University of Pannonia
Chemical Engineering and Matterial Sciences Doctoral School

Development of biocatalytic wastewater treatment technology

Ph.D THESES

Author:
Béla Varga

Supervisor:
Dr. Viola Somogyi
associate professor

University of Pannonia
Faculty of Engineering
Sustainability Solutions Research Laboratory

2023

Introduction

The removal of organic micropollutants in municipal wastewater treatment has not yet been solved, so the development of special technologies capable of reducing the environmental impacts caused by these pollutants is required. One such solution is the utilisation of oxidoreductase enzymes, such as laccase, to transform them into less toxic substances. However, in order to spread the use of the laccase enzyme in wastewater treatment on an industrial scale, it is necessary to develop immobilization methods that allow the enzyme to be used for longer periods and to maintain its stability during the treatment. Many different immobilisation and stabilisation methods have been developed for this purpose. The goal of my research was to develop methods that utilise biodegradable materials as a carrier so that their disposal after the end of life does not cause any environmental impact and is also suitable for retaining the reaction transformation products resulting from the treatment with laccase.

Methods

The doctoral work consists of two main areas according to tot he enzyme immobilisation method used. First, the immobilisation of laccase in calcium alginate by entrapment has been investigated. By mixing the enzyme into alginate, laccase containing alginate beads has been produced that can be removed by filtration. After adaptation of the immobilisation protocol to laccase, the most important properties from the point of operation have been determined such as specific activity, temperature and pH dependence, enzyme kinetic constants and its storage stability and reuse have been investigated.

Afterwards, the biocatalysts has been applied to transform a model micropollutant, diclofenac.

In the second phase of the research, a novel immobilisation method has been developed that is based on adsorption and cross-linking to form cross-linked laccase aggregates in the pores of a cellulose acetate microfiltration membrane. The parameters of the immobilisation protocol have been optimised by the response surface method and the enzyme catalytic membrane was characterised. For this purpose, same properties have been determined as in the case of alginate. Additionally, the effect of volumetric flow on the transformation of diclofenac was studied in a small membrane module as well as in a laboratory-scale membrane system with continuous recirculation of the pollutant-containing solution.

Theses

I. Laccase immobilised in alginate matrix

1. Laccase can be immobilised in a calcium-alginate matrix with high immobilisation efficiency**; however, the specific activity of the catalyst produced is low** due to the inhibitory effect of diffusion. The immobilisation efficiency of the entrapment in alginate beads is 98.2%, while the laccase corresponds only to a small portion of total mass (0.1% of wet mass and 3% of dry mass). The specific activity of the free laccase used for immobilisation is 457 U/mg, while the trapped enzyme has only 0.00026 U/mg apparent activity.

   Related publications: 4

2. During the utilisation of laccase immobilised in an alginate matrix, **the adsorption process has a great effect on the removal of diclofenac.** In the case of conversion of ABTS substrate, the products are accumulating in the alginate beads, which results in colouration of the beads and lower measurable activity. In the case of the transformation of the diclofenac, the main removal mechanism is the adsorption of the compound in the alginate matrix instead of the enzymatic transformation.

   Related publications: 4, 7

II. Laccase immobilised in the pores of a microfiltration membrane

3. I successfully immobilised laccase in cellulose acetate membrane with a two-step method. First, the laccase was adsorbed on the membrane, and then it was stabilized by making cross-linked enzyme-aggregated in/on the membrane.

   I concluded that during the adsorption of laccase on the membrane, **an increase in temperature could improve the immobilisation efficiency, but denaturation by high temperature must be considered.** By fine-tuning the parameters of the adsorption process, the highest immobilisation efficiency was achieved by the following conditions: pH=4, laccase concentration: 3 mg/ml, adsorption time: 9 hours, and temperature: 29°C.

I concluded that the effect **of the glutaraldehyde concentration during cross-linking is only significant in lower temperatures,** and the activity of the membrane could be improved by cooling to 4°C. The maximum membrane activity was achieved by cross-linking at 4°C with 100 mM glutaraldehyde concentration for 2 hours.

During the immobilisation with the developed method, intermolecular chemical bonds have been formed between enzyme molecules, which produce aggregates. Scanning Electron Microscopy has been used to confirm that process. As a result, I concluded that the average diameter of aggregates on the membrane is 0.39 μm.

I concluded **that the pore size and the membrane's permeability had been changed during the immobilisation process**. The pore size has been increased from 0.19 μm to 0.24 μm. The flow rate change depended on the pressure applied, but the average was a 21% decrease.

