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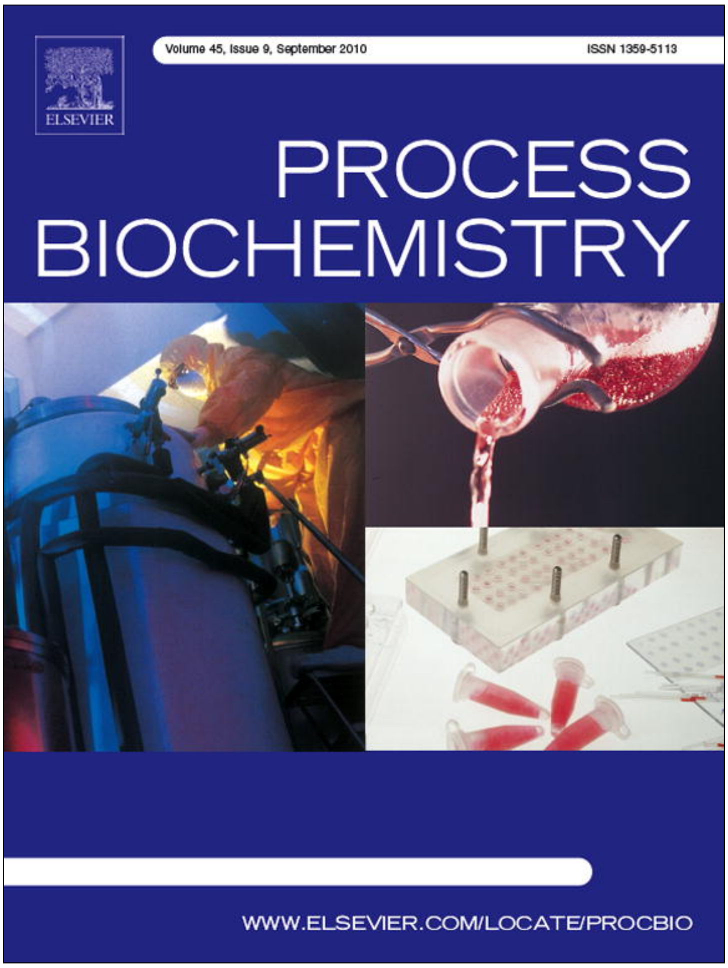
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Short communication

Bioremediation of phenol-contaminated water and soil using magnetic polymer beads

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ABSTRACT

In order to easily separate pollutant-absorbing polymer beads from contaminated soil or water, novel polymer beads containing magnetic particles were developed. The polymer beads containing 4.67% (w/w) magnetic particles exhibited an almost identical partitioning coefficient for phenol compared to that of the pure polymer. A 1.5 L phenol solution of 2000 mg/L added to a bioreactor was reduced to 481 mg/L phenol within 3 h by adding 100 g of these magnetic beads, and the phenol was completely degraded by microorganisms in 16 h. The magnetized beads were then readily removed from the bioreactor by a magnet with 10,000 G, and subsequently detached for re-use. 500 g of soil contaminated with 4 mg-phenol/g-soil was also contacted with 100 g beads, and greater than 97% removal of phenol from the soil was achieved within 1 day. The phenol-absorbing beads were easily separated from the soil by the magnet and transferred into a fermentor. The phenol was released from the beads and was degraded by the microorganism in 10 h. Modifying polymers to possess magnetic properties has greatly improved the ease of handling of these sequestering materials when decontaminating soil and water sources, in conjunction with contaminant release in partitioning bioreactors.

