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Treatment of phenol in synthetic saline wastewater by solvent extraction and two-phase membrane biodegradation

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ABSTRACT

Phenol in synthetic saline (100 g L^{-1} NaCl) and acidic (pH 3) wastewater was treated by a hybrid solvent extraction and two-phase membrane biodegradation process at 30 °C. Kerosene was adopted to be the organic solvent because it was biocompatible and had a suitable partition coefficient for phenol. Phenol in water was first extracted by kerosene in a batch stirred vessel and the loaded solvent was passed through the lumen of a polyvinylidene fluoride (PVDF) hollow-fiber membrane contactor; in the meantime, *Pseudomonas putida* BCRC 14365 in mineral salt medium was flowed across the shell, to which tetrasodium phyophosphate (1 g L^{-1}) was added as a dispersing agent. The effect of the initial phenol level in wastewater (110–2400 mg L^{-1}) on phenol removal and cell growth was experimentally studied. At a cell concentration of 0.023 g L^{-1}, it was shown that the removal of phenol from saline wastewater was more efficient at a level of 2000 mg L^{-1} when 0.02-m^2 membrane module was used. The effects of bigger membrane module size (0.19 m^2 area) and higher initial cell concentration (0.092–0.23 g L^{-1}) on the performance of such a hybrid process for the treatment of higher-level phenol in saline wastewater was also evaluated and discussed.

1. Introduction

Many of industrial effluents containing priority organic pollutants exhibit high salt concentrations and/or the extremes of pH, one or both of which prevents microbial growth or make it very difficult to sustain [1,2]. Lefebvre and Moletta [3] have made a literature survey on the treatment of organic pollutants in industrial saline wastewater. They reported that the main end markets for salt are the chemical process industry (mainly the chloralkali sector), road deicing and agro-food industries. Other non-negligible uses of salt are found in petroleum, textile and leather industries as well as for softening hard water. All these generate large amounts of saline wastewaters, rich in both salt and organic matter. When such industrial effluents are discharged into the environment without prior treatment, they cause severe damage by contamination of soils, surface water, and groundwater.

Physicochemical treatment of practical saline wastewaters is often suggested because such effluents are recalcitrant to biological treatment; however, the removal of organic matter from saline effluents is generally required prior to physicochemical processes. The main methods that have been studied are thermal processes such as solar and multiple-effect evaporation (to reduce volume of the effluents), coagulation–flocculation (to remove colloidal COD and turbidity), ion exchange (to remove salts), and some membrane processes such as UF (to remove suspended solids and colloidal COD), RO and electrodialysis (to remove salts). For removal of salts, the use of RO is particularly efficient, yet the large amounts of organic matter and suspended solids in effluents reduce the life time and the efficiency of membranes involved [3]. Hence, the optimal treatment of highly saline wastewater usually involves a biological treatment prior to salt removal.

On the other hand, although biological treatment is inhibited by high salt levels, it has proved feasible to use salt-adapted microorganisms capable of withstanding high salinities and of degrading the pollutants that are contained in effluent [4]. That is, the use of suitable microorganisms is also suggested in the treatment of saline effluents, prior to salt removal by physicochemical methods. However, the organic loading rate and salt level in the effluent should be equalized as far as possible, as these microorganisms are sensitive to environmental shocks [3]. Moreover, high amounts of salt are known to compromise the correct operation of conventional aerobic wastewater treatment processes only above chloride concentrations of 5–8 g L^{-1} [5].

The possibility of using hollow-fiber microporous membrane contactors as two-phase bioreactors for the degradation of phenol in saline and acidic solutions by *Pseudomonas putida* (*P. putida*) BCRC (Bioresource Collection Research Center) 14365 has been

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Nomenclature

S	phenol level at any time (mg L^{-1})
S_0	initial phenol level (mg L^{-1})
S_{cm}	phenol level in the cell medium (mg L^{-1})
$S_{cm,max}$	maximum phenol level in the cell medium (mg L^{-1})
t_{95}	time required for degrading 95% of the total phenol (h)
X	cell concentration (g L^{-1} or OD)
X_0	initial cell concentration (g L^{-1} or OD)

Greek letter

μ	specific cell growth rate defined in Eq. (3) (h^{-1})

evaluated at 30 °C [6]. After extraction of phenol from saline solutions into kerosene, kerosene was passed through the lumen of membrane module and the aqueous cell medium was flowed across the shell. Phenol was back-partitioned from kerosene to cell medium when cell medium is in contact with the loaded solvent through the mouth of the pores, and biodegradation occurs in the cell medium as long as the solvent is biocompatible. The proposed solvent extraction and two-phase membrane biodegradation process can treat 1000 mg L^{-1} phenol in wastewater containing NaCl up to 200 g L^{-1}, and the efficiency increases with increasing NaCl level due to salting-out effect for enhanced partition of phenol from wastewater to kerosene [6]. Except highly saline solutions, this hybrid process is suitable for the treatment of organic matter in acidic (pH < 3) and basic solutions because solvent extraction is an efficient way to separate organic matter from salts and ionic species (e.g., H^+, OH^-) in aqueous streams [7]. However, further studies are needed to extend the applicability of such a hybrid process including the treatment of increased level of organic matter in wastewater, and to establish the relationship among partition coefficient of organic matter and key operating parameters.

