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Biodegradation of toluene using *Candida tropicalis* immobilized on polymer matrices in fluidized bed bioreactors

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HIGHLIGHTS

- Biodegradation of gaseous toluene in bubble-column bioreactors.
- Whole-cell-immobilization of *Candida tropicalis* on polymer matrices.
- Polyethylene glycol and polyethylene glycol/activated carbon/alginate (PACA) bioreactor.
- Enhanced toluene removal in the PACA bioreactor with a CO_2 yield value of (0.6 g C_{CO_2}/g-$C_{toluene}$).
- Increase in both toluene mass transfer and activity of *C. tropicalis* in PACA.

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ABSTRACT

A yeast strain, *Candida tropicalis*, was whole-cell-immobilized on polymer matrices of polyethylene glycol (PEG) and polyethylene glycol/activated carbon/alginate (PACA). The polymer matrices were used as fluidized materials in bubble-column bioreactors for the biodegradation of toluene. Simultaneously, another bubble-column bioreactor using granular activated carbon (GAC) and a conventional compost biofilter were operated for comparison. In the compost biofilter, the toluene removal efficiency gradually deteriorated due to the limitation of microbial activity. The toluene removal in the GAC bioreactor was relatively high because of an increase of toluene mass transfer. However, low toluene removal efficiencies were observed in the PEG bioreactor, presumably because the synthetic polymer alone was not suitable for yeast cell immobilization. In the PACA bioreactor, toluene removal was found to be greater than 95% overall. The CO_2 yield coefficient calculated at the highest toluene loading condition for the PACA bioreactor was found to be higher than those observed in the other bioreactors. Furthermore, almost complete elimination capacities were observed in the PACA bioreactor at short-term toluene loading up to 180 g/m^3/h. In conclusion, the immobilization of *C. tropicalis* in the PACA matrix resulted in enhanced toluene biodegradation because of the increases of both mass transfer and microbial activity.

1. Introduction

Because volatile organic compounds (VOCs) are commonly classified as hazardous air pollutants, they need to be treated to meet intensified regulations before being discharged to air from various sources. Packed-bed bioreactors have been extensively applied for the removal of VOCs from waste air streams [1,2]. In practical applications, nevertheless, inactive microbial constituents continuously accumulate on the surface of packing materials and microbial activity declines during long-term operation make these biological treatment methods less attractive [3]. Several experimental studies showed that these problems can become severe when packed-bed bioreactors are subjected to high VOC loading rates over an extended period of operation [4–6]. Alternatively, suspended-growth bioreactors have been tested to provide reliable performance [7]. However, both low densities of active microorganisms over an extended period of bioreactor operation and low mass transfer rates of hydrophobic compounds from the gas to the liquid/microbial phases can be still limiting factors for the treatment of VOCs in suspended-growth bioreactors [8].

Immobilized cells in polymer matrices have recently been used for the biodegradation of VOCs due to a possibility of retaining high density of desired microorganisms within the system while maintaining their microbial activity. Immobilized cell systems have been used in bioreactors for the treatment of gaseous hydrogen sulfide [9]. Biodegradation of VOCs in waste gas streams has been demonstrated using immobilized bacterial cells on a fibrous matrix

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[10]. Furthermore, immobilized cell bioreactors have been found efficient both for the removal of VOCs during a long-term operation [11] and for short-term transient loading conditions [12]. For the whole-cell-immobilization, several polymer types, either natural polymers such as Ca-alginate and k-Carrageenan or synthetic polymers such as polyethylene glycol (PEG) and polyvinyl alcohols (PVA), have been used in many different applications [13–15]; yet, suitability and applicability of different polymer matrices have not been fully evaluated for the biodegradation of gaseous VOCs in suspended-growth bioreactors.

For the biodegradation of VOCs, many bacterial cultures have extensively been employed in bioreactors [10], but such bacterial systems are commonly known to be sensitive to operating conditions including VOC shock loading, pH, and temperature; and these likely cause operational problems in full-scale applications [16]. Yeast cultures may offer advantages in the degradation of VOCs because of tolerance to low pH and other unfavorable conditions [17]. It has been reported that yeast cultures were efficient for the biological removal of variety of chemicals with biodegradation rates equal to or greater than those observed in bacterial systems [18,19]. Recently, a yeast strain, *Candida tropicalis*, has been applied to enhance biodegradation of toluene in a fluidized bioreactor [20].

A combination of whole-cell-immobilized yeasts in a fluidized-bed bioreactor has yet to be fully explored for the biodegradation of VOCs in the gas phase. In this study, the yeast strain, *C. tropicalis*, was whole-cell-immobilized on two different polymer matrices; (1) PEG, a synthetic polymer and (2) polyethylene glycol + powdered activated carbon + alginate, a mixed polymer matrix with adsorptive materials, referred to as PACA. These yeast-immobilized bioreactors were tested for the biodegradation of toluene as a model VOC, in bubble-column, fluidized-bed bioreactors. Performance comparison was also conducted using both a conventional compost biofilter and a bubble-column bioreactor with granular activated carbon (GAC) as a fluidized material.

