Immobilization of Enzymes into nanofiber structure for Wastewater treatment

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Abstract

Wastewater treatment technology must deal with problematically cleanable substances. These substances include fats, proteins and endocrine disruptors, which can be partially or completely hydrolysed by enzymes such as lipases, proteases, laccases, and tyrosinases.

Direct application of biologically active substances has several disadvantages, such as storage, operational stability and significant operational costs, because the active substance must be repeatedly added into cleaning process. These drawbacks can be reduced by immobilization of biologically active enzymes into suitable inert structure like nanofibers, thanks to their high specific surface. Immobilized enzymes are active, stabilized and thus advantageous for long-term operation in cleaning process.

Actual activity of immobilized enzymes can be measured using fluorescence microscopy followed by image analysis. Moreover, dissolved and nanofiber-enzyme activity will be detected using standard methods. Efficiency of selected enzymes will be tested on concrete pollutants. Nanofibrous carrier should hold efficient quantity of immobilized enzymes with long time activity. Finally, we will test durability of carriers in unfavourable conditions directly in wastewater treatment plant. To conclude, this study is aimed to describe the effect of selected enzymes on particular pollutants and to determine their benefits and suitability for wastewater treatment applications.

Introduction

Over past century there has been an increasing amount of wastewaters, which cannot be cleaned by conventional wastewater processes. Among problematical substances (pollutants) are listed oils, fats, proteins and endocrine disruptors. Oils, fats, proteins form majority of pollutants in wastewaters from domestic and food industry, and are difficult to clean through conventional biological treatment due to their slow biodegradability. [1, 2]

Endocrine disruptors are chemicals that can or are suspected to mimic, or interfere with the action of endogenous hormones. These substances are common in household chemicals, pharmaceuticals, and substances used in industry. [3]

Enzymes are versatile catalysts that might bring better results than conventional wastewater cleaning methods. They catalyse specific reaction under moderate conditions (temperature, pH), and mainly without undesired side-reactions, which would otherwise increase reactant consummation and raise the cost of treatment. Previously named pollutants can be degraded or transformed by lipases (oils and fats), proteases, laccases and tyrosinases (endocrine disruptors). [4]

Direct application of enzymes is operationally and financially demandable, for this reason enzymes are immobilized into suitable biocompatible inert support. Immobilization also increase operational stability of enzymes and allows repeatable usage. Huge development of nanotechnology enables immobilization of enzymes on nanofibers. The premise of nanomaterials is to reduce diffusion limitations and maximize functional surface area to increase enzyme loading resulting in higher enzyme activity. [5]

Experiment and methods

Our aim is to evaluate activity of immobilised enzymes, which participate in degradation of oils, fats, proteins and endocrine disruptors. Lipase, protease, lacasse, tyrosinase are covalently immobilized on nanofibrous carriers, which are fibers with surface covered by nanofibers. Immobilized enzymes will be activated using suitable buffer (potassium phosphate buffer, or Tris-HCl) and fluorogennic substrate (1,2-Di-O-lauryl-rac-glycero-3-(glutaric acid 6-methylresorufin ester) (DGGR), or EnzChek® Lipase Substrate). Enzymes activity will be measured via fluorescence microscopy using suitable methods of image analysis. Evaluation of activity of enzymes immobilized on nanofibers has not been well described, so one of our goals is to modify conventional methods. Fluorescence microscopy will be accompanied by standard spectrophotometric method based on measuring bulk enzyme activity in solution. Effectiveness of carefully selected enzymes will be tested on concrete pollutants. Their concentration will be measured by chromatographic methods (HPLC for proteins, in case of endocrine disruptors GC MS MS).

Discussion

Different types of nanofibrous carriers (PES, PUR, and PVB) will be tested, and will be monitored for their suitability to carry optimal amount of immobilized enzymes and their long-term activity. Important will be the selection of most suitable nanofiber carrier, which will have the ability to carry optimal amount of covalently bind enzymes and it will be sufficiently resistant in hostile environment in application on wastewater treatment plant. In ideal case carrier should have moderate antibacterial effect, because creation of biofilm on carriers is undesired.

Conclusions

Here, I describe research that is aimed to optimize new wastewater cleaning technology for degradation of problematically cleanable substances. Technology is based on enzymes immobilized on nanofibrous carriers. Most important goal of research is to describe application of enzymes for concrete pollutants and to find suitable conditions of their usage in wastewater cleaning processes.

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