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Design and optimization of an enzymatic membrane reactor for tetracycline degradation

M. de Cazes[a], M.-P. Belleville[a], E. Petit[a], M. Llorca[b], S. Rodríguez-Mozaz[b], J. de Gunzburg[c], D. Barceló[b,d], J. Sanchez-Marcano[a,*]

[a] Université de Montpellier 2, Institut Européen des Membranes, UMR 5635 (CNRS-ENSCM-UM2), CC 047, 2 Place Eugène Bataillon, 34095 Montpellier Cedex 5, France
[b] Catalan Institute for Water Research (ICRA), Scientific and Technological Park of the University of Girona, H2O building, C/Emili Grahit 101, E-17003 Girona, Spain
[c] Da Volterra, Le Dorian, building B1, 172 rue de Charonne, 75011 Paris, France
[d] Water and Soil Quality Research Group, Dept. of Environmental Chemistry, IDAEA-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain

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ABSTRACT

The tetracycline, antibiotic considered as a recalcitrant pollutant, was successfully depleted from model aqueous solutions by immobilized laccase from *Trametes versicolor* in an enzymatic membrane reactor. The results obtained show that tetracycline is depleted from water solutions at room temperature and without adding any extra chemicals. The degradation of tetracycline in aqueous solutions at 20 mg L^{-1} during 24 h, with equivalent amounts of free or immobilized biocatalyst, allowed reaching a tetracycline degradation yield of 56% with an enzymatic membrane whereas it was only of 30% with free laccase. This result highlights the good reactivity and stability of the immobilized enzyme for the degradation of tetracycline. Moreover, the enzymatic membrane reactor was able to reach a constant degradation rate of 0.34 mg of tetracycline per hour during 10 days.

1. Introduction

With the background of an aging population and increasing urbanization, pharmaceutical products (PPs) and endocrine disrupting chemicals (EDCs) have been continuously released in the environment for a long time without being considered as priority pollutants to target.

As conventional wastewater treatment technologies are not efficient enough to completely remove pharmaceuticals from water, such products are currently found in water effluents from sewage facilities, as well as in surface water, in groundwater, adsorbed on sediments and even in drinking water [1–3]. Furthermore, ecotoxicity studies have demonstrated that PPs could affect the growth, reproduction and behavior of birds, fishes, invertebrates, plants and bacteria [4–6]. In particular, the presence of low concentrations of antibiotics in wastewaters could cause the development of antibiotic resistance by bacteria and then be an important source of public health problems in the future [7].

Indeed, lately important research efforts have been done in order to find a system to eliminate the PPs before rejecting the effluents to the environment. Among the different processes tested (physical adsorption, chemical or biological reactions) for the depletion of certain groups of pollutants [8–12], the use of biocatalysts such as laccases, glycosylases, proteases and lipases have been found to be particularly efficient [13]. In particular laccases are able to oxidize a wide range of pollutants at room temperature within a large range of pH using as oxidant the oxygen dissolved in water. Consequently, some reports have noticed the potential of laccase-catalyzed reactions for the removal of a large spectrum of pollutants [14–19].

To overcome the drawbacks related to enzymes cost, biocatalysts can be immobilized on a large variety of supports [16,18] as well as in membranes and used in enzymatic membrane reactors (EMRs) [20–22]. In EMRs the substrate solution flows through the membrane to the biocatalyst as a result of transmembrane pressure. Then the reaction takes place simultaneously with the mass transfer process through the membrane and the product is recovered in the permeate. Thus a precise control of the reaction with

* Corresponding author. Tel.: +33 467 149 149/+33 467 1419; fax: +33 467 149 119.
E-mail address: sanchez@iemm.univ-montp2.fr (J. Sanchez-Marcano).

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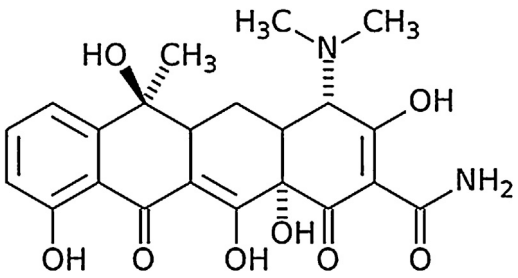

Fig. 1. Structure of tetracycline molecule.

minimized substrate and catalyst losses, faster reactions, higher yields and cleaner products can be expected [23].

This work describes the study of the potential enzymatic degradation of tetracycline (TC), a recalcitrant antibiotic present in some wastewaters. The laccase from *Trametes versicolor* was chosen as the biocatalyst because this biocatalyst has already demonstrated its activity for the degradation of this antibiotic [24]. For this purpose the enzyme was covalently linked onto a ceramic membrane previously coated with a polymer layer (i.e. gelatin). A comparison between free and immobilized enzymes was also carried out to evaluate the performances of the EMR and the stability of grafted enzymes. In addition different operating parameters like pH and temperature were studied in order to determine the optimal operating conditions for the tetracycline depletion. To our knowledge it is the first work reporting tetracycline degradation with immobilized laccase on ceramic membranes.

