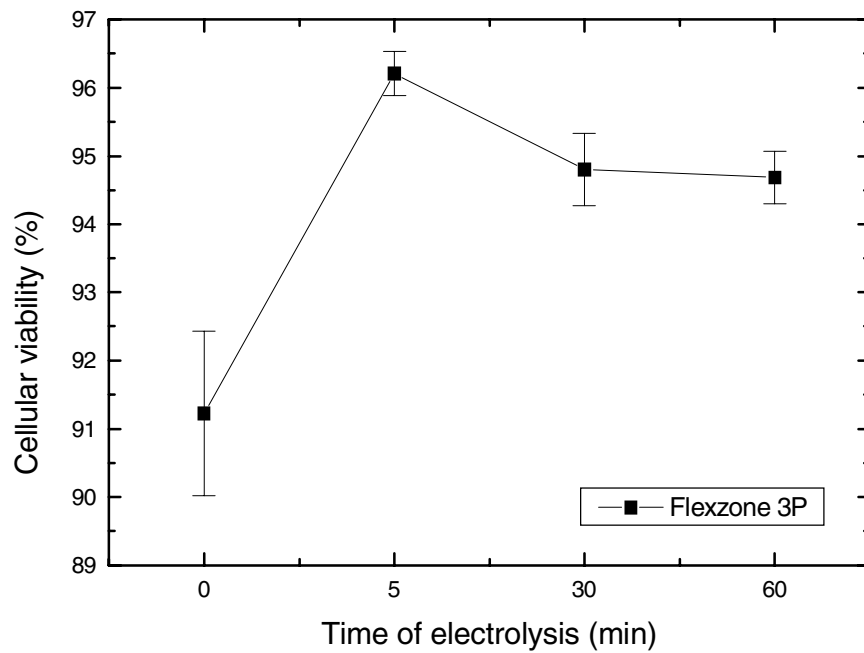
Figure 10 - Cellular viability (%) for *S. cerevisiae* in the simulated wastewater containing Flexzone 3P before (time zero) and after electrolysis. Yeasts were exposed to the simulated wastewater for 7 days.
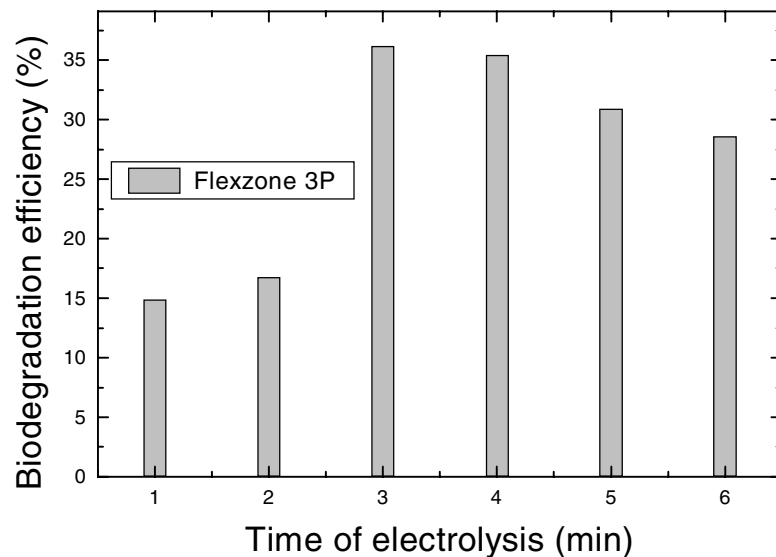

Figure 11 - Biodegradation efficiency of the simulated wastewater containing Flexzone 3P treated by electrolysis durations (1) only soil (2) soil and inoculum (3) soil, inoculum and untreated wastewater (4) soil, inoculum and wastewater treated during 5 min (5) soil, inoculum and wastewater treated during 30 min (6) soil, inoculum and wastewater treated during 60 min.

Inazaki, T. H. et al.

