Control of membrane-attached biofilms in extractive membrane bioreactors

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Abstract  Control of Membrane Attached Biofilm (MAB) formation and accumulation is a key aspect in the operation of Extractive Membrane Bioreactors (EMBs). In this work, MAB control was attempted in a novel EMB configuration which presents two innovative aspects: the presence of a biphasic biomedium and a contained liquid membrane module. This reactor, where the benefits of high shear forces and the use of a biphasic biomedium are effectively combined, was operated without any biofilm formation or reduction in organic substrate flux over time.

Keywords  Biofilm; membrane; bioreactor; flux; mass transfer

Introduction
In Extractive Membrane Bioreactors (EMBs) a non-porous membrane, usually silicone rubber, is used to extract organic substrates from wastewater into a controlled composition biomedium, where they are biodegraded (Livingston, 1994). Membrane-attached biofilms (MABs) form when suspended microorganisms in the biomedium spontaneously attach to the surface of the membranes (Freitas dos Santos and Livingston, 1995).

The organic substrate flux across the membrane is a key design parameter of EMBs, since a reduction in the flux will lead to a larger membrane area being necessary to achieve a fixed degree of pollutant removal from a given wastewater flow. The formation of biofilms has proved to be detrimental for the process performance due to severe mass transfer limitation of the organic substrate through the biofilm (Nicolella et al., 2000a, 2000b). In EMB, the resistance to organic substrate transfer from the wastewater to the biomedium through the liquid boundary layer on the tube side, the membrane, the biofilm and the liquid boundary layer on the shell side can be expressed by a resistances in series model (Nicolella et al., 2000a):

\[
\frac{1}{k_{So}} = \left[ \frac{1}{k_{Sw}} + \frac{R_{in}}{K_{ma}D_{Sm}} \ln \left( \frac{R_{ot}}{R_{in}} \right) \right] + \frac{R_{in}}{K_{ba}D_{sb}} \ln \left( \frac{R_{b}}{R_{rot}} \right) + \frac{1}{k_{Sb}}
\]

where \( k_{So} \) is overall mass transfer coefficient, \( k_{Sw} \) is the wastewater side mass transfer coefficient, \( k_{Sb} \) is the biomedium side mass transfer coefficient, \( D_{Sm} \) is the membrane diffusion coefficient for MCB, \( K_{ma} \) is the membrane/aqueous partition coefficient for MCB, \( K_{ba} \) is the biofilm/aqueous partition coefficient, \( R_{in} \) is the inner membrane radius, \( R_{ot} \) is the outer membrane radius and \( R_{b} \) is the radial coordinate of the biofilm/biomedium interface.

It is clearly shown by Eq. (1) that, if MABs form, they constitute a diffusional barrier, which creates an additional resistance (represented by the term \( [(R_{in}/K_{ba}D_{sb}) \ln(R_{b}/R_{ot})] \) in Eq. (1)) to organic substrate transfer from the wastewater to the biomedium. The elimination or reduction of this barrier is highly desirable to improve mass transfer performance of EMBs.

The EMB shares this problem with membrane oxygenation processes, where MABs also create mass transfer limitations (Debus and Wanner, 1992; Reij et al., 1995). More generally, excessive biofilm growth in technical equipment presents problems ranging from reduction of heat transfer fluxes, increasing fluid frictional resistances, and corrosion inside...
pipes, to problems in medical applications (Characklis and Marshall, 1989). Due to these adverse effects, several techniques have been used to minimise the accumulation of biofilms on surfaces (Flemming et al., 1996), but none of these techniques appears to be applicable in EMBs. There is therefore the need for new techniques to avoid or control the formation and accumulation of MABs.

In EMBs the hydrophobic nature of silicone rubber membranes and the high organic substrate concentration at the membrane/biomedium interface result in a “hospitable” environment for microbial growth in the proximity of the membrane wall. To reduce formation and accumulation of MABs, the membrane wall might be made less “hospitable” to microbial growth. In this work, this was attempted by increasing the wall temperature. Another option might be realised by creating other “hospitable” regions within the system which can compete with the membrane wall to favour microbial adhesion and growth.

