Electrolytic treatment of an effluent of a chemical industry for monitoring toxicity by *Saccharomyces cerevisiae*

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ABSTRACT

The electrolytic process was applied to the treatment of an effluent from a chemical industry aiming to evaluate the decreasing of toxicity. In the characterization of the effluent, it was evidenced high content of organic material as antiozotants and antioxidants that are toxic and extremely pollutant wastes. The influence of various factors was studied using steel electrodes. The results showed a decreasing of toxicity. In the first 10 min the toxicity was decreased remarkably. Electrolysis carried out changes in molecules of Flexzone 3P (n-phenil-n-isopropil-p-phenilenodiamine) and Flexzone 7P (n-fenil-n'-1,3-dimethilbutil-p-phenilenediamine), which leads to a lower toxicity. Finally, it was concluded that the electrolytic treatment was viable to improve the effluent biodegradation.

Key Words: electrolytic process, toxicity, bioindicator, effluent.

INTRODUCTION

The aim of treating effluent is the prevention of pollution and environmental degradation. In the treatment of residual water some critical issues should be addressed to improve the systems of industrial effluents such as detection and quantification of contaminants, removal of pollutants, treatment of residues and prevention of pollution (Smith, 1972).

Residual waters from chemical industries commonly present aromatic pollutants. These, most of the time, are resistant to biological degra-

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dation and cannot be removed from effluents, diminishing the efficiency of the treatment system of the industry (Chiang et al., 1997). Frequently in this case it is necessary to add high doses of chemical agents with the risk for secondary pollution.

In many situations the use of electricity in electrolytic cells has been considered for the elimination of polluents in effluents, contributing to the treatment of some organic industrial pollutants despite the difficulties to obtain complete oxidation of organic species. However, the electroxidation of organic compounds may act as a transformer of persistent compounds (low biodegradability) facilitating treatment through conventional, biological systems. Presently, it is recognized that the simple modification of the molecular structure of a compound can reduce dramatically its toxicity and increasing its biodegradability. In general, a persistent molecule has aromatic rings and its oxidation results in more biodegradable or biocompatible molecules (Angelis et al., 1998).

The toxicity of effluents is evaluated through standardized bioessays with water organisms used to indicate modification in the quality of the water. There are two kinds of toxicity to be addressed: the acute or lethal toxicity and the chronic toxicity with lethal or sublethal effects in the long run. Koch et al. (1993) after several analysis, have indicated yeast as an alternative organism for test of acute toxicity by environmental drugs and chemical substances as well as a weapon for preliminary exams and its inclusion in the group of tests for toxicity.

Yeasts are eucariote organisms and are a good model for the evaluation of the citotoxicity. Besides that, they are easily available in nature, playing an important role in many ecosystems. From the practical point of view they show various advantages as they are simple to cultivate and of easy maintenance of the control conditions preventing, in addition, problems in variability which is common in complex organisms (Soares & Calow, 1993).

Saccharomyces cerevisiae is one of the most studied and characterized microorganisms in the planet. It has been used in many studies as an excellent model of eucariotic and for studies on the forms and function of mitochondria. For these reasons the *Saccharomyces cerevisiae* has been largely used in biotechnology as a conductor for cloning (unique or multicopy) according to Schreuder et al. (1996).

The present study aims to reduce the toxicity of the effluent of a chemical industry that produces rubber antioxidant and antiazotant through the molecular transformation caused by the electrolytic process.

MATERIAL AND METHODS

1. Effluent

The choice electrolyzed effluent was that of a chemical industry of rubber antioxidant and antiozonant removed from the raw effluent, that

is, before conventional biological treatment. Further, it was simulated effluents with aromatic amines such as n-phenil-n-isopropil-p-phenilenodiamine (FLEXZONE 3P), n-phenil-n'-1,3-dimethilbutil-p-phnilenodiamine (FLEXZONE 7P) and also with antioxidants such as polymerized 1,2–dihidro-2,2,4–trimetlilquinoleine (NAUGARD Q) and AMINOX, the latter having not yet official designation and is the result of the reaction between acetone and diphenilamine. In each electrolysis it was used 40 ml of the effluent or solution with 5 repetitions at each time.

2. Electrolytic Process

The experimental sitemaps included a source of continuous current (Dawer), model FCC-3005D connected to composite steel electrodes with low concentration of heavy metals, which was verified by atomic absorption spectrophotometer, in percentage of mass:

aluminum – 0.0560, antimonium – 0.0023, carbon – 0.0655, cerium – 0.0001, lead – 0.0014, copper – 0.0111, sulfur – 0.0120, tin – 0.0385, iron – 99.5050, phosphorus – 0.0097, manganese – 0.2340, molybdenum – 0.0058, nickel – 0.0270, silicium – 0.0068, titanium – 0.0005, tungstenium – 0.0089 and vanadium – 0.0004.

