Chemosphere 91 (2013) 745–750

Contents lists available at SciVerse ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Use of microcapsules with electrostatically immobilized bacterial cells or enzyme extract to remove nonylphenol in wastewater sludge

Fu-Yin Hsu [a], Zheng-Yi Wang [b], Bea-Ven Chang [b,*]

[a] Institute of Bioscience and Biotechnology, National Taiwan Ocean University, Keelung, Taiwan
[b] Department of Microbiology, Soochow University, Taipei, Taiwan

HIGHLIGHTS

- We investigated the removal of nonylphenol (NP) by alginate microcapsules.
- Microcapsules increased the NP removal percentage in sludge.
- NP removal was greater with extract-containing microcapsules than cell-containing microcapsules.
- The removal percentage of toxic chemicals were BPF > BPA > NP > OTC > CTC > TC > BDE-15 > TBBPA > BDE-209.

ARTICLE INFO

Article history:
Received 2 August 2012
Received in revised form 8 February 2013
Accepted 9 February 2013
Available online 15 March 2013

Keywords:
Removal
Nonylphenol
Microcapsule
Bacterial cells
Enzyme extract
Sludge

ABSTRACT

We investigated the use of a high-voltage electrostatic system to immobilize bacterial cells or enzyme extract in alginate microcapsules for removing nonylphenol (NP) from wastewater sludge. With applied potential increased from 0 to 12 kV, the gel bead diameter decreased from 950 to 250 μm. The amount of bacterial cells or enzyme extract immobilized in alginate microcapsules was greater than that in suspension, for improved tolerance to environmental loadings. Removal of NP at 2.0–20.0 mg L^{-1} was greater with extract- than cell-containing microcapsules. The percentage of toxic chemicals (2.0 mg L^{-1}) removed with alginate microcapsules, in descending order of magnitude, was bisphenol-F > bisphenol-A > NP > oxytetracycline > chlortetracycline > tetracycline > dibromodiphenyl ethers > tetrabromobisphenol-A > decabromodiphenyl ether.

1. Introduction

In recent years, the expansion in both industrial and municipal wastewater treatment has markedly increased the volume of sludge generated, and increasingly stringent regulations have increased the complexity of disposal requirements. Although compounds are highly treatable in well-functioning wastewater treatment plants, many toxic-recalcitrant aromatic compounds such as nonylphenol (NP), bisphenol-A (BPA), bisphenol-F (BPF), tetrabromobisphenol-A (TBBPA), polybrominated diphenyl ethers (PBDEs), tetracycline antibiotics tetracycline (TC), chlortetracycline (CTC) and oxytetracycline (OTC) have been detected in effluent of sewage treatment plants (Ahel et al., 1994; de Wit, 2002; Halling-Sorensen et al., 2002; Chang et al., 2011).

Nonylphenol ethoxylates (NPEs) are the most common surfactants used in marketed products. The primary degradation of NPEs in wastewater treatment plants or in the environment generates more persistent, shorter-chain NPEs such as nonylphenol monoethoxylate (NPE1) and nonylphenol diethoxylate (NPE2) (Ahel et al., 1994). Because of their widespread presence in the environment and toxic activity even at low concentrations, these NPEs have received increased attention in regard to water-quality management and health.

Cost-effective and efficient methods are needed for removing toxic chemicals in sludge. Bioremediation is a popular and attractive technology that involves the metabolic potential of microorganisms to clean up the environment (Watanabe, 2001). The ability of bacteria to degrade toxic chemicals has been extensively studied with suspended-growth cells. In the presence of *Bacillus sphaericus* strain CT7 in sludge, the degradation half-life of NP was increased from 1.5 to 9.5 d with increasing NP concentrations from 1 to 25 mg L^{-1} (Chang et al., 2005). The toxic chemical degradation rate decreased with increasing chemical concentration because of competitive inhibition and the toxic effects of the chemical on microorganisms (Hsieh et al., 2008; Lu et al., 2012).

* Corresponding author. Tel.: +886 228806628; fax: +886 228831193.
E-mail address: bvchang@mail.scu.edu.tw (B.-V. Chang).

0045-6535/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.chemosphere.2013.02.019

From the patterns of ligninolytic enzymes, white-rot fungi can be divided into two groups: those producing only laccase and manganese-dependent peroxidase (MnP), and those also producing lignin peroxidase (LiP) depending on the ability to preferentially degrade lignin from woody plant-cell walls and mineralize a synthetic lignin preparation efficiently, respectively (Hatakka, 1994). *Pleurotus eryngii* is a representative species from the laccase-MnP group (Pelaez et al., 1995). Spent mushroom compost (SMC), a waste product of the mushroom industry, still contains many residual enzymes such as protease, cellulase, hemicellulase, LiP, MnP, and laccase (Lau et al., 2003). The use of SMC as a source material for ligninolytic enzymes is a win–win strategy, because it is easily obtained and saves on the disposal costs of waste removal. These features offer potential advantages for bioremediation and biological detoxification of hazardous and toxic compounds (Li et al., 2010; Liao et al., 2012). The use of SMC to remove organic toxic chemicals is enhanced by the addition of $CuSO_4$, $MnSO_4$, tartaric acid, or 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), with ABTS yielding a higher removal efficiency than the other additives (Liao et al., 2012).

Immobilization of bacterial cells or an enzyme extract is increasingly being used in biotechnology processes for biochemical conversion and bioremediation. Immobilized cells are useful for various transformation processes because as compared with suspended cells, they allow for more efficient use of the catalytic properties of enzymes (Chen et al., 2007). Calcium–alginate cross-linking is one of the most commonly used immobilization methods because the procedure is simple, has relatively mild effects, and is non-toxic to cells; however, the material is susceptible to biodegradation and has relatively low mechanical stability (Ha et al., 2009). Use of microcapsules is one of the preferred systems for cell culture and represents an exciting biotechnology approach to fermentation, especially for the production and intestinal delivery of therapeutic agents from genetically modified food-grade microorganisms (Qi et al., 2006). However, microencapsulation is not commonly used to remove toxic chemicals from wastewater sludge.

We investigated the use of a high-voltage electrostatic system to immobilize bacterial cells or an enzyme extract in alginate microcapsules for removing NP in activated sludge from wastewater systems. With batch experiments, we examined removal of NP and toxic chemicals such as BPA, BPF, NP, TC, CTC, OTC, TBBPA, dibromodiphenyl ether (BDE-15), and decabromodiphenyl ether (BDE-209). With bioreactor experiments, we evaluated the removal of NP after three separate additions of NP in sludge.

2. Materials and methods

2.1. Chemicals

NP reagent from Aldrich Chemical Co. (Milwaukee, WI) is a mixture of NP isomers, with 98.0% analytical standards. TBBPA of 97.0% analytical standard was from Aldrich Chemical Co.; BPA and BPF of 99.9% analytical standard; BDE-15 and BDE-209 of 98.0% purity; and TC, CTC, and OTC of 97.0% purity were from Sigma Chemical Co. (St. Louis, MO). Solvents were from Mallinckrodt (Paris, KY); all other chemicals were from Sigma Chemical Co.

2.2. Sampling and medium

Sludge samples were taken from the wastewater of the Dihus sewage-treatment plant, Taipei (total solids 0.87 g L^{-1}, pH 6.7). The basal medium contained the following (in mg L^{-1}): K_2HPO_4, 65.3; KH_2PO_4, 25.5; $Na_2HPO_4{\cdot}12H_2O$, 133.8; NH_4Cl, 5.1; $CaCl_2$, 82.5; $MgSO_4{\cdot}7H_2O$, 67.5; and $FeCl_3{\cdot}6H_2O$, 0.75. The pH of the basal medium was adjusted to 7.2 before autoclaving at 121 °C for 20 min.

2.3. Bacterial cells and enzyme extract

Bacterial cells included *B. sphaericus* strain CT7 and *Pseudomonas* sp. strain W4. *B. sphaericus* strain CT7, which can degrade NP, was isolated from sludge (Chang et al., 2005). *Pseudomonas* sp. strain W4, which can degrade BDE-15, was isolated from sediment (Huang et al., 2012). The bacterial cells were harvested after 48 h growth from 50 mL culture medium. The cultures were centrifuged (10000g × 10 min), rinsed, and re-suspended with phosphate buffer at pH 7.2.

Enzyme extract was from SMC of *P. eryngii*. SMC was produced in a mushroom cultivation factory in Chiayi, Taiwan, after the harvest of edible crops, with wheat bran and sawdust-based fermented compost. Enzyme extraction was performed in 1000-mL Erlenmeyer flasks containing 600 mL sodium acetate buffer (pH 5.0) and 120 g SMC for 3 h at 4 °C. The samples were centrifuged (10000g × 10 min), and the supernatant was partially purified by precipitation with ammonium sulfate and dialysis. SMC was a potential source of ligninolytic enzymes such as laccase, the crude extract from SMC were extracted and their laccase activities were measured (Baldrian, 2006; Li et al., 2010). Laccase analysis was performed as we previously described (Liao et al., 2012). The material was stored at −20 °C.

2.4. Preparation of microcapsules

Alginate solution was made by dissolving sodium alginate (4 wt.%) in 0.9 wt.% sodium chloride with stirring for 1 h at room temperature. Bacterial cells or enzyme extract solution was then added into the alginate solution. The final concentration of alginate was 2.0 wt.% in the mixture solution. The solution was prepared by dissolving calcium chloride (1.5 wt.%) with stirring at room temperature. An electrostatic droplet generator was used to prepare capsules (Tsai et al., 2010). The mixture was drawn into a 10-mL syringe fitted with a needle (23 G) and attached to a syringe pump that provided a steady solution flow rate of 25.2 mL h^{-1}. The positive electrode of the high-voltage electrostatic system was connected to the needle, and the negative electrode was placed in the collection solution 10 cm away from the needle tip. The size and shape of the microcapsules were characterized by use of a Leitz Laborlux II phase-contrast biological microscope (Germany).

The magnitude of the voltage generated between the needle and the gelling bath were important variables in determining alginate droplet size. The applied potential and gel bead diameter were inversely related. When the applied potential was increased from 0 to 12 kV, the gel bead diameter decreased from 950 to 250 μm. Thus, we used 12-kV potential to form microcapsules of 250-μm in diameter in the following experiments.

2.5. Removal experiments

We used two batch experiments. Experiment 1 involved bacterial cell-containing microcapsules. *B. sphaericus* strain CT7 was immobilized in alginate microcapsules to remove toxic chemicals NP, BPF, BPA, OTC, CTC, and TC; and *Pseudomonas* sp. strain W4 was immobilized in alginate microcapsules to remove BDE-15, TBBPA, and BDE-208. Experiment 2 involved extract-containing microcapsules and adding ABTS (1 mM) to remove toxic chemicals NP, BPF, BPA, NP, OTC, CTC, TC, BDE-15, TBBPA, and BDE-209.

The batch experiments involved use of 125-mL serum bottles containing 40 mL medium, 5 mL sludge, 5 g bacterial cell- or extract-containing microcapsules, and 2 mg L^{-1} toxic chemicals. We first compared NP degradation with suspended bacterial cells or