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ORIGINAL PAPER

Investigation of membrane bioreactor for in situ product removal based on silicone rubber membrane module

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Abstract
In many biotransformation productions performed in ordinary batch or fed-batch bioreactors, product inhibition of a production strain strongly decreases the yield and effectivity of the process. A way to overcome this effect is to apply extractive biotransformation, which means to continually remove the product from the fermentation broth. Nowadays, application of a membrane bioreactor with an immersed capillary membrane module is used as a promising solution for this case. In this work, we propose the membrane bioreactor for extractive bioproduction of chemical specialties consisting of a 3 L mixed tank bioreactor with an immersed extractive tubular membrane module. As the membrane material, silicone rubber tubes were chosen as it shows many advantages compared to other materials. As the model solute for the extraction, 2-phenylethanol (rose aroma) was chosen due to its strong inhibition effect on the production strain (*Saccharomyces cerevisiae*). The solute partition coefficient in the extraction system containing solute, water and silicone rubber was measured as well as the solute diffusion coefficient for the silicone rubber membrane. Three different membrane modules made of silicone rubber tubes were manufactured and tested in series of extraction experiments performed in the membrane bioreactor at different operation conditions including different biomass concentration, stirring rate, and aeration rate. Experimental data were compared with the prediction of mathematical model programmed in MATLAB with good accuracy.

Keywords Extractive biotransformation · Immersed module · Membrane extraction · Silicone rubber · 2-Phenylethanol

Introduction

A general bottleneck in biotechnological processes is the typically low product concentration due to the product inhibition of the production strain and it is common that several by-products are also produced. Due to these two factors, does downstream processing, isolation and purification, have an important impact on the economics of the production system. One way to overcome the product inhibition is to apply extractive biotransformation, which means to continually remove the product from the fermentation broth (Akacha and Gargouri 2015). Nowadays, application of membrane bioreactors with immersed capillary membrane module is a promising solution in this case. In this work, a membrane bioreactor for extractive biotransformation consisting of a 3 L mixed tank bioreactor with an immersed silicone rubber membrane module is proposed. As the model solute, 2-phenylethanol (PEA), a well-known rose aroma, was chosen due to its strong inhibition effect that allows producing about 4 g L^{-1} of PEA in an ordinary fed-batch bioreactor (Stark et al. 2003). There are several possibilities of PEA removal from the bioreactor (Hua and Xu 2011): two-phase extraction (Etschmann and Schrader 2006); adsorption (Mei et al. 2009); pervaporation (Etschmann et al. 2005), immobilized solvent extraction (Serp et al. 2003) or pertraction (Červeňanský et al. 2017). Membrane extraction is commonly used in many applications where product removal from the fermentation medium is required (Kubišová et al. 2002; Mihaľ et al. 2011, 2014); it was found to have many advantages compared to conventional extraction methods, e.g., absence of emulsions formation, density difference between liquids is not required, high interphase area, easy scale-up, etc. The mathematical description of this process is also well established (Gawronski and Wrzesinska 2000; Mihaľ et al. 2011).

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Application of silicone rubber (vinyl-methyl-polysiloxane) as a membrane material has several advantages: it is non-porous, hydrophobic and non-permeable to water, polar compounds and macromolecules but permeable to organic compounds (Doig et al. 1998); it is a cheap, flexible, chemically and thermally resistant material available in various thicknesses in sheet or tube form; it is possible to change its extractive properties by swelling with organic solvents (Doig et al. 1999). Several papers dealing with the application of a silicone rubber membrane for phenol removal from aqueous solutions can be found in literature (Xiao et al. 2009, 2013; Ren et al. 2017; Jin et al. 2018). In their paper, Brookes and Livingston (1995) investigated the extraction of organic pollutants (phenol, chlorobenzene, nitrobenzene) from dilute aqueous solution to another dilute aqueous solution through tubular silicone rubber membranes and determined the overall mass transfer coefficient of membrane transport. In the paper Doig et al. (1998), a silicone rubber membrane bioreactor was applied for baker's yeast-mediated reduction of geraniol to citronellol using hexadecane as an extractant and was compared with two-phase bioproduction. In his next paper, Doig et al. 1999 deal with testing the mass transfer characteristics of various organic solutes using the swollen form of silicone rubber in an aqueous organic extraction system for a wide spectrum of organic solvents. In our case, the silicone rubber membrane module has a promising potential for extractive bioproduction of PEA where the fermentation medium and extractant are separated with non-porous membrane and thus no pressure differences between media are necessary and no leakage or bulk flow of extractant to bioreactor occurs as was possible using porous polypropylene hollow fiber membrane modules applied in our previous works (Mihaľ et al. 2013, 2014).

In this work, the PEA partition coefficient in an extraction system containing PEA, water and silicone rubber membrane and the PEA diffusion coefficient for silicone rubber membrane were experimentally estimated. For better comprehension of the separation process, mathematical model of the membrane bioreactor was programmed in MATLAB and used to predict and verify the extraction capabilities of the silicone rubber membrane module. In the next step, three different membrane modules made of silicone rubber tubes: basic membrane module, membrane module swollen with dodecane and unswollen scaled-up membrane module, were manufactured and tested in a series of extraction experiments in the membrane bioreactor under different operation conditions, such as biomass concentration, stirring rate, and aeration rate. Experimental data were compared with the mathematical model prediction.

