Effect of Medium Composition and System Operation on Membrane-Attached Biofilm Morphology Alteration and System Performance in Membrane Bioreactor

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ABSTRACT

The formation and accumulation of membrane-attached biofilm (MAB) in membrane processes can prove to be detrimental to process performance due to mass transfer limitation. This effect demands countermeasures to minimize its accumulation. In this research, the influence of medium composition and system operation on morphology alteration of MAB and consequently their influence on mass transfer rate and overall system performance was investigated in a lab - scale extractive membrane bioreactor used for the extraction and degradation of phenol. The factors investigated included dilution rate, ammonium concentration, iron concentration and phenol feed concentration. The continuous feed of the growth medium and the change in dilution rate produced thinner but stronger and more controllable biofilm than that observed in a semi-batch experiment. Washout of the suspended cells was achieved by increasing the dilution rate to a value of 0.03 h^1 and from this moment on, visible cell growth occurred only within the biofilm. Complete absence of iron from the growth medium affected biofilm morphology whereas a decrease in ammonium concentration did not. However, in both cases phenol degradation efficiency was not affected. A feed concentration of 5 g/l resulted in large - scale detachment of biofilm but detached cells were well adapted to high phenol concentration environment and kept their ability to degrade transferred phenol. It is, therefore, concluded that this system is very robust to alteration in the nutrient feed and that once the biofilm is formed, alteration in its morphology and thickness did not have a significant effect on the system performance.

Key words: Membrane-attached biofilm, Membrane bioreactor

INTRODUCTION

Membrane bioreactors are applied for a variety of purposes, including waste gas and waste water management. During operation of these systems, biofilms may develop at the membrane/biomedium interface and participate in the separation/degradation processes. 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 (Flemming et al., 1996). In membrane processes, the surface properties of membranes may promote bacterial adhesion. The formation of membrane - attached biofilms (MABs) is documented in membrane processes such as extractive membrane bioreactors, EMB (Livingston, 1995a, 1995b) and bubbleless membrane bioreactors (Brindle and Stephenson, 1996). In these processes, MABs can prove to be detrimental to the process performance due to mass transfer limitation (Nicolella et al., 2000a). These effects demand countermeasures (Flemming et al., 1996) such as significant membrane pre-treatment costs or sophisticated process design (Nicolella et al., 2000b; Splendiani et al., 2003) and downtime for membrane cleaning or replacement (Flemming et al., 1996). The control of cell surface hydrophobicity as an important element in biofilm formation and growth in the membrane bioreactor has been reported elsewhere (Kuntiya et al., 2005).

In this work, the influence of medium composition and system operation on morphology alteration of membrane-attached biofilm and consequently its influence on mass transfer rate and overall system performance were investigated in a membrane bioreactor operating in a continuous mode. The aim is attempting to control the operating condition to minimize biofilm growth in a membrane process.

MATERIALS AND METHODS

Bacterial strain and inoculum preparation The microorganism used in this work was Pseudomonas spp. isolated collected from a soil sample in Thailand. The bacterium, which grows on phenol as a carbon source, was maintained at 4°C on nutrient agar slopes, supplemented with 0.2 g/l phenol, in order to prevent possible plasmid loss. To prepare an inoculum, the bacterium was grown in nutrient broth supplemented with 0.2 g/l phenol at ambient temperature (30-35°C) with orbital shaking (100-110 rpm) for 18-20 hours. Bacterial biomass was then harvested (16,000g, 10min, 4°C), washed once with and suspended in sterile distilled water giving an OD of 0.9 at 600 nm when determined spectrophotometrically before being used.

Mineral salt medium The mineral salt medium used was the same as that compounded by Ralston and Vela (1974) for detecting phenol - degrading bacteria, but bromothymol blue was not included. The composition of the medium was (g/l): $NaHCO_3$ 0.125; KH_2PO_4 0.1; NH_4Cl 0.07; Na_2SiO_3 0.02; $FeSO_4.7H_2O$; $MnCl_2.4H_2O$) 0.007; $ZnSO_4$. $7H_2O$ 0.0015; casamino acid 0.01. The only source of carbon supplied to the cells was phenol, transferred through the membrane. For the study on the influences of ammonium and iron concentrations on biofilm morphology and behavior, mineral medium with 0.0016 g/l ammonium chloride was fed to the system. The effect of iron was investigated by simply omitting iron sulfate from the medium.

Apparatus and experimental setup The membrane bioreactor used in this study, schematically represented in Figure 1, consisted of a single silicone rubber tube (3 mm i.d;

0.3 mm wall thickness and 200-240 mm length) held in a glass column (15 mm i.d., 300 mm length). The annular region outside the membrane was connected to a jacketed stirred-tank bioreactor, F (1L, Lh fermentation, UK). The nutrient solution coming from the fermentor (F) was recirculated on the shell side of the membrane reactor and the phenol solution from the feed vessel (PT) was recirculated inside the hollow membrane. The two flows were co-current. A pH controller (Anglicon Solo2) held the pH on the fermentor side at 7.8-8.2 via an addition of acid or alkali as necessary. The fermentor loop temperature was held constant at 30°C by running warm water from a water bath (Grant) through the jacket. Agitation was at 150 rpm (Lh fermentation agitator 502D) with aeration sufficient to maintain 6-7 mg/l dissolved oxygen (DO) in the fermentor. For continuous mode of operation, the mineral salt medium (MM) and feed outlet (Fo) were connected to the bioreactor by a multichannel pump (1).

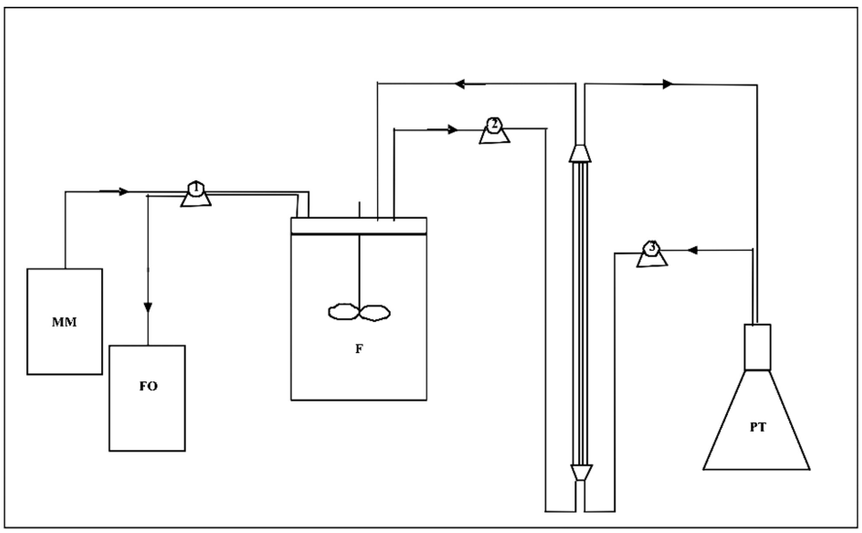


Figure 1. Simple schematic representation of the membrane bioreactor system. MM = mineral medium tank; FO = bioreactor outlet; F = bioreactor; PT = phenol feed tank; (1) = multichannel pump; (2), (3) = gear pump.

The continuous mode of operation was continued from the semi-batch operation (Kuntiya et al., 2005) by switching to a continuous supply of fresh mineral salt medium to the fermentor through a multi-channel peristaltic pump (pump 1 in Figure 1) and allowing medium to overflow. The flow rate of the pump was varied during the experiment. After inoculation of the bacteria in the fermentor, regular samples were taken from both sides of the reactor in order to obtain measurements of the biomass concentration in the fermentor, phenol concentration in the fermentor and in the feed vessel. The cell concentration and phenol and biomass concentrations were also measured in the samples taken from the fermenter outlet. For the study on the effects of ammonium and iron on growth and the performance of biofilm, analyses of ammonium and iron were also carried out on these outlet samples. Sampling and liquid volume restoration in the feed flask were done in the same manner as in semi-batch operation (Kuntiya et al., 2005). The effect of phenol feed concentration on biofilm/system

performance was also investigated by stepwise increase of the feed concentration up to 5 g/l. Table 1 summarizes the induced changes made to the system.

Table 1. Changes in dilution rate, medium composition and feed concentration (continuous experiment).

Condition	time period (hour)
Semi-batch experiment	10-49
Effect of dilution rate	
D = 0.03 h^{-1}	78-216
D = 0.06 h^{-1}	240-244
D = 0.015 h^{-1}	264-504
Effect of medium composition	
Ammonium	810-1,402
Iron	1,406-1,642
Operation under standard medium	1,552–2,483
Effect of phenol concentration in feed	
1.5 g/l	1,644-1,810
3 g/l	1,836-2,026
5 g/l	2,028-2,483

D = (F + R)/V, where D is the dilution rate; F is the flow rate from mineral medium tank, R is the return flow rate from the shell side of the membrane module, and V is the bioreactor volume. The volumes of piping and membrane tube were neglected in calculating V.

