Comparison between biological and chemical approaches towards the biodegradation of diesel/biodiesel blend

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1. Abstract

The rapid rise in crude oil prices combined with the predicted decrease in oil supplies in the near future has led to an increased notoriety of alternative fuels. There is the expansion of the market for fuels derived from renewable sources, mostly ethanol for automobiles and biodiesel for trucks, buses, tractors, and maritime transport. In addition to diversifying the energy matrix, biodiesel has some advantages from an environmental point of view in relation to petroleum diesel, such as higher biodegradability. Environmental accidents with oil and by-products cause considerable damage to the environment, generating a huge public concern, pushing for quick and cost-effective solutions. When released into the environment, the biodegradability of petroleum products depends not only on the intrinsic biodegradability of the pollutant, but also on the presence of adapted microorganisms. The objective of this study was to evaluate if the addiction of *Bacillus subtilis* inoculum and chemical surfactant Tween® 80 (separated and together) would assist the biodegradation of a 5% biodiesel/diesel blend in soil. The measurements of the bioremediation were obtained by respirometric tests and the CO_2 production data were fitted to mathematical model to better describe the biodegradation kinetics. As results, the CO_2 production by respirometric method showed that the addition of *Bacillus subitilis* inoculum and surfactant could either benefit or decrease the biodegradation rate of blends. Also, the modelled data provided new insights about the compounds behavior to predict and optimize the process.

Keywords: *Bacillus subtilis*, Tween® 80, respirometry, modelling.

2. Introduction

The development of alternative fuels, such as biodiesel, has gained notoriety for the last decades. The increased market value of crude oil, along with the projected decreases in its supply in the near future has urged the development of new energy sources. The environmental contamination due to combustion and accidental spills of petroleum byproducts has further expanded the need for environmentally safe biofuels.

Diesel oil is composed of a complex mixture of non-aqueous and hydrophobic compounds such as alkanes and branched chain hydrocarbons [1-2]. Biodiesel is derived from vegetable or animal oils with properties similar to those of petroleum diesel, being possible to make mixtures of these fuels and use them in cars without adjusting or replacing the engines.

A major environmental problem related to fuels refers to transport and storage processes, where spills can lead to contamination of soil and surface and groundwater. When in contact with the environment, the petroleum derivatives have slow biodegradation, since they constitute of non-polar compounds, that is to say, hydrophobic and recalcitrant.

To try to improve the biodegradation of these compounds one can make use of surfactants and microbial organisms. The non-ionic surfactant Tween® 80 results in the dissolution of hydrophobic substances in water, and thus, allows its biodegradation by microorganisms [3]. A microorganism that has shown good results in the decontamination of environments in cases of spills is the bacterium *Bacillus subtilis* [4-5]. In this sense, the biodegradation of the mixture of 5% biodiesel in diesel as well as the action of Tween® 80 surfactant and *Bacillus subtilis* bacterium in the biodegradation was investigated.

3. Methodology

3.1 Fuel Blends

Both pure diesel oil and biodiesel from soybean were acquired from a Brazilian refinery. Mixtures containing 5% of biodiesel in diesel were utilized in the assays.

3.2 Soil

Soil samples were collected according to Brazilian Standards NBR-14283 [6], in an area at University of State of São Paulo (UNESP), in Rio Claro - SP, 22º 23'S and 47º 32' W and sieved at 1.5 mm granulometry. They were characterized by the soil laboratory of the University of São Paulo (USP) according to the methodologies from Raij et al. [7] and Korndorfer et al. [8]. Microbial activity was determined by measuring respiratory [9].

3.3 Determination of CO_2 using Bartha´s method modified

Soil samples (50 g) were contaminated with B5 by adding 100 mL of fuel blends/kg of soil. The microbial activity, which was proportional to the biodegradation in our assays, was determined every 3 days (72 h) by measuring the amount of CO_2 released throughout 106 days of incubation at 28°C, following methodology described in Strotmann et al. [10]. Assays were run in triplicate, including the controls.

