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Influence of the Anaerobic Biodegradation of Different Types of Biodiesel on the Natural Attenuation of Benzene

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Abstract

In the present research work, different types of biodiesel were produced by a homogeneous alkali transesterification reaction using soybean oil, pork lard, and castor bean oil as raw materials, to evaluate how their different compositions may affect the biodegradability, namely, in the presence of benzene. Biodiesel was characterized according to the European standard EN 14214. The anaerobic biodegradation of the different types of biodiesel was examined as well as its influence on the biodegradation of benzene. Analyses were performed to determine the volume of methane (directly related to the anaerobic biodegradation of bio- diesel), the concentration of benzene over time, and the production of organic acids. The results showed methane production resulting from the anaerobic degradation of all biodiesel types. The differences between the degradation behavior of each fuel were negligible, contrary to what was expected; however, the amount of methane produced was low due to nutrient limitations. This fact was confirmed by the organic acid analysis as well as by the addition of new media. Anaerobic benzene biodegradation was found to be negatively impacted by the presence of all biodiesel types on average; therefore, the results of this study may impact management of sites that contain biodiesel and fuel hydrocarbon contamination.

Keywords Benzene · Biodiesel · Biodegradation · Contamination · Anaerobic

1 Introduction

In the current energy context, there is a large energy dependence on fossil fuels; the major source of energy production worldwide is oil, being mostly consumed on the transport sector (Moreira et al. 2010). The high dependence on this non-renewable fuel leads to the urgent need to create alternatives that should be focused on renewable energy sources, promoted by legal obligations for

their use.

In Portugal, the dependency on oil is high with the aggravating circumstance of being a non-endogenous resource of this country. Although renewable energy production takes place in Portugal, most is related to electricity production and there is still a minor production of biofuels for the transport sector (OECD 2013) that should substantially increase in the next years.

Within the context of biofuels, biodiesel—alkyl esters—produced from different triglyceride sources (e.g., vegetable oils, animal fats, or waste frying oils) might be used as an alternative automotive fuel to replace fossil diesel (Dias et al. 2013b). The common method for its production (implemented in most industrial plants), due to the associated low cost and simplicity, is transesterification, which consists on the reaction of triglycerides with an alcohol (usually methanol), in the presence of a catalyst, to produce fatty acid esters (biodiesel) and glycerol (Demirbas 2008; Gerpen et al. 2004). Amongst other methods that might be used for the production of alternative fuels to diesel are, more traditionally, biomass pyrolysis and microemulsification (Knothe et al. 2005; Mittelbach and Remschmidt 2004) and more recently non-catalytic transesterification (supercritical), Fischer-Tropsch synthesis, and hydrogenation (Dias et al. 2013a). The more advanced technologies are still less explored mostly due to high associated costs.

The most important factors affecting the transesterification reaction, which impact fuel quality (by affecting product conversion), are temperature (usually close to the boiling point of the alcohol used), time (from 1 to 24 h depending on raw material and type of catalyst), molar ratio of alcohol to raw material (alcohol in excess is required and 6:1 molar ratio is usually used), mixing intensity (as high as possible to promote mixture of the oil and alcohol phases), and amount and type of catalyst (common catalysts used are sodium and potassium hydroxides; amount of catalyst used might vary from 0.2 to 2 wt%, the typical value being 1 wt%) (Dias et al. 2008a; 2013b); the reaction can be either in batch or continuous (Dias et al. 2008a; 2013b; Gerpen et al. 2004; Knothe et al. 2005; Mittelbach and Remschmidt 2004). The optimization of the production process should take into account such parameters as well as the type of feedstock used (Ferrari et al. 2011).

The main advantages of biodiesel produced by transesterification are its ease of mixture with conventional diesel (completely miscible), without affecting engine characteristics, as well as its high biodegradability, low toxicity, and low emission profile compared to fossil diesel (Gerpen et al. 2004). Biodiesel might be used alone, with minor vehicle adaptations, but is mostly used in blends up to 20 % of biodiesel (B20) (Moreira et al. 2010). As with any other fuel, accidental spills of biodiesel or diesel/biodiesel blends into the subsurface may occur which can potentially lead to impacts on the environment and health. Therefore, a variety of studies have examined the aerobic biodegradation of biodiesel (DeMello et al. 2007; Lutz et al. 2006; Mariano et al. 2008; Owsianiak et al. 2009; Pasqualino et al. 2006; Sendzikiene et al. 2007). However, depending on the characteristics of the site and the amount spilled, anaerobic conditions may prevail over the aerobic ones. Al- though this is the case, few studies exist on the anaerobic biodegradation of biodiesel (Aktas et al. 2010; Corseuil et al. 2011b; Lapinskienne and Martinkus 2007; Ramos et al. 2013; Sorensen et al. 2011).

