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ScienceDirect
Chemosphere 71 (2008) 1446–1451
CHEMOSPHERE
www.elsevier.com/locate/chemosphere

The primary aerobic biodegradation of biodiesel B20

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Received 13 September 2007; received in revised form 4 December 2007; accepted 12 December 2007
Available online 11 February 2008

Abstract

We describe the primary aerobic biodegradation of a B20 fuel (20% soybean fatty acid methyl esters, 80% petroleum diesel) by unacclimated inocula from a rainwater detention pond. Biodegradation was rapid and essentially complete, with an overall median 'half-life', at ~100 ppm B20, of 6.8 days ($n = 34$). Using purge-and-trap and extraction methodologies, both coupled to GC/MS, and hexachloroethane and hexachlorobenzene as conserved internal markers in the B20, we followed the biodegradation of total detectable material, 76 individual analytes and eight undifferentiated groups of isomers, and calculated their half-lives under these conditions. The fatty acid methyl esters, *n*-alkanes and *iso*-alkanes, and simple and alkylated aromatic compounds were the most readily degraded compounds, followed by the naphthenes. The last (identified) compounds to be degraded were ethylalkanes, trisubstituted cyclohexanes and decalins, but even these disappeared with an apparent 'half-life' of <30 days.

Keywords: Biofuel; Environmental fate; Conserved internal markers

1. Introduction

Biodiesel, fatty acid methyl esters derived from triglycerides (Ma and Hanna, 1999), is viewed as a potential adjunct to petroleum based diesel, and both the US and the EU have plans for substantially increasing its use in the next few years. While biodiesel can be used alone, it is usually blended with petroleum diesel to produce an amended fuel. Current blends typically range from 2% to 20% biodiesel, and are known by the percentage of biodiesel with a B-prefix. Hence a product with 20% fatty acid methyl esters is known as B20.

Biodiesel and mixtures with petroleum diesel are widely believed to be environmentally preferable to the neat petroleum product, with rapid biodegradation in soil (Lapinskiene et al., 2006) and a somewhat lower acute toxicity to aquatic organisms (Khan et al., 2007). Indeed biodiesel has been suggested as an environmentally benign addition to help remediate coal tar polycyclic aromatic hydrocarbons in soils (Taylor and Jones, 2001), and crude oil (Pereira and Mudge, 2004) and heavy fuel oil (Fernandez-Alvarez et al., 2007) on beaches. Nevertheless there has been little examination of the biodegradation of biodiesel mixtures at the molecular level. DeMello et al. (2007) studied the biodegradation of biodiesel mixtures in the marine environment using one- and two-dimensional gas chromatography/flame ionization detection (GC/FID), and showed that the fatty acid methyl esters were degraded at about the same rate as the *n*-alkanes, and more rapidly than the other diesel components. Their elegant experiments also allowed evaporation to remove the more volatile components of the biodiesel mixtures.

Here we report the biodegradation of a biodiesel (B20) mixture by unacclimated freshwater microorganisms. The experiments used a concentration of 100 ppm (volume) of B20, which is in the range typically used in statutory biodegradation tests, such as the '301 tests' mandated by the Organization for Economic Cooperation and Development (1993). A major concern in experimental biodegradation

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doi:10.1016/j.chemosphere.2007.12.010

studies with hydrocarbons is the heterogeneities inherent in adding immiscible hydrocarbons to water; the B20 had to be added independently to each microcosm. Quantifying biodegradation is then confounded by subtly different amounts of hydrocarbon in different replicates. Furthermore, the volatility of diesel complicates experiments because the most volatile components are difficult to analyse by extraction with solvent because they tend to be lost, while the least volatile components cannot be analysed by purge-and-trap technologies. We therefore ran replicate experiments under two conditions. One series used sealed vials and analysis by purge-and-trap gas chromatography/mass spectrometry (GC/MS), the other used loosely stoppered flasks and extraction with methylene chloride followed by GC/MS. We added two halogenated compounds, hexachloroethane and hexachlorobenzene, to the B20 to serve as conserved internal markers (Mrsny et al., 1978; Prince and Douglas, 2005) to compensate for any heterogeneity in additions to our replicate microcosms. Biodegradation of both the fatty acid methyl esters and the petroleum diesel was essentially complete and relatively rapid (overall half-life 6.8 days). The biodegradation of 76 individual analytes and eight undifferentiated groups of isomers was followed, and their half-lives under these conditions calculated.

