<table>
<thead>
<tr>
<th>Title</th>
<th>Studies on the effect of concentration of a self-inhibitory substrate on biofilm reaction rate under co-diffusion and counter diffusion configurations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Authors(s)</td>
<td>Syron, Eoin; Kelly, Hugh; Casey, Eoin</td>
</tr>
<tr>
<td>Publication date</td>
<td>2009-06-15</td>
</tr>
<tr>
<td>Publication information</td>
<td>Journal of Membrane Science, 335 (1-2): 76-82</td>
</tr>
<tr>
<td>Publisher</td>
<td>Elsevier</td>
</tr>
<tr>
<td>Link to online version</td>
<td><a href="http://dx.doi.org/10.1016/j.memsci.2009.02.038">http://dx.doi.org/10.1016/j.memsci.2009.02.038</a></td>
</tr>
<tr>
<td>Item record/more information</td>
<td><a href="http://hdl.handle.net/10197/2758">http://hdl.handle.net/10197/2758</a></td>
</tr>
<tr>
<td>Publisher's statement</td>
<td>All rights reserved.</td>
</tr>
<tr>
<td>Publisher's version (DOI)</td>
<td>10.1016/j.memsci.2009.02.038</td>
</tr>
</tbody>
</table>
Studies on the effect of concentration of a self-inhibitory substrate on biofilm reaction rate under co-diffusion and counter diffusion configurations

Eoin Syron, Hugh Kelly and Eoin Casey*

School of Chemical and Bioprocess Engineering, University College Dublin, Ireland

*Corresponding Author: Eoin Casey, School of Chemical and Bioprocess Engineering, Engineering and Material Science Centre, University College Dublin, Belfield, Dublin 4, Ireland

E-mail: eoin.casey@ucd.ie
Telephone: +353 1 7161877
Fax: +353 1 7161177
Abstract

A simple mathematical model was developed to investigate the utilization rate of a self-inhibitory substrate in idealised biofilm reactors operating with either counter-diffusion or co-diffusion of oxygen and phenol. This study has implications for the development of membrane-supported biofilm technologies, such as the membrane-aerated biofilm reactor. An unsteady-state formulation of the model was used to investigate the effect of shock loads of phenol on biofilm performance. It was found that the counter-diffusion configuration may be advantageous under high phenol concentrations provided the biofilm thickness is above a critical value. The performance advantage of the counter-diffusion configuration is gained by the presence of an oxygen depleted layer, adjacent to the liquid-biofilm interface which acts as a diffusive barrier to phenol transport to the region of respiratory activity.

Keywords: biofilm; phenol; aeration; membrane; model; shock-load, inhibition
Introduction

There is an increasing interest in the membrane-aerated biofilm reactor (MABR) as a technology that can enhance the application of biofilms in wastewater treatment (33). In the MABR, the biofilm is naturally immobilized on an oxygen permeable membrane, counter-diffusion of oxygen and nutrients takes place creating a distinct stratification of respiratory activity within the biofilm. Several investigators have reported performance advantages of MABRs for simultaneous COD oxidation, nitrification, and denitrification (15, 30, 35, 39), high oxygen utilisation efficiencies (22) and high specific organic reaction rates (3, 21). Much of the recent research has focused on nitrogen removal (11, 17), however there is also interest in the use of the MABR for the aerobic treatment of xenobiotics. MABRs are viewed as particularly favourable in this context because bubbleless operation minimizes the air-stripping of compounds with high Henry’s law constants such as xylene (10) or acetonitrile (18). The MABR is also of interest because the creation of and ease of manipulation of a defined oxic/anoxic micro-environment can be advantageous for the degradation of compounds with problematic intermediates such as perchloroethylene (21).

To-date there have been no reports of the effect of substrate inhibition on the performance of the MABR. However, in an analogous reactor system, the extractive membrane bioreactor, for degradation of high strength phenol containing wastewater, it was observed (8) that the degradation ability of the biofilm was enhanced in comparison with those using freely suspended or Ca-alginate bead-immobilized cells, where substrate inhibition started to occur at relatively low phenol levels.
Mathematical models have previously been developed for the analysis of MABRs under steady-state and transient growth conditions (12, 19, 25, 32, 34, 36). The investigation of perturbations in nutrient concentration has only been recently reported (34), however shock loads of self-inhibitory carbon sources in a MABR have not been previously investigated and is the subject of the present article. Here, we investigate if (and under what conditions) the MABR could show superiority over conventional fixed film reactors in dealing with such pollutants, particularly under shock loading conditions.