Experimental

Analytical methods

The PEA concentration in samples was determined by an HPLC Infinity 1260 (Agilent Technologies, Santa Clara, USA) equipped with a SphereClone™ 5 µm ODS(2) 80 A, LC Column 150 × 4.6 mm (Phenomenex, Torrance, USA). A multi-wavelength detector was used at the fixed wavelength of 254 nm. The mobile phase was pumped through the column at 1 mL min^{-1} and consisted of methanol and water in the ratio of 80:20. The samples containing biomass were centrifuged for 10 min at 4000 rpm and filtered before the HPLC analysis.

Experimental equipment

Membrane bioreactor used in this work consisted of three parts: a mechanically stirred tank reactor, immersed capillary membrane module and glass vessel. The cover and bottom of the bioreactor was made of stainless steel, and the shell was made of glass. The working volume of the bioreactor was around 3 L, and its outer dimensions were 30 × 21 cm of height × width, respectively. The bioreactor was equipped with 6.7 cm six blades Rushton turbine stirrer and the regulation of the stirrer speed was in the range 200–1000 rpm. At the bottom of the bioreactor below the stirrer, a circular stainless steel sparger was installed and connected to the output of compressed air, the flow rate of which was measured by a rotameter with an integrated regulation valve. Liquid in the bioreactor was heated by the double bottom of the bioreactor connected to a thermostat. The bioreactor was equipped with a thermometer and pH and oxygen probe installed from the top of the bioreactor. At the bottom of the bioreactor, membrane modules manufactured in our laboratory were installed and tested.

Membrane modules were made of the silicone rubber tube DRINKTEC SILIKON 002x003 purchased from TOMIRTECH, s.r.o (Liptovský Mikuláš, Slovakia). The tube was of the VMQ type of silicone rubber made of vinyl-methyl-polysiloxane with an outer diameter of 3.1 mm and wall thickness of 0.5 mm. The first membrane module (M1) consisted of four separate 4 m-long tubes coiled around the baffle structure of the bioreactor. The ends of the tubes were connected to polypropylene tubes that led to the distributor and the collector placed above the cover of the bioreactor. Flexible tubes connected to the inlet and outlet of the membrane module were led to a glass vessel with the water phase stirred with an electromagnetic stirrer and pumped through the membrane module using a membrane pump (Liquiport, KNF Flodos,

Sursee, Switzerland) installed at the inlet of the membrane module.

The second tested membrane module (M1S) was made of the same tubes as M1, but treated by swelling in dodecane carried out by immersing separate silicone tubes into distilled water and pumping dodecane through the tubes for 12 h. Then, dodecane was drained out of the tubes and traces of dodecane were washed out with water. The swollen silicone tubes were then coiled and fixed around the baffle structure of the bioreactor, the tube terminals were connected to the distributor and the collector, and the membrane module was placed inside the bioreactor and flooded with distilled water.

Scale-up membrane module (M2) had three times higher contact area than membrane module M1. It consisted of four 12 m-long silicone rubber tubes (the same outer/inner diameter of tubes as M1) coiled around the baffle structure of the bioreactor and connected to the distributor and the collector in a similar way as M1. The characteristics of the tested membrane modules are summarized in Table 1. The detailed scheme of the experimental equipment can be found in our previous paper (Mihaľ et al. 2014).

Equilibrium experiments

In equilibrium experiments, distribution of PEA between the aqueous phase and silicone rubber or silicone rubber swollen with dodecane was investigated. At the beginning of the experiments, 1 mass% solution of PEA with distilled water was prepared. Then, about 0.2 g of silicone rubber tube (cut to small pieces, $3 \times 10 \times 0.5$ mm), water and PEA solution combined up to 1.3 mL of total volume were placed in eight 1.5 mL vials to reach the starting PEA concentration in water phase in the interval from 0.75 to 3.75 g L^{-1}. The vials were mixed in a vortex mixer for 48 h at room temperature (22 °C) to achieve equilibrium. About 400 µL of the water phase was then sampled and used for the analysis. The concentration in the silicone rubber was calculated from the material balance of the vial content. Swelling of silicone rubber was carried out by immersing the cut pieces of the silicone tube into dodecane for 12 h and their drying with filtration paper. Partition coefficients (k) were expressed as the ratio of PEA concentration in silicone rubber to that in aqueous phase.

Diffusion coefficient measurements

To create a functional membrane bioreactor with integrated membrane module and its accurate mathematical model, the diffusion coefficient of the transported solute in the material of the silicone rubber tube has to be known. For this purpose, the decrease of PEA concentration in a small volume of liquid caused by PEA absorption to the silicone rubber tube had to be measured. For the measurement, it was desirable to minimize the liquid volume and to maximize the mass of the silicone rubber tube immersed in the liquid. Intensive mixing of the liquid allowed to consider the liquid as ideally mixed and to neglect the resistance of PEA transport in the liquid film at the outer side of the silicone rubber tube.

For the experiment, a 62 cm-long silicone rubber tube was twisted to a spiral using copper wire filling the tube. The dimensions of the spiral were: diameter of about 1.5 cm and length of 6 cm. The ends of the tube were connected together in the inner sector of the spiral using a plastic connector. The spiral was placed in a graduated cylinder about 2 cm above its bottom. At the beginning of the experiment, about 35 mL of the PEA solution (PEA concentration of about 4.2 g L^{-1}) was poured inside the cylinder and the measurement started. The level of the liquid in the cylinder was about 2 cm over the spiral and the liquid was continuously stirred at 800 rpm. The measurement lasted for 180 min. The samples (400 µL) were taken every 10 or 20 min using a micropipette and placed in sealed vials. The PEA diffusion coefficient for the silicone rubber tube swollen with dodecane was measured in a similar way using a 46.7 cm-long normal silicone rubber tube swollen to the length of 62 cm. Swelling of the silicone tube was carried out by immersion in dodecane (with sealed terminals) for 12 h and drying with filtration paper. To get the PEA diffusion coefficient in the silicone rubber tube, the measured data were fitted with the calculated course of the

Table 1 Characteristics of the immersed capillary membrane module

Type of membrane module	M1	M1S	M2
Fiber material	Silicone rubber VMQ	Silicone rubber VMQ	Silicone rubber VMQ
Dimensions of contactor (mm)	150 × 100	150 × 100	150 × 100
Overall contact area[a] (m^2)	0.156	0.274	0.468
Number of fibers	4	4	4
Effective fiber length (m)	4	5.32	12
Outer/inner fiber diameter (mm)	3.1/2.1	4.1/2.8	3.1/2.1
Fiber wall thickness (mm)	0.5	0.65	0.5
Swollen with dodecane	Unswollen	Swollen	Unswollen

[a]Based on outer diameter

PEA absorption to the tube applying a routine programmed in MATLAB comprising the application of Fick's second law and the least square method.

Extraction experiments

The experimental apparatus was used to measure the extraction kinetics of PEA from its water solution in the bioreactor to the water phase placed in the reservoir. The extraction experiments E1–E6 were performed at different conditions in the bioreactor comprising aeration of the bioreactor or the presence of baker's yeasts in the PEA solution. At the beginning of the experiment, the reservoir was filled with about 0.3 L of distilled water mixed at 300 rpm by an electromagnetic stirrer. The water phase was then pumped from the reservoir to the membrane module and back to the reservoir. After the membrane module was deaerated, the flow rate of the liquid circulated through the module was set to the required value. Then, about 2.4 L of the prepared PEA solution (PEA concentration of around 3.5 g L^{-1}) with or without biomass (biomass concentration of 20 g L^{-1}) was poured to the bioreactor and the extraction started. If necessary, aeration of the PEA solution in the bioreactor was turned on and maintained at 800 L h^{-1}. For all extraction experiments, the liquid in the bioreactor was stirred at 500 rpm and tempered at 25 °C. Extraction experiments E1–E3 were performed using the M1 membrane module. Extraction experiments E4–E6 were applied using the swollen M1S membrane module. Experiment E4 was performed 3 days after the silicone rubber tubes were swollen with dodecane. Experiment E5 used module M1S after being stored for 10 months in distilled water to test its time stability. In experiment E6, the M1S module was used after it was kept immersed for 15 h in aerated distilled water to test the swollen membrane stability in contact with air bubbles. The extraction took 31 h for experiments E1–E4 and 6 h for experiments E5 and E6 to reach an equilibrium between the bioreactor and the reservoir. Samples (400 µL) were taken from the bioreactor and the reservoir.

Another set of extraction experiments was performed using the scaled-up membrane module M2. The experiments were started in a similar way to experiments E1–E6, but their duration was 8 h. They were performed at different biomass concentrations, stirrer speeds and air flow rates. Extraction experiment F0 was the basic experiment with 0 g L^{-1} biomass concentration, 300 rpm stirrer speed and 500 L h^{-1} air flow rate. In consequent experiments, F1A and F1B, the biomass concentration was changed to 20 and 40 g L^{-1}, respectively. In experiments F2A and F2B, the stirrer speed was changed to 200 and 500 rpm, respectively. In experiments F3A and F3B, the air flow rate was changed to 300 and 800 L h^{-1}, respectively. Other parameters of these experiments were kept at the settings of experiment F0. The characteristics of all performed extraction experiments as the type of the applied membrane module, biomass concentration (c_X), stirrer speed, air flow rate ($\dot{V}_{AIR}$), starting PEA mass in the bioreactor (m_{PEA}), circulation flow rate through the membrane module ($\dot{V}_{RES}$) and liquid volumes in the bioreactor (V_{STR}) and the reservoir (V_{RES}) are summarized in Table 2.

After the extraction experiments, the water phase was drained from the membrane module and from the bioreactor. Then, the vessels were three times flushed with distilled water and left flooded with water for 24 h to remove traces of biomass and to extract PEA trapped in the silicone rubber of the membrane module back to the water phase. After this step, the bioreactor and the membrane module were drained and then dried for 24 h with air. For the swollen membrane

Table 2 Characteristics of extraction experiments using membrane module M1, M1S and M2

Experiment	Membrane module	c_X (g L^{-1})	Stirrer speed (rpm)	$\dot{V}_{AIR}$ (L h^{-1})	m_{PEA} (g)	$\dot{V}_{RES}$ (L min^{-1})	V_{STR} (L)	V_{RES} (L)
E1	M1	0	500	0	8.06	0.29	2.304	0.294
E2	M1	20	500	0	8.02	0.29	2.367	0.294
E3	M1	20	500	800	8.02	0.29	2.355	0.294
E4	M1S	0	500	0	8.42	0.39	2.455	0.316
E5	M1S	0	500	0	8.51	0.39	2.46	0.316
E6	M1S	0	500	0	8.47	0.39	2.46	0.316
F0	M2	0	300	500	8.58	0.23	2.46	0.351
F1A	M2	20	300	500	8.55	0.23	2.46	0.352
F1B	M2	40	300	500	8.5	0.23	2.46	0.353
F2A	M2	0	200	500	8.58	0.228	2.46	0.354
F2B	M2	0	500	500	8.53	0.229	2.46	0.354
F3A	M2	0	300	300	8.54	0.227	2.46	0.353
F3B	M2	0	300	800	8.64	0.226	2.46	0.353

module, drying with air was not applied and the membrane module was left totally flooded from outside and inside with distilled water.