2. Materials and methods

Neat soybean biodiesel was a generous gift from J. Pierson of Pierson Oil, East Orange, NJ 07107 and M. Francaviglia of Sprague Energy Corp., White Plains, NY 10604. B20 was made in the laboratory by combining an aliquot with four volumes of a commercial diesel fuel. Hexachloroethane and hexachlorobenzene were added at approximately 1%. Water used as inoculum was collected from a New Jersey rainwater retention pond (~4000 m^2 surface area, up to 3 m deep) in November, and amended with 1% Bushnell Haas medium (Bushnell and Haas, 1941) to provide ~100 μM biologically available nitrogen and phosphorus. There were no detectable hydrocarbons in the water (detection limit ~2 ppb in 10 ml water). For the sealed vial microcosms, designed to prevent evaporation of the lightest hydrocarbons yet still provide enough oxygen for complete mineralization of the product, 10 ml of the amended pondwater were added to a 40 ml vial that already contained ~1 μl of B20. The vials were sealed with a Teflon membrane, and incubated with gentle swirling (100 rpm) or almost horizontal rotation (10 rpm) at laboratory room temperature (~21 °C). At designated times after their assembly, each vial was analysed by purge-and-trap gas chromatography coupled with mass spectrometry, as described previously (Prince et al., 2007). For the less volatile components, which could not be analysed by purge-and-trap methodologies, we added 100 ml of the amended pondwater to 250 ml Erlenmeyer flasks, which already contained ~10 μl of B20. The flasks were loosely capped, and incubated with gentle swirling (100 rpm). At designated times after their assembly, each flask was extracted three times with 2 ml methylene chloride, the extract dried with anhydrous sodium sulfate, and the volume reduced by evaporation until the predicted concentration of the B20, assuming no biodegradation, was ~10 μl/ml methylene chloride. This extract was analysed by GC/MS as previously described (Douglas et al., 1992). Both mass spectrometers were calibrated with perfluorotributylamine followed USEPA method 8270C. More than 80 compounds or groups of compounds were identified from their diagnostic ions and elution patterns (Prince et al., 2007; Douglas et al., 1992).

The percent depletion of total detectable material and individual B20 components was calculated using the equation:

$$\%\text{Loss} = [((A_0/C_0) - (A_s/C_s))/(A_0/C_0)] \times 100$$

where A_s and C_s are the concentrations of the target analyte and conserved compound in the sample, respectively, and A_0 and C_0 are the concentrations in the initial material. Hexachloroethane was used in the purge-and-trap analyses ($m/z = 117$, elution at 130.6 min), hexachlorobenzene in the extraction experiments ($m/z = 284$ at 29.0 min). Both halogenated compounds are known to be degraded by Cytochrome P450 isoforms under aerobic conditions (Takazawa and Strobel, 1986; Walsh et al., 2000), but this process yields characteristic products, tetrachloroethene and pentachlorobenzene, respectively, and these were not detected in our experiments. If degradation of the conserved compounds had occurred, our estimates of the biodegradation of the biodiesel components would be underestimates.

Current regulatory requirements in Canada (Environment Canada, 2000) and Europe (European Commission, 1996) focus on the half-life of chemical substances in the environment. We have calculated apparent half-lives, τ, for the disappearance of the total detectable B20 and the individual components at time t from the equation:

$$\tau = \ln 2 \cdot (-t/\ln(A_s/A_0)/(C_s/C_0)$$

as discussed in Prince et al. (2007).

3. Results

Fig. 1 shows a total ion chromatogram of the B20, with representative components identified. The majority of the prominent peaks are the petroleum *n*-alkanes, but the biodiesel components are readily identified, as are the two halogenated standards. Fig. 2 shows representative chromatograms from the experiment in Erlenmeyer flasks. Significant biodegradation occurred within two days, and in accord with the finding of DeMello et al. (2007), the fatty acid methyl esters (FAME in the figure) were degraded at approximately the same rate as the *n*-alkanes. By seven days the biodegradation in one of the three replicates sacrificed at this time was essentially complete, and only traces of hydrocarbon (1–2% of initial amount) were detectable in any of the replicates sacrificed at 31 days.