

DECOLORIZATION AND TOXICITY OF MUNICIPAL WASTE BY HORSERADISH (*Cochlearia armoracia*)

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The Municipal Station of Americana, SP, Brazil, treats a volume of 400 l s⁻¹ of effluent, of domestic and textile origin, and produces about 20 t of sludge per day. The plant horseradish, which contains high amount of peroxidases, was able to decolorize this effluent in 2 h and the solid waste in 2 days, at concentrations of 10 and 50%, respectively. However, there was an increase in the toxicity for the bioassays with *Hydra attenuata*, *Selenastrum capricornutum* and lettuce seeds, indicating formation of more toxic substances. Since horseradish showed the ability to decolorize these residues, it can be used as pre-treatment resulting in a sludge of less complex composition.

Keywords: textile dyes; bioassays; sludge.

INTRODUCTION

The textile plants produced a large volume of effluent, which might contain about fifteen percent of the total dyes used in the process and other products such as: starch, surfactants, dispersants, oil, emulsifiers, caustic soda, anti-foam, etc. The effluent treatment by aerobic process such as activated sludge is inefficient for removing the color of the effluent¹⁻³ since these substances are xenobiotics and microorganisms living in natural ecosystems don't have specific enzymes for its degradation⁴. This process also has the disadvantage of producing large amount of solid waste in the final of the process that might become another environmental problem⁴.

In an effluent containing many structural different dyes belonging to several chemical groups, is very difficult to isolate microorganisms that could degrade several classes of dyes⁵. In fact, in some cases a change in a position of a substituting group of the molecule might limit the degradation, and specificity between microorganism and molecule has been verified⁶. Enzymes with wide spectrum like peroxidases, laccases, mono and dioxygenases have been tested as alternative.

The anaerobic process promoted decolorization of dyes, especially azo dyes, but the degradation result in the production of amines as metabolite, which are carcinogenic and toxic⁷. The aeration subsequent showed these amines can be degraded in aerobic system⁸ and combined system have been tested⁹. The uses of white rot fungi have also been studied and have revealed their ability to decolorize many kinds of dyes¹⁰⁻¹². This degradation involves the action of extracellular lignolytic enzymes such as lignin peroxidase, manganese peroxidase and laccase¹³⁻¹⁴.

Horseradish peroxidase (HRP) is a heme-containing plant peroxidase that catalyzes oxidations of various substrates in the presence of hydrogen peroxide that leads to formation of the intermediate Compound I via a two-electron oxidation. Compound I can be reduced by an oxidable substrate to compound II. This intermediate reacts with another substrate molecule to produce the initial state of the enzyme¹⁵.

The horseradish plant was studied in the degradation of phenols¹⁶⁻¹⁷, chlorophenols¹⁸⁻²², PCBs¹⁵ and anilines²³, however chemical characteristics affected the degradation, since anilines

and phenols substituted with ions chlorine were more resistant to degradation^{19,23}. PCBs were degraded by HRP to several intermediates via dechlorination and hydroxylation followed by cleavage of the ring leading to complete degradation of these molecules¹⁵.

The decolorization of several dyes by horseradish was observed²⁴⁻²⁹. Ferreira-Leitão *et al.*²⁹ studied the degradation of the dye metileno blue (MB) by HRP and LiP and observed that 4.7% of the MB remained in the mixture for HRP and the degradation was 100% for LiP. In accord with Tonegawa *et al.*³⁰, the horseradish-peroxidase is able to degrade amines, which are known as metabolites of azo dyes degradation.

Enzyme immobilization in a matrix allows reusability over the process and has showed increase in the degradation efficiency³¹. About 79% of an azo dye remotion (Acid Black) was observed with acrylamide gel immobilized beads, while only 67% was found with free HRP and 54% with alginate matrix³¹.

Many treatments can be efficient in the decolorization, but it necessary to know if there is formation of toxic products during the process. A valuable technique to evaluate the efficiency of the process of degradation is the use of bioindicators³².

The Municipal Treatment Station of Americana, SP, is responsible for the treatment of effluents from 43 textiles industries together with 70% of city sewage and approximately 3/4 of total volume originates from industries. The treatment mainly involves biologic filters for effluent and anaerobic digestion for the sludge. Each day an average of 20 t of sludge with approximating 30% of solids is produced and the process has 50% of effectiveness.

In this work, the decolorization and toxicity of effluent and solid waste of Municipal Treatment Station of Americana, SP were evaluated after treatment by the plant horseradish.

EXPERIMENTAL PART

Horseradish

The horseradish material was donated by the Toyobo, Ltda. It was used in four different forms, the plant grinded in the laboratory (HLG), grinded in the industry (HIG), the bagasse resulting from the industrial peroxidase extraction process and the enzyme extracted by the industry.