Membrane extraction of PEA in the membrane bioreactor was simulated using a mathematical model programmed in MATLAB. Detailed information on the equations and derivation of the mathematical model for an immersed capillary membrane contactor used in a similar membrane bioreactor has been published in our previous paper (Mihaľ et al. 2013). The differences were caused by neglecting the PEA mass transfer resistance at the outer side of the membrane module and using different equations for the prediction of the particular mass transfer coefficient for the silicone rubber membrane taken from the paper by Doig et al. (1999). The measured PEA partition coefficients and PEA diffusion coefficients presented in this paper were also used in the mathematical model.

Results and discussions

Equilibrium experiments

The measured PEA partition coefficients (k) (PEA concentration in silicone rubber divided by the PEA concentration in water phase in equilibrium state) between silicone rubber and water are shown in Fig. 1.

For unswollen silicone rubber, the value of the PEA partition coefficient strongly depends on the PEA concentration in the water phase. At higher PEA concentrations, the k value stabilizes at the value of 0.75; at lower PEA concentrations, k strongly increases and is positive for PEA transport at low PEA concentrations in the bioreactor (higher k value induces higher PEA transport through the membrane). The same trend is also visible for silicone rubber tube swollen with dodecane, but the stabilized k value is about 0.6 at higher PEA concentrations and its increase at lower PEA concentrations is not as sharp as for normal silicone rubber. This observation is in good agreement with the PEA partition coefficient for dodecane of around 0.5 (Doig et al. 1999; Molinari et al. 1999; Červeňanský et al. 2017) which decreases the k value of the swollen silicone rubber compared to unswollen silicone rubber. The measured values of partition coefficient for unswollen and swollen silicone rubber are slightly higher compared to the value 0.5 measured by Doig et al. (1999) for PEA and dimethyl-siloxane type of silicone rubber with the same value measured also for silicone rubber swollen with hexadecane. For the mathematical model of the membrane bioreactor, the measured dependence of PEA partition coefficient on PEA concentration in the water phase was fitted using Eq. 1 for normal silicone rubber and Eq. 2 for swollen silicone rubber.

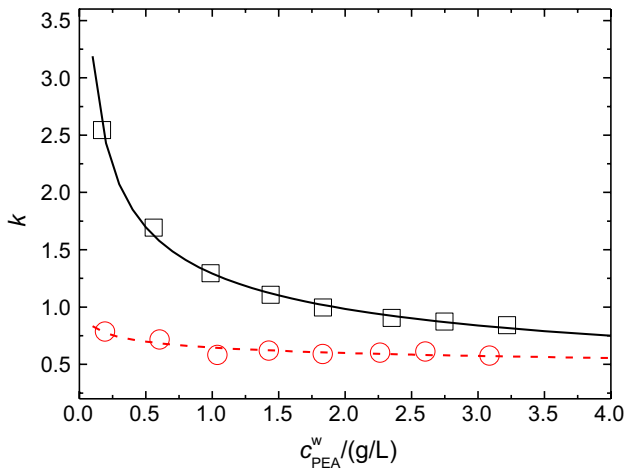

Fig. 1 Dependence of PEA partition coefficient in equilibrium system PEA–water–silicone rubber on PEA concentration in water phase. Equilibrium data (points) vs. calculated data (lines). Partition coefficient for unswollen silicone rubber (squares); partition coefficient for silicone rubber swollen with dodecane (circles)

$$k = 1.292 c^{w}_{\mathrm{PEA}}{}^{-0.392}, \tag{1}$$

$$k = 0.647 c^{w}_{\mathrm{PEA}}{}^{-0.11}. \tag{2}$$

Diffusion coefficient measurements

In literature, some correlations of diffusion coefficient of solutes in silicone rubber can be found (Lapack et al. 1994; Brookes and Livingston 1995), but their accuracy is questionable and a better approach is to experimentally determine its value. Fitting the value of the diffusion coefficient and comparing the calculated and measured data for PEA concentration decrease induced by PEA absorption to a silicone rubber tube are shown in Fig. 2. The PEA diffusion coefficient of 7.26×10^{-11} m^2 s^{-1} was obtained for normal silicone rubber and 2.97×10^{-10} m^2 s^{-1} for silicone rubber swollen with dodecane. Thus, swelling of silicone rubber with dodecane increases the PEA diffusion coefficient fourfold. During the swelling, the density of silicone rubber decreases from 1.15 to 0.98 g cm^{-3} and all its dimensions increase 1.33-fold. Molecules of dodecane fill the space between the vinyl-methyl-polysiloxane chains and decrease the density and viscosity of the material.

For comparison, according to the Wilke–Chang equation (Reid et al. 1987), the PEA diffusion coefficient in water is 9.59×10^{-10} m^2 s^{-1} and in dodecane it is 1.21×10^{-9} m^2 s^{-1}. So, the PEA diffusion coefficient measured in silicone rubber swollen with dodecane is threefold lower than that in water and fourfold lower than that in dodecane.