Phenol assay Phenol concentration in the bioreactor and in the feed vessel was measured by a colorimetric method based on the condensation of aminoantipyrine (Greenberg et al., 1992).

Ammonium assay Ammonium consumption was monitored through analysis of the outlet flow from the fermentor. The determination of the ammonium concentration in these samples was made by a colorimetric method known as the "phenate blue method" or the "indophenol blue method".

Iron assay The iron concentration of the samples was analyzed through flame spectrophotometry.

Cell density assay Suspended biomass concentration was determined by a spectrophotometer (PYE UNICAM PU 8600 UV/VIS, Phillips). The absorbance of samples was measured at 600 nm and the biomass concentration was calculated from a standard calibration curve.

Images Pictures of the biofilm were taken using a digital camera (Camedia, Olympus C-2500L).

Overall mass transfer coefficient (OMTC, Kov) determination The overall apparent mass transfer coefficient (K_{ov}) was calculated from equation : $N = K_{ov} (2 \pi r_i) (C_f - C_b)$, where N is the mass transfer rate per unit length, $g.m^{-1}s^{-1}$; K_{ov} the overall mass transfer coefficient; r_i the internal radius of the membrane tube, m; and C_f the phenol concentration in the wastewater, g/m^3.

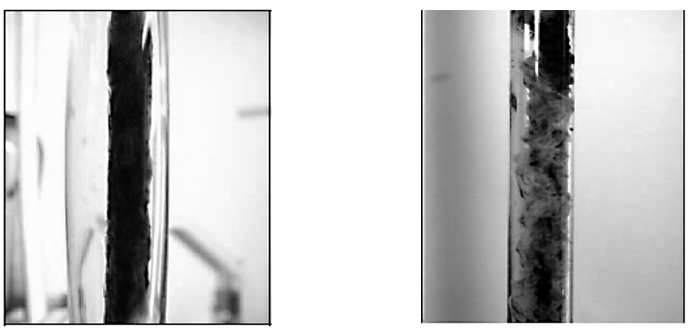
RESULTS

Biofilm morphology alteration

The change in biofilm morphology in response to a continuous mode of operation, medium composition and phenol feed concentration are shown in Figure 2, Figure 3 and Figure 4, respectively. The continuous addition of mineral medium produced the thinner, stronger and more controllable biofilm (Figure 2). Complete absence of iron affected biofilm morphology whereas a decrease in ammonium did not (Figure 3). A feed concentration of 5g/l resulted in a large - scale detachment (Figure 4).

(a) (b) (c)

Figure 2. Different stages of biofilm formation and its morphology alteration.
(a) fragile waving newly-formed biofilm in a semi-batch system (3d), D = 0.03 h^{-1}
(b) biofilm with random detachment in a continuous system (10d), D = 0.015 h^{-1}
(c) newly-formed white biofilm on top of the membrane tube in a continuous system (17d), D = 0.015 h^{-1}



(a) (b)

Figure 3. Morphological alteration of biofilm as affected by medium composition in a continuous system.
(a) biofilm grown under ammonium limiting for 21 days and non-restoring feed concentration condition for 18 days (55d);
(b) biofilm grown under non-iron condition for 10 days (65d).

(a) (b) (c)

Figure 4. Biofilm morphological alteration in response to the change in feed concentration.
(a) detached biofilm, 75d (feed concentration of 1.5 g/l);
(b) detached and growing biofilm, 84d (feed concentration of 3 g/l);
(c) biofilm with large detachment, 85d (feed concentration of 5 g/l).

Mass transfer coefficient and overall system performance

The change in biomass concentration in response to different dilution rates and mineral medium composition are shown in Figure 5 and Figure 6, respectively. Cell concentration was found to decrease dramatically in response to the dilution rate and wash out was observed (Figure 5). The decrease in ammonium concentration resulted in the sharp fall of biomass concentration and the same level of low biomass concentration was observed in the absence of iron from the medium (Figure 6).