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1. Introduction

Two-phase partitioning bioreactors (TPPBs) have been successfully applied to the biodegradation of various pollutants present at high concentrations [1–3]. Organic solvents have been traditionally used as a second phase in TPPBs, and they can protect cells from toxic substrates by lowering the concentration of pollutants in the aqueous phase and also enhancing the oxygen transfer rate in TPPBs [4]. A systematic procedure for the selection of an optimum organic solvent for use in TPPBs has previously been suggested [5]. According to the procedure, the organic solvent should be biocompatible, non-bioavailable, non-hazardous, inexpensive and have a high partitioning coefficient for the pollutant relative to the aqueous phase. A selection of organic solvents that meet these criteria can be readily prepared via this procedure in the case of pure cultures being used in TPPBs. Such a selection, however, may be much more complicated in the case of mixed cultures because each microorganism in the consortium may have different biological properties. We have recently shown the potential of replacing the liquid organic phase of TPPBs with inexpensive polymers as a second phase, which has the advantage of allowing the use of microbial consortia, while also eliminating operational challenges (e.g., emulsions) which sometimes arise in two-liquid phase TPPBs [6–8]. Various polymers in the shape of beads have been applied to the treatment of phenol [7,9], BTX [8,10] and polyaromatic hydrocarbons (PAHs) [11,12] in contaminated water [8] or soil [9,13]. In TPPBs with polymer beads as a second phase, the polymer beads absorb the target pollutants present in the TPPBs based on equilibrium considerations and release the pollutants to the cells in the aqueous phase based on metabolic demand. When using the polymer beads to decontaminate soil or water sources via direct contact and absorption, easy separation of the beads from the contaminated environment after absorbing the pollutants is essential. In previous work we have separated polymer beads from soil manually [13] or with a mesh screen [9,11]. However, these methods are likely acceptable only at the laboratory scale. Accordingly, a practical method which can be applied to actual site application should be developed in order to facilitate commercialization of the technology of TPPBs with polymer beads as the second phase for bioremediation. In this study, we are suggesting such a novel method, the co-blending of polymers with magnetic particles, for easy separation of polymer beads after absorbing pollutants from contaminated environments.

The generation and use of modified polymer beads (i.e., containing magnetic particles) possessing enhanced removal capabilities provides a novel and substantial improvement over our previous work in which we used as-received commercial polymers for bioremediation applications. To confirm the efficacy of our

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approach, in this study we demonstrated the feasibility of admixing and re-blending a thermoplastic polymer with magnetic particles, determined the proportion of magnetic beads that would be required to allow easy bead separation via a permanent magnet, determined the impact of the presence of magnetic particles on pollutant uptake performance by the polymers, and finally demonstrated the complete performance "cycle" of manufacturing magnetic polymer beads, decontaminating soil and water sources, and bioremediating the phenol-laden beads in a two-phase partitioning bioreactor.

2. Materials and methods

2.1. Preparation of polymer beads

Hytrel 8206 beads (HTBs) were kindly donated by Dupont Canada (Kingston, ON). HTB is a polyether-ester block copolymer thermoplastic elastomer consisting of a 50:50 blend of poly(butylene terephthalate):poly ether glycol. It has a melting temperature of 189 °C, a glass transition temperature of −59 °C, and the beads are rice-shaped, approximately 7 mm in length and 1.5 mm in diameter, with a density of 1.17 g/cm^3 [9]. Magnetic particles were prepared according to the co-precipitation method [14]. During this procedure evaporation of the ammonia solution caused the particles to aggregate, but the masses were broken up by being crushed in a mortar and pestle. Polymer beads containing magnetic particles were prepared as follows: 500 g of HTBs were mixed with 50 or 100 g of magnetic particles in a plastic bag with a zipper and shaken manually for 5 min. The mixture was put into a twin-screw extruder (Werner Pfleiderer ZSK-30, Germany) of which 6 sections of die were previously heated sequentially at 60, 200, 205, 205, 210 and 200 °C. The resultant polymer thread was pelletized to be cylindrical-shaped, 3–5 mm in length and 1.5 mm in diameter

2.2. Polymer characterization

The exact content of magnetic particles in the polymer beads was analyzed using a thermal gravity analyzer (TGA) (TA Instruments Q500, USA). Since the weight of samples for the TGA measurement is limited to about 10 mg, each small slice cut off from 10 arbitrarily chosen polymer beads was collectively placed on the TGA to measure the average content of magnetic particles. The density of the resultant polymer was determined using a mass cylinder and a microbalance. In order to observe the dispersion of magnetic particles, polymer beads containing magnetic particles were first melted to make a thin film of 2 mm thickness by compression moulding at 200 °C and pictures were taken with a fluorescence microscope (Leica DMLB2™, Germany).