In this study we selected phenol as a model pollutant because it is a common constituent in many industrial wastewaters, particularly petrochemicals. Phenol is toxic to fish at concentrations as low as 5-25 mg/L (28). Biological treatment of phenol containing wastewater is possible, but it inhibits the growth rate of those species that have the metabolic capability of using it as a substrate for growth (29). Shock loads of phenol containing wastewater have been responsible for severe disruptions in conventional industrial biological treatment facilities (14).

The objective of the present study was to develop a mathematical model to investigate the performance of a MABR under both steady-state and shock loading conditions with a self-inhibitory substrate (phenol). In the MABR phenol and oxygen are co-limiting substrates supplied from opposite sides (counter-diffusion). The model was adapted to also consider co-diffusion whereby oxygen and phenol are transported across the biofilm-liquid interface as would be the case in a conventional fixed film system. In this article, for convenience, we refer to this class of reactors as the conventional aerated biofilm reactor (CABR).
METHODS

A mathematical model was developed to consider diffusion and reaction of two co-limiting substrates, phenol and oxygen which are supplied from opposite sides of the biofilm in the MABR or from the same side in the case of the CABR.

Model assumptions

- The structure of a biofilm plays a major role in the mass transfer and reaction processes that occur therein. The heterogeneous structure of many biofilms permits convective transport within voids and water channels, whilst within the cell aggregates or clusters molecular diffusion is the predominant mode of mass transport (9). In the present work, as in most 1-dimensional biofilm kinetic models (24, 27), it is assumed that the biofilm is homogeneous, and that the mass transfer through the mass boundary layer and within the biofilm is diffusional and perpendicular to the biofilm surface. The reason is that spatially structured biofilm models present formidable modeling complexities (38).

Mass transport of substrates inside the biofilm is described using effective diffusion coefficients, assumed to be a fraction of their values in water. Here we use an empirical correlation (13) that relates biofilm density to the effective diffusion coefficient. With regard to the assumption of diffusional transport, it has been noted (20) that the limits of this assumption are expected to be of particular concern during the initial phase of biofilm growth, when only a part of the membrane will be covered by patches of biofilm, and heterogeneity will play a significant role in the description of the system.
In the case of thick biofilms, the role of heterogeneity will be less important, and the thickness of the biofilm will on average dampen the effect of local discontinuities and irregularities on the global behavior of the system.

- Reaction rates are described by an unstructured kinetic model that includes dual substrate limitation by phenol and oxygen and inhibition by phenol.

- The time variation of the biofilm thickness is slower than for reaction within the biofilm and while the model can be used to simulate different biofilm thicknesses, it does not explicitly include biofilm growth or decay processes within the model. This assumption is justified by comparing the time scale of biofilm growth (days) to the timescale for hydraulic retention time (hours), and as a consequence, time variations in the biofilm are neglected. Some other biofilm processes, such as consolidation(5), occur at a slower rate and may be important in describing long term operation, but, at present this aspect of biofilm behavior is restricted to complex spatially structured models(1).

- It is assumed that the MABR is operated at high wastewater recirculation flow rates, so that substrate concentration can be assumed uniform in the membrane module. As such, it is assumed that there are no axial gradients in substrate concentration in the membrane module and the biofilm thickness is uniform.
The effects of membrane module design are not considered. Such effects could include long-range interactions for example caused by the presence of neighboring fibers in a hollow-fiber membrane module. Although biofilm models have been developed to consider these types of processes, (4, 16), the assumption seems justifiable since full scale MABR design are insufficiently developed at present.

There is no diffusional resistance on the gas side of the membrane and this assumption is considered reasonable based on measurements taken in our laboratory for silicone, hollow fiber based designs(26).

pH is assumed uniform throughout the biofilm.

