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Experimental investigation of bio-removal of toxic organic pollutants from highly saline solutions in a triphasic system

Ruey-Shin Juang[a,b,*], Kuo-Jui Tseng[a]

[a] Department of Chemical Engineering and Materials Science, Yuan Ze University, Chung-Li 32003, Taiwan
[b] Fuel Cell Center, Yuan Ze University, Chung-Li 32003, Taiwan

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ABSTRACT

A combined solvent extraction and biphasic biodegradation process was proposed to remove organic pollutants from highly saline solutions, where the organic solvent is biocompatible and the organic–aqueous interface remains quasi-quiescent during the process. Phenol and *Pseudomonas putida* BCRC 14365 were selected as the model organic compound and biomass, respectively. The effects of added NaCl concentration (50–200 g/L) and pH (1.0–9.0) in synthetic solutions on phenol removal and cell growth were studied at 30 °C. The initial cell concentration was fixed at 0.025 g/L. Within the examined range, the adjustment of solution pH to 3.0 resulted in the best removal performance of 1100 mg/L phenol from such saline solutions (an apparent removal rate of about 20 mg/(L h)) when using kerosene as the organic solvent. The overall process appeared to be favored when the salt concentration in saline solution was in the range of 100–150 g/L. The application potentials of such a triphasic process for the removal of toxic organics (phenol) from highly saline and acidic wastewaters were finally demonstrated in fed-batch mode.

1. Introduction

Industrial wastewater sometimes contains organic pollutants, together with high salt levels and/or the extremes of pH, one or both of which prevent or at least hinder microbial growth [1]. The main end markets for salts are chemical process industries (mainly the chloralkali sector), road deicing, and agro-food industries. Other non-negligible uses of salts are encountered in petroleum, textile, and leather industries, as well as for softening hard water. Each of these sectors generates large amounts of saline effluents rich in both salts and organics [2]. Such effluents will cause severe damage by the contamination of soil, surface, and groundwater if they are discharged into the environment without prior treatment.

Practical saline/acidic effluents are often recalcitrant to biological treatment; therefore, physicochemical treatment is suggested. Reported techniques include ion exchange (to remove salts), thermal methods such as multiple-effect evaporation (to reduce the volume of the effluent), and membrane processes such as ultrafiltration (to remove suspended solids or colloidal COD), reverse osmosis, and electrodialysis (to remove salts). For salt removal, reverse osmosis is very efficient, yet high amounts of suspended solids and organics in effluent reduce the lifetime and efficiency of the membrane [2]. That is, the effective removal of organics from highly saline effluents is required prior to the physicochemical processes. On the other hand, it has also been proven feasible to use salt-adapted biomass capable of withstanding high salinity and of degrading the organics that are contained in the effluent [1,2]. The use of such biomass is thus permitted 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 the biomass is sensitive to environmental shocks [2]; moreover, high levels of salts are known to compromise the correct operation of traditional aerobic wastewater treatment only above chloride levels of 5–8 g/L [3].

It is known that solvent extraction (SX) is an effective way to separate water-soluble organics from salts and ions in saline solutions [4]. The organics are back-partitioned to the aqueous cell medium when the cell medium is in contact with the loaded organic solvent; biodegradation thus occurs in the cell medium as long as the solvent is biocompatible. This is the basis of the two-phase (biphasic) partitioning bioreactor, which was first proposed by Daugulis and his co-workers for the biodegradation of xenobiotic organics that exist in organic solvents [5]. In this bioreactor, a water-immiscible and biocompatible organic solvent that is allowed to float on the surface of the biomass-containing aqueous phase is used. The solvent can dissolve high levels of xenobiotic organics, which then partition to the aqueous phase at low levels. That is, the biomass experiences only low levels of the toxic organics, although large

* Corresponding author at: Department of Chemical Engineering and Materials Science, Yuan Ze University, 135 Yuan-Tung Road, Chung-Li 32003, Taoyuan, Taiwan. Tel.: +886 3 4638800x2555; fax: +886 3 4559373.
E-mail address: rsjuang@saturn.yzu.edu.tw (R.-S. Juang).

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amounts are added to the bioreactor. A local non-equilibrium is created when the biomass consumes some of the toxic organics, which causes more of the organics to be partitioned into the aqueous phase when the system attempts to maintain thermodynamic equilibrium [5–7].

An attempt was therefore made here to remove toxic organics from saline and acidic solutions by a triphasic process (i.e., coupling SX with biphasic biodegradation) in a continuous mode. In this process, the organics were extracted (partitioned) from the saline solution to an organic solvent in the first vessel and back-partitioned to the aqueous cell medium in the second vessel. Although the organic solvent could be toxic to the environment, it can be recycled as long as the volatility and water solubility is sufficiently low. To readily simulate such a triphasic process in the subsequent work, the aqueous–organic interfaces during experiments are kept quasi-quiescent; in this case, the interfacial area for mass transfer can be specified. Moreover, direct mixing of both sets of two immiscible phases may lead to the formation of emulsions, particularly when the effluent contains surface-active substances, making phase separation and subsequent treatment more difficult.

