Ecotoxicology and Environmental Safety 131 (2016) 127–134

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Influence of saponins on the biodegradation of halogenated phenols

Ewa Kaczorek [a,*], Wojciech Smułek [a], Agata Zdarta [a], Agata Sawczuk [a], Agnieszka Zgoła-Grześkowiak [b]

[a] Institute of Chemical Technology and Engineering, Poznan University of Technology, Berdychowo 4, 60-965 Poznan, Poland
[b] Institute of Chemistry and Technical Electrochemistry, Poznan University of Technology, Berdychowo 4, 60-965 Poznan, Poland

ARTICLE INFO

Article history:
Received 2 February 2016
Received in revised form
11 May 2016
Accepted 17 May 2016

Keywords:
Biodegradation
Cell surface properties
Halophenols
HPLC-MS/MS
Saponins

ABSTRACT

Biotransformation of aromatic compounds is a challenge due to their low aqueous solubility and sorptive losses. The main obstacle in this process is binding of organic pollutants to the microbial cell surface. To overcome these, we applied saponins from plant extract to the microbial culture, to increase pollutants solubility and enhance diffusive massive transfer. This study investigated the efficiency of *Quillaja saponaria* and *Sapindus mukorossi* saponins-rich extracts on biodegradation of halogenated phenols by *Raoultella planticola* WS2 and *Pseudomonas* sp. OS2, as an effect of cell surface modification of tested strains. Both strains display changes in inner membrane permeability and cell surface hydrophobicity in the presence of saponins during the process of halogenated phenols biotransformation. This allows them to more efficient pollutants removal from the environment. However, only in case of the *Pseudomonas* sp. OS2 the addition of surfactants to the culture improved effectiveness of bromo-, chloro- and fluorophenols biodegradation. Also introduction of surfactant allowed higher biodegradability of halogenated phenols and can shorten the process. Therefore this suggests that usage of plant saponins can indicate more successful halogenated phenols biodegradation for selected strains.

1. Introduction

The chemical and petroleum industries generate a number of highly toxic organic pollutants that contribute to harmful effects on the environment. Waste water from chemical industry often contains aromatic organic compounds, which often are resistant to biological degradation by naturally existing microbes and therefore remains in the environment. It makes these compounds able to transport over long distances and bioaccumulate in the tissues of humans and animals (Monsalvo et al., 2009).

Organic pollutants constitute a potential group of chemical compounds, which may pose a threat to human health and animals (Yong et al., 2009; Al-Khalid and El-Naas, 2012). Particularly dangerous to the environment are halogenated organic compounds, including halogenated phenols. Their sources are various industrial products, such as: herbicides, wood preservatives, paints, flame retardants or dyes (Sim et al., 2009). Furthermore, these compounds are difficult to remove from the environment and their biodegradation is relatively small (Juretic et al., 2014). These compounds are not only highly toxic but they also are characterized by mutagenicity and cancerogenicity properties (Pera-Titus et al., 2004). Nonetheless, they can be removed from the environment via biological methods, which are more efficient than chemical methods (Olaniran and Igbinosa, 2011). Biodegradation of halogenated aromatic compounds can be performed under aerobic or anaerobic conditions, depending on the type of microorganisms and the structure of pollutant. Microorganisms, mainly bacteria and fungi capable of including these compounds in their metabolic pathways are isolated from samples of soil and water from locations retaining in prolonged contact with the impurities (Travkin et al., 2006; Bergauer et al., 2005; Demnerova et al., 2005). Biodegradation pathways of halogenated aromatic compounds are similar to those for aromatic compounds in general (Commandeur and Parsons, 1990).

Many studies demonstrated that chlorophenol can be degraded by microorganisms present in water and sediments as well as in sludge (Crawford et al., 2007; Lallai and Mura, 2004; Liu et al., 2014; Karci, 2014; Moussavi et al., 2014). Furthermore, the biodegradation of halogenated phenols by bacteria strain may occur more efficiently, when is co-cultured with other bacteria or yeast (Ronen et al., 2005). According to some researchers, better results of biodegradation were observed when process was initiated by the less toxic compounds (for example phenol) as growth substrates – cometabolic degradation (Wang and Loh, 2000; Tobajas et al., 2012).

* Corresponding author.
E-mail address: ewa.kaczorek@put.poznan.pl (E. Kaczorek).

http://dx.doi.org/10.1016/j.ecoenv.2016.05.015
0147-6513/

The halogenated phenols can be degraded by different pathways, which are determined by the physical, chemical, and microbiological components of a particular environment. However, the pathways depend on number of halogen atoms in the molecule. In aerobic biodegradation of chlorophenol the ring is usually dihydroxylated by enzyme oxygenase in the first step. Two hydroxyl groups are positioned either ortho or para to one another on the ring (Pieper and Reineke, 2000). This is followed by cleavage of the ring.

Bioremediation of organic compounds can be enhanced by the addition of surfactants to polluted soils. On the one hand surfactants increase solubility and/or dispersion of organic compounds leading to an increase in the contact area between hydrocarbons and microorganisms (Zeng et al., 2011). On the other hand the surface active agents due to sorption on cell surface of microorganisms cause the modification of cell surface hydrophobicity as well as an increase in the permeability of biological membrane (Shoji et al., 2012). Modification of these properties may determine the effectiveness of biodegradation process of different pollutants. In the bioremediation both synthetic and natural surfactants are used. The use of surfactants in bioremediation process should be preceded by their selection. Some of them may have toxic effects by causing membrane disruption leading to cellular lysis. They can also increase membrane permeability causing metabolite leakage. These changes may lead to disorders related to the proper functioning of membrane (Singh et al., 2007).

Saponins are a surface active compounds, which are widely distributed in nature, most of all in the kingdom *Plantae*. They are known primarily with its pharmacological, hemolytic, emulsifying and foaming properties (Yücekutlu and Bildaci, 2008). They are glycosides – sugar derivatives. Furthermore, addition of saponins to polluted environment can cause increase of organic compounds biodegradation (Kobayashi et al., 2012; Choi et al., 2009; Tang et al., 2014; Huang et al., 2014).

The aim of this work was to investigate the three halogenated aromatic compounds biodegradation by two environmental bacterial strains: *Raoultella planticola* WS2 and *Pseudomonas* sp. OS2. The study focused on the biodegradation of 4-bromophenol, 4-chlorophenol and 4-fluorophenol. An important novelty point of the research was to determine the influence of natural surfactants on effectiveness of halogenated phenols removal from the environment. Two saponins extracts were used as surfactants. The first one was a commercial product – the extract from the bark of the *Quillaja saponaria* Molina. The second one was obtained from fruits of *Sapindus mukorossi* tree directly before experiments. Moreover, cell surface hydrophobicity, zeta potential and membrane permeability investigations of tested strains were conducted to explain the interaction in the system surfactant-halophenol-cell surface. This subject has not been investigated so far. What is more, the biodegradation of halogenated phenols is mostly described in cometabolism with intermediants of these compounds.

2. Materials and methods

2.1. Chemicals

The hydrocarbons and other fine chemicals employed in this study were of the highest purity grade (99%), produced by Merck (Germany). Haloaromatic compounds such as 4-bromophenol, 4-chlorophenol and 4-fluorophenol were obtained from Sigma-Aldrich. Halogenated phenol characteristic is shown in Table S1 (Supplementary materials).

The surface active agents used in this study are saponins which are natural glycoside-based non-ionic surfactants obtained from two different plants. The first one is a commercial product, an extract from the bark of the South American soap tree, *Quillaja saponaria* Molina (Sigma-Aldrich, USA, high grade purity). They are amphipathic glycosides belonging to non-ionic natural surfactants. Structurally they contain one or more hydrophilic glycoside moieties combined with a lipophilic triterpene or steroid derivative. The second one is obtained by special methanol extractions from fruits of *Sapindus mukorossi* tree (soap nut extract) and removal of both undesirable accompanying substances from the extracts and undesirable solvents (Smułek et al., 2016). Both surfactants contain triterpenoid saponins (Upadhyay and Singh, 2012; Guo and Kenne, 2000). These natural surfactants are characterized by lower toxicity and greater biodegradability compared to synthetic surfactants. Therefore, they can be used in bioremediation of pollutants as an alternative to them.

2.2. Microorganisms and growth conditions for the biodegradation test and measurement of cell surface changes

There were two environmental bacterial strains used in the experiments. The *Raoultella planticola* WS2 was isolated from soil contaminated with crude oil. Contaminated samples were collected from Polish northern areas. The *Pseudomonas* sp. OS2 was isolated from the activated sludge. Bacterial strains were identified using ID 32 GN biochemical tests (bio-Merieux, France) and molecular techniques. The 16S rRNA gene sequence of the tested strain has been deposited in the GeneBank database of NCBI under accession number **KP096507.1** (*Raoultella planticola* WS2) and **KP0965011.1** (*Pseudomonas* sp. OS2). The mineral salts medium (MSM) used throughout these studies was described previously (Kaczorek et al., 2010). A liquid culture was started by adding a loopful of cells from an agar plate into a 250 mL Erlenmeyer flask containing 50 mL of medium. After approximately 24 h 3–5 mL of this liquid culture was used for the inoculation of the final culture to reach an OD_{600} ~0.1 (10^8 cells per mL).

2.3. Cell surface properties

2.3.1. Microbial adhesion to hydrocarbons (MATH)

The cell surface hydrophobicity of the tested bacterial strains was assayed using the method of microbial adhesion to the hydrocarbon (Górna et al., 2011). It checked the influence of growth condition on cell surface modification of the two tested bacterial strains. *Raoultella planticola* WS2 and *Pseudomonas* sp. OS2 were grown on different carbon sources: 4-bromophenol, 4-chlorophenol and 4-fluorophenol at concentration 20 mg L^{-1}. Moreover, the addition of natural surfactants to the solution with halophenols was also analysed to determine the effect of this arrangement on the surface of bacteria. The surfactants concentration was determined on the basis of their critical micelle concentration (CMC). For commercial product it was 1.0 g L^{-1}, and for extract from fruits of *Sapindus mukorossi* tree 0.1 g L^{-1}. The growth temperature was 30 °C, and cells in the exponential growth phase were centrifuged at 8000 g for 5 min and washed twice with the MSM in order to remove residual hydrocarbons and surfactant. The cells were then re-suspended in the MSM to fit an optical density of ca. 1.0. Optical density was measured at 600 nm (OD_{600}) on a UV-Visible Spectrophotometer (Jasco, Germany). Next, 500 μL of hexadecane was added to 5 mL of microbial suspension and vortexed for 2 min. After 30 min the optical density of the aqueous phase was measured. Bacterial adhesion to hydrocarbon was calculated as: