Biological treatment of toluene contaminated wastewater by *Alcaligenese faecalis* in an extractive membrane bioreactor; experiments and modeling

S. N. Mehdizadeh, M. R. Mehrnia, K. Abdi and M. H. Sarrafzadeh

ABSTRACT

Conventional wastewater treatment methods are not efficient in treating wastewaters contaminated with volatile hydrocarbons such as benzene, toluene and xylenes (BTX). The aim of this study is to enhance the efficiency of an extractive membrane bioreactor (EMBR) in treating toluene contaminated wastewater by usage of pure culture of *Alcaligenese faecalis*. Toluene was used as a model of toxic contaminant because of its wide presence in wastewaters contaminated with petrol derivatives. The Haldane kinetic model adequately described the dynamic behavior of the toluene biodegradation by the strain of *A. faecalis* over a wide range of initial toluene concentrations (50–1,000 mg L^{-1}) with kinetic constants $\mu_{max} = 0.066\ h^{-1}$, $k_s = 91.7$ mg/L and $k_I = 278.2$. Overall mass transfer coefficient has been measured and described as resistance in the series model. No biofilm formed on the exterior surface of the membrane; however in previous works the layer of the biofilm on the exterior surface of the membrane acts as a mass transfer resistance. A mathematical model was developed to predict the pollutant concentration profile along the tube side of the membrane modules.

Key words | biodegradation kinetics, extractive membrane bioreactor, mathematical modeling, toluene, wastewater treatment

S. N. Mehdizadeh
M. R. Mehrnia (corresponding author)
M. H. Sarrafzadeh
Group of Biotechnology,
School of Chemical Engineering,
College of Engineering,
University of Tehran,
P.O. Box 11155-4563,
Tehran,
Iran
Email: *mmehrnia@ut.ac.ir*

K. Abdi
Department of Medicinal Chemistry,
Faculty of Pharmacy and Pharmaceutical Research Center,
Tehran University of medical sciences,
Tehran,
Iran

NOMENCLATURE

A	area (m^2)
C	concentration (kg/m^3)
F	wastewater flow rate (m^3/s)
K_{ov}	overall mass transfer coefficient (m/s)
k_s	Monod kinetics half rate concentration (mg/L)
k_I	substrate inhibition constant (mg/L)
L	membrane module length (m)
D_s	diffusivity of toluene in biomedia (m^2/s)
μ	specific rate of reaction (s^{-1})
R_{id}	inner radius of the membrane (m)
R_{od}	outer radius of the clean membrane (m)
R_{out}	outer radius of a membrane with attached biofilm (m)

INTRODUCTION

Water pollution by BTX is a serious problem because these substances are toxic, often carcinogenic for humans, and more soluble than other hydrocarbons in water. The main sources of water pollution by these aromatics are: (1) improper disposal and accidents during transportation in oil refineries and petrochemical plants, (2) storage tanks that release petroleum products such as diesel fuel, lubricating oil and gasoline, and (3) paint and pesticides manufacturers and other chemical industries (Farhadian *et al.* 2008).

There are various chemical, physical and biological methods used to treat organic contaminated wastewaters. Although, among all these technologies, biological methods are the most economical and energy efficient, conventional biotreatment methods suffer from lack of efficiency in remediation of BTX contaminated wastewaters. Since aeration in these processes causes the stripping of these volatile materials and creates air pollution, it appears that EMBR is potentially one of the most acceptable technologies for treating these wastewaters (Livingston 1994). The organic contaminated wastewater is passed over one side of the

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dense membrane, such as silicone or latex rubber which is impermeable to inorganic salts, acid bases (Figure 1). The pH of the wastewater does not affect the performance of the microbial active zone (Livingston *et al.* 1996). Organic molecules diffuse through the membrane and degrade on the other side by microorganisms (Livingston *et al.* 1998).

Mass transfer resistances have a significant role in the transfer rate of organics across the membrane. The resistance-in-series model can finely describe mass transfer across a dense membrane (Brookes & Livingston 1995). This model includes resistances of the liquid films on both sides of the membrane, biofilm resistance and membrane resistance (Livingston *et al.* 1998).

The aims of this study are to obtain kinetic data regarding the growth of a suspended microbial culture on toluene from batch experiments, and to study the biological treatment of toluene contaminated wastewater in an EMBR system.

MATERIALS

Microorganisms

The bacterium *Alcaligenese faecalis* PTCC 1624 (ATCC 8750) in lyophilized form was purchased from the Persian Type Culture collection of the Iranian Organization of Science and Technology. The culture was revived on a solid agar Petri dish and in liquid medium. Stock cultures were then obtained by standard spread plate microbial techniques. Prior to inoculation the cryogenically preserved bacteria were incubated with sterile medium containing casein peptone (10 g/L), yeast extract (5 g/L) and NaCl (10 g/L) in 250 mL Erlenmeyer flasks in order to increase cell density. The preculture was done in an incubator under 25 °C and at 150 rpm by shaking for 24 h. The final cell concentration OD_{600} of the preculture reached 1.28. For bacterial cultivation in shake-flasks and EMBR experiments, the precultured bacterial cells were inoculated in basal medium at inoculum size of 1/20 (v/v).

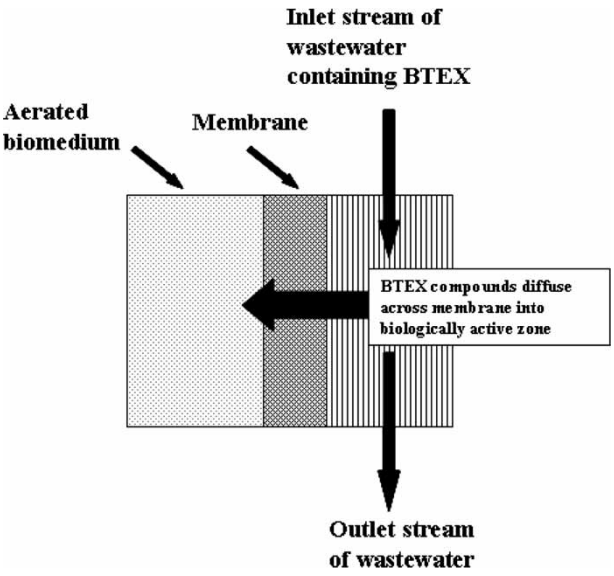

Figure 1 | The operational principle of EMBR (Livingston 1994).

Chemicals and growth medium

All the chemicals, including toluene, were of high purity and obtained from Merck Ltd. The bacteria were grown on a mineral medium prepared with deionised water and toluene as the sole carbon and energy source (Table 1). The concentrated mineral solutions were sterilized separately to avoid precipitation of salts during autoclaving of stock solutions (Table 2).

Table 1 | Composition of the synthetic medium

Component	Concentration (g/L)
K_2HPO_4	0.4
KH_2PO_4	0.2
NaCl	0.1
$MgSO_4$	0.1
$MnSO_4$	0.01
$Fe_2(SO_4)_3$	0.01
Na_2MoO_4	0.01
$(NH_4)_2SO_4$	0.4
Toluene	0.05–1

Table 2 | Concentrated mineral medium

Component	Concentration (g/L)
K_2HPO_4	40
KH_2PO_4	20
NaCl	10
$MgSO_4$	10
$MnSO_4$	1
$Fe_2(SO_4)_3$	1
Na_2MoO_4	1
$(NH_4)_2SO_4$	40

METHODS

Methods of analysis

Optical density of microbial suspension was measured against distilled water as reference at 600 nm using a UV–vis WTW specroflex 6600 spectrophotometer. The calibration curves were obtained by plotting the dry weight of biomass per litre against optical density of the suspension. The chemical oxygen demand (COD) of the synthetic wastewater was measured with Merck bar-coded COD vials. This method is in accordance with EPA Method 410.4 for colorimetric COD measurement.

Gas chromatography was used to measure toluene and hexadecane concentrations. Samples were analyzed on a Varian CP 3800 Gas Chromatograph (GC), which was fitted with a flame ionizing detector and a chrompack capillary column cpsil 8CB (30 m length, 0.53 mm I.D, 1.5 μm F.T). Helium was used as the carrier gas and nitrogen used as the makeup gas. The carrier gas pressure was constant at 10 psi and the flow of the makeup gas (N_2) was 25 mL/min. The injector and detector temperatures were set at 260 and 270 °C, respectively. The air and hydrogen flows were set at 300 and 30 mL/min. The column had an initial temperature of 70 °C, a final temperature of 200 °C and a temperature increase rate of 35 °C/min. Samples were prepared by a liquid–liquid suspended micro-extraction method. Aqueous samples were extracted with *n*-heptane (a 10 mL aqueous sample was stirred by magnetic stirrer and extracted to 100 μL *n*-heptane for 15 min). One μL of *n*-heptane suspended drop was removed and injected to the GC. Output from the GC was recorded on a personal computer to perform peak integration and analysis. Dissolved oxygen was monitored by a DO sensor installed in the bioreactor.

Shake-flasks experiments

In order to model the biodegradation process in EMBRs, it is essential to determine the capacity of the microorganisms for degradation and the degradation kinetics. The possibility of biodegradation of toluene at high initial concentrations (up to 1 g/L) was investigated. The initial concentrations of toluene were varied from 50 to 1,000 mg/L. One control batch without the addition of biomass was performed to determine losses due to volatilization. After 170 h 7.5% of the toluene initial concentration was reduced. For each concentration, 12 flasks were kept in an incubator–shaker at 150 rpm. One sample of 10 mL was taken from each flask. Each batch degradation was carried out in a sterilized 500 mL air-sealed bottle with a Teflon screwed cap containing 100 mL of media. A sufficient amount of headspace was provided to avoid oxygen limiting conditions. After inoculation, each bottle was put on a rotary shaker and maintained at 25 °C. Each bottle was periodically removed from the shaker for aqueous phase sampling. All the transfers were made in a UV chamber, and glass wares and medium were properly autoclaved.

Extractive membrane bioreactor system

The experimental set-up of an extractive membrane bioreactor is presented in Figure 2. A 1.3 L commercial fermentor was connected to the membrane modules via a flow of biomedium. The temperature in the bioreactor was controlled at 25 °C and the pH set at 7 and the stirrer speed was 200 rpm. The air flow rate was 2 L/min. The initial toluene concentration was 300 mg/L, which is lower than the toluene solubility in water at 25 °C. Four 1-m pieces of polydimethyl-siloxan (PDMS) tubes with inner diameter of 2.5 and 0.2 mm wall thickness were installed in the glass shell, with two separated inlets, to assemble the membrane module. The inner diameter of the shell is 0.03 and its length is 1 metre. To investigate the biofilm formation, the experiments were done for 9 days and sampling was done every 3 days. To maintain an almost constant toluene concentration in the wastewater, it was prepared daily to avoid changes in concentration due to evaporation.

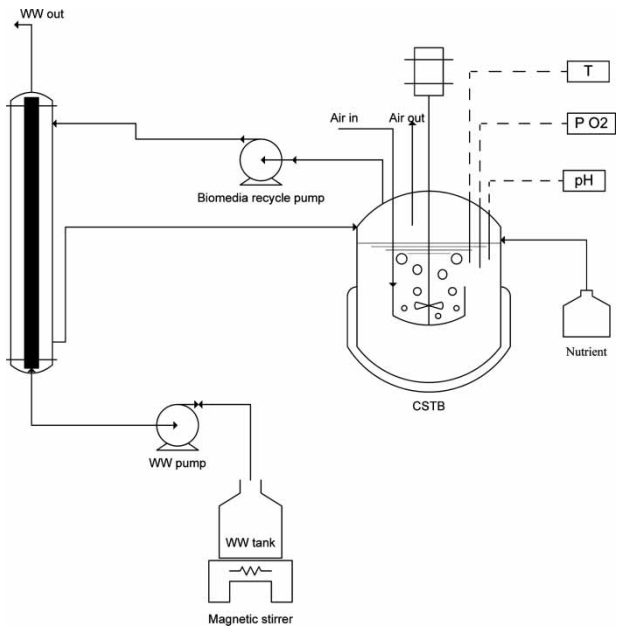

Figure 2 | Experimental set-up.

To verify the mathematical model, four single tube membrane modules with different lengths were used. The inner diameter of the shell is 0.02 m and the lengths of these modules were 0.5, 0.6, 0.7 and 1 m.

Mathematical model

An extractive membrane bioreactor system has two main parts: a bioreactor and a membrane module. Most microbial reaction occurs in the biomedia on the membrane exterior and the bioreactor is a place where oxygen and essential nutrients are supplied for microorganisms. The model is developed with reference to a cylindrical coordinate system. In this system z represents the vertical axis, r the radial axis and θ the angular direction. The cylindrical tube (within the tube is sliced horizontally into 'n' slices), numbered from = 1 to n where the position '0' is located at the contaminated wastewater influent and the end of the 'n-th' section is located at the wastewater effluent.

Each slice is assumed to be a continuous stirred tank, the whole tube is like several CSTRs in series and the mass balance of toluene in the tube side at steady state condition leads to Equation (1):

$$0 = F(C_{s,t,j-1} - C_{s,t,j}) - K_{ov}A_{t,j}(C_{stj} - C_{s,\mathrm{bio},j}(R_{od})) \quad j = 2, n \tag{1}$$

'n' is the number of tanks in series and K_{ov} the overall mass transfer coefficient accounts for mass-transfer resistance in the tube side liquid film and in the membrane (Brookes & Livingston 1995), as shown in Equation (2).

$$\frac{1}{K_{ov}} = \frac{1}{K_{s,t}} + \frac{R_{id}\ln(R_{od}/R_{in})}{D_{mem}K_{mem/aq}} \tag{2}$$

The biofilm and biomedium can be modeled by fractional continuity equation (Bird *et al.* 1960). Diffusion and reaction of toluene are taken in to account in the model. By assuming incompressible fluid and constant diffusion, the following equation was obtained.

$$\frac{\partial C}{\partial t} + \left[V_r\frac{\partial C}{\partial r} + V_z\frac{\partial C}{\partial z} + V_\theta\frac{\partial C}{\partial \theta}\right] = D_s\left(\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial C}{\partial r}\right) + \frac{1}{r^2}\frac{\partial^2 C}{\partial \theta^2} + \frac{\partial^2 C}{\partial z^2}\right) + R_s = 0 \tag{3}$$

Model assumptions. (1) The system is steady state. (2) The mass transfer in the biomedia and the membrane occurs only by diffusion. (3) Haldane kinetics is used to describe the toluene biodegradation and cell growth. (4) No biofilm is formed. (5) There is no accumulation of toluene in the silicon rubber and there is no swelling of the membrane due to absorption of toluene. (6) The effective permeability of the toluene remains constant throughout the silicone tube and it is independent of the concentration. (7) Biomass concentration remains constant during the experiments (for 4–6 h). (8) There is no diffusion of the substrate in z and θ directions. (9) The microbial suspension is completely mixed and reducing the liquid resistance equal to zero on the outer surface of the membrane. (10) Kinetic parameters and mass transfer coefficients remain constant.

Thus Equation (3) changes into Equation (4) for each CSTR:

$$D_s\left(\frac{d^2C_{s,\mathrm{bio},i}(r)}{dr^2} + \frac{1}{r}\frac{dC_{s,\mathrm{bio},i}(r)}{dr}\right) + \mu_{s,\mathrm{bio},i}(r)X = 0 \quad j = 1, n \tag{4}$$

Boundary conditions:

$$\text{At } r = R_{od} \quad D_s\frac{dC_{s,\mathrm{bio},j}(R_{od})}{dr} = -K_{ov}(C_{s,t,j} - C_{s,\mathrm{bio},j}(R_{od})) \tag{5}$$

$$\text{At } r = R_{out} \quad D_s\frac{dC_{s,\mathrm{bio},j}(R_{out})}{dr} = 0 \tag{6}$$

RESULTS AND DISCUSSION

Microbial growth kinetics

Owing to the simplicity, shake-flask experiments were conducted to examine the effect of initial concentration on the degradation behavior of toluene using bacterial strain *A. faecalis* at 25 °C. The batch experiments were repeated and the results were found reproducible within an acceptable range. Biomass growth as a function of time can be seen in Figure 3. Higher toluene initial concentration yields higher biomass concentration at the end of the exponential growth phase.

In order to evaluate growth kinetics, the biomass growth data from different initial toluene degradation batch experiments were plotted on a semi-logarithmic graph. After a

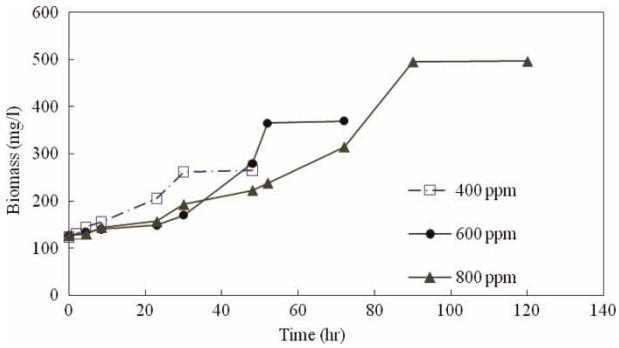

Figure 3 | Biomass concentration as a function of time, three toluene initial concentrations (400, 600, 800 ppm).

short lag phase, linear plots were obtained at all initial concentrations, which indicated that the cultures were growing exponentially in this region. The specific growth rates (μ) were calculated from the slope of the logarithm of the biomass concentration vs. time curves in the linear range.

Toluene has a toxic nature and an inhibitory effect on cell growth and the Andrews–Haldane growth kinetics equation can represent the growth kinetics on this substrate. Cell Growth Kinetics in a batch reactor during the exponential phase, is modeled by the following equation:

$$\frac{dX}{dt} = \mu X \tag{7}$$

Haldane's inhibitory growth kinetics is as follows (Littlejones & Daugulis 2008):

$$\mu = \frac{\mu_{max} S}{k_s + S + (S^2/k_I)} \tag{8}$$

In these expressions, S is the liquid substrate concentration; μ, the specific growth rate; X, the biomass concentration; μ_{max}, the maximum specific growth rate; k_s, the Monod half-saturation constant and k_I is the substrate inhibition constant. Term k_s is that value of the limiting nutrient concentration at which the specific growth rate is half of its maximum value.

Experimentally obtained specific growth rates were plotted as a function of toluene initial concentrations and fit to Equation (8). Kinetic parameters were estimated by nonlinear least square methods. The following model equation and regression coefficient were obtained:

$$\mu = \frac{0.066S}{S + 91.74 + (S^2/278.2)}, \quad R^2 = 0.97 \tag{9}$$

The variation of experimental specific growth rate as a function of toluene concentration and the fitted curve (Haldane model) is presented in Figure 4. The value of $Y_{x/s}$ was calculated directly from experimental data (cell mass produced vs. total mass of substrate consumed). This value is smaller than values obtained by other researchers due to the high substrate concentrations in this study. Values of the kinetic constants obtained in this work are compared with other published data in Table 3. The values obtained in this work are in the range of the literature values.

The specific growth rate increased up to 180 mg toluene L^{-1}. Further increases in toluene concentration inhibited cell growth, although inhibitory concentrations were far from the application range of biological treatment processes.

Mass transfer

Partition coefficient

To determine the membrane-aqueous partition coefficient of toluene at 25 °C empirically, the method of Brookes & Livingston (1995) was used. $K_{mem/aq}$ is the slope of the following equation:

$$\frac{C_0}{C_{eq}} - 1 = K\frac{V_{mem}}{V_{aq}} \tag{10}$$

V_{aq} is the liquid solution volume, V_{mem} is the volume of the membrane piece, C_0 is the initial concentration of the organic pollutant and C_{eq} is the equilibrium concentration of solute in the aqueous phase. Figure 5 shows the plot for estimation of $K_{mem/aqueous}$ for toluene at 25 °C. For the used type of silicon, the calculated partition coefficient was 227.4.

Overall mass transfer coefficient measurement

Experiments for determination of the overall mass transfer coefficient were done 12 h after inoculation. Hence the

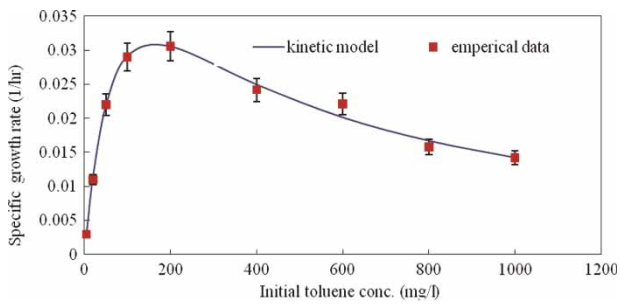

Figure 4 | Specific growth rate as a function of toluene initial concentration.

Table 3 | Biodegradation kinetic parameter values for toluene

Microorgansm	Maximum toluene conc. (mg/L)	$Y_{x/s}$	μ_{max} (1/h)	k_s (mg/L)	k_i (mg/L)	References
Alcaligenese faecalis	1,000	0.44	0.066	91.74	278.2	This study
Pseudomonas putida O1	70	0.65	1.56	15.07	44.43	Oh *et al.* (1994)
Pseudomonas putida F1	43	1.28	0.86	13.8	–	Reardon *et al.* (2000)
Consortium	80	1.25	0.6	34.12	–	Littlejones & Daugulis (2008)
Ralstonia sp. YABE411	47.87		0.062	3.022	–	Lin & Cheng (2007)
Pseudomonas sp. YATO411	49.87		0.063	0.845	–	Lin & Cheng (2007)
Pseudomonas	49.87		0.897	0.845	–	Lin & Cheng (2007)

microorganisms were in the growth phase. 500 mL of wastewater with 400 mg/L toluene concentration was prepared and circulated within the membrane tubing. The wastewater flow was laminar ($Re = 70.5$) and transfer of toluene across the membrane controlled by diffusion. The flux of toluene across the membrane can be described by Equation (11):

$$J = -\frac{dC}{dt}\frac{V_w}{A} \tag{11}$$

Also, according to the overall mass transfer equation, we have:

$$J = -K_{ov}(C - C_l) \tag{12}$$

By equalizing Equations (11) and (12) and providing a simple integration, we get the following equation:

$$\ln\left(\frac{C_0 - C_l}{C - C_l}\right) = \frac{A}{V_w}K_{ov}(t - t_0) \tag{13}$$

It was assumed that there was no gradient of pollutant concentration in the axial direction of the membrane tube, and cell concentration remained constant during the time of sampling (4–6 h). The amount of toluene in the biomedia was less than 10 mg/L and due to the circulation of the biomedia between membrane module and fermentor, it remained in this range during the tests. The value of K_{ov} was obtained from the linear regression of $\ln((C_0 - C_l)/(C - C_l))$ versus time when C_l reached equilibrium (Figure 6).

C_0 and C are toluene concentration at t_0 and t; C_l is the toluene concentration in biomedia; A is the membrane area and V_w is the volume of wastewater. The empirical value of the overall mass transfer coefficient was calculated to be 1.33×10^{-6}.