The electrode consisted of two plaques of steel maintained 15 mm apart by a Nylon ® screw.

During the electrolysis the electrode was immersed in an electrolytic basin containing 40 ml of the effluent or solution with constant steering in a stationary system, that is, after each time of electrolysis the effluent or solution was changed. The process was repeated five times for each period of electrolysis. The charge applied was of 0.5 A and the tension ranged from 7.0 V to 8.0 V. The electric tension during the electrolysis was recorded directly in the source of power.

After the electrolysis the temperature was measured in the effluents and ranged from 25°C to 28°C varying in a rate of 3.6°C h^{-1}. The increase observed in the temperature did not cause any modification in the viability of microorganisms or in the chemical properties of the effluents.

The conductivity of the raw effluents and simulated effluents electrolyzed was determined after each period and the result ranged from 5.7 to 6.0 ms/cm and the pH 5.6 to 6.4. The DBO of the raw effluent was 205.8 for 95.6 mg/L and the DQO of 1290,1 for 374.3 mg/L.

After the electrolysis aliquots of 10 ml were removed for each period of electrolysis for test of toxicity (repeated 5 times).

3. Microorganisms

The *Sacharomyces cerevisiae* was selected for the test of toxicity. The yeast was cultivated from purification of yeast contained in biological ferment Fleischmann Royal ®, available at supermarkets.

YEPD (agar, extract of yeast, peptone, dextrose) formulated according to Lodder (1971) was the culture medium for growth and counting of the units that formed the colonies by millimeter (UFC/ml) of the *S. cerevisiae*

4. Purification of yeast

Purification was obtained through by suspension and homogenization of 1 g of biological ferment in 200 ml of sterile 0.85% saline. From this suspension dilutions were made and afterwards inoculation in Petri dish with YEPD. The dish was incubated at 34°C for 72h.

After this period the selected yeast colonies were removed and suspended again in 10 ml of sterilized 0.85%. Aliquots of 2 ml of this solution were poured in Erlenmeyer containing 100 ml of liquid YEPD. This culture was kept in the homogenizer at 150 oscillation/minute for 24h thus obtaining viable cells of yeast.

The cultures were centrifuged at 5000 rpm for 15 minutes. The sediment was washed in sterilized distilled water and suspended again in 300 ml of sterilized distilled water. Finally, the cells were ready to be inoculated in the electrolyzed effluent.

5. Toxicity test

In order to evaluate the biological toxicity of the raw effluent, before and after the electrolytic process, it was used the *Sacharomyces cerevisiae,* which is a bioindicator easily obtainable, resistant to acid solutions and that has a cell wall more resistant than that of bacteria.

The toxicity was evaluated through the cell viability of the *Sacharomyces cerevisiae* in the raw effluents electrolyzed (in the different periods) in the non-electrolyzed effluents and in the simulated effluents containing aromatic amines (Flexzone 7P and 3P) and the antioxidants (Aminox and Naugard Q), electrolyzed in the different period or non electrolyzed.

In this regard, solutions of different volumetric concentration with 1.0 ml of the suspension were prepared containing the microorganisms for 9.0 ml of the studied solution in each period of the electrolysis. The tubes were then homogenized and incubed at 28°C for 48 h.

Afterwards, cells were counted and stained with eritrosine B in a Neubauer chamber for determination of cell viability. The percentage of toxicity was obtained taking into consideration the total cell viability as 100%.

RESULTS

Toxicity of the raw effluent

Results of toxicity of the raw effluent indicated that with increase of the period of electrolysis, the effluent became less toxic or more biodegradable since a greater viable cell count was found, which can be observed in FIGURE 1. After 20 minutes it was not observed toxic effect to the *S. cerevisiae*.

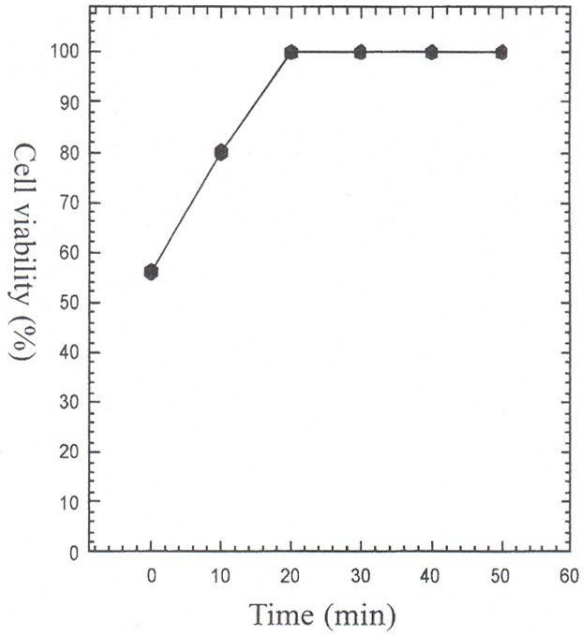

FIGURE 1 – Representation of cell viability for *S. cerevisiae* along the period of electrolysis of the raw effluent.

