Recycling of immobilized cells for aerobic biodegradation of phenol in a fluidized bed bioreactor

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ABSTRACT

Biodegradation is an environmentally friendly and cost-effective alternative that proved to be efficient for the removal of toxic phenol compounds from aqueous solutions. However, it has been reported that phenol is inhibitory to bacterial growth at concentrations above 0.05 g/L. This study was undertaken to study the degradation of phenol at initial concentrations of 20 mg/L by *Bacillus* cells individually immobilized in two different matrices including polyvinyl alcohol-sodium alginate (PVA-SA) and polyvinyl alcohol-guar gum (PVA-GG). Results of batch experiments demonstrated that complete removal of phenol was obtained using immobilized cells in the first cycle after 270 and 300 min using cells immobilized in PVA-SA and PVA-GG. Additional cycles were conducted to evaluate the validity of recycling the beads of immobilized cells for phenol biodegradation. Results revealed that the phenol percentage removals were 96, 90, 83, and 75% for the second, third, fourth, and fifth cycles, respectively after 270 min. However, complete removal of phenol was obtained at extended time durations up to 300, 360, and 390 for the second, third, and fourth cycles, respectively. Also, the potential of immobilized cells versus free cells for the degradation of higher phenol concentration up to 50 mg/L was investigated.

Keywords: Immobilized cells, Aerobic biotreatment, Phenol, Wastewater, and *Bacillus subtilis*

1. INTRODUCTION

Phenol was one of the first compounds inscribed into the list of priority pollutants by the US Environmental Protection Agency (US EPA) since it quickly penetrates the skin and may cause severe irritation to the eyes and respiratory tract. The concentration of phenols in wastewater may vary from 10 to 300 mg/L [1, 2]. The removal of phenol from industrial effluents is of great practical significance for environmental protection [3-5]. Intensive attention has been paid to degradation of toxic pollutants by microorganisms and their transformation into compounds inoffensively with formation of new cellular mass. Bacteria are often the dominant hydrocarbon degrader in aquatic systems [6, 7]. However, difficulty arises in biological treatment due to the toxicity of refractory organic toxic compound such as phenol to the microbial population. So, the introduction of new and improved biotechnologies that enable engineers and scientists to tackle the more contemporary environmental problems such as detoxification of hazardous compounds through the use of living microorganisms would be necessity [8, 9]. Immobilized cells have the potential to degrade toxic refractory compounds faster than conventional treatment systems since high densities of specialized

microorganisms are used in immobilized cells (IC) systems. The technique of immobilized cells not only simplifies separation and recovery of microbial cells but also makes the application reusable which reduces the overall cost [10]. Compared with free cells (FC) technology, immobilized cells (IC) show many advantages, such as the stability of biocatalyst can be greater for immobilized cells, protection of biomass from organic overloads and relatively severe environmental conditions such as extended pH; the immobilized cells exhibited higher tolerances in acidic (pH 4-5) and highly saline (10 % NaCl) environments than those of free cells [9, 11].
This study aimed to investigate the potential of immobilized *Bacillus subtilis* cells for phenol degradation in aqueous solution using two different types of bio-carriers in spouted bed bioreactor.

2. MATERIALS AND METHODS

Bio-carrier materials

The materials that used as bio-carriers in this study included natural polymers cross-linked with polyvinyl alcohol (PVA) to improve and increase the mechanical stability of beads because natural polymeric carriers are abundant but they are less stable in wastewater than synthetic polymers [12]. Table1 presents the types and sources of the utilized polymers.

Table 1 Polymers used as bio-carriers in the study

Name	Abbreviation & Chemical formula	Source	E. Code*
Polyvinyl alcohol	PVA $(C_2H_4O)n$	raw material of vinylon	———
Sodium alginate	SA $(NaC_6H_7O_6)_n$	Algal polysaccharides derivatives (Marine-Seaweed algae)	E401
Guar Gum	GG	Obtained from non-marine botanical resources	E412

*(E. Number): is a code for a substance that can be used as food additives within European Union & Switzerland.

