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Fabrication of novel rhamnolipid-oxygen-releasing beads for bioremediation of groundwater containing high concentrations of BTEX

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ABSTRACT

In this work, biosurfactant-containing oxygen-releasing beads (BS-ORBs) were prepared and used for the bioremediation of BTEX in groundwater. The response surface methodology (RSM) was utilized to optimize the BS-ORBs. The results reveal that the optimal proportions of polyvinyl alcohol (PVA), biochar, CaO_2, alginate, biosurfactant, and citrate were 33%, 32.5%, 13%, 13%, 5.8%, and 2.6% (w/w), respectively. The integrality ratio of optimized BS-ORB was 3.81%. The regression equation obtained from RSM accurately predicted the integrality ratio ($R^2 = 0.994$). The BS-ORBs continuously released oxygen (>0.5 mg/L) for 128 days. The concentration of the biosurfactant was maintained above 30 mg/L for 58 days. Batch experiments indicated that adding BS-ORB improved the bioremedation of the groundwater that contained high levels of BTEX (120 mg/L) for three cycles. These results indicate that the BS-ORBs are a potential oxygen-releasing material for the biomediation of groundwater that is contaminated with a high concentration of BTEX.

1. Introduction

Groundwater is often contaminated by BTEX because of leaks from petroleum tanks and pipelines (Gross et al., 2013). The compounds that comprise BTEX are acutely toxic and carcinogenic and pose high risks to human health and the ecosystem (Durmusoglu et al., 2010). Bioremediation is a green and low-cost technique for the bioremediation of contaminated groundwater (Newcombe and Crowley, 1999; Shahsavari et al., 2013). However, both the low dissolved oxygen concentration and low solubility of BTEX often limit the effectiveness of bioremediation (Lee et al., 2014).

Many investigations have demonstrated that surfactants can form micelles at the critical micelle concentration (CMC) and increase the apparent solubility of hydrophobic compounds, improving their biodegradation (Calvo et al., 2009; He et al., 2008; Li et al., 2016). However, many synthetic surfactants are themselves toxic and bio-recalcitrant (Mukherjee and Bordoloi, 2012; Souza et al., 2014). They also contaminate groundwater after their application. Biosurfactants that are produced by microbes have the advantage of exhibiting low toxicity and high biodegradability (Cameotra and Singh, 2008; Johann et al., 2016). They may therefore be suitable for preparing oxygen-releasing beads (ORB).

Biochar is a low-cost agricultural soil ameliorant in Taiwan. In our earlier work, the polyvinyl alcohol (PVA), CaO_2, citrate and biochar were used to prepare oxygen-releasing beads (Wu et al., 2015) for use in groundwater. However, information on the effects of biosurfactants on the oxygen-releasing ability and the integration of ORBs are lacking.

Conventionally, the one-factor-at-a-time method is used to optimize the proportions of prepared materials with various functions. The optimization becomes more complex as more materials are used. The response surface methodology (RSM) is a statistical method for designing experiments; establishing models; assessing the influences of various variables; optimizing the settings for desired response, and reducing the number of experimental runs that must be performed to evaluate the interactions among factors (Box and Wilson, 1951). The RSM has been utilized for optimizing processes in various fields (Montgomery, 2008).

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Since different materials are used in the preparation of BS-ORBs, the RSM should be used to optimize the proportions of materials used for preparing BS-ORBs to maximize the integrity ratio.

BTEX are hydrophobic compounds. The biodegradation of BTEX are often limited because of their low aqueous solubility. The addition of surfactant reportedly increases the solubility of BTEX, thus enhances their biodegradation in groundwater (Mulligan et al., 2001; Souza et al., 2014). However, the related study on surfactant-containing ORBs are lack. In this study, the novel BS-ORBs were synthesized and was used to enhance the bioremediation of high concentration of BTEX in groundwater.

In this work, the biosurfactant-containing oxygen-releasing beads (BS-ORBs) are prepared and their effectiveness in the bioremediation of BTEX-contaminated groundwater is assessed. The selected biosurfactant was *Rhamnolipid*. RSM was used to determine the optimal ratio of materials in the preparation of BS-ORBs.

2. Materials and methods

2.1. Preparation of BS-ORBs

Bamboo biochar was obtained by the pyrolysis of bamboo coal (Kwan-E Agri-Chem Industry, Taiwan) in a furnace with a programmable controller (CWF-1200, Carbolite, UK) at 350 °C for 2 h. The biochar was ground and passed through a 100 mesh sieve (particle size < 0.149 mm) before use. The pH of the bamboo biochar was 7.5 and its specific surface area was 1.793 $m^2\ g^{-1}$ (Wu et al., 2015).

