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Effect of cadmium ion on biodegradation of decabromodiphenyl ether (BDE-209) by *Pseudomonas aeruginosa*

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HIGHLIGHTS

- BDE-209 degradation by *P. aeruginosa* in the presence of Cd was investigated.
- Degradation was accelerated by the increase of CSH induced by low level of Cd.
- Higher membrane permeability caused by low level of Cd promoted degradation.
- Depression of cell growth and metabolism by high content Cd inhibited degradation.

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ABSTRACT

The influence of Cd(II) ions on the degradation of decabromodiphenyl ether (BDE-209) by an aerobic degrading strain, *Pseudomonas aeruginosa*, was investigated. The results demonstrated that the strain *P. aeruginosa* exhibited a high level of resistance against cadmium toxicity, and Cd(II) ions of different concentrations possessed mixed reactions on BDE-209 bioremoval. The degradation efficiency was stimulated at low concentrations of Cd(II) ions ($\leq$1 mg L^{-1}) but inhibited at higher levels ($\geq$5 mg L^{-1}). Subsequent analyses revealed that the increase of cell hydrophobicity and membrane permeability were two main factors for Cd(II) ions of low concentrations to accelerate BDE-209 degradation. However, inhibition effect by high concentrations of Cd(II) ions was mainly attributed to the negative impact of metals on growth and metabolism of the strain. It was also showed through cellular distribution of BDE-209 that different concentration of Cd(II) ions affected the amount of BDE-209 inside or outside the cell at different incubation time.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are a class of synthetic organic compounds substituted with up to ten bromine atoms per molecule. They have been extensively used as flame retardants in, for example, plastics, electronic appliances, furniture and vehicles [1,2]. As a result of massive industrial use, PBDEs have been detected in a wide variety of environmental media such as air [3], soil [4], sediment and sewage sludge [5] as well as biological samples including birds [6], fish [7], human blood serum and breast milk [8]. PBDEs are found even in the Arctic biosphere [9]. Because of the characteristics of PBDEs, such as persistence, bioaccumulation, and probable toxicity and carcinogenic/mutagenic effects, PBDEs pollution has become a pressing problem facing the world. It is crucial to develop effective technologies for PBDEs elimination from the environment.

A number of physicochemical methods have been tried for remediating PBDEs contamination [10–12]. Biological treatments have attracted more attention due to their low cost, and environmentally benign nature. Recent work on biotransformation has demonstrated that PBDEs were debrominated to lower-brominated congeners by a variety of anaerobic bacteria [13–16]. However, the information on aerobic biotransformation of PBDEs is limited at present, especially biodegradation of decabromodiphenyl ether (BDE-209). In 2011, Deng et al. isolated an indigenous strain DB-1 from PBDEs polluted sediment which could degrade BDE-209 to lower-brominated PBDEs using lactate, pyruvate and acetate as carbon sources under aerobic condition [17]. Most of the studies on aerobic biotransformation of PBDEs were focused on the lower-brominated PBDE congeners, such as mono- through hexa-BDEs [18,19].

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Although a great deal of attention has been paid to PBDEs elimination from the environmental media by biological treatments recently, it is worth noting that bioremoval of PBDEs-metal combined pollutants is also critical since organisms and environments are frequently exposed simultaneously or sequentially to a variety of pollutants via multiple exposure routes. And especially, most of PBDEs are released into the environment due to improper handling and disposal of electronic waste, implying that abundant heavy metals co-exist with PBDEs. To date, a number of reports have collectively showed that various metals, such as Ni, Cu, Zn, Pb, Hg and Cr posed certain effects on the degradation of organic compounds [20–22]. Nevertheless, it is evident that bioremoval of PBDEs-metal combined pollutants has received very limited attention. At present, there are only a few reports on the effect of individual heavy metals on the biodegradation of PBDEs.

Cadmium is one of the most ubiquitous pollutants released from chemical industries, automobiles, and many other inventories. It is also among the most abundant heavy metals co-existed with PBDEs in environment. Thereby, taking an investigation on the effect of Cd(II) ions on BDE-209 biodegradation is necessary for us to make a further understanding of the characteristics and mechanisms involved in PBDEs removal in the heavy metal co-existed polluted environment.

The main objective of the present work was to study the influences of Cd(II) ions on degradation of BDE-209, focusing on the degradation efficiency of BDE-209 by *Pseudomonas aeruginos*, in the presence of Cd(II) ions. Meanwhile, the effect of Cd(II) ions on cell surface characteristics and ATP activity of *P. aeruginosa* during degradation of BDE-209 was discussed to explore the possible mechanism of Cd(II) involvement. Moreover, the cellular distribution of BDE-209 under the influence of Cd(II) ions during degradation was also investigated.

2. Materials and methods

2.1. Chemicals

BDE-209 with a purity of >98% and *o*-nitrophenyl-β-D-galactoside (ONPG) were purchased from Sigma–Aldrich (St. Louis, MO, USA) and the standard of BDE-209 was purchased from Accustandard. A stock solution (1000 mg L^{-1}) of Cd(II) ions was prepared by dissolving $Cd(NO_3)_2$ (AR grade) in distilled water. The stock solution was then appropriately diluted to obtain the test solutions of desired strength. Other commercial chemicals were of analytical reagent grade and purchased from Guangzhou Chemical Reagent Factory.

2.2. Strain and culture medium

P. aeruginosa, a potential aerobic strain for BDE-209 biodegradation used in this work, was isolated from an e-waste dismantling area in Guiyu town of Guangdong province, China by our lab members.

Two kinds of culture media were used in this study.

Nutrient medium was used for strain culture, and its composition was as follows (g L^{-1}): glucose 5, peptone 2, and yeast powder 1.

Mineral salt medium (MSM) was used as the degradation medium, and its composition consisted of (g L^{-1}): NH_4NO_3 1, KH_2PO_4 1.5, K_2HPO_4 3, and 2 mL L^{-1} trace elements (g L^{-1}: $MgSO_4$ 4, $CuSO_4$ 1, $MnSO_4$, $FeSO_4{\cdot}7H_2O$ 1, $CaCl_2$ 1), pH was adjusted to 7.5.

All the media were previously sterilized in an autoclave at 121 °C for 30 min.

2.3. Microbial cultivation

P. aeruginosa was grown in 500 mL Erlenmeyer flask filled with 200 mL of liquid nutrient medium and incubated at 30 °C for 24 h under a rotary shaker at 150 r min^{-1}. Then, the cells were harvested and separated from the medium by centrifugation at 6000 r min^{-1} for 10 min. The separated biomass was washed three times with 0.05 mol L^{-1} sterile phosphate buffer (pH 7.3) and suspended in the same solution.

2.4. Preparation of intracellular enzymes

Bacterial cells, suspended in the ice-cold 0.05 mol L^{-1} sterile phosphate buffer, were subjected to 150 rounds of sonication in an ice-water bath for 3 s followed by cooling for another 3 s. The debris was removed by centrifugation at 8000 r min^{-1} for 10 min. The supernatant was filtered with 0.22 μm pore-size filters and the filtrate was considered the crude extract of intracellular enzyme. The protein was measured by Bradford method at 595 nm by UV–vis spectrophotometer using BSA (bovine serum albumin) as a standard [23].

2.5. Effect of Cd(II) ions on the growth of P. aeruginosa

Cells were pre-grown in 500 mL Erlenmeyer flasks containing 200 mL nutrient medium at 30 °C for 24 h under a rotary shaker at 150 r min^{-1} until a mid-exponential growth phase was reached. For the generation of growth curves, 1% pre-grown cells were inoculated into 100 mL Erlenmeyer flasks containing 40 mL nutrient medium, 1 mg L^{-1} BDE-209 and Cd(II) ions. The concentrations of Cd(II) ions were adjusted to 0.5, 1, 5, 10 mg L^{-1}, respectively. The controls used included cultures containing nutrient medium without BDE-209 and Cd(II) ions. Samples were withdrawn periodically to determine OD at 600 nm.

2.6. Effect of Cd(II) ions on degradation of BDE-209 by cells of P. aeruginosa and intracellular enzymes

In order to investigate the effect of heavy metal on BDE-209 degradation, $Cd(NO_3)_2$ was added into flasks and the final concentrations of Cd(II) ions were set at 0.5, 1, 5, and 10 mg L^{-1}, respectively. *P. aeruginosa* was inoculated on 20 mL MSM containing 1 mg L^{-1} BDE-209 and 5 mg L^{-1} glucose. Besides, intracellular enzymes were added to solution containing 1 mg L^{-1} BDE-209. Flasks with intracellular enzymes did not inoculate with *P. aeruginosa*. The concentrations of enzymes were determined by the weight of *P. aeruginosa*. BDE-209 biodegradation was carried out at 30 °C and 150 r min^{-1} in the dark for the desired time to determine residual concentrations of BDE-209. Un-inoculated flasks were served as control.

2.7. Effect of Cd(II) ions on cell surface hydrophobicity of P. aeruginosa

Cells of *P. aeruginosa* after undergoing the biodegradation in the presence of Cd(II) ions at various concentrations as described in Section 2.6 were withdrawn periodically to determine the relative cell surface hydrophobicity (CSH) using the microbial adherence to hydrocarbon (MATH) method with certain modifications [24].

A modified MATH method using xylene was basically performed throughout the study to assess the relative CSH. Four milliliters of the washed cells were added to acid-washed test tubes and overlaid with 4 mL of xylene. The resultant aqueous/organic mixtures were mixed with a vortex mixer for 1 min and the mixtures were allowed to settle for 15 min. The aqueous phase was then carefully removed

using a Pasteur pipette and the OD at 600 nm was read. Hydrophobicity expressed as adherence to xylene was calculated according to the formula: MATH = 100% × [(OD_{600} of washed cells) − (OD_{600} of aqueous layer following extraction with xylene)/(OD_{600} of washed cells)].

2.8. Effect of Cd(II) ions on membrance permeability of P. aeruginosa

Membrance permeability of *P. aeruginosa* was determined by measuring the release of β-galactosidase activity into the culture medium using ONPG as a substrate [25]. Bacteria grown to logarithmic phase in nutrient medium were collected, washed and resuspended in 0.9% sodium chloride solution. Bacterial suspension of 1 mL was pipetted into 10 mL lactose induction medium (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. After that, Bacteria were collected, washed and resuspended in β-galactosidase buffer (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), the OD at 630 nm was adjusted to about 0.3.

Bacterial suspension of 10 mL was pipetted into the test tube followed by adding 1 mL ONPG of 1 g L^{-1}, then 1 mg L^{-1} BDE-209 and $Cd(NO_3)_2$ at various concentrations as described in Section 2.6 were added. Test tubes were incubated with water bath heating at 37 °C and the production of o-nitrophenol over time was monitored with a spectrophotometer at 420 nm. The system without pollutants was served as control.

2.9. Effect of Cd(II) ions on ATPase activity of P. aeruginosa

To investigate the influence of Cd(II) ion concentrations on ATPase activity of *P. aeruginosa*, $Cd(NO_3)_2$ at various concentrations as described in Section 2.6 was added to the MSM containing 1 mg L^{-1} BDE-209 and 5 mg L^{-1} glucose. Flasks were inoculated with consortium at an optical density value of 0.45–0.47 at 600 nm (OD_{600}) on a rotary shaker at 30 °C and 150 r min^{-1} in the dark. After 24 h, bacterial cells were harvested to extract crude enzymes and detect ATPase activity. The ATPase activity of cell lysates was quantitated using a spectrophotometer at 660 nm with A007 enzyme kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu Province, China). One unit of ATPase activity was defined as the amount of inorganic phosphorus produced by ATP decomposition per hour per mg protein.

2.10. Analytical methods of BDE-209

Extraction: After designated time of incubation, the whole samples were extracted with an equal volume of n-hexane/dichloromethane for twice with ultrasonic-assistance. Each time the mixture was vigorously shaken for 30 min and allowed to set until phase separation. Two organic parts were collected and treated with anhydrous sodium sulfate to remove water. Then the solution was concentrated by using a rotary evaporator at 40 °C. The resulting residue was dissolved in 10 mL n-hexane for GC–MS analyses.

GC–MS analysis: BDE-209 was analyzed using GC–MS (7890A-5975C, Agilent Technology, USA) equipped with a type DB-5 ms GC column (15 m × 0.25 mm × 0.1 μm). Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}. The column temperature program started at 110 °C, held for 2 min, then the oven was heated to 310 °C at a rate of 15 °C min^{-1} and held for 5 min. Splitless injection of 1 μL sample was performed automatically at 280 °C. The temperature of ion source was set at 150 °C, and auxiliary temperature was 290 °C. The mass spectrometry used negative chemical ionization (NCI) mode with methane as reagent gas and the ionization energy was 70 eV.

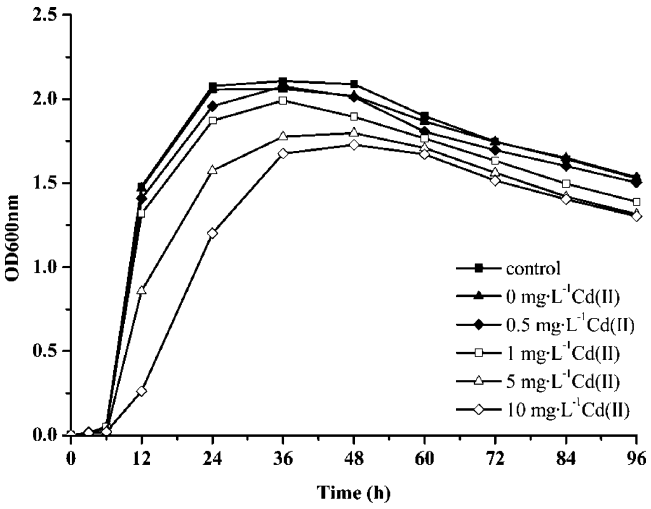

Fig. 1. Effect of Cd(II) ions on the growth of *P. aeruginosa*.

2.11. Statistical analysis

All of the experiments were performed in triplicates and the results presented were average values of the three replicates. The standard deviations for all measurements ranged from 2.0% to 8.5%.

3. Results and discussion

3.1. Effect of Cd(II) ions on the growth of P. aeruginosa

Metal ions may inhibit the metabolic activity of microorganisms involved in the degradation of organic compounds and thus affect the biodegradation rate [26]. The extent of inhibition depends on the concentration and availability of heavy metals and the action of complex processes controlled by multiple factors, including the nature of metals, media and microbial species [27].

In the growth curve of *P. aeruginosa*, as shown in Fig. 1, the lag phase of *P. aeruginosa* was relatively shortlived (0–6 h) and the strain entered logarithmic phase rapidly. After 24 h the bacteria reached stationary phase, and then entered decline phase after 48 h. The presence of 1 mg L^{-1} BDE-209 did not pose significant effect on bacteria growth. As for the effect of Cd(II) ions, *P. aeruginosa* growth was inhibited at higher concentrations (≥5 mg L^{-1}), but was only slightly influenced at low levels (≤1 mg L^{-1}). Addition of high concentrations of Cd(II) ions delayed bacteria entering into logarithmic growth phase and biomass declined by 15.6–20.4% at 36 h. Compared with some studies, the inhibitory effect of Cd(II) ions (10 mg L^{-1}) on *P. aeruginosa* growth was small. Hong et al. demonstrated that 10 mg L^{-1} Cd(II) ions inhibited the growth of *S. wittichii RW1*, and biomass declined by more than 50% [21]. By contrast, *P. aeruginosa* exhibited a higher level of resistance against cadmium toxicity. Possible reason was that *P. aeruginosa* used in this research lived long in the environment surrounded with electronic wastes and was therefore more tolerant of cadmium toxicity, implying that *P. aeruginosa* is able to be employed for degradation of organic compounds in the presence of Cd(II) ions.

3.2. Effect of Cd(II) ions on degradation of BDE-209

Based on our preliminary work, *P. aeruginosa* could effectively transform BDE-209 and the intracellular enzyme played a major role in the course of degradation. In order to explore

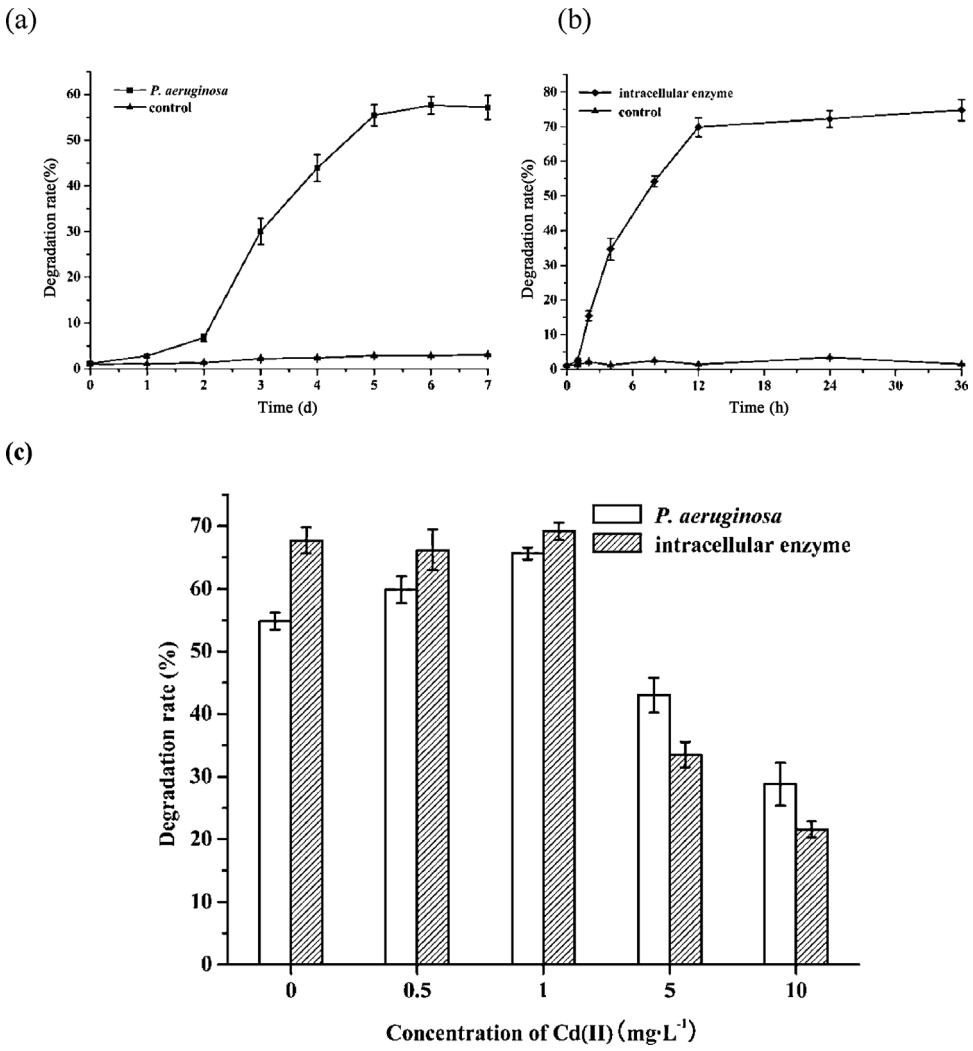

Fig. 2. Degradation efficiency of BDE-209 (1 mg L^{-1}) under different conditions. (a) degraded by *P. aeruginosa* in 7 d; (b) degraded by intracellular enzyme in 36 h; (c) degraded by *P. aeruginosa* and intracellular enzyme separately under different concentrations of Cd(II) ions. Cells and intracellular enzyme were incubated for 120 and 12 h, respectively.

the interrelation between strain and intracellular enzyme in transformation of BDE-209 in the presence of Cd(II) ions, further study was conducted.

The degradation curves by cells and intracellular enzyme of *P. aeruginosa* were presented in Fig. 2(a) and (b). It was seen that the degradation speed of *P. aeruginosa* cells was much slower than that of intracellular enzyme. The degradation rate of strain cells increased very slowly during the first 2 d, and ascended sharply from the 3rd d and then tended to stabilize after 5 d. On the contrary, the degradation rate by intracellular enzyme had almost no lag phase and kept ascending until 12 h. The result was ascribed to the fact that insoluble BDE-209 had to be first adsorbed on the cell surface, and then entered into the cell for utilization, while intracellular enzyme could contact and transform BDE-209 directly. This result also indicated that the degradation efficiency by intracellular enzyme was better than *P. aeruginosa* cells.

As illustrated in Fig. 2(c), the influence of Cd(II) ions on degradation of BDE-209 varied with its concentration. Degradation by *P. aeruginosa* cells was stimulated at low concentrations of Cd(II) ions ($\leq$1 mg L^{-1}), while inhibited at higher levels ($\geq$5 mg L^{-1}). The similar inhibitory result was observed when dealing with BDE-209 using intracellular enzyme with higher levels of Cd(II) ions present, but no significant impact on degradation was exhibited with low concentrations of Cd(II) ions. Possible explanations for this result were (i) Some heavy metals, such as cadmium, when their concentration was low, could promote the activity of the bacteria, thus increasing the utilization of BDE-209; (ii) Functional enzyme for the degradation of BDE-209 was synthesized faster in cells at low concentration of Cd(II) ions; (iii) Cell-surface characteristics changed in favor of BDE-209 adsorption and entering into the body for utilization by the cells when heavy metals existed, which was supported by our subsequent experiment in Section 3.3. It was also found that although Cd(II) ions posed certain inhibition on BDE-209 degradation in both cells and enzyme containing systems, the degradation rate by *P. aeruginosa* cells decreased less than that of enzyme. This suggested that the strain was better able to protect itself from detrimental effect when it was exposed to BDE-209-Cd(II) ions co-contaminated environment, while the enzyme contacted with contaminant directly and therefore was inactivated more easily, resulting in greater negative impact on degradation.

3.3. Effect of Cd(II) ions on cell-surface characteristics of *P. aeruginosa* during degradation of BDE-209

In order to explore whether the degradation efficiency of BDE-209 was affected by cell-surface characteristics of *P. aeruginosa*, CSH and membrane permeability was investigated.

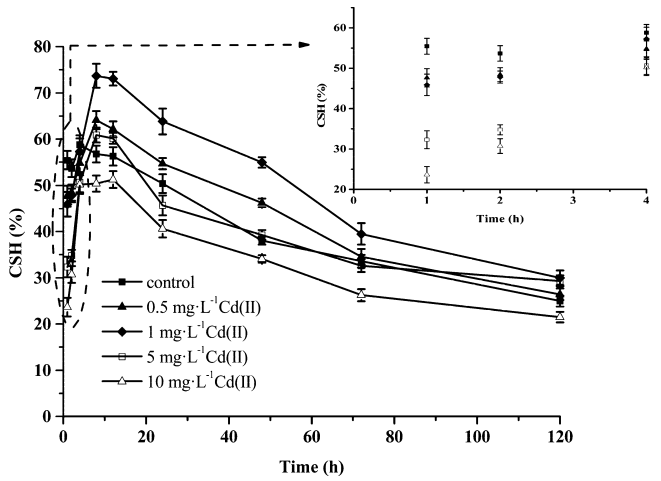

Fig. 3. CSH of *P. aeruginosa* during degradation of 1 mg L^{-1} BDE-209 under different concentrations of Cd(II) ions over incubation time.

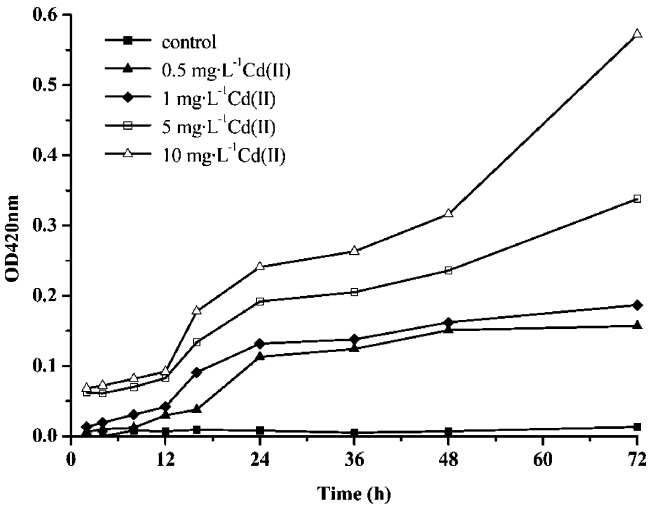

Fig. 4. Membrane permeability of *P. aeruginosa* during degradation of 1 mg L^{-1} BDE-209 under different concentrations of Cd(II) ions over incubation time. Membrane permeability was determined optically at 420 nm by measuring the release of β-galactosidase activity.

3.3.1. *Effect of Cd(II) ions on CSH of P. aeruginosa*

During degradation of BDE-209 under the influence of Cd(II) ions at concentrations of 0–10 mg L^{-1}, CSH alteration of *P. aeruginosa* was recorded and plotted in Fig. 3. At the beginning of degradation (1 h), CSH decreased with increase of Cd(II) ions concentration. This phenomenon suggested that vast of Cd(II) ions was adsorbed on the cell surface and occupied the adsorbing sites during a short time, which was consistent with our previous work that *P. aeruginosa* had good adsorption capability of Cd(II) ions at concentrations 0–10 mg L^{-1}, thus increasing the hydrophilic performance of *P. aeruginosa*. Subsequently, the CSH of the strains in the system with/without Cd(II) ions all increased rapidly and reached the maximum level at the 8th h. Compared with the control, the strains in the system containing low concentration of Cd(II) ions had higher CSH which rose by 7.4% at 0.5 mg L^{-1} and 16.9% at 1 mg L^{-1} Cd(II) ions, respectively. The explanations for this phenomenon are (i) Complexing of Cd(II) ions with some cell surfactant protein changed surface charges of cells and membrane potential, thereby converted hydrophilic groups into hydrophobic groups on the cell surface and enhanced hydrophobicity of strains [28]; (ii) The production of extracellular polymeric substances (EPS) were restrained with addition of low concentration of Cd(II) ions. And it was confirmed that the total content of EPS showed a negative effect on the relative hydrophobicity [29]; (iii) It was found that biosurfactant was produced by *P. aeruginosa* [30], and addition of low concentration of Cd(II) ions increased production of biosurfactants, which promoted the transfer of hydrophobic substrate. The impact of high concentration of Cd(II) ions on hydrophobicity of *P. aeruginosa* during degradation of BDE-209 was ignorable, compared with what it had on degradation rate.

Direct contact mode is one of the mechanisms involved in hydrophobic organic compounds uptake. It is believed that direct adherence to the surface of compounds is the first step in the process of removing hydrophobic organic pollutants by microbes. The bacterial CSH is one of the most important factors governing bacterial adhesion, uptake and degradation of hydrophobic organic compounds [31]. Zhang et al. demonstrated that there was a relationship between high CSH and strong degradation ability [32]. The results in current study indicated that the hydrophobicity of *P. aeruginosa* was improved by addition of Cd(II) ions at low concentrations. Thus, it was confirmed that change of hydrophobicity was one of the main factors for affecting degradation rate of BDE-209 by *P. aeruginosa* in the presence of low concentration Cd(II) ions.