2.3. Preparation of phenol-contaminated water and soil

For the treatment of phenol-contaminated water, phenol was dissolved in a mineral medium (described below) and the resultant phenol solution of 2000 mg/L was used to represent highly contaminated water.

Phenol-contaminated soil was prepared based on a previous study [9]. Soil samples were collected onsite from Gangneung-Wonju National University, Korea, and sterilized in an autoclave. The moisture and organic content were measured by drying the soils at 100 °C for 24 h and 600 °C for 24 h, respectively (Lenton furnace, UK) and were 9.4% and 3.3%, respectively. 500 g of soil was contaminated by evenly spraying 50 mL of 40 g/L phenol on the soil which was then placed in a 2 L flask. After 100 g of polymer beads with 10% magnetic particles (PBMs10) were put into the flask, it was manually shaken for 5 min and this shaking was repeated every 8 h. 1 g soil samples were taken periodically from three different points in the flask and placed in a vial containing 10 mL of distilled water. The vial was shaken vigorously for 5 min and the phenol concentration in the supernatant was measured. The phenol-contaminated soil without PBMs10 was treated under the same condition and its phenol concentration was also measured. By comparing these two phenol concentrations, the decrease of phenol concentration in the soil with PBMs10 was determined. PBMs10 was separated using a permanent magnet rod with 10,000 G (Jungil Magent, Korea), 30 cm in length and 26.5 cm in diameter after the 24 h contacting period.

2.4. Removal capacity and partitioning coefficient measurements

In order to compare the removal capacities for phenol, three different polymer beads were tested, which were HTBs, polymer beads with 10% magnetic particles (PBMs10) and polymer beads with 20% magnetic particles (PBMs20). 3.5 g of each polymer was put into a flask containing 70 mL of 2000 mg/L of phenol in tap water. The flask was tightly sealed with a rubber stopper. Two flasks without polymer beads were also monitored as controls. After 24 h of incubation at 30 °C in a shaking incubator (180 rpm), aqueous samples were taken and assayed. All experiments were performed in duplicate and phenol concentrations in the aqueous solution were measured twice according to the 4-aminoantipyrine method [15] using a spectrophotometer (Biochrom UltroSpec 3000, UK). The calibration curve for phenol was previously prepared and was linear up to 20 mg/L of phenol. Partitioning coefficients were determined for HTBs and PBMs10. The experimental procedure was identical to that for removal capacity described above except that each 3.5 g of polymer beads was put into a flask containing 70 mL of 250, 500, 1000, 2000, 3000, 4000 and 5000 mg/L of phenol. By correlating phenol concentration at equilibrium and the amount of phenol absorbed into the polymers per gram of polymer beads, the partitioning coefficients were determined.

Table 1
The absorption of phenol using three different polymers.

Polymer beads	Density (g/cm^3)	Removal capacity (mg-phenol/g-bead)	Reduced removal capacity (mg-phenol/g-polymer)
HTBs	1.17	27.74	27.74
PBMs10	1.12	26.40	27.69
PBMs20	1.23	23.52	25.04

2.5. Microorganism and culture conditions

Pseudomonas fluorescence KNU417 (abbreviated as KNU417), capable of utilization phenol as a sole carbon and energy source, was able to degrade up to 700 mg/L of phenol in 65 h but could not degrade phenol when present at greater than 1000 mg/L [16]. This microorganism is a good model to demonstrate the usefulness of TPPBs because of its sensitivity to high phenol concentrations. KNU417 was pre-cultured on glucose and yeast extract with mineral salts as described previously [16]. After 24 h of pre-culture, the cells were centrifuged (Hanil combi-514R, Korea) and washed with distilled water several times. The cells were then inoculated into a 250 mL flask containing 100 mL of mineral medium supplemented with 300 mg/L phenol as the sole carbon and energy source. Detailed mineral composition and culture condition are also described in the same reference. 2 mL of supernatant were taken every 3 h from the flask for determining cell mass and phenol concentrations. When the phenol was completely degraded, the microorganisms were centrifuged and were ready to be inoculated into the TPPB. This procedure is aimed at microbial adaptation [16], shortening the required time remarkably before cells start to degrade substrate.

2.6. Batch operation of TPPB

Prior to batch operation, 1.5 L of 2000 mg/L phenol solution was placed in a 5 L fermentor (Kobiotech, KF-5L(D)FERMEN, Korea) and operated with 1 vvm of aeration, 400 rpm of agitation, 30 °C and pH 6.5 for 24 h to ensure negligible abiotic loss of phenol. For the treatment of phenol-contaminated water, 100 g of PBMs10 were added to the fermentor containing 1.5 L of 2000 mg/L of phenol solution operated under the same conditions. 3 h after the PBMs10 addition, phenol-adapted cells were inoculated into the fermentor. The initial cell concentration was about 0.3 g/L. Aqueous samples were taken periodically from the fermentor to measure cell mass and aqueous phenol concentration. When the phenol concentration in the aqueous phase was less than 5 mg/L, a calculated amount of phenol was dissolved in the 10 mL of withdrawn aqueous sample and fed back to the fermentor to restore the phenol concentration back to approximately 2000 mg/L. The cell mass was measured using a UV–VIS spectrophotometer (Jasco V-550, Japan) at 660 nm. After complete phenol degradation, the beads were readily removed from the aqueous phase by a magnet and were subsequently detached for further use.

For the treatment of phenol-contaminated soil, after PBMs10 were separated with the magnet rod from the soil, they were detached from the magnet, and transferred to the bioreactor with 1 L of mineral medium, to which 0.3 g/L of phenol-adapted cell had been already inoculated. Liquid samples were periodically taken from the bioreactor to measure cell mass and phenol concentration.

3. Results and discussion

3.1. Characterization of novel polymer beads

The PBMs10 are dark brown while HTBs and magnetic particles are creamy yellow and black, respectively. The content of magnetic particles in PBMs10 and PBMs20 were measured to be 4.67% and 6.08%, respectively, using TGA. The loss relative to the amount of magnetic particles added was due to the fact that some magnetic particles were attached to the plastic bag and hopper of the extruder, which is often observed during extruder operation. The density of PBMs10 and PBMS20 are 1.12 and 1.23 g/cm^3, respectively, while that of HTBs is 1.17 g/cm^3 as shown in Table 1. To test for physical stability, PBMs10 and PBMs20 were placed in tap water for 7 days in a shaking incubator and no noticeable changes in shape

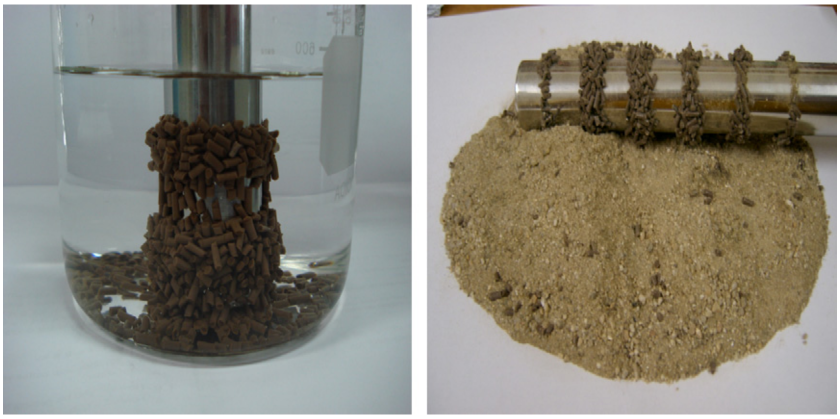

Fig. 1. Separation of PBMs10 from water (left) and soil (right) by a magnet.

or physical properties were found. A microscopic image was taken of the PBMs10 and it showed good dispersion of magnetic particles within the polymer.