Metabolic lag, i.e. the delay that occurs before there is a change in the metabolic state of a microorganism in response to changes in local substrate conditions was not described in the present model. Reports of metabolic lag have tended to focus on transient effects where there is a change between starvation conditions and nutrient rich conditions e.g. (23). In the present model we simulate responses between two different bulk phenol concentrations. Incorporation of the lag effect, using an approach described by (37), could be implemented in future versions of this model but this would add much additional complexity.
**Model Equations**

A differential unsteady-state mass balance on phenol gives;

\[
\frac{\partial S}{\partial t} = D_{\text{eff},S} \frac{\partial^2 S}{\partial y^2} - \left( \frac{X \mu}{Y_{X/S}} \right)
\]

A differential unsteady-state mass balance on oxygen gives;

\[
\frac{\partial O}{\partial t} = D_{\text{eff},O} \frac{\partial^2 O}{\partial y^2} - \left( \frac{X \mu}{Y_{X/O}} \right)
\]

Where \( S \) and \( O \) are the spatial average concentrations of phenol and oxygen respectively.

**Boundary Conditions (MABR)**

At the liquid-biofilm interface (\( y=L \))

\[
\frac{\partial O}{\partial y} \bigg|_{y=L} = \frac{k_i L}{D_O} \left[ O_{B,L} - O_{\text{bulk}} \right]
\]

\[
\frac{\partial S}{\partial y} \bigg|_{y=L} = \frac{k_i L}{D_S} \left[ S_{L} - S_{\text{bulk}} \right]
\]

The boundary condition for oxygen at the biofilm-liquid interface allowed transport of oxygen into the bulk liquid in cases where oxygen was not fully depleted within the biofilm. The bulk liquid oxygen concentration \( (O_{\text{bulk}}) \) was set at zero which is reasonable for most cases because oxygen becomes fully depleted within the biofilm. In practice, for MABRs, it is desirable for this to be the case as it ensures high oxygen transfer efficiencies, one of the main advantages of the MABR.

Two possibilities were considered for phenol at the biofilm-membrane interface. In the first case it was assumed that the membrane was impermeable to phenol, in which case \( k_M \)
was zero. Alternatively, the possibility of phenol diffusion through the membrane was considered which is possible in an open ended membrane lumen, particularly if the biofilm is thin.

\[
\frac{\partial S}{\partial y} \bigg|_{y=0} = \frac{k_{yL}}{D_{yf,S}} \left[ S_{BO} - S_M \right]
\]

\[
O \bigg|_{y=0} = O_M
\]

**Boundary Conditions (CABR)**

\[
\frac{\partial S}{\partial y} \bigg|_{y=L} = \frac{k_{yL}}{D_S} \left[ S_{BL} - S_{bulk} \right]
\]

\[
O \bigg|_{y=L} = O_{bulk}
\]

**Reaction kinetics**

Dual substrate kinetics are assumed with an inhibition term for phenol according to a previously described model (7)

\[
\mu = \mu_{\text{max}} \left( \frac{S}{S + K_S + S^2 / K_i} \right) \left( \frac{O}{K_a + O} \right)
\]

**Shock loads**

The boundary conditions for phenol were modified to simulate a shock load. The Heaviside function \((H)\) was applied such that at \(t > 0\) a sudden increase in the bulk phenol concentration was imposed. The Heaviside function is defined as \(H(x) = 1\), if \(x \geq 0\), \(H(x)\)
\( = 0 \) for \( x < 0 \). The initial conditions for the shock load simulation were the steady state

values of \( O \) and \( S \)

\[ O = O_{ss}, \quad S = S_{ss}, \quad t = 0 \]

**Solution**

The equations and boundary conditions were solved in MatLab (MathWorks Inc, Massachusetts, USA) using a solver for initial-boundary value problems for parabolic-elliptic partial differential equations in 1-dimension. The sources of all parameters used are shown in Table 1. The solution generates the concentration profiles and from these profiles the substrate fluxes at the interfaces were calculated using finite differences.