3.3.2. *Effect of Cd(II) ions on membrane permeability of P. aeruginosa*

The inner layer of Gram-negative bacteria consists of phosphatidyl glycerol and cardiolipin [33]. β-galactosidase will be released when change in membrane permeability occurs. As shown in Fig. 4, when cells were treated with different concentrations of Cd(II) ions, a lag time of about 12 h was followed by a progressive release of β-galactosidase for up to 24 h to reach a steady state. However, the higher concentration of Cd(II) ions posed greater effect on membrane permeability than the low levels. The release of β-galactosidase accelerated again after 48 h in the presence of high concentrations of Cd(II) ions, especially at 10 mg L^{-1} Cd(II) ions. This behavior could be explained that cells ruptured and the holes on the cell walls of the bacteria actually appeared, after cells were exposed to high concentration of heavy metals for a long time, resulting in rapid increase of membrane permeability [28]. These results indicated that the release of β-galactosidase caused by Cd(II) ions was time- and dose-dependant. Thus, it is reasonable to consider that Cd(II) ions can increase the membrane permeability of *P. aeruginosa*. BDE-209 was easily transported into the cell and more intracellular enzyme leaked to the outside of cell to contact with organic pollutants when the membrane permeability increased. Therefore, change in membrane permeability was another factor to affect degradation rate of BDE-209 by *P. aeruginosa* with co-existence of Cd(II) ions.

3.4. *Effect of Cd(II) ions on ATP activity of P. aeruginosa*

The results obtained in Section 3.3 indicated that promotion of BDE-209 degradation by *P. aeruginosa*, co-existed with low concentration Cd(II) ions, was closely related to the changes of hydrophobicity and membrane permeability of strains. However, it was worth noting that although Cd(II) ions of high concentration enhanced permeation and exerted certain impact on hydrophobicity of strains, they played an inhibition role in BDE-209 bio-removal. This would be due to that the bacterial cells were damaged and metabolism was negatively affected at higher concentration of Cd(II) ions, resulting in the decrease of BDE-209 degradation efficiency.

ATPase plays important roles in various biological functions, including energy metabolism and nutrition substance transportation. The effects of Cd(II) ions on ATPase activity of *P. aeruginosa*

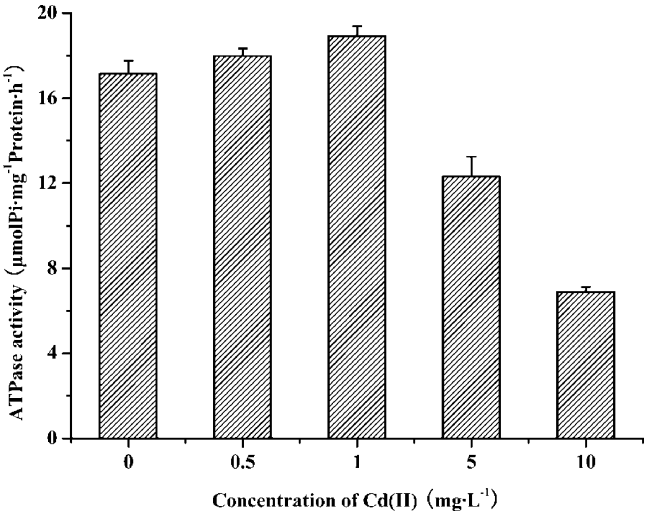

Fig. 5. Effect of Cd(II) ions concentration on the ATPase activity of *P. aeruginosa*. Cells were incubated with Cd(II) ions concentration at 0, 0.5, 1, 5 and 10 mg L^{-1}, respectively for 24 h.

were summarized in Fig. 5. ATPase activity decreased significantly ($P < 0.05$) when strains were grown in medium with 10 mg L^{-1} Cd(II) ions compared with control, while it was only slightly influenced by low level of Cd(II) ions, implying that high concentration of Cd(II) ions inhibited ATPase activity of *P. aeruginosa*. Thus, it could be concluded that the inhibition in BDE-209 degradation by high concentration of Cd(II) ions was mainly attributed to the negative impact of Cd(II) ions on the growth and ATPase activity of *P. aeruginosa*.

