Biocatalytic membranes through aqueous phase separation

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Abstract

Hypothesis: Test Polymer membranes play a critical role in water treatment, chemical industry, and medicine. Unfortunately, the current standard for polymer membrane production requires unsustainable and harmful organic solvents. Aqueous phase separation (APS) has recently been proposed as a method to produce membranes in a more sustainable manner through induced polyelectrolyte complexation in aqueous solutions.

Experiments: We demonstrate that APS has another natural advantage that goes beyond sustainability: the easy incorporation of enzymes in the membrane structure. Biocatalytic membranes hold great promise in for example biorefinery, but the most common current post-production processes to immobilize enzymes on the membrane surface are complicated and expensive.

Findings: In this study we demonstrated the first biocatalytic membrane produced via APS. We demonstrate an easy procedure to incorporate lysozyme in polyelectrolyte complex membranes made via APS. Our functionalized membranes have the same structure, water permeability (in the range of high nanofiltration, low ultrafiltration), and retention as membranes without lysozyme. Lysozyme is antibacterial by catalysing the hydrolysis of specific peptidoglycan bonds in bacteria walls. We demonstrate that the functionalized membranes are also capable of catalysing this reaction. The membranes remain enzymatically active for a period of at least one week. This opens new routes to produce polymer membranes with added biological function.

Abbreviations: NIPS, Non-solvent induced phase separation; APS, aqueous phase separation; PEC, polyelectrolyte complex; PAH, poly(allyl hydrochloride); PSS, poly(sodium 4-styrene sulfonate); mQ, ultrapure Milli-Q water; SEM, scanning electron microscopy; PEM, polyelectrolyte multilayer; PWP, pure water permeability; BSA, bovine serum albumin; PEG, poly(ethylene glycol).

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1. Introduction

Polymer membranes are ubiquitous in water purification and industrial separation techniques. In the face of global water shortages as well as developing (bio)technology, the demand for more, better, and more versatile membranes continues to increase [1–3]. In addition, there are investigations into making membrane production processes more sustainable [4–7]. The common production method for polymer membranes is non-solvent induced phase separation (NIPS), also known as immersion precipitation [8,9]. In this process, polymers are typically first dissolved in an aprotic organic solvent and then cast on a substrate (e.g., a glass plate) as a thin liquid film. This film is subsequently submerged in a non-solvent bath, where the polymer then precipitates as the solvent migrates out of the polymer solution. The interface of the polymer solution and the non-solvent often forms a dense selective polymer layer while the polymer structure between the interface and the substrate will be more porous. This membrane asymmetry allows for higher water permeabilities due to the porous structure while maintaining desirable selective properties due to the dense selective layer. A disadvantage of NIPS is the requirement of repro-toxic organic solvents like N-methyl-2-pyrrolidone, which has recently been added to the restricted substances list by the European Commission. Therefore, alternative production methods have been proposed that use different, more sustainable solvents [10–12]. Still, it is difficult to find a more sustainable solvent than water.

In 2019 aqueous phase separation (APS) has first been demonstrated as a membrane production method in which both phases of the phase inversion are aqueous solutions [13–20]. Of specific interest is complexation-driven APS, where oppositely charged polyelectrolytes are first mixed in an aqueous solution under conditions that prevent polyelectrolyte complexation (e.g., high salinity or an extreme pH). Similar to NIPS, the viscous polyelectrolyte solution is then cast on a substrate and submerged in a different aqueous solution with conditions at which polyelectrolyte complexation occurs (e.g., low ionic strength or an opposite pH). Membranes produced via APS are also typically asymmetric. The membrane properties can be tuned by the manipulation of the precipitation conditions such as salt concentration or pH [13–15,17,18]. Since the complete membrane production process via APS occurs in aqueous environments, this method does not require the use of organic solvents.

In this study we suggest an additional advantage of APS: aqueous environments are more favorable to biomacromolecules such as enzymes, whereas organic solvents typically lead to enzyme denaturation and inactivation. For this reason, APS could allow easy incorporation of enzymes directly in the membrane production process instead of attaching enzymes as a post-production modification. In this way, existing APS techniques can be modified to create biocatalytic membranes. Currently there are no reported APS systems that include enzymes as either part of their structure or as added functionality.

Biocatalytic membranes combine e.g., enzyme functionality with separation properties of polymer membranes [21,22]. Biocatalytic membranes potentially provide substantial benefits to the field of membrane technology due to the large variety of functionalities that biomacromolecules can give to membranes [23,24]. For example, biocatalytic membranes using hydrolase and oxidoreductase enzymes have been suggested for the removal of micropollutants and to reduce fouling [23,25]. Despite the potential of biocatalytic membranes, there are many limitations to their production and widespread use. Currently, enzymes are typically immobilized on the membrane surface after fabrication of the membrane [21,22]. The enzyme immobilization can require various additional processing steps. In addition, enzymes can lead to a decrease in membrane permeability and selectivity, enzyme immobilization or membrane production conditions can deactivate the enzyme, or enzymes can leach out of the membrane [21,22].

Here, we demonstrate the incorporation of the enzyme lysozyme in polyelectrolyte complex (PEC) membranes produced via APS. For this reason we have adapted a previously reported APS system consisting of poly(allylamine hydrochloride) (PAH) and poly(sodium 4-styrenesulfonate) (PSS) [13]. We propose a modification to existing APS protocols that allows for easy addition of enzymes directly to the casting solution, with the result that functional enzymes are incorporated in the membrane structure.

The enzyme chosen for this study is lysozyme. Lysozyme is an antibacterial enzyme that is part of the innate immune system and is found in saliva, tears, and other mucus secretions [26,27]. Its main function is destroying gram-positive bacteria by catalyzing the hydrolysis of specific peptidoglycan bonds in bacterial cell walls [28,29]. Lysozyme is interesting for membrane technology due to its capacity to reduce membrane fouling by preventing or reducing the formation of a bacterial biofilm on the membrane [30].

We demonstrate that the incorporation of lysozyme into PAH/PSS membranes produced via APS can be highly straightforward, especially when compared to the existing methods for functionalizing membranes produced with traditional membrane fabrication processes. Moreover, the resulting membranes demonstrated enzymatic properties which persisted for at least one week. There was no detectable lysozyme found leaching out of the membrane during the membrane production process or during up to one week of storage. In addition, the membrane permeability, retention, and structure are not affected by the presence of lysozyme. The relative ease of production and biocatalytic activity opens a new route to produce sustainable membranes via APS with added biological functionality.

2. Materials & methods

2.1. Materials

PAH 40% solution with a molecular weight (MW) of 150 kDa was purchased from Nittobo. PSS 30% solution with a MW of 200 kDa (product number 561967), lyophilized hen-egg lysozyme (product number L876), lyophilized micrococcus lysodeikticus (product number M3770), 50% glutaraldehyde solution (product number 340855), NaOH pellets, fuming HCl solution, and NaCl were purchased from Merck. Ultrapure Milli-Q water (mQ) is filtered by an Advantage A10 water purification system (Millipore), otherwise ‘water’ refers to demineralized water.

2.2. Membrane production

PSS stock solutions were diluted from 30 to 15 wt% with mQ or with 0.75 g/l lysozyme in mQ solution. PAH stock solutions were diluted from 40 to 15 wt% with mQ and 10 M NaOH to reach 7.5 wt% NaOH. These 15 wt% PAH and PSS solutions are then mixed 1:1:1 (mass ratio) respectively to achieve a 2:1 positive:neutral monomer ratio. The polyelectrolyte solutions are mixed and degassed to remove air bubbles, resulting in a viscous 15% mixed polymer solution.

The polymer solution is then cast on a glass substrate using a casting knife with a 600 μm gap before being submerged in 250 ml precipitation solution consisting of 2% fuming HCl, 0.5 M NaCl, and 0.1% crosslinker glutaraldehyde in mQ. Precipitation took place for 15 min before the membranes were placed in water. In
water, the membranes spontaneously detach from the glass plate within a few minutes. Membranes were stored in water until further analysis.

2.3. Membrane characteristics evaluation

The membrane morphology was determined by scanning electron microscopy (SEM). First, the membranes were submerged in a 20% glycerol solution for at least 4 h. The samples were then air dried and submerged in liquid nitrogen for 10 s. For cross sections, frozen membranes were cracked. All samples were sputter-coated with 5 nm of Pt/Pd with a Q150T Plus (Quorum Technologies) before imaging with a JSM-6010LA electron microscope (JEOL).

