Enhancing polyhydroxyalkanoate productivity with cell-retention membrane bioreactors

Burniol-Figols, Anna; Pinelo, Manuel; Skiadas, Ioannis V.; Gavala, Hariklia N.

Published in:
Biochemical Engineering Journal

Link to article, DOI:
10.1016/j.bej.2020.107687

Publication date:
2020

Document Version
Peer reviewed version

Enhancing polyhydroxyalkanoate productivity with cell-retention membrane bioreactors

Anna Burniol-Figols, Manuel Pinelo, Ioannis V. Skiadas and Hariklia N. Gavala*

Technical University of Denmark (DTU), Dept. of Chemical and Biochemical Engineering, Søltofts Plads, Building 228A, 2800 Kgs. Lyngby (Denmark)

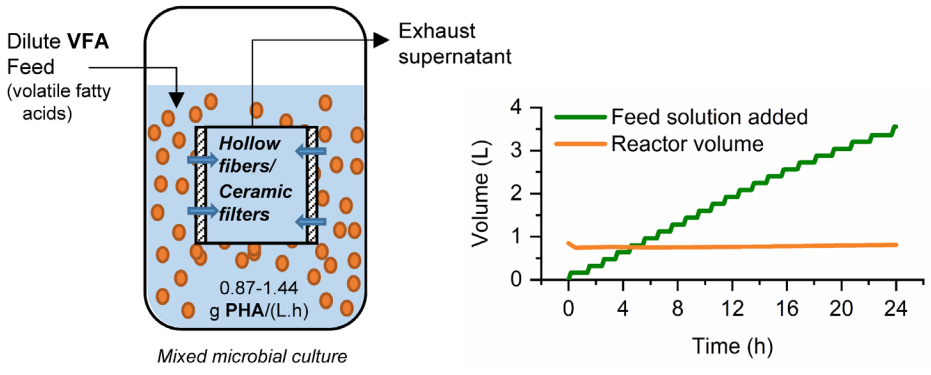
*Corresponding author: hnga@kt.dtu.dk; hari_gavala@yahoo.com

Accepted manuscript. Biochemical Engineering Journal. https://doi.org/10.1016/j.bej.2020.107687

Abstract

One of the factors limiting the economic viability of polyhydroxyalkanoates (PHA) is the low volumetric productivity obtained with second-generation feedstocks, resulting from their low carbon concentration. In the present study, the use of membrane bioreactors (MBRs) was evaluated as a strategy to retain the microbial cells in the reactor and to enable a repeated supply of substrate without increasing the reactor volume. Two immersed MBR systems were studied: classical pressure-driven MBRs (hollow fibers and ceramic filters), and a novel diffusion-based MBR. In the latter, the rate of volatile fatty acid (VFA) diffusion across the membranes was lower than the VFA consumption rate of the culture, and thus, not suitable to attain high productivities. Possible research directions to increase substrate diffusion are suggested. On the other hand, pressure-driven configurations led to high values of productivity (0.87-1.44 g PHA/(L.h)) during a fed-batch PHA accumulation using mixed microbial consortia. No flux reduction was observed in a 24 h fed-batch process, which allowed for a reduction of up to 82 % of the reactor volume, demonstrating the potential of this strategy. Hollow fibers and ceramic filters offered similar results during the fed-batch, but they presented different limitations and advantages.

Graphical abstract



1 Introduction

The interest in biodegradable and biobased plastics has increased intensely during the last decades, due to their potential to mitigate some of the environmental impacts caused by petroleum-derived plastics. Among them, polyhydroxyalkanoates (PHA) have received significant attention given their high biodegradability, wide range of applications, and possible raw materials [1]. Yet, PHA represent only a minor percentage of the bioplastics market, which is to a big extend, due to their high production costs [1].

Microbial cultivation techniques allowing for high values of PHA productivity (frequently in the range of 2-3 g PHA/(L.h)) were already reported during the 90s [2,3]. These strategies implied the use of axenic cultures, highly purified and concentrated feedstocks, and pure O_2 supply. All of which are factors that translate into high operational costs. Over the years, the focus has shifted to organic waste streams which, besides reducing costs, would bring improvements in terms of sustainability [1,2,4]. For similar reasons, the use of mixed microbial consortia (MMC) is emerging, owing to their low operational demands [1,5].

Values of volumetric PHA productivity obtained with second-generation feedstocks have rarely surpassed values of 1 g PHA/(L.h), neither in pure cultures or MMC [2,4,5]. Hence, there is a need for developing efficient cultivation techniques to produce PHA from these substrates. One of the main challenges is the low carbon concentration in such feedstocks, which causes volume increase during fed-batch cultivations [1,3,4]. A way to circumvent this issue is the use of membrane bioreactors (MBRs) with cell-retention systems, which enable removal and addition of feed while keeping the cells in the bioreactor.

MBRs have been extensively applied in wastewater treatment [6] and increasingly in other biotechnological applications [7], but little research has been performed on the frame of PHA production. A few studies have explored the use of external loop MBRs in PHA production bioreactors with pure cultures [8–11]. In such systems, the fermentation broth is recirculated through an external cross-flow filtration module, where part of the supernatant is removed (Figure 1). High PHA productivities were

reported, up to 4.6 g PHA/(L.h) in Ahn et al.[8]. However, external loop MBRs rely on high cross-flow velocities to control membrane fouling, which result in high operational costs [6,12]. Moreover, cell recirculation can lead to shear stress or oxygen limitation, resulting in cell death or reduced cell growth [6,7,13].

Operational costs are generally lower in immersed MBR configurations (iMBR), in which the filtration modules are submerged in the cell broth and aeration can provide fouling control [7,12] (Figure 1). To the extent of our knowledge, iMBRs have only been tested for PHA production in the study of Kumar et al. [14], resulting in a two-fold increase in the cell concentration. Yet, the productivity of the system was low (< 0.1 g PHA/(L.h)) due to the low PHA yield of the strain.

Both immersed and external loop MBRs are based on the application of a pressure differential as the driving force to achieve liquid permeation across a membrane (commonly by applying suction on the permeate side) [6,15] (Figure 1). More recently, an alternative MBR strategy has been suggested, using concentration gradients as the driving force [6,15] (Figure 1). In such systems, the microorganisms are separated from the feed medium by means of a membrane, which allows diffusion of the substrate to the cell compartment due to the difference of concentration. Experience on these systems is very limited, and so far only focused on anaerobic processes [15]. Membrane fouling - the main challenge of MBRs - can still occur, but it is less likely, as the magnitude of the force bringing the cells and other foulants to the surface of the membrane is smaller.

In the present study, immersed pressure-driven and diffusion-based MBR configurations were tested for the first time in the frame of PHA production using MMC. The main objective was to provide a proof of concept of their utilisation to overcome the volume limitations imposed by diluted feed solutions. Despite they are both immersed systems, the abbreviation iMBR is kept for conventional immersed pressure-driven MBRs, while dMBR is used to refer to diffusion-based MBR configurations.

2 Materials and methods

2.1 PHA-producing culture

PHA producing culture was enriched and maintained in a Sequential Batch Reactor (SBR) of 1.7 L working volume, run under aerobic feast-famine regime as described in [16]. Briefly, the reactor followed a cyclic operation, where every 12 h, half of the volume of the reactor (850 mL) was replaced with fresh medium containing crude glycerol, butyrate, propionate, and acetate, to a substrate concentration of 45 C_{mM} at the beginning of each cycle. The effluent at the end the cycle was used as inoculum for the PHA accumulation without any prior sedimentation. The culture was dominated by the genera *Thauera* and *Ammaricoccus* [16].

2.2 Feed solution

The feed for the PHA accumulation was a synthetic mixture of VFA, simulating the concentration of VFA obtained experimentally after optimisation of crude glycerol fermentation in continuous mode (operated at pH 6.4, HRT of 10 h and 50 g/L of glycerol) [17]. The fermentation effluent contained butyrate (6.4 g/L), propionate (1.8 g/L), acetate (1.1 g/L) and lactate (0.75 g/L). Soluble ammoniacal nitrogen ($N\text{-}NH_3$) and phosphate (PO_4^{3-}) were 10.8 and 501 mg/L, respectively, corresponding to a nitrogen/carbon ratio (N/C) of 1.8 N_{mmol}/C_{mol}, and a phosphorous/carbon ratio (P/C) of 11.9 P_{mmol}/C_{mol}.

For the experiments described in section 2.3 (effect of N/C ratio) and due to volume limitations (see below), the VFA concentration was 10 times higher than in the model effluent. The proportion of each VFA and the P/C ratio was the same as in the model effluent. Different N/C ratios were tested (explained in section 2.3). For the experiments described in section 2.4 and 2.7, the VFA concentration and the P/C ratio was the same as in the model effluent and the N/C ratio was adjusted to 15 N_{mmol}/C_{mol}.

For all experiments, the VFA solution was prepared in BA Medium [17] and adjusted to pH 6.4 with NaOH. N/C and P/C ratios were adjusted with NH_4Cl and K_2HPO_4. Antifoam 204 (Sigma Aldrich) and allylthiourea were added to a final concentration of 85 µL/L and 5 mg/L.

2.3 Fed-batch PHA accumulation: effect of the nitrogen/carbon ratio

PHA accumulation experiments were performed in a Multifors reactor (1.4 L) (Infors HT, Switzerland) with a starting volume of 650 mL of inoculum. The culture was fed with a VFA medium (section 2.2), in which the VFA concentration was 10 times higher than in the model effluent to circumvent the volume limitation of the reactors. Four experiments were performed at different N/C ratios (N_{mmol}/C_{mol}): 1.8 (as in the model effluent), 15, 30 and 50. The experiments with N/C ratios of 1.8 and 15 ran simultaneously in parallel reactors using inoculum from the same SBR cycle. The experiments at N/C ratios of 30 and 50 also ran simultaneously on another SBR cycle.

Experiments were performed in fed-batch mode for 24 h. Medium was initially added to a VFA concentration of 90 C_{mM} (14 mL of medium). Thereafter, the same feeding volume was repeated whenever butyrate was exhausted, manifested by an increase of more than 8 % in the dissolved oxygen saturation (pO_2) with respect to the pO_2 immediately after substrate addition. Aeration, agitation, temperature and pH were kept at 1 L/min, 750 rpm, 30 °C and pH 8, respectively.

2.4 Diffusion-based MBR configuration (dMBR)

VFA diffusion was tested in two membrane materials: PSf (polysulfone) and PVDF (poly(vinylidene fluoride)), both with a poly(propylene) support layer. The PSf membrane had a pore size of 0.2 µm (GRM0.2PP, Alfa Laval, Sweden – 270 µm thickness). Two pore sizes (0.15 and 0.45 µm) were tested for the PVDF membrane (FSM0.15PP and FSM0.45PP, Alfa Laval, Sweden - 285 µm thickness). Before use, the membranes were cleaned with distilled water (dH_2O) and dilute NaOH (pH 10) at 45 °C according to the manufacturer instructions.

The membranes were placed in an aluminium support inside the bioreactor, separating the bioreactor in two concentric compartments (Figure 2). The internal compartment contained the microbial culture. Aeration (1 L/min), agitation (750 rpm) and pH control (pH 8) were performed in this compartment. The feed was supplied in the external compartment, which was cell free. The membranes were placed with the active membrane surface in contact with the external compartment. The total active surface of the membrane was 312 cm^2.

To test VFA diffusion across the membranes, the external compartment was filled with 160 mL of medium at pH 6.4 (section 2.2). The internal compartment was loaded with 650 mL of autoclaved inoculum (to avoid VFA consumption). The VFA diffusion rate (in C_{mmols}/h) was calculated from the slope of the VFA amount detected in the inner compartment vs. time during the first 30 min, and thus corresponded to the initial and maximum VFA diffusion rate.

2.5 Immersed pressure-driven MBR configurations (iMBR)

2.5.1 Hollow fibers

Hollow fiber modules were purchased from Zena Membranes (Czech Republic). They consisted of poly(propylene) fibers with an average pore size of 0.1 µm and an external and internal diameter of 310 and 240 µm, respectively. Two types of membrane configurations were tested: a rod configuration (Figure 3 A) and a loop configuration (Figure 3 B). The rod consisted of 800 fibers of 10 cm length, and the loop was made of 400 fibers of 20 cm. Both configurations had a filtration surface of 779 cm^2 and were connected to a poly(vinyl chloride) (PVC) pipe placed in a 12 mm port in the bioreactor. In order to minimise the internal volume of the PVC pipe, a tube of 2 mm diameter was placed inside, directly connecting the outlet of the pipe to base of the pipe. For the loop configuration, a plastic net was fixed between the agitation shaft and the filter module to avoid damage by the impellers (Figure 3 B).

Before the first use, the fibers were soaked in 2-propanol for 30 min and rinsed with dH_2O. The fibers were always kept in dH_2O to prevent drying. In between experiments, the fibers were cleaned with NaOH

0.1 wt%, citric acid 2 wt% and dH_2O. For each reagent, the cleaning consisted of three steps: soaking, filtering and backflushing (30 min each). dH_2O permeability was measured before each experiment.

2.5.2 Ceramic filters

The ceramic filtration module was built with 8 ceramic cylinders (silicone carbide) of 10 mm external diameter, 6 mm internal diameter and 12 cm length (LiqTech International, Denmark). The active filtration layer was on the external part of the cylinders (0.5 µm pore size) (Figure 4). The filters were fit in a stainless steel support secured with threaded rods and sealed with silicone. The upper part of the support connected the internal volume of all filters to a single steel outlet tube fixed to one of the ports of the bioreactor. The active surface area of the module was 276 cm^2. No pre-treatment was applied before the first use. In between experiments, the filters were cleaned by soaking, filtering and backflushing for 30 min with NaOH 2 wt% (30 °C), followed by 30 min backflushing air at 1.5 bar. The cleaning procedure was then repeated with dH_2O. dH_2O permeability was measured before each experiment.

2.6 Evaluation of fluxes in iMBR configurations

Filtrations were carried out using a peristaltic pump (Masterflex L/S 7518-00), in which the relation between rpm and flow (mL/min) was calibrated with dH_2O at room temperature without being coupled to any filtration device. Critical and limiting fluxes were assessed by the flux stepping method [18]. The setpoint of the pump was increased step-wise from 8-25 mL/min, recording the permeate flow and the transmembrane pressure (TMP) (measured between the peristaltic pump and the filter with a gauge pressure manometer (GM520, Professional Instruments – measuring range ± 350 mbar) as indicated in Figures 3 and 4). For each setpoint, the filtration proceeded until 160 mL of permeate had been removed (target volume exchange in fed-batch as explained in section 3.2.2). Thereafter, the permeate was returned to the bioreactor through the filter in reverse flow. The reverse flow setpoint was the same as the filtration flow for setpoints higher than 16 mL/min. For lower filtration flows, the reverse flow was constant at 16 mL/min.

This cycle of filtration and backflushing was repeated 5 times for each setpoint. Flow-TMP graphs were constructed using the TMP read at the end of the 5th filtration of each setpoint flow.

During these tests, aeration, agitation, temperature and pH were maintained as during cultivation (section 2.3). The experiments were performed with dH_2O and cell cultures at different concentrations (1.7 and 16 g/L), corresponding to the ones observed at time 0 h and 20 h during the fed-batch with an N/C ratio of 15 N_{mmol}/C_{mol} (section 3.1).

2.7 Fed-batch PHA accumulation in iMBRs

PHA accumulation was performed similarly to the description in section 2.3. The key difference was that when butyrate depletion was detected, 160 mL of cell-free liquid were removed though the filtration modules (hollow fibers loop or ceramic filters). Thereafter, 160 mL of fresh feed (at the concentration of the model effluent, section 2.2) were added, keeping the volume constant at 750 mL throughout the 24 h fed-batch (except for sampling and acid/base addition). Feed addition was done through the filter in reverse flow to reduce membrane fouling. The batch started with 850 mL of inoculum from the SBR and thus the first filtration removed slightly more permeate (260 mL) to reach the 750 mL after the first feeding. The initial concentration of VFA after the first feeding was equivalent to the one provided in experiments in section 2.3 (90 C_{mM}).

Based on the results presented in section 3.2.2, the filtration flow was set at 16 mL/min for the hollow fiber module (loop configuration). For the ceramic module, the filtration flow was set at 16 mL/min for the first filtration and at 13 mL/min for the subsequent ones. Feed addition occurred at 16 mL/min in reverse flow. In order to avoid accumulation of feed in the inner space of the filters after feed addition, about 7 mL of permeate were returned to the reactor in reverse flow.

2.8 Analytical methods

Samples taken from the bioreactors were centrifuged at 8000 g for 5 min. The supernatant was filtered through 0.45 µm Nylon filters and analysed for VFA and N-NH_3 concentration as described in [19]. The pellet was washed with distilled water and centrifuged again twice before freeze-drying. The PHA content of the biomass pellet was determined by GC chromatography as described in [19], using methyl-3-hydroxybutyrate (99 %, J&K Scientific®) and methyl-3-hydroxyvalerate (≥ 98 %, Sigma-Aldrich®) as calibration standards [20]. Total biomass concentration was estimated by measurement of the total suspended solids (TSS) in fresh samples, performed according to standard protocols [21] using glass fiber filters of 0.7 µm pore size (Merk Millipore). PHA concentration (g/L) was obtained by multiplying the TSS (g/L) by the PHA content (wt%). Active biomass concentration (cells excluding PHA or X in g/L) were then estimated by subtracting the calculated PHA (g/L) from the TSS (g/L).