Table 1. Compositions of respirometrics flasks

Treatment	Composition	Sample	*B. subtilis*	Tween® 80 (10% v/v)	H_2Od
		Volume (mL)			
CSoil	Soil + distilled water	-	-	-	14
Cb	Soil + inoculum + distilled water	-	3	-	11
Cs	Soil + surfactant + distilled water	-	-	6	8
Cbs	Soil + inoculum + surfactant + distilled water	-	3	6	5
B5	Soil + B5 + distilled water	5	-	-	9
B5b	Soil + B5 + inoculum + distilled water	5	3	-	6
B5s	Soil + B5 + surfactant + distilled water	5	-	6	3
B5bs	Soil + B5 + inoculum + surfactant	5	3	6	-

3.4 Kinect Models

Blends degradation kinetics on soil was performed according to the CO_2 production profile expected in respirometry, proposed by Montagnolli et al. [11], adapted from Schmidt et al. [12].

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

$$R = Rmax / (1+ ((Rmax – R0) /R0) *exp (-r*t)) \qquad \text{Eq. (1)}$$

where R is the CO_2 produced, Rmax is the maximum of CO_2 produced, R0 is the initial CO_2 produced, r is the specific production rate, and t is the time in which the biodegradation occurs. These parameters allowed an in-depth analysis of changes in the biodegradation kinetics according to the biodiesel concentration.

4. Results

4.1 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 our assays had high levels of organic matter, and micronutrients such as iron and manganese when compared to other studies on the biodegradation of biodiesel and diesel blends [13].

Table 2. 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.L¹))	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 [14].

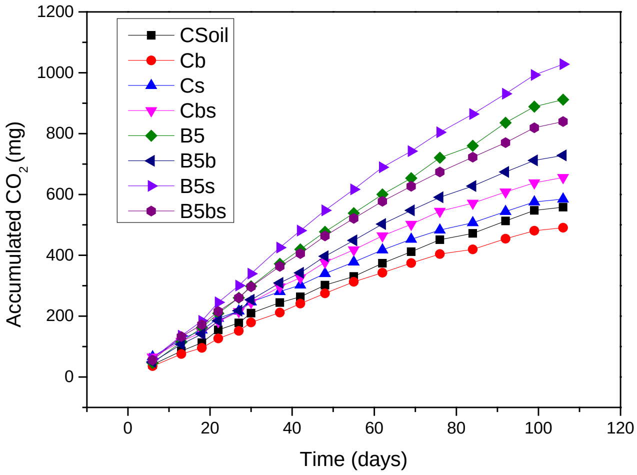
The sandy soil (Table 2) had a lower water retention capacity and high susceptibility to erosion. Nevertheless, microbial growth was promoted in our assays due to high levels of organic matter, CEC, sum of bases and micronutrients like iron and manganese when compared to the values found by Junior et al. [13] of the soil used for the biodegradation of biodiesel blends in diesel by *Candida viswanathii*.

There was a noticeably high development of filamentous fungi during the incubation period. Macroscopic micellar structures in respirometric flaks were observed, possibly linked to organisms that were partially responsible for the acquired biodegradation rates. The fungal growth was expected, as the given pH conditions promoted their growth [15].

4.2 CO_2 evolution

The accumulated CO_2 of B5, B5b, B5s, B5bs and their respective controls are presented in Fig. 1. The addition of blends increased the soil microbial activity, especially in B50 mixtures. The CO_2 production values of soil control (CSoil) were subtracted from the blends dataset.

Fig. 1 Biodegradation rates of samples containing 5% of biodiesel in diesel in soil. CSoil: soil control, Cb: *B. subtilis* inoculum control, Cs: surfactant control, Cbs: *B. subitilis* inoculum + surfactant control, B5: 5% of biodiesel in diesel, B5b: 5% of biodiesel in diesel + *B. subitilis* inoculum, B5s: 5% of biodiesel in diesel + chemical surfactant, B5bs: 5% of biodiesel in diesel + *B. subitilis* inoculum + chemical surfactant.



The study showed that the addition of *B. subtilis* inoculum and surfactant impacted the biodegradation of diesel/biodiesel blends and that the bacteria inoculum was not a good adjuvant for the biodegradation process analyzed in our study. It also revealed that, the respirometry accompanied by mathematical modeling are good methods for elucidating questions related to biodegradation of diesel/biodiesel blends.