Some two-phase alternatives can be referred for treatment of high-level organic matter in saline solution. Collins and Daugulis [8] have studied the biodegradation of phenol up to 10 g L^{-1} in the so-called two-phase partitioning bioreactors, which uses a water-immiscible and biocompatible organic solvent (2-undecanone) that is allowed to float on the surface of a biomass-containing aqueous phase. Solvent is used to dissolve large amounts of organic matter, which then partition to aqueous phase at low levels. When cells consume some of the substrate, disequilibrium is created, which causes more of the organic matter to be partitioned to aqueous phase as the system tries to maintain thermodynamic equilibrium [9]. Because cells are basically colloidal substances, direct mixing of the two immiscible phases may lead to the formation of emulsions or the third phase due to the existence of metabolic products, particularly at high cell densities. This may make phase separation and subsequent treatment (e.g., solvent recovery) more difficult. On the other hand, an extractive membrane bioreactor (EMBR) has been proposed to solve the problems outlined above [1,10,11], which uses a dense membrane that is virtually permeable to organic compounds but non-permeable to water or ionic species. The membrane separates wastewater from cell medium where biodegradation occurs under controlled conditions, making it useful for the treatment of saline effluents. The permeated organic matter is swept off by either cell medium or organic solvent, constituting the so-called one- or two-phase EMBR. Splendiani et al. [11] have used two-phase EMBR to remove monochlorobenzene from water using single polydimethylsiloxane fiber and to degrade it by *Burkholderia* sp. strain using perfluoromethyldecalin as the organic solvent. However, both types of EMBR processes are extremely time-consuming due to slow mass transfer within the dense membrane.

In this work, the effect of initial phenol level on the removal of phenol from saline and acidic solutions by solvent extraction coupled with biodegradation in membrane contactors was studied. Phenol was selected as model organic matter because it is one of the most common pollutants even at an extremely low level [12], and *P. putida* was used due to its high biodegradation efficiency [13,14]. Experiments were carried out using polyvinylidene fluoride (PVDF) hollow fibers. The wastewater contained 110–2400 mg L^{-1} phenol and 100 g L^{-1} NaCl at pH 3, whereas the cell medium consisted of *P. putida* BCRC 14365 and 1 g L^{-1} dispersing agent, tetrasodium phyophosphate (TSP). An operating temperature of 30 °C was selected since it is optimal for *P. putida* growth [14]. The possibility for improved treatment of higher-level phenol in saline wastewater by changing membrane module size (0.02–0.19 m^2 area), initial cell concentration (0.023–0.23 g L^{-1}), and cell medium volume were finally evaluated.

2. Materials and methods

2.1. Microorganism, nutrient medium, and solutions

P. putida BCRC 14365 used was obtained from the Food Industry Research and Development Institute, Hsinchu, Taiwan. The stock cultures were stored at 4 °C. The nutrient medium contained 3 g L^{-1} beef extract, 5 g L^{-1} peptone, and the mineral salt (MS) medium at pH 7. The compositions of MS medium (in g L^{-1}) were KH_2PO_4 (0.42), K_2HPO_4 (0.375), $(NH_4)_2SO_4$ (0.244), NaCl (0.015), $CaCl_2{\cdot}2H_2O$ (0.015), $MgSO_4{\cdot}7H_2O$ (0.05), and $FeCl_3{\cdot}6H_2O$ (0.054). The phosphate buffer (pH 7) was prepared by mixing equal volumes of 0.375 g L^{-1} K_2HPO_4 and 0.465 g L^{-1} KH_2PO_4 solutions in deionized water (Millipore, Milli-Q). All these inorganic chemicals were supplied by Merck Co. as analytical reagent grade. Prior to use, the MS medium and phosphate buffer were sterilized in an autoclave at 121 °C for 15 min.

The organic solvent kerosene (Union Chemical Co., Taiwan) was washed twice with 20 vol% H_2SO_4 to remove possible aromatics impurities and then with deionized water (Millipore Milli-Q) three times before use. Kerosene was chosen because it is cheap, stable, and biocompatible. The partition coefficients of 1000 mg L^{-1} phenol, defined below, between kerosene and wastewater as well as kerosene and cell medium were measured to be 0.50 and 0.35, respectively, at 30 °C.

$$\text{partition coefficient} = \frac{\text{equilibrium phenol level in the organic phase}}{\text{equilibrium phenol level in the aqueous phase}} \quad (1)$$

The wastewater was prepared by dissolving 110–2400 mg L^{-1} phenol (Merck Co.) and 100 g L^{-1} NaCl in deionized water, in which the pH was adjusted to be 3 by adding 0.1 M HCl. The cell medium contained MS medium at pH 7, to which 1 g L^{-1} TSP (RDH Co.) was added as a dispersing agent. The solution pH was measured using a pH meter (Horiba F-23, Japan). The initial cell concentration was fixed at 0.023 g L^{-1} in cell medium, otherwise stated elsewhere.

