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Toxicity Studies Applied to Evaluate the Zeolites Adsorbent for Treating Methylene Blue in Wastewater

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Introduction

Dyes are widely used by textile industry for coloring products. Azo dyes are one of the most important classes of dyes once it accounts with 50% up to 65% of the commercial products (Chung, 1983; Kunz et al., 2002a, 2002b). If the color of effluent from textile activities seems to be the major problem, related to bad acceptance of water, the damage to the biological systems are worst once these wastewater introduce a variety of organic compounds, including toxic and mutagenic substances, to the natural reservoir of water.

According to Guaratini and Zanoni (2000), a dye is a colored substance used to impart permanent color to other substances and its most important use is coloring textile fibers and fabrics. These authors carried out an extensive review of technologies for dying treatment ant they took into consideration the toxicological and ecotoxicological aspects.

Acute toxicity of azo dyes measured by the european criteria for classification of dangerous substances is rather low, amounting to LD50 values of 250 to 2000 mg per kg body weight. (Novotny et al., 2006). However, the degradation products of azo dyes and their impurities include aromatic amines that are compounds of concern due to their potential carcinogenicity. Relatated to genotoxicity, Ames test using variants in vitro metabolic activation with S9 for Salmonella strains is the regular assay for aquatic environment in Southamerica, including Brazil.

Attempts have been paid to improve the treating condition for waters and several advanced techniques are being developed. Sevimli and Kinaci (2002) compared the Fenton and ozonization processes for textile dying degradation. Ionizing radiation processing have been studied for some usual dyes and for raw textile effluents (Koreia). Very few papers combined the efficacy of the developing technology and their biological aspects. The importance of ecotoxicological and mutagenic aspects for suitable developing of treatment technologies is a growing understanding (Borrely et al., 2000).

Methylene blue active substance (MB) is a cationic aromatic dye, which is water soluble with molecular formula $C_{16}H_{18}N_3SCl$ and molecular weight of 319.85 g/mol. This compound is environmentally important since it has several applications as microbial dye agent, chemical color indicator, chemical active reagent and even as a medicine. Methylene blue dye may cause ecological disturbance for aquatic environment and affect human health by eye burns, breathing disease and it may produce burning sensation if ingested; nausea, vomiting, profuse sweating, mental confusion, etc. This dye is applied for dying cotton, wood and silk. Therefore the treatment of effluent containing such dye is of interest due to its impacts on receiving waters (Fungaro et al., 2005).

The aim of the present paper is to evaluate the capability of zeolites for the decoloration of methylene blue and to evaluate how much this treatment will interfere with the possible biological effects. This compound was chosen as a standard for zeolites adsorption while kinetics data will be helpful for several dyes solution to be studied. This paper presents the zeolites efficacy evaluated for toxicity reduction using two lived organisms (*Daphnia similis* crustacean and *Vibrio fischeri* bacteria) and color removal as well.

Proposal of treatment – reciclying fly ash for wastewater improvement through zeolites

Fly ash is produced by burning coal in coal-fired power stations and zeolite synthesized from fly ash is being proposed as an adsorbent for wastewater treatment. This technique is important not only for wastewater technology but also for residues reuse a management.

The wastewater containing various dyes are difficult to treat due to their complex aromatic structure, stable under the influence of light and oxidizing agents, and reactive dyes are especially resistant to aerobic digestion (Orthman et al., 2003). Of the numerous techniques studied, adsorption in particular is an effective process for the removal of dyes from waste effluents. Currently and commonly using activated carbon which presents a high adsorption capability (Pelekani and Snoeyink. 2000; Walker and Weatherley, 2000; Meshko et al., 2001). However, because of the relatively high cost of activated carbon, there have been attempts to utilize low-cost adsorbents (Sanghi and Bhattacharya, 2002).

It is possible to convert fly ash into zeolitic products by hydrothermal treatment with alkaline medium. The product has a significantly increased surface area and cation exchange capacity when compared to the raw ash (Henmi, 1987; Querol et al., 1997; 2001; Murayama et al., 2002).

Zeolitic materials have been used as low-cost adsorbents for the removal of metal from aqueous solution (Singer and Berkgaut, 1995; Lin and Hsi, 1995; Amrhein et al., 1996; Querol et al., 2001, 2002). Metals adsorption onto zeolites synthesized from Brazilian coal ashes was investigated (Fungaro and Silva, 2002; Fungaro et al., 2004). However, few investigations have focused on the organic component of potential waste streams (Woolard et al., 2002; Fungaro et al., 2005).