Table 3 shows the accumulated CO_2 in the last day of the respirometric assays to easily compare the biodegradation rates in the different treatments. Our results are further discussed based on the values of CSoil subtracted from the accumulated CO_2 datasets. The CSoil values were subtracted as they represent the basal respiration of the native soil microbiota in each assay.

Table 3. Accumulated CO_2 produced by the end of the respirometric test

Treatment	Accumulated CO_2 (mg/50g)
Cb	490.6581
Cs	584.8312
Cbs	655.6531
B5	911.7468
B5b	728.3539

B5s	1027.99
B5bs	839.6561

Considering the different treatments for each sample, the highest CO_2 production by the treatments was ascendingly ordered as: B5s> B5> B5bs> B5b. The surfactant presented higher CO_2 production, being 11.29% more than in the pure sample, 29.11% in the sample with inoculum and 18.3% in the sample with both (Table 3). This is due to the ability to change the surface and interfacial properties of a liquid by decreasing the surface tension between the contaminant and the medium [16-17].

A common feature of all petroleum fractions is their low solubility in water, which hampers the microbial action in degrading them [18-21]. Thus, the action of the surfactant provided better contact of the microorganisms with the oil and then its use in the microbial metabolism. Other studies have shown a positive effect of another surfactant, Corexit, on the biodegradation of oil by mixed bacterial communities [22].

Therefore, the action of the *B. subtilis* inoculum was detrimental to the biodegradation of the sample possibly because it generated competition with the microbiota already present in the fuels and soil used in the test.

4.3 Kinect of Biodegradation

The Figure 2 shows the kinetic model according to Schmidt et al. [12] and modified by Montagnolli et al. [11]. Considering the data for modeling according to Montagnolli [11], it is possible to estimate the total time of biodegradation. Tendency for stabilizing the biodegradation process initiates after 138 days for B5, 115 days for B5b, 135 for B5s and 127 days for B5bs.

Fig. 2 Kinetics model in CO_2 evolution results. B5: blend containing 5% of biodiesel in diesel; B5b: blend containing 5% of biodiesel in diesel + inoculum of *B. subtilis*; B5s: blend containing 5% of biodiesel in diesel + surfactant and B5bs: blend containing 5% of biodiesel in diesel + inoculum of *B. subtilis* + surfactant.

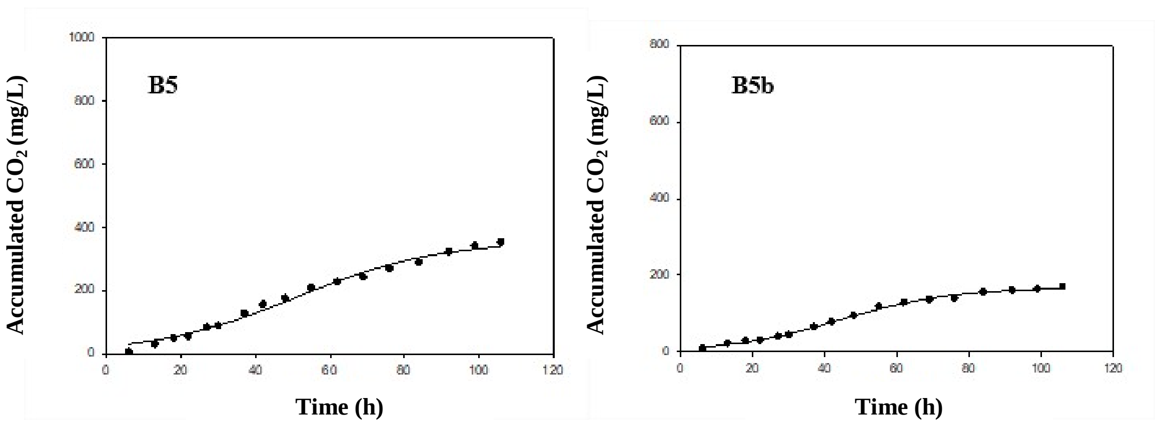
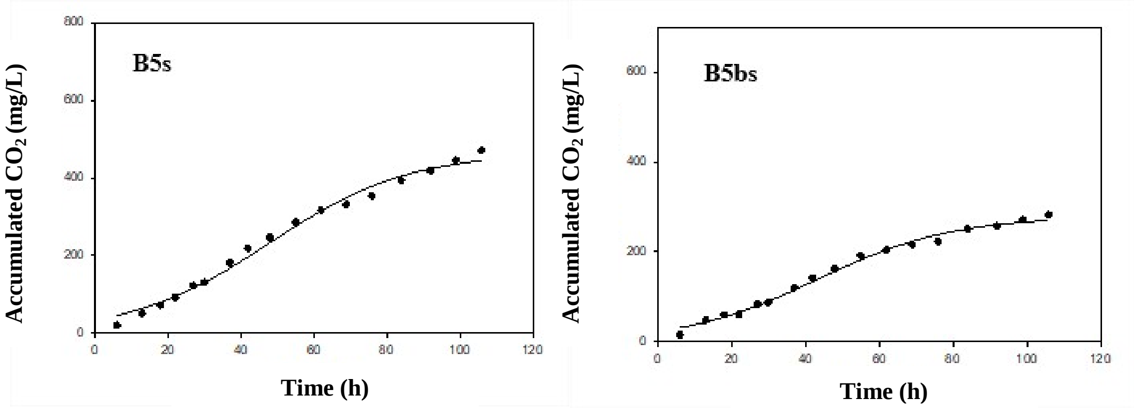


