Journal of Hazardous Materials 321 (2017) 9–17

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

Physiology and bioprocess of single cell of *Stenotrophomonas maltophilia* in bioremediation of co-existed benzo[a]pyrene and copper

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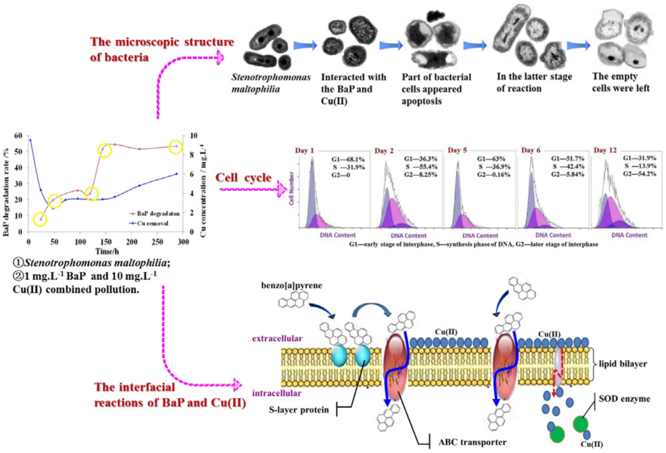
HIGHLIGHTS

- BaP damaged the cell membrane and influenced the membrane potential.
- *S.maltophilia* resisted upsetting situation by encouraging early apoptosis.
- Some cells suicide to facilitate the metabolism of BaP by the hardy bacteria.
- The bacterial cell division and reproduction was blocked because of BaP and Cu.
- The expression of proteins related to transport of BaP were evident different.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 28 June 2016
Received in revised form 19 August 2016
Accepted 1 September 2016
Available online 2 September 2016

Keywords:
Cell response
Combined pollutants
Benzo[a]pyrene
Copper
Microorganism

ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) and heavy metals are generally present in mixtures in the e-waste dismantling areas, posing serious health risk to the local people. Bioremediation has been considered as a promising approach for PAHs and heavy metals removal. In this study, we applied flow cytometry to obtain a better understanding of membrane potential (MP), apoptosis and cell cycle of *Stenotrophomonas maltophilia* affected by combined pollutants of benzo[a]pyrene(BaP) and Cu(II). The results showed that BaP was the main factor damaging the cell membrane and influencing the MP. *S. maltophilia* could even protect against upsetting situation by encouraging early apoptosis, whereby compromised cells committed suicide, as a result, which, in turn, facilitated the metabolism of the bacteria

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http://dx.doi.org/10.1016/j.jhazmat.2016.09.002

with high-vitality. Furthermore, even if the bacterial cell division was blocked and stopped reproduction, a large number of key enzymes inside cells could still be used for degradation of BaP. The expression of protein related to the transport and metabolism of BaP, regulation of redox reactions and phosphorylation in bacterial cell during bioprocess were evident different.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) and heavy metals are the main contaminants in the e-waste dismantling areas. They are generally present in mixtures, resulting in complex and diversified pollution consequences. It has been proved that these pollutants were transferred to the food chain, and human health was threatened since high levels of various heavy metals and PAHs were detected in the blood, placentas, hair, etc., of residents living in/near e-waste sites [1–3]. Although there have been many available techniques devoted to heavy metals and persistent organic pollutants (POPs) removal, the current technology for the treatment of coexisted contaminants, such as those in e-waste sites, is very sparse [4,5]. Microbe is the most common and tenacious species in the environment, and bioremediation technology has been considered as a promising approach for PAHs and heavy metals removal [6–8].

Microorganism is one of the important factors influencing the process and efficiency of bioremediation [9–11]. To survive the stress of pollutants, the microbe has developed elaborate mechanism to perceive external signals and adjust metabolic pathways. In this process, the cell response in physiological-biochemical characteristics was the important signal for removal of PAHs and heavy metals. Especially cell membrane was both the initial interface and also the major site for metabolic reactions of various substances, and it would affect the biosorption and transport of pollutants [12]. Moreover, the cell activity and reproductive growth were also the keys to ensure the efficiency of decomposition and transformation of contaminants.

The single cell analysis may give high resolution insights into whole cell culture concerning the cell status of viability and metabolic activity [13–15]. To gain a deeper insight into single cell performance during biological removal processes, appropriate method to characterize the physiology of a particular bacterial population has to be developed. As a diagnostic tool, flow cytometry (FCM) has found recently more and more important application in microbial processes [16,17]. For strengthening the biological removal it is very important to monitor the stress response related to cell viability as such information determines the efficiency of the bioprocess. This can be done by measuring cell integrity, membrane potential (MP), cell apoptosis and cell cycle. Change in MP is therefore one of the most sensitive indicators for culture status estimation, whereas cell integrity measurement relates to more distinctive parameters like compromised cell membrane and cell wall structures [18]. And the alteration of cell cycle and apoptosis can reflect the microbial reproductive activity, which is useful to evaluate the reacting process. Besides, the studies on protein expression levels offered by quantitative proteomics grants the technology great potential in studying microbial processes under environmental conditions. For example, proteomics can detect the shift in oxidation/reduction potential (e.g., from iron reduction to sulfate reduction) at bioremediation sites by detecting changes in community proteome information from contributing bacterial populations [19]. Also, the mechanisms of biodegradation of aromatic compound could be elucidated through analyzing the specific proteins induction in the enzymatic pathways in response to aromatic compound exposure [20]. Finally, proteomics can also be used to study the importance of key enzymes during microbial degradation of contaminants, and to verify the validity of biomarkers for contaminant removal [21,22].

In our previous studies, *Stenotrophomonas maltophilia* has been proved to be a potential strain for BaP biodegradation and Cu(II) biosorption, which was isolated from the contaminated site. The research found that BaP (67.1%) and Cu(II) (56.7%) were rapidly removed by *S. maltophilia* on the 1st d, the BaP inside the cell was utilized as carbon source to generate certain more bioavailable substances, such as phthalic acid. And part of Cu(II) on the cell were reduced to Cu and Cu(I) [23–25]. Thus, as the key player, the physiology and bioprocess of microbial cell is very important to be understood since these are beneficial to control the efficiency of BaP/Cu(II) removal. Although previous studies have derived the variation of cell permeability and microstructure during removal of BaP and Cu(II) by *S. maltophilia* [23], the current study was aimed to further investigate the cell response of *S. maltophilia* against BaP and Cu(II), to analyze various BaP/Cu(II)-induced proteomes to confirm metabolism, transportation and transformation of these pollutants, and to reveal the removal mechanisms of the combined pollutant on the cell interface.

2. Materials and methods

2.1. *Strain, medium and chemicals*

2.1.1. *Strain*

S. maltophilia was used for the present study. It was isolated from an e-waste dismantling area in the town of Guiyu in Guangdong Province, China. It is the gram-negative bacteria and belongs to aerobic microorganism.

2.1.2. *Medium and chemicals*

BaP was obtained from Sigma–Aldrich (St. Louis, MO, USA) and was prepared by dissolving an appropriate amount of the standard in methanol at a final concentration of 100 mg L^{-1}. The solution was kept in a brown bottle at 4 °C and wrapped to avoid any light exposure prior to use. A stock solution of Cu(II) (1000 mg L^{-1}) was prepared by dissolving $Cu(NO_3)_2$ (AR grade) in distilled water and sterilized by passing the solution through Millipore membrane filters (0.22 mm). Mineral salt medium (MSM) was used as the degradation medium, and its composition was as follows (g L^{-1}): $(NH_4)_2SO_4$ [1], K_2HPO_4 [7], KH_2PO_4 [3], $MgSO_4{\cdot}7H_2O$ (0.1), sodium citrate (0.5), pH 7.0–7.2. All the mediums were previously sterilized in an autoclave at 121 °C for 30 min.

2.2. *Cultivation of bacterial consortia*

A single colony from the slants was incubated in nutrient broth (composition: peptone, 10 g L^{-1}; sodium chloride, 5 g L^{-1}; and beef extract, 3 g L^{-1}) and grown 24 h with shaking at 30 °C. After growth, the bacterial culture was centrifuged (6000 r min^{-1}, 10 min, 25 °C). The obtained pellet was washed twice using sterile water, and then suspended in certain amount of MSM solution to be made into 0.05 g mL^{-1} bacterial suspension for the biodegradation/biosorption experiments.