2.2. Free suspension cultivation

P. putida cells were activated at 30 °C in nutrient medium, into which 100 mg L^{-1} phenol was added for adaptation for 24 h. The cells collected after centrifugation at 6000 rpm for 10 min was re-suspended in phosphate buffer and re-centrifuged. After cleaning, the activated cells were inoculated into the culture medium (250 mL) in 500-mL Erlenmeyer flasks to give an initial

Table 1
Specifications of the hollow-fiber membrane modules adopted in this work

Parameter	Small module	Big module
Part number	Asahi Kasei Microza UMP-0047R	Asahi Kasei Microza UMP-1147R
Number of fibers	21	200
Fiber internal diameter	1.4 mm	1.4 mm
Fiber outer diameter	2.2 mm	2.2 mm
Fiber wall thickness	0.40 mm	0.4 mm
Effective membrane area	0.02 m^2	0.19 m^2
Effective fiber length	314 mm	546 mm
Effective pore size	0.2 μm	0.2 μm
Membrane material	PVDF	PVDF
Overall length	410 mm	640 mm
Overall diameter	16 mm	45 mm

cell concentration of 1.3×10^8 cells mL^{-1}. This corresponds to an optical density at 600 nm (OD_{600}) of 0.055 or a dry cell weight of 0.023 g L^{-1}. After inoculation, the flask was capped with cotton plugs and placed in a shaker controlled at 120 rpm and 30 °C.

The number of colonies and dry cell weight were determined as follows. Serial dilution of the culture was conducted and 0.1 mL of the diluted culture was spread on a plate. The plate was incubated at 30 °C for 24 h and the number of colonies was counted. In a separate experiment, cells in the cultures (40 mL) with different concentrations were harvested by centrifugation (6000 rpm) at 4 °C for 10 min several times and washed with deionized water. The cells were then dried at 60 °C for 24 h until a constant weight was reached.

2.3. Hollow-fiber modules and the related experiments

Two microporous PVDF hollow fibers, Microza UMP-0047R (small module) and UMP-1147R (big module), were used here, which were manufactured by Asahi Kasei Chemicals Co., Japan. They are encased in a cylindrical shell in an analogous manner to the shell-and-tube heat exchanger. The properties of these two modules are listed in Table 1. Liquids like water with high surface tension do not readily wet PVDF fibers, thus pure ethanol was used to wet them [15]. After wetting for 6 h, the modules were flushed with deionized water to rinse away any remaining ethanol before use.

Fig. 1 shows the experimental setup of combining solvent extraction and two-phase biodegradation in hollow-fiber membrane contactors. After activated cells were inoculated in culture medium to the required concentration, they were pumped through the shell of the module at 2.0–5.6 mL min^{-1} using a peristaltic pump (Cole-Parmer, Masterflex 7518-00). In the meantime, phenol in saline wastewater was first extracted by kerosene and the loaded solvent was pumped through the lumen at 2.0–5.6 mL min^{-1}. This flow rate was selected such that both phases could flow steadily. Both solutions were circulated in the module and totally recycled to their original flasks, where they were agitated at 300 rpm by magnetic stirrers to provide required aeration (in cell medium) and partitioning (in organic solvent). Temperature was controlled at 30 °C in water bath. Phenol was back-partitioned from kerosene to cell medium through the pores of the membranes, and biodegradation takes place in suspensions or within the biofilm formed on the outer surface of membrane. Unless specified elsewhere, the working volumes for wastewater, organic solvent, and cell medium were all 0.5 L. Samples were taken from these external flasks (wastewater 1 mL, cell medium 1 mL, kerosene 3 mL) at regular intervals.

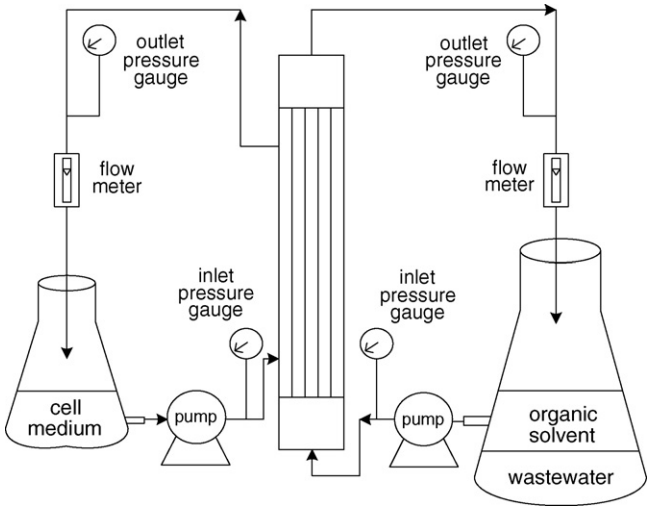

Fig. 1. Experimental setup for the removal of phenol from saline wastewater by a hybrid solvent extraction and two-phase membrane biodegradation process.

2.4. Post-treatment of membrane bioreactors

To avoid possible contamination of the medium in the followed experiments, the membrane bioreactors used were immediately sterilized and cleaned after experiments. They were flushed with 70% ethanol and deionized water for 1 h each at higher flow rates (lumen 15 mL min^{-1}, shell 15 mL min^{-1}). They were then washed with a 0.5% w/v Terg-A-Zyme solution (Alconox Inc., USA) at the above flow rates for 1 h and at moderate flow rates (lumen 10 mL min^{-1}, shell 10 mL min^{-1}) for 3 h. After washing, the former solution is yellow and the latter becomes colorless. Finally, the module was rinsed in order with deionized water, 0.5 M NaOH, and 0.1 M NaOH for 0.5 h each at the aforementioned high flow rates.