In the presence of hydrophobic solvents in the bioreactor, microbial adhesion to solvent droplets might also occur (Nakahara et al., 1977), favoured by changes of cellular hydrophobicity during cellular cycle (Allison et al., 1990). Microbial adhesion to hydrophobic liquid droplets has been reported for many biphasic aqueous/organic bioreactors consisting of two liquid phases (water and organic solvent) in direct contact and dispersed by mixing (Efroymon and Alexander, 1991; Ascon-Cabrera and Lebeault, 1993, 1995). This behaviour might be exploited in EMBs to avoid MAB accumulation using a biphasic aqueous/organic bioreactor where solvent droplets constitute a large hydrophobic surface area available for preferential microbial adhesion. In addition the tangential flow along the membrane might create significant shear stresses, which can also be exploited to avoid or limit the formation and accumulation of MABs.

In this work the effect of shear stress on MAB accumulation and organic substrate flux was studied under different operating conditions in a lab-scale EMB. A new EMB configuration was also proposed which combines the benefits of high shear forces and the use of a biphasic bioreactor to control the formation and accumulation of MABs.

**Materials and methods**

The experiments performed in this work aimed to investigate the evolution over time of biofilm thickness and organic substrate flux for different EMB configurations and operating conditions. Experiments were performed using a single tube EMB and an Extractive Membrane Biphasic Bioreactor (EMBB), whose configurations are described below.

**Experimental set-up**

*Extractive Membrane Biphasic Bioreactor.* The EMBB, schematically represented in Figure 1, incorporated a membrane module, a bioreactor and a two-phase separator. The wastewater was recirculated from the membrane module to a well mixed vessel, where raw wastewater was continuously fed. The biomedium was recirculated from the membrane module to a well mixed bioreactor where temperature, pH and dissolved oxygen were controlled, and fresh nutrient salts medium was continuously added. A biphasic aqueous/organic bioreactor was obtained by using different fractions of silicone oil as the organic phase. The bioreactor overflow was pumped to a two-phase gravity separator (5 l volume) where the organic phase separated at the top and was recirculated to the bioreactor, whilst the aqueous phase collected at the bottom of the separator was disposed. The operating conditions and the characteristics of the EMBB system are shown in Table 1.

Two EMBB configurations were used for the experiments:

**EMBB 1:** the membrane module comprised a silicone rubber membrane tube fitted coaxially in a glass shell. Biomedium and wastewater recirculated in the shell and membrane tube respectively.
EMBB 2: the membrane module is a contained liquid membrane module, comprising two silicone rubber tubes fitted in a glass shell. The shell was filled with silicone oil. The membrane tubes used in this case had diameter and wall thickness as reported in Table 1 and were provided with four equally spaced protruding ridges (1.5 $\times$ 1 mm) running along the length of the tubes. The maximum distance between the two tubes was around 6 mm. Wastewater and biomedium streams flowed in one of the two membrane tubes respectively. This EMBB configuration is called Extractive Liquid Membrane Biphasic Bioreactor (ELMBB).

**Extractive Membrane Bioreactor.** The EMB configuration was similar to that of the EMBB represented in Figure 1, with the exception of the two-phase separator, which was not present.

The wastewater was recirculated from the membrane module to a well mixed vessel, where raw wastewater was continuously fed. Temperature of the wastewater recirculation vessel was varied using a hot plate and measured by a thermometer. The biomedium was recirculated from the membrane module to a well mixed bioreactor where temperature, pH and dissolved oxygen were controlled, and fresh nutrient salts medium was continuously added. The operating conditions and the characteristics of the EMB system are shown in Table 1.

**Table 1** Characteristics and operating conditions of EMB and EMBB

<table>
<thead>
<tr>
<th>Characteristics and conditions</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane tube length</td>
<td>300</td>
<td>mm</td>
</tr>
<tr>
<td>Membrane tube inner diameter</td>
<td>3</td>
<td>mm</td>
</tr>
<tr>
<td>Membrane thickness</td>
<td>0.3</td>
<td>mm</td>
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<tr>
<td>Shell column diameter</td>
<td>16</td>
<td>mm</td>
</tr>
<tr>
<td>Feed flow rate</td>
<td>0.1</td>
<td>l/h</td>
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<tr>
<td>Feed MCB concentration</td>
<td>200</td>
<td>mg/l</td>
</tr>
<tr>
<td>Nutrient flow rate</td>
<td>0.02</td>
<td>l/h</td>
</tr>
</tbody>
</table>
Two EMB configurations were used for the experiments:

