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Biological treatment of transformer oil using commercial mixtures of microorganisms

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ABSTRACT

The biodegradation of six PCB congeners (IUPAC nos. 28, 52, 101, 138, 153, and 168) present in transformer oil using different commercial mixtures of microorganisms (Sybron 1000, Biozyn 301, Biozyn 300, DBC 5, ChZR, NS 20-10, NS 20-20, and NS 20) under anoxic, oxic, or anoxic/oxic treatments was investigated at laboratory scale. Overall, PCB congener biodegradation was observed in all treatments in the ranges of 0–99%, 2–97% and 40–94% under anoxic, oxic, or anoxic/oxic treatments, respectively. The highest biodegradation of total PCB congeners occurred using Sybron under oxic and anoxic conditions (76.0% and 91.3% reduction, respectively; initial PCB concentration, 1417.1 mg l^{-1}). Also, the highest biodegradation extent of the PCB congeners occurred when the commercially available mixture of microorganisms, Sybron, was used (84.7% reduction) under combined anoxic/oxic conditions. Demonstration that biodegradation of most of the individual PCB congeners was achieved by the commercial mixtures of microorganisms in this study suggests that PCB-impacted environments can sustain populations of these PCB-metabolizing organisms. This is particularly relevant for the development of biostimulation or bioaugmentation strategies for the bioremediation of PCB-contaminated wastes.

1. Introduction

Polychlorinated biphenyls (PCBs) are man-made contaminants that have been found in air (Asher et al., 2007; Cetin et al., 2007), water (Wurl and Obbard, 2006), soil (Wyrzykowska et al., 2007), sediment (Blais et al., 2003; Robinson et al., 2008) and biota (Asher et al., 2007; Bouwman et al., 2008; Mendoza et al., 2006), as well as in human adipose tissue (Decastro et al., 2006; Huang et al., 2007). Though PCBs were banned from the U.S. in 1977, our biosphere contains approximately 7.5 $\times$ 10^8 kg of released PCBs (Borja et al., 2005; De et al., 2006). It is recognized that long-term exposure to PCBs can provoke toxic effects in humans and wildlife (IARC, 1997; Li, 2007). In addition, PCBs have been identified as potential endocrine disruptors, resulting in renewed research interest (Decastro et al., 2006; Oskam et al., 2005). In spite of present and future restrictions on the production and use of PCBs (EC, 1991, 1996, 1997, 2006; EPA, 2006), they will not be immediately removed from the environment since they have a very high bioaccumulation potential (Gregoraszczuk et al., 2003; Huang et al., 2007) and are resistant to environmental degradation processes (Abramowicz, 1990; Dai et al., 2002). Thus, the investigation of treatment options is of current concern.

Several physical and bioremediation technologies are available (Aslund et al., 2007; De et al., 2006; Katsumata et al., 2007; Lin et al., 2006; Manzano et al., 2004), although, in general, physical techniques are extremely costly and generate toxic by-products. Thus, bioremediation appears to be the most environmentally friendly solution. Biological methods have been utilized in water (Katsumata et al., 2007) and soil remediation of PCBs (Liu and Yu, 2006; Manzano et al., 2004; Michel et al., 2001; Varanasi et al., 2007). Two distinct biological systems capable of biodegrading PCBs have been identified: aerobic oxidative processes and anaerobic reductive processes (Abramowicz, 1990; Patureau and Trably, 2006). The aerobic bacterial biodegradation of PCBs has been well studied (Abramowicz, 1995). Several microorganisms have been isolated that can aerobically degrade PCBs, preferentially degrading the lowest chlorinated congeners (Moeder et al., 2005; Pieper, 2005). These organisms attack PCBs via the well-known 2,3-dioxygenase pathway, converting PCB congeners to the corresponding chlorobenzoic acids (Seeger et al., 1997). Anaerobic bacteria attack the highest chlorinated PCB congeners through reductive dechlorination (Nollet et al., 2005; Rodrigues et al., 2001). In general, this microbial process effects the preferential removal of *meta* and *para* chlorines, resulting in a depletion of highly chlorinated PCB

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congeners with corresponding increases in lower chlorinated, *ortho*-substituted PCB congeners (Abramowicz, 1990, 1995).

