Bioremediation of diesel/biodiesel blends in soil: a respirometric approach

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ABSTRACT

Many bacteria and fungi are able to metabolize oil; however, the environmental pollution caused by accidental spillage is still a problem to be solved. Previous work has shown that a blend of biodiesel in diesel could increase the rate of biodegradation but it is not a rule. In this paper, we analyzed the rate of biodegradation of 5, 25 and 50% blends of soy biodiesel in diesel into soil (B5, B25 and B50) using (i) the Bartha and Pramer's respirometric method and (ii) the dehydrogenase method. Respirometric analysis shown that after 106 days of incubation the CO_2 evolved by both B5 and B25 were similar whereas the blend 50% (B50) produced 60% more CO_2 than both of them. Dehydrogenase activity was higher with the increase in biodiesel amount and the activity of all blends where higher than the control as well. These results shows that there is a correlation between the amount of biodiesel in diesel and the increase of microorganisms' activity when released in the soil.

Keywords: Biodiesel. Diesel. Microbiota. Soil

1. Introduction

With the rapid rise in the price of crude oil and the projected decreases in oil supplies in the near future, alternative fuels are receiving notoriety. One of these alternative fuels is biodiesel. It is chemically defined as a mixture of monoalkyl esters of fatty acids, derived from transesterification of vegetable oils and animal fats [1]. Since it possesses properties similar to mineral diesel, biodiesel can be blended with diesel without requiring changes in engines or other adaptation, reducing emissions of pollutants into the air [2-4].

The diesel oil is a complex mixture of non-aqueous and hydrophobic compounds such as alkanes, branched chain hydrocarbons and aromatic compounds in the C_9-C_{28} range. These compounds can harness the local biota due to their toxicity [5,6]. The processes of extraction, refining, transport, and storage of petroleum and its derivatives are prone to leaking and/or spilling accidents, resulting in environmental contamination [7]. In addition, the low accessibility of hydrophobic compounds to microbial cells causes their slow biodegradation [8].

When released into environment, the biodegradability of petroleum products depends not only on the intrinsic biodegradability properties of the pollutant, but also on the presence of adapted microorganisms [9,10]. From an environmental perspective, one of biodiesel advantages over petroleum diesel is its major biodegradability. This biodegradability is related to the absence of aromatic molecules, presence of high-energy ester bridges, and hygroscopic properties [7].

Studies on bioremediation of diesel/biodiesels blends [11, 12-18], have shown that such mixtures are more easily biodegraded and less toxic than diesel oil. Additionally, biodiesel degradation may contribute to accelerate the biodegradation by promoting the microbial growth in contaminated soil with a synergetic action.

In this work, we aimed to determine the biodegradability of diesel/biodiesel blends in soil by both the Bartha and Pramer's respirometric and the dehydrogenase methods.

2. Methodology

2.1 Fuel Blends

Both pure diesel oil and biodiesel from soybean were acquired from a Brazilian refinery. The mixtures containing 5% (B5), 25% (B25) and 50% (B50) of biodiesel in diesel were utilized in the assays.

2.2 Soil

Soil samples were collected according to Brazilian Standards NBR-14283 [19], at São Paulo State University (UNESP), in Rio Claro - SP, 22º 23'S and 47º 32' W and sieved in a granulometric device with opening size of 1.5 mm. The samples had their physical-chemical properties quantified according to Raij et al. [20] and Korndorfer et al. [21].

Microbial activity was determined by measuring respiratory [22] and dehydrogenase activity [23].

2.3 Determination of CO_2 using Bartha's method modified

Fifty grams of soil samples were contaminated with B5, B25, and B50 by adding 100 mL of fuel blends/kg of soil. The microbial activity (biodegradation) was determined at intervals of 3 days (72 h) by measuring the amount of CO_2 released/106 days of incubation at 28° C, following methodology described in Strotmann et al. [22]. Assays were run in triplicate, including the controls. All soil samples had the same moisture.

2.4 Kinect Models

The kinetic of blends biodegradation was calculated according to the CO_2 production, as reported by Schmidt et al. [24] and modified by Montagnolli et al. [25].

The Eq. (1) below refers to the amount of accumulated CO_2:

$$\mathbf{B} = \mathbf{Bmax} /(1+((Bmax - B0)/B0)*\exp(-r*t))$$

where B is the CO_2 produced, Bmax is the maximum of CO_2 produced, B0 is the initial CO_2 produced, r is the specific production rate, and t is the time in which the biodegradation occurs.

2.5 Dehydrogenase activity

The dehydrogenase activity was determined after 30 days of incubation according to Thalmann [23] with modifications. Two grams of soil samples were taken from the respirometric flasks and mixed with 2 mL of TTC (2,3,5-triphenyltetrazolium chloride) 1% (w/v) diluted in Sorensen´s buffer (Na_2HPO_4

– KH_2PO_4), pH 7.2 [26]. The mixture was transferred to test tubes (50 mL) and incubated at 30º C/48 hours.

After incubation, 16 mL of acetone were added to stop the enzyme reaction and, after homogenization, the tube was left in the dark for 2 hours. The soil suspension was filtered and the absorbance measured at 482 nm. A standard curve was built to quantify the values of 1,3,5-triphenyltetrazolium formazan (TPF mL^{-1}) formed in each sample.

2.6 Statistical analysis

The results were statistically analyzed by ANOVA with a confidence level of 95%. The normalized data underwent Tukey test aided by the BioEstat 5.0 software suite.

3. Results and discussion

Results of soil analysis and biodegradation of biodiesel/diesel are described.

3.1 Soil analyses

The soil physicochemical characteristics and texture are summarized in Table 1. The soil was classified with low water retention capacity and high susceptibility to erosion. It was composed of it 676 $g.kg^{-1}$ of sandy, 232 $g.kg^{-1}$ of clay and 92 $g.kg^{-1}$ of silt. The soil used in the assays had high levels of organic matter, and micronutrients such as iron and manganese when compared to the values found in other studies of biodegradation of biodiesel and diesel blends [11].

Table 1. Soil sample characteristics

Parameter analysed and methodology	Results	Parameter analysed and methodology	Results
pH $CaCl_2$	4.6	**K (Resin)**	1.2 $mmolc.dm^{-3}$
Al (Colorimetric (KCl 1 $mol.L^1$))	4 $mmolc.dm^{-3}$	**m**	20%
Boron (hot water)	0.19 $mg.dm^{-3}$	**Manganese (DTPA)**	34.1 $mg.dm^{-3}$
BS	24%	**Mg (Resin)**	5 $mmolc.dm^{-3}$
Ca (Resin)	10 $mmolc.dm^{-3}$	**Organic matter (Colorimetric)**	40 $g.dm^{-3}$
CEC	68.2 $mmolc.dm^{-3}$	**P (Resin)**	6 $mg.dm^{-3}$
Cupper (DTPA)	1.7 $mg.dm^{-3}$	**S (Calcium phosphate 0,01 $mol L^1$)**	<4 $mg.dm^{-3}$
H+Al (SMP)	52 $mmolc.dm^{-3}$	**SB**	16.2 $mmolc.dm^{-3}$
Iron (DTPA)	60 $mg.dm^{-3}$	**Zinc (DTPA)**	1 $mg.dm^{-3}$

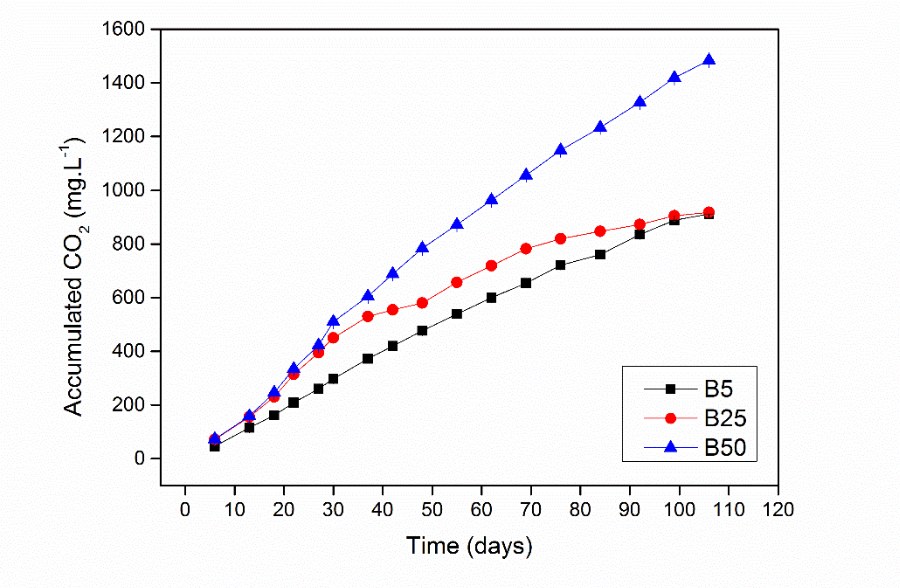
Legend: P = phosphor; S = sulfur; K = potassium; Mg = magnesium; SB = sum of the bases; CEC = cation-exchange capacity; BS = base saturation; m = aluminum saturation; $mmolc.dm^{-3}$ = millimoles per cubic decimeter; DTPA = diethylenetriaminepentaacetic acid; SMP is a buffer solution [20].

During the incubation period it was possible to see the development of filamentous fungi in respirometric flaks possibly related and partially responsible for the observed biodegradation. The fungal growth was expected, as the given pH conditions promotes their growth [27].

3.2 CO_2 evolution

The accumulated CO_2 of B5, B25 and B50 blends are presented in Fig. 1. The addition of blends increased the soil microbial activity, especially when the mixture was B50. The CO_2 production values of soil control (SC) was subtracted from the blends dataset.

Fig. 1 Accumulated CO_2 of B5, B25 and B50 samples.



Legend: B5 (blend containing 5% of biodiesel in diesel), B25 (blend containing 25% of biodiesel in diesel) and B50 (blend containing 50% of biodiesel in diesel)

In the first 20 days the CO_2 production was very similar for all blends. This is expected because there was a soil microbiota adaptation delay to the diesel/biodiesel presence.

In B50 blend, there is an evident increase in CO_2 evolution, producing a 550 $mg.L^{-1}$ of CO_2 surplus compared to B5 and B25 samples after 106 days. The B50 blend had a similar production of CO_2 compared to B25 blend during the first 30 days. Afterwards, CO_2 production of B25 decreased, probably after the easily biodegradable part of biodiesel was already consumed.

The treatment B25 showed a sharper increase than B5. This was probably caused by the organic matter presence in biodiesel stimulated the microbial degradative metabolism. This has also been demonstrated in a study by Mariano et al. [17] where the biodegradation of B20 and B100 was higher than pure diesel. At the end of the test the B5 and B25's CO_2 production was similar. It was important to observe that the increase from 5% to 25% of biodiesel in the blend did not increase the CO_2 production at 106 days. At the end of the test, the B5 and B25 produced 911 and 917 mg L^{-1} of CO_2, respectively.

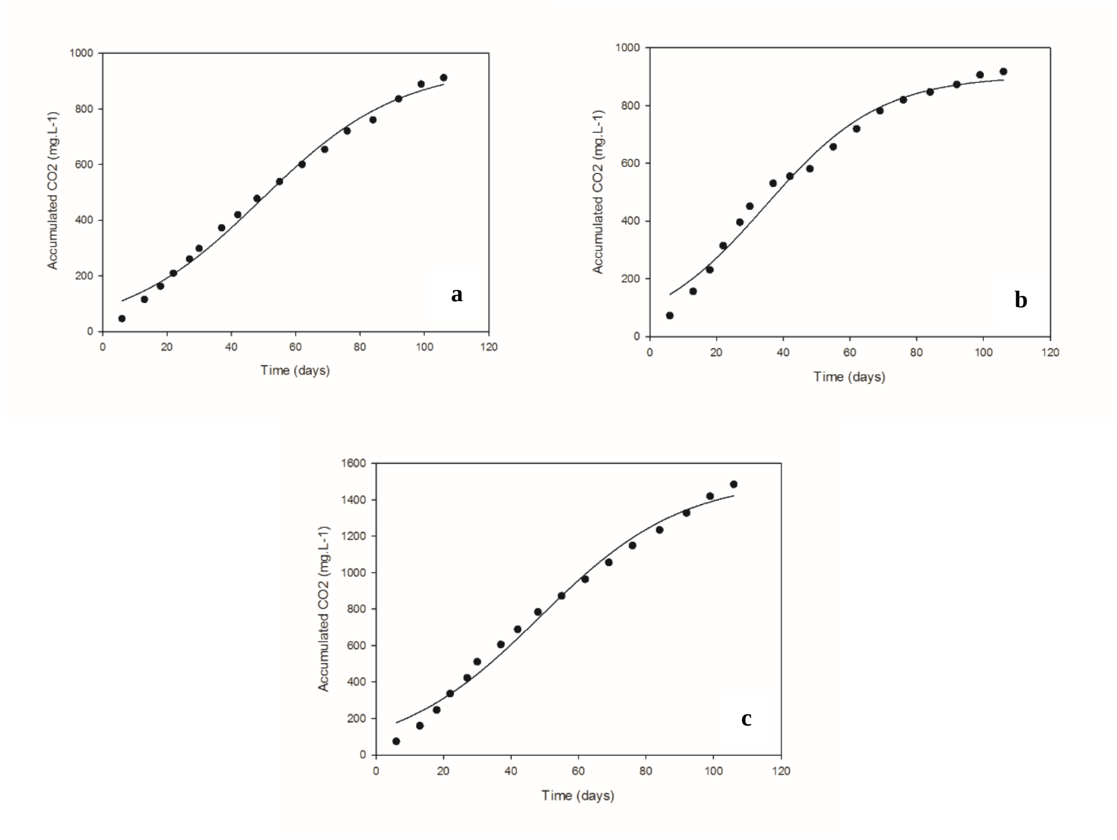
The blend B5 produced the lowest amount of CO_2, what can be explained by the large amount of diesel in the blend, which suppressed the stimulation of indigenous soil microorganisms. Zhang et al. [28] claim that higher biodegradability of biodiesel is due to natural products consisting purely of fatty acids, which are biologically active, being recognized and attacked immediately by enzymes such as Acetyl-CoA dehydrogenase.

Some studies showed a positive synergistic effect of adding biodiesel to diesel in terms of biodegradation, because the fatty acids present in their formulation are a source of energy promote the degradation of diesel [28-29].

3.2.1 Kinect of Biodegradation

The Figure 2 shows the kinect model according to Schmidt et al. [24] and modified by Montagnolli et al. [25]. Considering the data for modeling according to Montagnolli et al. [25], it is possible to estimate the expected time of biodegradation. Tendency for stabilizing the biodegradation process initiates after 241 days of incubation for B5, 195 days for B25 and 248 days for B50.

Fig 2 Kinetics model in CO_2 evolution results.



Legend: (a) B5 (blend containing 5% of biodiesel in diesel), (b) B25 (blend containing 25% of biodiesel in diesel) and (c) B50 (blend containing 50% of biodiesel in diesel)

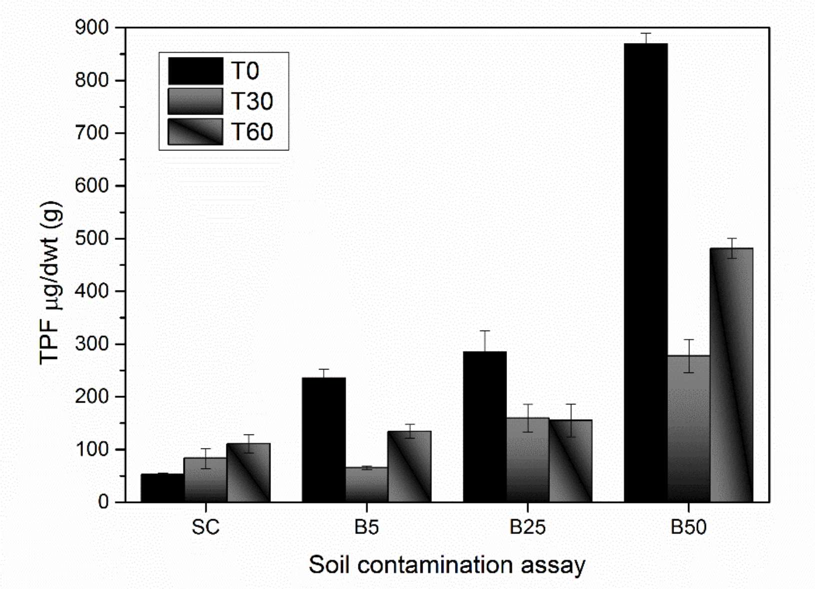
The B25 blend is in a stabilization process of biodegradation in the end of the test, so the model calculates 195 days to complete the process. Furthermore, the B5 and B50 mixtures are still undergoing biodegradation, which take longer to stabilize the biodegradation.

The longest time for B50 can be explained by the easily biodegradable of organic matter present in biodiesel that has to be degraded. The second longest time for B5 probably because by the larger amount of diesel, what is harder to biodegrade than biodiesel and may not stimulate the unsuitable microbiota in our experiments.

3.3 Dehydrogenase activity

Figure 2 shows the activity of soil dehydrogenase in soil contaminated with B5, B25 and B50 blends. From this data, we can note the influence of these blends in the activity in soil. Table 2 shows the statistical analysis in 30 and 60 days of CO_2 production test and 0, 30 and 60 days for dehydrogenase activity.

Fig. 3 Soil dehydrogenase activity in contaminated soil.



Legend: T0: in the initial time, T30: after 30 hours and T60: after 60 hour. SC is soil control, B5 (blend containing 5% of biodiesel in diesel), B25 (blend containing 25% of biodiesel in diesel) and B50 (blend containing 50% of biodiesel in diesel). The error bars represent standard deviation (replicates = 3)

All the initial time measurements showed a high activity for dehydrogenase. Samples B5 and B25 had no significant difference among them but showed an increase of 77% and 80% in relation the SC (Figure 3). The B50 activity was 94% higher than SC, and had 3 times the activity of B25. In 30 days the B5% had a slightly lower activity than the SC.

In T30 the B25 showed an increase of 48% and the B50% an increase of 70% of soil enzymatic above the SC. When comparing B25 and B50, we observe that the greater amount of biodiesel caused a 42% increase in the dehydrogenase activity. In 60 days of test, the only sample that differed statistically was the B50, being 77% higher than the SC (Table 2).

The amount of biodiesel and the production of CO_2 obtained had a correlated increase on both days. In 30 days of test the accumulated CO_2 increased 33% from B5 to B25 and 25% from B25 to B50. And in 60 days it increased 16% from B5 to B25 and 25% from B25 to B50.

Treatment	**CO_2 production**		**Dehydrogenase activity**		
	30 days	**60 days**	**0 day**	**30 days**	**60 days**
SC			52.67[a]	83.14[a]	110.93[a]
B5	298.11[a]	600.16[a]	236.05[b]	64.90[a]	134.59[a]
B25	450.71[b]	718.78[b]	285.45[b]	159.38[b]	155.32[a]

B50	510.44[c]	963.49[c]	869.79[c]	276.98[c]	480.94[b]

Table 2. CO_2 production in 30 and 60 days; dehydrogenase activity in 0, 30 and 60 days.

Legend: SC is soil control, B5 (blend containing 5% of biodiesel in diesel), B25 (blend containing 25% of biodiesel in diesel) and B50 (blend containing 50% of biodiesel in diesel) *different letters (a, b and c) in the same column are statistically different among treatments using the Tukey test P<0.05 level.

These results suggest that the addition of organic matter and nutrients present in biodiesel was decisive for the observed increase in microbial metabolism. This corroborates with results found by Meyer et al. [30] in which the increase in amount of nutrients like nitrogen and phosphorus increased the microbial metabolism. Lapinskiene et al. [31] also reported that increasing the percentage of biodiesel in soil from 1% to 12% increased dehydrogenase activity over the 6 days of incubation.

The treatments with the highest respiration rates were also those with the highest dehydrogenase activity (B50 > B25 > B5). A wide variety of dehydrogenases group act in the oxidation processes of organic compounds. Therefore, the dehydrogenase activity can be used also as an indicator of biodegradation activity [32].

The dehydrogenase activity provided a quick information about the impact of blends on soil microbiota activity, while the respirometric method allowed to monitor the biodegradation throughout a longer period of evaluation. Thus, these methods can be conciliated in biodegradation studies to provide us a better evaluation of results obtained in tests.

4. Discussion

There are many investigations about biodegradation of diesel/biodiesel blends, in which an increase in biodegradability when added biodiesel was demonstrated [11-18]. This increase can be attributed to the fact that fatty acid methyl ester (FAME) present in biodiesel are a better carbon source compared to petroleum hydrocarbons to support microbial growth [33]. Also, this increased mineralization rate can be attributed to better solubility of hydrocarbons in the presence of biodiesel [34, 35].

In this study, treatments containing higher biodiesel amount showed higher CO_2 production and also with higher dehydrogenase activity (B50>B25>B5). Dehydrogenase activity can be used as an indicator of contamination, as well as the biodegradation activity, since a large group of dehydrogenases act on oxidation processes of organic compounds [32]. Lapinskiene et al. (2006) found a similar result, reporting that the activity of dehydrogenases constantly decreases with increasing concentration of diesel fuel.

According to Pasqualino et al. (2006) and Zhang et al. (1998) there is a positive synergistic effect of adding biodiesel to diesel, because the microorganisms utilizes the fatty acids of biodiesel as a source of energy to promote the degradation of diesel. Prince et al. (2008) in a study of aerobic biodegradation of a blend containing 20% of biodiesel in diesel found that methyl esters of fatty acids were degraded in a similar speed of n-alkanes present in the diesel fuel, checking again a relation between the degradation of fatty acids and of hydrocarbons.

5. Conclusion

With the results obtained in Bartha and Pramer respirometric tests and dehydrogenase tests it was concluded that there is a correlation between the amount of biodiesel added to diesel fuel and the increase in microbial activity when exposed in the soil. Based in actual data provided in this paper, it is clear that addition of biodiesel in diesel stimulates the microbial activity in soil.

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