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Polymer membranes for active degradation of complex fouling mixtures

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ABSTRACT

Immobilization of different digestive enzymes consisting of proteases, amylases and lipases on polyvinylidene fluoride resulted in biocatalytic active polymer membranes with self-cleaning capability. Activation of enzymes and therefore "switching on" the membrane system was achieved by adjusting pH and temperature leading to an active degradation of fouled substances on its surface. Fouling and self-cleaning experiments with solutions of protein, lipid, carbohydrate, and a mixture were performed and resulted in high recovery of water permeance (99%, 72%, 77%, and 68%, respectively). Furthermore, real samples including river water (75% after first cycle), and household sewage (62%) were examined, as well as first investigations in longtime performance, and stability were performed. Comprehensive membrane characterization was conducted by investigation of the immobilized enzyme concentration, enzyme activity, fouling tests and water permeation monitoring, mercury porosimetry, X-ray photoelectron spectroscopy, scanning electron microscopy, and finally, zeta potential, as well as water contact angle measurements.

1. Introduction

1.1. Membrane technology and fouling

Polymer membranes made of synthetic organic macromolecules have a steadily increasing importance in technical applications due to their high physical and chemical stability [1–3]. Commonly used materials are polyethersulfone (PES) and polyvinylidene fluoride (PVDF), next to polyethylene (PE), polyacrylonitrile (PAN), or polysulfone (PSf). Many modern filtration technologies make use of polymer membranes, *e.g.* waste water treatment [4,5], hemodialysis [6], or dairy industry [7]. One main issue in using membranes is *membrane fouling*. This phenomenon is associated with the decrease of filtration performance or a necessary increase in driving force (*e.g.* pressure) in order to continue the filtration process [8–11]. A major problem for the regularly cleaning of a fouled membrane is the use of aggressive or environmentally harmful chemicals (typically strong oxidants such as hypochlorite or citric acid) [12]. Numerous approaches to improve the anti-fouling properties of membranes have been developed, particularly to tailor the membrane surface in terms of hydrophilicity, surface charge, or affinities. Commonly used strategies are the direct chemical modification of the polymer material [13], as well as the use of hydrophilic monomers (copolymerization or grafting reactions) [14–17], and hydrophilic polymers (polymer blend) [18–20]. In our previous studies, irradiation-based approaches have been used to modify membrane surfaces, *e.g.* with plasma [21] or electron irradiation [22–25]. In the case of electron irradiation, the mechanical stability of tested membranes could be preserved [22,24].

1.2. Utilization of enzymes

The use of enzyme solutions to clean organically fouled membranes is an efficient and environmentally friendly method that is already used in actual processes [26–28]. A disadvantage of using native enzyme solutions is the relatively high sensitivity of enzymes towards changes in operating parameters. Thus, *immobilization* can increase the stability of enzymes [29]. At the same time, a potential risk of permeate contamination is reduced and a simple separation from the filtration mixture is promoted [30]. As a result, not only long-term stability is increased, but also resource consumption is lowered [31].

1.3. Self-cleaning membranes

The principle of *self-cleaning membranes* (Fig. 1) is based on the following aspects:

- Immobilization of digestive enzymes on a membrane surface puts both enzymes and fouling substances adsorbed on the surface in contact with each other.
- The fouling layer that builds up during filtration can then be removed enzymatically *via* hydrolysis by activating the digestive enzymes through adjusting the ambient conditions (pH, temperature,

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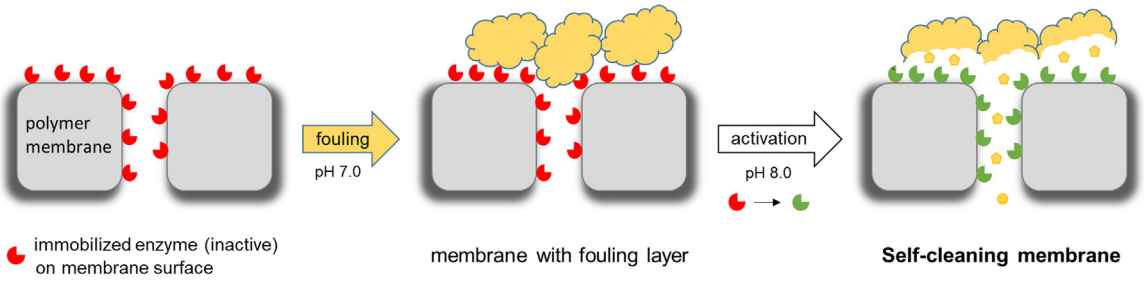

Fig. 1. Schematic principle of self-cleaning membranes. Digestive enzymes immobilized on the membrane surface are activated by changing the buffer pH within a range of 7.5–8.5 and digest the fouling-causing substrates.

buffer) [32].

