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Enhanced degradation of prometryn and other *s*-triazine herbicides in pure cultures and wastewater by polyvinyl alcohol-sodium alginate immobilized *Leucobacter* sp. JW-1

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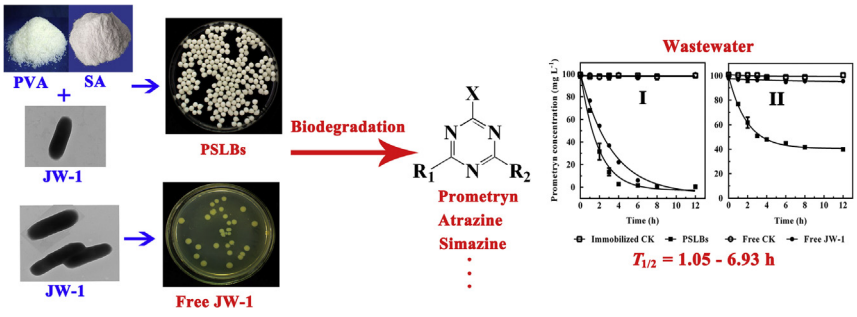
HIGHLIGHTS

- PSLBs exhibited greater removal efficiencies of prometryn than free JW-1 cells in wastewater.
- PSLBs showed better tolerance to pH, temperature, and salinity variability than free JW-1 cells
- PSLBs can efficiently degrade ten *s*-triazine herbicides.

GRAPHICAL ABSTRACT



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ABSTRACT

The *s*-triazine herbicides, such as prometryn, have been widely used in agriculture and have raised much public concern over their contamination of water and soil. *Leucobacter* sp. JW-1 cells were immobilized in polyvinyl alcohol sodium alginate (PVA-SA) beads and then used to degrade prometryn. Orthogonal array experiments showed that the optimal immobilization conditions of PVA-SA immobilized *Leucobacter* sp. JW-1 beads (PSLBs) were 3% JW-1 cells (w/v, wet weight), 10–12% (w/v) PVA, 2–3% (w/v) calcium chloride, and an immobilization time of 24–36 h. The PSLBs were more tolerance to pH, temperature and salinity changes than free JW-1 cells and were stable and effective for degrading prometryn through six reuse cycles without losing their degradation capacity. The half-life of prometryn degradation by PSLBs at 100 mg L^{-1} in pesticide plant wastewaters were 1.1–6.9 h. The rate constants of prometryn degradation by PSLBs in wastewaters ranged from 304 to 576 mg L^{-1} day^{-1}, which were approximately 1.25–118 times those of degradation by free JW-1 cells. The PSLBs degraded 99.9% of atrazine, 99.9% of ametryn, 97.8% of propazine, 100.0% of simetryn, 77.9% of simazine, 98.9% of terbuthylazine, 95.2% of prometon, 98.9% of atraton, and 31.6% of terbumeton at an initial concentration of 50 mg L^{-1} of each herbicide in 2 days. This study indicates that PSLBs persistently biodegrade *s*-triazine herbicides better than JW-1 free cells, and can be an efficient, safe and reusable biomaterial for the removal of *s*-triazine herbicides from contaminated sites.

1. Introduction

Bioremediation is an efficient, low-cost and environmentally friendly technique used to clean up chemically contaminated environments.

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The capacities of free microbial cells to degrade hazardous chemicals have generally been well proven under laboratory conditions. However, free microbial cells can face tremendous stresses such as survival, proliferation, mechanical disturbances, nutrition utilization, low adaptation, and competition derived from indigenous microorganisms in natural environments (Siripattanakul et al., 2009; Stelting et al., 2014). Immobilization techniques have been well established to overcome those problems. Immobilized microbial cells can be shielded from the stress of high pollutant concentrations, predators and competition with indigenous microorganisms (Kumar et al., 2012). Meanwhile, immobilization techniques can provide higher bacterial cell densities, prevent cell washout during continuous degradation, and increase mechanical strength (Huang et al., 2015). The merits of immobilized microbial cells thus include the alleviation of environmental stress to free cells (Huang et al., 2015).

The characteristics and pathways of pollutant degradation, including pesticides (Abigail and Das, 2015; More et al., 2015), surfactants (Siripattanakul et al., 2008), polycyclic aromatic hydrocarbons (Chen et al., 2017), and hydrocarbons (Siripattanakul et al., 2008), by immobilized microorganisms have been well documented in previous studies. Immobilization techniques have also used in the biodegradation of *s*-triazine herbicides. For instance, *Agrobacterium radiobacter* J14a was immobilized in polyvinyl alcohol (PVA) to degrade atrazine in a sand column (Siripattanakul et al., 2009). Sodium alginate (SA) was used as a gel matrix to immobilize *Arthrobacter* sp. DNS10, which could degrade atrazine (Zhang et al., 2015). *Pichia kudriavzevii* Atz-EN-01 was immobilized by a mixed matrix (PVA and SA) for the degradation of atrazine (Abigail and Das, 2015).