2.5. Analysis of cells and phenol

Biomass concentration in cell medium was determined by measuring OD_{600} using a JASCO UV/visible spectrophotometer (UV-550, Japan), in which 1-cm path length square quartz cuvette with culture medium was used as reference. Aqueous samples (wastewater and cell medium) were subjected to filtration through a Millipore PVDF filter (0.2 μm) before HPLC analysis of phenol on a Phenomenex C18 column (particle size, 5 μm). A mixture of acetonitrile (50 vol%) and water that flowed at 1 mL min^{-1} was used as the mobile phase. The wavelength of UV detector (JASCO 975) was 270 nm and an aliquot of the sample (10 μL) was injected. Each experiment was at least duplicated under identical conditions. The detection limit of phenol was around 4 mg L^{-1} and the reproducibility of concentration measurements was within 5%. Here, the organic sample (3 mL) was pre-mixed with deionized water (3 mL) for 24 h, and the aqueous layer was analyzed as described above. Phenol concentration in kerosene was obtained based on the measured aqueous-phase concentration and its partition coefficient between kerosene and water.

3. Results and discussion

3.1. Effect of initial phenol level on biodegradation

Figs. 2 and 3 show the effects of initial phenol level (S_0, 1000–2400 mg L^{-1}) in saline solution containing 100 g L^{-1} NaCl at pH 3 on the biodegradation of phenol by *P. putida* containing 1 g L^{-1} TSP in MS medium using the small module (UMP-0047R). In these figures and hereinafter, the time changes of phenol levels in

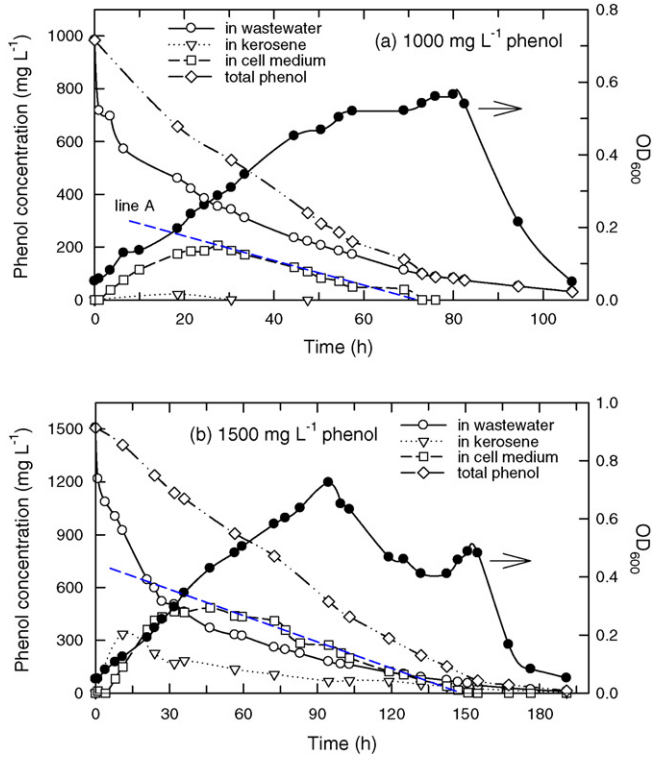

Fig. 2. Effect of initial phenol level in wastewater (1000 and 1500 mg L^{-1}) on phenol biodegradation in small module UMP-0047R (wastewater: 0.5 L, 100 g L^{-1} NaCl, pH 3; kerosene: 0.5 L; cell medium: 0.5 L, OD_{600} = 0.055, pH 7).

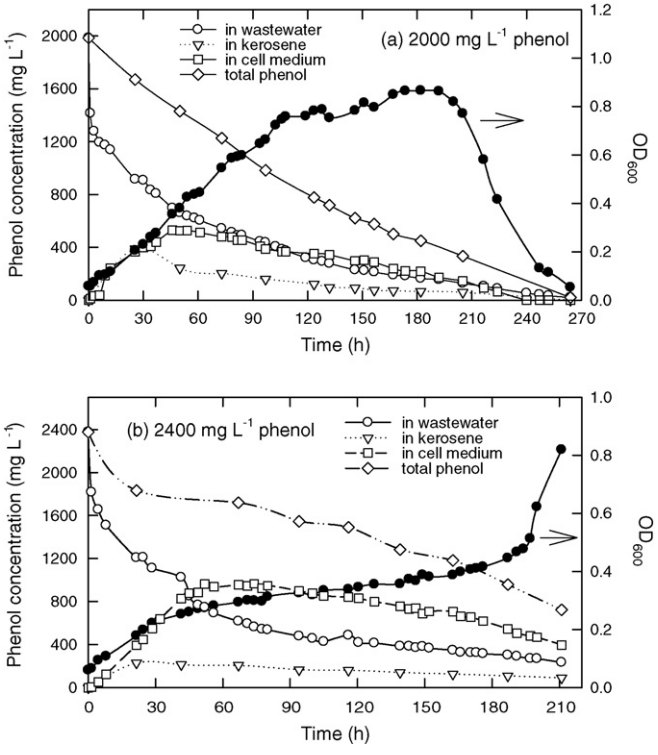

Fig. 3. Effect of initial phenol level in wastewater (2000 and 2400 mg L^{-1}) on phenol biodegradation in small module UMP-0047R (wastewater: 0.5 L, 100 g L^{-1} NaCl, pH 3; kerosene: 0.5 L; cell medium: 0.5 L, OD_{600} = 0.055, pH 7).

wastewater, kerosene, and cell medium, as well as the total phenol level and cell growth in suspended cell medium are provided.