**RESULTS**

In membrane-aerated biofilms, the organic substrate diffuses into the biofilm from the wastewater, while oxygen is supplied across the membrane-biofilm interface. The potential rate limiting steps within the biofilm are: (i) diffusional mass transfer of oxygen; (ii) diffusional mass transfer of organic substrate; (iii) utilization rate. Reaction takes place in a region of the biofilm where both substrates are present simultaneously. Figure 1 illustrates representative steady-state concentration profiles of phenol and oxygen in a MABR under two different conditions corresponding to air and pure oxygen. It is apparent that, for the conditions selected, a significant portion of the biofilm is oxygen depleted. It is a unique characteristic of MABRs that the oxygen depleted layer adjacent to the biofilm-liquid interface acts as a diffusive barrier to the carbon source. In the context of a self-inhibitory
substrate this effect can be exploited in the MABR to reduce the apparent concentration of the carbon source in the reaction zone. Figure 1 also shows dimensionless activity, defined as the ratio between the predicted average growth rate for a given set of conditions and the maximum possible growth rate if all substrates are available in excess. As the oxygen concentration at the membrane interface increases, the region of maximum respiratory activity, shown here by the dimensionless activity profile, shifts towards the biofilm liquid interface. Under oxygen limited conditions, activity is located adjacent to the membrane and the anoxic layer adjacent to the liquid acts as a diffusive barrier to phenol.

A characteristic of counter-diffusion biofilm systems, such as the MABR, is the decline in substrate utilization rate as thickness increases. This effect has been previously observed in experimental systems (6) and by mathematical modelling (31). In Figure 2 this effect is apparent for a representative set of conditions (50mg/L and an oxygen concentration of 8 mg/L). The results in this figure show the overall utilization rate for a given overall biofilm thickness rather than the utilization rate with a particular depth of the biofilm. At low biofilm thicknesses, phenol utilization rate is limited only by the quantity of biomass present for both the MABR and CABR and there are no apparent differences in phenol utilization rate. However if the membrane is permeable to phenol then the rate of phenol transfer into the biofilm comprises both utilization and transfer into the membrane lumen as can be seen by the total transfer rate curve in Figure 2. In the case of the MABR, there is a critical thickness, above which, the region of respiratory activity moves away from the membrane interface and the flux of phenol through the membrane diminishes significantly. In the CABR oxygen limitation commences at a biofilm thickness of
approximately 50\(\mu\)m, for the conditions simulated in Figure 2 and when the thickness exceeds this value, no further increases in phenol utilization rate occurs. It should be noted that for the simulation in figure it was assumed that that steady state biofilm thicknesses of up to 500\(\mu\)m are achievable for these conditions.

The effect of phenol concentration on utilization rate is shown in figure 3 at a bulk oxygen concentration of 8 mg/L and for fixed biofilm thicknesses of 100\(\mu\)m, 300\(\mu\)m and 500\(\mu\)m. It is clear that there are differences between the CABR and MABR in terms of the effect of biofilm thickness. The CABR shows superior performance at low bulk concentrations of phenol, but as the bulk phenol concentration rises above 50mg/L, phenol inhibition reduces the utilization rate. Above a biofilm thickness of approximately 100 \(\mu\)m, thickness has no effect on CABR performance. In contrast, the MABR performance peak is dependent on both biofilm thickness and phenol concentration. As the biofilm thickness increases, the maximum phenol utilization rate is achieved at progressively higher bulk liquid concentrations. Above the peak value, the performance of the MABR depends on the permeability of the membrane to phenol.

The simulation results in Figure 4 illustrate the differences between a membrane that is impermeable or permeable to phenol. If phenol is transferred through the membrane, the flux of phenol through the biofilm can act to minimize the inhibitory effect of phenol at high concentrations. The effect of intramembrane oxygen pressure is also shown in figure 4 and it can be seen that at high bulk phenol concentrations, high utilization rates can be achieved by operating at a higher intramembrane oxygen pressure.
The effects of a transient phenol shock load is shown in Figure 5 for a representative set of conditions. Here the initial conditions were set to allow meaningful comparison between the CABR and MABR, i.e. the bulk liquid concentration was set at a value which gives the same phenol removal rate at a biofilm thickness of 500μm and under identical oxygen concentrations at the respective boundaries. The shock load represents a step increase in the bulk phenol concentration from 240 mg/L to 1000 mg/L for a duration of 100 seconds.

The shock load results in a 40% increase in the phenol utilization rate in the MABR and a 40% decrease in the utilization rate in the CABR. In both cases there is a dramatic increase in the availability of phenol, however in the case of the CABR the concentration of phenol in the active region is close to the bulk concentration and at a level which is severely inhibitory. In the MABR, because of the diffusional resistance to transport of phenol, the actual concentration of phenol in the reaction zone is significantly less than that in the bulk and the increased availability of phenol results in an increased reaction rate. It should be noted that in the MABR a significant fraction of the additional phenol supplied as a result of the shock load could potentially be transferred through the entire biofilm into the membrane lumen. The extent of this transfer is dependent primarily on the mass transfer resistances of both the membrane and the biofilm, the latter resistance is controlled by the thickness and the effective diffusivity of the biofilm. The recovery time, i.e., the time taken to return to steady state value is noticeably longer in the MABR than the CABR due again to the location of the active zone within the biofilm. When the bulk phenol returns to normal levels, the transfer of excess phenol in the membrane aerated biofilm to the bulk liquid is retarded by a layer of biofilm that adds a significant mass transfer resistance.
DISCUSSION

Biological treatment of phenolic wastewater has been a subject of significant research effort over the past number of years. Although physico-chemical methods are widely used for the treatment of phenol, those methods have been criticized for reasons of cost and the tendency for formation of secondary toxic materials such as chlorinated phenols (2). One of the most problematic areas in biological treatment of phenol relates to shock loads whereby the microorganisms suddenly experience levels of phenol that are severely inhibitory. In most biological treatment systems, including activated sludge and the various fixed film processes, the phenol concentration in the bulk liquid is at a similar level to that in contact with the phenol degrading micro-organisms. In contrast, due to spatial stratification of microbial activity in membrane-aerated biofilms, this is not necessarily the case. Provided the biofilm thickness is sufficiently high, an oxygen depleted layer of the biofilm acts as a diffusive barrier to phenol and consequently the phenol concentration in the active region of the biofilm is significantly lower than the bulk liquid. It is important to highlight, when making comparisons between the two different systems, that under otherwise identical operating conditions, the membrane-aerated and conventional-aerated biofilms would, not necessarily have the same biofilm thicknesses under practical conditions. The simulation of biofilm growth is beyond the scope of the present study, but nevertheless, this point should be considered when evaluating the potential benefits of the MABR in this context.
An often stated advantage of the MABR is their ability to minimise stripping of volatile organics compounds (VOCs) during aerobic treatment. This may be the case under optimised conditions, however if the biofilm thickness is at a suboptimum level or if a shock load is encountered, it is likely that a significant quantity of VOC will be transferred into the membrane lumen. If the membrane lumen is open-ended, as is often the case in MABRs, stripping of the VOC is possible. In the simulations presented in this study we considered scenarios where the membrane was either permeable or non-permeable to phenol. As can be seen from Figures 2, 4 and 5, the effect of phenol transfer through the membrane can be significant and should be considered in future experimental studies.

The results of the simulations presented in Figure 5 show that the response of the MABR to a shock load is predicted to be significantly different to that of a conventionally aerated biofilm. The comparative difference clearly depends on the magnitude and duration of the shock loading. In this work we simulated a short shock loading period where the duration of the shock is of the same time-scale as the utilization rate of phenol and significantly shorter than the hydraulic retention time. Shock loadings of longer duration, of the same timescale as the hydraulic retention time, are expected to have an effect similar to the steady state solution. Consideration for adaptation of the microorganisms to higher phenol concentrations during extended or repeated shock loads must be considered in practical systems.

The results in figure 5 show that, following the step change in bulk phenol concentration a significant fraction of the additional phenol is transferred through the biofilm and into the membrane lumen. The phenol transfer rate exceeds its utilization rate.
for a duration of approximately 100 seconds. While this transfer of phenol to the gas phase may be viewed as undesirable from a practical viewpoint, it actually reduces the phenol concentration in the biofilm and hence partly mitigates the effect of the shock load on growth inhibition.

CONCLUSIONS
The aim of this study was to apply mathematical modelling to compare reaction rates of membrane aerated biofilms to conventionally aerated biofilms when treating a wastewater consisting predominantly of a constituent that is self-inhibitory. In general, the MABR seems to be particularly advantageous, compared to the co-diffusion system, in dealing with high bulk liquid concentrations and in coping with shock loading conditions, provided the biofilm thickness is above a critical value and the phenol concentration is sufficiently high. The performance advantage is gained by the presence of an oxygen depleted layer, adjacent to the liquid-biofilm interface which acts as a diffusive barrier to phenol transport to the region of respiratory activity. The model results are valid provided the biofilm is homogeneous, the liquid compartment is well mixed and that the effects of metabolic lag are negligible. The results presented here are the first that we are aware of that show the merit of the MABR in treating a self inhibitory substrate, and it is expected that the model will serve as a framework that prompts the development of an experimental program to further investigate the ideas presented here.
**Nomenclature**