**EMB 1**: biomedium and wastewater recirculated in the shell and in the membrane tube respectively.

**EMB 2**: biomedium and wastewater recirculated in the membrane tube and in the shell respectively.

A synthetic wastewater containing monochloro-benzene (MCB) was used throughout the experiments. The nutrient salts medium composition is given by Jorge and Livingston (2000). A mixed microbial culture was used to degrade MCB. Biofilm thickness was continuously monitored and recorded by a computer controlled video-imaging technique (Zhang et al., 1998) during the experiments where the biomedium was pumped in the shell side of the membrane module. MCB concentrations in the inlet and outlet wastewater streams and in the aqueous phase of the biomedium were periodically measured by gas chromatography according to the procedure described by Zhang et al. (1998).

The overall mass transfer coefficient and the flux of organic substrate across the membrane were calculated according to Nicolella et al. (2000a).

**Experimental procedure**

Different experimental runs were performed under varying operating conditions in both EMB and EMBB. A description of all the experimental runs is given in Table 2. MCB concentration in the feed wastewater, feed wastewater flow rate and nutrient flow rate were constant in all experiments (see Table 1).

The effect of shear stress on the evolution over time of biofilm thickness and organic substrate flux was studied in the EMB by varying biomedium recirculation flow rate. Biomedium was pumped either in the shell (Runs 1 and 2) or in the membrane tube (Run 3). An experimental run (Run 4) was performed by heating the wastewater at about 70ºC to evaluate the effect of temperature on MAB formation and accumulation.

In the EMBB experiments, a biphasic biomedium was recirculated from the bioreactor to the membrane module at high flow rates in order to combine high shear and presence of hydrophobic droplets to control the MAB formation. First the biphasic biomedium was recirculated in the shell side of a conventional shell-tube membrane module using two different volumetric fractions of silicone oil (Runs 5 and 6). Then, to increase the shear stress at the biomedium/membrane interface, the biphasic biomedium was recirculated inside the membrane tube (Run 7). Since this configuration presented problems related to solvent breakthrough in the wastewater, a contained liquid membrane module was set-up as described in the previous section (Run 8).

### Table 2 Description of experimental runs

<table>
<thead>
<tr>
<th>Run</th>
<th>Membrane module configuration</th>
<th>Reynolds number in the biomedium side</th>
<th>Reynolds number in the wastewater side</th>
<th>Wastewater side temperature</th>
<th>Volume fraction of silicone oil in the bioreactor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EMB 11</td>
<td>2000</td>
<td>12000</td>
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</tr>
<tr>
<td>2</td>
<td>EMB 1</td>
<td>9000</td>
<td>12000</td>
<td>ambient</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>EMB 2</td>
<td>3800</td>
<td>2200</td>
<td>ambient</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
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<td>12000</td>
<td>60ºC</td>
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</tr>
<tr>
<td>5</td>
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<td>9000</td>
<td>12000</td>
<td>ambient</td>
<td>30</td>
</tr>
<tr>
<td>6</td>
<td>EMBB 1</td>
<td>9000</td>
<td>12000</td>
<td>ambient</td>
<td>60</td>
</tr>
<tr>
<td>7</td>
<td>EMBB 1</td>
<td>12000</td>
<td>3500</td>
<td>ambient</td>
<td>30</td>
</tr>
<tr>
<td>8</td>
<td>EMBB 2</td>
<td>9000</td>
<td>5000</td>
<td>ambient</td>
<td>50</td>
</tr>
</tbody>
</table>
In the EMBB experiments, a biphasic biomedium was recirculated from the bioreactor to the membrane module at high flow rates in order to combine high shear and presence of hydrophobic droplets to control the MAB formation. First the biphasic biomedium was recirculated in the shell side of a conventional shell-tube membrane module using two different volumetric fraction of silicone oil (Runs 5 and 6). Then, to increase the shear stress at the biomedium/membrane interface, the biphasic biomedium was recirculated inside the membrane tube (Run 7). Since this configuration presented problems related to solvent breakthrough in the wastewater, a contained liquid membrane module was set-up as described in the previous section (Run 8).

Results and discussion

EMBB operation

A two-phase separator was used to separate water and silicone oil in the reactor overflow. The biomass suspended in the reactor overflow tended to accumulate at the interface between silicone oil and water in the separator. However, the interface between the two phases was well defined, allowing the recovery of silicone oil, which was periodically reintroduced in the reactor.

Three EMBB experiments (Run 5–7) were performed using a single tube membrane module. In one of these experiments (Run 7) the biphasic biomedium was recirculated in the membrane tube to obtain higher liquid velocity (4 m/s in Run 7, and 0.2 and 0.7 m/s in Run 5 and 6 respectively), i.e. higher shear stress on the membrane wall (240 N/m² in Run 7, and 0.6 and 7 N/m² in Run 5 and 6 respectively, as calculated through Eq. (4)). This experiment was unsuccessful because, due to high trans-membrane pressure at the membrane module inlet (estimated as 0.25 $10^5$ Pa using the Darcy-Weisbach formula for the head loss in the shell and in the tube), silicone oil broke through the membrane into the wastewater. This inconvenience led to the design of a contained liquid membrane module, as described in the experimental section.

Biofilm accumulation

The formation and accumulation of MABs was studied in the EMB by measuring the evolution over time of biofilm thickness under varying operating conditions, namely biomedium recirculation flow rate and wastewater temperature. The results of these experiments are presented in Figure 2, which shows the biofilm thickness as a function of time in different experimental runs, as listed in Table 2.

To evaluate the effect of increasing liquid shear stress on MAB accumulation, two experimental runs (Run 1 and Run 2) were performed in EMB configuration 1 (biomedium in shell, wastewater in membrane tube) at different biomedium recirculation flow rates. As
can be observed in Figure 2, biofilms grew more rapidly in the run at high Reynolds number (Run 2, Re=9000 corresponding to a liquid velocity of 0.7 m/s and shear stress of 7.2 N/m²) than in the run at low Reynolds number (Run 1, Re=2000 corresponding to a liquid velocity of 0.2 m/s and shear stress of 0.4 N/m²). According to the analysis of rate limiting phenomena in MAB performed in a previous work (Nicolella et al., 2000a), this behaviour can be attributed to better hydrodynamic conditions for oxygen transfer at biofilm/biomedium interface. From the results of Figure 2, it can be concluded that, in the range of operating conditions tested in this work, formation and accumulation of MABs in EMBs cannot be controlled by increasing the shear forces of the tangential flow on the membrane surface. The shear loss rate is far lower than the growth rate (Nicolella et al., 2000a) and any modification of the hydrodynamic conditions at MAB boundaries will affect the mass transfer rate of soluble substrate rather than the adhesion and detachment rate of particulate matter.

The effect of liquid shear stress on biofilm detachment rate in EMBs cannot easily be compared to that found in other systems, since contrasting results have been reported for different biofilm reactors. Rittmann (1982) found that biofilm loss due to shear stress was a major component of the overall specific loss rate for fluidisation of sand-type particles covered by biofilm, whilst no significant influence of shear stress on detachment rate was observed in rototorque biofilm reactors (Peyton and Characklis, 1992) and annular biofilm reactors (Peyton, 1996). Contrary to general beliefs, Kwok et al. (1998) observed that in biofilm airlift suspension reactors the biomass detachment decreased with increasing detachment force, and concluded that, according to the hypothesis postulated by van Loosdrecht et al. (1995), compact, dense biofilms develop when shear forces are high.

To evaluate whether thermal effects would displace the microorganisms from attaching to the membrane wall, an experiment (Run 4) was carried out by heating the wastewater at 70°C. This resulted in a calculated membrane wall temperature at the biomedium interface of around 55°C. The hydrodynamic conditions (Reynolds number on membrane tube and shell side) of this experiment were the same as in Run 2. As shown by Figure 2 the evolution over time of biofilm thickness was similar in Run 2 and Run 4, so it can be concluded that wastewater temperature had no effect on biofilm accumulation.

MABs also formed in EMBB during Run 5 and 6, when the biphasic biomedium was recirculated from the shell of the membrane module to the bioreactor. The biofilm accumulated up to a thickness of 2.1 mm in Run 6.

Biofilm thickness could not be measured during Run 3, 7 and 8, since in those cases the biomedium/membrane interface was inside the membrane tube. Evidence of biofilm formation was visually observed during Run 3 when the image of membrane tube given by the video camera used for biofilm thickness measurement became darker and darker over time. No evidence of biofilm formation was found during Run 8, where the membrane tube remained clear throughout the experiments.

Overall mass transfer coefficient (OMTC)
The organic substrate OMTC was estimated in all experimental runs but Run 4, when, due to the high wastewater temperature and high volatility of MCB, the measurement of MCB concentration in the wastewater recirculation vessel was not reproducible. All results of OMTC measurements are presented in Figure 3, which shows the OMTC as a function of time for different experimental runs.

OMTC decreased with time in all experiments where MAB formed. This was attributed to increased resistance to organic substrate diffusion caused by the biofilm (Nicolella et al., 2000a). It can be concluded from these results that the formation of MABs is detrimental for EMB performance. It should be observed that the reduction of OMTC is more noticeable in
EMB experiments, where OMTC was reduced from $2.8 \times 10^{-5}$ to $0.3 \times 10^{-5}$ m/s (Run 1), than in EMBB experiments, where OMTC was reduced from $2.3 \times 10^{-5}$ to $1.0 \times 10^{-5}$ m/s (Run 6). This might be due to the different structure of biofilms formed in EMB and EMBB experiments. The biofilm formed during Run 1 and 2 appeared to be more compact and uniform compared to that formed during Run 5 and Run 6, which presented a discontinuous structure comprised of silicone oil droplets and biofilm patches. Visual observations of biofilm structures were supported by the measurements of biofilm densities at the end of the experiments, which ranged from 37 to 44 kg/m$^3$ in Run 1 and 2 respectively and gave 22 kg/m$^3$ for Run 6.

The presence of silicone oil droplets in the biofilm formed in the EMBB experiments (Run 5 and Run 6) leads to an increase of i) partition coefficient of MCB between water and biofilm and ii) diffusion coefficient of MCB in the biofilm. The partition coefficient of MCB between silicone oil and water measured in this work was 122, much higher than partition coefficient between water and a biofilm formed in the presence of an aqueous biomedium, which, as measured by Zhang et al. (1998), is unitary. The diffusion coefficient of MCB in silicone oil was estimated through the Scheibel’s equation (Perry and Green, 1997) as $6 \times 10^{-9}$, whilst the diffusion coefficient of MCB in water is $1.5 \times 10^{-9}$ (Brookes and Livingston, 1995). According to the resistances-in-series model represented by Eq. (1), the mass transfer resistance in the biofilm (represented by the term $[R_{in}/K_{mb}D_{Sb}] \ln(R_b/R_{ot})$) in Eq. (1) decreases with increasing partition coefficient between water and biofilm ($K_{mb}$) and with increasing diffusion coefficient of MCB in the biofilm ($D_{Sb}$). The mass transfer in the biofilm formed in the presence of a biphasic biomedium (which contains silicone oil droplets) is therefore expected to be faster than that in the biofilm formed in the presence of an aqueous biomedium.

During EMB experiments, the shear stress at the membrane/biomedium interface was increased from 7 N/m$^2$ in Run 2 to 32 N/m$^2$ in Run 3, when the biomedium was recirculated in the membrane tube. This was not enough to prevent biofilm formation, and a 30% decrease of OMTC was observed during the first 5 days of Run 3.

The OMTC did not decrease over time during the EMBB experiment with contained liquid membrane (Run 8) and remained almost constant at a value higher ($2.1 \times 10^{-5}$ m/s) than those measured in the single-tube EMB and EMBB. This confirms that MABs did not form in the EMBB with contained liquid membrane. In this system, (i) high shear stress at the biomedium/membrane interface, obtained by recirculating the biomedium at high flow rate in the membrane tube ($Re_b=9000$ on the biomedium side, corresponding to a liquid velocity of 3 m/s and a shear stress on the membrane wall of 170 N/m$^2$), and (ii) a biphasic biomedium, which provides a mobile support in the form of liquid droplets as a preferential alternative to the membrane walls for biomass adhesion, are effectively combined to avoid formation and accumulation of MABs.

Conclusions

The evolution over time of MAB thickness and organic substrate mass transfer coefficient, measured in a lab-scale single-tube EMB when varying operating conditions and using either a monophasic or a biphasic biomedium, showed that the separate use of high shear stress at the biomedium/membrane interface and a biphasic biomedium (providing a mobile support for microbial adhesion), is not effective in avoiding or reducing MAB formation and accumulation. However, these experimental trials, led to the successful design of a novel Extractive Liquid Membrane Biphasic Bioreactor which was operated without any membrane-MAB formation or reduction in organic substrate flux across the membrane.
References