It is recognized that the formation of membrane-attached biofilm is natural for biological processing in membrane units [16–19], because of numerous potential attachment and adhesion mechanisms of bacteria [20,21]. Hence, organic matter is degraded not only in suspensions but also within the biofilm. However, a comparatively thinner biofilm is generally formed in this two-phase biodegradation process due to the larger extent of membrane swelling by organic solvent, in contrast to other aqueous one-phase biological processes [6,20,21]. Moreover, 1 g L^{-1} TSP was used as a dispersing agent here to retard the attachment of bacteria and to protect against biofouling on the solid matrices [22]. The contribution of biofilm to the overall degradation is hence assumed to be negligibly small. In this regard, the present two-phase biodegradation in membrane contactors includes the following steps:

$$\text{phenol in kerosene} \overset{\text{back-partition}}{\longrightarrow} \text{phenol in cell medium}$$

$$\overset{\text{degradation}}{\longrightarrow} \text{final products}(H_2O, CO_2)$$

Because the flow rate of shell- or lumen-side streams (2.0–5.6 mL min^{-1}) has little effect on biodegradation (not shown), mass transfer and partition of phenol from wastewater to kerosene as well as mass transfer and back-partition of phenol from kerosene to cell medium is not the rate-controlling step. It is also expected that mass transfer of phenol through the kerosene-filled pores of the membrane is faster than biodegradation of phenol. Thus, phenol level in the cell medium would reach a maximum, as commonly observed in typical consecutive reactions. To increase the contact of both solutions in membrane contactors, a flow rate of 2.0 mL min^{-1} was applied throughout the work.

Under the conditions studied, it is seen that biomass in the cell medium starts to grow, although gradually, at the early stage of biodegradation process. However, the suspended cells grow still slow during 30–190 h of operation when S_0 = 2400 mg L^{-1} (Fig. 3b); within that period, phenol level in the cell medium (S_{cm}) is higher than about 650 mg L^{-1}. In this case, cells are likely attached to the outer surface of the fibers to successively form an immobilized biofilm during this period.

To discuss the effect of operating parameters more definitely, two quantities are compared here. One is the maximum phenol level in cell medium ($S_{cm,max}$) in the whole process. Because phenol that was transferred from kerosene to cell medium is the unique carbon source for cell growth, $S_{cm,max}$ is an important index to the performance in the present two-phase biodegradation system. The other quantity is the apparent degradation rate of phenol in cell medium (mg L^{-1} h^{-1}), defined by

$$\text{degradation rate} = -\frac{dS_{cm}}{dt} \quad (2)$$

Here, the apparent degradation rate is calculated based on the time changes of S_{cm} after $S_{cm,max}$ was reached, as indicated by the straight line A in Fig. 2a and b.

Generally speaking, the time profiles of S_{cm} and total phenol level have roughly equivalent slopes within that period. In this regard, the times required for removing 95% of the total phenol (t_{95}) are also compared, which is roughly estimated by extrapolating the straight line to zero phenol level if necessary. It is seen that $S_{cm,max}$ (also, the time required to reach this value) is 210 mg L^{-1} (near 27 h), 480 mg L^{-1} (near 43 h), 530 mg L^{-1} (near 48 h), and 960 mg L^{-1} (near 52 h) when S_0 is 1000, 1500, 2000, and 2400 mg L^{-1}, respectively; also, t_{95} is found to be about 73, 155, 250, and 282 h, respectively, under such conditions.

It has been actually reported that the substrate inhibition kinetics of phenol in MS medium alone at pH 7 (without extra salts) by

suspended *P. putida* BCRC 14365 at 30 °C can be described by [14]:

$$\mu = \frac{1}{X}\frac{dX}{dt} = \frac{0.33S}{13.9 + S + (S^2/669)} \quad (3)$$

where S is the phenol level (mg L^{-1}), X is the cell concentration (g L^{-1} or OD_{600}), and μ is the specific cell growth rate (h^{-1}). The value of μ is determined at the exponential phase of the growth curve. This means that the maximum growth rate occurs when S_0 = 100 mg L^{-1}; above that, *P. putida* starts to be inhibited by phenol, and cannot grow when the phenol level in suspension exceeds about 750 mg L^{-1} at X_0 = 0.023 g L^{-1} [14]. The substrate inhibitory effect at such a high $S_{cm,max}$ value of 960 mg L^{-1} can explain the unusual behavior of cell growth when S_0 = 2400 mg L^{-1} (Fig. 3b).

On the other hand, the effects of S_0 in wastewater (110–500 mg L^{-1}) on biodegradation using the small module are shown in Figs. 4 and 5. Phenol in the cell medium is undetectable at such low S_0 values, and t_{95} is around 83 and 94 h when S_0 is 330 and 500 mg L^{-1}, respectively. Evidently, the present process is not favored to treat low-level phenol waters, due to the lack of phenol in suspension as the carbon source for cell growth. The use of other biocompatible solvents with different partition coefficients may overcome this problem.

The variations of S_0 on the degradation rate as well as the maximum cell concentration ($OD_{600,max}$) are shown in Fig. 5b, indicating that $OD_{600,max}$ increases with increasing S_0. Moreover, the present two-phase process reveals better degradation rate when S_0 = 2000 mg L^{-1} under the conditions studied. As indicated above, $S_{cm,max}$ is 530 mg L^{-1} when S_0 = 2000 mg L^{-1}. Thus, a $S_{cm,max}$ value of 530 mg L^{-1} allows for the growth of *P. putida*, which corresponds to a specific cell growth rate of 0.18 h^{-1} calculated according to Eq. (3). This is about 70% of the maximum cell growth rate (=0.26 h^{-1}) occurring when S_0 = 100 mg L^{-1}. It appears that the ratio of 70% can be adopted as a measure (also, 530 vs. 750 mg L^{-1}) to evaluate the performance of such a two-phase biodegradation process. In other words, $S_{cm,max}$ is the most important and simplest parameter for this purpose, which can be controlled/adjusted by selecting suitable organic solvents.