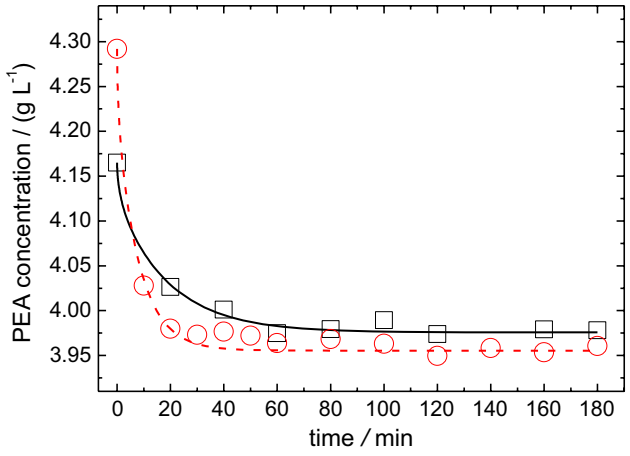

Fig. 2 PEA diffusion coefficient measurements for PEA diffusion into the silicone rubber. Dependence of PEA concentration decrease on time in PEA solution placed in the graduated cylinder during the experiment. Measured data (points) vs. calculated data (lines). Measurement for normal silicone rubber (squares, solid line); measurement for silicone rubber swollen with dodecane (circles, dashed line)

Extraction experiments

To verify the prediction of the mathematical model with experimental data and to observe the influence of biomass, aeration and modification of the membrane (by swelling it with dodecane) on the extraction process, a set of four extraction experiments (E1–E4) was carried out under different conditions. The extraction kinetics of PEA for experiments E1 and E2 are shown in Fig. 3 and for experiment E3 in Fig. 4. Empty points show the experimental data measured by the analysis of samples taken from the bioreactor and the reservoir. Lines represent the prediction of the mathematical model.

The first extraction experiment (E1) was performed with PEA solution without biomass and without aeration in the bioreactor (Fig. 3a). It can be observed that the equilibrium between the PEA solution in the bioreactor and the water phase in the reservoir was reached in almost 20 h. The accuracy of the simulated data considering the predicted ones was very good. Experiment E2 (Fig. 3b) was performed in the presence of biomass with a concentration of 20 g L^{-1} in the PEA solution and without aeration in the bioreactor. The course of the extraction kinetics is practically the same as for experiment E1, but there is a visible small deviation between the mathematical model prediction and the experiment probably caused by the presence of biomass, as the applied mathematical model does not involve it. However, the observed deviation can be considered as negligible

Figure 4 shows the measured extraction kinetics of extraction experiment E3 performed in the presence of aeration and biomass in the bioreactor, which simulated the conditions in the bioreactor during real PEA production. As it can be seen, the difference between the mathematical model and the experiment is quite visible but not critical. During the experiment, it was observed that aeration induced air bubbles that were notably sticking to the tubes of the membrane module, especially between the module and the glass wall of the bioreactor. The bubbles were dynamically vibrating, moving along the tubes, sticking and unsticking. So, some notable part of the membrane module was constantly blocked by these bubbles and thus was not available for PEA transport. According to the simulation of these three experiments, the overall PEA mass transfer coefficient reached the value of around 9.19×10^{-8} m s^{-1}.

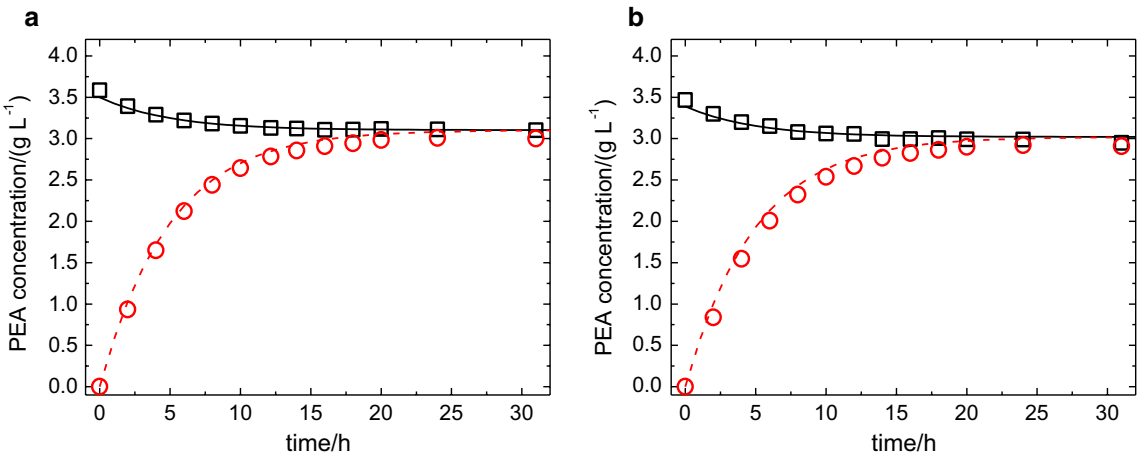

Fig. 3 Extraction kinetics of PEA from water solution in the bioreactor to the water phase in the reservoir performed in membrane bioreactor with M1 membrane module. Comparison of experimental data (points) with mathematical model prediction (lines) for extraction experiment E1 performed without aeration and biomass in the bioreactor (**a**); and for extraction experiment E2 performed without aeration and with biomass in the bioreactor (**b**). Concentration in bioreactor (square), concentration in reservoir (circle)

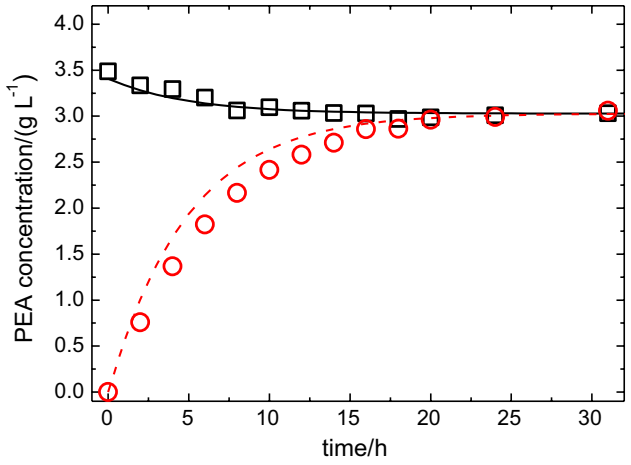

Fig. 4 Extraction kinetics of PEA from water solution in the bioreactor to the water phase in the reservoir performed in membrane bioreactor with M1 membrane module. Comparison of experimental data (points) with mathematical model prediction (lines) for extraction experiment E3 performed with aeration and biomass in the bioreactor. Concentration in bioreactor (square), concentration in reservoir (circle)

