Chemosphere 161 (2016) 382–389

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Anaerobic biodegradation of soybean biodiesel and diesel blends under sulfate-reducing conditions

Shuyun Wu [a], Mohamad H. Yassine [b], Makram T. Suidan [c, *], Albert D. Venosa [d]

[a] Department of Biomedical, Chemical, and Environmental Engineering, University of Cincinnati, Cincinnati, OH 45220, United States
[b] Department of Mathematics and Natural Sciences, College of Arts and Sciences, Gulf University for Science and Technology, Hawally 32093, Kuwait
[c] Faculty of Engineering and Architecture, American University of Beirut, Beirut, Lebanon
[d] U.S Environmental Protection Agency (retired), National Risk Management Research Laboratory, 26 W. Martin Luther King Drive, Cincinnati, OH 45268, United States

HIGHLIGHTS

- Sulfate-reducing biodegradation of biodiesel and diesel blends were characterized.
- Saturated FAME: degradation rate decreased with an increasing carbon chain length.
- Unsaturated FAME: degradation rate increased with an increasing double bonds.
- Degradation rate was affected by the bioavailability of FAMEs in aquatic system.
- Degradation rate was affected by the inhibition of long-chain fatty acids.

ARTICLE INFO

Article history:
Received 2 January 2016
Received in revised form
13 June 2016
Accepted 21 June 2016
Available online 20 July 2016

Handling Editor: Y. Liu

Keywords:
Biodiesel
FAMEs
Alkane
Anaerobic biodegradation
Sulfate-reduction

ABSTRACT

Biotransformation of soybean biodiesel and its biodiesel/petrodiesel blends were investigated under sulfate-reducing conditions. Three blends of biodiesel, B100, B50, and B0, were treated using microbial cultures pre-acclimated to B100 (biodiesel only) and B80 (80% biodiesel and 20% petrodiesel). Results indicate that the biodiesel could be effectively biodegraded in the presence or absence of petrodiesel, whereas petrodiesel could not be biodegraded at all under sulfate-reducing conditions. The kinetics of biodegradation of individual Fatty Acid Methyl Ester (FAME) compounds and their accompanying sulfate-reduction rates were studied using a serum bottle test. As for the biodegradation of individual FAME compounds, the biodegradation rates for the saturated FAMEs decreased with increasing carbon chain length. For unsaturated FAMEs, biodegradation rates increased with increasing number of double bonds. The presence of petrodiesel had a greater effect on the rate of biodegradation of biodiesel than on the extent of removal.

1. Introduction

Biodiesel is a mixture of monoalkyl esters of long-chain fatty acids derived from transesterification of vegetable oils or animal lipids. It has been attracting great interest as an alternative fuel because of its potential as a renewable, easily biodegradable energy source that can contribute to decreased greenhouse gas (GHG) emissions. Since the passage of the Energy Policy Act of 2005, the annual production of biodiesel increased more than 18-fold by 2013. The Energy Independence and Security Act of 2007 increased the volume of renewable fuel required to be blended into transportation fuel from 9 billion gallons in 2008 to 36 billion gallons by 2022. With the increasing usage of biodiesel, a thorough understanding of its fate in the environment is required since biofuels pose similar environmental risks as fossil fuels when released to the environment. However, the susceptibility of these fuel blends to biological degradation is insufficiently understood and the reported studies about the biodegradation of biodiesel blends have principally focused on aerobic processes (DeMello et al., 2007; Owsianiak et al., 2009; Yassine et al., 2013), whereas the anaerobic conditions

* Corresponding author.
E-mail address: msuidan@aub.edu.lb (M.T. Suidan).

