The Role of Bacteria in the Breakdown of Carcinogenic Substances (PCBs) in Wastewater for Safe Recycling Purposes – A Review

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Abstract

Water is one of the most essential natural resources for daily human activities, yet it is so scarce. Treated wastewater and untreated sewage contain bacteria that can be advantageous to the recycling process. The composition of effluent originates from various industries, such as pharmaceutical industries, mining industries, agricultural sector, household waste, chemical industries, and various manufacturing industries, including the oil manufacturers. These introduce potential health-threatening compounds to wastewater. These compounds that remain for a long time without being broken down or changed in their chemical composition and/or structure in the environment are referred to as recalcitrant compounds. Polychlorinated Biphenyls (PCBs) are examples of such compounds. These compounds have serious negative health effects on humans (act as mutagens and carcinogens). The capacity of Polychlorinated Biphenyl’s (PCBs) degradation by bacteria (biodegradation) depends on the diversity and characteristics of naturally occurring populations and their response to environmental conditions. Conventional physical and chemical methods that are used to decontaminate wastewater contaminated by these substances are time and energy consuming. The use of Moringa oleifera seeds, which is currently under trial, is also very costly and not sustainable. This threatens the economic security of most developing countries. Biodegradation is the metabolic ability of microorganisms to transform or mineralize organic contaminants into less harmful, non-hazardous substances, which are then integrated into natural biochemical cycles. This process is not only cost effective, but it is also environmentally friendly. Wastewater that has gone through effective biodegradation can be fully recycled, thus conserving raw water and at the same time ensuring safe available water for all.

Key words: Recycling; wastewater; polychlorinated biphenyls (PCB); water conservation; safer recycled water; biodegradation; sustainability

Introduction

Sustainable development requires the promotion of environmental management and the constant search for new technologies to treat vast quantities of waste, which includes treatment of wastewater for purposes of recycling (Khadrhaoui and Belaid, 2012). The scarcity of water as a natural resource, resulting from the aggravating effects of global warming, has resulted in great global concern to recycle and conserve water, especially in sub-Saharan Africa where the problem of water scarcity has affected most countries (Ateba and Maribeng, 2011). South Africa is faced with freshwater scarcity that is exacerbated by its increasing demand, pollution, unsustainable use, and climate change (DWA, 2012).

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The presence of chemicals in the environment calls for quantification of such in order to come up with risk analyses posed by those chemicals (Guillen et al., 2012; Urbaniak, 2013). According to Guillen et al., (2012), substances such as pharmaceuticals, perfluorinated acids and perfluorosulfonates, PAHs, PCBs, pesticides, and surfactants are mostly found in wastewater. Ying et al. (2009) also noted that the presence of pharmaceutically active compounds in wastewater are a major concern. Several methods may be used to quantitatively determine these substances from wastewater, which are mainly from sewage treatment plants (Ying et al., 2009). According to Ying et al., (2009), although most research done regarding the removal of these substances was carried out, it was mainly on activated sludge and not done on wastewater. Some strains of organism Acinetobacter have the ability to degrade pollutants such as biphenyls from wastewater (Gumaa et al., 2010). Enterobacter cloacae secrete an emulsifier that increases the hydrophobicity of the bacterial cell surface and neutralises the surface charge of cells (Pacw-Plociniczak et al., 2011). This, as a result, increases the ability of the bacteria to degrade PCBs (Pacwa-Plociniczak et al., 2011; Fakruddin, 2012). Biosurfactants are also effective at the extremes of temperature, pH, and salinity (Kapadia and Yagnik, 2013; Fakruddin, 2012), a property that is essential in biodegradation of PCBs, as they are hydrophobic organic compounds (Pacwa-Plociniczak et al., 2011). This property makes these recalcitrant compounds removable through physic-chemical means or treatments, limited bioavailability to microorganisms, and limited availability to oxidative and reductive chemicals when applied in treatments (Pacwa-Plociniczak et al., 2011).

**Polychlorinated Biphenyls (PCBs)**

Polychlorinated biphenyls (PCBs) consist of two benzene rings with a carbon-to-carbon bond between carbon 1 on one ring and carbon 1 on the second ring (EPA, 2013). The PCBs have varying numbers of chlorines in their structure (Barbalace, 2003; Anyasi and Atagana, 2011; EPA, 2013) the diagram below.

![Structure of PCBs (Barbalace, 2003)](image)

Toxicity of PCBs is dependent upon the number of chlorines present on the biphenyl structure and their position, that is, the co-planar congeners (Barbalace, 2003; Anyasi and Atagana, 2011). The PCB congeners that have been seen to be highly toxic were those that had chlorine atoms attached to the 3, 4-ortho positions, followed by those with 5-10 chlorine atoms in the para and meta positions (Anyasi and Atagana, 2011).

In terms of structural relationship to toxicity, PCBs fall into two distinct categories, referred to as coplanar, or non-ortho-substituted "arene" substitution patterns, and noncoplanar, or ortho-substituted congeners (Jensen, 2006). The coplanar group members have a fairly rigid structure with the biphenyl rings in the same plane. This gives the molecule a structure similar to polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans and allows it to act in the same way as these molecules as an agonist of the aryl hydrocarbon receptor (AhR) in organisms (Arsalan et al., 2007). These types of PCBs are considered contributors to overall dioxin toxicity (Arsalan et al., 2007).

Noncoplanar PCBs, with chlorine atoms at the ortho positions, have not been found to activate the AhR and are not considered part of the dioxin group; however, studies have indicated some neurotoxic and immunotoxic effects, but at levels much higher than normally associated with dioxins, and thus, of much less concern to regulatory bodies (Rudel et al., 2008; Wethington and Hornbuckle, 2005).

PCBs are very stable compounds and do not decompose readily (Rudel et al., 2008). This is due to their chemical inability to oxidize and reduce in the natural environment. Furthermore, PCBs have a long half life (8 to 15 years) and are insoluble in water, which contributes to their stability and recalcitrant property (Rudel et al., 2008). The biodegradability (and solubility in water) of PCBs is also dependent upon the number of chlorine molecules it has (Anyasi and Atagana, 2011; EPA, 2013). The more chlorine molecules contained in a
compound renders that compound less biodegradable (EPA, 2013). PCBs are mostly hydrophobic and some are less hydrophilic (Nwinyi, 2011; Muir and Lohmann, 2013). These properties result in bioaccumulation of these compounds, as they do no dissolve in water and thus, render them difficult to be biodegraded (Schafer and Kegley, 2002; Barbalace, 2003; EPA, 2013; Muir and Lohmann, 2013).

**Elimination of PCBs**

Although the Stockholm Convention on Persistent Organic Pollutants (POPs) (of which PCBs are part of) signed in 2001 was aimed at eliminating and/or restricting the production and use of POPs (Lammel et al., 2009), more of these ubiquitous substances were still being introduced into the environment through various human activities (Teran et al., 2012). Water has become a widely used environmental matrix for monitoring POPs (Muir and Lohmann, 2013; van Leeuwen et al., 2013), although more studies on PCBs have been carried out on contaminated soils than water (Leigh et al., 2006).

The destruction of PCBs by chemical, thermal, and biochemical processes is extremely difficult and presents the risk of generating extremely toxic dibenzodioxins and dibenzofurans through partial oxidation (Leigh et al., 2006; Arsalan et al., 2007; US EPA, 2013).

**Generalized Structure of PCBs (US EPA, 2013)**

Effects of Polychlorinated Biphenyls (PCBs) on Human Health

PCB mixtures have been associated with cancer incidents in animals from a long time back (Pavuk et al., 2004; Ross, 2004; Lynch et al., 2012). PCBs were found to induce liver tumours, thyroid adenomas, intestinal metaplasia, and adenocarcinomas in rats and mice (Pavuk et al., 2004). Exposure to some environmental chemicals such as DDT and PCBs have been associated with a drop in sperm count, breast cancer, testicular cancer, and hypospadias, which are all associated with endocrine disruption caused by these chemicals (Rogan and Ragan, 2007). This comes as a result of some PCB congeners being able to occupy thyroid receptors, thus interrupting its action (Ross, 2004; Rogan and Ragan, 2007).

PCBs accumulate in the fats of organisms and get passed on from one organism to the other in food chains (Zhao et al., 2009; Man et al., 2011), thus causing bioaccumulation. The PCBs get entry into the human body and animals through the skin, lungs, and gastrointestinal tract (Anyasi and Atagana, 2011). This gets distributed to various parts of the body via blood and gets to accumulate in different tissues (Anyasi and Atagana, 2011; Man et al., 2011). The effects of PCBs on humans depend on age, sex, and part of the body affected by the chemicals (Anyasi and Atagana, 2011). The liver, as the major organ for removal of toxins in the body, is usually highly affected by PCBs (Pavuk et al., 2004; Anyasi and Atagana, 2011). Humans become exposed to PCBs through consumption of contaminated fish, meat, and dairy products (Lynch et al., 2012) and also through grains grown in PCB contaminated soils (Anyasi and Atagana, 2011; Lynch et al., 2012). PCBs have been isolated from human milk and serum (Linderholm et al., 2010; Man et al., 2011) and have been found to have effects on breastfed children leading to low IQ and endocrine related ailments (Linderholm et al., 2010; Man et al., 2011; Lynch et al., 2012). Some studies have shown an increase in cancer mortality in workers exposed to PCBs (Anyasi and Atagana, 2011).

**Biodegradation**

Biodegradation is the metabolic ability of microorganisms to transform or mineralize organic
contaminants into less harmful, non-hazardous substances, which are integrated into natural biochemical cycles (Leigh et al., 2006; Dhall et al., 2012). Specific bacteria, having bio-degradative potential for various chemical substances in wastewater and raw water, may be used to treat water (Dhall et al., 2012) for purposes of safely recycling. Bacteria, unlike other organisms, have the ability to interact better with man-made and naturally occurring compounds, which results in such compounds being changed structurally and eventually getting degraded (Anyasi and Atagana, 2011). This is, in a way, a better cleanup strategy that can be used in the cleanup of wastewater, as it is environmentally friendly (Anyasi and Atagana, 2011). Use of mixed population of microbes is usually recommended, as it has been seen to yield faster results as the two different microbes attack different parts through different mechanisms resulting in effective breakdown of a compound (Farhadian et al., 2007; Martinkova et al., 2009). This activity also creates a condition of co-metabolism (Martinkova et al., 2009).

Although PCBs are not readily biodegradable, some bacteria species, such as *Vibrio cholera*, *Acinetobacter Iwoffnii*, *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Rhodococcus* sp., *Bacillus* sp., and *Burkholderia* sp., have been found to have a role in the biodegradation of these compounds (Petric et al., 2007, Hamzah et al., 2010; Nwinyi, 2011, Anyasi and Atagana, 2011; Blokesch, 2012). This may be achieved through co-metabolism and mineralisation (Anyasi and Atagana, 2011; Blokesch, 2012) and the use of a metabolic pathway similar in all these bacteria, which comprises four steps catalysed by enzymes BphA, BphB, BphC, and BphD (Petric et al., 2007). The pathway, according to Petric et al., 2007, is initiated by insertion of two oxygen atoms at the carbon positions 2, 3 of one aromatic ring. This is then followed by dehydrogenation meta-cleavage and hydrolysis which results in the formation of a 5-carbon compound, thus a biphenyl catabolic pathway (Petric et al., 2007). Due to the hydrophobic moiety of PCBs, the presence of surfactants has been found to be essential in the biodegradation (Das and Chandran, 2011).

Surfactants produced by bacteria are essential in bioremediation (Salihu et al., 2009; Das & Chandran, 2011). They play an important role in the metabolism of these organisms (Das & Chandran, 2011). The surfactants are amphiphilic compounds (Gumaa et al., 2010). The hydrophilic moiety can be a carbohydrate, amino acid, phosphate group, or some other compound (Gumaa et al., 2010). The fact that these chemical substances have characteristics of biocompatibility, specificity, and digestibility (Kapadia and Yagnik, 2013) makes them advantageous for use in biodegradation (Fakruddin, 2012; Martins et al., 2012).

**Biosurfactants**

Biosurfactants have a wide range of structures, and some examples and their sources are given in Figure 1.

Another property of surfactants that has been found to be important in biodegradation is their ability to permit microorganisms to grow on water immiscible substrates by reducing surface tension at the phase boundary (Fakruddin, 2012). This makes the substrate more readily available for uptake and metabolism (Fakruddin, 2012; Pacwa-Plociniczak et al., 2011; Kapadia and Yagnik, 2013). This characteristic of bioavailability and increase in cell surface interaction is important in biodegradation (Pacwa-Plociniczak et al., 2011; Kapadia and Yagnik, 2013), as it enhances hydrocarbon mobilization, solubilisation, or emulsification. This can be summarised as shown in Figure 2.

Biosurfactants are also effective at the extremes of temperature, pH, and salinity (Kapadia and Yagnik, 2013; Fakruddin, 2012), a property that is essential in biodegradation of PAHs and PCBs, as they are hydrophobic organic compounds (Pacwa-Plociniczak et al., 2011).

**Mechanisms Involved in Biodegradation of Xenobiotic Compounds**

Xenobiotic compound, owing to its recalcitrant nature, is hard to break down and degrade (Heider et al., 2008). The complexity of its chemical composition adds to this recalcitrant property (Pacwa-Plociniczak, 2011).
For breaking down such compounds, the enzymes act on certain groups present in the compound (Heider, 2008). For example, in the halocarbons, the halogen group is targeted, with enzymes such as oxygenases playing a major role (Pacwa-Plociniczak, 2011).

The bonds like ester-, amide-, or ether bonds present in the compounds are first attacked, leading to breaking down of compounds (Abor-Amer, 2011; Cycon et al., 2013). In some cases, the aliphatic chains, and in aromatic compounds the aromatic components, may be targeted (Abor-Amer, 2011). The site and mode of attack depends on the action of the enzyme, its concentration, and the favourable conditions (Abor-Amer, 2011). Often it is seen that the xenobiotics do not act as a source of energy to microbes, and as a result, they are not degraded (Abor-Amer, 2011). The presence of a suitable substrate induces its breakdown (Cycon et al., 2013). These substrates are known as co-metabolites, and the process of degradation is known as co-metabolism (Cycon et al., 2013). Gratuitous metabolism is another process in which the xenobiotics serve as substrates and are acted upon to release energy (Pacwa-Plociniczak et al., 2011).

This cannot be achieved through the use of *Moringa oleifera* and related species in treating wastewater to remove PCBs. The removal of these compounds has not been fully studied with the use of the plant protein, although the protein has been found to be slightly efficient with the reduction of fecal coliforms and other bacteria (Dalen et al., 2009; Kawo and Daneji, 2011). The use of *Moringa oleifera* seed powder in water treatment has been found to target mainly microorganisms, thus reducing turbidity (Mumuni et al., 2013). Although this mode of water treatment has been used, especially in rural areas of the developing countries, the synthetic polymers, aluminium sulphate, ferric chloride and poly aluminium chlorides used together with this powder have been reported to be unsafe (Danel et al., 2009; Mumuni et al., 2013; Lea, 2014). The action of *Moringa oleifera* seed powder has been reported to be based
on the ability of the protein contained in the seeds to be able to form coagulants that reduce water turbidity by acting on coliforms (Mangale et al., 2012). The bacteria found to be mainly involved in biodegradation of POPs and PCBs has been found mostly not to be coliforms (Anyasi and Atagana, 2011; Blokesch, 2012). The remaining bacteria, after treating water with Moringa seed powder, that is the $10^4 – 10^5$ of bacteria left (Mangale et al., 2012), may be those that are capable of biodegradation (although no report to that effect has been found). Tree growth and productivity relies on environmental conditions and thus, production and maintaining the Moringa tree species may not be sustainable given the global warming related environmental problems. The indoor growth of plants compared to bacteria (microorganisms in general) is quite costly, although Lea (2014) argues that propagation is affordable.

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