Phenol was selected as the model organic compound here because it is a common representative of toxic organics even at extremely low levels [8]. *Pseudomonas putida* (*P. putida*) was used due to its high removal efficiency [9,10]. The feasibility of using a triphasic process for the removal of phenol in such highly saline and acidic solutions was investigated. First, six organic solvents were screened *via* biodegradability tests, and the effects of operating parameters on the performance were studied. The synthetic wastewater contained 1100 mg/L phenol and 50–200 g/L NaCl in pH range of 1.0–9.0. The initial concentration of *P. putida* BCRC (Bioresource Collection Research Center) 14,365 in mineral salt (MS) medium was fixed at 0.025 g/L. An operating temperature of 30 °C was chosen, which is suitable for *P. putida* growth [9]. This triphasic process could be an interesting test for this purpose because the toxic organic pollutants were initially present in the organic solvent in the previous studies using biphasic partitioning bioreactors [5–7,11–17].

2. Materials and methods

2.1. Microorganism, nutrient medium, and solutions

P. putida BCRC 14365 (ATCC 31800, source: wastewater from textile chemical plant, Welford, SC) used was obtained from the Food Industry Research and Development Institute, Hsinchu, Taiwan. The stock cultures were stored at −80 °C and were streaked on nutrient agar 24 h before experiments. The nutrient medium contained 3 g/L beef extract, 5 g/L peptone, and the MS medium at pH 7.0. The compositions of MS medium (in g/L) 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) [9]. A phosphate buffer of pH 7.0 was prepared by mixing equal volumes of 0.375 g/L K_2HPO_4 and 0.465 g/L KH_2PO_4 solutions in deionized water (Millipore, Milli-Q). All inorganic chemicals used were supplied by Merck Co. (Germany) as analytical reagent grade. Prior to use, the MS medium and phosphate buffer were sterilized in an autoclave at 121 °C for 30 min.

Analytical reagent grade organic solvents including 1-hexanol, 1-octanol, 1-decanol, 2-undecanone, and decane were selected here for biodegradability tests because they have been commonly used in biphasic bioprocesses [11–13]. All these solvents were offered from Merck Co. and used as received. Kerosene (Union Chemical Co., Taiwan) was washed twice with 20 vol.% H_2SO_4 to decolorize and remove possible aromatics and then with deionized water three times before use. The wastewater was prepared by dissolving 1100 mg/L phenol (Merck) and 50–200 g/L NaCl in deionized water, the pH of which was adjusted to be 1.0–9.0 by adding 0.1 M of HCl or NaOH solution. The cell medium consisted of MS medium at pH 7.0. All aqueous pH values were measured using a pH meter (Horiba F-23, Japan).

2.2. Free suspension cultivation

P. putida cells were cultured at 30 °C in a nutrient medium, into which 100 mg/L phenol was added for adaptation for 24 h. The cells collected after centrifugation at 6000 × *g* for 10 min were resuspended in phosphate buffer and re-centrifuged. After cleaning, the cells were inoculated into the MS medium (350 mL) in 500-mL Erlenmeyer flasks in an incubator to give an initial concentration of 1.3×10^8 cells/mL. This corresponds to an optical density (OD) at 600 nm of 0.064 or a dried cell weight of 0.025 g/L. After inoculation, the flask was capped with cotton plugs and placed in a shaker controlled at 120 rpm and 30 °C.

2.3. Solvent selection

Biodegradability tests were conducted as follows [11,12]. The activated cells were placed in 100-mL glass flasks after they were inoculated in the MS medium (50 mL) to a concentration of 0.025 g/L, to which 5 mL of the tested solvent and 550 mg/L phenol were added. These flasks were closed with a Teflon sieve and controlled at 30 °C in a shaker bath at 110 rpm. In some experiments, the mixtures of two solvents with an equal-volume fraction were tested. Aqueous samples (1 mL) were taken at regular time intervals to monitor the biodegradation of phenol and the growth of *P. putida*. A control test was also carried out under similar conditions in the presence of biomass but in the absence of organic solvent.

The biodegradability of the organic solvent(s) that showed acceptable phenol biodegradation was further examined. For each organic solvent, the biodegradation of phenol and/or the growth of cells were compared using three different carbon sources, 500 mg/L phenol, 5 vol.% organic solvent, and the combination of both. For simplicity, an organic solvent was considered to be non-biodegradable if the biomass did not apparently grow on that single carbon source.

2.4. Measurements of partition coefficients

Equal volumes (20 mL) of the aqueous solutions initially containing 1100 mg/L phenol and the organic solvents were placed in 50-mL Erlenmeyer flasks that were closed with a Teflon sieve. They were agitated at 100 rpm by a magnetic stirrer for 24 h in a water bath at 30 °C. After phase equilibrium, the concentration of phenol in aqueous solution was measured by HPLC, as stated in Section 2.6, and that in organic solvent was obtained by mass balance. Thus, the partition coefficient of phenol was calculated by

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

2.5. Experiments in the triphasic process

Fig. 1 shows the experimental setup of the triphasic process and the dimensions of the glass vessels used. After the activated cells were inoculated in the MS medium to a concentration of 0.025 g/L, they were placed in a second vessel; in the meantime, saline solution containing phenol was poured into the first vessel and agitated by a magnetic stirrer at 100 rpm. The organic solvent was gradually added into the system and was circulated at a flow rate of