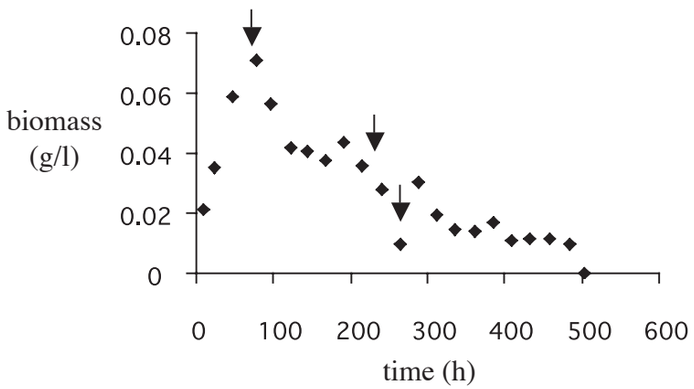


Figure 5. Change in biomass concentration in response to different dilution rates.
Note: Arrows, from left to right, indicate the starting time where the system was operated with D = 0.03, 0.06, and 0.015 h^{-1}, respectively.

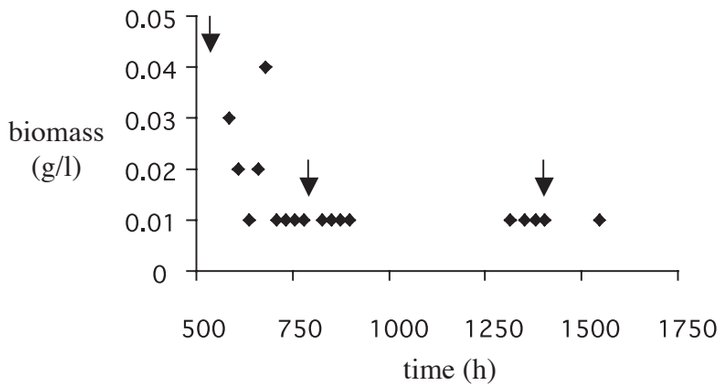


Figure 6. Change in biomass concentration in response to change in mineral medium composition.
Note: Arrows, from left to right, indicate the time where the mineral medium contained ammonium concentration of 0.07 g/l, of 0.0016 g/l, and no iron, respectively.

The overall change in Kov and phenol flux from the time where medium composition was changed until the end of experiment are shown in Figure 7 and Figure 8, respectively. Only minor fluctuation in Kov was observed over time (Figure 7). The flux increased in response to the increase in feed concentration (Figure 8) but no significant change in system performance in terms of degradation efficiency was observed throughout the experiment. Phenol was never detected in the system.

DISCUSSIONS AND CONCLUSIONS

Biofilm morphology alteration

The system was first operated in a semi-batch mode in order to allow growth and formation of the biofilm; the latter was visible on the membrane surface after 3 days of the operation. The biofilm grew slowly and formed a homogeneous, thin, and lightly-yellowish layer with a fragile waving appearance (Figure 2a). The system was then switched to continuous mode of operation by continuously feeding the mineral medium into the bioreactor via a multi-channel peristaltic pump and allowing the medium to overflow. With the continuous addition of the mineral medium, the biofilm continued to develop and to maintain the same appearance; random clearer areas, which then were renewed following biofilm detachment were observed from time to time (Figure 2b). By comparing this appearance to that observed in the semi-batch experiments reported by Kuntiya et al., (2005), this led to the hypothesis that the continuous feed of the biomedium and the change in dilution rate produced a biofilm of different morphology. It looked thinner, stronger and more controllable. Apart from the initial growth during the semi-batch mode of operation to grow the biofilm, no significant change in biofilm thickness was observed when the dilution rate was changed, the estimated thinckness was around 1-1.6 mm.

The change in ammonium concentration in the mineral medium did not produce any observable variation in biofilm morphology. The biofilm retained its colour and appearance which supported the idea that the ammonium was not limiting. However, during days 37-55, the phenol concentration in the feed was not restored, but allowed to decrease until it reached approximately 0.35 g/l, and a change in biofilm structure was observed. At this time, the system had been operated with D 0.015 h^{-1} for 45 days and ammonium in the mineral medium had been reduced from 0.07 g/l to 0.0016 g/l for 21 days. The biofilm thickness increased with a darker colour, which might be due to the lack of carbon source or ageing of the biofilm. Interestingly, when the phenol feed concentration was restored on day 56, a new white and cotton-like biofilm started to form above the old dark one (Figure 2c), suggesting that phenol limitation had caused the change in biofilm structure.