Immobilization protocol

● ***Polyvinyl alcohol – Sodium alginate matrix***

Sodium alginate (SA) solution was prepared in sterilized distilled water and combined with PVA. Biomass inoculum (5 ml) was added to the PVA-SA slurry, and stirred for 10 minutes to get a uniform mixture taking into consideration that no bubbles were entrapped inside.
The slurry was injected using sterile hypodermic syringe. The alginate solution was dropped into ice cold mixture of calcium chloride (4%) and boric acid (6%). Beads were formed in $CaCl_2$ solution that was incubated overnight for curing. The cured beads were washed with sterile distilled water 3 to 4 times. When the beads were not being used, they were preserved in 0.9 % sodium chloride in the refrigerator. This procedure was adopted according to [13].

● ***Polyvinyl alcohol – guar gum matrix***

6 g PVA and 2 g GG were dissolved in 100 ml distilled water and blended by a magnetic stirrer at 70 °C for 30 min. After the mixture cooled to room temperature, it was inoculated with 5 ml of biomass cells (pure or mixed culture) and was stirred by a magnetic stirrer for 15 min. The obtained solution was poured into sterile micro-plates to form beads with immobilized cells. Keeping them in the freezer for 12 h and then thawing them. The freezing-thawing procedure was repeated for 3 times to improve the stability of beads [13]. Generally, polymer gel of guar gum is more brittle than alginate gels. Fig. 1 presents samples of the prepared beads.

Fig.1 Samples of the prepared beads

Experimental system configuration and set up

The experimental system consisted of a specially designed fluidized bed bioreactor, known as spouted bed bioreactor (SBBR) made of Perspex column (inner diameter 50 mm, height 70 cm) with 45°

conical base. The SBBR was outfitted with a Perspex jacket (inner diameter 80 mm) for temperature control. A water bath was designed to continuously circulate the water at a desired temperature of 30°C. The water bath consisted of 6 Liter-cylindrical Perspex tank, occupied with heater and water pump to circulate the water into the reactor jacket. The aqueous solutions were fed to the SBBR via a peristaltic pump (Type: Thomas 3386 Mini variable speed pump). In order to provide an intense mixing and maintain aerobic environment into the spouted bed bioreactor, air was injected from the bottom of the reactor by an air pump through a 6 mm-orifice. A flow meter was provided to control the air flow into the system. The experimental setup is given in Fig. 2. The spouted bed bioreactor operated in an up flow co-current air/water mode at a temperature of 30°C. The experimental work was accomplished in a batch mode. The system was used to assess the effects of the detention time, contaminants initial concentration, effect of inoculum type and concentration, free versus immobilized cells, type of immobilized beads matrix, number of effective biodegradation cycles.

Due to the fact that high concentration of phenol up to 50 mg/L may inhibit the free bacterial cells, a set of experiments was conducted using higher initial concentration of 50 mg/L with immobilized cells to investigate the effect of relatively high of phenol on their activity for biodegradation.

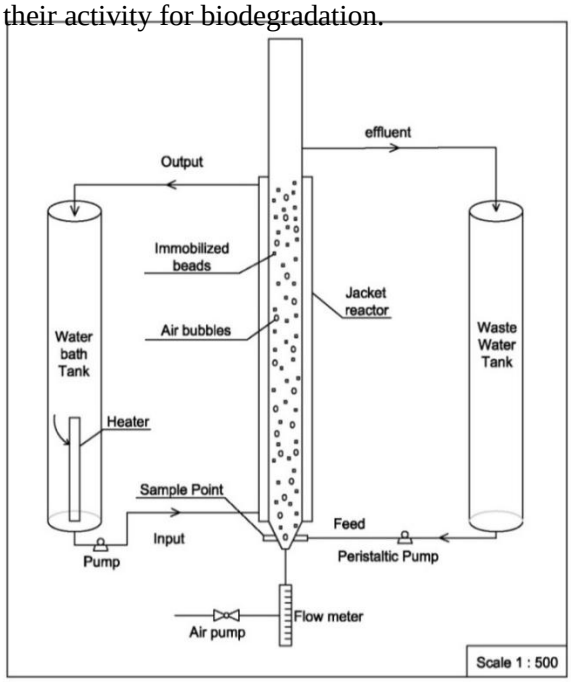

Fig. 2 Schematic diagram of the pilot-plant

Analytical analysis and methodologies

Phenol concentrations in aqueous samples were determined by T80 UV-VIS Spectrophotometer at 270 nm. Chemical oxygen demand (COD) concentrations in aqueous samples were measured using the COD analyzer (Model: Lovibond, RD 125). The concentration of the free cells (FCs) biomass was measured using the volatile suspended solids (VSS) measurement according to the procedure reported in the *Standard Methods* [14]. For counting the immobilized cells, the beads were dissolved by immersing them in 4 % $NaHCO_3$ solution for 30 min. Samples of microorganisms residing in the wastewater were used without additional treatment. Routine counts of biomass cells in wastewater or within the beads were counted by the Plate Count Method (CFU/mL) in a series of dilutions (in 0.85 % saline) and traditional approach of volatile suspended solids (VSS, g/L) [15].

3. RESULTS AND DISCUSSION

Assessment of biomass concentration

A preliminary set of experiments were conducted to investigate the effect of inoculum concentration on the pollutants biodegradation. Results revealed that the concentration of inoculums was found to have a notable impact on the degradation of phenol as given in Fig. 3.

The effect of inoculum concentration as a volume ratio for the selected isolates; *Bacillus* was studied for phenol degradation using three different volume ratios including 3, 5 and 7 % with 20 mg/L initial concentration of phenol.

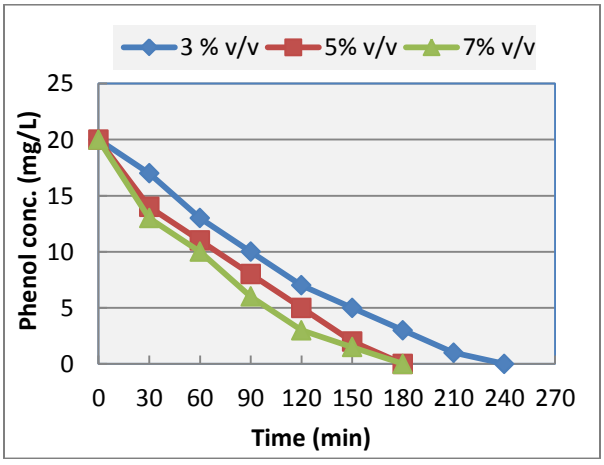

Fig. 3 Effect of inoculum volume on phenol degradation by *Bacillus*

Results revealed that Bacillus completely degraded phenol in 240, 180 and 180 min at 3, 5 and 7 % v/v, respectively. Accordingly, 5% was selected as the optimum cell concentration to prepare the beads.

Effect of beads concentration on biodegradation rate

The concentration of beads (immobilized cells) in the bioreactor plays an important role in the biodegradation process. Fig. 4 shows the effect of beads concentration (as volume percentage) on the biodegradation rate of phenol. The degradation rates of phenol using 25% (v/v) of PVA-SA and PVA-GG were 5.16 and 4.60 mg/L.h, respectively. Whereby, they were 4.69 and 4.27 mg/L.h, respectively using 10% (v/v). It is well observed that the degradation rates using 25 % (v/v) of beads are higher than the degradation rate using 10% (v/v). Since the concentration of beads in the bioreactor can be related directly to the concentration of the bacterial biomass immobilized in the beads, it is expected that as the beads amount increases, the biodegradation rate will increase.