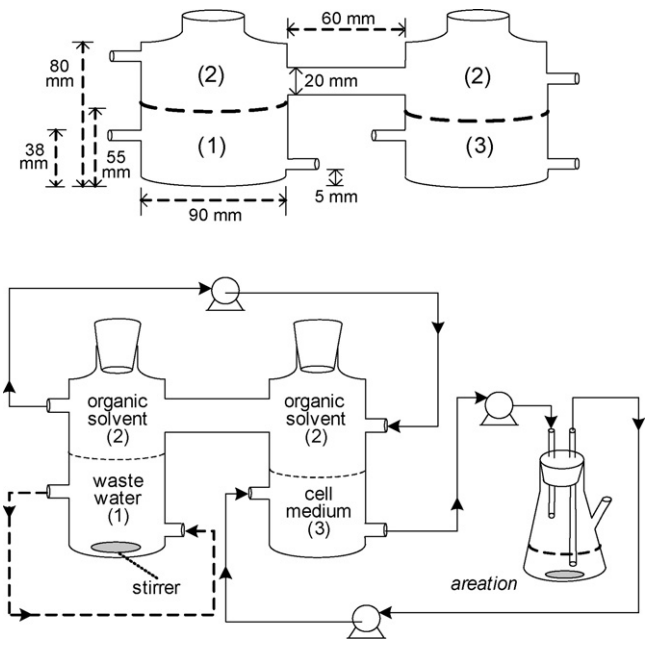

Fig. 1. Dimensions of the vessels and the experimental setup of triphasic process for the removal of toxic organic pollutants from highly saline solutions.

80 mL/min using a peristaltic pump (Cole-Parmer, USA). This flow rate was selected because the aqueous–organic interfaces in both vessels remained quasi-quiescent. In this work, the cell medium was pumped at a flow rate of 60 mL/min to an external flask that was agitated by a magnetic stirrer at 300 rpm to provide necessary aeration, and the medium was totally recycled to the second vessel at the same flow rate.

The temperature was controlled at 30 °C by immersing the entire device in a water bath. Unless specified elsewhere, the working volumes of wastewater, organic solvent, and cell medium were each 350 mL. Samples were taken (wastewater 1 mL, cell medium 1 mL, organic phase 3 mL) at preset time intervals. It should be noted that the sample withdrawn from the cell medium was immediately mixed with 0.3 mL of 3 M HCl to stop further biodegradation.

Some fed-batch experiments were finally conducted following the dashed line around the first vessel of Fig. 1. Two feeding strategies were attempted by monitoring either biomass concentration in the cell medium or phenol level in the wastewater. At selected timing, 10 mL of 35 g/L phenol solution and 10 mL of fresh MS medium (free of bacteria) were periodically and simultaneously added to the wastewater and cell medium, respectively.

2.6. Analysis of cells and phenol

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 that contained 3 g/L beef extract, 5 g/L peptone, and 15 g/L agar. The plate was then incubated at 30 °C for 24 h, and the number of colonies was counted. In separate experiments, cells in the cultures (40 mL) with different cell concentrations were harvested by centrifugation (6000 × g) at 4 °C for 10 min and washed with deionized water. The cells were then dried at 60 °C for 24 h until a constant weight was reached.

Biomass concentration in the cell medium was determined by measuring OD at 600 nm using a Jasco UV/visible spectrophotometer (UV-550, Japan). The aqueous samples from 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). An aliquot of the sample (10 μL) was injected. A mixture of acetonitrile (50 vol.%) and water that flowed at 1.0 mL/min was used as the mobile phase. The temperature was fixed at 40 °C, and the wavelength of UV detector (Jasco 975) was at 280 nm. On the other hand, the organic sample (3 mL) was premixed with deionized water (3 mL) for 24 h, and the concentration of phenol in aqueous layer was analyzed as described above. Phenol concentration in the organic solvent was obtained according to the measured aqueous-phase concentration and its partition coefficient between the organic solvent and water. Each experiment was at least duplicated under identical conditions. The detection limit of phenol was about 4 mg/L, and the reproducibility of concentration measurements was within 5%.

3. Results and discussion

3.1. Selection of suitable organic solvents

Fig. 2 shows the results of biodegradability tests. It is found that, compared to the control test (i.e., the absence of organic solvents), biodegradation of phenol is acceptable in the presence of 1-decanol, kerosene, and an equal-volume mixture of both. That is, phenol can be completely biodegraded within a reasonably short period under such conditions.