On the few existing studies on anaerobic conditions (Aktas et al. 2010; Corseuil et al. 2011b; Lapinskienne and Martinkus 2007; Ramos et al. 2013; Sorensen et al. 2011), some focused on evaluating the degradation in terms of LCFA (long-chain fatty acids), nitrates, and sulfates (Corseuil et al. 2011b; Liu and Suflita 1994), and also on biodiesel impacts, for example, on metal corrosion (Aktas et al. 2010). One recent study examined the efficacy of ammonium acetate for the biostimulation of anaerobic BTEX removal in the field at a B20 contaminated site (Ramos et al.

2013). Corseuil et al. (2011b) studied the use of soybean and castor bean oil biodiesel and found that unsaturated/less viscous bio- diesel tends to be more easily degraded; however, it is not known whether other types of biodiesel will show similar behavior. In addition, limited research has been done to examine the impact of biodiesel on the anaerobic biodegradation of benzene. Previous work on the influence of biodiesel on anaerobic benzene biodegradation showed the negative impact of one type of bio- diesel (soybean biodiesel) but did not monitor for methane production or test other biodiesel sources (Corseuil et al. 2011b). Another work monitored for methane production but did not identify the source of biodiesel in the B20 blend (Ramos et al. 2013). Anaerobic benzene degradation is important due to its presence in fuel hydrocarbons, toxicity, and the difficulty in its degradation under methanogenic conditions (Da Silva and Alvarez 2004; Edwards et al. 1992; Grbic-Galic and Vogel 1987). It is, however, expected, that the anaerobic biodegradation of different types of biodiesel and the resulting impact on benzene degradation presents a high degree of complexity (Corseuil et al. 2011b; Sorensen et al. 2011).

Therefore, the aims of the study were to perform anaerobic biodegradation of the different types of bio- diesel in the presence and absence of benzene while monitoring methane production and determining organic acid production, and evaluate how different biodiesel compositions affect the biodegradability, namely in the presence of benzene.

2 Materials and Methods

2.1 Materials

The vegetable oils and animal fat used were commercial. The soybean oil used was from the brand OliSoja, the pork lard was from the brand Monteiro Carnes and the castor bean oil was from Fagron. The reagents used during biodiesel synthesis and purification procedures were methanol 99.5 % (analytical grade, Fisher Scientific), sodium hydroxide 97 % powder (reagent grade, Aldrich), potassium hydroxide >99 % (ACS, P.A., Merck), and hydrochloric acid 37 % (ACS reagent, Aldrich). Benzene standard used during anaerobic degradation studies was ≥99.5 % (Panreac Química SAU) and Methane standard for GC analysis was 99.9995 % (Linde). All other chemicals used were of reagent grade. Sediment samples used were collected from a lagoon downgradient from the *Estarreja* Chemical Complex (NW Portugal), with the following coordinates: 40° 46′ 34.6″ N, 8° 36′ 68.4″ W. The site has a long history of contamination, including benzene and other hydrocarbons (Ordens 2008).

2.2 Biodiesel Production

Biodiesel was produced by a homogeneous alkali transesterification reaction, followed by purification steps. The conditions used varied depending upon the raw material, being selected based on previous work (Dias et al. 2008a; 2008b; 2013b).

Biodiesel was produced in duplicates and the results regarding the properties are mean values with relative percentage differences always less than 5 % of the mean.

2.3 Analytical Methods

2.3.1 Raw Materials and Biodiesel Characterization

The acid value of the raw materials was determined by volumetric titration according to the standard NP EN ISO 660 (2002). Other properties of such raw materials might be inferred from previous studies (Dias et al. 2008a; Dias et al. 2008b; Dias et al. 2013b).

