original article

Isolation and identification of aerobic polychlorinated biphenyls degrading bacteria

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ABSTRACT

Aims: The purpose of this study was to isolate and identify aerobic polychlorinated biphenyls (PCBs) degrading bacteria.

Materials and Methods: This study was performed in lab scale aerobic sequencing batch biofilm reactor. Polyurethane foams were used as bio-carrier and synthetic wastewater was prepared with PCBs in transformer oil as the main substrate (20-700 μg/l) and acetone as a solvent for PCBs as well as microelements. After achieving to adequate microbial population and acclimation of microorganisms to PCB compounds with high efficiency of PCB removal, identification of degrading microbial species was performed by 16s rRNA gene sequencing of isolated bacteria.

Results: Gene sequencing results of the isolated bacteria showed that *Rhodococcus* spp., *Pseudomonas* spp., *Pseudoxanthomonas* spp., *Agromyces* spp., and *Brevibacillus* spp. were dominant PCB-degrading bacteria.

Conclusion: PCB compounds can be degraded by some microorganisms under aerobic or anaerobic conditions or at least be reduced to low chlorinated congeners, despite their chemical stability and toxicity. Based on the results of the study, five bacterial species capable of degrading PCBs in transformer oil have been identified.

Key words: Aerobic bacteria, biodegradation, polychlorinated biphenyls

INTRODUCTION

Polychlorinated biphenyls (PCBs) are synthetic aromatic compounds containing two benzene rings with one to ten chlorine atoms. There are 209 different compounds called congeners that vary in the number and position of their chlorines.[1] PCBs are poorly soluble in water but lipophilicity of PCBs contributes to their accumulation in oils and fats and magnification in food chain.[2] Due to their chemical and physical stabilities, PCBs were extensively used in a wide range of industrial applications such as transformer oils, hydraulic fluids, capacitor dielectrics, heat exchanger, plasticizers, waxes, and pesticide extender.[3]

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Owing to their toxicity and persistence in the environment, production and usage of PCBs were banned in the USA since 1979. However, because of their widespread use, PCBs have been largely released in the environment and are today detected in every compartment of the ecosystem, such as air, water, soil, sediments, and living organisms. Since PCBs are lipophilic in nature, their bioaccumulation in cells and food chain makes a threat for animal and human health[4] and concern about environmental pollution with these persistent organic pollutants is increasing.[3] These compounds are absorbed through the skin, lungs, and gastrointestinal tract in human and animals. Studies on animals showed that PCBs are carcinogenic. Short-term consumption of food containing large amount of PCBs can lead to liver damage and death in animals.[2] In humans, PCBs have been identified as potential carcinogens and teratogens.[5] Some epidemiological studies on workers exposed to PCBs showed that exposure to this contaminant has been eventuated to liver damage and malignant melanoma.[2] Researches have also showed that exposure to high concentration of PCBs causes a variety of adverse health effects, including skin disease (chlorance), liver damage (e.g., clinical hepatitis), and non-cancer short-term effects such as body weight loss, impaired immune function, and damage to the central nervous system.[1] Therefore, the investigation of treatment options for removal of PCBs from waste chemicals is of major importance.[6] Several physicochemical processes such as incineration, photolysis, reductive dehalogenation in presence of metals or strong base for destruction of PCBs have been proposed.[7,8] However, incineration as a conventional disposal method with high efficiency could emit toxic compounds and is extremely costly.[9] Since microorganisms play a fundamental role in the removal of waste chemical compounds and persistent organic pollutants,[10] recently, microbial degradation of PCBs is considered as one of the most effective methods for removing the compound from the environment.[1,11] Studies on biodegradation of PCBs have showed that the compounds can be converted to mineral or lower toxicity materials.[2] Two distinct mechanisms for the microbial degradation of PCBs have been identified: Oxidative degradation by aerobic bacteria and reductive dehalogenation by anaerobic ones.[12] The purpose of this study was to isolate and identify aerobic PCB-degrading bacteria which have ability to metabolize PCBs contaminated transformer oils.

MATERIALS AND METHODS

Set-up and adaptation

This study was performed in lab scale aerobic sequencing batch biofilm reactor [Figure 1]. Polyurethane foams were used as bio-carrier and reactor was inoculated with a mixture of thickened activated sludge from a municipal wastewater treatment and transformer contaminated soil microbial consortium has already been acclimatized

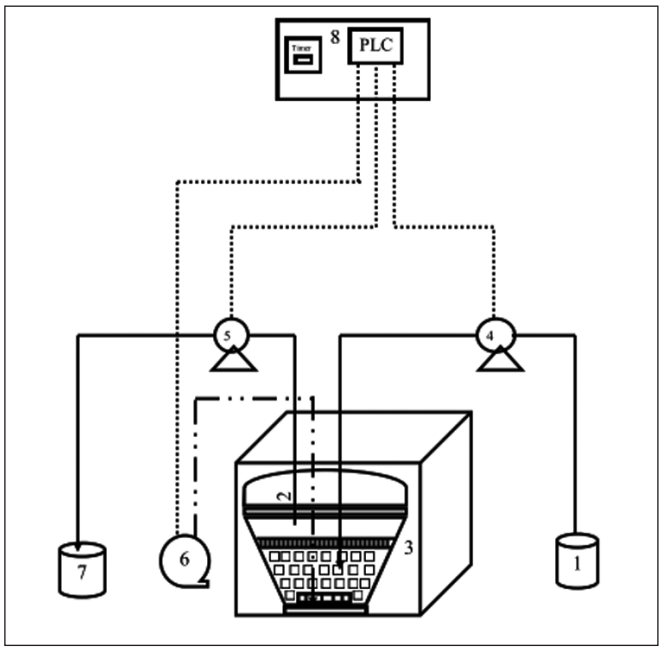

Figure 1: Scheme of aerobic sequencing batch biofilm reactor containing immobilized biomass: (1) Substrate tank, (2) SBBR, (3) water bath, (4) feed pump, (5) discharge pump, (6) air pump, (7) treated effluent, and (8) programmable logic controller (PLC) and timers

to PCB in a batch experiment. Synthetic wastewater was prepared with PCBs in transformer oil as the main substrate (20-700 μg/l) and acetone as a solvent for PCBs as well as microelements. The composition of mineral medium was as follows: NH_4Cl, 0.16 g/l; K_2HPO_4, 0.58 g/l; KH_2PO_4, 0.23 g/l; $MgSO_4 \cdot 7H_2O$, 0.09 g/l; $CaCl_2 \cdot 2H_2O$, 0.07 g/l; and trace elements (1 ml/l wastewater) as follows: $FeCl_3 \cdot 6H_2O$, 1.5 g/l; H_3BO_3, 0.15 g/l; $CuSO_4 \cdot 5H_2O$, 0.03 g/l; KI, 0.03 g/l; $MnCl_2 \cdot 4H_2O$, 0.12 g/l; $Na_2MoO_4 \cdot 2H_2O$, 0.06 g/l; $ZnSO_4 \cdot 7H_2O$, 0.12 g/l; and $CoCl_2 \cdot 6H_2O$, 0.15 g/l.[13] After achieving to adequate microbial population and acclimation of microorganisms to PCB compounds with high efficiency of PCB removal, identification of degrading microbial species was performed. Biodegradation of PCBs in transformer oil was confirmed and quantified with gas chromatography (AGILENT 6890 N, USA) equipped with an electron capture detector and a capillary column (30 m × 0.32 mm) with HP-5 phase after extraction. Purification and separation of PCB compounds in transformer oils and effluent samples were conducted by liquid–liquid extraction using *n*-hexane.

Culture media

Culture medium was similar to nutrient composition of the input to reactor in which 15 g/l agar was also added to media and mixed by heat. After media were autoclaved and cooled to 50°C, PCB compound was added to the culture medium and was inserted to the plates before hardening.[14,15]

Molecular identification of microorganisms

Biofilm samples were collected at the end of the experiment by scraping biomass from the bio-carriers and vortexing in 5 ml of sterile water for 5 min to homogenize the bacteria. Aliquots of biofilm samples were then spread on synthetic wastewater agar plates and incubated at 30°C. After 2-7 days of incubation, all bacterial colonies were characterized based on the colony and cell morphology. Each distinct colony was suspended in 100 µl of deionized water and genomic DNA was extracted by boiling for 15 min and centrifuging at 13,000 rpm for 5 min. The supernatant was used for polymerase chain reaction (PCR) amplification of 16s rDNA with Eubac27F and 1492R1 primers,[16] which amplifies a ~1,420 base pair (bp) fragments of 16s rRNA gene. The PCR amplification was conducted in a final volume of 50 µl containing 2 µl of template DNA, 0.2 µM of each primer, 0.2 mM of each dNTPs, 5 µl of 10× PCR buffer, and 1.5 units of Taq DNA polymerase. PCR was performed with an initial denaturation for 5 min at 95°C followed by 30 cycles of 94°C for 45 s, 55°C for 1 min, 72°C for 1.30 min and, a final extension at 72°C for 5 min. The PCR products were analyzed by agarose gel electrophoresis using 1.5% gel containing ethidium bromide together with DNA molecular weight marker. Gels were viewed on a UV transilluminator (UV TECH, France) and sizes of DNA fragment were compared with 100 bp ladder DNA. The DNA sequencing of the amplified gene was performed, and DNA sequence-analysis was undertaken by BLAST algorithms and databases from the National Center for Biotechnology (www.ncbi.nlm.nih.gov).