Water contact angle (θ°) for the membranes used in the diffusion set-up was measured by the sessile drop method [22] in a goniometer (OCA20, Dataphysics Instruments, Germany). For each membrane, the reported values correspond to the average θ° measured in five random locations, recorded immediately after dropping 1 µL of MiliQ® water at room temperature onto the membrane. Membrane surface charge was assessed by the zeta potential, measured in a SurPASS electrokinetic analyzer (Anton Parr, Austria) as described in [23].

3 Results

In order to maximise productivity in a MBR configuration, two aspects have to be met. The first is that the culture is able to sustain PHA accumulation and reach high cell densities. This aspect is evaluated in section 3.1. The second aspect is that the MBR configuration is able to operate for the whole PHA accumulation period and meet the requirements of the culture. This is presented in section 3.2.

3.1 Effect of the nitrogen/carbon ratio on culture saturation

The main advantage of an MBR is the possibility of operating at a constant volume. Extended PHA accumulation batches (maximising the g PHA/g initial biomass) would be desirable to fully exploit this advantage and minimise the volume of the reactor producing inoculum (SBR). However, it is very common that PHA production ceases when cells reach maximum PHA accumulation capacity. This is a result of operation under severe nitrogen limiting conditions, which maximise PHA accumulation and prevent cell growth [1,24]. Recent studies have reported the possibility of extending the PHA accumulation and increase the PHA productivity by supplying limiting rather than starving nutrient conditions, which allow cell growth and PHA accumulation to occur simultaneously [24].

In the present study, four nitrogen to carbon (N/C) ratios were tested during fed-batch PHA accumulation. Feed addition occurred whenever butyrate was exhausted, as previous studies on the same culture evidenced that net PHA production drastically reduced during consumption of the remaining VFA (acetate and propionate) [16]. The culture produced a copolymer (poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)) referred simply as PHA in the text and figures.

The four N/C ratios tested (expressed in terms of N_{mmols}/C_{mol}) resulted in 4 different scenarios in terms of nitrogen availability (Figure 5 A). In the two lowest ratios (1.8 and 15) all the nitrogen provided was consumed and both cultures were nitrogen limited for the whole batch. However, the degree of limitation was more severe in the first case, which could be considered nitrogen starved. For the N/C ratio of 30, the culture had nitrogen in excess for the first 12 h, but thereafter became nitrogen limited. At an N/C ratio of 50, the culture had excess of nitrogen throughout the cultivation.

With the N/C ratio in the model effluent (1.8 N_{mmol}/C_{mol}), substrate consumption and PHA production considerably slowed down after 6 h (Figure 5 B and C). Cell growth was almost negligible (Figure 5 E), what probably led to a saturation of the cells once they had reached its maximum PHA content of about 70 % (Figure 5 G).

For the other three N/C ratios tested, the culture had a sustained substrate consumption throughout the 24 h (Figure 5 B). For all parameters evaluated, these three experiments behaved almost identically for the first 12 hours (Figure 5). At this point, about 8 g of PHA had been produced (Figure 5 C) at a yield of 0.7-0.8 C_{mol} PHA/C_{mol} S (Figure 5 H). The productivity observed for these three experiments during the first 12 h more than doubled the one observed for the N/C ratio of 1.8 (1.0 vs 0.43 g PHA/(L.h)) (Figure 5 D).

From 12 h to 24 h, the behaviour of the culture differed slightly according to the nitrogen loading. Higher nitrogen concentrations enabled a higher production of active biomass (Figure 5 E), which translated into a slightly higher substrate consumption (Figure 5 B). For the nitrogen limited case (N/C ratio of 15), this did not have negative effects on the PHA yield or PHA content. At the end of the batch, these parameters presented values of 0.72 C_{mol} PHA/C_{mol} S and 78 %, respectively, very close to those obtained in nitrogen starving conditions (Figure 5 H and G). On the other hand, for the N/C ratios of 30 and 50, the higher biomass yield resulted in a decrease in the PHA yield and PHA content, especially remarkable for the N/C ratio of 50 (nitrogen excess throughout the batch). The latter presented a PHA yield of 0.45 C_{mol} PHA/C_{mol} S and a decrease of the PHA content from 70 % at 12 h to 60 % at 24 h (Figure 5 H and G). This decrease in the PHA content indicated that the growth response had taken over the PHA accumulation of the culture.

The N/C ratio of 30 presented only slightly lower values than the N/C ratio of 15 for all parameters. The differences were usually not significant when considering the measurements' standard deviation, but could already indicate that the growth response was starting to take over at this nitrogen loading. Therefore, a N/C ratio of 15 was chosen for further experiments in MBR configurations.

In terms of productivity, the values calculated for the whole 24 h batch were considerably lower than obtained during the first 12 h (Figure 5 D). The reason behind that was an important decrease in the specific substrate consumption rate (q_s) with time (Figure 5 F). Hence, even though the productivity could be greatly enhanced by supplying extra nitrogen and allowing cell division to occur, PHA accumulation rates decreased for all the N/C ratios tested. For the N/C ratio of 15, the productivity decreased from 1.00

g PHA/(L.h) at 12 h to 0.78 g PHA/(L.h) after 24 h, being this the scenario with the least drop in productivity (Figure 5 D).

3.2 Evaluation of MBR configurations

3.2.1 Evaluation of the dMBR configuration

For this configuration, microfiltration membranes were used to create a cell-free compartment, from where clarified broth could be withdrawn without removing cells from the bioreactor (Figure 2). The same compartment could be used to supply fresh medium containing VFA, which could diffuse across the membrane to the cell-containing compartment to support PHA accumulation.

In order to maximise PHA productivity with this configuration, VFA diffusion across the membrane had to be at least equal to the substrate consumption rate of the culture (r_s). Therefore, VFA diffusion was measured in different membrane materials (PSf and PVDF) and compared to r_s (36.7 C_{mmols} VFA/h in the experiments in section 3.1). This minimum threshold of VFA diffusion was not reached for any of the membranes (Figure 6 A), and thus the configuration would not be able to sustain the PHA accumulation process.

For all experiments, the diffusion rate of each of the VFA (butyrate, propionate, acetate and lactate) was proportional to their concentration, suggesting no preferential membrane selectivity. At a similar pore size, the PSf membrane (0.2 µm) presented a much higher diffusion rate than the PVDF membrane (0.15 µm) (19 vs. 5 C_{mmols} VFA/h). With the first, the diffusion was comparable to the achieved with the PVDF membrane at 0.45 µm (18 C_{mmols} VFA/h). Hence, PSf resulted in a higher diffusion rate.

The hydrophilicity of the materials was measured by contact angle measurements (Figure 6 B), were values below 90° are generally accepted to correspond to hydrophilic surfaces, while hydrophobic for higher values [22]. According to this, PSf was hydrophilic, but PVDF was slightly hydrophobic, with values around 95° and no significant differences between pore sizes ($p = 0.1$).

290 All membranes presented a negative surface charge at the pH of the feed solution (pH 6.4) (Figure 6 C).
291 At this pH, the VFA also have a negative charge. Thus, charge repulsion could have hindered diffusion
292 across the membrane. For the PVDF membranes, the charge was more negative for the 0.15 μm pore
293 membrane compared to the 0.45 μm, which could be a possible explanation for the reduced VFA
294 diffusion obtained at the lower pore size.

295 Based on these results, the higher VFA diffusion rates obtained with PSf compared to PVDF at a similar
296 pore size could be related to the higher hydrophilicity of this membrane combined with the lower negative
297 charge density.

298 **3.2.2 Evaluation of fluxes iMBR configurations**

299 The main limitation of iMBRs is the occurrence of fouling and the consequent reduction of the membrane
300 performance. This phenomenon has led to the definition of two flux concepts: the critical flux and the
301 limiting flux [18]. The critical flux is the one at which irreversible membrane fouling starts to occur.
302 Assuming weak form behaviour, this can be defined as the point at which the relation between the
303 transmembrane pressure (TMP) and flux starts to deviate from linearity, or the point at which the TMP
304 increases at a constant flux [18]. The limiting flux is the highest flux that can be maintained in a given
305 system. Below this flux, fouling might be forming, but it is still possible to maintain the flux by
306 increasing the TMP. Above the limiting flux, the fouling saturates the filtration capacity of the membrane.
307 Here, the discussion is done in terms of flow (instead of flux) for the ease of comparison between setups
308 with different filtration areas.