3.2. *Demonstration of polymer bead separation*

In order to demonstrate the removal of polymer beads from water and soil, the magnet was applied to a 1 L flask containing 500 mL water and 50 g of magnetized beads and to 250 g soils mixed with 25 g magnetized beads, respectively, as shown in Fig. 1. Complete separation of the beads from water required 5 immersions of the magnet taking 45 s for complete transfer to another flask. Five immersions were required because, as the magnetized beads with their higher density had settled to the bottom of the Erlenmeyer flask, only the tip of the magnet was available for attraction, not the entire length, as the magnet had to be introduced vertically into the flask. Some detachment of the beads, as well as attachment to other beads rather than the magnet, also occurred during removal of the magnet from the water. It was also confirmed that the magnetic polymers were easily separated without interferences of cells and salts in the medium in an actual culture broth (not shown). In the case of soil, more than 90% of the magnetic beads were removed by 4 applications of the magnet, rolling it on and in the soil, within 40 s. Another 2 applications were required for complete removal, which required an additional 35 s.

The results indicate that the use of magnetic polymer beads is promising for the purposes of bead separation. Detaching the magnetic polymer beads from the magnet is as important as attaching them, for actual application to large quantities of contaminated water and soil, and the use of an electromagnet whose magnetic field can be switched off provides such easy detachment. The electromagnet, however, requires a larger surface area relative to a permanent magnet to provide the same attractive force towards the magnetic beads, which is not likely to be suitable for a flask or a lab fermentor. Accordingly, it is believed that electromagnets can be successfully applied at the pilot or industrial scale rather than lab scale experiments, and this is the focus of an ongoing study.

3.3. *Removal capacity*

If the proportion of polymer beads in a bioreactor is substantial, the operation of the bioreactor can be negatively affected [17]. HTBs showed outstanding removal capacity for phenol compared to other polymers in a previous study [7]. The higher the removal capacity, the smaller the amount of polymer beads that is required to reach a desired phenol concentration. Accordingly, the novel polymer beads proposed in this study should have comparable removal capacity for phenol relative to HTBs alone. Table 1 shows the removal capacities of the three different polymer beads for phenol. The results indicate that HTBs have a removal capacity of 27.74 mg/g for phenol. The PBMs10 shows 4.8% lower removal capacity relative to that of HTBs while PBMs20 exhibited a 15.2% lower capacity. The reduced removal capacities of PBMs10 and PBMs20, which were 27.69 and 25.04 mg-phenol/g-polymer, respectively, are thus reduced with increasing content of magnetic particles. PBMs10, however, shows almost the same removal capacity as the original polymer beads (HTBs) suggesting that PBMs10 is comparable to HTBs for phenol removal in bioremediation applications. In the process of extruding and pelletizing, PBMs10 showed a relatively regular shape and smooth surface while PBMs20 did not. In addition, PBMs10 are readily attracted to the magnet, not requiring a higher content of magnetic particles. Therefore, PBMs10 beads were used in the subsequent experiments.

3.4. *Partitioning coefficient*

In a manner similar to organic solvents as a second phase in conventional TPPBs, polymer beads can act as a reservoir which absorbs pollutants to reduce their concentration in an aqueous phase and continuously supplies the pollutant to the microorganism based on the microbial demand. As a first step in assessing the application of PBMs10 for use in a TPPB, the partitioning coefficient should be determined by measuring the phenol concentrations in the polymer beads and aqueous phase at equilibrium. In this section, the volume of polymer bead was used instead of bead mass for convenience but the volume can be readily converted to mass via the density of the beads. Fig. 2 shows the correlation of phenol concentration in the aqueous phase and the amount of phenol absorbed per gram of polymer beads at equilibrium, corresponding to the partitioning coefficient. The figure shows that the partitioning coefficient of HTBs was 52.08 while that of PBMs10 was 48.07. The results imply that PBMs10 have a slightly lower removal capacity for phenol than HTBs, which means that more PBMs10 are required to obtain the same removal efficiency as HTBs. The difference is, however, only 7.6%.

