

*Full Length Research Paper*

# **Aerobic biodegradation of a mixture of chlorinated organics in contaminated water**

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**The environmental persistence, toxicity and/or carcinogenicity of chlorinated aliphatic compounds (CAHs) and their potential for bioaccumulation in food chains has made them of serious environmental concern. The frequency of a mixture of these compounds encountered in most contaminated sites has warranted investigation into their fate in contaminated sites. In this study, therefore, the biodegradation of a mixture of CAHs; namely, carbon tetrachloride (CCl<sub>4</sub>), 1,2-dichloroethane (DCA) and dichloromethane (DCM), in contaminated water microcosms was investigated. The mixture of CAHs investigated was observed to be simultaneously degraded in both microcosms with up to 86.28% CCl<sub>4</sub>, 44.64% DCM and 52.34% DCA degradation observed in the untreated microcosms. The degradation rate constants of the CAHs ranged variously between 0.168 – 1.234 week<sup>-1</sup> for CCl<sub>4</sub>; 0.175 – 0.832 week<sup>-1</sup> for DCM; and 0.232 – 0.588 week<sup>-1</sup> for DCA in both water microcosms with higher degradation generally observed in New Germany Wastewater compared to those in Northern Wastewater. Findings from this study also suggest that biostimulation and/or bioaugmentation is required to speed up the biodegradation process, depending on the available nutrients and the presence or absence of microbial population capable of CAHs' metabolism at the contaminated sites.**

**Key words:** Bioaugmentation, biodegradation, biostimulation, chlorinated aliphatic hydrocarbons, microcosms.

## **INTRODUCTION**

Chlorinated aliphatic hydrocarbons (CAHs) are an extremely diverse group of organic chemicals. They are widely used industrially and agriculturally as degreasing agents, solvents, detergents, paint removers and chemical intermediates in the production of a whole range of products (Leisinger et al., 1994). Their environmental persistence, toxicity and/or carcinogenicity and potential for bioaccumulation in food chains are of serious environmental concern. Also, the possible existence of CAHs as Dense Non-Aqueous Phase Liquids (DNAPLs) makes them more difficult to clean up at contaminated sites compared to other hydrocarbons. These chemical properties of CAHs along with their toxicity present the potential for adverse health effects and ecosystem perturbations upon exposure (Chang and Alvarez-Cohen, 1996). They

are known to cause considerable environmental pollution and human health problems as a result of their persistence and toxicity (Mohn and Tiedje, 1992; Squillace et al., 1999). The hazards presented by these compounds in particular have prompted investigation into their fate in subsurface waters and in the soil environment (Lee et al., 1998; Poelarends et al., 1998; van den Wijngaard et al., 1993). There is therefore a continued effort to find appropriate remediating approach for the removal of CAHs in various contaminated sites.

The use of CAHs as carbon and energy sources by microorganisms is common, and has been extensively documented (Grosser et al., 1991; Lee et al., 1998; Bradley and Chapelle, 2000). Microorganisms have been shown to evolve an extensive range of enzymes and pathways that enable them to degrade a wide array of these compounds (Klier et al., 1999; Krooneman et al., 2000). However, most of the previous studies have focused on the microbial degradation of CAHs individually, whereas, a mixture of these compounds are often

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frequently encountered in most contaminated sites, thus increasing their level of recalcitrance. The objective of this study therefore, was to evaluate the biodegradation of a mixture of CAHs; namely, carbon tetrachloride (CCl<sub>4</sub>), 1,2-dichloroethane (DCA) and dichloromethane (DCM), in contaminated water microcosms. Effect of biostimulation and bioaugmentation on the degradation process was also investigated. This is expected to provide a predictive model for an efficient way of removing this group of compounds from sites contaminated with a mixture of CAHs.