\[ D_S \] phenol diffusion coefficient in water (m\(^2\) s\(^{-1}\))
\[ D_O \] oxygen diffusion coefficient in water (m\(^2\) s\(^{-1}\))
\[ D_{\text{eff},S} \] effective phenol diffusion coefficient in the biofilm (m\(^2\) s\(^{-1}\))
\[ D_{\text{eff},O} \] effective oxygen diffusion coefficient in the biofilm (m\(^2\) s\(^{-1}\))
\[ k_M \] membrane mass transfer coefficient (m s\(^{-1}\))
\[ K_O \] half-saturation coefficient for oxygen (kg m\(^{-3}\))
\[ K_I \] inhibitory constant for phenol (kg m\(^{-3}\))
\[ K_P \] half-saturation coefficient for phenol (kg m\(^{-3}\))
\[ L \] biofilm thickness (m)
\[ O \] Spatial average oxygen concentration (kg m\(^{-3}\))
\[ O_{\text{bulk}} \] bulk liquid oxygen concentration, CABR (kg m\(^{-3}\))
\[ O_{BL} \] oxygen concentration at the biofilm-liquid interface (kg m\(^{-3}\))
\[ S \] Spatial average phenol concentration (kg m\(^{-3}\))
\[ S_{BL} \] phenol concentration at the biofilm-liquid interface (kg m\(^{-3}\))
\[ S_{BO} \] phenol concentration at the biofilm-membrane interface (kg m\(^{-3}\))
\[ S_{\text{Bulk}} \] bulk liquid phenol concentration (kg m\(^{-3}\))
\[ S_M \] equilibrium phenol concentration in the membrane (kg m\(^{-3}\))
\[ X \] the biomass concentration (biofilm density).
\[ y \] distance in biofilm (m)
\[ Y_{X/S} \] biomass yield coefficient for phenol (kg kg\(^{-1}\))
\[ Y_{X/O} \] biomass yield coefficient for oxygen (kg kg\(^{-1}\))
\[ \mu \] specific growth rate (hr\(^{-1}\))
\[ \mu_{\text{max}} \] maximum specific growth rate (hr\(^{-1}\))
REFERENCES


FIGURE LEGENDS

Figure 1 Representative steady-state concentration profiles of phenol (---), oxygen (---) and dimensionless activity (‘‘) in an MABR with biofilm thickness of 500 μm. Bulk phenol concentration is 50mg/L and oxygen concentration at the biofilm-membrane interface is 8mg/L or 40mg/L corresponding to A and B respectively.

Figure 2 Simulated overall phenol utilisation rates for the MABR (O) and CABR (■) for a range of biofilm thicknesses, a bulk phenol concentration of 50mg/L and an oxygen concentration of 8 mg/L. For the scenario where the MABR membrane is permeable to phenol, the total phenol transfer rate through the biofilm-liquid interface is shown as (●);this includes phenol utilization rate and any additional transfer of phenol into the membrane lumen.

Figure 3 A: Phenol utilisation rates in the CABR as a function of bulk phenol concentration at biofilm thickness of 100μm(■), 300μm(□) and 500μm(□). B: Phenol utilisation rates in the MABR as a function of bulk phenol concentration at biofilm thickness of 100μm(■), 300μm(□) and 500μm(□).

Figure 4 Phenol utilisation rates in the MABR as a function of bulk phenol concentration. In the case of a phenol permeable membrane simulations are shown for either air(■) or oxygen(■).In the case of a phenol impermeable membrane simulations are shown for either air(■) or oxygen(□).

Figure 5 Transient simulation results showing the phenol utilisation rate following a 100 second shock load of phenol at a concentration of 1000 mg/L for both MABR (---) and CABR (‘‘). Prior to and after the shock load the bulk phenol concentration was 240 mg/L. In all cases the oxygen concentration at the respective boundary was 8mg/L and biofilm thickness was 500 μm. For a membrane that is permeable to phenol, the total flux of phenol through the biofilm-liquid interface are shown as (---).