



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

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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-quiet 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.

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### 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

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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 contained 1100 mg/L phenol and 50–200 g/L NaCl in pH range of 1.0–9.0. The compositions of MS medium (in g/L) were KH<sub>2</sub>PO<sub>4</sub> (0.42), K<sub>2</sub>HPO<sub>4</sub> (0.375), (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (0.244), NaCl (0.015), CaCl<sub>2</sub>·2H<sub>2</sub>O (0.015), MgSO<sub>4</sub>·7H<sub>2</sub>O (0.05), and FeCl<sub>3</sub>·6H<sub>2</sub>O (0.054) [9]. A phosphate buffer of pH 7.0 was prepared by mixing equal volumes of 0.375 g/L K<sub>2</sub>HPO<sub>4</sub> and 0.465 g/L KH<sub>2</sub>PO<sub>4</sub> 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<sub>2</sub>SO<sub>4</sub> 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 re-suspended 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<sup>8</sup> 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

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