Toxicity of the simulated effluents

The toxicity essay allowed the observation of the behavior of the electrolytic process as a procedure to diminish toxic and persistent substances and to make them more biodegradable.

To evaluate the biological toxicity the *S. cerevisiae* was inoculated in the simulated effluent containing aromatic amines (Flexzone 3P and Flexzone 7P) and antioxidant (Aminox and Naugard Q) before and after the electrolysis in the 10th, 20th, 30th, 40th and 50th minutes thus determining the cell viability.

As for the Flexzone 3P, FIGURE 2 shows that the cell viability increased along the time of electrolysis. The reason for that was that the persistent substances in the composition of the Flexzone 3P used for preparing the simulated effluent became more biodegradable due to the elec-

trolyze and the byproducts resulting showed less toxicity to *Sacharomyces cerevisiae,* that is, became more biocompatible. Results for Flexzone 7P showed an increase in toxicity for *S. cerevisiae* in the first 10 minutes. Most probably there were a transformation of the molecular structure so that the byproducts showed a great toxicity regarding *S. cerevisiae.* However, after 10 minutes there was an increased cell viability due to another byproduct which was less toxic and compatible to the microorganism in longer periods of electrolysis (FIGURE 2).

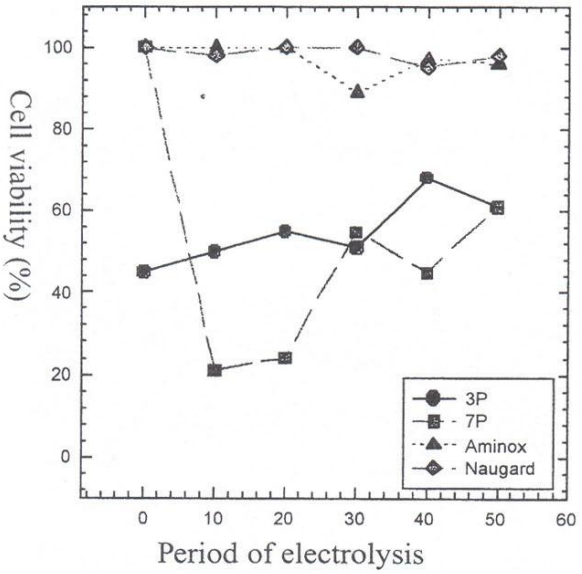

FIGURE 2 – Cell viability of *S. cerevisiae* in terms of period of electrolysis for Flexzone 3P, Flexzone 7P, Aminox and Naugard Q.

The behavior of Aminox was similar to that of Flexzone 7P. However, the increase in toxicity at 30 minutes was small and, later on, the toxicity diminished with the increase in the period of electrolysis (FIGURE 2).

Naugard Q did not show increase in toxicity and was indifferent to *S. cerevisiae* in any period of electrolysis (FIGURE 2).

The results of toxicity of the antioxidant substances Aminox and Naugard Q showed that electrolysis presents poor or no influence at all in the decrease or increase in toxicity since these substances are non-toxic regarding *S. cerevisiae*.

DISCUSSION

The cell viability of the raw effluent increased with the period of electrolysis because the effluent became less toxic. Ribeiro et al. (2000) evaluated the toxicity of some fungicides such as penconazol, cymoxanil and dichlofluamid, using yeast of *Kluyveromyces marxianus, Pichia anomala, Candida utilis, Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* as bioindicators. The yeast showing greater sensibility to fungicides were *C. utilis* and *S. cerevisiae*. The authors suggested that yeast could be used for quantification of toxicity and also as an alternative for complementary tests of toxicity.

The persistent substances in the composition of Flexzone 3P became more biodegradable after the electrolysis. The resulting subproducsts became more biocompatible to microorganisms. Presently, it is known that a simple modification in the molecular structure can markedly reduce the toxicity, increasing the biodegradability of a compound. The persistent molecule has, in general, aromatic rings and its oxidation induces the formation of molecules more biodegradable or biocompatible (Angelis et al., 1998).

As per antioxidant substances (Aminox and Naugard Q) the electrolysis showed minimal or no influence in the diminution or increase in toxicity since these two substances demonstrated not to be toxic to *S. cerevisiae*.

Flexzone 7P, after 10 minutes of electrolysis, showed toxicity to microorganisms but the toxicity decreased along the electrolysis period.

It is possible to conclude that the electrolytic process is an efficient method in the treatment of effluents since it speed up the biodegradability probably by transforming the persistent substances in the residual waters of industries that produce rubber antioxidants in substances less toxic and, thus, more biocompatible with the environment. Moraes (2000) has also observed these effects of the electrolytic process in effluents of oil refinery and his results lead to the same conclusion that the electrolytic treatment significantly reduces the toxicity of some effluents.

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