RESULTS

At the end of the experiment, seven different bacterial colonies were developed on PCB agar culture with regard to apparent characteristics were isolated [Table 1]. 16s rRNA gene sequence analysis of seven isolated bacterial colonies grown in the presence of PCBs showed the presence of five bacterial strains of aerobic PCB-degrading bacteria [Table 2]. Overall, PCBs biodegradation was observed in the range of 99.2-99.7% for PCBs concentration from 20 to 700 µg/l.

Table 1: Isolated bacterial characteristics of degrading PCBs

Number	Colony morphology	Cell morphology	Gram staining
S1	Large, milk-like mucoid colonies	Bacilli	Gram positive
S2	Very small, milk-like colonies	Cocci	Gram positive
S3	Small white colonies	Bacilli	Gram positive
S4	Large, brown colonies	Coccobacilli	Gram negative
S5	Large mucoid, milk-like colonies	Coccobacilli	Gram negative
S6	Pink colonies	Cocci	Gram positive
S7	Mucoid colonies	Coccobacilli	Gram positive

Table 2: Gene sequencing results of isolated bacteria

Isolate	Closest relative based on GenBank
S1,S3	*Brevibacillus* spp.
S2	*Agromyces* spp.
S4	*Pseudoxanthomonas* spp.
S5	*Pseudomonas* spp.
S6	*Rhodococcus* spp.

DISCUSSION

Several microorganisms have been isolated and characterized that can degrade PCBs preferentially the lowest chlorinated congeners in aerobic conditions.[8] In 1983, Furukawa first studied microbial degradation of PCBs and identified the number of PCB-degrading bacteria.[12] In this work, a preliminary characterization of dominant PCB-degrading bacteria was performed; based on 16sRNA gene sequence, five PCB-degrading bacterial strains were found to be mainly gram-positive bacteria as shown in Table 2. PCB-degrading bacterial strains including gram-negative bacteria such as *Pseudomonas*, *Sphingomonas*, *Achromobacter*, *Alcaligenes*, *Comamonas*, and gram-positive bacteria such as *Corynebacterium*, *Rhodococcus*, and *Bacillus* have also been described by others.[5,12] Fedi *et al*.[17] (2001) reported 15 bacterial strains using biphenyl as sole carbon and energy source belonged to *Pseudomonas* spp., *Alcaligenes* spp., *Comamonas* spp., *Ralstonia* spp. Aerobic oxidative degradation consist of two group of genes: First group responsible for converting PCB congeners to chlorobenzoic acid and second group is responsible for degrading chlorobenzoic acid.[2] Mostly, bacteria degrade PCBs by upper biphenyl pathway that is initiated by 2,3-biphenyl dioxygenase. This enzyme can be caused to cleave PCB compound at 2,3 positions.[18] When biphenyl is used by bacteria, the ring meta-cleavage, yellow compound is produced. This has been observed in most bacteria especially by the *Pseudomonas* spp.[2] In the study about 5 months after reactor performance and achieving to maximum efficiency removal, the yellow color was observed in the effluent of the reactors. The effluent samples were analyzed by gas chromatography and the presence of chlorobenzoic confirmed mineralization of PCBs. Wagner *et al*.[19] (1998) studied biodegradability of PCB compounds by aerobic bacteria with a batch system and observed the yellow color after 5 months. Gene sequencing results of the isolated bacteria showed that *Rhodococcus* spp., *Pseudomonas* spp., *Pseudoxanthomonas* spp., *Agromyces* spp., and *Brevibacillus* spp. were dominant PCB-degrading bacteria. Chang *et al*.[20] (2005) isolated *Pseudoxanthomonas* spp. from oil contaminated wastewater. The degradation of PCB by immobilized cells of *Pseudomonas* spp. on Siran carrier also resulted in 52-99% after 3 weeks.[21] Baxter *et al*. reported 76% and 88% decreasing in Aroclor 1242 for *Nocardia* spp. and *Pseudomonas* spp., respectively, during

52-day incubation.[6] PCBs biodegradation was observed in the range of 99.2-99.7% for PCBs concentration from 20 to 700 μg/l. Boyle *et al*. also reported between 12.8% and 24.5% of degradation when *Comamonas testosterone* and *Rhodococcus rhodochrous* were inoculated at a concentration of either 5 ppm or 10 ppm of Aroclor 1242, respectively.[1] In another study, Tartakovsky *et al*.[22] (2001) showed the presence of *Pseudomonas* and *Xanthomonas* spp. in the bioaugmented reactor, and *Pseudomonas*, *Rhodococcus*, and *Alcaligenes* spp. in the non-bioaugmented reactor. In this study, we found other bacterial strains, such as *Brevibacillus* and *Agromyces* spp. that could contribute to the removal of PCB contaminated transformer oil.

In conclusion, PCB compounds can be degraded by some microorganisms under aerobic or anaerobic conditions or at least be reduced to low chlorinated congeners, despite their chemical stability and toxicity. Based on the results of the study, five bacterial species capable of degrading PCBs in transformer oil have been identified.

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