Swelling of the silicone tubes with dodecane led to an increase of about 1.33-fold in all dimensions of the tubes. It means that the outer diameter of the tube expanded from 3.1 to 4.1 mm, inner diameter expanded from 2.1 to 2.8 mm, tube thickness expanded from 0.5 mm to 0.65 mm and the length of the tubes expanded from 4 to 5.32 m. Accordingly, the increase of all tube dimensions increased also the surface area of the tubes but not by 1.33-fold, but by 1.33^2-fold, which is 1.769-fold. So, the overall contact area available for PEA transport was increased from 0.156 to 0.276 m^2. This fact, considering also the fourfold increase of the PEA diffusion coefficient, accelerated the PEA transport and allowed reaching the equilibrium of PEA concentration in the bioreactor and the reservoir in only 5 h in the extraction experiment E4 (Fig. 5a) compared to 20 h in the previous extraction experiments (E1–E3), despite the increased thickness of the tubes and decreased PEA partition coefficient, which is unfavorable for the PEA transport through the silicone membrane. According to the mathematical model prediction for experiment E4, the overall PEA mass transfer coefficient reached the value of around 1.94×10^{-7} m s^{-1}, which is a twofold higher value than that calculated for the unswollen membrane module M1.

Figure 5b shows the comparison of extraction kinetics of experiments E5 and E6 with the mathematical model prediction. Experiments E5 and E6 took 6 h in comparison to experiment E4 (31 h) and were better focused on reaching the equilibrium PEA concentration between the bioreactor and the reservoir. Both experiments were performed using swollen silicone rubber membrane module M1S stored for 10 months flooded in distilled water (experiment E5) and consequently by its 15 h storage in aerated distillate water (experiment E6) to test the time stability and stripping stability of dodecane in swollen silicone rubber tubes. When comparing the extraction kinetics of experiment E5 (squares) with the mathematical prediction (lines), small differences due to the inaccuracy of the mathematical model can be observed but the equilibrium is reached in 5 h equally as in experiment E4 (Fig. 5a). Therefore, it can be stated that silicone rubber tube swollen with dodecane is stable when stored flooded in distilled water and its storage has no impact

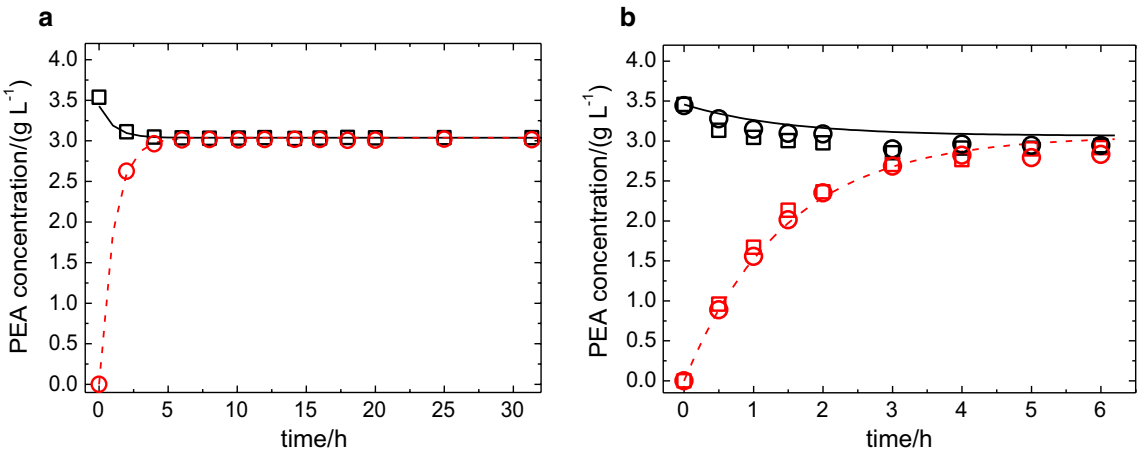

Fig. 5 Extraction kinetics of PEA from water solution in the bioreactor to the water phase in the reservoir performed in membrane bioreactor with M1S membrane module. Comparison of experimental data (points) with mathematical model prediction (lines) for extraction experiment E4 performed without aeration and biomass in the bioreactor (**a**); and for extraction experiment E5 and E6 performed without aeration and biomass in the bioreactor and after 10 months of storage in distilled water (E5, squares) or after consequential 15 h of storage in bubbled distilled water (E6, circles) (**b**). Concentration in bioreactor (black square, circle), concentration in reservoir (red square, circle)

on the extraction capability of the membrane module. When the swollen tube was left on air at laboratory temperature, dodecane evaporated out of the tube in 3 days and the silicone rubber tube shrunk to its original dimensions before swelling. During the aeration of the membrane bioreactor, a part of the bubbles were stuck to the surface of the membrane module and continually became loose and regenerated, so dodecane could be partially stripped off by air. The fact that dodecane was removed from the swollen tubes of the membrane module by air bubbles was confirmed by the dodecane smell observed at the air output of the bioreactor. Due to the negligible dodecane solubility in distilled water (3.7×10^{-3} mg L^{-1} at 25 °C), the majority of dodecane was probably stripped off directly from the surface of the silicone rubber tubes rather than from distilled water. Figure 5b shows the extraction kinetics of experiment E6 (circles), where the membrane module M2 was used in the bioreactor flooded with aerated distilled water for 15 h. From the comparison with experiment E5, it can be observed that the equilibrium was still not reached after 6 h due to slower extraction, which means that aeration has negative impact on the extraction capability of PEA for the membrane module swollen with dodecane. With the increasing time of aeration, the impact of aeration would be more observable, and due to the contraction of the tubes, the membrane module would be threatened by rupture or throttle of tubes.