2. Materials and methods

2.1. Culture of C. tropicalis

The yeast strain, *C. tropicalis*, was obtained from the Korean Culture Center of Microorganisms (KCCM 50075). The strain was incubated on yeast/mold agar plates before transferring to fresh media. After several cycles of regeneration, the strain was transferred into a 120-mL serum bottle containing 50 mL of a sterilized nutrient solution. The nutrient solution consisted of a hydrocarbon minimal medium (HCMM), and the chemical composition of HCMM has been reported elsewhere [20].

The yeast strain was cultured in the serum bottle in the presence of toluene vapor as a sole carbon and energy source. Ten micro-liters of pure toluene were injected into the serum bottle, and the culture was incubated in a shaker at 20 °C. The toluene concentration in the headspace of the bottle was regularly monitored as described below. The culture was transferred to another bottle containing fresh nutrient solution, and incubated until the optical density (OD_{600}) reached 1.5. After several cycles of transfer and incubation, the enriched culture was used either to inoculate bioreactors or to be immobilized in different polymer matrices.

2.2. Bioreactor media

In this study, four different media were applied; (1) compost in a packed-bed biofilter, (2) GAC as a fluidized material in a bubble-column bioreactor, (3) PEG and (4) PACA, each as a yeast-immobilized matrix in a bubble-column bioreactor. The compost, originally made with a mixture of yard waste and dewatered municipal sludge, was purchased from a local gardening store. The compost was sieved to remove large particles and mixed by hand with 200 mL of the enriched yeast culture before being packed in a column. The GAC used in the study was purchased from an activated carbon company (Samchully Co., Korea), air-dried, and passed through a 0.6 mm sieve. The bulk density of the GAC was 450 kg/m^3 with a BET (Brunauer–Emmett–Teller isotherm) specific surface area of 950 m^2/g as provided by the manufacturer.

In order to immobilize the yeast cells in the synthetic polymer, PEG, 180 g (161 mL, 18% (w/v)) of polyethylene glycol diacrylate (MW 700, Sigma–Aldrich, USA) was dissolved in the HCMM nutrient solution, and then 2.5 g of potassium persulfate was added and thoroughly mixed at 25 °C. After complete dissolution of potassium persulfate, 100 mL of 2% methylenebis acrylamide was added. Then, 2.5 g of N,N,N′,N′-tetramethylethylenediamine (TMEDA, Alfa Aesar®, UK) was added. The pH of the solution was adjusted to 6 using 10% acetic acid. In the final step, a volume of 200 mL of enriched *C. tropicalis* culture was added in the mixture. The mixture was immediately filled in a plastic tube with an internal diameter of 5 mm, and dried at room temperature for 30 min. The solidified matrix was extruded from the tube and cut into small pieces in cylindrical shapes with a volume of each PEG matrix of 1 mL and a density of 0.85 g/mL.

The yeast culture was also immobilized in another polymer matrix of polyethylene glycol, powdered activated carbon, and alginate, which was referred to as the PACA matrix in this study. The powered activated carbon was prepared by pulverizing the GAC described above. For the PACA matrix, 1 g of sodium alginate (Yakuri Chemicals Co., Japan) was dissolved in a 20 mL of water at 60 °C, and then 18 g of PEG diacrylate described above was added and dissolved. A 30 μL of TMEDA was then added, and pH was adjusted to 6 using 10% of acetic acid. After adjusting pH, 1 g of PACA was added, and the volume of the mixture was brought up to 50 mL. Another volume of 50 mL of the enriched yeast culture was added to the mixture. The final solution was immediately filled in another plastic tube with an internal diameter of 5 mm, and solidified for 30 min at 30 °C. The solidified matrix was extruded and cut into small pieces, following the same procedure as described above for the PEG matrix.

2.3. Bioreactor systems and operation

In this study, three bubble-column fluidized bioreactors and one compost biofilter were tested individually as shown in Fig. 1. Each bioreactor was consisted of an acrylic pipe with an internal diameter of 8 cm and a height of 40 cm. After purification using a filter (HEPA-Vent, Whatman, USA) to prevent microbial contamination, a compressed air stream was mixed with pure, research-grade toluene, which was injected using a syringe pump (KD Scientific, USA). The toluene-loaded air stream was continuously fed through a diffuser placed at the bottom of each bioreactor. An image of bubbles generated through the diffuser was acquired using a photograph taken with a digital camera, and the bubble size in the image was compared with a graduated ruler attached to the outside of the column. More than 200 bubbles in the digital image were analyzed, and their sizes ranged from 3.6 to 4.8 mm. In the fluidized-column bioreactors, the bubble swirl rose through the column filled with the yeast culture and the media. All bioreactor components and pipelines were sterilized using isopropyl alcohol before being inoculated with the yeast strain. The liquid solutions containing the HCMM nutrients were autoclaved before use in the bioreactors.

In the compost biofilter system, the compressed air was first passed through a water-filled impinger as a humidifier, and it was contaminated with toluene. The toluene-laden air stream was supplied to the bottom of the column. The column was packed with the compost at an initial moisture content of 38%. The biofilter