In this regard, the biodegradability of 1-decanol or kerosene itself was further examined. It is clear from Fig. 3a that 1-decanol could act as a carbon source for cell growth, but this is not the case for kerosene, which can justifiably be considered non-biodegradable because no cell growth occurs during the experiment. As shown in Fig. 3b and c, the presence of 1-decanol enhances the growth of *P. putida*. Thus, 1-decanol is not adequate

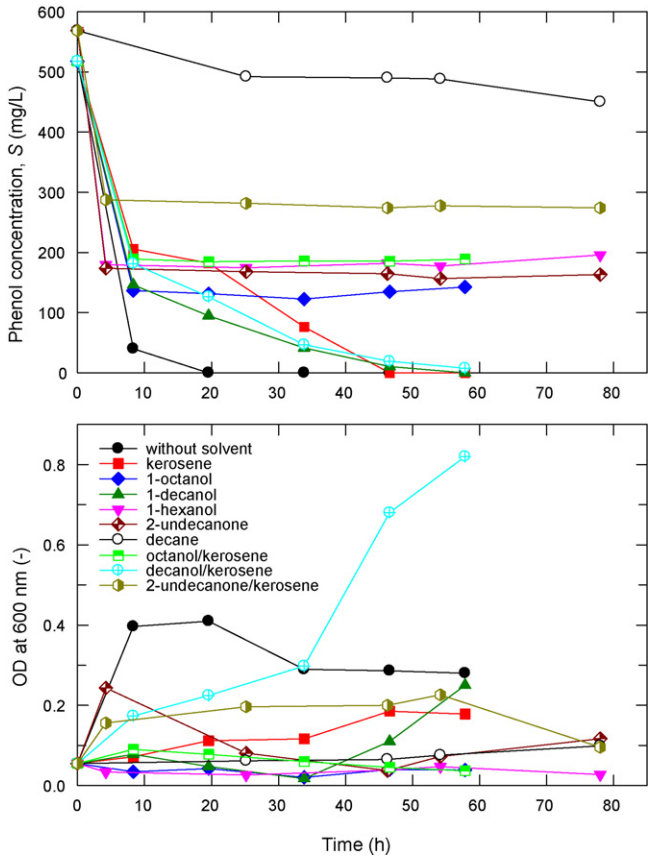

Fig. 2. Phenol biodegradation and cell growth in a mixture of MS medium (50 mL) and 5 mL of various single organic solvents and equal-volume binary mixtures (1 OD = 0.39 g dried cell/L).

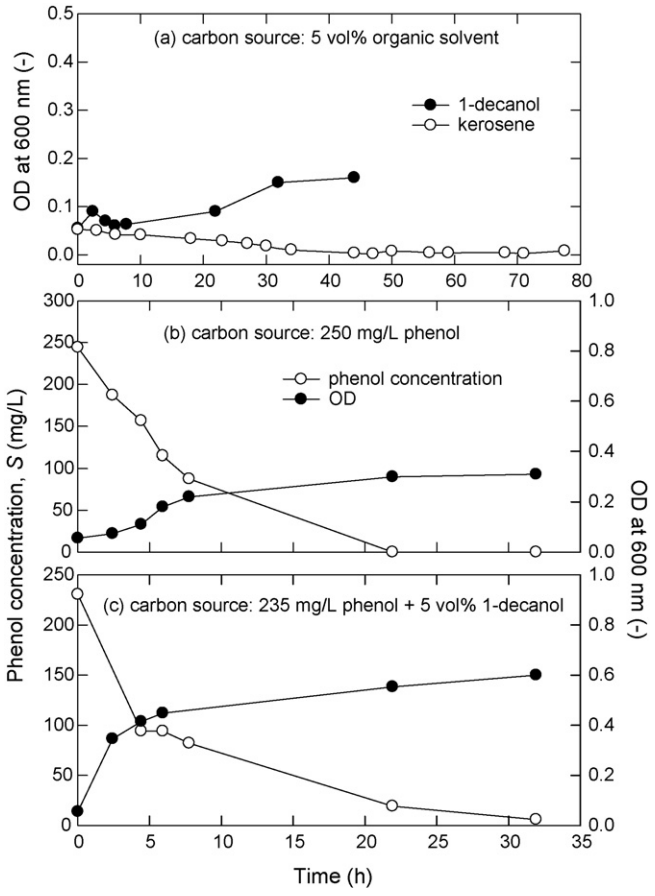

Fig. 3. Growth of *P. putida* in the MS medium (50 mL) containing the carbon sources of (a) 5 vol.% solvent, (b) 250 mg/L phenol, and (c) 235 mg/L phenol and 5 vol.% 1-decanol (1 OD = 0.39 g dried cell/L).

as an organic solvent for this purpose. In fact, 250 mg/L phenol and 5 vol.% 1-decanol contribute nearly the same extent of cell growth. In this regard, kerosene is chosen as the solvent in the present triphasic process.

In addition to the above properties, the low water solubility, suitable density (about 0.8 g/mL), low volatility (vapor pressure of 0.1 Torr at 37 °C), and low cost of kerosene make it practically promising [18]. Table 1 lists the partition coefficients of phenol between kerosene and various aqueous media that are considered to be adequate for this purpose. For example, the partition coefficients of 1100 mg/L phenol between kerosene and wastewater as well as kerosene and cell medium are 0.50 and 0.35, respectively, at 30 °C.

Table 1
Partition coefficients of phenol (1100 mg/L) between equal volumes of kerosene and various aqueous solutions at 30 °C.