In batch operation for treating phenol-contaminated water, it is useful to calculate the resultant phenol concentration present after adding polymer beads or the amount of polymer beads required to obtain a desired phenol concentration. These estimations are possible via the definition of partitioning coefficient given below.

$$\frac{x}{M} = a\frac{CV - x}{V} \tag{1}$$

where x, M and a are the total amount of phenol absorbed into polymer beads at equilibrium (mg), the volume of polymer beads (cm^3) and the partitioning coefficient (−), respectively. C and V represent

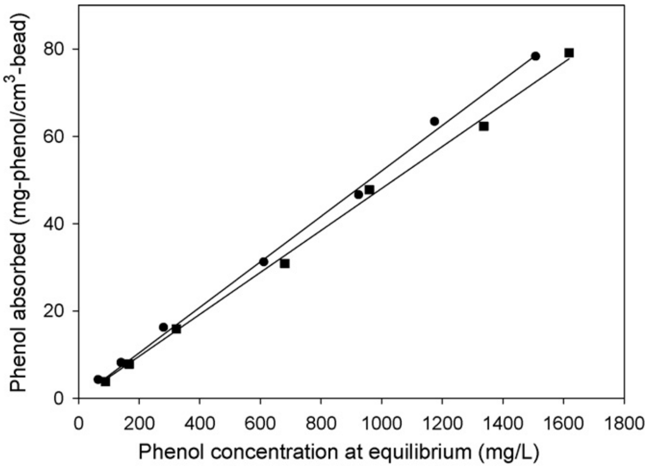

Fig. 2. Partition coefficients of Hytrel 8206 and PBMs10. ●, Hytrel 8006; ■:, PBMs10

initial phenol concentrations in the aqueous phase (mg/L) and the volume of the aqueous phase (L), respectively. Using Eq. (1), it is possible to estimate the aqueous phenol concentration at equilibrium. For example, if 100 g of beads (or 89.3 cm^3) are added to the 1.5 L of 2000 mg/L, the resultant phenol concentration is estimated to be 518 mg/L.

3.5. Batch operation for biodegradation

In a previous study, 200 g of HTBs were used to treat 3 L of 1850 mg/L phenol solution [7]. In this study, the polymer beads were added directly to the fermentor and 14 h was provided for equilibrium to be established before cell inoculation. Since it took only 3 h to reach 95% of the equilibrated phenol concentration after adding PBMs10 (data not shown) in this study, KNU417 adapted to 300 mg/L phenol was inoculated 3 h after 100 g of PBMs10 addition.

The phenol concentration was expected to be 518 mg/L as stated above but the actual value was measured to be 481 mg/L as shown in Fig. 3, corresponding to 7.1% error. The lower phenol concentration in the aqueous phase or the higher removal capacity of PBMs10 than expected was likely due to the mineral salts medium and microorganisms in the fermentor. In order to investigate the effect of cell mass on the removal capacity, the cell mass in mineral medium was set to 0.0, 0.5, 1.0, 2.0 and 3.0 g/L. The resulting removal capacities were in the range of 26.2–26.9 mg-phenol/g-bead without any trend (data not shown). Accordingly, it could be said that cell mass causes no effect on the removal capacity of PBMs10 for phenol. However, in the case of the concentration of mineral medium, as shown in Fig. 4, the removal capacity of PBMs10 was increased by increasing the strength of the mineral salt medium. The strength of the mineral medium in Fig. 4 refers to the multiple of the salts concentration used in the mineral medium, for example, 2 means double the salts concentration of the mineral medium whose original composition was identical to that for adaptation. The result also shows that there is a saturated or maximum removal capacity of polymer beads towards phenol with respect to the concentration of mineral medium. Since the pH values for 0.5–5.0 times the medium strength was around 6.3 while that of tap water was 6.6, pH is not thought to affect the removal capacity noticeably. Therefore, it is believed that the increase of removal capacity may be due to the interaction between mineral salts, phenol and the polymer beads, which is the subject of an ongoing study. Since the mineral salt composition of the aqueous phase continuously changes during bioreactor operation, the originally estimated partitioning coefficient may not necessarily give exact information on the phenol concentrations in the aqueous phase and polymer beads over the course of the experiment. In addition, the cell mass and some degradation intermediates may also affect the partitioning coefficient. Therefore, it is reasonable to suggest that the partitioning coefficient has some range rather than a specific and constant value during TPPB operation that allows estimation of the range of phenol concentrations in both phases.