The majority of reported PCB-degrading bacteria are Gram-negative, including *Pseudomonas*, *Alcaligenes*, *Acinetobacter,* and *Achromobacter* (Master et al., 2005; Master and Mohn, 2001; Ohtsubo et al., 2006; Yang et al., 2007), but Gram-positive bacteria, including *Rhodococcus*, *Arthrobacter*, and *Corynebacterium* species have also been reported to be capable of metabolizing PCBs (Kitagawa et al., 2001; McKay et al., 1997; Rybkina et al., 2003; Saagua et al., 1998), using PCBs as their source of carbon and energy. The biodegradation of PCBs in real contaminated sites is often limited by the low content of autochthonous microbial communities capable of metabolizing these recalcitrant contaminants. In such cases inoculation may be one viable option for successful bioremediation; nevertheless, little is still known about the use of bioaugmentation in real contaminated sites (Di Toro et al., 2006). Recent advances in the degradation of PCBs have sought PCB-degrading communities, which can be surprisingly diverse (Vasilyeva and Strijakova, 2007). In general, pure cultures of PCB-degrading bacteria (Evans et al., 1996; Fava and Bertin, 1999), consortia of specialized bacteria (Abramowicz, 1995; Fava and Bertin, 1999; Klasson et al., 1996; Rojas-Avelizapa et al., 2003), and genetically engineered bacteria able to avoid the accumulation of potentially toxic or dead-end intermediates of target pollutants (Reineke, 1998) have been applied for the bioaugmentation of PCB-contaminated ecosystems. Although positive results have been obtained (Evans et al., 1996; Fava and Bertin, 1999), several failures have also been recorded (Harkness et al., 1993; Morgan and Watkinson, 1989). One marginally investigated approach is the amendment of the PCB-contaminated ecosystems with unspecified, naturally established complex consortia of microorganisms, such as those occurring in sludge, manure, or compost (Dejonghe et al., 2001). These sources normally contain such a high diversity of microorganisms (bacteria, fungi, etc.) that the species necessary to biodegrade the pollutants and/or their metabolites may be present. Further, the addition of such a rich consortium of different microorganisms might result in the establishment of new and fruitful interactions (at the catabolic and genetic level) between different microorganisms occurring at the augmented ecosystem, and this in turn might result in an improved removal of pollutants (Dejonghe et al., 2001). These sources of microorganisms can also carry a variety of essential nutrients that might strongly contribute to sustain survival and colonization of inoculated species in the contaminated site (Antizar-Ladislao et al., 2004, 2008). Therefore, complex consortia of microorganisms appear to be of special interest for bioaugmentation of complex PCB-contaminated ecosystems, although little is still known about the potential of such sources of microbial consortia (Antizar-Ladislao et al., 2004; Dejonghe et al., 2001; Kastner and Mahro, 1996) and about commercial mixtures of microorganisms in the biodegradation of real PCB-contaminated ecosystems (Di Toro et al., 2006).

This study explores the ability of commercial mixtures of microorganisms to metabolize PCBs. It is hypothesized that such mixtures of microorganisms can be applied as part of an environmental management strategy to reduce or eliminate hazardous materials (e.g., PCBs) from a polluted ecosystem. To the best of our knowledge, this is the first study in which the ability of commercial mixtures of microorganisms specifically prepared to metabolize PAHs is evaluated to treat transformer oil.

2. Materials and methods

2.1. Transformer oil

The waste transformer oil used in this study was obtained from the Paraffine Institute of Warsaw (Poland), and consisted of 70% of PCB congeners. The initial concentration of the six selected PCB congeners was: PCB28, 368.2 mg l^{-1}; PCB52, 691.0 mg l^{-1}; PCB101, 123.8 mg l^{-1}; PCB138, 92.7 mg l^{-1}; PCB153, 17.1 mg l^{-1}; and PCB180, 134.3 mg l^{-1}.