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Peroxidase activity

The peroxidase activity was evaluated in the plant extract (10 g of plant in 100 mL of water) by the methods of syringaldazine³³ and O-dianisidine³³. The reactions were carried out in triplicates and the absorbance was evaluated at 460 nm after 10 min of reaction. For the Syringaldazine method, it was used 0.6 mL of plant extract, which was added with 100 μ L of a 0.1% solution of syringaldazine in ethanol absolute, 200 μ L of citric acid/sodium hydrogenphosphate buffer solution at pH 5.0 and 100 μ L of H₂O₂ 2 nM. For the O-Dianisidine Method, 0.6 mL of enzymatic extract were added with 100 μ L of 1 mM solution of o-dianisidine, 200 μ L of citric acid/sodium hydrogenphosphate buffer solution at pH 5.0 and 100 μ L of H₂O₂ 2 nM. Peroxidase activity is expressed in International Unit per gram (UI g⁻¹) that is equivalent to mmol min⁻¹ of syringaldazine or o-dianisidine oxidized.

Decolorization of the effluent

The effluent was sampled (3 L) after complete treatment in the Municipal Station. Erlenmeyer flasks containing 30 mL of the effluent was mixed with 3 g of horseradish or 3 mL of extract. Hydrogen peroxide was added to a final concentration of 20 mM in accord with Dec and Bollag¹⁸. The decolorization was evaluated by spectrophotometry and the percentage of decolorization was calculated as recommended by Glenn and Gold³⁴, who suggest that if the maximum/minimum absorbance rate was kept constant there was not decolorization but adsorption effect.

Decolorization of the sludge

The sludge was sampled (3 kg) after the anaerobic digestion, addition of polymers and centrifugation, ready to leave the treatment station. The decolorization of the sludge was done by homogenization with the root grinded in the lab (HLG), grinded by the industrial process for extraction of peroxidases (HIG) and the bagasse, which is the residue of the enzyme extraction process. It was used 100 g of sludge mixed with the plant material in three proportion, 1:1, 3:1 and 9:1 (w/w), and H₂O₂ was added to a final concentration of 20 mM. The decolorization was evaluated by reflectance in Spectrophotometer Minolta Chroma Meter Cr-200 that give 3 parameters, "L", "a" and "b" that correspond to different wave of light reflected by the Spectrophotometer: "L" (color varying between black to white), "a" (green to red) and "b" (blue to yellow). Increase in the parameters L, a and b indicate decolorization³⁵.

Toxicity tests

For *Hydra attenuata*, three organisms were placed into each of three (12-well microplate) wells into which 6 dilutions of effluent and sludge extracted (100, 50, 25, 12.5, 6.25, 3.125 and 1.56%). Lethal and sublethal effects were observed after 24, 48, 72 and 96 h of exposure³⁶.

For algal tests, similar dilutions of the effluent and sludge extracted were used and 2.5 ml were placed into scintillation vials with a 10⁴ cells⁻¹ inoculum of *Selenastrum capricornutum* (Printz). After 72 h of incubation at 24 °C at 4000 (\pm 100) lux, growth inhibition was evaluated by counting the number of cells present in each vial with a Neubauer measuring chamber under a microscope at 400 X³⁷.

The lettuce seed root growth inhibition test was performed with 20 seeds in a polystyrene Petri dish, containing a filter paper embedded in 2 mL of each sample dilution. Root lengths were measured after 72 h of exposition³⁸.

Data analysis

Comparisons were made with standard measurement endpoints including NOEC (No observed effect concentration), LOEC (Lowest observed effect concentration) for *Hydra* and the determination of 50% effects (IC₅₀ for the algal assay and *Lactuca* assay). IC₅₀s were calculated using the EcoTox-Statistics Version 1.1 program.

RESULTS

Peroxidase activity

Grinded roots in lab showed higher peroxidase activity compared to the industry, probably due to some problem during the transportation to the lab. The bagasse showed lower activity and the enzyme extract showed the highest activity (Table 1). The two methods used for analyses showed similar values for peroxidase activity in HIG and extract (Table 1), but for HLG and bagasse the O-dianisidine values were higher than syringaldazine.

