Enzymatic frontiers: Optimizing effluent degradation by laccase for environmental sustainability

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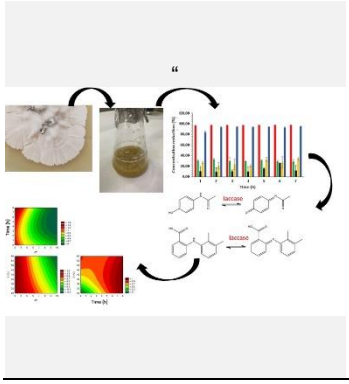

New effluent treatments that enable the reduction of waste and the reduction of environmental pollutants have grown substantially; mainly, biological treatments have proven effective in treating effluents containing drugs, dyes, etc. In this context, a laccase-rich extract (an oxidoreductase) was obtained and showed a specific activity = 0.016 U/mg. This extract was applied in the effluent treatment composed of paracetamol, diclofenac, mefenamic acid, ibuprofen, sulfamethoxazole, and trimethoprim. It was possible to observe a greater efficiency in the degradation of paracetamol and mefenamic acid. Afterward, the optimization of the treatment of an effluent containing paracetamol and mefenamic acid was carried out, making it possible to determine the ideal working condition (pH = 4.2, t = 7 hours, T <30ºC). This condition reduced paracetamol and mefenamic acid concentrations by more than 90% and 65%, respectively.

Introduction

Due to increasing concern about the impacts of effluents from the most diverse industrial processes and from homes on the environment, many studies are being carried out aiming at the introduction of new treatments that enable good removal of compounds such as phenolics. Azo compounds, pesticides, and hormones [1-3].

These compounds lead to various environmental damages for aquatic organisms and humans, emphasizing the development of allergies, nervous system problems, and cancer [3]. Therefore, reducing them to acceptable levels must be done to minimize damage to the environment and human health and help develop various diseases.

In this scenario, the use of techniques that involve the degradation or adsorption of these compounds stands out, such as, for example, the use of Fenton's reagent, aerobic and anaerobic degradation by microorganisms capable of producing enzymes such as oxidoreductases, membrane filtration, electrochemical processes, flocculation, use of adsorbents, oxidation, ozonation methods, among others [1-3]. Biological treatments have gained prominence in recent years due to their high efficiency, good competitiveness, and efficiency compared to other treatments [4,5].

In this context, considering the importance of carrying out treatments that efficiently remove these compounds, this work evaluated the efficiency of carrying out enzymatic treatment of synthetic effluent-containing drugs with an enzymatic extract enriched in laccase (an oxidoreductase) obtained from the endophytic *Xylaria* sp fungus.

Material and Methods

Initially, the process of obtaining the enzyme extract enriched in laccase was carried out. For this, the fungus *Xylaria* sp. was subjected to 3 consecutive subcultures in previously autoclaved potato dextrose agar medium. Each growth was carried out at 23ºC for one week. After this step, the fungus was grown in a specific medium for the production of laccase composed of malt extract, Tween 80, $CuSO_4$, KH_2PO_4, Na_2HPO_4, $MgSO_4.7H_2O$, peptone, and glucose. The fermentation time was 11 days, the temperature was 29ºC, and the pH was 6.7. The process was carried out using a rotation of 140 rpm and was subsequently centrifuged at 6,000 rpm for 40 min.

The supernatant (rich in laccase) was used during the treatment of synthetic effluent containing paracetamol, diclofenac, mefenamic acid, ibuprofen, sulfamethoxazole, and trimethoprim with concentrations of 50 ppm. The enzymatic activity in each step was set at 0.4 U/mg. After verifying the degradation efficiency of paracetamol and mefenamic acid, their treatment conditions were optimized regarding pH, temperature, and treatment time. All concentration data were determined via HPLC using a C18 Phenomenex. The data were analyzed using the Statistica program, making it possible to verify the influence of each variable and determine the ideal condition for treating the effluent.

Results and Discussion

Initially, the enzyme extract was obtained, showing an enzymatic activity of 0.255 U/mL and a specific activity of 0.016 U/mg. It was used in the reaction stage to treat the effluent of the drugs. Analyzing the results, it is possible to observe a greater efficiency in the degradation by oxidation of paracetamol and mefenamic acid (Figure 1).