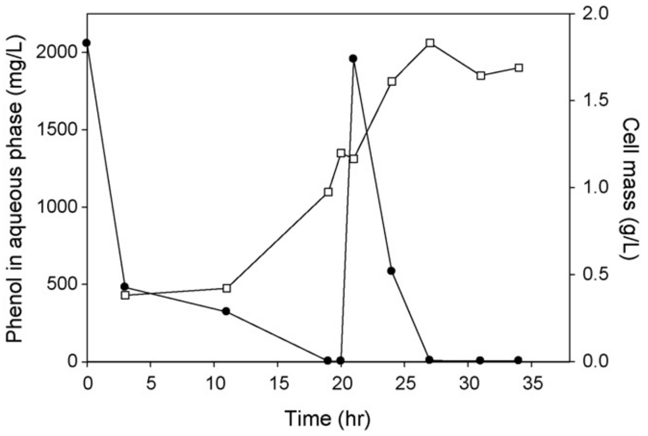

Fig. 3. Treatment of phenol-contaminated water in the TPPB with PBMs10 as a second phase. ●, phenol; □, cell mass

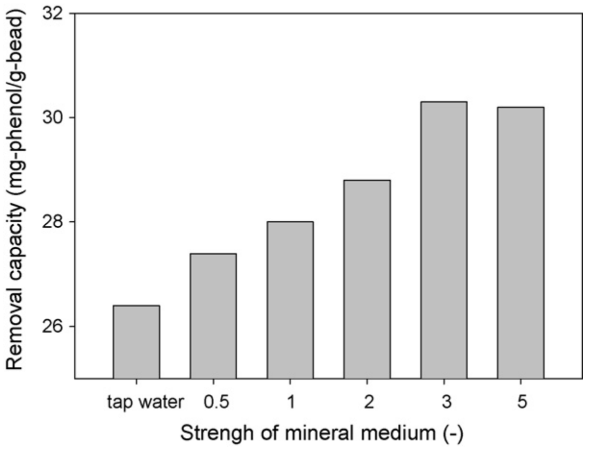

Fig. 4. The effect of the strength of mineral medium on the removal capacity of PBMs10.

Fig. 3 shows that over 99% of the initial 2000 mg/L of phenol was degraded in 17 h and the cell yield was 0.41 while over 99% of the second 2000 mg/L of phenol addition was degraded in 6 h with a growth yield of 0.26. The reason for the lower growth yield in the second feed may be due to the exposure of the cells to the high concentration of phenol. Unlike the first addition of 2000 mg/L where the cells experienced at most 481 mg/L of phenol, the cells were potentially inhibited by the second 2000 mg/L of phenol and a slight decrease in cell mass was observed around 20 h of operation. Another possible reason is that more energy is required to overcome the effect of substrate inhibition, phenol [18], which would lead to lower cell yield. However, the degradation of the second 2000 mg/L was much faster than the first one because cell mass was considerably higher in the second period.