The membrane pure water permeability (PWP) was determined by placing 3.0 cm² cut-outs of flat membranes (Fig. 1B) in dead-end filtration cells (Amicon) where water was used as a feed. The feed vessel was pressurized by nitrogen gas and the feed pressure was maintained at 1 bar. The permeate mass was measured as a function of time and calculated to provide the pure water permeability in $\text{bar}^{-1} \text{cm}^2 \text{min}^{-1}$ with $\text{bar}$ the pressure of the feed and $h$ the time in hours.

The retention was measured in a similar set-up, except water was replaced with a 1 g/L bovine serum albumin (BSA) solution or a solution of 1 g/L poly(ethylene glycol) (PEG) of 2, 6, 10, 20, and 35 kDa. PEG and BSA (66.5 kDa) concentrations in the feed and permeate were compared to determine the retention. The BSA concentrations were determined with a 2401PC UV–vis spectrophotometer (Shimadzu). The PEG concentrations were determined via gel permeation chromatography with a 1260 Infinity (Agilent) chromatographer using a 100 Å, 10 Polymer Standards Service Suprema 8x300 mm and 30 Â, 10 μm column and a 50 mg/L NaNa₄ eluent at 1 ml/minute.

2.4. Lysozyme concentration determination in solution

To determine the release of lysozyme from the membrane, 10 cm² of membrane with or without lysozyme was stored in 1 ml of mQ. At day 1, 2, 4, and 7 the supernatant was sampled and the mQ replaced. The supernatant samples were centrifuged for 30 min and evaluated for their absorbance at 281.5 nm, which is a characteristic absorbance peak for lysozyme. The absorbance of the supernatant for the membrane with lysozyme is compared to that of the membrane without lysozyme, as well as the theoretical absorbance that would be expected with lysozyme release based on the measured absorbance spectrum of lysozyme.

2.5. Lysozyme enzymatic activity

Lysozyme activity of the membrane with and without lysozyme was determined via a lysozyme activity protocol. For this protocol, a 0.15 mg/ml suspension of lyophilized *micrococcus lysodeikticus* is prepared in a 50 mM potassium phosphate buffer at pH 6.2. Membrane cut-outs of 1 x 1 cm (Supplementary Fig. S2) with and without lysozyme were incubated in the suspension and as dead-end measurements the suspension was evaluated for absorbance at 450 nm. Active lysozyme cleaves the micrococcus lysodeikticus in the suspension leading to a decrease in absorbance at 450 nm over time. In the absence of active lysozyme, the decrease in absorbance is much slower. The activity is expressed in units (U) calculated via:

$$\text{Units} = \frac{A(T) - A(B)}{0.001 \times S}$$  \hspace{1cm} (1)

where $A(T)$ and $A(B)$ are the differences in absorbance at 450 nm per minute of the substrate suspension of the membrane with lysozyme and the membrane without lysozyme control respectively. The factor of 0.001 is part of the unit definition. The factor $S$ represents membrane surface area, which is $1 \text{cm}^2$ ($1 \text{cm} \times 1 \text{cm}$ membrane) unless otherwise specified. One piece of membrane was submersed in 2 ml of bacteria suspension.

3. Results & discussion

APS allows for membrane production to occur completely in an aqueous environment without requiring an organic solvent. This is not only an advantage because of sustainability, but also opens up a natural possibility to incorporate biomacromolecules in the membranes. These biomacromolecules would, with production methods like NIPS, be destroyed or denatured and rendered inactive. Incorporating biomacromolecules like enzymes can functionalize the polymer membranes.

We incorporate the antibacterial enzyme lysozyme by first mixing it with the anionic polyelectrolyte PSS before mixing with the highly alkaline PAH solution. At this high pH, PAH is uncharged. This viscous alkaline PAH/PSS/lysozyme) solution is cast and coagulated in an acidic precipitation bath resulting in a PEC membrane, as schematically illustrated in Fig. 1A. We first evaluate whether the membrane properties such as morphology (via SEM), water permeability, and retention change by the addition of lysozyme. Then we investigate whether lysozyme leaches out of the membrane. Finally, we demonstrate that the membranes have biocatalytic properties.

3.1. Membrane characteristics

The PAH/PSS membranes with and without lysozyme appear as slightly elastic white sheets that can be cut in the desired shape (Fig. 1B) regardless of the absence (left) or presence (right) of lysozyme. They can be comfortably handled with tweezers or hands without breaking. There was no noticeable difference in handling the two membranes. The morphology of the membrane structure was further evaluated with SEM. The surface of both membranes without (Fig. 1C) and with (Fig. 1E) lysozyme appear homogeneous without clear pores, where we stress that smaller pores (<50 nm) would not be visible at these magnifications. Cross-sections of the membranes (Fig. 1D and F) show a typical asymmetric membrane structure for phase separation processes consisting of a dense selective layer supported by a porous structure. The thickness of the selective layer of membranes was 42 ± 10 μm (n = 5) without lysozyme or 39 ± 5 μm (n = 5) with lysozyme. Additional SEM images at different magnifications are available in Supplementary Fig. S1.