309 The ultimate objective of the fed-batch cultivation using MBRs was to remove supernatant every time
310 that butyrate was exhausted and replace it with fresh VFA medium. In order to minimise the time that the
311 culture was in absence of butyrate, the filtration time interval had to be as short as possible, but without
312 surpassing the limiting flow, or in the ideal case, the critical flow. The target was to remove 160 mL of
313 supernatant (about 1/5 of the working volume) in 10 min (16 mL/min). By replacing this volume, the
314 initial concentration of substrate after each feeding would be about 90 C_{mM} of VFA, which was found not

315 inhibitory for the culture in previous studies [16]. The two submerged membrane types (hollow fibers and
316 ceramic filters) were tested at flows between 8-25 mL/min, to determine the critical and limiting fluxes.
317 Two cell concentrations were evaluated (1.7 and 16 g/L), corresponding to the cell concentration at the
318 beginning of the fed-batch and after 20 h, respectively (section 3.1).

319 ***Hollow fibers***

320 Two types of hollow fiber configurations were tested: a rod configuration and a loop configuration
321 (described in Figure 3). With the rod configuration, the target flow of 16 mL/min (12.3 L/(h.m²)) could
322 not be attained, even with the initial cell concentration of the fed-batch (1.7 g/L) (Figure 7 A). The pump
323 could not maintain flows over 10 mL/min, which was identified as the limiting flow of the system.

324 The loop configuration was much less prone to fouling. The peristaltic pump could always maintain the
325 setpoint flows at the tested range, even with a concentrated cell culture (16 g/L) (Figure 7 B). In other
326 words, the limiting flow was not reached. At the cell concentration of the beginning of the batch (1.7 g/L),
327 the system did not show significant increases in the TMP in consecutive filtrations at the same setpoint
328 flow (Figure 7 D). Moreover, the relation between TMP and flow was linear for the whole range (Figure 7
329 C). Hence, the results did not show signs of having reached a critical flow.

330 With the cell culture of 16 g/L, the critical flow could be identified at 19 mL/min (14.6 L/(h.m²)). At this
331 point, the TMP vs. flow curve started to deviate from linearity (Figure 7 C), and a slight increase in the
332 TMP in consecutive filtrations could be observed (Figure 7 D). Based on these results, operation of the
333 hollow fiber loop module at 16 mL/min (12.3 L/(h.m²)) was considered for the fed-batch PHA
334 accumulation experiments presented in section 3.3.

335 ***Ceramic filters***

336 At the initial cell concentration (1.7 g/L), the ceramic filtration module presented a very similar behaviour
337 to the hollow fiber loop. Neither a limiting flow nor a clear critical flow could be identified (Figure 8).
338 The pump could always maintain the setpoint flow (Figure 8 A) and there were no clear deviations from

linearity in the curve of TMP vs. flow (Figure 8 B). No significant increase in the TMP was observed in successive filtrations at the same setpoint (Figure 8 C).

At the high cell concentration (16 g/L) though, important fouling was observed. Already at 13 mL/min, the TMP increased considerably from the first to the fifth filtration (Figure 8 C), and the TMP increased exponentially in relation to the flow (Figure 8 C). The critical flow was identified at about 13 mL/min (28.2 L/(h.m²)), although lower flows should be tested to better identify the linear region of the curve of TMP vs. flow in Figure 8 B. At 13 mL/min irreversible fouling started to occur, but it was not until much higher flows (25 mL/min) where flow reduction was observed (Figure 8 A) (limiting flow).

Given that the system presented clear fouling at the target flow (16 mL/min), an additional anti-fouling strategy was considered, consisting of backflushing air through the filter. This is a common cleaning strategy [6], which can be applied in non-filtration periods for fed-batch processes [25]. The filters presented a very high resistance to air and very little air flow could be obtained at pressures lower than 1.5 bar. At this pressure, 250 mL/min of air could be transferred. However, when this strategy was tested with the cell culture, it led to intense foam formation, which could not be controlled even with addition of antifoam at concentrations above the recommended by the supplier (Antifoam 204 Sigma-Aldrich at 0.2%). Hence, air backflushing could not be applied as additional antifouling strategy.

In order to avoid fouling and eventual saturation of the filters, operation below the critical flux is preferred. For the present case, a sub-critical flux would represent a decrease in the productivity of the system, as it would extend substrate-limiting periods. Based on these results the fed-batch PHA accumulation with ceramic filters operated at 13 mL/min (28.2 L/(h.m²)), which was already at the critical flow, but far below the limiting flow. A flow of 16 mL/min was applied to the first filtration of the batch, as this was below the critical flow observed for the cell concentration at the beginning of the batch.

3.3 Fed-batch PHA accumulation in iMBRs

Hollow fibers (loop configuration) and ceramic filters were tested in a fed-batch PHA accumulation regime using synthetic VFA medium. The two systems presented very similar results (Figure 9).

The filters were used to remove permeate during the fed-batch (1/5 of the working volume) whenever butyrate was consumed. Fresh feed solution was then provided through the filter in reverse flow. During a 24 h experiment, this procedure occurred over 20 times, without observing any reduction on the filtration flow (Figure 9 A and D). Both iMBRs allowed to feed over 3.5 L of substrate through the reactor, while keeping the working volume at 0.75 L. As a result, they enabled an 82 % reduction on the working volume, compared to a scenario where these experiments would be performed without a cell-retention system. The concentration of PHA at this point was 18.5 g/L (Figure 9 B and E).

The use of submerged filtration during PHA accumulation resulted in high values of productivity (0.87-1.44 g PHA/(L.h)) (Figure 9 C and F). However, a decrease of PHA productivity was observed during the fed-batch, especially after 12 h, as already observed in the experiments without cell-retention systems presented in section 3.1. After 12 h, the productivity was 1.20 and 1.27 g PHA/(L.h), for hollow fibers and ceramic filters, respectively. At 24 h, the values decreased to 0.89 and 0.87 g PHA/(L.h), respectively. If stopped after 12 h, the bioreactors would present a total TSS concentration of about 20 g/L with a PHA content of 70 % PHA (14 g PHA/L) (Figure 9 B and D). At this point, the use of iMBRs would represent a 75 % reduction of the working volume with respect to a system without cell recycling (about 2 L of feed had been circulated).

Overall, despite the iMBRs were capable of sustainable operation for 24 h, such long cultivations would not appear as a clear choice looking exclusively at the values of productivity during PHA accumulation (Figure 9 C and F). However, the perspective might change when taking into consideration the volume and duration of the cycle of the SBR (innoculum reactor), and the overall productivity of the process; as extended PHA accumulation batches would bring a reduction of the inoculum need and thus a reduction of the SBR reactor.

It is important to mention that the results presented for the hollow fibers module were obtained with a periodical rotation of the hollow fiber module during the fed-batch to release the cell cake. When this procedure was not done, considerable cell deposition was observed on the hollow fibers, and in the zones in contact with the wall of the reactor. The cake did not affect the performance of the membrane, which could still maintain the flow during the whole 24 h fed-batch, but it compromised the performance of the culture and the determination of microbial biomass. By slightly rotating the filter, cell deposits were released by the turbulence created by the agitation and aeration. Immediately after rotating, the concentration of suspended cells would increase by 13 %, and a drop in the oxygen saturation was observed, indicating an increase in the metabolism. It is a known fact that oxygen and substrate can become limiting in the inner layers of the cake, in which the cells are at risk of death [26]. In the present setup, cake formation led to a decrease of 46 % in the substrate consumed and PHA produced (when considering the whole 24 h batch). Such an important effect could be a result of the limited space between the fiber loop and the wall of the reactor (Figure 3 B), which allowed little movement of the fibers.

4 Discussion

4.1 Perspectives on the use of dMBRs for PHA production

With the reactor design and membranes studied in this work, diffusion of VFA across the membrane did not meet the substrate requirements of the culture (section 3.2.1). At the highest porosity tested, PSf (0.2 µm) and PVDF (0.45 µm) allowed for a diffusion rate between 18-19 C_{mmol} VFA/h (corresponding to a flux of 576-610 $C_{mmol}/(h.m^2)$) (Figure 6). This value should have been double in order to meet the substrate requirements of the present culture (36 C_{mmol} VFA/h).

Diffusion rate of VFA across the membranes is proportional to the membrane surface and the concentration difference between the two sides of the membrane [15]. Thus, the strategy could still be valuable in a reactor design with double surface of contact. In the same line, substrate requirements could be met with a feed solution of approximately double VFA concentration (assuming a proportional

concentration polarisation effect). This would correspond to a concentration of about 20 g/L of VFA, which is common in many fermentation effluents [5]. However, even in that scenario, the concentration of VFA in the feed side would decrease as acids diffuse to the cell compartment, decreasing the transmembrane gradient. Hence, the VFA diffusion rate would decrease with time, eventually leading again to a situation where substrate requirements would not be met. A possible way to achieve a constant VFA flux would be a continuous recirculation of broth from the fermentation bioreactor to the feed compartment of the PHA accumulating reactor, similar to what was suggested by Du and Yu [27].

In regards to membrane materials, the results showed higher diffusion rates with PSf compared to PVDF, with the first being more hydrophilic. Higher diffusion for hydrophilic materials was also reported by Du and Yu [27] and Youngsukkasem et al. [28], which are, to our knowledge, the only previous reports evaluating VFA diffusion studies in a similar context. Moreover, the present study showed that VFA diffusion was highly correlated with the negative charge density on the membrane surface (Figure 6), possibly justified by repulsive forces with the ionised carboxyl groups of VFA at pH 6.4. To reverse these interactions, the pH should be lower than the isoelectric point of the membranes (about pH 4 in Figure 6 C), or lower than the pKa of the acids (3.8 – 4.9). Given that these are rather low values of pH to carry out a biological process, it would be interesting to study other hydrophilic membrane materials with positive or neutral surface charge at a higher pH range.

Small pore sizes are generally preferred in iMBR applications, as they minimise pore clogging by microbial cells [26]. Yet, our results showed that smaller pore size had an important effect on the charge density and led to lower diffusion rates. Therefore, bigger pore sizes might be beneficial for diffusion-based applications.

4.2 Perspectives on the use of iMBRs for PHA production

This section discusses the main challenges and opportunities identified with the use of iMBR configurations (hollow fibers and ceramic filters) for PHA production, summarised in Table 1.

Two types of hollow fiber configurations were tested in this study: a rod configuration and a loop configuration (Figure 3). As described in section 3.2.2, the rod configuration was more prone to fouling than the loop. In the rod, the fibers were very compact, allowing little circulation of liquid in between. Conversely, the loop configuration allowed for a more free movement of air and liquid in between the fibers, which provided membrane scouring and alleviated fouling. However, this looser configuration also led to higher fiber surface, creating a cell-retention net. Cells would get trapped in this fiber net, and between the fibers and the reactor wall, resulting in cell deposits. As explained in section 3.3, this did not lead to problems of membrane operation, but it led to an important decrease on the substrate consumption rate (46 %), most likely caused by substrate and oxygen limitation in the inner layers of the cell cake. In the current setup, this could be solved by rotating the fibers, what changed the flow patterns between the fibers and released the cell cake. This aspect would be of critical relevance when considering a scale-up of the process, especially considering that the product (PHA) would also be trapped.

Table 1: Main opportunities and challenges of hollow fibers (loop configuration) and ceramic filters applied as submerged filtration systems during PHA accumulation.

	Hollow fibers	Ceramic filters
Advantages /opportunities	• Stable permeate flux during 24 h fed-batch process. • Low relation footprint/filtration area.	• Stable permeate flux during 24 h fed-batch process. • Not prone to cell cake formation. • More resistant to chemical cleaning. Possibility to couple to PHA purification through chemical digestion methods.*
Disadvantages /challenges	• Prone to cake formation. The fiber net traps cells leading to reduced cell activity and lower PHA production.	• High relation footprint/filtration area.

*According to literature information. Not tested in this study.

Cake formation was not an issue in ceramic filters. Their tubular smooth surface was not prone to accumulate cells under the turbulent flow pattern in the bioreactor. However, the ceramic filters presented a lower critical flow compared to the hollow fiber loop (13 vs. 19 mL/min) (section 3.2.2). This was a result of the three times bigger filtration surface provided by the hollow fibers (779 cm^2 vs. 276 cm^2). In terms of flux, the critical point was higher for the ceramic filters (28.2 vs. 14.6 $L/(h.m^2)$). Space limitations in the bioreactor impeded the possibility of increasing the surface of filtration. Given the lesser propensity of ceramic filters to cell cake formation, it would be interesting to explore filter configurations that could increase the filtration area without increasing the footprint.

A successful fed-batch operation with effluent removal and no flow reduction could be attained with both the hollow fiber loop and the ceramic filters (section 3.3). Both systems operated below their limiting flow allowed a rapid exchange of exhausted supernatant for fresh medium (aprox. 10 min). However, to minimise periods without substrate, the ceramic module was operated above its critical flow towards the end of the batch, when high cell concentrations were reached (according to the results in Figure 8 section 3.2.2). At this point of the discussion, it is interesting to introduce the concept of the sustainable flux [18]. Operation below the critical flux, where no fouling occurs, is a good strategy to prolong membrane performance and to minimise the costs of membrane cleaning. However, operation at such fluxes might not be feasible for certain processes. Instead, it might be feasible to operate at fluxes with a low fouling rate, in which the cost and downtime from membrane cleaning are reasonable. In this respect, it would be interesting to consider the integration of membrane cleaning and PHA recovery. In recent years, PHA recovery methods based on the use of chemicals to digest and solubilise non-PHA material have gained interest. Such processes involve the same type of chemicals used for membrane cleaning (alkalis, acids or oxidizing agents) [1,6,20], and thus integration of both operations could be envisioned. In this regard, ceramic membranes would offer an additional advantage, given their higher resistance to chemicals and overall robustness [7].

The main objective of this study was not to define a sustainable flux, which should be studied in real effluents and considering a long-term performance of the systems, but to perform a proof of concept of the utilisation of MBRs for PHA accumulation. In this sense, both iMBR configurations proved valuable to overcome the limitations of dilute feeding solutions, reaching high PHA productivities (0.87-1.44 g PHA/(L.h)). These values are among the highest reported for PHA production processes in MMC - generally below 0.5 g/(L.h) [5] - showing the great potential of this cultivation strategy. Besides being valuable for fed-batch cultivations, iMBR could also be an asset in PHA production in continuous mode, as they could offer the possibility of uncoupling the hydraulic retention time from the cell retention time and cell growth.

5 Conclusions

One of the main limitations to obtain high PHA productivities with second-generation substrates is the dilute nature of the feed solutions. The present study evaluated for the first time the use of membrane bioreactors (MBRs) in the frame of PHA production in mixed microbial consortia. The main conclusions of the work were:

- In pressure-driven immersed MBR configurations (iMBR), both hollow fibers and ceramic filters allowed to feed over 3.5 L of substrate in a fed-batch period of 24 h, while keeping a constant reactor volume of 0.75 L, representing an 82 % reduction of the reactor volume.
- A high volumetric productivity could be attained in iMBR configurations (0.87-1.44 g PHA/(L.h)) despite the use of a diluted substrate feed (approximately 10 g/L of volatile fatty acids).
- Hollow fibers allowed to operate at higher flows, due to their lower ratio of surface/footprint. However, they presented a risk of cell deposition, resulting in a decreased metabolic activity and PHA production rate.

- Ceramic filters were less prone to cell deposition and fouling, but they had a higher footprint, leading to a lower critical flow with the surface available in the bioreactor.
- VFA diffusion across PSf and PDVF microfiltration membranes was not enough to satisfy the substrate requirements of the culture in a novel diffusion-based MBR configuration.

Acknowledgements

This work was financed by the Technical University of Denmark. The authors thank Mingbo Ji for the contact angle and the zeta potential measurements.

References

[1] C. Kourmentza, J. Plácido, N. Venetsaneas, A. Burniol-Figols, C. Varrone, H.N. Gavala, M.A.M. Reis, Recent Advances and Challenges towards Sustainable Polyhydroxyalkanoate (PHA) Production, Bioengineering. 4 (2017) 1–43. https://doi.org/10.3390/bioengineering4020055.

[2] W. Blunt, D.B. Levin, N. Cicek, Bioreactor Operating Strategies for Improved Polyhydroxyalkanoate (PHA) Productivity, Polymers (Basel). 10 (2018) 1197. https://doi.org/10.3390/polym10111197.

[3] J.L. Ienczak, W. Schmidell, G.M.F. De Aragão, High-cell-density culture strategies for polyhydroxyalkanoate production: A review, J. Ind. Microbiol. Biotechnol. 40 (2013) 275–286. https://doi.org/10.1007/s10295-013-1236-z.

[4] M. Koller, L. Maršálek, M.M. de Sousa Dias, G. Braunegg, Producing microbial polyhydroxyalkanoate (PHA) biopolyesters in a sustainable manner, N. Biotechnol. 37 (2017) 24–38. https://doi.org/10.1016/j.nbt.2016.05.001.

