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Triphenyltin biodegradation and intracellular material release by *Brevibacillus brevis*

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HIGHLIGHTS

- Triphenyltin, diphenyltin and monophenyltin were degraded by *Brevibacillus brevis*.
- Metabolites, ion and protein release, membrane permeability, viability were detected.
- Triphenyltin could be degraded simultaneously to diphenyltin and monophenyltin.
- Triphenyltin increased Cl^-, Na^+, Ca^{2+} and protein release, and membrane permeability.
- *B. brevis* metabolically released Cl^- and Na^+, and passively diffused Ca^{2+}.

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ABSTRACT

Triphenyltin (TPT) is an endocrine disruptor that has polluted the global environment, and thus far, information regarding the mechanisms of TPT biodegradation and intracellular material release is limited. Here, TPT biodegradation was conducted by using *Brevibacillus brevis*. Degradation affecting factors, metabolite formation, ion and protein release, membrane permeability, and cell viability after degradation were investigated to reveal the biodegradation mechanisms. The results showed that TPT could be degraded simultaneously to diphenyltin and monophenyltin, with diphenyltin further degraded to monophenyltin, and ultimately to inorganic tin. During degradation process, *B. brevis* metabolically released Cl^- and Na^+, and passively diffused Ca^{2+}. Protein release and membrane permeability were also enhanced by TPT exposure. pH ranging from 6.0 to 7.5 and relatively high biomass dosage in mineral salt medium improved TPT degradation. Biodegradation efficiency of 0.5 mg L^{-1} TPT by 0.3 g L^{-1} *B. brevis* at 25 °C for 5 d was up to 80%.

1. Introduction

Starting from the 1940s, the usage of organotins (OTs) had increased many folds; these synthetic and multipurpose chemicals (Jadhav et al., 2011) had been extensively used as heat and light stabilizers in the manufacture of poly vinyl chloride (Adams et al., 2011), and as active ingredients of biocides, and fungicides (Antes et al., 2011) and especially antifouling paints. With the increase of marine traffic, dredging activity and construction of aquatic infrastructure, harmful levels of OTs in waters and sediments are present. Among these OTs, triphenyltin (TPT) and its degradation products are present in all compartments of the natural environment. As an endocrine disruptor highly toxic to many different non-target organisms, TPT can cause high larval mortality and imposex (Jadhav et al., 2011). Worse still, TPT bioaccumulation through food chain poses potential health threat to human beings.

Over the recent decades, TPT analytic methods, distribution and ecotoxicity were well determined (Yu et al., 2011; Zuo et al., 2012), while its degradation was less concerned. Among the physical and chemical methods (Zhao et al., 2011), OH-radical, direct photolysis (Palm et al., 2003) and thermal decomposition (Mesubi and Olatunji, 1983) have been investigated for TPT elimination. However, high cost, energy and chemical requirement in these methods limited their application. Therefore, more cost-effective and efficient methods in TPT degradation need to be developed.

Studies attempting to investigate the effects of pH, temperature and environmental factors on TPT biodegradation illustrated that some supplemental substrates and certain environmental factors seemed to have encouraged the biodegradation efficiencies

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(Stasinakis et al., 2005; Heroult et al., 2008). For example, suitable kinds and levels of surfactants, nutrients, oxygen and metals obviously enhanced TPT biodegradation (Ye et al., 2013a). As one of the trisubstituted OTs, there is an increased concern about the biodegradation of TPT organic groups. Sequential dephenylation was inferred via the calculation of mass balance in batch reactors during TPT degradation process (Stasinakis et al., 2005). After biosorption by *Brevibacillus brevis*, TPT was further transformed to diphenyltin (DPT), monophenyltin (MPT) and tin intracellularly (Ye et al., 2013a). The degradation of DPT and tributyltin (TBT) was also performed through a single successive loss of an organic group (Heroult et al., 2008). Although these reports on biodegradation process of phenyltins (PTs) are of great significance in elucidating the fate and effect of TPT in environment, there is still a paucity of information about quantitative analysis of TPT degradation products, evaluation of factors favouring the biodegradation and cellular physiological–biochemical responses during TPT degradation process, which has limited the further application of OT biodegradation in polluted environments.

The present work aimed to effectively degrade TPT by *B. brevis*, and enhance the biodegradation by exploring the effect of initial pH, biomass dosage, degradation media, bacterium age and some potential metabolites in this process. Additionally, DPT and MPT formation, ion and protein release, cellular membrane permeability and viable count after TPT degradation were investigated to reveal the mechanisms of TPT biodegradation.

2. Materials and methods

2.1. Strain and chemicals

B. brevis was isolated from the sediment samples collected at a town called Guiyu in Guangdong Province, China, in which primitive e-waste processing and recycling activities are extensively involved (Ye et al., 2013a).

TPT was obtained from Sigma–Aldrich (St. Louis, MO, USA). Beef extract medium (BEM) contained (in g L^{-1}) 3 beef extract, 10 peptone and 5 NaCl. The concentrations of $Na_2HPO_4{\cdot}12H_2O$, KH_2PO_4, NaCl, NH_4NO_3, $MgSO_4$ and $ZnSO_4$ in mineral salt medium (MSM) were 100, 50, 40, 20, 5 and 3 mg L^{-1}, respectively.

2.2. Microbial culture

B. brevis was inoculated into BEM at 30 °C on a rotary shaker at 100 rpm for 24 h. Subsequently, the cells were separated by centrifugation at 3500*g* for 5 min, and washed three times with sterile distilled water before used in further experiments.

2.3. Biodegradation experiments

The flask with 20 mL MSM containing 0.5 mg L^{-1} TPT and 0.3 g L^{-1} *B. brevis* was inoculated in the dark at 25 °C on a rotary shaker at 100 rpm for 5 d to elucidate the best conditions and reveal the mechanism of TPT biodegradation.

The initial pH at 5.0–9.0 was adjusted by adding 0.1 M HCl or NaOH to investigate its influence on cellular membrane permeability, cell lethality and TPT degradation. The effect of 5 different media, namely MSM, distilled water, phosphate buffer solution (PBS) with pH 6.5, sterile and non-sterile water of Pearl River, on TPT biodegradation was conducted. Water quality (in mg L^{-1}) of Pearl River was as follows: DO, COD_{Mn}, COD_{Cr}, NH_4^+-N, TN, TP, Zn, As, Cr(VI) and volatile phenol were 5.1, 3.1, 7.8, 0.75, 3.14, 0.157, 0.023, 0.002, 0.004 and 0.001, respectively. Growth curve of *B. brevis* cultured in BEM from 3 h to 6 d was determined by detecting the optical density of culture medium at 600 nm. Meanwhile, *B. brevis* harvested from BEM at 0.5, 1, 2, 3, 4, 5 and 6 d, respectively, was used to reveal the effect of culture time on TPT biodegradation. Based on initial concentration of TPT, ethanol, acetic acid, acetone and benzene in the range of 5–100 μM were added into MSM, respectively, to illustrate the impact of potential organic metabolites on TPT biodegradation.

To reveal metabolites of TPT biodegradation, *B. brevis* at different dosage up to 0.5 g L^{-1} degraded 0.5 mg L^{-1} TPT, DPT and MPT for 5 d, separately. Meanwhile, *B. brevis* at 0.3 g L^{-1} was inoculated in MSM with different TPT concentration up to 1.0 mg L^{-1}, respectively, to ascertain correlation between TPT biodegradation and cellular physiological–biochemical responses, including cell membrane permeability, intra- and extracellular protein concentration, and ion release. Three samples for each experiment were taken and the mean values were used in calculations.

2.4. Extraction and derivatization of PTs

After biodegradation, 2 mL HCl at 1 M and 10 mL hexane were added into 20 mL solution. PTs in the mixture were sonicated for 20 min in an ultrasonic bath and allowed to settle until phase separation. After the organic phase was removed, 10 mL hexane was added into the aqueous phase, and then the operation was repeated again. The organic part was collected, followed by concentrating using a rotary evaporator at 30 °C. The residues were dissolved by 5 mL methanol and derivatised in pH 4.5 acetate buffer with 2 mL of 2% sodium diethyl dithiocarbonate. The recoveries of TPT, DPT and MPT were 96%, 93% and 91%, separately.

2.5. Analytical methods of PTs

TPT and its metabolites were analyzed according to previously published methods (Ye et al., 2013a) by gas chromatography–mass spectrometry (GC–MS) (QP2010, Shimadzu) equipped with a Rxi-5MS GC column (30 m × 0.25 mm × 0.25 μm). The detection limits of TPT, DPT and MPT were 250, 110 and 110 ng L^{-1}, respectively.

2.6. Cellular membrane permeability

Membrane permeability of *B. brevis* was determined by measuring the concentration of β-galactosidase released into the culture medium using *o*-nitrophenyl-β-D-galactoside (ONPG) as a substrate (Shi et al., 2013). Cells inoculated in nutrient medium for 2 d were collected, washed and suspended in 0.9% NaCl solution. The suspension was added into lactose induction medium (in g L^{-1}: KH_2PO_4 3, $Na_2HPO_4{\cdot}7H_2O$ 12.8, NaCl 0.5, NH_4Cl 1, $MgSO_4$ 0.5, $CaCl_2$ 0.01, lactose 5) and incubated at 37 °C. Subsequently, cells were collected, washed and suspended in β-galactosidase buffer (in g L^{-1}: KH_2PO_4 0.24, $Na_2HPO_4{\cdot}12H_2O$ 2.9, NaCl 8, KCl 0.2, $MgSO_4{\cdot}7H_2O$ 0.25, β-mercaptoethanol 3.9 mL). After then, cells were used to treat 0.5 mg L^{-1} TPT in the presence of 50 mg L^{-1} ONPG. The production of *o*-nitrophenol was detected at 504 nm.

2.7. Analytical methods of ions

After biodegradation, the solution was centrifuged at 3500*g* for 10 min. The resultant supernatant was filtered using a 0.22-μm polyether sulphone filter. Afterwards, the concentrations of Cl^-, Na^+ and Ca^{2+} were detected by an ICS-900 ion chromatography system (Dionex, Sunnyvale, CA, USA).