Aqueous solution	Partition coefficient
Deionized water (without salt)	0.35
Cell medium (MS medium at pH 7.0)	0.33
Saline solution (100 g/L NaCl, pH 1.0)	0.51
Saline solution (100 g/L NaCl, pH 3.0)	0.50
Saline solution (100 g/L NaCl, pH 9.0)	0.45
Saline solution (150 g/L NaCl, pH 3.0)	0.66
Saline solution (200 g/L NaCl, pH 3.0)	1.98

3.2. Effect of wastewater pH on phenol biodegradation

Fig. 4 shows the effect of wastewater pH on the biodegradation of 1100 mg/L phenol in highly saline solutions (100 g/L NaCl) by *P. putida* in MS medium. It is clear that *P. putida* starts to grow in the cell medium, although gradually, at the early stage of this triphasic process. The cells exhibit a typical growth curve, and the lag phase does not exceed about 5 h. Phenol level in the cell medium (S_{cm}) increases during the lag phase and reaches a maximum near the beginning of the exponential phase of cell growth. After the lag phase, cells begin to grow quickly at the expense of the fast biodegradation of phenol.

To discuss more definitively the effects of operating parameters such as wastewater pH and salt level on phenol removal, two quantities were compared. One is the maximum phenol level in the cell medium ($S_{cm,max}$). Because phenol that is transferred to the cell medium is the sole carbon source for cell growth, the magnitude of $S_{cm,max}$ and the time required to reach this value (t_{max}) are important indices of system performance. The other quantities are the apparent (R_{app}, average basis) and maximum removal rates (R_{max}, instantaneous basis) of phenol (mg/(L h)) defined by

$$R_{app} = -\left(\frac{S_{tot}\big|_{t=t_{100}} - S_{tot}\big|_{t=0}}{t_{100}}\right) = \frac{S_0}{t_{100}} \quad (2)$$

$$R_{max} = -\left(\frac{dS_{tot}}{dt}\right)_{max} \quad (3)$$

where S_0 and S_{tot} are the total phenol levels in the system at time $t = 0$ and $t = t$, respectively, and t_{100} is the time required for complete removal of phenol from the system (i.e., $S_{tot} = 0$). In general, t_{100} can be directly obtained from the experimental data or estimated by extrapolating S_{tot} to zero in the plots of S_{tot} versus t.

The values of $S_{cm,max}$, t_{max}, R_{app}, and R_{max} obtained at different wastewater pH are listed in Table 2. It is seen that $S_{cm,max}$ gradually decreases with increasing wastewater pH but sharply reduces at pH 9.0. In addition, the t_{max} values are comparable under the studied pH ranges. Phenol in water has a pK_a of 9.86 [19]. About 12% of phenol exists in the form of sodium salt in wastewater at pH 9.0. The slower mass transfer observed at pH 9.0 is thus related to the dissociation of phenol and to the consequent reduction in the partition coefficient (the order of magnitude of partition coefficient reduction is consistent with the fraction of phenol sodium salt, as shown in Table 1) and in the driving force for mass transfer [13]. The comparatively low OD at 600 nm, as shown in Fig. 4d, supports this argument. On the other hand, a wastewater pH of 3.0 is suggested according to the values of R_{app} or R_{max} (Table 2), although a wastewater pH of 1.0 is also acceptable.

It was reported that there is a substrate inhibitory effect when phenol in MS medium alone (without salts and solvents) is

Table 2
The apparent and maximum rates of phenol removal obtained using the present triphasic process (initial phenol level S_0 = 1100 mg/L; initial cell concentration 0.025 g/L).

Saline solutions		$S_{cm,max}$ (mg/L)	t_{max} (h)	R_{app} (mg/(L h))	R_{max} (mg/(L h))
pH	NaCl concentration (g/L)				
1.0	100	400	9	16.6	44.4
3.0	100	340	8	19.3	50.7
5.0	100	330	7	12.8	38.1
7.0	100	270	8	10.9	34.8
9.0	100	100	8	8.7	29.6
3.0	50	180	8	13.0	40.8
3.0	150	370	8	19.6	44.0
3.0	200	570	20	12.9	42.9