2. Experimental

2.1. Products

Commercial powder of laccase from *T. versicolor* (activity $\geq$10 U mg^{-1}, ref. 51639), tetracycline ($\geq$98.0%, ref. 87128) (Fig. 1), gelatin, glutaraldehyde and ABTS ($\geq$98.0%, ref. 11557) were purchased from Sigma-Aldrich. Mono-channel ceramic membranes (α-alumina) were supplied by Pall-Exekia (pore diameter of 0.2 μm, 15 cm long, external/internal diameter of 1 and 0.7 cm, effective area of 28.6×10^{-4} m^2). Tetracycline solutions were prepared at 20 mg L^{-1} in osmosed water (pH = 6) or in different 50 mM citrate/phosphate buffers (pH 3 to 7). The TC is usually encountered in wastewaters (hospital and wastewaters treatment plants) in a concentration range from 1 to 900 μg L^{-1} [25]. The model solutions tested in this work were prepared with buffers or osmosed water and pure TC at 20 mg L^{-1}. This concentration is high compared to the actual concentrations noted above. It was arbitrarily chosen in order to have a good precision on depletion rates while allowing the identification of the degradation products.

2.2. Immobilization protocol

Active membranes were prepared according to a 3-step procedure developed by Belleville et al. [26] and adapted for laccase immobilization by Chea et al. [14]. First, the wet ceramic supports (α-alumina tubular membranes from Pall-Exekia, France (15 cm of length, 1 cm of external diameter and 0.7 cm of internal diameter, 0.2 μm of mean pore size) were coated with a gelatin layer by filtrating a gelatin solution at 1 g L^{-1}. Then the bio-polymer layer was activated by a glutaradehyde solution (4% (w/v) for 1 h and finally, the laccase (10 g L^{-1} solution) was let to react with free aldhehyde groups of glutaraldehyde during 2 h. All the solutions were prepared in a 50 mM phosphate buffer pH 7 and after each step, the excess solution was removed by rinsing 4 times the membrane with the same phosphate buffer. The active membranes were then stored in a desiccator with P_2O_5 until being used. Blank membranes were prepared with the same method but without enzymes.

2.3. Pilot unit

The pilot unit used for EMR runs is shown in Fig. 2. It was built with stainless steel and PTFE in order to avoid adsorption problems. The EMR can be operated with or without recirculating the retentate (Fig. 2). In this exploratory work we carried out experiments in batch configuration (the permeate valve was kept close except during sample withdrawing and permeate flux measurements). The temperature, the trans-membrane pressure and the flow rate can be measured and controlled with sensors and a cryothermostat. Since the feeding tank was open to atmospheric pressure, there was no need to add extra oxygen in the water. It was indeed proved that adding oxygen by sending pressurized air into water had no significant impact on dissolved oxygen concentration level.

2.4. Enzymatic degradation

In order to determine the optimal operating conditions and to compare the activity of free and immobilized enzymes, several experiments were carried out with 100 mL of tetracycline solutions (20 mg L^{-1}) prepared either in 50 mM citrate-phosphate buffer (pH 3 to 7) or in osmosed water (pH 6) at 25 °C for 24 h in a stirred tank bioreactor. In the case of free enzyme experiments an amount of commercial powder was added to the tetracycline solution, whereas for immobilized enzymes we used small pieces of a crushed enzymatic membrane (EM). The amount of crushed EM used for the reaction corresponds to the same concentration of enzymes used for free enzymes experiments. Before degradation experiments the small portions of the crushed membrane were washed with osmosed water in order to eliminate potential free laccases that might not have been rinsed properly during the grafting step. Tetracycline auto-degradation and adsorption were estimated

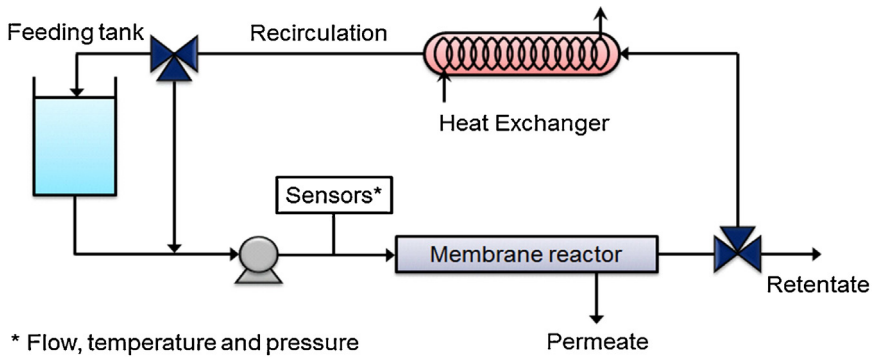

Fig. 2. Enzymatic membrane bioreactor and pilot unit.