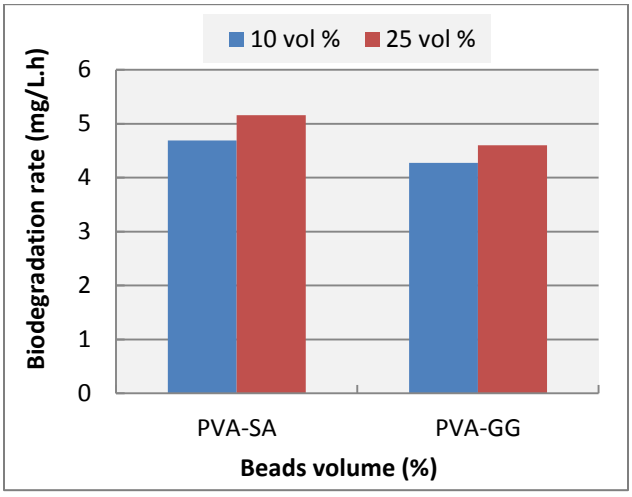

Fig. 4 Biodegradation rate of phenol by *Bacillus* immobilized in two different bio-carriers using 20 mg/L initial phenol concentration

The potential of using immobilized cells for phenol degradation

Results demonstrated complete degradation of phenol can be obtained using immobilized cells of *Bacillus*. On the other hand, the feasibility of reusing immobilized cells for phenol degradation was evaluated. The immobilized cell beads were reused in consecutive degradation experiments, and the phenol was almost degraded at high rates even for the excessive cycles up to 5 cycles in this study as shown in Figs. 5 and 6. Results revealed that the immobilized cells could be reused without reduction of their degradation capability. This phenomenon could reduce expenses during operational periods [16].

The use of natural gums such as gaur gam (GG) may be, however, limited by their mechanical strength and the lack of open spaces to accommodate active cell growth resulting in their rupture and cell release into the growth medium especially for long periods of use [17].Therefore, their biodegradation rates are relatively less than alginate gels (sodium alginate in this study).

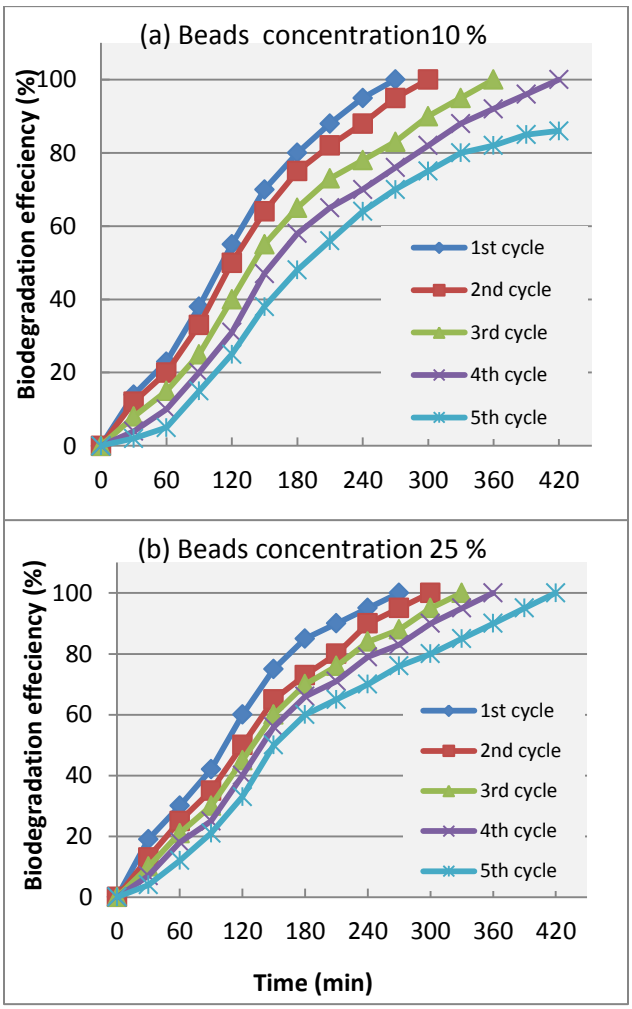

Fig. 5 Profiles of phenol degradation by *Bacillus* cells immobilized in PVA-SA

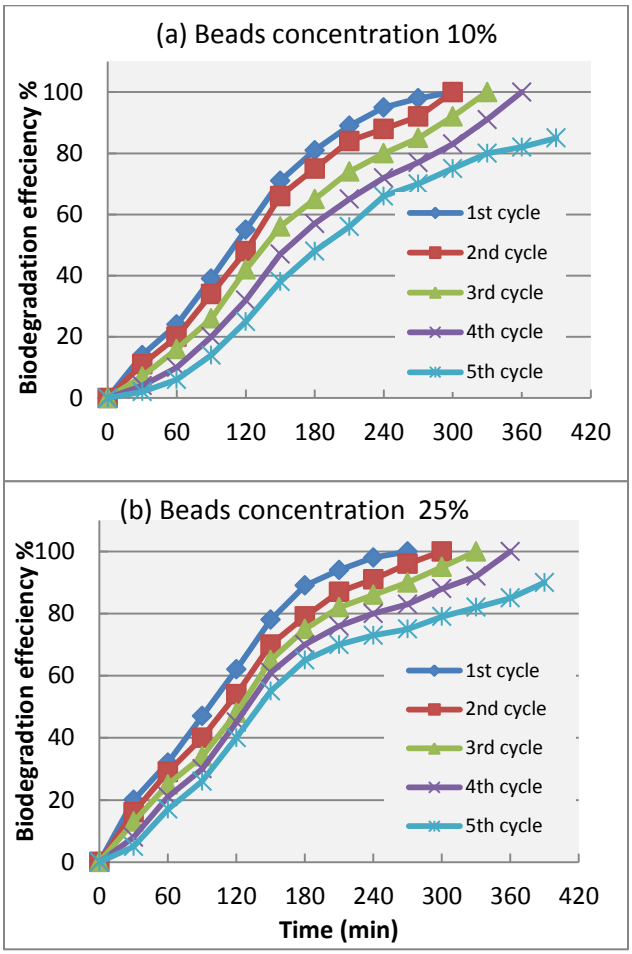

Fig. 6 Profiles of phenol degradation by Bacillus cells immobilized in PVA-GG

Additionally, no inhibition of immobilized cells was observed when using a relatively high concentration of phenol up to 50 mg/L and their degradation rate didn't affected by the high concentration of phenol compared to the free cells as given in Fig. 7.

Use inactive beads (blank beads) for phenol removal

In order to assess if there is any abiotic process such as adsorption might be associated with the biodegradation of phenol, a set of control experiments were performed using blank beads without biomass cells. Results revealed that no change in phenol concentration was observed during 24h, indicating the absence of any abiotic process. This finding was in a good agreement with the observation reported by Khattar et al. [18] who examined the effects of using agar-blank beads on Cr removal from aqueous solution.

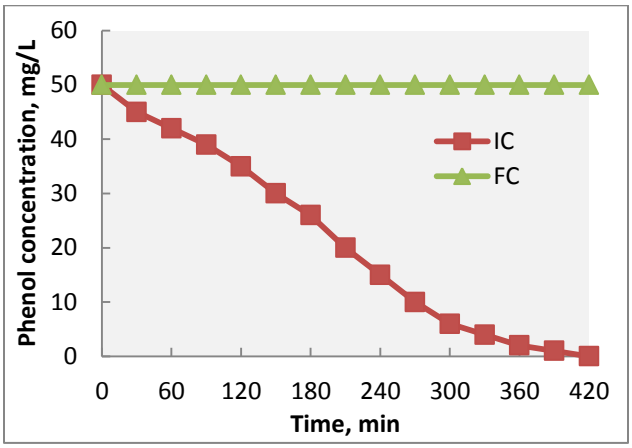

Fig. 7 Profiles of phenol biodegradation using immobilized cells versus free cells at 50 mg/L initial concentration of phenol

4. CONCLUSION

The biodegradation of phenol using *Bacillus* cells individually immobilized in PVA-SA and PVA-GG matrices was evaluated in a spouted bed bioreactor. Results revealed that complete removal of phenol can be obtained after 270 and 300 min using cells immobilized in PVA-SA and PVA-GG, respectively at the first cycle. Excessive cycles up to 5 cycles were investigated and the experimental results indicated the potential efficiency of the immobilized cells to be recycled for complete removal of phenol at different time durations. Also, results demonstrated that the cells concentration affected the biodegradation rate.

5. REFERENCES

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