## MATERIALS AND METHODS

### Sample collection and microcosms set-up

Wastewater samples used in this study were collected from the Northern and New Germany Wastewater Treatment Plants and stored at 4°C, prior to use. Microcosms were constructed with 150 ml of the water samples in 250 ml serum bottles as previously described (Olaniran et al., 2006). The microcosms were fortified with a mixture of CAHs, each to a final concentration of 0.5 mM. For the biostimulation experiment, glucose, yeast extract and fructose were added separately to each reaction mixture at a final concentration of 1% (w/v). The bacterial consortia used in the bioaugmentation experiment were developed from pure strains of bacteria previously isolated from contaminated sites and the standardized culture (OD of 1 at λ<sub>600</sub>) of the consortia was added separately to each reaction mixture at 1% (v/v) concentration. Abiotic (no cells plus DCA) was used as negative control.

### Degradation kinetic studies

The amount of the different CAHs degraded was determined at the different sampling times from the headspace sample analysis in a gas chromatograph (Varian model 3700). Samples were analyzed by injecting 500 μl headspace samples directly into the gas chromatograph, using a gas-tight syringe (Hamilton) with the injector and detector at 200°C and the column at 100°C as described elsewhere (Coleman et al., 2002). The concentration of the different CAHs in the microcosms was determined by comparison to a standard curve derived from known quantities of the compounds in serum bottles with the same gas and liquid volumes as the experimental bottles as previously described (Olaniran et al., 2006). The degradation rate constant in each microcosm enrichment was estimated in accordance with the method of LaGrega et al. (1994).

### Wastewater analysis

The wastewater samples were analysed for pH, calcium, magnesium, sodium, iron, nitrate, nitrite, phosphate, sulphate, soluble organic carbon and total Kjeldahl nitrogen using standard methods (Black et al., 1965).

### Identification of the bacterial isolates

The bacterial isolates used in the bioaugmentation experiment were previously isolated from contaminated sites and identified based on the amplification, sequencing and analysis of their 16S rRNA genes as described elsewhere (Olaniran et al., 2007). The 16S rRNA gene sequences of the bacterial isolates were compared to those in the GenBank database (<http://www.ncbi.nlm.nih.gov>) by using BLAST

**Table 1.** Physico-chemical properties of the wastewater samples used in this study.

| Major cations/anions (mg/L) | NGWW <sup>a</sup> | NWW <sup>b</sup> |
|-----------------------------|-------------------|------------------|
| Calcium                     | 20.8              | 20.9             |
| Magnesium                   | 5.59              | 7.18             |
| Sodium                      | 101               | 76.8             |
| Potassium                   | 18.5              | 15.9             |
| Iron                        | 0.03              | 0.03             |
| Nitrate (soluble)           | 7.70              | 3.95             |
| Nitrite (soluble)           | <0.05             | 3.29             |
| Sulphate (soluble)          | 40.6              | 30.0             |
| Soluble Organic Carbon      | 16.3              | 23.2             |
| Phosphate                   | 2660              | 2230             |
| Total Kjeldahl Nitrogen     | <3.0              | 5.25             |

<sup>a</sup>New Germany Wastewater; <sup>b</sup>Northern Wastewater.

search tool (Altschul et al., 1997) to determine the most similar sequences.

## RESULTS AND DISCUSSION

This study was undertaken to investigate the biodegradation of a mixture of CAHs by indigenous microorganisms present in contaminated water as well as to determine the effects of biostimulation and bioaugmentation on the biodegradation process. The mixture of CAHs investigated in this study was observed to be simultaneously degraded in both microcosms constructed with the New Germany Wastewater (NGWW) and Northern Wastewater (NWW), with up to 86.28% CCl<sub>4</sub>, 44.64% DCM and 52.34% DCA degradation observed in the untreated microcosms. The degradation rate constants of the CAHs ranged variously between 0.168 – 1.234 week<sup>-1</sup> for CCl<sub>4</sub>; 0.175 – 0.832 week<sup>-1</sup> for DCM; and 0.232 – 0.588 week<sup>-1</sup> for DCA in both water microcosms (Table 2) with higher degradation generally observed in NGWW compared to those in NWW. The presence and bioavailability of nutrients which are required for growth of microorganisms has been shown to increase microbial activities and subsequently increasing biodehalogenation (Mohn and Tiedje, 1992). Hence, the higher concentrations of nitrate, phosphate, sulphate, sodium and potassium in NGWW than in NWW could account for the higher degradation of CAHs observed in microcosms constructed with NGWW (Table 1).