Biodegradation and biofilm formation

In order to investigate the biodegradation of toluene, 500 mL of fresh synthetic wastewater with 400 mg/L toluene was prepared and circulated in the membrane tubing. Figure 7 shows the time course profile of toluene degradation and cell growth in the EMBR. The dramatic decline of toluene concentration occurred in the initial hours due to its transfer into the biomedia and consumption of toluene by the bacterial culture. After the growth phase of the bacterial population, the cell concentration increased and the toluene decreased slowly. This is because of deduction in dissolved oxygen and nitrogen source in the biomedia.

Biofilm formation in the EMBR system was studied during the nine-day experiments. In this set of experiments

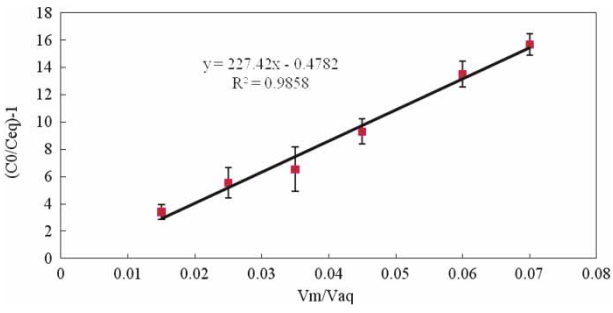

Figure 5 | Estimation of $K_{mem/aq}$ at 25 °C for toluene.

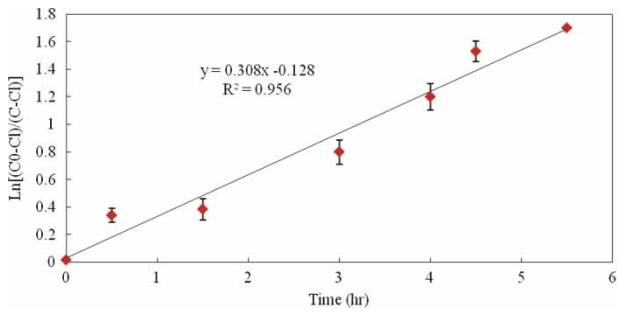

Figure 6 | Estimation of overall mass transfer coefficient of toluene in shell and tube membrane module.

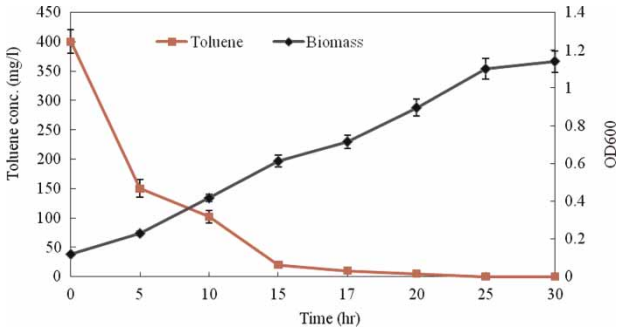

Figure 7 | Time course profile of toluene biodegradation in an EMBR.

a synthetic wastewater with initial COD of 1,250 mg/L was prepared daily and passed through the membrane tubing continuously with no circulating flow back to the feed tank. Samples were collected 1 h after substitution of fresh wastewater to ensure steady state conditions. Sampling was done from the outlet flow of wastewater from the membrane tubing. The result of the above mentioned experiments, for two different flow rates of wastewater, are presented in Figure 8. The outlet COD of the stream with 9.9 mL/min was more than the outlet COD of the stream which flowed at the rate of 3.3 mL/min. In EMBRs with continuous wastewater streams, lower flow rate is preferable because organic compounds have more contact time to transfer across the membrane. There were no considerable changes in the outlet COD during the nine days.

This leads to the conclusion that *A. faecalis* could not form biofilm on silicone membrane, and this is advantageous for the EMBR system. Biofilm formation causes reduction of organic compound flux across the membrane. Also, a rise in the biofilm thickness caused a gradual reduction in the optical density of the biomedia (Zhang *et al.* 1998). However, the results of the experiments showed roughly constant flux and steady increase in the optical density of the biomedia.