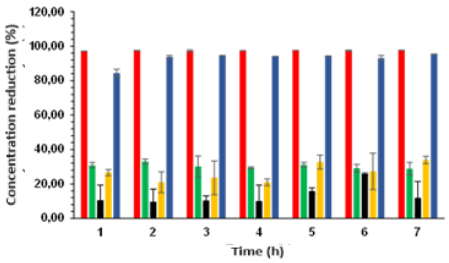

Figure 1. Degradation (%) of each drug (■ Paracetamol, ■ Sulfamethoxazole, ■ Diclofenac, ■ Ibuprofen e ■ mefenamic acid).

The greater efficiency observed can be attributed to laccase's greater capacity to oxidize phenolic and amino compounds. After this stage, an effluent treatment containing paracetamol and mefenamic acid was optimized. For this, statistical planning was used to reduce the number of experiments (Table 1). Through analysis of the results, it was found that times above 7 h, slightly acidic pH, and temperatures between 20 and 40ºC favor the degradation process by oxidation (Figure 2), and the ideal treatment condition was the use of pH equal to 5.4, for 7.36 hours, and a temperature range between 23.2 and 43.9°C (Figure 3).

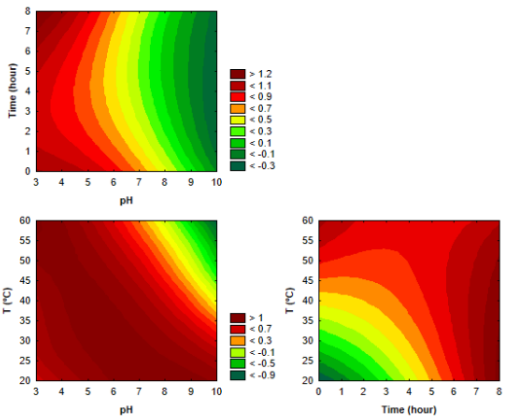

Figure 2. Contour surfaces obtained during the evaluation of the enzymatic degradation of drugs

Table 1. Paracetamol concentration reduction data in each condition

pH	Time (h)	T (ºC)	pH	Time (h)	T (ºC)	reduction [paracetamol] (%)	reduction [mefenamic acid] (%)
6.5	-1.7	0	6.5	0.64	40	63.27	10.7
-1	-1	1	5	2	50	83.79	30.6
-1	-1	-1	5	2	30	40.33	47
1	-1	1	8	2	50	44.62	2.84
1	-1	-1	8	2	30	51.41	2.61
0	0	-1.7	6.5	4	23.3	81.53	8.39
0	0	1.68	6.5	4	56.8	23.61	6.61
0	0	0	6.5	4	40	67.59	4.48
0	0	0	6.5	4	40	66.3	6.8
-1.68	0	0	3.98	4	40	67.22	44.5
1.68	0	0	9.02	4	40	11.78	5.96
-1	1	-1	5	6	30	93	65.9
1	1	-1	8	6	30	76.06	4.41
-1	1	1	5	6	50	93.44	42.5
1	1	1	8	6	50	43.97	1.03
0	1.68	0	6.5	7.36	40	85.87	12.4

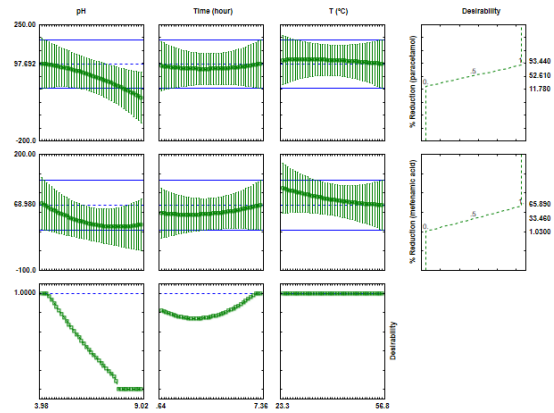

Figure 3. Optimization of effluent treatment conditions.

Conclusions

During the development of the present work, it was possible to observe the efficiency of enzymatic degradation of drugs via oxidation promoted by the laccase enzyme (an oxidoreductase). Greater efficiency in the degradation of phenolic and amino compounds such as paracetamol and mefenamic acid was also verified, and the optimization of its degradation condition aimed at large-scale applications.

Acknowledgments

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