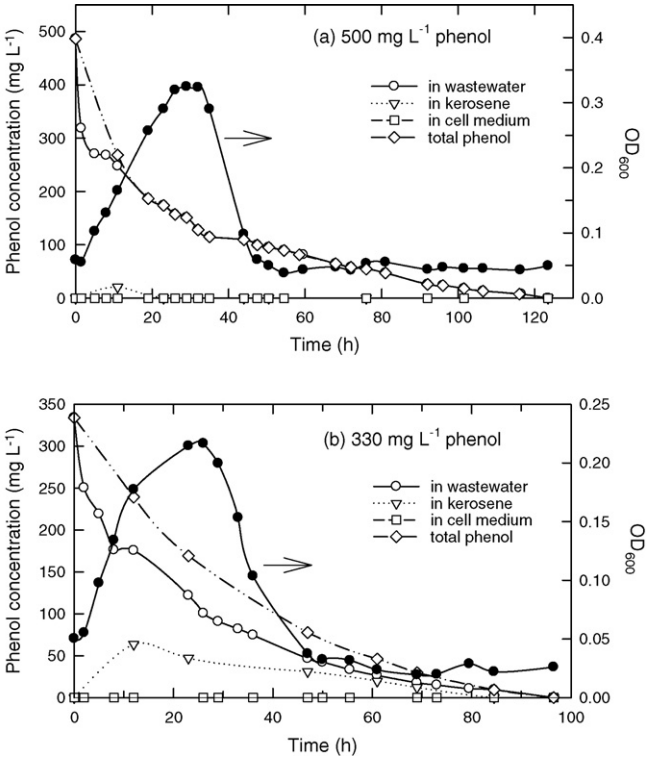

Fig. 4. Effect of initial phenol level in wastewater (500 and 330 mg L^{-1}) on phenol biodegradation in small module UMP-0047R (wastewater: 0.5 L, 100 g L^{-1} NaCl, pH 3; kerosene: 0.5 L; cell medium: 0.5 L, OD_{600} = 0.055, pH 7).

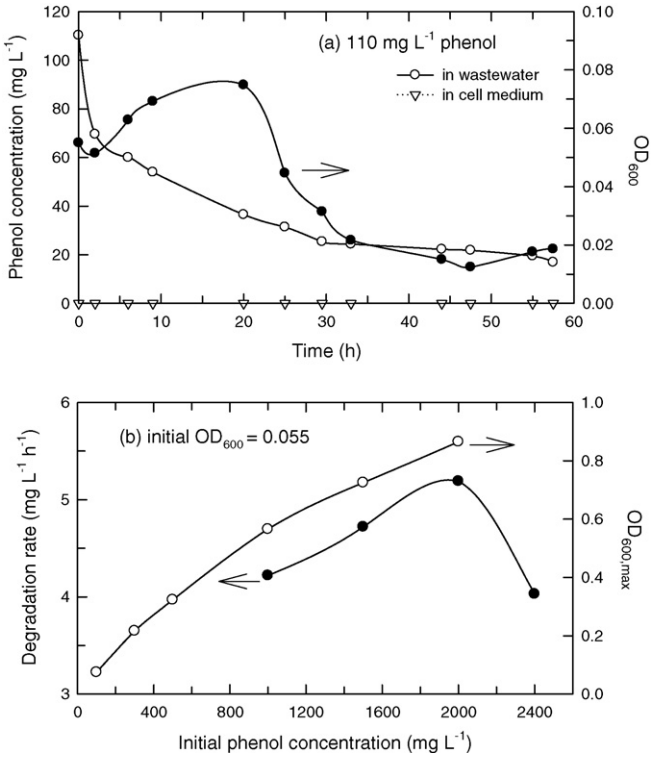

Fig. 5. Effect of initial phenol level in wastewater (100–2400 mg L^{-1}) on apparent degradation rate and the maximum OD_{600} in small module UMP-0047R (wastewater: 0.5 L, 100 g L^{-1} NaCl, pH 3; kerosene: 0.5 L; cell medium: 0.5 L, OD_{600} = 0.055, pH 7).

3.2. Effect of membrane module size on phenol biodegradation

In this work, attempts were made to improve biodegradation of phenol at higher levels including the use of bigger membrane modules and higher initial cell concentrations. Fig. 6 shows the results using the big module (UMP-1147R). The value of $S_{cm,max}$ is 32 mg L^{-1} (5 h), 385 mg L^{-1} (50 h), and 730 mg L^{-1} (34 h) when S_0 is 1000, 1500, and 2000 mg L^{-1}, respectively; also, t_{95} is about 58, 184, and 186 h, respectively.

It is expected that mass transfer of phenol from loaded kerosene to cell medium is faster when a bigger module is used, because the module has larger contact area between kerosene and cell medium. However, the improvement is apparent only at S_0 = 1000 mg L^{-1} under the conditions studied compared to the use of small module (Figs. 2a, b, and 3a). As indicated above, the overall process is determined by degradation instead of mass transfer (as well as back-partition) of phenol from kerosene to cell medium. This is the reason why $S_{cm,max}$ is higher using the bigger module when S_0 = 2000 mg L^{-1}; however, the time required for removing phenol can be reduced particularly at such high phenol levels because $S_{cm,max}$ is reached more quickly.