Figure 12 - Biodegradation of wastewater containing Flexzone 3P during 44 days (a) Accumulated CO_2 evolution (b) CO_2 evolution per day. Legend: (1) only soil (2) soil and inoculum (3) soil, inoculum and untreated wastewater (4) soil, inoculum and wastewater treated during 5 min (5) soil, inoculum and wastewater treated during 30 min (6) soil, inoculum and wastewater treated during 60 min.
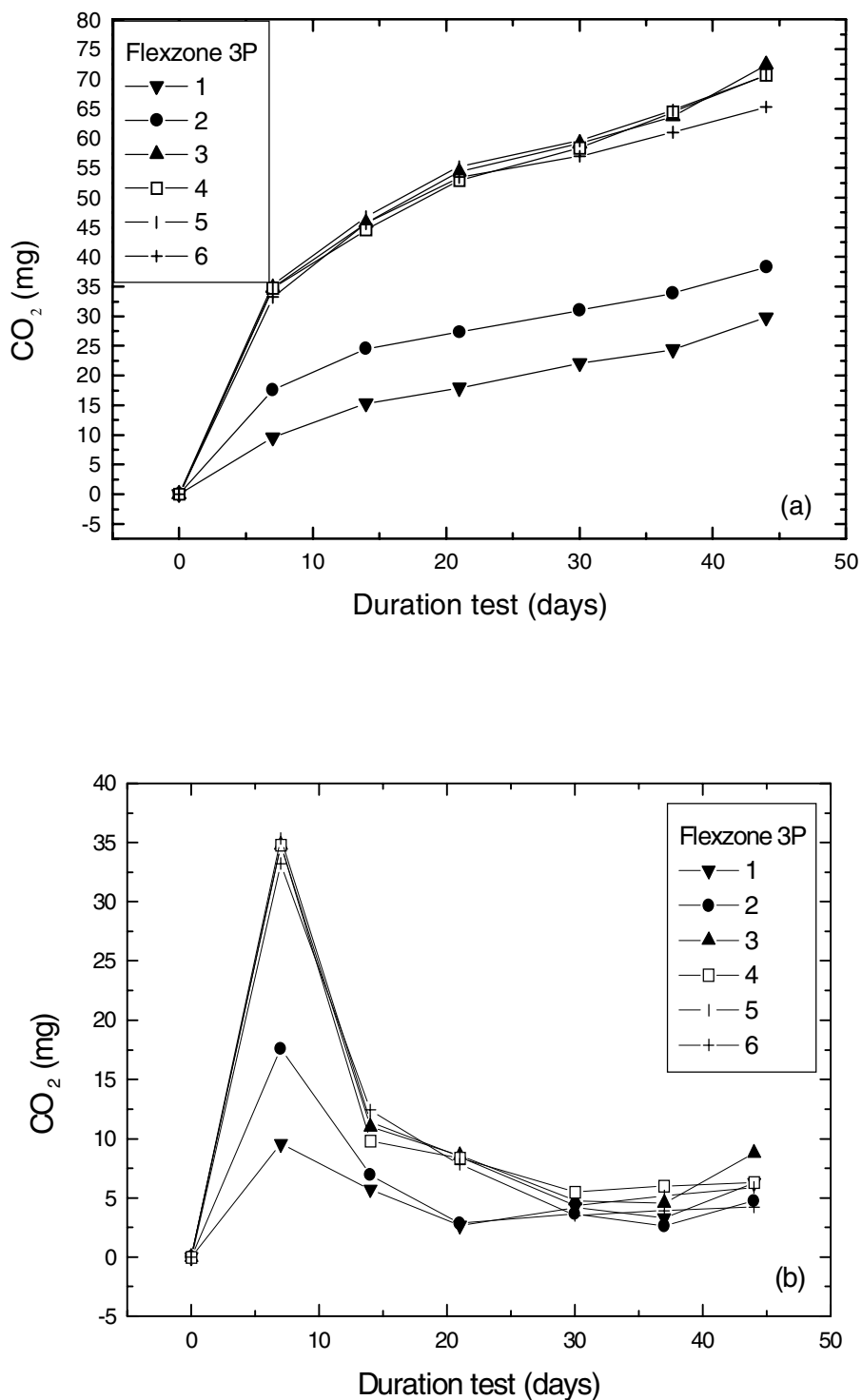
CONCLUSIONS

The electrochemical treatment of simulated wastewater containing Flexzone 3P was investigated. Anode of Ti/TiRuO$_2$ was employed for the experiment. It was found that the increasing electrolysis time improved the treatment. After 60 min of electrolysis, the concentration of Flexzone 3P was reduced by 65%. Gas chromatography and UV-visible spectrophotometry results showed that electrochemical process was effective in the degradation of Flexzone 3P. The toxicity test with *S. cerevisiae* also showed that the process could reduce the toxicity of the test compound. The untreated wastewater showed degradation by inoculum because it was previously adapted to the test compound. The electrolysis duration of 5 min showed better result for the Bartha respirometric test than other electrolysis durations.

ACKNOWLEDGEMENTS

RESUMO

O presente trabalho apresenta os resultados dos tratamentos eletrolíticos (05; 15; 30; 45 e 60 min) no efluente simulado com o composto Flexzone 3P, utilizando-se eletrodos de Ti/TiRuO$_2$, sob 0,025 A cm^{-2}. Após as eletrólises, foram feitas análises de pH, condutividade, espectrofotometria no UV-vis, análise por cromatografia gasosa, ensaios de toxicidade utilizando *Saccharomyces cerevisiae* e ensaio de respirometria de Bartha. O pH dos efluentes foi diminuindo à medida que aumentou- se o tempo de eletrólise, ao contrário da condutividade, a qual apresentou pequeno aumento. Verificou-se que após 60 min de tratamento foi obtida diminuição da concentração do composto Flexzone 3P em 65,1%. Analisando-se os espectros de UV-vis e cromatogramas do efluente após realização do tratamento eletrolítico, concluiu-se que ocorreram oxidações e transformações de funções orgânicas do composto, durante os tratamentos. Após a realização do tratamento eletrolítico, o efluente simulado apresentou diminuição da toxicidade a *S. cerevisiae*, após 5 min de eletrólise. O tempo de eletrólise de 5 min foi o que apresentou melhor resultado de biodegradação do que os demais períodos de tratamento, no ensaio de respirometria para o efluente com o composto Flexzone 3P.

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