Once the research aimed the zeolites adsorption potential for dye removal, Methylene blue was chosen due to its known strong adsorption onto solids and often serves as a model compound for removing organic contaminants from aqueous solutions. The kinetic data and equilibrium data of adsorption studies were processed to understand the adsorption mechanism of the dye molecules onto the synthetic zeolite. To confirm the improvement of the dye solutions after zeolite adsorption treatment, acute toxicity has been evaluated for MB solution before and after the treatment. This toxicity evaluation was carried out with *Daphnia similis* cladocera and *Vibrio fischeri* bacteria.

Three batch treatment of MB with zeolites were carried out with the toxicity assays for *Daphnia similis* and *Vibrio fischeri*. The results were based on the percentage of reducing effects into the test-organisms after the zeolite adsorption.

Material and Methods

Materials

The samples of fly ash from cyclone filter were obtained from a coal-fired power plant located at Figueira County, in Paraná State, Brazil. The chemical composition of fly ash was determined by X-ray fluorescence analysis (XRFS RIX 3000 - Rigaku) and the sample had a very low SiO_2/Al_2O_3 ratio compared to the most coal fly ashes (1.2 w/w). This feature coupled with the relatively low impurities content (Fe, Ca and S oxides) confers a high potential for the use of this sample as a starting material for the synthesis of low-Si zeolites (Zeng et al., 2002).

Methylene blue (Merck) was studied in water solution for zeolites adsorption and toxicity evaluation. It was prepared a stock solution (3.2 g L^{-1}) deionized water (Millipore Milli-Q) and the solutions for adsorption tests were prepared by diluting.

Zeolite synthesis

Coal fly ash was used as starting material for zeolite synthesis by means of hydrothermal treatment. In synthesis experiment, 20 g of ash was heated to 100 ^{o}C in oven for 24 h with 160 mL of 3.5 mol L^{-1}

NaOH solution. The zeolitic material was repeatedly washed with deionized water and dried at 100 ^oC for 24 h. X-ray diffracion analysis (Rigaku model RINT-2000) revealed that the obtained product was NaP1 zeolite with traces of hydroxysodalite, quartz and mullite. The cation exchange capacity value of 1.1 meq g^{-1} was determined using ammonium solutions (Scott et al., 2002).

Adsorption studies

The adsorption was performed by batch experiments. Kinetics studies were carried out by agitating 100 ml of dye solution of known initial dye concentration with 1 g of zeolite at room temperature (25 ^oC) at a constant agitation speed of 120 rpm. Samples were pipetted out using a 10 mL-syringe at different time intervals. The collected samples were then centrifuged and the concentration in the supernatant solution was analyzed using a UV spectrophotometer (Cary 1E – Varian) by measuring absorbance at λ_{max} = 650 nm and pH=5. Adsorption isotherms were carried out by contacting 1 g of zeolite with 100 mL of methylene blue over the concentration ranging from 3.2 to 96 mg L^{-1}. The time for agitation was 10 minutes, enough for reaching equilibrium.

Toxicity evaluation

Daphnia similis and *Vibrio fischeri* luminescent bacteria were the tested-organisms for the evaluation of methylene blue acute toxicity. Three experiments were performed for MB solutions adsorption and toxicity.

Acute toxicity for crustacean assays were performed with *D. similis* (ABNT, 2004), exposure for 24 hours and 48 hours at 20^oC $\pm$ 0.5. The water hardness was adjusted and fixed to 46 mg/L $CaCo_3$ for the toxicity tests and for the culture of this lived organism. At least five concentrations of solutions were analyzed and the results were expressed as EC 50 for 48 h. Once this parameter is inversely proportional, these values were transformed into Toxic Units (TU) for the calculation of adsorption efficacy.

Acute toxicity for luminescent bacteria was carried out with Microtox System – model 500 (Azur Environment, USA). The bacterial luminescence was measured after 15 minutes exposition and the lost sign was related to the effect according to the dye concentration. The assay results were expressed as EC 50, which is the effective concentration that causes 50% of light inhibition after 15 min exposure. The assay considered the suitable correction when required due to hard blue color of the untreated samples

These assays were performed according to ABNT and ISO methods and when finished exposition the results were transformed from EC50 to Toxic Units. The efficacy was calculated as the percentage of toxicity reduction.