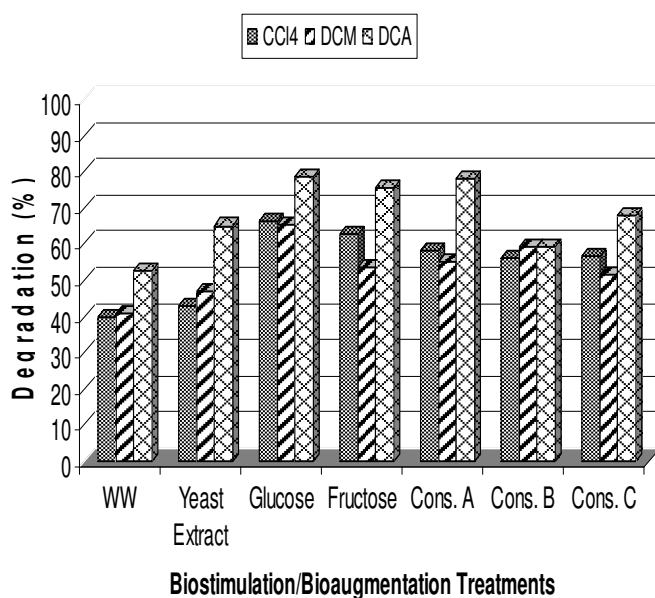
Biostimulation was found to enhance the degradation of the mixture of CAHs, resulting in 3.28 – 26.43% increase for CCl<sub>4</sub>; 6.1 – 24.68% increase for DCM and 12.72 – 26.36% increase for DCA degradation in the microcosms constructed with the NWW (Figure 1). Similarly, up to 11.25% increase for CCl<sub>4</sub>; 41.68% increase for DCM and 32.67% increase for DCA degradation was achieved after three weeks in the microcosms constructed with NGWW (Figure 2). Of the nutrients added, glucose seemed to

**Table 2.** Degradation rate constants of CAHs in water microcosms under various biostimulation and bioaugmentation treatments ( $Wk^{-1}$ ).

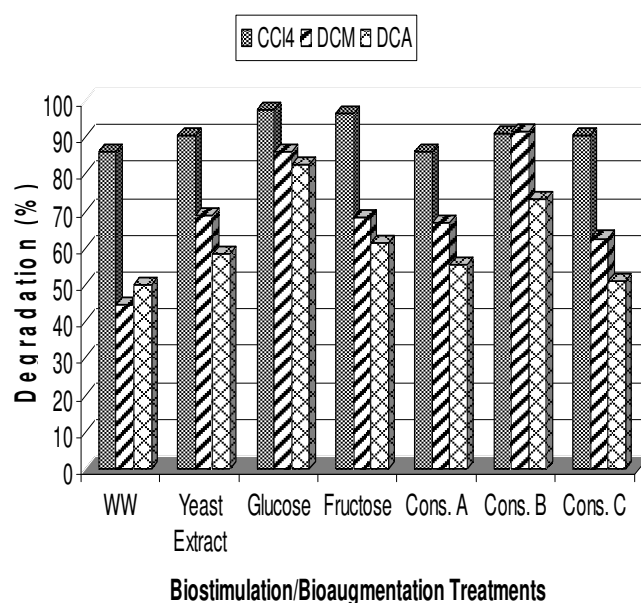
| Treatment     | CCl <sub>4</sub> |                   | DCM   |       | DCA   |       |
|---------------|------------------|-------------------|-------|-------|-------|-------|
|               | NWW <sup>a</sup> | NGWW <sup>b</sup> | NWW   | NGWW  | NWW   | NGWW  |
| NONE          | 0.168            | 0.662             | 0.175 | 0.197 | 0.247 | 0.232 |
| Yeast Extract | 0.187            | 0.787             | 0.211 | 0.389 | 0.351 | 0.292 |
| Glucose       | 0.361            | 1.234             | 0.354 | 0.663 | 0.515 | 0.588 |
| Fructose      | 0.331            | 1.144             | 0.255 | 0.385 | 0.468 | 0.317 |
| Cons. A       | 0.292            | 0.662             | 0.266 | 0.369 | 0.505 | 0.270 |
| Cons. B       | 0.276            | 0.805             | 0.299 | 0.832 | 0.297 | 0.438 |
| Cons. C       | 0.280            | 0.789             | 0.240 | 0.328 | 0.376 | 0.238 |

<sup>a</sup>Northern Wastewater; <sup>b</sup>New Germany Wastewater.