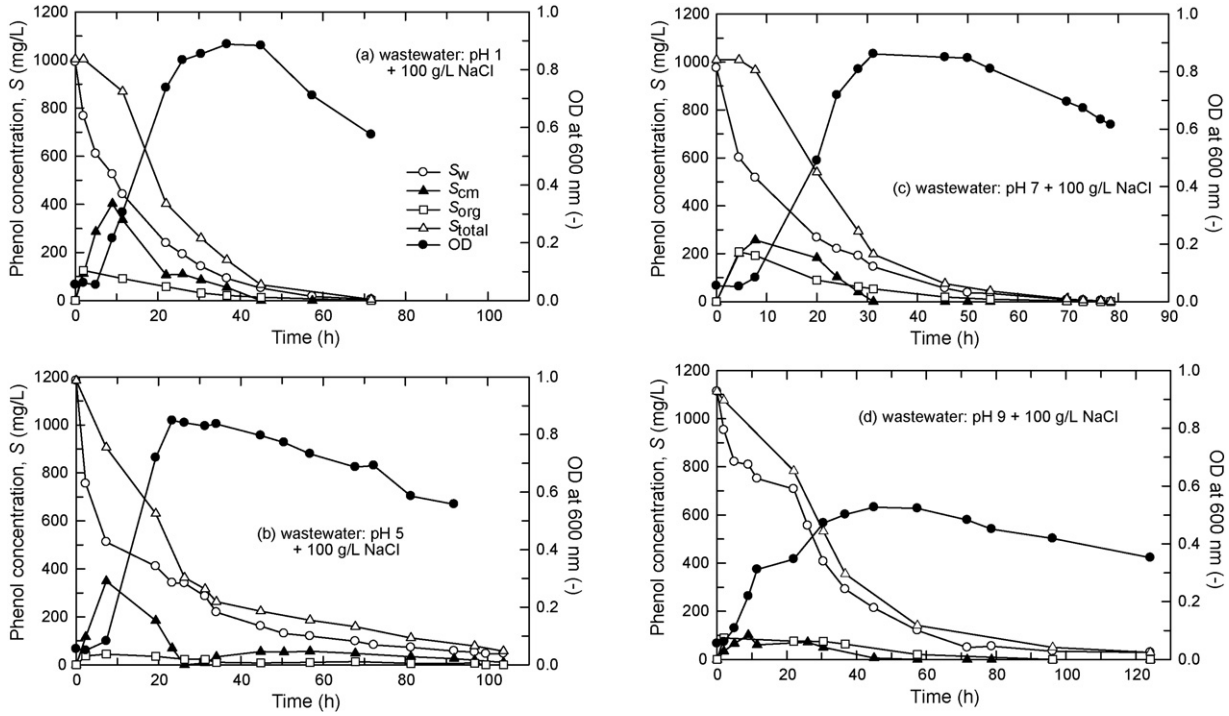

Fig. 4. Effect of the pH of wastewater containing 100 g/L NaCl on phenol biodegradation and cell growth (a) pH 1.0, (b) pH 5.0, (c) pH 7.0, and (d) pH 9.0 (1 OD = 0.39 g dried cell/L).

degraded at 30 °C and pH 7.0 by suspended *P. putida* BCRC 14365 [9]. The maximum cell growth rate occurs at a phenol level of 100 mg/L; above that, *P. putida* starts to be inhibited and cannot grow as phenol level exceeds about 650 mg/L. Thus, $S_{cm,max}$ value of 400 mg/L obtained in the present triphasic system still allow *P. putida* cells to grow. That is, the $S_{cm,max}$ value is proposed to judge whether or not the triphasic system works. It is expected that the $S_{cm,max}$ value can be controlled *via* the selection of suitable solvents.

On the other hand, biphasic biodegradation process can also be operated in microporous hollow-fiber membrane contactors [20,21]. For treating toxic organics in saline and acidic wastewaters, for example, phenol in wastewater was first extracted by kerosene in a batch vessel, and the loaded solvent was passed through the lumen of polyvinylidene fluoride hollow fibers (pore size 0.2 μm, area 0.02 m^2); simultaneously, *P. putida* BCRC 14365 in the MS medium was flowed across the shell. Under comparable conditions in the present triphasic system including S_0, initial cell concentration, and wastewater pH, such a membrane-based triphasic process gave higher $S_{cm,max}$ (400–500 mg/L) and larger t_{max} (~50 h). This is because of the existence of much larger mass transfer resistance through the micropores of the membrane. In fact, the wastewater pH of 3.0 was also optimized in such a membrane-based triphasic process [20], although it was judged on the basis of the time required for complete removal of phenol (t_{100}), instead of R_{app} or R_{max}.

3.3. Effect of salt level in the wastewater on phenol biodegradation

Fig. 5 shows the effects of salt level in the wastewater (50–200 g/L NaCl) on the biodegradation of 1100 mg/L phenol in acidic solutions (pH 3.0) by *P. putida* in the MS medium. Evidently, $S_{cm,max}$ increases with increasing salt level, which is consistent with the trend of the partition coefficient (Table 1). For the wastewater containing 200 g/L NaCl, $S_{cm,max}$ is much higher (570 mg/L), thereby leading to a much larger t_{max} (20 h). This is likely because the bacterial strain needs 3 days before becoming acclimated to such a high phenol concentration (membrane changes, specific protein expression, etc.) or because it has high partition coefficient due to the salting-out effect [3,19,20]. Thus, a salt level of 100–150 g/L in wastewater is preferred for the present triphasic process according to the results of R_{app} or R_{max} (Table 2).

In the biodegradation of phenol at pH 7.0 by suspended *P. putida*, it was found that cells cannot grow on 100 mg/L phenol when the solution contains NaCl of more than 25 g/L, likely due to salinity toxicity (osmotic effect) [22,23]. The present triphasic process should include extraction (partition) of phenol from wastewater to kerosene, stripping (back-partition) of phenol from the loaded solvent to the cell medium, and the biodegradation of phenol in the cell medium. The improved removal efficiency of organics with sufficiently high salt levels (e.g., 200 g/L) is a result of preventing inorganic salt ions from partitioning to the organic solvent.