Formation and thickness increase of the biofilm, reduced the efficiency of the EMBR. In this situation,

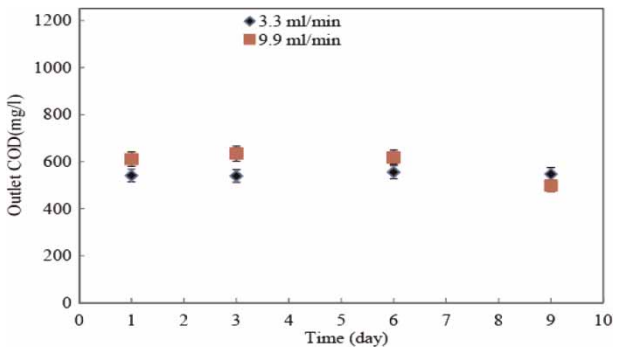

Figure 8 | Investigation of biofilm formation through measurement of the outlet COD.

Table 4 | Parameters used in the simulation

Parameters	Value	Parameters	Value
F (m^3/s)	1.24×10^{-7}	K_{ov} (m^2/s)	1.33×10^{-6}
μ_{max} (1/h)	0.066	D_s (m^2/s)[a]	9.86×10^{-10}
k_s (mg/L)	91.7	L (m)	1
k_I (mg/L)	278.2	R_{id} (m)	1.25×10^{-3}
$Y_{x/s}$	0.44	R_{od} (m)	1.45×10^{-3}
$X_{suspension}$ (mg/L)	250	R_{out} (m)	0.01

[a]Cocchini *et al.* (2002).

larger membrane modules and periodic washing of the membranes are needed which impose more cost on the process. Usage of *A. faecalis* eliminates mass transfer resistance of the biofilm and enhances the system efficiency and cuts down the process costs.

Model verification

The aim of this section is to verify the accuracy of the mathematical model, stated previously, to predict the pollutant concentration along the membrane tubing. Equations (1)–(6) have been solved numerically with the finite difference method. Parameters of the model are shown in Table 4. The kinetic model is the same as Equation (9).

Biofilm resists mass transfer and causes reduction of both pollutant flux across the membrane and the optical density of the biomedia (Zhang *et al.* 1998). In order to investigate biofilm formation, a 9-day experiment was carried out. During this period no biofilm formed on the silicone membrane exterior, toluene flux remained almost unchanged and optical density permanently increased. Thus, it is advantageous to use *A. faecalis* in EMBRs to reduce mass transfer resistance.

The results of the model and average experimental data are shown in Figure 9. This figure shows that the experimental results and the model are coincident. This leads to the conclusion that the model captures all the main phenomena which determine the system performance. Thus, when oxygen mass-transfer resistance does not limit the reaction rate of the system, this model is applicable in the chosen range of operating conditions.

CONCLUSION

Biological methods are very sensitive to high pollutant concentrations, especially in the case of toxic contaminants.

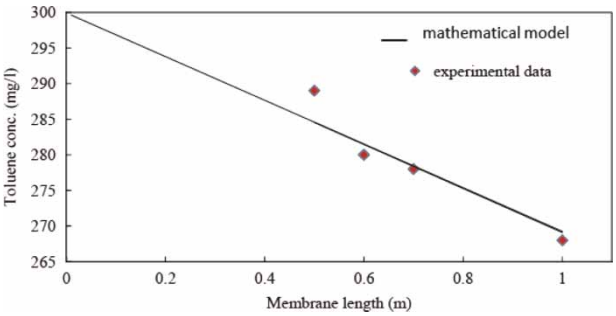

Figure 9 | Comparison between the model and experimental results.

Over the concentration ranges tested, *A. faecalis* could degrade high toluene concentrations up to 1 g/L. It was concluded that this microorganism is a suitable choice for biological processes in which the risk of surges in pollutant load is high. The Haldane–Andrews model described the growth kinetics of *A. faecalis* on toluene as the substrate. It does not form biofilm on the surface of the silicone membrane in an extractive membrane bioreactor. The suggested mathematical model predicted pollutant concentrations in effluent streams efficiently.

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