Cons. A is a mixture of isolates 006, 007, 413, AO1, and New 6; Cons. B is a mixture of isolates 004, 007, 413, AO1 and New 6; and Cons. C is a mixture of isolates 004, 006, 413, AO1 and New 6.



**Figure 1.** Biodegradation profiles of the different CAHs present in the mixture in Northern Wastewater under various treatment conditions. Cons. A is a mixture of isolates 006, 007, 413, AO1, and New 6; Cons. B is a mixture of isolates 004, 007, 413, AO1 and New 6; and Cons. C is a mixture of isolates 004, 006, 413, AO1 and New 6.



**Figure 2.** Biodegradation profiles of the different CAHs present in the mixture in New Germany Wastewater under various treatment conditions. Cons. A is a mixture of isolates 006, 007, 413, AO1, and New 6; Cons. B is a mixture of isolates 004, 007, 413, AO1 and New 6; and Cons. C is a mixture of isolates 004, 006, 413, AO1 and New 6.

have the most effect in enhancing the degradation of CAHs, followed by fructose in both microcosms. This is very significant as these substrates are water soluble, easily metabolizable and non-toxic (Gao et al., 1997). It is therefore believed that these substrates could serve as safer and more acceptable alternatives for the co-metabolic degradation of CAHs in contaminated aquatic systems.

There is increasing evidence pointing to the use of microorganisms from the same ecological niche to overcome the ecological barriers that mitigate against the

success of bioaugmentation options in bioremediation processes (Fantroussi and Agathos, 2005). Addition of a bacterial consortium, representing a significant component of the community in a bioreactor treating wastewater was reported to reduce the Chemical Oxygen Demand of the total pollution load by approximately 85% (Van der Gast et al., 2004). This suggests that bioaugmentation with selected strains can lead to more effective treatment than the application of uncharacterized communities. Also, microbial consortium containing *Dehalococcoides* have been previously applied for effective remediation of

chloroethene contaminants (Adamson et al., 2003; Major et al., 2002). The bacteria used in the bioaugmentation experiments in this study were previously isolated from contaminated sites and characterized for CAHs' degradation. Analysis of their 16S rRNA genes identified them to belong to *Bacillus* spp. with the exception of isolate New 6 whose sequence was found to be identical to *Alcaligenaceae* bacterium Lm-2-1 (Accession Number EF025348). Addition of the different bacterial consortium developed from pure strains of these known CAHs-degrading bacteria was found to have a positive impact on the degradation process, resulting in 18.63, 47.11 and 22.9% increased degradation of  $\text{CCl}_4$  (Figure 1), DCM (Figure 2) and DCA (Figure 2), respectively. This is not surprising though, since addition of indigenous degrader population that was exposed to the contaminant has been suggested to be very effective in bioaugmentation (Gentry et al., 2004). Thus, the strategy of using tailor-made consortia seems to be a promising bioaugmentation approach for effective bioremediation applications.

In conclusion, this study has clearly demonstrated that simultaneous aerobic degradation of a mixture of CAHs by microorganisms indigenous to contaminated wastewater is possible. However, the degradation of the individual compound in the mixture varies and is dependent on the microbial group present, amount of nutrients available for microbial growth and activities, and the number and positioning of chloro-substituents attached to the carbon moieties. Findings from this study also suggest that biostimulation and/or bioaugmentation is required to speed up the degradation process, depending on the available nutrients and the presence or absence of microbial population capable of CAHs' metabolism at the contaminated site. Identification of the microbial phylotypes playing active role in the degradation of this mixture of CAHs and optimization of the conditions for more enhanced degradation of the compounds is worth investigating.

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