Investigation with SEM found no observable difference in membrane structure. Both membranes showed structures consisting of a dense selective layer and an underlying porous structure (Fig. 1D and F) consistent with previously reported PEC membranes produced via APS [13–18]. These structures are also consistent with what is otherwise expected from NIPS(-like) production processes [31]. There was no significant difference in the thickness of the selective layer or overall morphology of the membrane structure comparing membranes with and without lysozyme.

Enzyme loading has often been associated with a decrease in membrane function due to disruption of the membrane structure [21,22,32]. To study the effect of lysozyme incorporation in the membranes on the functioning of the membranes, the PWP and retention properties of membranes with and without lysozyme were compared. PWP was tested by measuring water permeation over time under controlled pressure. Retention and molecular weight cut-off were determined by comparing PEG (2 to 35 kDa) or BSA (66 kDa) concentrations of the permeate and the feed.
In Fig. 2, we show the PWP and BSA retention properties of PAH/PSS membranes with and without lysozyme. The PWP of membranes was $11 \pm 2 \text{L m}^{-2} \text{bar}^{-1} \text{h}^{-1}$ without lysozyme and $12 \pm 2 \text{L m}^{-2} \text{bar}^{-1} \text{h}^{-1}$ with lysozyme. The BSA retention of membranes without lysozyme was $95 \pm 1\%$ while the BSA retention of membranes with lysozyme was $93 \pm 1\%$. The retention of the PEG particles up to 35 kDa was negligible (data not shown). The differences between the membranes for both PWP and BSA retention are not statistically significant.

The PWP values fall within the expected range of high permeabilities for nanofiltration membranes to low permeabilities for ultrafiltration membranes, while the observed protein retention would fit with tight ultrafiltration membranes. Permeabilities of PAH/PSS PEC membranes have been reported to vary strongly depending on production parameters. By varying polyelectrolyte solution concentration, polyelectrolyte molecular weight, and salinity of the precipitation bath, the PWP of PAH/PSS membranes could be varied from $2400$ to $2 \text{L m}^{-2} \text{bar}^{-1} \text{h}^{-1}$ [13]. Other reported APS systems use alternative polyelectrolyte pairs and/or phase inversions, making direct comparisons difficult. Noticeable differences are found in the casting solution total polyelectrolyte concentration, casting thickness, casting substrate, and precipitation bath composition (pH, ionic strength, ion species, crosslinker concentration) [13–19]. It is likely that the exact production parameters have a large influence on the properties of the membranes, though the exact details are not yet fully understood. Interestingly, the thickness of the selective layer of our PAH/PSS membranes is approximately an order of magnitude larger than that of a previous reported PAH/PSS membrane, though with a very similar PWP and BSA retention [13].

Our APS-produced PAH/PSS membranes containing lysozyme are easy to produce and do not show a decrease in permeability or BSA retention when compared to membranes without lysozyme at the reported loading density (Fig. 2). In contrast, other membranes functionalized with enzymes have previously reported a decrease in permeabilities of up to 90% [32–36]. The incorporation of lysozyme-containing nanotubes has been reported to lead to a doubling of the permeability but with decreased retention [37]. A decrease in retention properties as a result of enzyme loading
has been more commonly observed for other biocatalytic membranes [21].

3.2. Lysozyme stability in PAH/PSS membranes

Biocatalytic membranes rely on the continued presence of enzymes for their catalytic functionality. Enzymes that are physically or covalently bound to membranes may leach from the membrane over time resulting in a loss of biocatalytic activity or can become inactive. To test whether lysozyme leaches out of our PAH/PSS membranes, we stored membranes in ultrapure water and evaluated the absorbance of the storage water at 281.5 nm, a characteristic absorbance wavelength of lysozyme. We compare this absorbance with the storage water of lysozyme-free membranes after 1, 2, 4, and 7 days using UV–vis absorbance spectrophotometry. From this comparison we determined whether lysozyme remained in the membrane.