Sludge samples were obtained from the oil cracking wastewater treatment plant of Nan-Ya Plastics (Six Naphtha Cracking Industry Site at Mailiao of Yunlin County, Taiwan). The *Pseudomonas* sp. YATO411 and *Mycobacterium* sp. CHXY119 were isolated from sludge after long term acclimation in the laboratory and then identified based on 16S rDNA (Lin and Cheng, 2007).

First, BS-ORBs were prepared as follows. A gelling agent (PVA/alginate = 3:1 w/w) was heated in an autoclave (121 °C, 1 h). After it was cooled, the gelling agent was mixed with an oxygen-releasing agent (CaO_2, 5 g), a pH-buffering agent (citrate, 2.5 g), a supporter (bamboo biochar, 13.24 g), 10 mL of BTEX degrader (*Mycobacterium* sp. CHXY119 (3.2×10^7 CFU/mL) and *Pseudomonas* sp. YATO411 (4.5×10^7 CFU/mL)), biosurfactant, and PVA, to form the ORBs (with a diameter of 1 cm) using Chinese Herbal Medicine Pill Machine (USK-47, Yu Sheng Guang food machines, Taiwan). The ORBs were mixed with various doses of PAV and biosurfactant to prepare BS-ORBs. Fig. 1 displays the preparation procedure.

2.2. Integrality experiment

BS-ORBs were added to a 250 mL flask that contained 100 mL of mineral solution and shaken in a water bath (20 °C, 150 rpm). After seven days, the BS-ORBs were weighed. The initial mass was compared with the mass on day seven. This test was carried out in triplicate. The integrality ratio was calculated using Eq. (1) (Wu et al., 2015):

$$\text{Integrality ratio}(\%) = \left[1 - \left(\frac{W_0 - W_{day7}}{W_0}\right)\right] \times 100\% \quad (1)$$

where W_0 is the initial weight of the BS-ORBs (g) and W_{day7} is the weight of BS-ORB (g) that were sampled on day seven in the integrality experiment.

2.3. Effects of PVA and biosurfactant on integrality of BS-ORBs

The effects of PVA and biosurfactant doses on the integrality of BS-ORBs were evaluated. RSM was used to design the experiment to maximize the integrality ratio of BS-ORBs (Table 1). Table 1 presents the RSM design. In this design, the CaO_2, citrate, and biochar were fixed at 5, 2.5, 13.24 g, respectively, based on our previous studies (Wu et al., 2015). The selected independent variables were PVA dose (X_1) and biosurfactant dose (X_2).

A factorial 2^3 central composite design was used with integrality ratio (Y) as an output that depended on two variables (X_1 and X_2). The experimental design involved 14 runs, which were performed under the conditions that are listed in Table 1. The integrality ratio was also estimated using Eq. (2) (a second-order response surface model) (Montgomery, 2008).

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i<j} \sum_{i=1}^{k} \beta_{ij} X_i X_j + \varepsilon \quad (2)$$

where Y is the predicted response (integrality ratio); the β terms are parameters (regression coefficients) whose values are to be calculated; β_{ij} denotes an interaction between two factors; β_{ii} represents pure second-order or quadratic effects; k is the number of experimentally considered factors, and ε is a random experimental error. The significance of each variable (factor) was calculated by performing Student's *t*-test. The results were obtained using the Statistica 6.0 software package (Statsoft, Tulsa, USA). An analysis of variance (ANOVA) was carried out to calculate the response surface, its contours, and multiple linear regression equation. The critical integrality was estimated from an established regression model and was evaluated based on a hump in the three-dimensional graph.

2.4. Releasing characteristics of oxygen and biosurfactant

The oxygen and biosurfactant release experiments were performed in an acrylic column reactor (diameter: 4 cm, H: 25.5 cm, total volume: 314 mL). The flow rate and hydraulic retention time were 0.21 mL $min^{-1}$and 1.24 day, respectively. The influent was a mineral solution. Each liter of the influent mineral solution contained 0.25 mg $CoCl_2$, $6H_2O$, 0.25 mg $CuCl_2 \cdot 2H_2O$, 1 mg

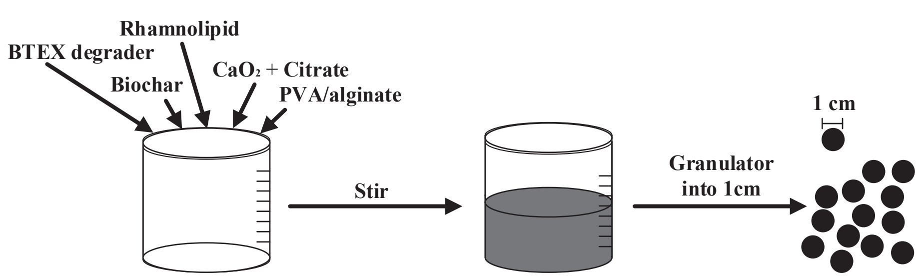

Fig. 1. The preparation procedure of BS-ORBs flowchart.