Membrane module M2 contains three times more silicone rubber tubes than membrane module M1, so its tubes are much closer to each other and a part of its surface is in mutual contact and thus probably limited for mass transfer. PEA mass transfer through the walls of the tubes of membrane module M2 can be then reduced compared to M1, especially if a higher concentration of biomass is present in the fermentation medium clogging small gaps between the tubes. The potential difference in mass transfer can be observed when comparing the extraction kinetics with mathematical model prediction. Figure 6 displays the impact of different biomass concentrations in the membrane bioreactor on PEA extraction kinetics with scale-up membrane module (M2). The extraction experiment F0 was performed with biomass concentration of 0 g L^{-1}, and experiments F1A and F1B were carried out at the biomass concentration of 20 and 40 g L^{-1}, respectively. Prediction of the mathematical model was created using the settings of the basic experiment F0. No significant impact of dense body of membrane module (tubes for M2S were wound around the baffle in denser configuration compared to M1) or biomass can be observed in Fig. 6 and the difference between the mathematical model prediction and experimental data is acceptable. Despite the active area of the scaled-up membrane module M2 being 1.7-fold higher and the thickness of the tubes being 1.33-fold lower than for the swollen membrane module M2S, the extraction capability of the M2 membrane module was lower due to the decreased PEA diffusion coefficient in silicone rubber.

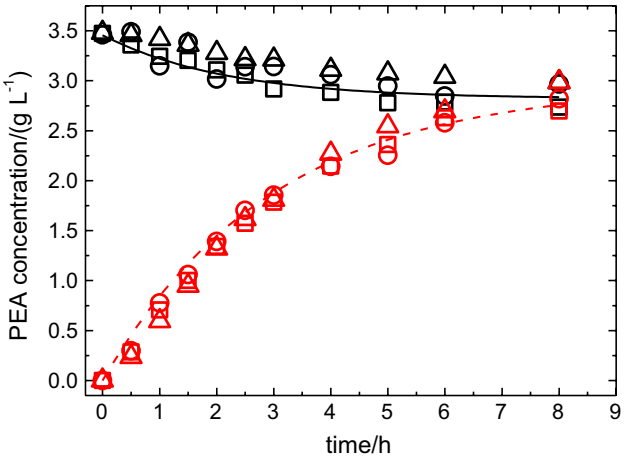

Fig. 6 Extraction kinetics of PEA from water solution in the bioreactor to the water phase in the reservoir performed in membrane bioreactor with M2 membrane module. Comparison of experimental data (points) with mathematical model prediction (lines) for extraction experiments F0, F1A and F1B performed at a stirrer speed of 300 rpm, air flow rate of 500 L h^{-1} and different biomass concentrations: 0 g L^{-1} (F0, squares), 20 g L^{-1} (F1A, circles) and 40 g L^{-1} (F1B, triangles). Concentration in bioreactor (black square, circle, triangle), concentration in reservoir (red square, circle, triangle)

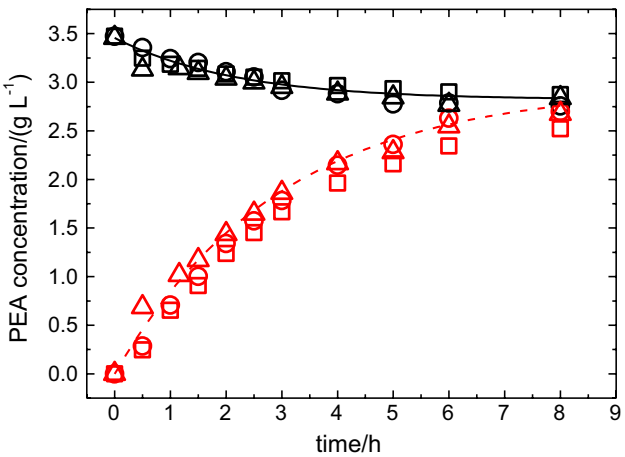

Fig. 7 Extraction kinetics of PEA from water solution in the bioreactor to the water phase in the reservoir performed in membrane bioreactor with M2 membrane module at different stirrer speeds. Comparison of experimental data (points) with mathematical model prediction (lines) for extraction experiments F0, F2A and F2B performed at biomass concentration of 0 g L^{-1}, air flow rate of 500 L h^{-1} and different stirrer speeds: 200 rpm (F2A, squares), 300 rpm (F0, circles) and 500 rpm (F2B, triangles). Concentration in bioreactor (black square, circle, triangle), concentration in reservoir (red square, circle, triangle)

Determination of the influence of stirrer speed in the membrane bioreactor was another problem (Fig. 7); the

extraction kinetics of experiments F2A, F0 and F2B with stirrer speed of 200, 300 and 500 rpm, respectively, were compared. The mathematical model assumed the whole membrane bioreactor interior as ideally mixed and the outer tube resistance as negligible. To keep the terms, the interior of the bioreactor should be mixed at high frequency which produces high sheer stress in the biomass, high energy consumption and generation of surplus heat that has to be removed from the bioreactor. On the contrary, low stirring frequency has negative impact on the mass transfer through the laminar film on the outer side of the tubes and the transport capability of the membrane module is then not fully utilized. Thus, the optimal stirring rate has to be found.