[5] F. Valentino, F. Morgan-Sagastume, S. Campanari, M. Villano, A. Werker, M. Majone, Carbon recovery from wastewater through bioconversion into biodegradable polymers, N. Biotechnol. 37 (2016) 9–23. https://doi.org/10.1016/j.nbt.2016.05.007.

[6] S. Judd, The MBR book: principles and applications of membrane bioreactors for water and wastewater treatment., Second edi, Elsevier, 2010. ISBN: 9780080966823.

[7] F. Carstensen, A. Apel, M. Wessling, In situ product recovery: Submerged membranes vs. external loop membranes, J. Memb. Sci. 394–395 (2012) 1–36. https://doi.org/10.1016/j.memsci.2011.11.029.

[8] W.S. Ahn, S.J. Park, S.Y. Lee, Production of poly(3-hydroxybutyrate) from whey by cell recycle fed-batch culture of recombinant *Escherichia coli* , Biotechnol. Lett. 23 (2001) 235–240. https://doi.org/10.1023/A:1005633418161.

[9] C. Haas, T. El-najjar, N. Virgolini, M. Smerilli, M. Neureiter, High cell-density production of poly(3-hydroxybutyrate) in a membrane bioreactor, N. Biotechnol. 37 (2017) 117–122. https://doi.org/10.1016/j.nbt.2016.06.1461.

[10] J.L. Ienczak, M. Schmidt, L.K. Quines, Poly(3-Hydroxybutyrate) Production in Repeated fed-Batch with Cell Recycle Using a Medium with low Carbon Source Concentration, Appl. Biochem. Biotechnol. 178 (2016) 408–417. https://doi.org/10.1007/s12010-015-1883-9.

[11] M. Schmidt, J. Lutz, L. Kelin, K. Zanfonato, W. Schmidell, G. Maria, F. De Aragão, Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) production in a system with external cell recycle and limited nitrogen feeding during the production phase, Biochem. Eng. J. 112 (2016) 130–135. https://doi.org/10.1016/j.bej.2016.04.013.

[12] M. Gander, B. Jefferson, S. Judd, Aerobic MBRs for domestic wastewater treatment: A review with cost considerations, Sep. Purif. Technol. 18 (2000) 119–130. https://doi.org/10.1016/S1383-5866(99)00056-8.

[13] L.H. de Andrade, F.D. dos S. Mendes, J.C. Espindola, M. Amaral, Internal versus external submerged membrane bioreactor configurations for dairy wastewater treatment, Desalin. Water Treat. 52 (2014) 16–18. https://doi.org/10.1080/19443994.2013.799048.

[14] P. Kumar, H. Jun, B. Soo, Co-production of polyhydroxyalkanoates and carotenoids through bioconversion of glycerol by *Paracoccus* sp. strain LL1, Int. J. Biol. Macromol. 107 (2018) 2552–2558. https://doi.org/10.1016/j.ijbiomac.2017.10.147.

[15] A. Mahboubi, P. Ylitervo, W. Doyen, H. De Wever, M.J. Taherzadeh, Reverse membrane bioreactor: Introduction to a new technology for biofuel production, Biotechnol. Adv. 34 (2016) 954–975. https://doi.org/10.1016/j.biotechadv.2016.05.009.

[16] A. Burniol-Figols, C. Varrone, S.B. Le, A.E. Daugaard, I. V. Skiadas, H.N. Gavala, Combined polyhydroxyalkanoates (PHA) and 1,3-propanediol production from crude glycerol: Selective conversion of volatile fatty acids into PHA by mixed microbial consortia, Water Res. 136 (2018) 180–191. https://doi.org/10.1016/j.watres.2018.02.029.

[17] C. Varrone, I. V. Skiadas, H.N. Gavala, Effect of hydraulic retention time on the modelling and optimization of joint 1,3 PDO and BuA production from 2G glycerol in a chemostat process, Chem. Eng. J. 347 (2018) 525–534. https://doi.org/10.1016/j.cej.2018.04.071.

[18] P. Bacchin, P. Aimar, R.W. Field, Critical and sustainable fluxes: Theory, experiments and applications, J. Memb. Sci. 281 (2006) 42–69.

https://doi.org/10.1016/j.memsci.2006.04.014.

[19] A. Burniol-Figols, C. Varrone, A.E. Daugaard, S.B. Le, I. V. Skiadas, H.N. Gavala, Polyhydroxyalkanoates (PHA) production from fermented crude glycerol: Study on the conversion of 1,3-propanediol to PHA in mixed microbial consortia, Water Res. 128 (2018) 255–266. https://doi.org/10.1016/j.watres.2017.10.046.

[20] A. Burniol-Figols, I. V. Skiadas, A.E. Daugaard, H.N. Gavala, Polyhydroxyalkanoate (PHA) purification through dilute aqueous ammonia digestion at elevated temperatures, J. Chem. Technol. Biotechnol. 95 (2020) 1519-1532. https://doi.org/https://doi.org/10.1002/jctb.6345.

[21] APHA, AWWA, WEF, Standard Methods for the Examination of Water & Wastewater, 21st editi, American Public Health Association (APHA), Washington, DC, USA, 2005. ISBN: 0875530478.

[22] R.S. Hebbar, A.M. Isloor, A.F. Ismail, Contact Angle Measurements, in: N. Hilal, A.F. Ismail, T. Matsuura, D. Oatley-Radcliffe (Eds.), Membr. Charact., Elsevier B.V., 2017. ISBN: 9780444637765: pp. 219–255. https://doi.org/10.1016/B978-0-444-63776-5.00012-7.

[23] Y. Zhang, J. Wang, F. Gao, Y. Chen, H. Zhang, A comparison study: The different impacts of sodium hypochlorite on PVDF and PSF ultrafiltration (UF) membranes, Water Res. 109 (2017) 227–236. https://doi.org/10.1016/j.watres.2016.11.022.

[24] F. Valentino, L. Karabegovic, M. Majone, F. Morgan-Sagastume, A. Werker, Polyhydroxyalkanoate (PHA) storage within a mixed-culture biomass with simultaneous growth as a function of accumulation substrate nitrogen and phosphorus levels, Water

584 Res. 77 (2015) 49–63. https://doi.org/10.1016/j.watres.2015.03.016.

585 [25] T. Suzuki, A Dense Cell Retention Culture System Using a Stirred Ceramic Membrane
586 Reactor, Biotechnol. Bioeng. 44 (1994) 1186–1192.
587 https://doi.org/10.1002/bit.260441005.

588 [26] O.T. Iorhemen, R.A. Hamza, J.H. Tay, Membrane Bioreactor (MBR) Technology for
589 Wastewater Treatment and Reclamation : Membrane Fouling, Membranes. 6 (2016) 1–29.
590 https://doi.org/10.3390/membranes6020033.

591 [27] G. Du, J. Yu, Green Technology for Conversion of Food Scraps to Biodegradable
592 Thermoplastic Polyhydroxyalkanoates, 36 (2002) 5511–5516.
593 https://doi.org/10.1021/es011110o.

594 [28] S. Youngsukkasem, H. Barghi, S.K. Rakshit, M.J. Taherzadeh, Rapid Biogas Production
595 by Compact Multi-Layer Membrane Bioreactor: Efficiency of Synthetic Polymeric
596 Membranes, Energies. 6 (2013) 6211–6224. https://doi.org/10.3390/en6126211.

597

Figures

Figure 1: Schematic representation of different membrane bioreactor (MBR) configurations. Alternative reactor designs are possible for each configuration. Modified from [15].

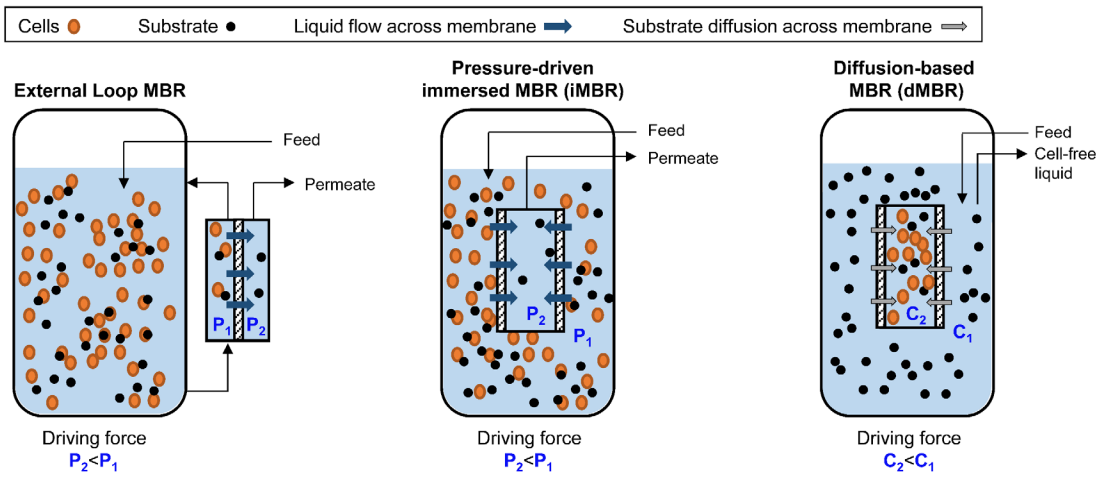


Figure 2: Diffusion-based membrane bioreactor (dMBR). A: Membrane support. B: Schematic representation of the diffusion membrane inside the bioreactor. Blue arrows indicate diffusion across the membrane.