On the other hand, this work has demonstrated that the present triphasic process is able to readily remove the priority organic pollutants such as phenol and trichloroethylene from industrial effluents with sufficiently high salinity, acidity, or alkalinity as long as adequate water-insoluble organic solvent is available. More definitively, adequate organic solvent, which should be screened by experimentation, means that it must be biocompatible, non-biodegradable, and have a suitable partition coefficient for the organic pollutant with respect to the specific aqueous medium. This solvent could control the remaining concentration of organic pollutant in the cell medium, making substrate inhibitory effect negligibly small and biodegradation process feasible. The so-called optimal partition coefficient varies with biodegradation kinetics of organic pollutant (or growth kinetics of the cells) and wastewater compositions in the present triphasic system; however, the partition coefficient of an organic solvent can be adjusted by adding various fractions of the second miscible solvent. In other words, such a triphasic process readily treats highly polluted wastewater in cyclic batch mode [5,6,14]: no dilution of the wastewater is required. However, problems encountered in

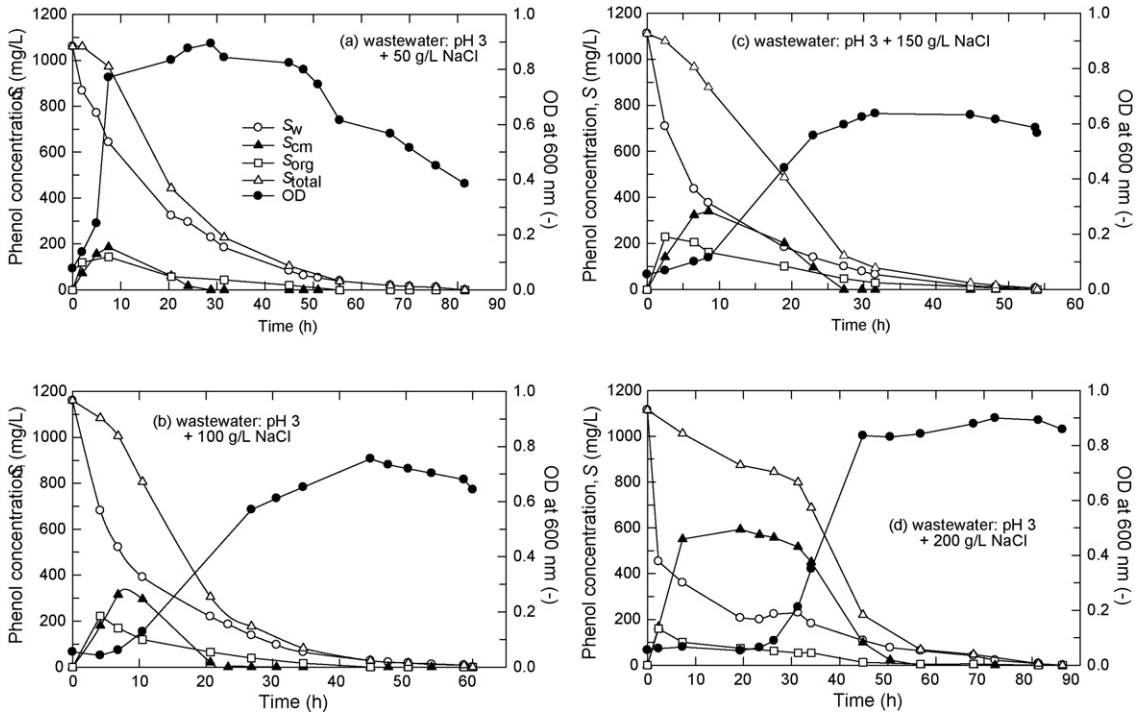

Fig. 5. Effect of salt level in the wastewater at pH 3.0 on phenol biodegradation and cell growth (a) 50 g/L NaCl, (b) 100 g/L NaCl, (c) 150 g/L NaCl, and (d) 200 g/L NaCl (1 OD = 0.39 g dried cell/L).

common liquid–liquid systems such as emulsion formation, phase separation, and biomass contamination should be avoided.

3.4. Fed-batch triphasic process

As described above, the possibility of using the present triphasic process in a fed-batch mode was tested [5,6,14]. Figs. 6 and 7 show the results for periodical feeding when OD at 600 nm was lower than 0.7–0.8 and S_w was larger than 10 mg/L. In the first mode (Fig. 6), cells grow well, although the total phenol level in the system (S_{tot}) continuously increases with the time of feeding because phenol cannot be completely degraded in each batch. This problem could be readily overcome as long as the response (i.e., OD in cell medium) is adjusted to a low criterion, for example, 0.4–0.5; in this case, the degradation time is sufficient, as seen between the third and fourth feeding.

Conversely, another lag phase of cell growth occurs after the second feeding in the second mode (Fig. 7) although an efficient degradation is obtained. The reasons leading to such a good degradation rate remain unclear at this stage compared to the results between the first and second feeding. Regardless, the treatment capacity is significantly reduced, and the behavior is more difficult to predict, in contrast with the case illustrated in Fig. 6. In other words, the efficient biodegradation and easy monitoring (optical density) would make the triphasic process practically promising for the removal of toxic organics from highly saline and acidic/basic effluents.