From the comparison of extraction kinetics shown in Fig. 7, a small deviation is noticeable for experiment F2A performed at the stirrer speed of 200 rpm compared to the mathematical model prediction and extraction kinetics of experiments F0 and F2B carried out at 300 and 500 rpm, respectively. For experiment F2A, the bioreactor and reservoir PEA concentrations are quite far from reaching the equilibrium concentration in 8 h, which indicates that a stirrer speed of 200 rpm is not suitable for sufficient bioreactor mixing and a higher mixing rate should be used to secure PEA mass transfer, especially when a real fermentation medium is used.

The last set of extraction experiments was focused on the impact of air flow rate in the membrane bioreactor on the PEA transport when the scaled-up membrane module was used. During the bioreactor aeration, the surface of the membrane module is covered with air bubbles, which decreases the active area of silicone rubber tubes for mass transfer. The higher the aeration rate, the greater is the tube surface area covered. In Fig. 8, the comparison of extraction kinetics for experiments F3A, F0 and F3B performed at the air flow rate of 300, 500 and 800 L h^{-1}, respectively, is shown together with the comparison to the mathematical model prediction for experiment F0. The trend of extraction kinetics for experiment F3A and F0 is quite similar to the simulated prediction. But evident slowdown of the extraction kinetics is visible for experiment F3B at the aeration rate of 800 L h^{-1}, where at the end of the experiment, at time 8 h, the equilibrium was not reached. This fact can be caused by reduction of the surface area of the membrane module available for the PEA transport by the bubbles of air stuck to its surface. For higher aeration rate than that used in experiment F3B, higher slowdown of the extraction kinetics can be expected. This must be considered during real extractive fermentation, as it may result in insufficient removal of the produced compound which is a problem, especially in case of product inhibition.

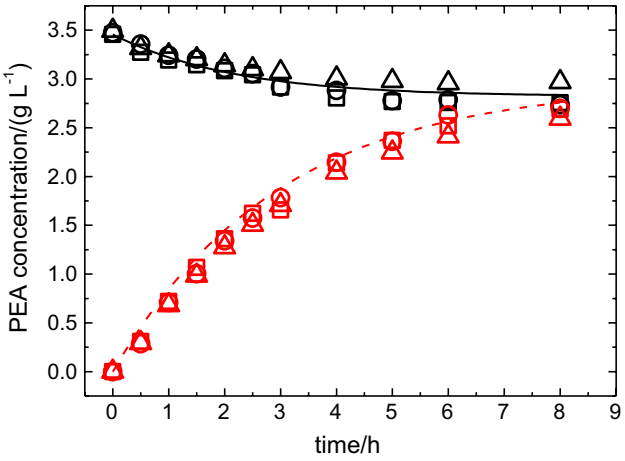

Fig. 8 Extraction kinetics of PEA from water solution in the bioreactor to the water phase in the reservoir performed in the membrane bioreactor with M2 membrane module at different air flow rates. Comparison of experimental data (points) with mathematical model prediction (lines) for extraction experiments F0, F3A and F3B performed at biomass concentration of 0 g L^{-1}, stirrer speed of 300 rpm and different air flow rates: 300 L h^{-1} (F3A, squares), 500 L h^{-1} (F0, circles) and 800 L h^{-1} (F3B, triangles). Concentration in bioreactor (black square, circle, triangle), concentration in reservoir (red square, circle, triangle)

Conclusions

The presented silicone rubber membrane module has been proven to be well applicable for potential in situ water–water extraction of PEA from the fermentation medium during extractive biotransformation. The presence of biomass, aeration and stirring in the bioreactor have only minor impact on PEA transport through the membrane in the investigated range of operation parameters in case of low stirring rate and high air flow rate. Swelling of silicone rubber tubes of the membrane module with dodecane decreases the PEA partition coefficient, but increases the PEA diffusion coefficient for silicone rubber, thus increasing the PEA mass transport through the membrane. Swelling also extends the dimensions of silicone rubber tubes 1.33-fold, which enlarges the active area of the membrane module by 1.77-fold. Experiments show that swollen membrane module is stable during its long-term storage flooded in distilled water but unstable in aerated water, which strips off dodecane from the tubes and thus changes its extraction properties and geometry. The mathematical model of the membrane bioreactor with integrated silicone membrane module was verified on a set of extraction experiments for three types of membrane modules: the unswollen silicone rubber membrane module (M1), silicone rubber membrane module swollen with dodecane (M1S) and for scaled-up unswollen membrane module (M2). Due to many benefits, great potential application of the membrane bioreactor with integrated silicone rubber

membrane module is in water–water extractive bioproduction of PEA interconnected with PEA adsorption to adsorption column. As silicone rubber is a non-porous hydrophobic material permeable to PEA, but non-permeable to biomass, water and other polar compounds, its application is beneficial in case PEA adsorption from water phase with minimum inpurities is required by an adsorption process. For further study, it is necessary to test the proposed membrane bioreactor on a real microbial PEA production (using *Saccharomyces cerevisiae*) linked with continual PEA extraction and possible PEA adsorption. The created mathematical model of the membrane bioreactor will be employed for correct setup of the extractive biotransformation experiment.

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