Related publications: 3, 8, 13

4. I concluded that **the conversion of the substrate with the enzymatic membrane produced depends on the volumetric flow rate**. In the case of the ABTS reaction, the conversion of the substrate and the volumetric flow is inversely proportional in the range of 0.4 - 1.9 l/hour. In the case of lower flow rates (0.02 - 0.07 l/h), what I used for diclofenac conversion, a minimum flow rate value can be determined for the maximal efficiency. Related publications: 3, 13

5. Some of **the low-solubility reaction products of diclofenac transformation, catalysed by free or immobilised laccase, could be removed by microfiltration**.

   I concluded that in the chemical reaction catalysed by oxidoreductase enzymes, based on the analysis of literature information, I found that the **toxicity of the resulting reaction products can vary depending on the given component, therefore it is advisable to develop immobilization methods that are also suitable for removing these substances**. The resulting reaction products in the reaction catalysed by laccase are usually coupled molecules, which can be separated by adsorption or membrane separation processes.

   Some reaction products of the laccase catalysed transformation result in a yellow discolouration, with light absorbance around 450 nm. Precipitates are formed from these since the water solubility of these products is considerably lower than the initial compound. These compounds cannot be eliminated during the catalysis with one-time dead-end filtration but recirculating the solution can be removed.

   Related publications: 1, 3, 5, 6, 8

Publications

Publication related to the PhD dissertation:

1. **Varga, B.,** Somogyi, V., Meiczinger, M., Kováts, N., Domokos, E. (2019) Enzymatic treatment and subsequent toxicity of organic micropollutants using oxidoreductases - A Review, *Journal Of Cleaner Production*, 221, 306-322. Q1, IF: 7,246

2. Meiczinger, M., **Varga, B.,** Wolmarans, L., Hajba, L., Somogyi, V. (2022) Stability improvement of laccase for micropollutant removal of pharmaceutical origins from municipal wastewater, *Clean Technologies and Environmental Policy*, 23, 3213-3223, Q2, IF=4,7

3. **Varga, B.,** Meiczinger, M., Jakab, M., Somogyi, V. (2023) Design and Optimization of Laccase Immobilization in Cellulose Acetate Microfiltration Membrane for Micropollutant Remediation, *Catalysts*, 13(2):222, Q2, IF=4,501.

Publication in Hungarian Journals:

4. **Varga, B.,** Somogyi, V., Meiczinger, M., Domokos, E. (2019) Immobilization of laccase in alginate beads, *Hungarian Journal of Industry and Chemistry*, 47(2)

Publication in conference proceedings:

5. **Varga, B.** Somogyi, V. Meiczinger, M., Domokos, E. (2017) Review on products of enzymatic degradation of micropollutants in wastewater, 12th Conference On Sustainable Developement of Energy, Water and Environment Systems, Dubrovnik
6. **Varga, B.**, Somogyi, V., Meiczinger, M., Nagy, E., Domokos, E. (2018) Investigation of Diclofenac Degradation in Different Matrices, Conference On Sustainable Developement of Energy, Water and Environment Systems, Novi Sad

Publications on conferences, abstract:

7. **Varga**, B. Meiczinger, M., Somogyi, V., Nathnakumar, K., Nagy, E., Domokos, E., Lal, B. (2018) Degradation of diclofenac with laccase enzyme immobilized in alginate beads, 10th Eastern European Young Water Professionals Conference, Zágráb
8. **Varga, B.,** Wolmarans, L., Meiczinger, M., Somogyi, V. (2019) Immobilization of Laccase Inside a Microfiltration Membrane for Removal of Diclofenac, ANQUE-ICCE-CIBIQ, Santander
9. Meiczinger, M., **Varga, B.,** Nagy., E., Somogyi, V. (2019) Investigation of Immobilization Efficiency of Laccase by Different Technique in Biocatalytic Membrane Reactor ANQUE-ICCE-CIBIQ, Santander
10. **Varga, B.** (2019)**,** Biokatalitikus technológia fejlesztése mikroszennyezők eltávolítására, Green Solution Veszprém

11. **Varga, B.,** Somogyi, V., Meiczinger, M., Domokos, E. (2018) Diklofenák eltávolítása enzimkatalitikus eljárással különböző közegekben, Környezetmérnöki Konferencia és Szakmai Nap, Debrecen
12. **Varga, B.** (2018) Diklofenák eltávolítása lakkáz enzimmel különböző közegekben, Víz- és szennyvízkezelés az iparban (VSZI'18), Zalakaros
13. **Varga, B.**, Meiczinger, M., Somogyi, V. (2021) Keresztkötött lakkáz aggregátumok létrehozása mikroszűrő membrán pórusaiban, II. FKF Szimpózium, Budapest