Since no stoichiometric data were available for iron consumption, its effect on biofilm morphology was studied by simply removing it from the mineral feed. Medium without iron was supplied to the system at D = 0.1 h^{-1}, for 6 days (days 59-65), during which the biofilm appeared to be very thin and turned black in colour (Figure 3a), which was probably due to the lack of iron and again ageing of the biofilm. Surprisingly, when iron was restored to the system, the new white biofilm formed above the old dark one still kept growing (Figure 3b). Samples taken during this period showed that the iron concentration in solution was negligible, leading to the conclusion that the system was already working at or close to limiting iron concentration. From these preliminary observations on the effect of medium composition on the biofilm, it was concluded that medium composition had an influence on biofilm morphology and probably on its consequent behaviour. More studies are therefore required to clarify the role of the two nutrients, ammonium and iron, on biofilm morphology and its uptake by *Pseudomonas* spp. used in this research.

The study on the effect of phenol feed concentration on biofilm morphology was started by first increasing it from 0.75–1.5 g/l during days 68-75. The increase in feed concentration of this magnitude caused no effect on biofilm morphology except on day 75 when detachment was observed (Figure 4a). This increase in feed concentration was not believed to be the reason for the detachment as, for example, in the semi-batch experiment where a feed concentration up to 2.2 g/l (Kuntiya et al., 2005) was used without causing any problem to the system. It could again be the nature of this *Pseudomonas* biofilm which appeared to be fragile which led it to detach after a certain time of growth. A further increase in feed concentration to 3 g/l from day 77 to day 84 caused no significant change to biofilm morphology and its thickness still increased (Figure 4b). However, when the feed concentration was increased to 5 g/l on day 85, large portions of biofilm were released to the flowing biomedium, leaving some part of the membrane surface almost clean (Figure 4c). This detachment was probably due to stresses induced by phenol toxicity, which had been known to attack cell membranes, or it might influence the production of exopolysaccharide necessary for biofilm integrity. The detachment when the biofilm was exposed to high phenol concentration might be also related to the sloughing process, described by Peyton and Characklis (1993). Detachment was accompanied by a consequent increase in biomedium turbidity. Under this induced stress, the performance of the system was still not affected and phenol was never detected in the bioreactor. Biofilm cells released from the membrane surface were proved to be viable and were probably a mutant which had adapted to different environmental conditions (such as nutrient starvation, and high phenol feed concentrations).

Mass transfer coefficient and overall system performance

Considering the specific growth rate obtained from shake - flask batch experiment using mineral salt medium (= 0.04 h^{-1}, data not shown), the first dilution rate of 0.03 h^{-1} was used, as dictated by the size of tubing and the pump used. This dilution rate was slightly lower than the 'predicted' wash out dilution rate, 0.04 h^{-1}. The system was operated with this dilution rate for 7 days, a period in which the suspended cell concentration in the bioreactor fell to 0.04 g/l. The dilution rate was then increased to 0.06 h^{-1}, by which after 1 day of operation, the cell concentration was found to decrease dramatically (Figure 5). This was expected since the dilution rate used was well above the predicted wash out value. The final dilution rate (0.015 h^{-1}) was operated for 11 days, a period in which cells in the bioreactor did not re-establish their concentration. The cell concentration continued to decrease until no cells were detected in the bioreactor apart from instantaneous peaks which could be observed at certain times, probably as a result of biofilm detachment. These observations showed that cellular growth and phenol degradation occurred only within the biofilm in this system operating under the described condition.

When ammonium in the medium was reduced from 0.07 g/l to 0.0016 g/l, the biomass concentration fell sharply (Figure 6) due to the low level of nitrogen source for growth. The same level of low biomass concentration was observed in the absence of iron from the medium. No significant change in system performance in terms of phenol degradation efficiency was observed when ammonium was reduced from 0.07 g/l to 0.0016 g/l, and iron was not incorporated into the biomedium. Phenol was never detected in the bioreactor.

Minor fluctuation in Kov was observed throughout the experiment (Figure 7). As the system was operated non-stop, changes in medium composition and operating condition as summarized in Table 1, including biofilm age, may have synergistic influences on Kov. There was no dramatic change of flux during the first 1,000 hours of operation, and the increases in flux at hours 1,644, 1,836, and 2,028 were the result of increasing the feed concentration to 1.5, 3, and 5 g/l, respectively (Figure 8).

In an overview of overall changes in Kov and phenol flux, it can be noted that even with the many changes made to the system, there was no dramatic change in the overall mass transfer coefficient even though the flux did change dramatically. It is, therefore, concluded that this system is very robust to alteration in the nutrient feed and that once the biofilm is formed, alteration in its morphology and thickness did not have any significant effect on the system performance. The biofilm grew back to its original appearance as the phenol concentration in feed decreased. Phenol removal was stable throughout the experiment without any degradation problem due to biofilm formation.

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