Table 1. Peroxidase activity of Horseradish grinded in the Lab (HLG), in the industry (HIG), plant extract and the extracted bagasse

Treatment	Peroxidase activity (U g ⁻¹)	
	Syringaldazine method	O-Dianisidine method
HLG	4.969 x 10 ⁻¹	7.52 x 10 ⁻¹
Plant Extract	9.750 x 10 ⁻¹	9.108 x 10 ⁻¹
HIG	2.125 x 10 ⁻¹	2.985 x 10 ⁻¹
Bagasse	8.012 x 10 ⁻³	1.078 x 10 ⁻²

Decolorization of the effluent

The effluent was decolorized equally, by visual observation, in all treatments. There was influence of the plant color in the lectures, mainly for HIG and bagasse. Although the bagasse had the lowest peroxidase activity, it seems to have the same efficiency for decolorization of the effluent. The complete decolorization occurred after 2h of exposition to the treatments. There was no observed color adsorption by the solid material and the difference between two absorbance measurements, as recommended by Glenn and Gold³⁴, also indicate that no adsorption had occurred.

Table 2. Percentage of decolorization in the effluent treated with horseradish grinded at the Lab (HLG), in the industry (HIG), plant extract and the extracted bagasse

Treatment	Decolorization (%)	
	680 nm	583 nm
HLG	30.0	28.99
Plant Extract	31.2	32.54
HIG	2.0	4.14
Bagasse	2.0	8.28

Decolorization of the sludge

The decolorization of the sludge visually occurred in the proportions of 1:1 w/w (50% of horseradish) in 2 days. The decolorization with horseradish at 10 and 25% depends on continuous homogenization of the material, due the decolorization

was restricted to the region of contact with the horseradish grinded material. Decolorization was observed by reflectance (Table 3) with decrease in the L, a and b parameters showing clarification.

Table 3. Decolorization by reflectance of the sludge treated with horseradish grinded in the Lab (HLG), in the industry (HIG) and the extracted bagasse at 50, 25 and 10%

Treatment	Time = 0			After Time = 5 days		
	L	a	b	L	a	b
HLG 50 %	24.78	0.13	2.7	27.96	0.34	4.61
HLG 25 %	24.18	-0.04	1.19	26.55	0.04	3.63
HLG 10 %	24.66	0.05	1.13	22.31	-0.41	0.2
HIG 50 %	26.86	0.44	4.03	32.54	0.83	6.43
HIG 25 %	26.15	0.18	2.48	26.41	-0.35	2.16
HIG 10 %	23.51	-0.06	0.88	23.14	-0.59	0.31
Bagasse 50%	26.46	0.43	3.38	29.58	0.43	5.54
Bagasse 25 %	23.64	0.11	1.51	27.04	-0.08	3.56
Bagasse 10 %	24.29	0.21	1.73	22.08	-0.46	0.04

Toxicity tests

The non-treated effluent (control) showed lower toxicity effects than the decolorized effluent, for *Hydra* and *Selenastrum* (Table 4), however the treatments did not cause any inhibition for lettuce growth roots. In the algal test there were not toxicity for the effluent control, but after the treatment with horseradish the toxicity was high and the dilution of 2.76% of HLG inhibited 50% of the algal growth (Table 4).

For the sludge, the toxicity also increased after the treatment. The IC_{50} decreased about 9 times for algal and 2.7-6.5 times for lettuce root growth, meaning high increase in the toxicity compared to the control (Table 5).

The increase in toxicity may be due the incomplete degradation of the several products present in the sludge. The low degradation efficiency of products might be caused by adsorption of their metabolites to enzyme during the reaction resulting in the enzyme inactivation³⁰. This could be minimized by addition of polyethylene

Table 4. Toxicity for *Selenastrum* and *Hydra* of the effluent after treatment with horseradish grinded in the Lab (HLG), in the industry (HIG), plant extract and extracted bagasse

Treatment	Algal test	<i>Hydra</i> test	
	IC_{50} (%)	NOEC	LOEC
Control	No-effect	12.5	25
HLG	2.76	3.12	6.25
Plant Extract	1.70	3.12	6.25
HIG	1.86	1.56	3.12
Bagasse	1.70	12.5	25

Table 5. Toxicity (IC_{50}) for *Selenastrum* and lettuce roots of the sludge after treatment with horseradish grinded in the Lab (HLG), in the industry (HIG) and the extracted bagasse

Treatment	IC_{50} (%)	
	Algal test	Lettuce seeds test
Control	9.30	82.72
HLG	0.88	30.61
HIG	0.60	17.49
Bagasse	0.26	12.80

glycol and surfactants or the use of immobilized enzyme, which had showed decrease in the formation of the product-enzyme conjugates²¹.

CONCLUSION

The horseradish decolorizes the effluent and sludge from the municipal treatment station of Americana, but after the treatment there was an increase in the toxicity of these residues, showing that the degradation leads to formation of toxic substances. Thereafter, horseradish could be used in the initial steps of the effluent treatment for decolorization followed by a conventional treatment for further degradation and elimination of the toxicity.

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