The *s*-triazine herbicide prometryn, 2,4-bis (isopropylamino)-6-(methylthio)-*s*-triazine, is widely used as a pre- or post-emergency herbicide to control broadleaf and grassy weeds in agriculture and nonagricultural soils (Zhou et al., 2009). Water and soil have been contaminated due to widespread applications of prometryn and its moderate persistence in the environment (Zhou et al., 2009). Prometryn has been frequently detected in surface water and groundwater (Papadopoulou-Mourkidou et al., 2004). The concentrations of prometryn varied from 0.031 to 4.40 μg L^{-1} in the surface waters of the Greek part of the Evros basin (Vryzas et al., 2011) and exceeded 1.00 μg L^{-1} in the groundwater of the Axios river basin (Papadopoulou-Mourkidou et al., 2004). Prometryn could cause damage to the caudal kidney of carp at a concentration of 0.51 μg L^{-1} (Velisek et al., 2013) and induce acute and chronic toxicity to humans through the food chain (Wang et al., 2014). Prometryn is listed as an endocrine disruptor in the priority list of the European Union (Database, 2013). Although prometryn had been banned in Europe since 2004, it is still being widely used in Australia, Canada, China, South Africa, and the United States (Velisek et al., 2015; Zhou et al., 2012), accentuating the importance of prometryn biodegradation in water and soil. Therefore, biodegradation-utilizing immobilized strains that efficiently remove or detoxify prometryn residues has attracted growing attention in the safe uses of prometryn and the remediation of its pollution.

Our previous studies have demonstrated that *Leucobacter* sp. JW-1 rapidly degraded prometryn and that its degradation rate constant was approximately 3 times that from the conversion of atrazine by the model strain *Pseudomonas* sp. ADP (Liu et al., 2017). Although numerous immobilized strains have been reported to degrade pollutants, it would be still valuable to utilize carrier materials to immobilize novel fast degradation strains to detoxify *s*-triazine herbicides. To the best of our knowledge, no report of prometryn biodegradation by immobilized strains has been found in the literature.

The aims of this study were to (i) investigate the effects of immobilization conditions on prometryn biodegradation; (ii) evaluate the degradation capacities of PVA-SA immobilized *Leucobacter* sp. JW-1 beads (PSLBs) and free JW-1 cells under different stress conditions; and (iii) compare bioremediation efficiency of PSLBs and free JW-1 cells in practical wastewater. We hypothesized that the efficiency of prometryn degradation by PSLBs was greater than that by free *Leucobacter* sp. JW-1 cells in water. We immobilized *Leucobacter* sp. JW-1 cells to enhance the ability of JW-1 to degrade prometryn. Different stress conditions, including pH, temperature, salt concentrations, and types of practical wastewaters, were used to examine the differences in prometryn degradation by PSLBs and free JW-1 cells. We show that immobilized *Leucobacter* sp. JW-1 cells have better application potential than free cells in the treatment of *s*-triazine herbicide-containing wastewater.

2. Materials and methods

2.1. Chemicals and medium

Atrazine (97% purity), ametryn (97% purity), prometryn (96% purity), propazine (98.5% purity), simazine (97% purity), simetryn (96% purity), and terbuthylazine (97% purity) were obtained from the Binnong Technology Co. Ltd., Binzhou, China. Prometon (purity, 99%), atraton (purity, 99%), and terbumeton (purity, 98.5%) were purchased from Dr. Ehrenstorfer GmbH, Augsburg, Germany. PVA (1750 ± 50 polymerization degrees), SA, boric acid and $CaCl_2$ were purchased from the Xilong Chemical Co., Ltd., Shantou, China. All other reagents were of analytical grade.

Mineral salt medium (MSM) was prepared by dissolving 0.4 g of $MgSO_4 \cdot 7H_2O$, 0.2 g of K_2HPO_4, 0.2 g of $(NH_4)_2SO_4$, 0.08 g of $CaSO_4$, and 0.002 g of $FeSO_4 \cdot 7H_2O$ in 1000 mL of distilled water at pH 7.0. Beef extract peptone medium (BEPM) contained 5 g of beef extract paste, 10 g of peptone, and 5 g of sodium chloride in 1000 mL of distilled water at pH 7.5.

2.2. Bacterial strain and culture conditions

Leucobacter sp. JW-1 was isolated from activated sludge from a wastewater treatment plant and could utilize prometryn as a sole source of carbon and energy in pure culture (Liu et al., 2017). Favorable growth conditions for JW-1 were pH 7.5 and 30 °C. The 16S rDNA gene sequence of strain JW-1 has been deposited in GeneBank under the accession number KT439069. The strain JW-1 was preserved in the China General Microbiological Culture Collection Center (CGMCC), Beijing, China under the CGMCC number 11754. To obtain wet JW-1 cells, we precultured JW-1 in 500-mL Erlenmeyer flasks containing 200 mL of BEPM (pH 7.5) at 30 °C and 150 rpm on a rotary shaker. After incubation for 40 h, the wet JW-1 cells were collected by centrifugation (3380 ×*g*, 10 min) and then immediately washed thrice (3 × 20 mL) with 0.9% sterilized sodium chloride solution for later use.

2.3. Orthogonal array experimental design for prometryn degradation

Immobilized materials can affect the degradation capacity of JW-1 cells. Multiple parameters were optimized to best immobilize JW-1 cells to degrade prometryn. The effects of different immobilization parameters, including PVA concentration (A), $CaCl_2$ concentration (B), the wet weight of JW-1 cells (C), and immobilization time (D), on the efficiency of prometryn degradation were investigated. An L_9 (3^4) orthogonal array table (Table 1) was designed by SPPS19.0 software (IBM SPSS, New York, USA) to optimize the immobilization parameters according to the orthogonal experimental design method. Each prometryn degradation test was performed according to the orthogonal array table.

A solution containing PVA (8, 10, and 12%, w/v) and SA (2%, w/v), as gel matrix solution (A), was added to 100 mL of distilled water in a 250-mL Erlenmeyer flask. The crosslinking solution (B) was prepared in 100 mL of distilled water containing boric acid (5%, w/v) and $CaCl_2$ (1, 2, and 3%, w/v). The pH value was adjusted to 7.0 with sodium bicarbonate. Solutions A and B were autoclaved at 121 °C for 20 min and then cooled to room temperature. JW-1 cells (1, 2, and 3%, wet weight, w/