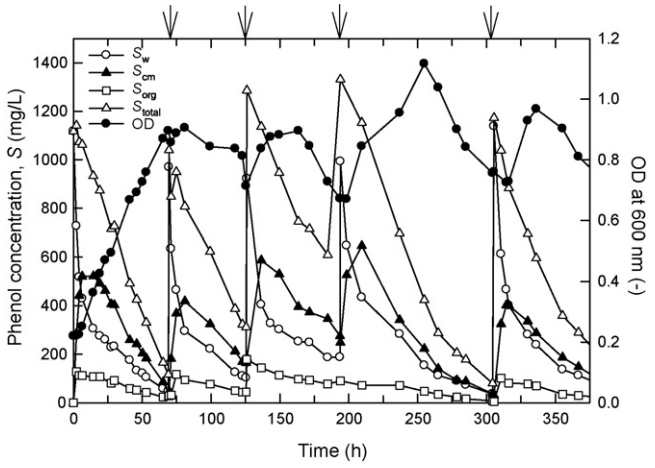

Fig. 6. Fed-batch experiments based on the response of biomass concentration in the cell medium for the wastewater containing 100 g/L NaCl at pH 3 (1 OD = 0.39 g dried cell/L).

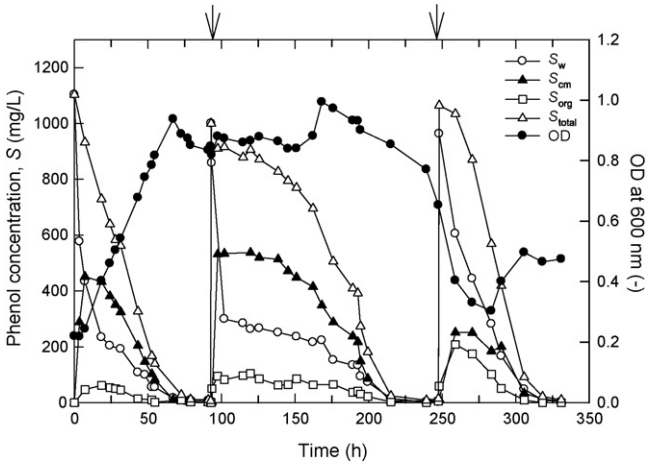

Fig. 7. Fed-batch experiments based on the response of phenol level in the wastewater S_w for the wastewater containing 100 g/L NaCl at pH 3 (1 OD = 0.39 g dried cell/L).

4. Conclusions

The feasibility of using a triphasic process (solvent extraction + biphasic biodegradation) for the removal of phenol from highly saline solutions by *P. putida* BCRC 14365 in a mineral salt medium was evaluated at 30 °C. The following results were obtained:

1. Of the organic solvents studied (1-hexanol, 1-octanol, 1-decanol, 2-undecanone, decane, and kerosene), biodegradability tests showed that kerosene was preferred. In addition, the low water solubility, suitable density, low volatility, and low cost of kerosene made it practically promising for recycling use.
2. The maximum phenol level in the cell medium ($S_{cm,max}$) and the maximum removal rate (R_{max}) were the crucial parameters in characterizing the present process. $S_{cm,max}$ was used to judge whether or not the system worked, and R_{max} was used to determine the efficiency. In general, a system with a much lower $S_{cm,max}$ (e.g., the case when wastewater pH was not lower than or not far from the pK_a of the organics) was not favored. However, $S_{cm,max}$ should not exceed the level where the cells started to be inhibited and could not grow.
3. The values of $S_{cm,max}$ and the partition coefficient showed the same trends with salt concentration. The much high partition coefficient of phenol due to the salting-out effect when wastewater contained 200 g/L NaCl led to a high $S_{cm,max}$ and a longer degradation process. In this regard, a salt level of 100–150 g/L in wastewater was preferred for the present triphasic process according to the results of degradation rates.
4. Fed-batch tests showed that periodic feeding of fresh wastewater was better achieved by monitoring cell concentration in the cell medium instead of the phenol level in the wastewater; in this situation, the treatment capacity was remarkably higher. Such an efficient bio-removal makes the triphasic process promising for removing toxic organics from highly saline and strongly acidic/basic solutions, provided that biocompatible solvents were available. However, further studies are necessary to establish a method for determining an optimal partition coefficient of the organics for a given treatment process.

Notation

MS	mineral salt
OD	optical density of cell medium at 600 nm
R_{app}	apparent removal rate of phenol defined in Eq. (2) (mg/(L h))
R_{max}	maximum removal rate of phenol defined in Eq. (3) (mg/(L h))
S_0	initial phenol level in the system (mg/L)
S_{cm}	phenol level in the cell medium at any time (mg/L)
$S_{cm,max}$	maximum phenol level in the cell medium during the process (mg/L)
S_{org}	phenol level in the organic phase at any time (mg/L)
S_{tot}	total phenol level in the system at any time (mg/L)
S_w	phenol level in the wastewater at any time (mg/L)
SX	solvent extraction
t_{max}	time required to reach the $S_{cm,max}$ value (h)
t_{100}	time required for complete removal of phenol in the system (h)

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