For both membranes with and without lysozyme we observed an increased absorbance at 281.5 nm in the aqueous storage medium (Fig. 3). While the average absorbance of the storage water of the membrane containing lysozyme was consistently lower than the absorbance of the storage water without lysozyme, there was no statistically significant difference (t-test, p < 0.05) between the two conditions.

The observed presence of absorbance at 281.5 nm can be explained by the presence of soluble PAH/PSS complexes. Due to the processing of the PAH/PSS membranes in 1 cm² segments, trace amounts of PAH/PSS PECs remain on the membrane surface. The absorbance detected is consistent with the absorbance spectrum for PAH/PSS PECs while the characteristic absorbance spectrum for lysozyme is not detected (Supplementary Fig. S5). When lysozyme is incorporated in PAH/PSS PECs, the amount of lysozyme released in the aqueous phase was determined. Less than 1% of lysozyme was released in the aqueous phase (Supplementary Fig. S4). Only at salt concentrations above 0.5 M NaCl we observed a significant lysozyme release. Less than 1% of lysozyme was released in the aqueous phase (Supplementary Fig. S4). Only at salt concentrations above 0.5 M NaCl we observed a significant lysozyme release. Less than 1% of lysozyme was released in the aqueous phase (Supplementary Fig. S4).

3.3. Lysozyme activity in PAH/PSS membranes

Lysozyme is an antibacterial enzyme that operates by degrading the cell walls of gram-positive bacteria. To study whether lysozyme remains active when incorporated into the PAH/PSS membranes, the enzymatic activity of lysozyme-containing membranes was investigated on a substrate of lyophilized gram-positive micrococcus lysodeikticus bacteria. We evaluated the enzymatic activity of the lysozyme-containing membranes on the day of membrane fabrication, as well as after one week of storage in water to determine the enzyme stability over time.

The enzymatic activity of PAH/PSS membranes with and without lysozyme is shown in Fig. 4. The activity of lysozyme is characterized by a decrease in solution turbidity at 450 nm caused by the degradation of the bacterial substrate by lysozyme (Fig. 4A and B). Lysozyme-containing membranes had a lysozyme load of 4.49 ± 0.41 μg/cm² (n = 5). Using formula 1, the activity of lysozyme-containing membranes was calculated (Fig. 4C). For the membranes, the activity was 2.47 ± 0.49 U/cm² on the day of membrane preparation and 1.23 ± 0.47 U/cm² after one week in storage. PAH/PSS membranes without lysozyme did not display any enzymatic activity. The fresh lysozyme in solution had an activity of 2.22 ± 0.05 U/μg. After one week, the activity of the lysozyme in solution had decreased to 1.67 ± 0.04 U/μg.

The activity of membranes without lysozyme is similar to the activity of the substrate without added enzyme (Fig. 4A), indicating that the PAH/PSS membranes do not have an inherent catalytic activity on the bacterial substrate that can be mistaken for lysozyme's enzymatic activity. Membranes containing lysozyme show a decrease in absorbance at 450 nm, indicating the presence of active lysozyme. The activity of lysozyme-containing membranes per mass of lysozyme (0.55 U/μg) is lower than that of lysozyme in solution (2.22 U/μg).

The decrease in enzymatic activity of lysozyme-loaded membranes compared to lysozyme in solution is likely the result of diffusion limitations of the bacterial substrate posed by the membrane matrix (Supplementary Fig. S5) [38]. Lysozyme molecules located deeper in the membrane structure might not react with the relatively large bacterial substrate that is unable to penetrate the membrane structure. The activity of lysozyme is approximately halved by the high and low pH conditions similar to those of the membrane production process when incubated at low pH, high pH, or both in sequence (Supplementary Fig. S6).

For membranes with lysozyme immobilized on the surface, a large drop in activity has been previously reported [39]. Here, membranes were functionalized with 2.5 mg/cm² of lysozyme.
resulting in an activity of 1.5 to 4.8 U/cm². In contrast, our PAH/PSS PEC membranes were loaded with 4.49 mg/cm² of lysozyme resulting in an activity of 2.5 U/cm² [39]. This comparison demonstrates that our method for the incorporation of lysozyme can be done with 500x less lysozyme, while still resulting in a comparable enzymatic activity.