Fig. 2 (continued)



The values of R^2, the estimated time for biodegradation (RRminT) and the corresponding maximum CO_2 production by respirometric systems (Rmax) are shown in Table 4.

Table 4. The corresponding maximum CO_2 production by respirometric systems (Rmax), the estimated time for biodegradation (RRminT) and values of R^2

Treatment	Rmax (mg)	RRminT (days)	R^2
B5	358.14	138	0.98
B5b	167.86	115	0.99
B5s	465.99	135	0.98
B5bs	278.75	127	0.99

Legend: B5: blend containing 5% of biodiesel in diesel; B5b: blend containing 5% of biodiesel in diesel + inoculum of *B. subtilis*; B5s: blend containing 5% of biodiesel in diesel + surfactant and B5bs: blend containing 5% of biodiesel in diesel + inoculum of *B. subtilis* + surfactant.

The value of Rmax is established when the curve generated by the model stabilizes and no more significant increase occurs in parameter R, defined in the equation as accumulated CO_2 production, over time. Values of $R < 0.99 \times$ Rmax were considered as a significant increase. The biodegradation end time was then considered as the CO_2 production time equal to $R = 0.95 \times$ Rmax [11].

According to Table 11, the highest CO_2 production in the Bartha assays were B5s and B5. These are also the ones that took the longest time for the end of the biodegradation by the bacteria, (135 and 138 days, respectively). These samples also had the highest rates of maximum biodegradation, being 465.99 mg for the B5s and 358.14 mg for the B5 assay.

As previously described, the addition of bacteria "impaired" the biodegradation of fuels, further confirmed by the kinetic modelling of the lowest values of Rmax, respectively 167,86 and 278,75 for B5b and B5bs, and the lowest RRminT, 115 days for B5b and 127 days for B5bs.

Overall, a long period was required for the B5-containing assays to stabilize their biodegradation. This suggests that the higher amount of diesel in these samples (95%) was resistant to microbial biodegradation. According to Meyer et al. [23], this is due to the presence of aliphatic compounds and aromatic hydrocarbons, which led to a more difficult biodegradation than the long chain esters present in biodiesel. Also, according to Owsianiak et al. [24], mixtures with less than 10% biodiesel in diesel cause microorganisms to preferentially degrade the fatty acid methyl ester (FAME) of the biodiesel, triggering the deceleration of the assimilation of the n-alkanes of the diesel. Our kinetic studies corroborate with a study realized by DeMello et al. [25], in which they report a delay in starting the biodegradation of diesel/biodiesel mixtures caused by the presence of fatty acid methyl esters. Such esters may delay the onset of biodegradation of some fossil diesel compounds, although they are, together with n-alkanes, readily biodegradable fractions.

5. Conclusions

With the results obtained at this research, we can conclude that the addition of surfactant Tween 80 (10% v/v) for the mixture of 5% of biodiesel in diesel was beneficial for its biodegradation, being more suitable than the addition of *B. subtilis* inoculum.

6. References

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