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Solubilization and degradation of polychlorinated biphenyls (PCBs) by naturally occurring facultative anaerobic bacteria

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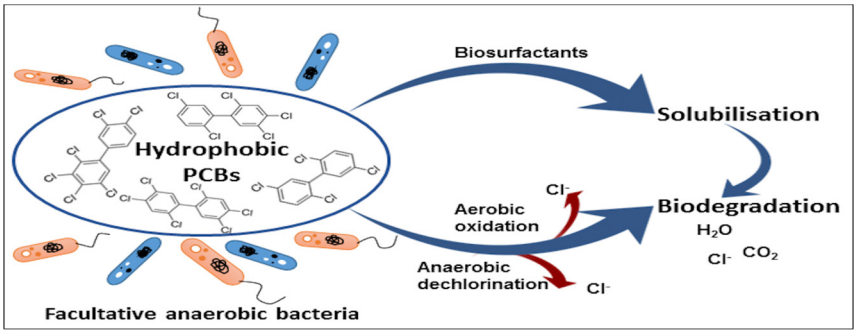
HIGHLIGHTS

- The best strains degraded PCBs under both anaerobic and aerobic conditions.
- Natural bacterial isolates exhibited concomitant PCB solubilization and degradation.
- PCB solubility positively correlated with potential biosurfactant production.
- Highest chlorine removal was achieved under two stage anaerobic-aerobic conditions.
- *Lysinibacillus* sp. demonstrated the highest PCB solubility and chloride build up.

GRAPHICAL ABSTRACT



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ABSTRACT

A combination of solubilization and degradation is essential for the bioremediation of environments contaminated with complex polychlorinated biphenyls (PCB) mixtures. However, the application of facultative anaerobic microorganisms that can both solubilize and breakdown hydrophobic PCBs in aqueous media under both anaerobic and aerobic conditions, has not been reported widely. In this comprehensive study, four bacteria discovered from soil and sediments and identified as *Achromobacter* sp. NP03, *Ochrobactrum* sp. NP04, *Lysinibacillus* sp. NP05 and *Pseudomonas* sp. NP06, were investigated for their PCB degradation efficiencies. Aroclor 1260 (50 mg/L), a commercial and highly chlorinated PCB mixture was exposed to the different bacterial strains under aerobic, anaerobic and two stage anaerobic–aerobic conditions. The results confirmed that all four facultative anaerobic microorganisms were capable of degrading PCBs under both anaerobic and aerobic conditions. The highest chlorine removal (9.16 ± 0.8 mg/L), PCB solubility (14.7 ± 0.93 mg/L) and growth rates as OD_{600} (2.63 ± 0.22) were obtained for *Lysinibacillus* sp. NP05 under two stage anaerobic-aerobic conditions. The presence of biosurfactants in the culture medium suggested their role in solubility of PCBs. Overall, the positive results obtained suggest that high PCB hydrolysis can be achieved using suitable facultative anaerobic microorganisms under two stage anaerobic-aerobic conditions. Such facultative microbial strains capable of solubilization as well as degradation of PCBs under both anaerobic and aerobic conditions provide an efficient and effective alternative to commonly used bioaugmentation methods utilizing specific obligate aerobic and anaerobic microorganisms, separately.

1. Introduction

The diversity and magnitude of synthetic toxic chemicals released into the environment are creating long-term human and ecosystem

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health impacts. Polychlorinated biphenyls (PCBs) are one such toxic chemical group consisting of 209 different chlorinated organic compounds. PCBs are persistent in the environment due to low reactivity, high chemical stability and extreme hydrophobicity (Beyer and Biziuk, 2009). Conversely, high lipophilicity makes PCBs soluble in fats. Such characteristics results in bioaccumulation, bioconcentration and biomagnification along the food chains leading to numerous health implications in humans and animals (ATSDR, 2000). Due to their persistence in the environment, sites contaminated with PCBs such as electricity distribution stations, service areas and dumpsites as well as sediments in the nearby waterbodies are still posing significant threats to human and ecosystem health.

Microorganisms play an important role in the removal of toxic chemical compounds from the environment. Biological conversion of highly and moderately chlorinated PCB congeners into less chlorinated congeners has been reported to take place through dechlorination under anaerobic conditions (Praveckova et al., 2015; Agullo et al., 2017). In comparison, lower and moderately chlorinated congeners can be degraded by oxidative bacteria under aerobic conditions through upper and lower biphenyl degradation pathways (Field and Sierra-Alvarez, 2008). Therefore, to achieve complete degradation, one of the most promising bioremediation strategies is to combine the anaerobic dechlorination and aerobic oxidation (Passatore et al., 2014). Although numerous sediment and soil based studies have been conducted either under anaerobic or aerobic conditions separately, using potential PCB degrading bacteria (Adrian et al., 2009; Payne et al., 2011; Wang and He, 2013; Murinova et al., 2014), studies based on combined anaerobic-aerobic conditions are limited (Evans et al., 1996; Master et al., 2002; Long et al., 2015). Studies by Evans et al. (1996) and Master et al. (2002) used two separate groups of bacteria capable of reductive dechlorination and aerobic oxidation, respectively, to degrade PCBs in contaminated soil slurry. No studies appear to have been undertaken so far on PCB degradation using facultative anaerobic microorganisms under two-stage anaerobic-aerobic conditions. However, there is a recent study based on facultative anaerobic bacteria mediated *in situ* delignification and enhanced gas release under microaerophilic conditions in soil containing lignocellulose (Rashid et al., 2017).

Past research literature on biochemical pathways and intracellular localization of enzymes responsible for PCB degradation suggest that PCBs have to be solubilized first for easier passage through the cell wall and into the cytoplasm prior to being metabolized. Therefore, an increase in the rate of solubilization could accelerate the entrance of PCBs into the cells and their subsequent degradation (Ohtsubo et al., 2004). Most of the research studies undertaken so far on PCB solubility have been based on the addition of chemical or biological surfactants with limited investigation into the actual application of microorganisms producing surfactants (Singer et al., 2000; Fava and Di Gioia, 2001; Occulti et al., 2008; Viisimaa et al., 2013). Chemical surfactants have the advantage of being economical, but are often toxic to biological systems (Abraham et al., 2002). In comparison, biosurfactants generally exhibit higher interfacial tension reduction activities compared to chemical surfactants, and are less toxic and readily biodegradable (Viisimaa et al., 2013). However, the main disadvantage in the use of commercially available biosurfactants is the high cost (Aparna et al., 2012).

Therefore, the use of suitable biosurfactant producing microbial strains, which are also capable of degrading PCBs under both, anaerobic and aerobic conditions, would be an attractive alternative to the use of either chemical or biological surfactants or PCB degrading aerobic and anaerobic bacterial groups, separately. Indeed, the application of biosurfactant-producing and pollutant-degrading microorganisms offers the dual advantage of a continuous supply of biodegradable surfactants and the ability to degrade pollutants (Megharaj et al., 2011).

The aim of this study was to isolate potential naturally occurring facultative anaerobic bacteria from soil and sediment environments and to investigate their capability for degrading Aroclor 1260, a complex and widely used commercial PCB mixture, under comparative anaerobic, aerobic and two stage anaerobic-aerobic conditions while solubilizing a hydrophobic PCB mixture. The outcomes of the study are expected to contribute to the development of more efficient and effective bacterial mediated bioremediation treatment of PCB contaminated soils and sediments.

2. Materials and methods

2.1. PCB source

Aroclor 1260 was selected as the commercial grade PCB source for this study and obtained as a GC/FID grade technical mixture from AccuStandard Inc. (New Haven, CT, USA). Aroclor 1260 represents a complex PCB mixture consisting of about 75 different penta to nona chloro biphenyls with an average of 6.3 chlorines per biphenyl molecule (Bedard et al., 2007). Aroclor 1260 was prepared as a 50 mg/mL stock in GCMS grade acetone, before use.

2.2. Screening, enrichment and identification of possible PCB degrading microorganisms

2.2.1. Sampling of soil and sediments

In order to isolate possible facultative anaerobic PCB degrading bacteria, six soil samples around the Brisbane City area and six sediment samples from Brisbane River (27.4745° S, 153.0293° E) and Coombabah Lake, Gold Coast (27.54° S, 153.22° E), Australia were collected into sterile glass bottles and transported on ice to the laboratory. Soil and sediment samples were separately homogenised and 50 g from each composite soil and sediment mixtures were added to duplicate 250 mL Erlenmeyer flasks. After contamination with Aroclor 1260 to obtain 50 mg/kg PCB concentration, flasks were incubated stationary under aerobic conditions at room temperature (23 °C ± 1 °C) for one month. Similarly, 50 g of each composite soil and sediment mixture were added to duplicate 50 mL polypropylene vials with screw caps, contaminated with Aroclor 1260 to obtain 50 mg/L concentration and incubated stationary inside the anaerobic chamber (COY lab products) main compartment at room temperature (23 °C ± 1 °C) for one month. The atmosphere inside the anaerobic chamber was maintained constant at 4.9% H_2, 10.7% CO_2 and 84.4% N_2.

2.2.2. Selective enrichment of possible PCB degrading bacteria

Isolation of microorganisms capable of utilizing PCBs was carried out through a series of selective enrichments using DSMZ medium 465a (Atlas, 2005) as the base minimal salt medium (MSM) with the following modifications. After autoclaving the medium, instead of 0.5 g/L hydroxybiphenyl in ethanol, Aroclor 1260 in GCMS grade acetone was added as the sole carbon and energy source to give 50 mg/L final PCB concentration. Four 250 mL Erlenmeyer flasks containing 100 mL of sterile MSM medium were prepared. Two flasks were inoculated with 10 g of composite soil, and the other two were inoculated with 10 g of sediment previously contaminated with Aroclor 1260. Two flasks (one with soil and the other with sediment) were incubated aerobically in a platform shaker maintained at 150 rpm and 28 °C. The other two remaining flasks were incubated anaerobically under static conditions in an incubator kept inside the anaerobic chamber with the temperature kept at 28 °C. Four serial transfers (10% of the enrichment medium) were carried out from each flask at weekly intervals into fresh sterile MSM containing 50 mg/L Aroclor 1260. From the final flask, supernatant was plated on nutrient agar (CM003, Oxoid) and incubated overnight at 28 °C. Morphologically different colonies were isolated and streaked on fresh nutrient agar plates.

2.2.3. Characterization of potential PCB degrading bacteria

Bacterial isolates obtained from selective enrichment were inoculated in duplicate on minimal salt agar plates containing 50 mg/L of