http://dx.doi.org/10.1016/j.chemosphere.2016.06.078
0045-6535/

prevail in subsurface environments where unintended fuel releases frequently occur. Biodiesel is often used in the form of fuel blends with petrodiesel and the physicochemical properties of their mixtures are more indeterminate and differ from those of the neat fuels when they are blended together. Therefore, understanding the biodegradation patterns of biodiesel and petrodiesel blends under anaerobic conditions is very important. Recent attempts to investigate the biodegradation of biodiesel and petrodiesel blends under anaerobic conditions have been very modest (Aktas et al., 2010; Sørensen et al., 2011; Wu et al., 2015). Aktas et al. (2010) evaluated anaerobic biodegradation of biodiesel by five anaerobic inocula and found that biodiesel could be easily hydrolyzed and converted to a variety of fatty acid intermediates within one month under sulfate reduction and methanogenic conditions. Sørensen et al., 2011 reported that the activity of all three groups of anaerobes (methanogens, sulfate- and nitrate-reducers) was stimulated by the presence of biodiesel. Wu et al. (2015) investigated the biodegradation of biodiesel and petrodiesel blends under methanogenic conditions and observed that when biodiesel was blended with low concentrations of petrodiesel, the petrodiesel did not impact the biodegradation of biodiesel much. Significant effects on the biodegradation rate and extent of transformation started appearing when petrodiesel and biodiesel were present in equal volumetric proportions (B50). There are no reported studies of the anaerobic biodegradation of biodiesel under iron reducing conditions.

Petrodiesel is composed mostly of saturated and aromatic petroleum hydrocarbons. It exhibits low biochemical reactivity and for many decades was thought to undergo biodegradation only in the presence of molecular oxygen. Biodegradation of petrodiesel under strictly anaerobic conditions has been frequently reported in the past decade (Rueter et al., 1994; Widdel and Rabus, 2001; Wentzel et al., 2007). Boopathy (2004) evaluated the biodegradation of petrodiesel under various anaerobic conditions in soil columns and found that the highest petrodiesel biodegradation rate was under mixed electron acceptor conditions followed by sulfate-reducing, nitrate-reducing, and finally methanogenic conditions. However, even under optimum conditions, anaerobic biodegradation was reported to require in excess of 310 days to achieve 81% transformation of petrodiesel fuel. Mukherji et al. (2004) reported that the maximum degradation of petrodiesel by a culture isolated from deep-sea sediment was only 18% over 50 days under anoxic nitrate-reducing conditions. It appears that the biodegradation of petrodiesel under anaerobic conditions mostly occurs under sulfate-reducing conditions either after long exposure times or when present in low environmental concentrations. Some studies have shown that adding biodiesel to the fuel blends could promote and enhance the biodegradation of petrodiesel under aerobic conditions (Miller and Mudge., 1997; Zhang et al., 1998; Pasqualino et al., 2006; DeMello et al., 2007 and Yassine et al., 2013). Miller and Mudge (1997) suggested that this enhancement was achieved through co-solubilization while Zhang et al. (1998) and Pasqualino et al. (2006) claimed it was achieved by co-metabolism. Whether biodiesel blending would promote the biodegradation of petrodiesel under anaerobic conditions is still unknown, and the biodegradation of biodiesel/petrodiesel blends under anaerobic conditions has been rarely investigated to date. The objective of this study was to investigate anaerobic biodegradation of soybean biodiesel and petrodiesel blends in a sulfate-reducing environment, which is a prevalent condition in anaerobic sediments. Serum Bottle Reactor (SBR) tests of pure unblended biodiesel (B100), pure unblended petrodiesel (B0), and B50 were conducted to determine the anaerobic biodegradation kinetics of individual FAME compounds in biodiesel and the associated sulfate utilization rate.

2. Materials and methods

2.1. Chemicals

Unblended soybean biodiesel (B100) was purchased from Peter Cramer North America (Cincinnati, OH) with FAMEs mole fractions of 0.145 C16:0-methyl ester (ME), 0.055 C18:0-ME, 0.206 C18:1-ME, 0.518 C18:2-ME, and 0.0759 C18:3-ME. Low-sulfur petrodiesel (B0) was purchased from a local BP petrodiesel station (Cincinnati, OH) with a mole fraction of 0.165 nC10-nC23 n-alkanes. B50 fuel blend was blended in our laboratory by volumetric splash mixing. Palmitic acid methyl ester (99%), palmitoleic acid methyl ester (99%), stearic acid methyl ester (99%), oleic acid methyl ester (99%), linoleic acid methyl ester (99%), linolenic acid methyl ester (99%), and n-alkanes standard mixture (nC10-nC30), were all purchased from Sigma Aldrich (USA).