Composition derives from the methyl ester analysis in the GC according to EN 14103 (2003).

The biodiesel quality was evaluated according to the European biodiesel standard EN 14214. The following properties were determined: (i) acid value, by volumetric titration according to the standard EN 14104 (2003); (ii) kinematic viscosity, determined at 40 °C using glass capillary viscometers according to the standard ISO 3104 (1994); (iii) flash point, using a rapid equilibrium closed cup method, according to the standard ISO 3679 (2004); (iv) ester and linolenic acid methyl ester con- tents, by gas chromatography (GC) according to the standard EN 14103 (2003); (v) iodine value, determined from ester content according to annex B of EN 14214 (2003); and, oxidation stability, determined at 110 °C, according to the standard EN 14112 (2003), using a Rancimat equipment (Metrohm).

GC analyses were conducted on a Dani GC 1000 DPC gas chromatograph (DANI Instruments S.p.A.) with an AT-WAX (Heliflex capillary, Alltech) column according to (Dias et al. 2008a, 2013b).

2.3.2 Determination of Methane, Benzene, and Organic Acids

Methane and benzene were analyzed using headspace samples (0.1 or 0.5 mL collected using a Pressure-Lok® analytical syringe (VICI)) by gas chromatography. A Shimadzu GC-2014 equipment was used, being equipped with a flame ionization detector and a 1 % SP-1000 on a 60/80 Carbopack B packed column (Supelco). The following temperature program was used: 60 °C for 3 min, 20 °C /min rate until 200 °C, followed by an 8 min hold. The injector and detector temperatures are as previously described (Freedman and Gossett 1989). The concentrations of the aqueous substrate solutions were determined from headspace samples using Henry's constants, as previously described (USEPA 2005).

Organic acids (acetate, propionate, formate, pyruvate, valerate, malonate, maleate, oxalate, and citrate) were analyzed by ion chromatography using a Dionex IonPac AS11-HC 4×250 mm, a suppressor (ASRS®300 4 mm), and an eluent generator cartridge (Dionex, RFIC^{TM}). The conditions were as follows: pre-run of 8 min followed by 20 min with 30 mM NaOH and finally 10 min with 60 mM NaOH; the flow rate used was 1.5 mL min^{-1}, as previously described (Pereira et al.2013).

2.3.3 Microcosm Preparation

Microcosms were prepared and sealed (Teflon-lined septa with aluminum crimp caps) in an anaerobic glove box (Bactron Anaerobic Chamber model II) with 20 g of wet sediment and 80 mL of synthetic groundwater in 125 mL serum bottles. The synthetic groundwater contained the following compounds: $NaCO_3$ (410 mg/L), KH_2PO_4 (531 mg/L), K_2SO_4 (40 mg/L), NH_4Cl (16 mg/L), $MgCl_2 \cdot 6H_2O$ (12 mg/L), and $CaCl_2$ (6.7 mg/L) as previously described (Da Silva et al. 2005; Von

Gunten and Zobrist 1993) and supplemented with resazurin (0.5 mg/L) as redox indicator. The bottles were subsequently removed from the chamber and purged with N_2 followed by the addition of 1.5 mL of CO_2. The initial pH of the microcosms was approximately 7.5.

Microcosms were amended with each type of biodiesel alone (soybean B100 (100 % biodiesel), pork lard B100, and castor bean oil B100), benzene, or biodiesel of each type plus benzene, as follows: set 1 (60 mg/L B100 soybean), set 2 (60 mg/L B100 pork lard), and set 3 (60 mg/L B100 castor bean oil) were prepared in duplicate; sets 4 (3–4 mg/L benzene), 5, 6, and 7 (3–4 mg/L benzene+60 mg/L soybean, pork lard, or castor bean oil B100, respectively) were prepared in triplicate. Controls were also prepared in duplicate without the addition of biodiesel or benzene ('As Is'). In addition, microcosms were setup in duplicate using lactate (150 mg/L) as a readily degradable substrate. Estarreja sediment was effective in producing methane from lactate (Danko et al. 2012). The concentration used for lactate was the same amount of COD as for the biodiesel. Abiotic controls were prepared by autoclaving (20 min, 121 °C). The incubation period was approximately 200 days. Microcosms were quiescently incubated at room temperature (20–25 °C), upside down and in the dark. Methane production was evaluated over time.

3 Results and Discussion

3.1 Raw Material and Biodiesel Properties

The most relevant properties of the raw materials used are presented in Table 1. The soybean oil composition (inferred from the methyl ester profile) shows that 15.4 wt% is saturated fatty acids and, mostly, linoleic acid is present, which agrees with the reported values for this type of oil (Dias et al. 2008a; Rossel 1986), being the only oil between the studied ones with around 4 wt% of a polyunsaturated fatty acid (C18:3) although with a slightly lower value of C18:3, compared with the literature (between 5.5 and 9.5 wt%) (Rossel 1986), which might indicate some degree of degradation, also reflected by the acid value, that is also slightly higher than the value reported for soybean virgin oil (Dias et al. 2008a). It should be emphasized that such differences should not affect the behavior of the fuel, since it is still high-quality oil. The lard biodiesel presented a similar composition as others reported in the literature with 34.5 wt% of saturated methyl esters and relatively low acid value (Dias et al. 2008b; 2013a). The castor oil presents a unique composition, with around 90 wt% of ricinoleic acid. The composition values agree with range reported in the literature (Rossel 1986) and the acid value is relatively low, which would be expected for refined oil. The iodine values clearly reflect the degree of unsaturation of the raw materials and agree with previous studies (Dias et al. 2008a, b; 2013b).

The key quality parameters of the biodiesel produced are presented in Table 2. The flash point was similar between the different biodiesel types and it is an indicator of the fuel safety for handling and storage (values higher than 101 °C are demanded by EN 14214). The other measured properties clearly distinguish the three types of fuels. Biodiesel from pork lard presented the lowest oxidation stability, indicating that it might be more prone to degradation whereas the ricin oil present- ed clearly the highest oxidation stability, even though the acid value was found to be high in this product. In addition, the kinematic viscosity of castor oil biodiesel is more than five times higher than that of the other types of biodiesel, which might also interfere with the biodegradation of this type of biodiesel (decreasing its bioavailability). The low oxidation stability of the soybean oil biodiesel might be related to what was previously refereed in terms of the C18:3 content and

might also be reflected in the ability of this oil to biodegrade. In general, biodiesel properties are within the range of values found in previous studies (Dias et al. 2008a; Dias et al. 2008b; Dias et al. 2013b).

3.2 Anaerobic Biodegradation of Biodiesel (B100) from Soybean Oil, Pork Lard, and Castor Oil

Experiments were conducted to examine the impact of the different characteristics of biodiesel on its anaerobic biodegradation. For that, the amount of methane produced from the anaerobic degradation of the different biodiesel types in each microcosm (soybean oil biodiesel microcosm (MSO), pork lard biodiesel microcosm (MPL) and castor oil biodiesel microcosm (BCO)) was measured. All microcosms contained sediment, groundwater, and resazurin. The different biodiesel types were added to each bottle (approximately 60 mg/L) and, in addition, lactate-only bottles were used (approximately 150 mg/L) to serve as a control and to gauge microbial activity, as previously referred. All batch experiments were initially pink, indicating that the incubations were not anaerobic. After a period be- tween 3 weeks and 1 month, all of the batch reactors for all of the different types of tests, except for the autoclaved controls, turned clear, indicating that anaerobic conditions were present. Soon afterwards, methane production was observed in the live incubations (Fig. 1). Similar behavior was observed for the lactate controls (data is further analyzed in section 3.3).

Previous work with soybean oil-based biodiesel indicated that both nitrate and sulfate were completely consumed after 35 days (Corseuil et al. 2011b). The autoclaved bottles remained pink and methane production was not observed; therefore, the degradation of the different types of biodiesel must have occurred via biological processes and primarily under anaerobic conditions.

Even though the specific characteristics of each type of biodiesel are different (Tables 1 and 2), Fig. 1 shows little differences in methane production between them. Due to the different composition of each biodiesel and specifically, the unique composition of castor bean oil (with a very high percentage of methyl ricinoleate and also a very high viscosity), it would be expected that the biodegradation would show a different trend compared to the other types of biodiesel, due to lower bioavailability, as observed in previous studies (Corseuil et al. 2011b). However, the biodegradation rates based on methane production between the three biodiesel types in this study were very similar. The reason why this behavior was observed is further explored in the next section.

3.3 Effects of the Presence of Biodiesel in the Anaerobic Degradation of Benzene

Experiments were conducted to evaluate the impact of the three different biodiesel types on benzene biodegradation. This is important since benzene is often the contaminant of concern at hydrocarbon contaminated sites and previous work on the influence of biodiesel on anaerobic benzene biodegradation showed the negative impact of soybean oil biodiesel (Corseuil et al. 2011b). This study aims to present further information, namely considering the behavior of other types of bio- diesel and also by measuring the methane produced during degradation. The results of the biodiesel impact on benzene biodegradation are shown in Table 3. Over the 150-day period of monitoring, approximately 18 % of the initial amount of benzene was removed through biodegradation. Such removal is not surprising considering that the anaerobic biodegradation of benzene is notoriously difficult and may require very long periods of time for total removal (Da Silva and Alvarez 2004; Edwards et al. 1992; Grbic-Galic and Vogel 1987). On the other hand,

less biodegradation on average was observed for benzene with biodiesel made from pork lard, castor oil, or soybean oil. This suggests that the presence of biodiesel negatively impacted benzene degradation, which agrees with previous results using soy- bean oil biodiesel (Corseuil et al. 2011b). Adding to that previous study, this work also demonstrates these negative effects with two additional types of biodiesel (pork lard and castor oil). Taking into account the different types of biodiesel, there were differences observed be- tween the amounts of benzene removed, with the highest removal being observed in the presence of pork lard and the lowest for castor oil. This might have been caused by the higher viscosity and lower bioavailability of castor oil biodiesel compared to the other biodiesel types.

The negative influence of biodiesel in benzene bio- degradation may have been caused by a variety of mechanisms, as suggested by Corseuil et al. (2011b). Biodegradation of aromatics, such as benzene and toluene, is known to be negatively impacted by the presence of ethanol and other compounds (Ma et al. 2013; Osterreicher-Cunha et al. 2009). For example, enzymes involved in aromatic biodegradation could be repressed (catabolic repression) or feedback inhibition (metabolic flux dilution) could occur in the presence of other compounds such as acetate, ethanol, or phenol (Duetz et al. 1994; Lovanh and Alvarez 2004; Lovanh et al. 2002; Ma et al. 2013). These compounds can be groundwater contaminants and/or products of anaerobic processes. Also, the biodegradation of ethanol has been shown to rapidly deplete electron acceptors, which could have been used for benzene, toluene, and xylene biodegradation (Da Silva and Alvarez 2002). In addition, the thermodynamics of the system could interfere with benzene biodegradation. For example, acetate concentrations above 64 mg/L (Corseuil et al. 2011a) and 75 mg/L (Ramos et al. 2013) were found to be responsible for the inhibition of anaerobic biodegradation of benzene. Also, ethanol has been shown to decrease the relative abundance of benzene, toluene, and xylene degrading bacteria (Cápiro et al. 2008).

The amount of methane produced in this study from microcosms was much lower than expected based on the amount of biodiesel added to the microcosms since the expected amount was between approximately 4000 and 5000 μL. Therefore, different reasons for the low activity were explored. After approximately day 190, aliquots from the microcosms were removed and the pH was checked. The values for pH for all the bottles ranged from 7.2 to 7.4, which is within the normal range for microbial activity. Next, organic acids were determined since they, along with methane, can be products of the anaerobic biodegradation of biodiesel. The results indicate that several acids were produced, including acetate, propionate, formate, and malonate; however, at low concentrations, of less than 100 mg/L (Table 4). An electron balance was performed based on the amounts of biodiesel added and on the amounts of methane and organic acids produced. Accordingly, the estimated COD recovery of the products was 40.3 %, 28.9 %, and 29.4 % for BCO, BSO, and BPL, respectively. Since little methane and organic acids were produced, the results retained from the analysis of this study cannot draw definite conclusions on why this behavior occurred, although possible reasons are explored in the following paragraphs.

One possible explanation for the relatively low recovery is due to the presence of long-chain fatty acids (LCFA), which are biodiesel and lipid biodegradation products. LCFA overloading has been shown to lead to volatile fatty acid (VFA) and LCFA accumulation that may significantly reduce methane production due to imbalances between acid consuming and methane producing microorganisms (Alves et al. 2009; Angelidaki and Ahring 1992; Eiroa et al. 2012; Rinzema et al. 1994). Overloading of LCFA can cause concentrations of total volatile acids to be above 2000 mg COD/L or more and/or acetate concentrations above 500 mg COD/ L (Cavaleiro et al. 2001; Cavaleiro et al. 2009; Eiroa et al. 2012). As the total calculated amounts of COD (<55 mg COD/L)

and acetate (<15 mg COD/L) produced in this study for microcosms containing biodiesel are much less, they should not be inhibitory.

Results of VFA analysis, methane produced, and the COD balance suggested that some micro- or macronutrient(s) limitation was affecting microbial activity. To evaluate this fact, approximately 8 mL of media (10 vol%) was removed from the microcosms containing lactate. Subsequently, the same volume of O_2 free synthetic groundwater as described in the materials and methods (prepared in an anaerobic glove box) was added back to these bottles and purged with N_2 followed by an addition of CO_2 (as described in Section 2).

After 3 days of incubation, methane concentrations were checked and although the two microcosms reached different maximum values of methane production, there is no doubt that a very significant increase in methane production occurred in both cases (Fig. 2). The differences observed may be due to the heterogeneity of the soil. Production continued thereafter for approximately a total of 70 days. The amount of methane produced was approximately 35 times higher (on average) than the amount produced prior to the introduction of new media. This suggests that the microbial activity was, in fact, limited by some macro- or micronutrient(s) deficiency rather than inhibition by LCFA. Nutrient limitation is known to affect biodegradation and microbial community development and the addition of nutrients was found to increase anaerobic activity by as much as 50 % or more in different studies (Feng et al. 2010; Jansen et al. 2007; Kayhanian and Rich 1995; Monanakrishna et al. 2010). This is expected since micro- and macronutrients are key components of enzymes, proteins, etc. (Takashima and Speece 1990). These results may also help to explain the differences observed in this study, namely the apparent low level of biodiesel biodegradation and the non-existent differences between the different biodiesel types, compared to other studies (Corseuil et al. 2011b) on the anaerobic biodegradation of biodiesel.

4 Conclusion

This study showed the anaerobic degradation of different types of biodiesel (from soybean oil, pork lard, and castor oil) through methane production and also by the production of several organic acids, including acetate. The differences between the degradation behavior of each fuel were negligible, contrary to what was expected; however, the amount of methane produced during biodegradation from the microcosms was low, apparently due to micro- and/ or macronutrient limitation that was confirmed upon the addition of new media. Nevertheless, the results showed that under the examined conditions, the different types of biodiesel negatively impacted benzene biodegradation. This suggests that sites containing mixtures of these compounds may need active steps to ensure that benzene does not migrate away from these areas. In addition, this study suggests that the comparison between biodiesel from different raw materials under anaerobic degradation is even more complex than expected, and, therefore, it is important to find other ways to obtain further information on the relationships between different properties of bio- diesel and its anaerobic degradation.

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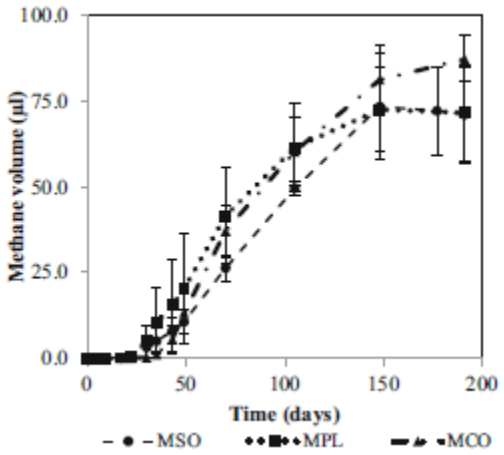

Fig. 1 Methane production during anaerobic degradation of the different types of biodiesel; *MSO* soybean oil biodiesel microcosm, *MPL* pork lard biodiesel microcosm, *MCO* castor oil bio- diesel microcosm

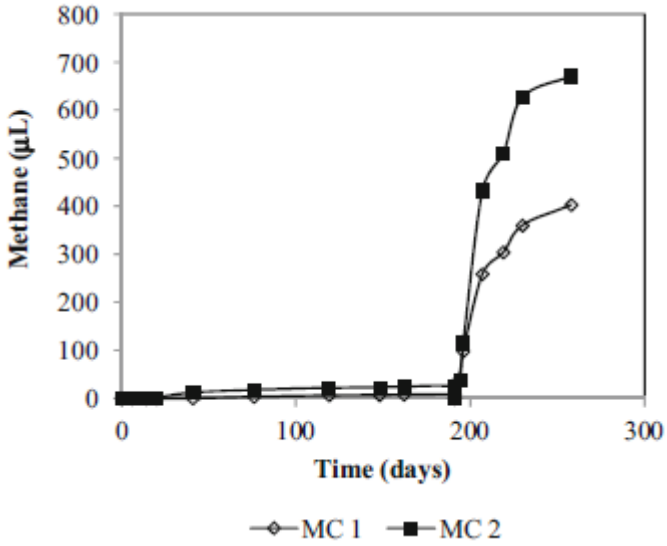

Fig. 2 Methane production from lactate-containing microcosms (*MC1* and *MC2*) after the addition of new media. Bottles were purged of residual methane concentrations at day 190 and

given new groundwater

Table 1 Raw material properties

Raw material	Acid value (mg KOH g^{-1})	Iodine value (cg $I_2 g^{-1}$)	Composition (methyl ester profile, wt%)
Soybean oil	0.61	127	C16:0=11.7
			C18:0=3.7
			C18:1=26.5
			C18:2=54.1
			C18:3=4.0
Pork lard	0.76	69	C16:0=22.0
			C18:0=11.3
			C18:1=47.7
			C18:2=13.3
			C18:3=0.8
			Others=5.0
Castor bean oil	0.52	88	C16:0=1.1
			C18:0=1.2
			C18:1=2.9
			C18:1-OH=90.5
			C18:2=4.3

Mean values of two replicates, relative percentage differences always less than 5 % of the mean

Table 2 Quality properties of soybean oil biodiesel (BSO), pork lard biodiesel (BPL), and castor oil biodiesel (BCO)

Property[a]	Biodiesel type		
	BSO	BPL	BCO
Methyl ester content (wt%)	96.0	93.9	84.5
Acid value (mg KOH g^{-1})	0.5	0.5	3.5
Kinematic viscosity, 40 °C ($mm^2 s^{-1}$)	4.51	4.78	16.70
Oxidation stability, 110 °C (h)	1.2	0.1	14.9
Flash point (°C)	174	175	171

[a] Mean values of two replicates, relative percentage differences always less than 5 % of the mean

Table 3 Benzene biodegradation in the presence of biodiesel

Experiment	Benzene removal (%)
BSO+benzene	10.78±0.65
BPL+benzene	14.35±1.63
BCO+benzene	6.67±0.99
Benzene only	17.74±2.29

Removal percentage considering the difference between the concentration at the beginning and end of the experiment (approximately day 150)

BSO biodiesel from soybean oil, *BPL* biodiesel from pork lard, *BCO* biodiesel from castor oil

Table 4 Organic acid analysis

Substrate	Organic acid (mg/L)[a]				
	Acetate	Propionate	Formate	Pyruvate	Malonate
Castor oil	11.5–15.5	ND	0.5–2.7	ND	52.8–77.8
Castor oil with benzene	6.8–8.3	ND	ND	ND	26.8–28.9
Soybean oil	2.3–6.4	ND	ND	ND	64.8–68.9
Soybean oil with benzene	4.2–9.2	ND	0.6–1.4	4.0–7.1	28.5–38.3
Pork lard	2.0–8.5	ND	0.5–4.0	1.1–4.9	53.0–72.9
Pork lard oil with benzene	5.0–6.9	ND	0.8–3.2	ND	32.9–44.7
Lactate	38.6–51.9	34.1–52.0	ND	0.5–3.5	38.4–50.0

ND not detected (detection limit 0.2 mg/L)

[a] Concentration data ranges (subtracted from the controls); most relevant acids presented, others measured were below detection limits