2.2. Microorganisms and culture conditions

Eight commercial mixtures of microorganisms (bacterial strains) specifically prepared to metabolize various organic contaminants present in soils and water (Table 1) were purchased from Bioarcus Company (Poland), and used as received. The ability of these mixtures to metabolize PCBs present in waste transformer oil was thus investigated. Commercial mixtures of microorganisms were used in the solid form as received. For each experiment, 1 g of each microbial mixture was added to each culture.

2.3. Experimental set-up

Twenty-four different conditions were investigated in triplicate in glass reactors. Each reactor held 1 g of microbial mixture as supplied in 1000 ml mineral solution augmented with mineral oil at 0.4% v/v.

2.3.1. Mineral media

Mineral medium in oxic conditions: NH_4Cl 1 mg l^{-1}, KH_2PO_4 1.15 mg l^{-1}, $Na_2HPO_4{\cdot}7H_2O$ 1.15 mg l^{-1}, $MgSO_4$ 0.5 mg l^{-1}, Na_2CO_3 0.02 mg l^{-1}. Mineral medium in anoxic conditions: $(NH_4)_2SO_4$ 1.0 mg l^{-1}, KH_2PO_4 0.2 mg l^{-1}, $Na_2HPO_4{\cdot}7H_2O$ 1.15 mg l^{-1}, $MgSO_4$ 0.02 mg l^{-1}, Na_2CO_3 0.02 mg l^{-1}, $FeSO_4{\cdot}7H_2O$ 0.01 mg l^{-1}. Anoxic mineral medium was supplemented with two different electron acceptors: sulphate (4.5 mg l^{-1}) and nitrate (5.0 mg l^{-1}) salts. Mineral medium in anoxic/oxic conditions: $(NH_4)_2SO_4$ 1.0 mg l^{-1}, KH_2PO_4 0.2 mg l^{-1}, $Na_2HPO_4{\cdot}7H_2O$ 1.15 mg l^{-1}, $MgSO_4$ 0.02 mg l^{-1}, Na_2CO_3 0.02 mg l^{-1}, $FeSO_4{\cdot}7H_2O$ 0.01 mg l^{-1}. In anoxic/oxic conditions mineral medium was supplemented with two different electron acceptors: sulphate (4.5 mg l^{-1}) and nitrate (5.0 mg l^{-1}) salts.

2.3.2. Incubation conditions

Oxic cultures were incubated at 30 °C on a rotary shaker set at 220 rpm for 21 days. For the anoxic conditions, oxygen present in solution was displaced by nitrogen prior to closing the flasks. These cultures were incubated in the reactors at 30 °C in the dark on a rotary shaker set to 220 rpm for three months.

The performance of the commercial mixtures of microorganisms was also investigated under combined anoxic/oxic conditions. Thus, the cultures were first incubated under anoxic conditions for three months as described above, and then the same cultures were incubated under oxic conditions for three weeks, as described.

Table 1
The commercially available biological mixtures used in the experiments

Series of biological mixtures[a]	Name of biological mixture	Types of the pollutants oriented for the biological mixture investigation
Sybron	Sybron 1000	Polyaromatic hydrocarbons (PAH), phenols
Biozyn	Biozyn 301	Aliphatic hydrocarbons, PAH, phenols, lubricants, grease
	Biozyn 300	Surfactants, fat, aliphatic hydrocarbons, PAH, wastes of wood and paper industry
DBC	DBC 5	Aliphatic hydrocarbons, PAH
Enviro-Zyme 214	ChZR	Organic compounds, industrial waste
NS 20	NS 20-10	Aliphatic hydrocarbons, fat, benzoic acid, alcohols
	NS 20-20	PAH, lignin, cresols, naphthalene, wastes of wood industry
	NS 20	Aliphatic hydrocarbons, PAH, biphenyl, mineral oil, chlorophenols

[a] Commercial mixtures of the microorganisms were purchased from Bioarcus Company, Poland.