Results and Discussion

Kinetic and Equilibrium Adsorption Studies

The effect of contact time on adsorption process was investigated at various initial dye concentrations. It can be seen from Fig. 1a that the adsorption of methylene blue on zeolite occurred very quickly within the first 10 min after which a maximum value of adsorption capacity was attained.

The rapid uptake of dye particles for the first 10 min is due to the occurrence of solute transfer only due to adsorbate and adsorbent interactions with negligible interference due to solute–solute interactions (Varshney et al., 1996). The equilibrium of the adsorption process was attained so fast that the kinetic data could not be modeled.

Equilibrium data of methylene blue onto zeolite synthesized from fly ash are shown in Fig. 1b. The isotherm shapes are largely determined by the adsorption mechanism and can therefore be used to diagnose the nature of the adsorption (Giles et al., 1960). The adsorption isotherm for solution may be classified into four main classes relating to their shapes termed S, L, H and C and subgroups 1, 2, 3, 4 or max. The equilibrium isotherm has the shape of L3 type curve and indicates that the second layer of dye can form readily.

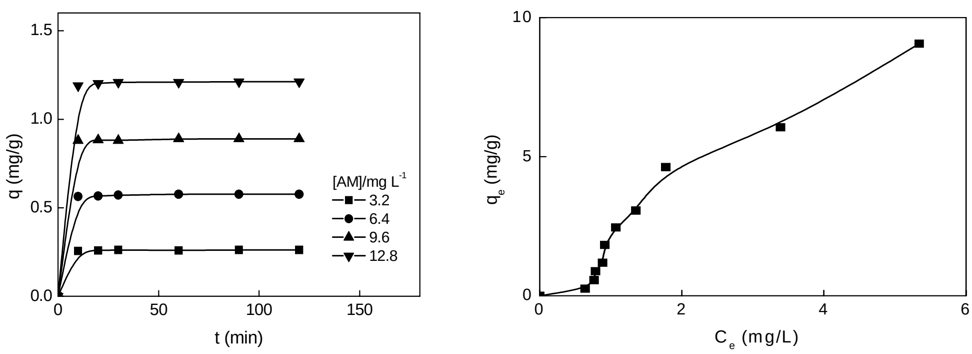

Fig. 1. Adsorption of methylene blue onto zeolite. (a) Kinetic studies; (**b**) Adsorption isotherm.

Toxicity Evaluation Studies

The acute toxicity results are summarized at Table 1 where it can be noted that the effective concentration that caused effect (immobilization) for daphnids varied from 0.16 ppm up to 0.43 ppm and the effective concentrations that promoted 50% of light reduction from *V. fischeri* were 16.58 ppm up to 18.64.

The MB acute toxicity was observed through the *V. fischeri* EC_{50} (%, 15min) between 15.55 mg/L and 18.64 mg/L, which increased to 29.67 mg/L and 43.90 mg/L after zeolites adsorption, 2.35 times less toxic after treatment. The MB EC_{50} (%, 48h) values obtained for *D. similis* were 0.16 mg/L up to 0.43

mg/L. After zeolites adsorption treatment these values were 26.10 mg/L up to 47.80 mg/L. The efficacy for acute toxicity removal for bacteria and for daphnids were 55,42% and 99,18%, respectively.

Table 1 - Efficacy of zeolite adsorption for MB acute toxicity removal.

EC-50 Untreated (15 min, % v/v)	EC-50 adsorbed (15 min, % v/v)	TU Untreated	TU adsorbed	Removal (%)
17.23 (*)	34.74	5.80	2.87	50.51
18.64 (*)	43.9	5.36	2.27	57.65
16.58 (*)	39.5	6.03	2.51	58.37
15.55 (*)	29.67	6.43	3.37	47.59
0.19 (**)	26.10	526.31	3.83	99.27
0.16 (**)	47.80	625.0	2.09	99.66
0.43 (**)	31.47	232.56	3.17	98.63

(*) 15 min - *V. fischeri* and (**) 48h - *D.similis*

If we consider the health risks when 15% to 30% of the textile dyes are incorporated by the waterways due to not suitable treatment, this idea to transform a residue as a support for wastewater improvement is good. On the other hand, the developing technologies must to prove its efficacy through biological assays cause it is very possible partial degradation or combination of compounds in water be more toxic than it was originally.