3.5. Effect of Cd(II) ions on cellular distribution of BDE-209

The effect of Cd(II) ions on cellular distribution of BDE-209 during degradation was also investigated and the result was illustrated in Fig. 6. It can be seen that the amount of BDE-209 inside/outside cells changed under different dosage of Cd(II) ions. BDE-209 adsorbed on the cell surface was considered as intracellular contaminant, based on that adsorption was commonly known as the first step for contaminant to enter into the cell, and that it was difficult and inaccurate to measure the mere amount of BDE-209 on the cell surface. As illustrated in Fig. 6(a), extracellular BDE-209 decreased rapidly on the first day, due to the adsorption effect of the cells. Then, extracellular BDE-209 declined slowly in the next few days and then ascended gradually. However, this rising trend came more quickly, when higher concentration of Cd(II) ions existed, on d 4 at 5 mg L^{-1} Cd(II) ions and d 3 at 10 mg L^{-1} Cd(II) ions, respectively. The explanation for this phenomenon was that some bacteria cells ruptured after several days' incubation and BDE-209 was released. The more Cd(II) ions existed, the more cells ruptured and the quicker this rupture occurred.

As depicted in Fig. 6(b), intracellular BDE-209 increased rapidly on the first day of degradation, which was consistent with the changes of extracellular BDE-209. During the last 2 days (d 6–d 7), the residual BDE-209 inside cells continuously declined and this downtrend had an obvious negative correlation with the concentration of Cd(II) ions. This suggested that the intracellular BDE-209 was released as the cells ruptured during the last period of the experiment, and besides, the higher the concentration of Cd(II) ions, the more BDE-209 was released. From d 2 to d 5, intracellular BDE-209 continued to decrease, but no significant correlation between concentrations of Cd(II) ions and BDE-209 was observed. This was probably because part of BDE-209 inside the cells was decomposed by intracellular enzyme, and the amount of residual BDE-209 was also influenced by many other factors such as adsorption and transformation.

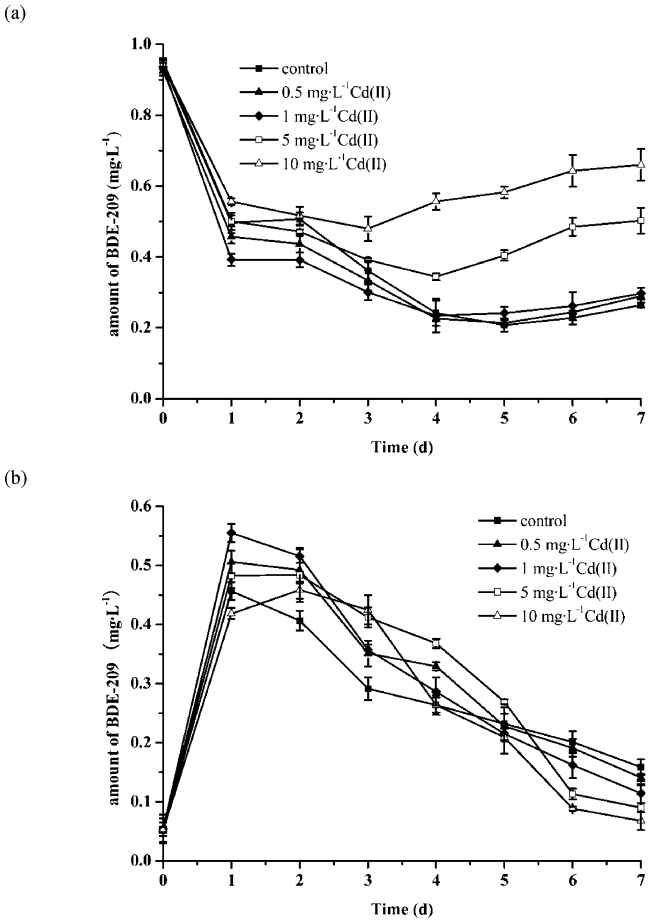

Fig. 6. Cellular distribution of BDE-209 during degradation of 1 mg L^{-1} BDE-209 under different concentrations of Cd(II) ions over incubation time. (a) the amount of BDE-209 outside the cells;(b) the amount of BDE-209 inside the cells.

4. Conclusions

This study demonstrated that the presence of Cd(II) ions significantly affected biotransformation of BDE-209 by *P. aeruginosa*. Cd(II) ions of low concentration ($\leq$1 mg L^{-1}) enhanced degradation rate, which was ascribed to the increase of hydrophobicity and membrane permeability of *P. aeruginosa* in favour of BDE-209 utilization by strain. However, high concentration of Cd(II) ions ($\geq$5 mg L^{-1}) inhibited degradation of BDE-209, which was mainly due to the impact of toxic heavy metal on the growth and metabolism of strain. Research on cellular distribution of BDE-209 showed that different concentrations of Cd(II) ions affected the amount of BDE-209 in/outside the cells at different incubation time.

Acknowledgments

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