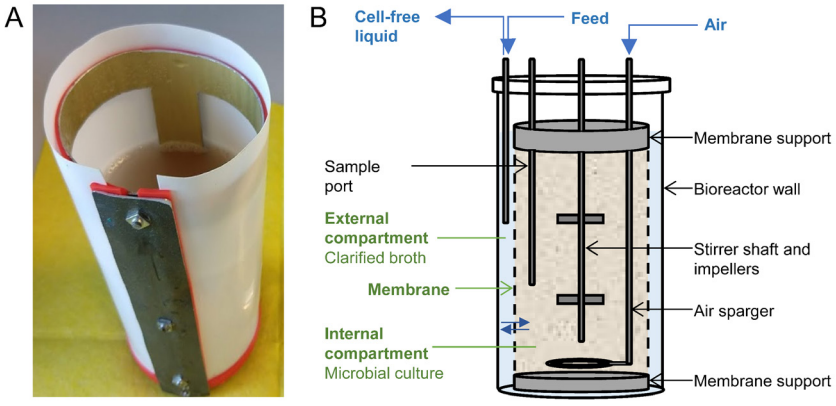


Figure 3: Hollow fiber configurations. A: Rod configuration. B: Loop configuration placement in the bioreactor. C: Schematic representation of the hollow fiber rod module inside the bioreactor.

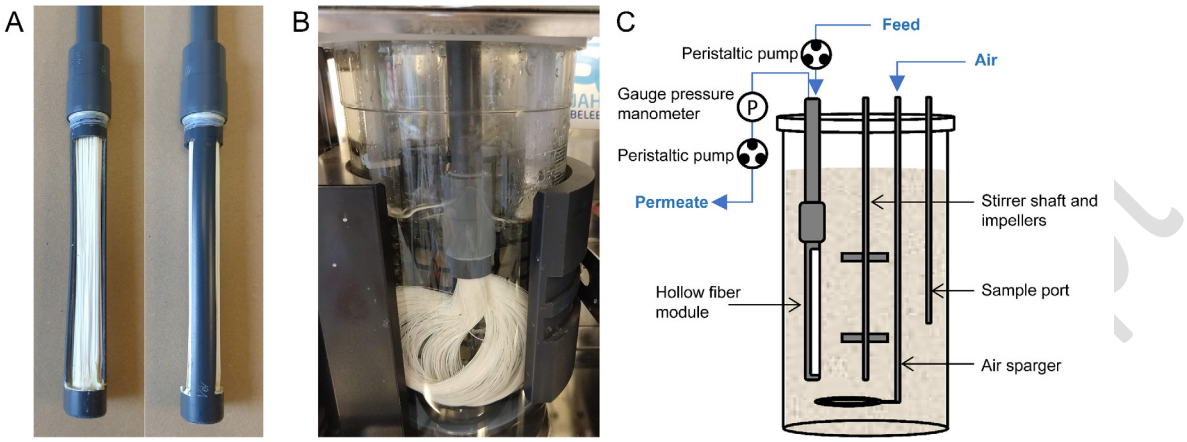


Figure 4: A: Ceramic filtration module. B: Schematic representation of the ceramic filtration module inside the bioreactor

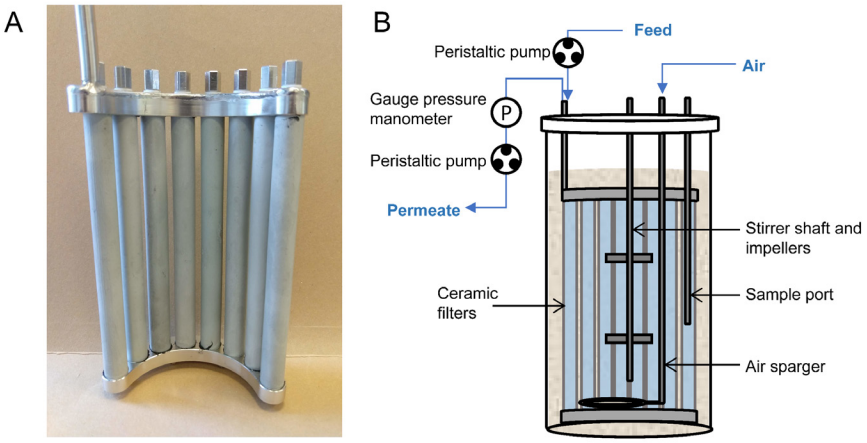


Figure 5: Fed-batch PHA accumulation experiments at different nitrogen/carbon ratios. *In order to better reflect the obtainable productivity in a membrane bioreactor configuration with constant volume, volumetric productivity was calculated considering a constant volume equal to the initial volume.

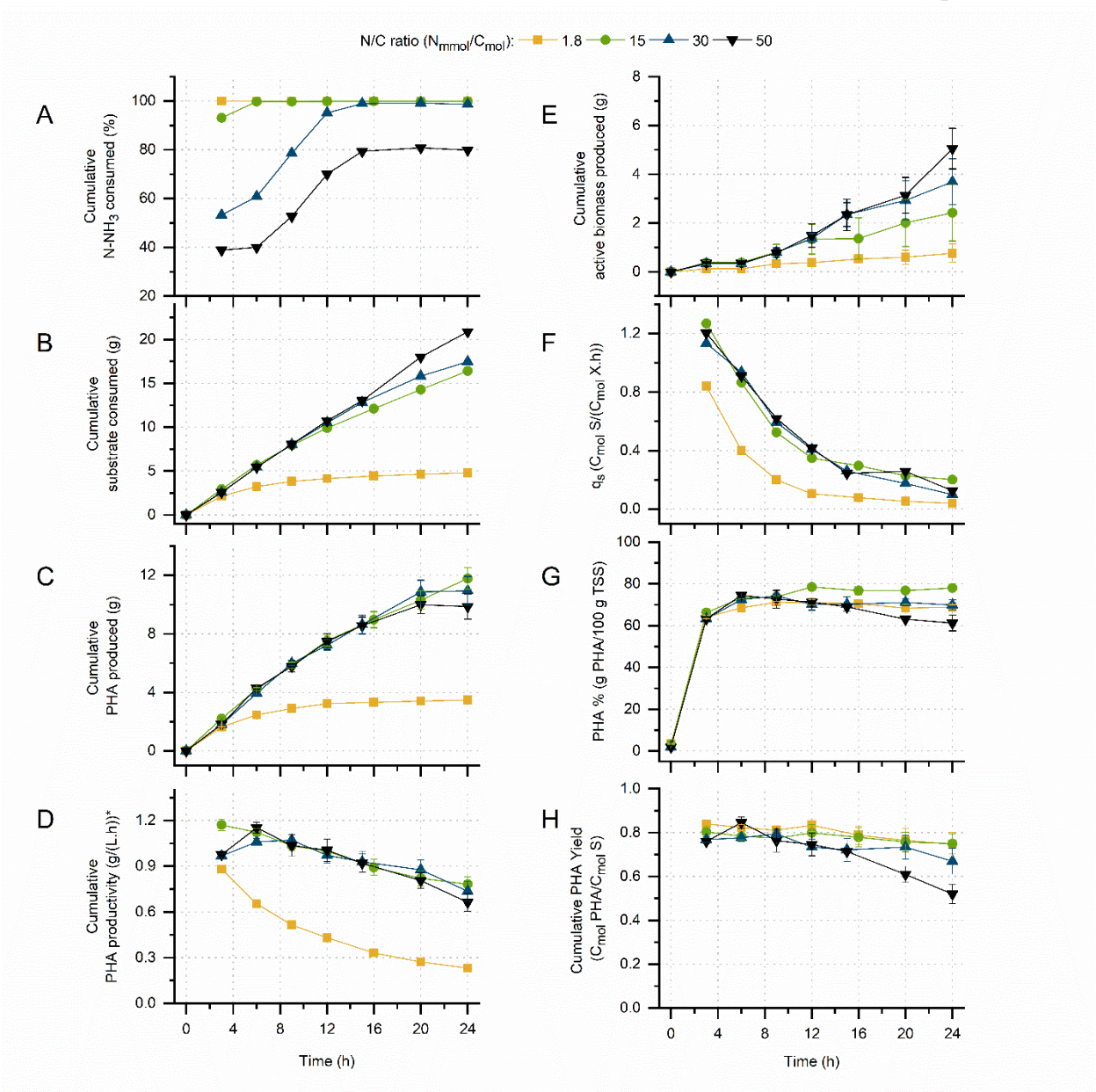


Figure 6: Results of the diffusion-based MBR with PSf and PVDF membranes at different pore size. A:VFA diffusion across the membranes (C_{mmols}/h). The dashed line across the graph indicates the substrate consumption rate of the culture (r_s) (C_{mmols}/h). B: Water contact angle. C: Zeta Potential.