Figures 2 and 3 represent the raw EC 50 (mg L^{-1} or %) numbers for both tested organisms, and the efficacy for acute toxicity removal (average) for bacteria and for Daphnis were 55,42% and 99,18%, respectively.

Important is to note that this coloring agent was less toxic to the bacteria than to Daphnis, although these number can not be directly compared due to individual characteristics of the organisms, route of action to cause the effects to cells, time exposure etc, and besides the zeolite as methylene blue adsorbent agent accounted for at least 50% removal of the total acute toxicity

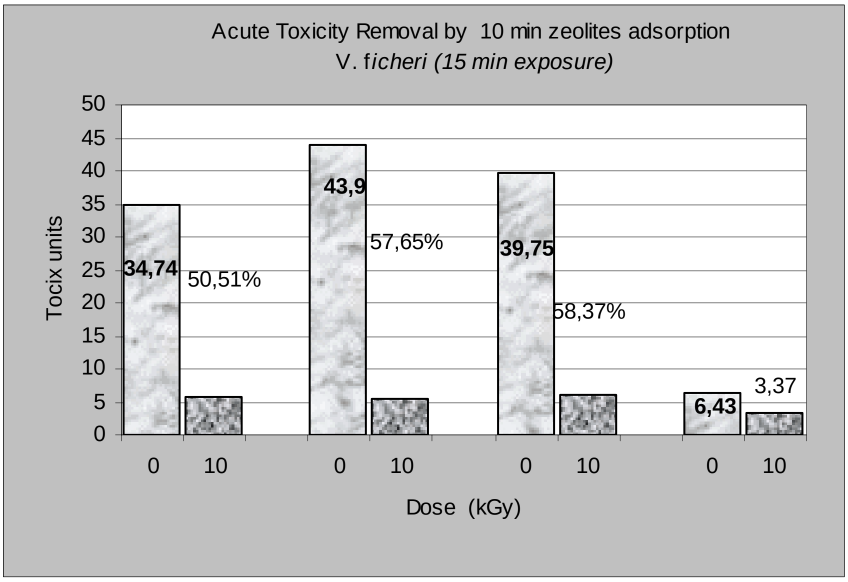

Figure 2 – Zeolite efficiency for acute toxicity removal with bacteria.

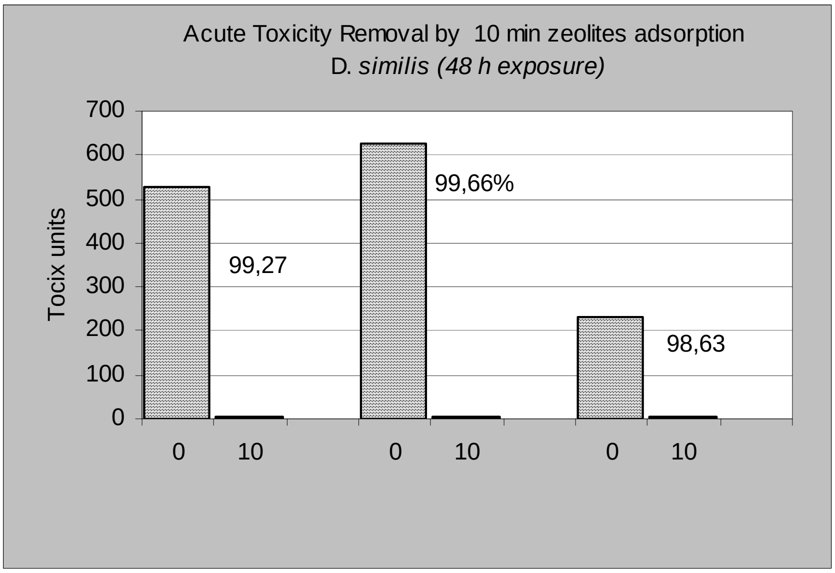

Figure 3 – Zeolite efficiency for acute toxicity removal with daphnids.

Conclusion

The zeolites adsorption proved to be a possible treatment for dyes with 80-94% of color removal and the methylene blue adsorption as a model agent accounted with 55% and 99% efficacy for acute toxicity removal. Both values are very important and the zeolite as a decolouring method was quite enough for this studied product. The toxicity assays results revealed that more than a biological assay must be applied when wastes treatment technologies are being developed.

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