2.2. Culture acclimation

Two 12-L laboratory-scale continuous flow stirred-tank reactors (CSTR) with a solids retention time of 40 days were used to enrich for bacterial cultures capable of biodegrading biodiesel under sulfate reducing conditions at room temperature 22 °C. The cultures were obtained from an anaerobic digester at a local wastewater treatment plant in Cincinnati, OH. A schematic of the bioreactor is provided in Fig. S1 in the supplementary material section. 1.4 g/L organic feed of B100 (biodiesel only) and B80 (80% biodiesel and 20% petrodiesel) were respectively delivered to the bioreactors through Hamilton syringe pumps. It was not possible to enrich for B50 due to the inhibitory effect of petrodiesel. At the onset of this study sodium and ammonium sulfate were added to the nutrient solution to convert the redox conditions from methanogenic to sulfate-reducing. Sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$), which inhibits sulfate-reducing bacteria (Pareek et al., 2000), was not added to the nutrient solution. In a sulfate-reducing environment, sulfide is produced via the reduction of sulfate. High concentrations of sulfide have been reported to inhibit sulfate-reducing microorganisms (Maillacheruvu et al., 1993). To minimize the potential for inhibition and to avoid sequestration of heavy metals in the nutrient solution via sulfide precipitation, excess ferrous iron in the form of $FeCl_2 \cdot 4H_2O$ was added to the nutrient feed. The bioreactors received a separate combined feed of essential nutrients solution and vitamins minimal medium as the following final concentrations: 1351 mg/L Na_2SO_4, 7691 mg/L $FeSO_4.7H_2O$, 450 mg/L NH_4SO_4, 119 mg/L $MgSO_4 \cdot 6H_2O$, 70.8 mg/L $CaCl_2 \cdot 2H_2O$, 2666 mg/L $FeCl_2 \cdot 4H_2O$, 9.59 mg/L $CuSO_4 \cdot 5H_2O$, 13 mg/L $MnSO_4 \cdot H_2O$, 10.5 mg/L $ZnCl_2$, 9.16 mg/L $CoCl_2 \cdot 6H2O$, 0.64 mg/L $B(OH)_3$, 9.58 mg/L $NiCl_2.6H_2O$, 0.24 mg/L 4-aminobenzoic acid (99%), 0.096 mg/L biotin, 0.0048 mg/L cyanocobalamin, 0.096 mg/L, folic acid dihydrate (99%), 0.24 mg/L nicotinic acid (98%), 0.24 mg/L pantothenic acid Ca-salt hydrate (98%), 0.48 mg/L pyridoxine hydrochloride (98%), 0.24 mg/L riboflavin (98%), 0.24 mg/L thiamine hydrochloride (99%), 0.24 mg/L thioctic acid (98%). The same nutrients growth medium was used in the batch experiments. A buffer solution containing 3250 mg/L Na_2CO_3, 3250 mg/L K_2CO_3, 208 mg/L KH_2PO_4 provided a strong buffering capacity to maintain the pH in the bioreactor at 7.0 ± 0.2. The flow rate of the nutrient and buffer solutions was monitored on a daily basis. Water quality variables including pH, target analytes in effluent including FAMEs and n-alkanes, volatile fatty acids (VFAs), influent sulfate, effluent sulfate, total and volatile suspended solids (TSS/VSS), and effluent chemical oxygen demand (COD) were monitored routinely. The gas meter showed no gas production, because the carbon dioxide produced by sulfate reduction remained in the aqueous phase, in equilibrium, with carbonate and bicarbonate. Methane was detected in the