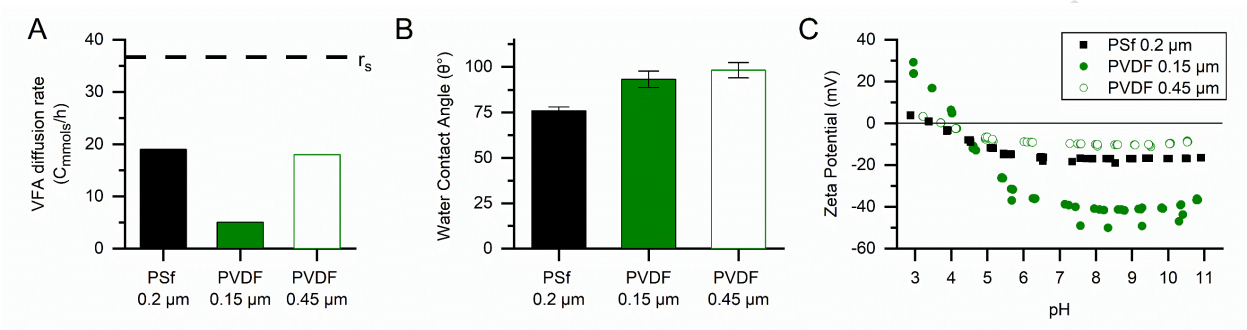


Figure 7: Evaluation of critical and limiting flux for the hollow fiber modules. A: Rod configuration. B, C and D: Loop configuration. In graphs A, B and C, the values of flow and transmembrane pressure (TMP) are the ones recorded at the end of the 5th filtration at each flow setpoint.

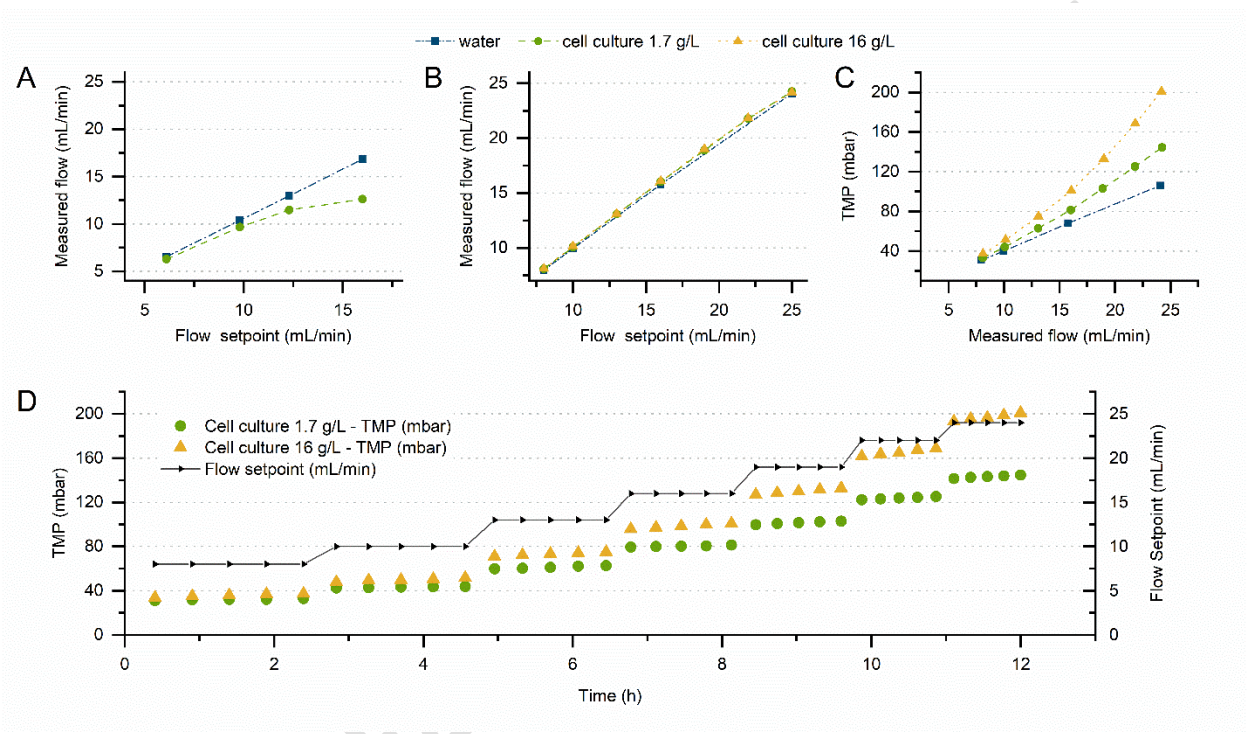


Figure 8: Evaluation of critical and limiting flux for the ceramic filtration module. In graphs A and B, the values of flow and transmembrane pressure (TMP) are the ones recorded at the end of the 5th filtration at each flow setpoint.

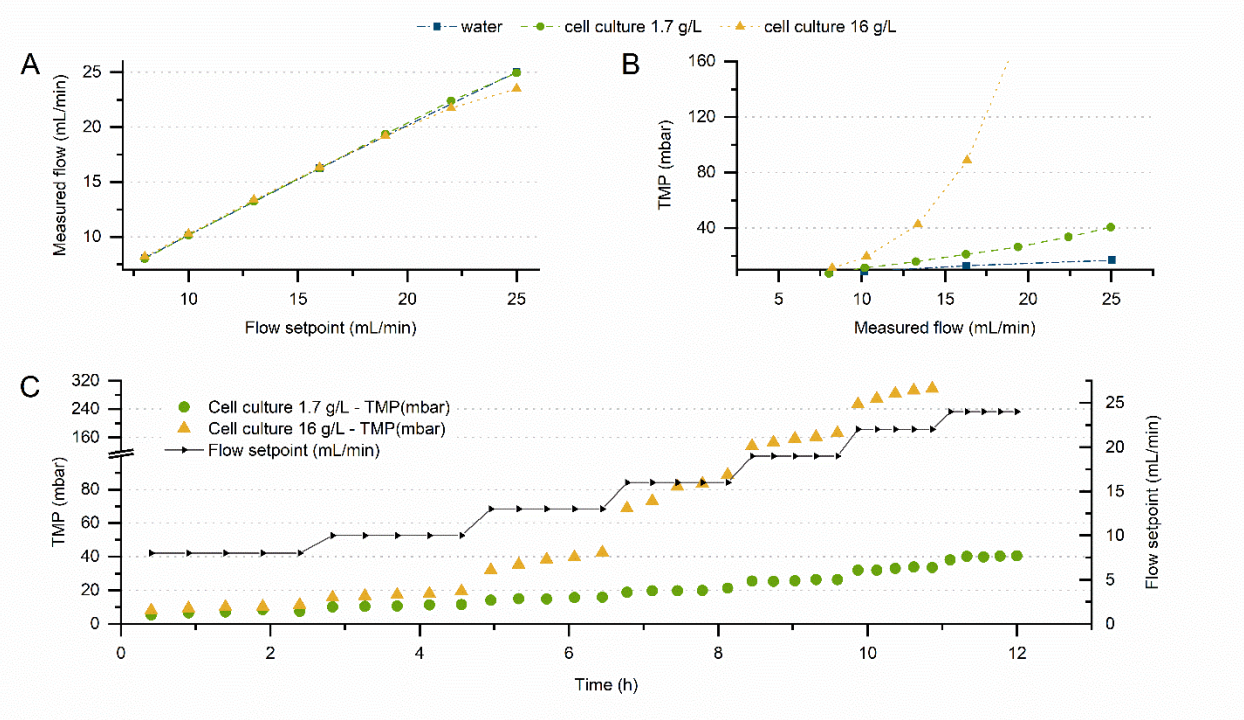


Figure 9: Fed-batch PHA accumulation in iMBR (pressure-driven immersed bioreactors): loop hollow fibers (left) and ceramic filters (right).