3.3. Effect of cell concentration on phenol biodegradation

Fig. 7 shows the effects of initial cell concentration (X_0) on the biodegradation of 2000 mg L^{-1} phenol by *P. putida* using the

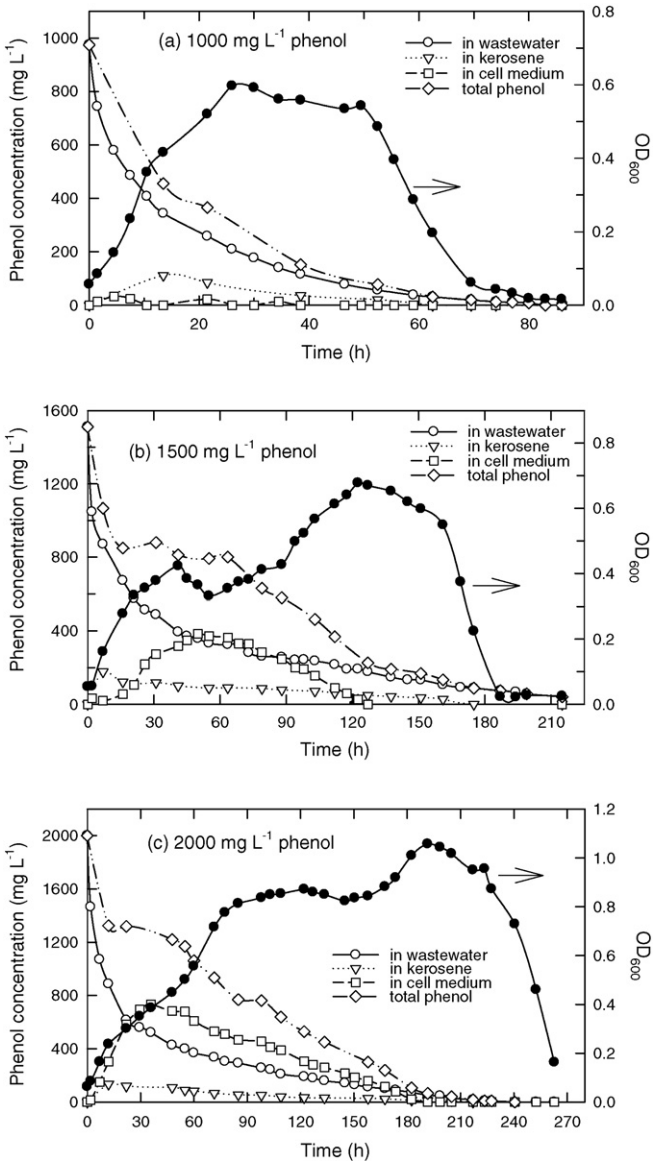

Fig. 6. Effect of initial phenol level in wastewater (1000–2000 mg L^{-1}) on phenol biodegradation in big module UMP-1147R (wastewater: 0.5 L, 100 g L^{-1} NaCl, pH 3; kerosene: 0.5 L; cell medium: 0.5 L, OD_{600} = 0.055, pH 7).

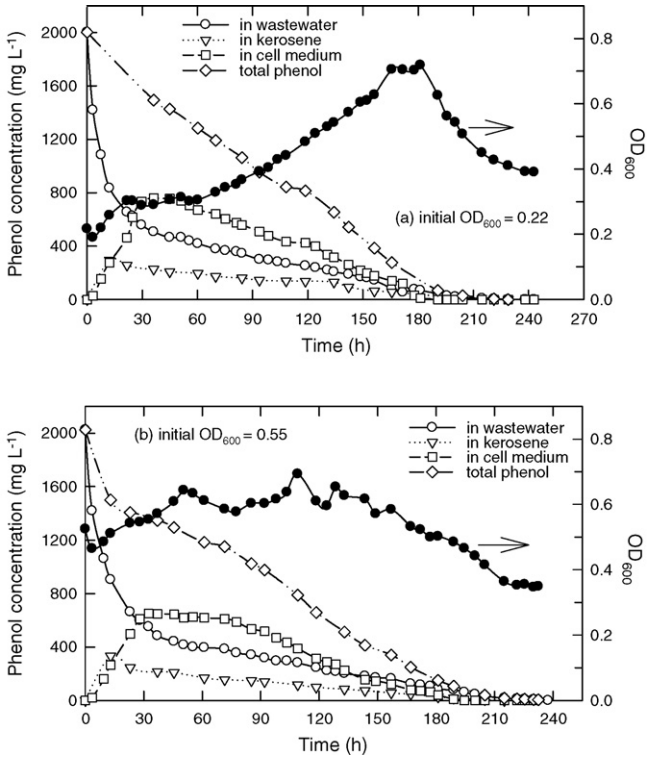

Fig. 7. Effect of initial cell concentration in cell medium (OD_{600} = 0.22 and 0.55) on phenol biodegradation in big module UMP-1147R (wastewater: 0.5 L, 2000 mg L^{-1} phenol, 100 g L^{-1} NaCl, pH 3; kerosene: 0.5 L; cell medium: 0.5 L, pH 7).

big module. The value of $S_{cm,max}$ is 730 mg L^{-1} (34 h), 760 mg L^{-1} (36 h), and 650 mg L^{-1} (32 h) when the initial OD_{600} is 0.055, 0.22, and 0.55, respectively. In addition, t_{95} is about 185, 186, and 184 h, respectively. Evidently, the 10-fold increase in cell concentration has little effect on the performance of this two-phase biodegradation process. This may be due to either the limitation of oxygen transfer and supply [9] or the possible release of inhibitory compounds from phenol biodegradation at high X_0.

The above argument is also supported from Fig. 8, which shows the effect of cell medium volume on the biodegradation of 2000 mg L^{-1} phenol by *P. putida* using the big module. This is the dilution effect because the total amount of cells is kept constant. It is found that $S_{cm,max}$ is 155 mg L^{-1} (35 h) and 760 mg L^{-1} (36 h) when the initial OD_{600} and cell medium volume is 0.055 and 2 L as well as 0.22 and 0.5 L, respectively; also, t_{95} is around 81 and 186 h, respectively.

3.4. Comments on the proposed two-phase treatment of saline wastewater

The present results reveal that the combination of solvent extraction and two-phase biodegradation in membrane contactors enables us to treat organic pollutants in the wastewaters with sufficiently high salinity and acidity as long as the qualified water-insoluble organic solvents are available. It can overcome or avoid the problems encountered in two-phase partitioning bioreactor such as emulsion formation, phase separation, and cells contamination [8], as well as in two-phase EMBR such as time-consuming. To treat water with high-level organic matter, however, the present process could be effectively enhanced by selecting qualified

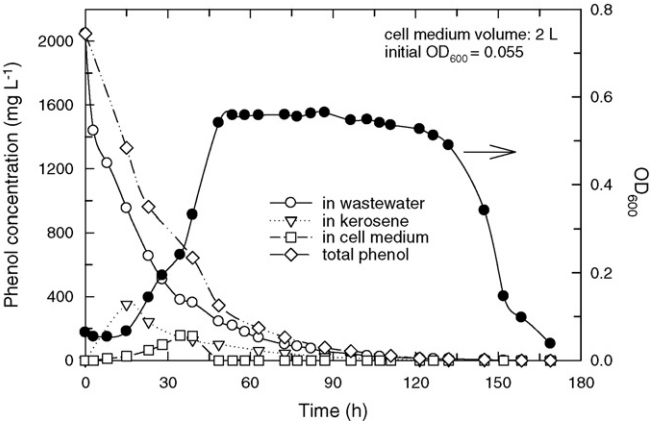

Fig. 8. Effect of cell medium volume on biodegradation rate of phenol in big module UMP-1147R (wastewater: 0.5 L, 2000 mg L^{-1} phenol, 100 g L^{-1} NaCl, pH 3; kerosene: 0.5 L; cell medium: 2 L, OD_{600} = 0.055, pH 7).

solvents, in contrast to the use of bigger membrane modules and higher cell concentrations. The qualified solvent should be biocompatible and has a suitable partition coefficient for organic matter with respect to specific aqueous solution. That is, such a solvent can control and adjust the concentration of organic matter in cell medium, making substrate inhibitory effect small and biodegradation feasible [9].

Further studies are necessary to establish the screening strategy for organic solvents because the optimal partition coefficient varies with the growth kinetics of biomass (or biodegradation kinetics of the organics), membrane module size, and even wastewater composition. The partition coefficient of organic matter for a given solvent can be also adjusted by mixing various fractions of the second miscible solvent. In a word, such a two-phase hybrid process treats highly polluted effluents in a cyclic batch mode and no dilution of the wastewater is required.

4. Conclusions

The possibility of the use of combined solvent extraction and two-phase biodegradation in hollow-fiber membrane contactors for the removal of phenol from saline wastewater was evaluated by *P. putida* BCRC 14365 at 30 °C. Phenol-loaded kerosene was passed through the lumen of the membrane module and the cell medium was flowed across the shell. The following results were obtained:

1. Back-partition and mass transfer of phenol from kerosene to cell medium was faster than the followed biodegradation in membrane contactors. The level of phenol transferred from kerosene to cell medium (S_{cm}) thus reached a maximum during the overall process, as found in typical consecutive reactions.
2. The present process was not favored to treat low-level phenol water (110 mg L^{-1}), due to the lack of phenol in the cell medium as carbon source for cell growth. The maximum S_{cm} ($S_{cm,max}$) was a simple index to judge the feasibility of the process. In addition, a threshold $S_{cm,max}$ value (in this case, 750 mg L^{-1}) could be estimated according to the growth kinetics of suspended cells; above that threshold value, the cells would attach on the membrane surface, thereby allowing to treating high-level effluents (e.g., 2400 mg L^{-1}).
3. For a phenol level of 2000 mg L^{-1}, an increase in initial cell concentration from 0.023 to 0.23 g L^{-1} did not improve biodegradation, possibly due to the limitation of oxygen supply or possible release of inhibitory compounds from phenol biodegradation. However, the time required for phenol removal reduced when the module area increased from 0.02 to 0.19 m^2 because the $S_{cm,max}$ not only increased but also reached more quickly. When the volume of each solution was 0.5 L, 95% of 2000 mg L^{-1} phenol in wastewater could be removed within 186 h using the bigger module.
4. Further studies are necessary to establish the screening strategy for organic solvent because the optimal partition coefficient would vary with the growth kinetics of cells, membrane module size, and even wastewater composition.

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