The activity of lysozyme-containing membranes decreases by 50.2% over the course of one week, compared to an activity decrease of 24.8% for lysozyme in solution. A decrease in activity can be caused by inactivation of the enzyme over time as both the membrane and the lysozyme solution were stored in ultrapure water. Alternatively, the activity of lysozyme has been reported to decrease when complexed with PSS in solution [40]. We also observed a decrease in the activity of lysozyme when evaluated in the presence of PAH or PSS in solution (Supplementary Fig. S7).

Decreased enzymatic activity has not been observed for all enzyme-polyelectrolyte systems, suggesting that for future APS systems the polyelectrolyte pairs can be chosen specifically so that they conserve or even enhance the activity of the functional enzymes [41].

Enzymes have also been incorporated in polyelectrolyte multilayer (PEM) membranes. With PEMs, oppositely charged polyelectrolytes are deposited on a support membrane to create alternating layers. Instead of exclusively using polyelectrolytes, biomolecules such as enzymes or nucleic acids can be assembled into the PEM structure [42]. In several studies, enzymes were incorporated in a PEM and enzymatic activity was still observed [43,44]. A study incorporating the protein-digesting enzyme trypsin in a PEM nanofiltration membrane reported that the relative activity of trypsin in solution was equal to that of trypsin embedded in two types of PEMs [44]. The incorporation of enzymes in PEMs can result in enhanced enzyme stability; while a decrease in enzymatic activity similar to that of our membranes was observed, this decrease took 10–12 days as opposed to our observed 7 days [44]. However, the increase in enzyme stability comes at the cost of enzymes leaching from the PEMs over time, which was not observed in our system [45]. Loading PEMs with biomolecules is often done in the context of drug delivery where loss of the loaded compound over time is a desired property of the system [42]. PEM fabrication is often time-consuming and additional chemical processing steps are often needed to guarantee successful enzyme loading.

The immobilization of lysozyme (or other enzymes) on various films and membranes via traditional methods can also take many additional processing steps and require different chemical treatments and take up to multiple days [21,38,39,46–51]. In one study where lysozyme was added directly to the membrane casting solu-
tion (more similar to membranes produced via APS), the lysozyme required (covalent) grafting to a protective agent via additional processing steps [37]. In contrast, incorporating lysozyme in PEC membranes produced via APS is as simple as diluting commercially available solutions of the anionic polyelectrolyte with a lysozyme solution (in ultrapure water) in place of diluting with ultrapure water. This process takes no additional time (excluding preparation of the lysozyme solution) and no additional chemical steps (Fig. 1A). The ease of enzyme loading is a substantial benefit of APS over other biocatalytic membrane production techniques.

4. Conclusion & outlook

APS is a new membrane production method first reported in 2019. Since the first publications, various different polyelectrolyte pairings have been reported that can result in stable APS membranes [13–20]. In this study we demonstrated the first biocatalytic membrane produced via APS.

We found that the enzyme lysozyme can be incorporated into PAH/PSS membranes produced via APS by a single addition step during production and requires no additional post-production modification process. The APS-produced PAH/PSS membranes functionalized with lysozyme show enzymatic activity consistent with that of lysozyme in solution. The enzymatic activity remains for at least one week. In addition, the membrane characteristics such as the morphology, pure water permeability, and BSA retention are unaffected by the addition of lysozyme at the tested lysozyme loading concentration. We envision that biocatalytic membranes produced via APS, based on the proof of concept reported here, can compete with biocatalytic membranes produced via traditional methods.

There are many enzymes which have been explored in membrane technology with the potential to provide interesting functionality. Biocatalytic membranes produced via traditional methods like NIPS have been modified with enzymes such as oxidoreductases, hydrolases, laccases, and horseradish peroxidases are currently immobilized on membrane surfaces in order to counteract membrane fouling or degrade various micro pollutants [23,52–55]. Applications of biocatalytic membranes are also suggested in biomedical engineering. Hybrid transplanted tissue survival containing polymer membranes is often hampered by the slow growth of blood vessels which may be remediated by incorporating proteins such as vascular endothelial growth factor in the membrane structure [56,57,58]. In addition, biocatalytic membranes have also been suggested for use in dialysis [59,60]. The advent of laboratory-driven gain-of-function evolution may even open up the production of enzymes that do not occur naturally for customized biocatalytic activity [61–63].

CRediT authorship contribution statement

Jéré J. van Lente: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing – original draft, Visualization. M. Irshad Baig: Methodology, Writing – review & editing. Wiebe M. de Vos: Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. Saskia Lindhoud: Conceptualization, Resources, Data curation, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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