Fig. 5(a) shows that over 97% of 500 g of phenol-contaminated soil was remediated by 100 g of PBMs10 in 24 h. The modified polymer beads were then separated by the magnet and transferred to the fermentor with 1 L of mineral medium to which phenol-adapted cells had been previously inoculated 5 min earlier. The phenol concentration in the aqueous phase gradually increased to 233.5 mg/L in the first 3 h although cell mass also steadily increased as shown

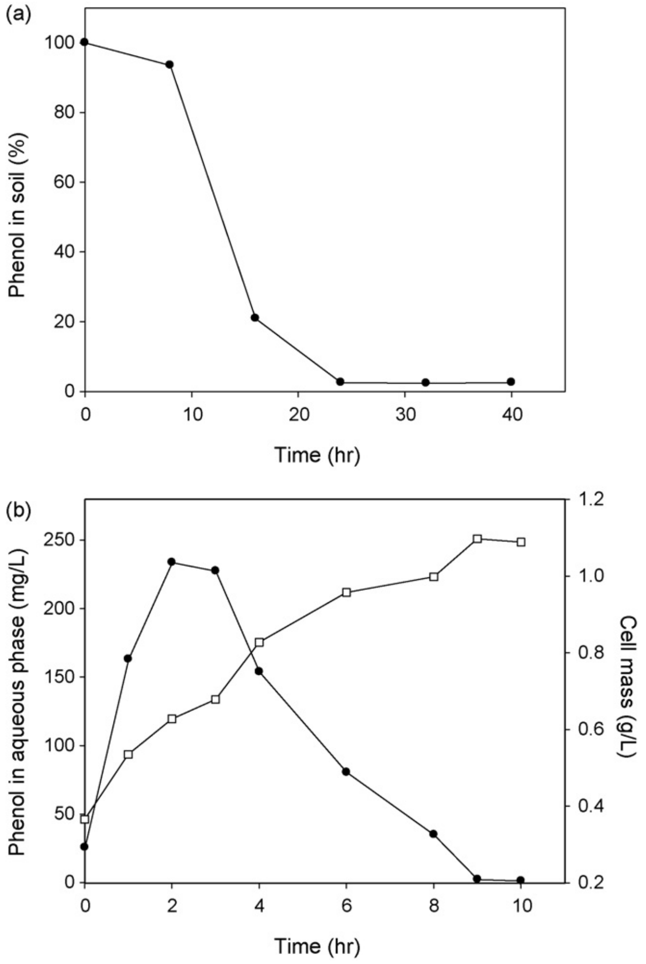

Fig. 5. Removal and biodegradation of phenol from phenol-contaminated soil. (a) Phenol removal from soil using PBMs10. (b) Biodegradation of phenol released from PBMs10. ●, phenol; □, cell mass.

in Fig. 5(b). The reason for initial increase in phenol concentration may be due to the higher phenol release rate from the polymer beads relative to the phenol degradation rate by the cells. When the phenol level in the aqueous phase was 233.5 mg/L, corresponding to 8.6 mg-phenol/cm^3-bead via the partitioning coefficient, 1193.5 mg of phenol remained in the fermentor or 746.5 mg of phenol was degraded by the cells in the first 3 h. The period in which a small change in aqueous phenol concentration occurred may be due to the brief time when the phenol release rate to the aqueous phase was similar to the phenol degradation rate by cells. After this period, the phenol degradation rate exceeded the phenol release rate, which decreased the phenol concentration in the aqueous phase. Since the phenol concentration in the aqueous phase was not high enough to cause an inhibition effect on the cells, it is reasonable to assume the cell yield remained at 0.41. Therefore, the total cell increase of 731.7 mg corresponding to 1784.1 mg of phenol degraded in the bioreactor means the total removal efficiency is 89.2% on the basis of initial 2000 mg input.

4. Conclusions

Novel polymer beads containing magnetic particles were manufactured in this study and 4.67% magnetic particles were found to be enough for easy separation from phenol-contaminated water or soil by means of a magnet. The novel polymer beads possessed almost 93% of the removal capacity of the original polymer beads (Hytrel 8206) and had a partitioning coefficient of 48.07 for phenol relative to water. The use of the novel polymer beads for bioremediation applications is promising as it can improve separation of the polymer from the contaminated source, while having only a modest effect on the removal capacity. The effect of mineral medium and cell mass on the partitioning coefficient will be investigated further to predict more accurately the relationship between phenol concentrations in the aqueous and polymer phases. In addition, an electromagnet will be applied to the clean-up of contaminated water and soil with various pollutants as well as phenol at pilot scale in an ongoing study.

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