In this study, *porcine pancreatin* was used which is an inexpensive mixture of enzymes. It is composed of various proteases, lipases, amylases, and other enzymes [33] and is widely used in the pharmaceutical, cosmetics, and food industries [34–38]. To achieve higher levels of enzyme activities, also purified porcine pancreatic α-amylase (PPA), purified porcine pancreatic lipase (PPL), and a comparable lipase from the yeast *Candida rugosa* [39] were investigated.

1.4. Enzyme immobilization

Various methods for enzyme immobilization are discussed in the literature [31]. In this study a standard approach using the carbodiimide EDC (1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride) and NHS (*N*-hydroxysuccinimide) was performed [40–42]. Prior electron-beam induced grafting reaction with AEMA (2-aminoethyl methacrylate hydrochloride) was used to introduce functional groups ($-NH_2$) onto the membrane surface for subsequent chemical immobilization with EDC/NHS, resulting in strong covalent amide bonds [40,43].

1.5. This study

In previous publications enzyme immobilization for pollutant degradation [44,45], and enzyme immobilization for self-cleaning applications using pancreatin [32,46,47] were described. Pancreatin was immobilized on commercially available hydrophilized PES membranes, initial investigations regarding fouling and self-cleaning experiments were performed, and good cleaning potentials towards proteins and lipids were observed [47]. So far, however, immobilization of amylases and thus the investigation of self-cleaning in carbohydrate fouling was not successful, although carbohydrates (polysaccharides) play an important role in many industrial processes [48–52].

The linkage of amylase is therefore imperative to develop a fully self-cleaning membrane towards the three main groups of biomolecules – proteins, carbohydrates and lipids. The aim of this work was the successful immobilization of active amylase next to protease and lipase on PVDF polymer membranes, as well as the subsequent investigation in fouling and self-cleaning experiments. In addition, the lipase and protease activity should be increased compared to preliminary works, and finally, a combination of different enzymes should be immobilized. Determination of enzyme activity was done by using progress curve approach (see Section 2). All so-prepared membranes were comprehensively characterized. For each enzyme group, self-cleaning capability was studied in fouling experiments with individual components, and a mixture, as well as real samples including river water and household sewage. Furthermore, initial long-time storage under different conditions followed by activity tests was investigated.

2. Experimental

2.1. Chemicals and materials

2.1.1. Polymer membrane:

Commercially available polyvinylidene fluoride flat sheet membrane (PVDF, Roti®; 0.45 µm) was purchased from Carl Roth (Karlsruhe, Germany).

2.1.2. Enzymes:

Porcine pancreatin (P), porcine pancreatic α-amylase (PPA), porcine pancreatic lipase type II (PPL), and *Candida rugosa* lipase type VII (candida lipase, CL) were purchased from Sigma Aldrich (St. Louis, Mo., USA).

2.1.3. Chemicals:

The following chemicals were obtained from Sigma Aldrich (St. Louis, USA) for immobilization reaction: *N*-hydroxysuccinimide (NHS), and 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC). Furthermore, 2-aminoethyl methacrylate hydrochloride (AEMA, Acros Organics) from Thermo Fisher Scientific (Geel, Belgium), as well as HCl and NaOH from Merck (Merck Millipore, Billerica, USA) were used. Deionized water in Millipore® quality was used for all steps. All chemicals were of analytical grade and used without further purification.

2.1.4. Buffers:

Phosphate buffered saline (PBS, tablets), disodium hydrogen phosphate dihydrate, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and calcium chloride dihydrate were purchased from Sigma Aldrich (St. Louis, USA).

2.1.5. Enzyme kinetics:

4-Nitrophenol (PNP) from Fluka Analytical (Munich, Germany) and dimethyl sulfoxide (DMSO), 4-Nitroaniline (PNA), 4-Nitrophenyl palmitate (PNP-palmitate) and 4-Nitrophenyl-α-D-maltohexaoside (PNPG6) from Sigma Aldrich (St. Louis, USA) were used. In addition, *N*-α-benzoyl-D,L-arginine-4-nitroanilide hydrochloride (BAPNA) from Panreac AppliChem (Castellar del Valles, Barcelona, Spain) was used. Bicinchoninic acid (BCA, Pierce, IL, USA) as protein determination kit was provided by Thermo Fisher Scientific (Geel, Belgium).

2.1.6. Fouling tests:

For protein fouling bovine serum albumin (BSA, 66 kDa) from Sigma Aldrich (St. Louis, USA) and for carbohydrate fouling sodium alginate (alginate, M_w N/A) from MP Biomedicals (Solon, Ohio, USA) were used. For lipid fouling linseed oil (Kunella Feinkost, Cottbus, Germany) was purchased in a grocery store and sodium dodecyl sulfate (SDS) from Carl Roth (Karlsruhe, Germany) was used.

Additionally, river water (river *Parthe*, Adenauerallee/Brandenburger Straße, GPS: 51.353921, 12.396539, 07.10.2017, 14:30, overcast, stored at 4 °C, TIC: 16.4 mg/L, NPOC: 5.2 mg/L, TC: