

# Biodegradation of Biodiesel in Laboratory and Field in a Clayey Residual Soil

A. Thomé, A.W.S. Trentin, I. Cecchin, C. Reginato

**Abstract.** This study compared the process of biotic and abiotic degradation of biodiesel in a clayey soil in a laboratory environment (*ex situ*) and infiel environment (*in situ*). The experiment carried out *ex situ* had controlled temperature and humidity, while the experiment *in situ* was directly influenced by external factors such as rainfall and temperature. The soil was collected deformed and contaminated with 4% of biodiesel in relation to dry weight (m/m). The bioreactors were molded under the same initial parameter conditions of the field. The experiment lasted for 120 days and contaminant reduction measurements were made throughout the experiment. At the end of the experiment, there was a reduction of 89% in the contaminant initially added to the soil in the *ex situ* experiment, whereas in the *in situ* experiment there was a reduction of 32%. The main conclusion is that the laboratory experiment provided better results of biodegradation, since it was possible to perform an effective control of abiotic factors, such as temperature, humidity and pH. However, these results are not representative of the actual behavior in the field.

**Keywords:** abiotic factors, bioremediation, leaching, natural attenuation.

## 1. Introduction

Monitored Natural Attenuation (MNA) is by definition a process that combines all possible reactions that may reduce a contaminant concentration in the environment, such as physical or chemical reactions and biological process. The success of a biodegradation process depends on the intrinsic ability of the system to create and maintain the conditions that promote the biodegradation of pollutants at a sufficiently high rate. (EPA, 1999; Mulligan & Yong, 2004; Bento *et al.*, 2005; Das & Chandran, 2011; Declercq *et al.*, 2012; Sihag *et al.*, 2014; Agnello *et al.*, 2016; Guarino *et al.*, 2017).

Bioremediation techniques can be categorized as *ex situ* when they occur in controlled environments, such as in laboratories, or *in situ* when they occur in the field and environmental conditions are not controlled (Frutos *et al.*, 2012; Smith *et al.*, 2015; Azubuike *et al.*, 2016). Different studies have been carried out to evaluate the effectiveness of *ex situ* bioremediation (Dott *et al.*, 1995; Pasqualino *et al.*, 2006; DeMello *et al.*, 2007; Mariano *et al.*, 2008; Owsiania *et al.*, 2009; Aktas *et al.*, 2010; Corseuil *et al.*, 2011; Sorensen *et al.*, 2011; Borges *et al.*, 2014; Agnello *et al.*, 2016; Guarino *et al.*, 2017; Decesaro *et al.*, 2017), or *in situ* bioremediation (Dott *et al.*, 1995; Sendzikiene *et al.*, 2007; Ramos *et al.*, 2013). However, a direct comparison, with the same soil conditions and the same contaminant, of the monitored natural attenuation technique when applied

*in situ* and *ex situ* was not found. The influence of infiltration of water in the soil due to rainfall and the influence of the variation of ambient temperature on the biodegradation of biodiesel in residual clayey soil are not yet known. Therefore, studies seeking to understand the response of the monitored natural attenuation technique when applied *in situ* and *ex situ* environments are important for the scientific and technical remediation field.

The objective of this study is to evaluate biodegradation by monitored natural attenuation in experiments in a controlled environment (*ex situ*) and non-controlled (external) environments (*in situ*) using a simulated contamination of biodiesel in a residual clayey soil.

## 2. Materials and Methods

### 2.1. Soil

The clayey soil was obtained from the Geotechnical Experimental Site at the University of Passo Fundo, Southern Brazil. It was collected at 2 meters depth (B horizon) in an open pit, and represents a typical Brazilian basaltic residual soil. Table 1 shows the geotechnical and physical properties of the soil (ASTM, 2017a; ASTM, 2017b; ASTM, 2017c; ASTM, 2017d; ASTM, 2017e; ASTM, 2018a; ASTM, 2019). The soil is classified as an Oxisol (Streck *et al.*, 2008) and is a high plasticity clay (CH) according to the Unified Soil Classification System (ASTM, 2017f). As can be seen in Table 1, the soil has a relatively

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**Table 1** - Geotechnical and physicochemical characteristics of the clayey soil.

Parameter (unit)	Value
Clay (%)	68
Silt (%)	5
Fine sand (%)	27
Liquid limit (%)	53.0
Plastic limit (%)	42.0
Particle unit weight (kN/m <sup>3</sup> )	26.7
Natural moisture content (%)	34
Natural unit weight (kN/m <sup>3</sup> )	16.3
Void ratio	1.2
Degree of saturation (%)	75.7
Porosity (%)	54
pH	5.4
Organic matter (%)	< 0.8
Cation exchange capacity-CEC (cmolc/dm <sup>3</sup> )	8.6
Hydraulic conductivity (m/s)	1.39 x 10 <sup>-5</sup>

acidic pH, high clay content and a low Cation Exchange Capacity (CEC) (ASTM, 2018b; ASTM, 2018c). These characteristics are representative of a typical soil with a predominance of clay-mineral kaolinite due to its structural conformation. X-ray diffraction analysis indicated that the clay fraction is composed of approximately 70% kaolinite and 30% oxides (Fe and Al). The organic content of the soil is low due to the greater depth at which the soil was collected (ASTM, 2014). The soil is very porous, with a high

void ratio, characteristic of residual clays, and these characteristics allow a high permeability.

## 2.2. Contaminant characteristics (biodiesel)

Pure soybean biodiesel (B100) was used in this study to simulate organic contamination in a typical fuel spill/leak situation. Biodiesel has a high flash point ( $104.2 \pm 5.0$  °C) and 77% less particulate matter emissions when compared to regular diesel. Biodiesel also has a higher level of biodegradability and a lower volatility than regular diesel. It is insoluble in water and has a density of 889 kg/m<sup>3</sup>. Moreover, it does not contain sulphur and aromatic compounds (Silva & Corseuil, 2012; Meneghetti *et al.*, 2012; Thomé *et al.*, 2014), but contains mainly methyl esters highly hydrophobic due to the presence of long carbon chains, which results in a very low solubility in water (Wedel, 1999). Table 2 presents the main characteristics of the methyl esters present in the regular soy biodiesel.

## 2.3. Assembly of the experiment *ex situ* (laboratory)

The disturbed soil sample was contaminated with 4.0% biodiesel over the dry weight of soil (m/m), equivalent to a soil contamination of 40 g/kg. Previous studies in laboratory have shown that values above this value cause leaching of the contaminant (Cecchin *et al.*, 2016; Thomé *et al.*, 2017). The collected soil was dry until reaching 20% of moisture content. It was sieved in a 2 mm sieve (ASTM No.10) for homogenization, and then water was added until it reached a 30% moisture. After homogenized, the 4% of biodiesel was added, and the soil returned to the field moisture content of 34%. The experiment was set up in aluminum cylinders (24 cm in diameter and 24 cm in height), with a volume of 108 cm<sup>3</sup> (Fig. 1). The bottom of the cylin-

**Table 2** - Physicochemical properties of methyl esters of soybean oil.

Properties	Compound				
	Methyl Palminate	Methyl Stearate	Methyl Oleate	Methyl Linoleate	Methyl Linolenate
Molecular formula	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	C <sub>19</sub> H <sub>32</sub> O <sub>2</sub>
Molecular mass (g mol <sup>-1</sup> )	270.46	298.51	296.49	294.49	291.46
FP (°C)	30.5 <sup>(2)</sup>	39.0 <sup>(2)</sup>	-20.0 <sup>(2)</sup>	-35.0 <sup>(2)</sup>	-52.0 <sup>(3)</sup>
BP (°C)	415-418 <sup>747(2)</sup>	442-443 <sup>747(2)</sup>	218.5 <sup>20(2)</sup>	215.0 <sup>20(2)</sup>	182.0 <sup>3(4)</sup>
Relative density	0.852 <sup>(5)</sup>	0.850 <sup>(5)</sup>	0.874	0.889	0.895 <sup>(5)</sup>
Solubility Water (25 °C) (mg L <sup>-1</sup> )	Insoluble <sup>(6)</sup>	Insoluble	Insoluble	Insoluble <sup>(6)</sup>	Insoluble <sup>(6)</sup>
Vapor pressure (atm)	4.99 × 10 <sup>-8</sup>	5.85 × 10 <sup>-9</sup>	9.72 × 10 <sup>-9</sup>	1.15 × 10 <sup>-8(7)</sup>	1.14 × 10 <sup>-8(7)</sup>
Henry's constant	1.37 × 10 <sup>-1</sup>	2.34 × 10 <sup>-1</sup>	3.23 × 10 <sup>-2</sup>	6.54 × 10 <sup>-3(7)</sup>	1.39 × 10 <sup>-3(7)</sup>
Log K <sub>ow</sub> (25 °C)	7.38	8.35	7.45	6.82 <sup>(7)</sup>	6.29 <sup>(7)</sup>
Log K <sub>oc</sub> (25 °C)	4.26	4.79	4.79	-	-

Note: FP: Fusion Point; BP: Boiling Point.

Source: TOXNET (2018);<sup>(1)</sup>NIST (2018);<sup>(2)</sup>Knothe (2005);<sup>(3)</sup>Foon *et al.* (2006);<sup>(4)</sup>SCIENCE LAB.COM (2018);<sup>(5)</sup>SIGMA-ALDRICH (2018);<sup>(6)</sup>TGSC (2008);<sup>(7)</sup>Krop *et al.* (1997).

ders was filled with inert sand to level the useful volume of all cylinders used. The soil volume was calculated according to the useful area of the cylinder, considering the soil field characteristics for moisture (34%), natural unit weight ( $16.3 \text{ kN/m}^3$ ) and void ratio ( $e = 1.19$ ). The total soil mass contained in each bioreactor was divided into 5 equal parts; for each part, compaction was performed within the bioreactor vessel at the natural field density. Bioreactors were assembled and kept in a laboratory with a controlled temperature of  $24^\circ\text{C}$  during the whole time.

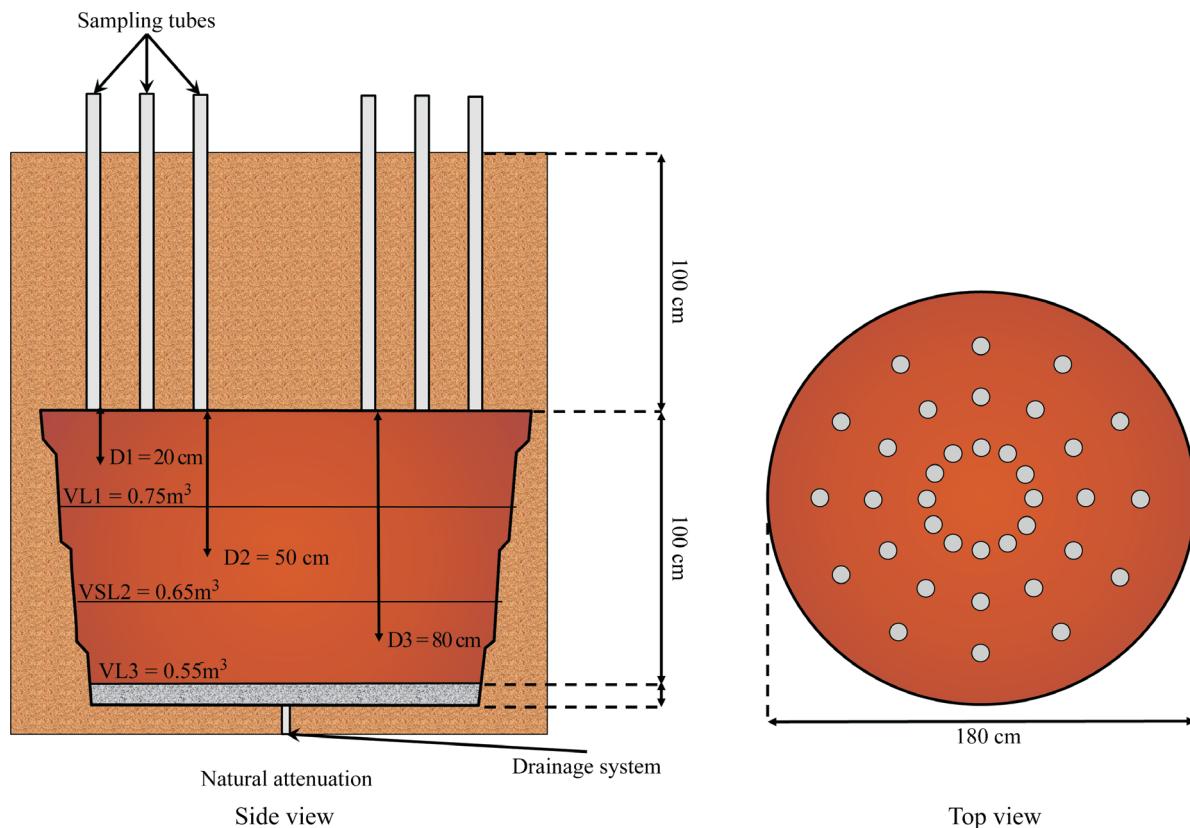
Samples were collected at 15, 30, 45, 60, 90 and 120 days. It was used a hollow metal rod of 1 cm in diameter (direct push) to collect the samples. The metal rod was carried to the depth at which it reached the drain. Upon removal from the test specimen, the contained soil therein was homogenized, and 15 g were separated to verify the biodiesel content at that time, in triplicate. After the samples were collected, the empty site was filled with clean sterile sand.

#### 2.4. Assembling the experiment *in situ* (field)

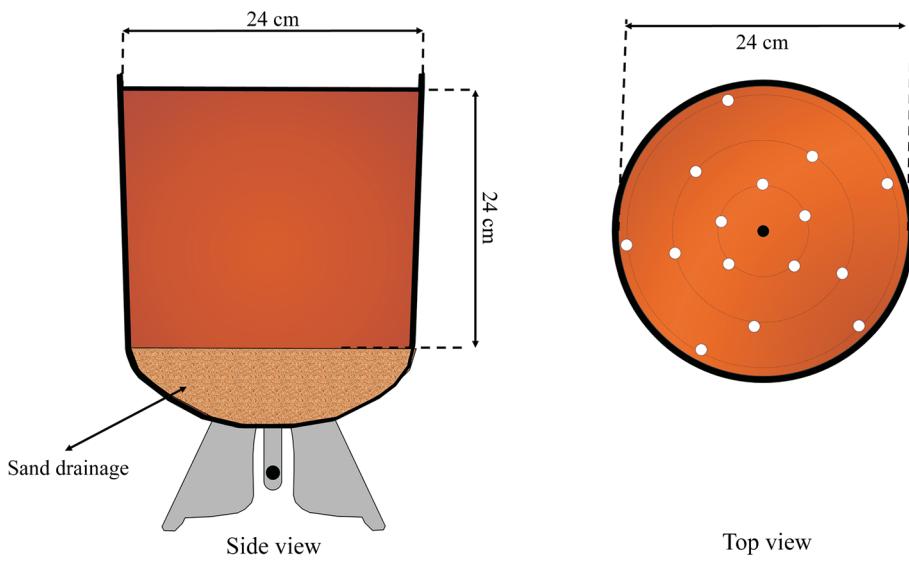
The assembly of this reactor included the use of a polyethylene box with a  $2 \text{ m}^3$  capacity. The total volume of contaminated soil used was  $1.95 \text{ m}^3$ , with  $0.75 \text{ m}^3$  in the layer 1 (VL1),  $0.65 \text{ m}^3$  in layer 2 (VL2) and  $0.55 \text{ m}^3$  in layer 3 (VL3) (Fig. 2). At the base of the reactor, a percolated drainage system was installed, providing leachate material

collected on rainy days. The molding process was similar to that of the laboratory experiment. The soil was homogenized and mixed with water until it reached 30% moisture content, and then 4% of contaminant (97.4 kg) was added and homogenized to achieve the natural soil moisture in the field, *i.e.*, 34%. Considering the large volume of soil, the soil compaction process was carried out in layers of 10 cm thickness. This was performed so that the soil density control was more efficient and became as close as possible to the natural field density ( $16.3 \text{ kN/m}^3$ ) and the natural void ration ( $e = 1.19$ ).

A 2 meter deep trench was excavated on a slope of the experimental field, and the box was placed at the bottom. At soil sampling, guide tubes were left at pre-determined points, and a backfill was performed with local natural soil (Fig. 2). Due to the large size of the bioreactor, three sampling depths were defined in relation to the natural soil surface, that is, depth 1 (D1) at 0.2 m from the bioreactor surface, depth 2 (D2) at 0.5 m, and depth 3 (D3) at 0.8 m. The soils collected at each depth were homogenized and taken to the laboratory to determine the residual contaminant content. This determination was performed in triplicate. The samplings occurred in the same period of the laboratory study (*ex situ*), that is, at 15, 30, 45, 60, 90 and 120 days. As a response variable, the percentage of contaminant reduction was evaluated over the experimental time at each depth.



**Figure 1** - Illustration of the bioreactor used in the laboratory experiment (*ex situ*).



**Figure 2** - Illustration of the bioreactor used in the field experiment (*in situ*).

In the case of application *in situ*, elements such as soil temperature, humidity and infiltration were not controlled. The field experiment was set up in January, which is summer in the Southern Hemisphere. By the Köppen classification, Passo Fundo (28°13'36" S, 52°23'13" W and 687 m altitude) is located in the fundamental temperate climate zone (C), presenting a humid (f) and a subtropical specific variety (Cfa). Thus, the local climate is described as humid subtropical (Cfa), with rainfall well distributed during the year, and average temperature of the hottest month exceeding 22 °C. Table 3 shows the mean, minimum and maximum temperatures and mean rainfall in the months during which the experiment was performed (EMBRAPA, 2018).

## 2.5. Analyses of experiments *ex situ* and *in situ*

The residual free phase contaminant content for the *ex situ* experiment and for the *in situ* experiment was determined by the quantification of oils and greases after the homogenization of soil samples. The extraction was performed using a UNIQUE® probe ultrasound, according to the methodology of USEPA 3550B.

In both experiments, the residual content of contaminant extracted was calculated by Eq. 1.

$$RC = \frac{W_{B+C} - B_w}{D_w} \quad (1)$$

onde  $RC$  = residual of contaminant present in test specimen (g/kg),  $D_w$  = quantity of soil in dry weight used in the analysis (g),  $B_w$  = weight of the beaker (g) e  $W_{B+C}$ : weight of the beaker plus the weight of the contaminant extracted from the soil (g).

## 3. Discussion

### 3.1. Evaluation of the residual content of experiment *ex situ* (laboratory)

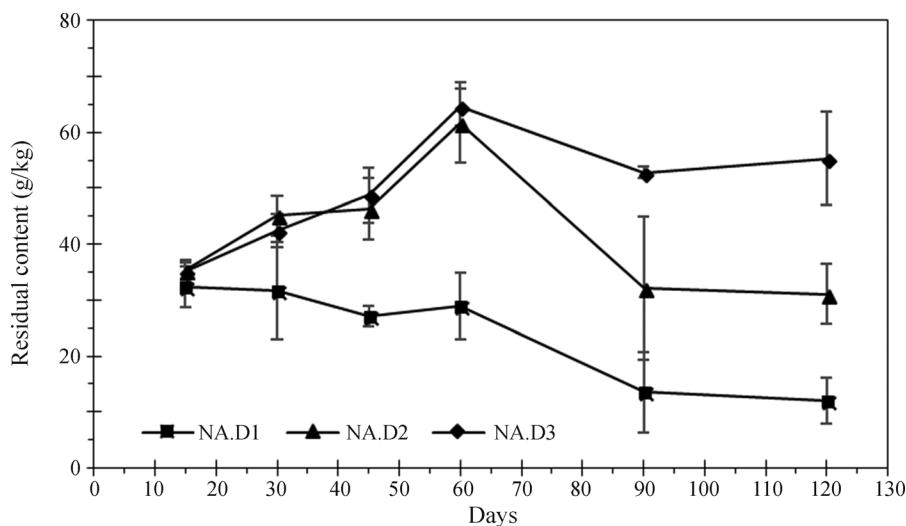
Figure 3 shows the residual biodiesel content in the different sample periods of the experiment *ex situ*, and the data presented represent the mean of the values obtained from both bioreactors used in the experiment.

Considering the initial value of contaminant added to the soil (40 g/kg), the contents of the contaminant decreased with time. After 15 days of experiment, the residual content was 24 g/kg, which represents a decrease of 40% in the initial contamination, the same value observed in the sampling performed in the period of 30 days of experiment.

The values observed in the samplings carried out after 45 and 60 days of experiment presented the same percentage of decrease in contaminant (60%), remaining with

**Table 3** - Average, minimum and maximum temperatures and average rainfall during the field experiment (*in situ*).

	January	February	March	April
Mean temperature (°C)	22.1	21.9	20.6	17.6
Higher temperature (°C)	28.3	28.0	26.7	23.7
Lower temperature (°C)	17.5	17.5	16.3	13.5
Rainfall (mm)	143.4	148.3	121.3	118.2



**Figure 3** - Mean residual biodiesel content in the different sampling periods in the laboratory experiment.

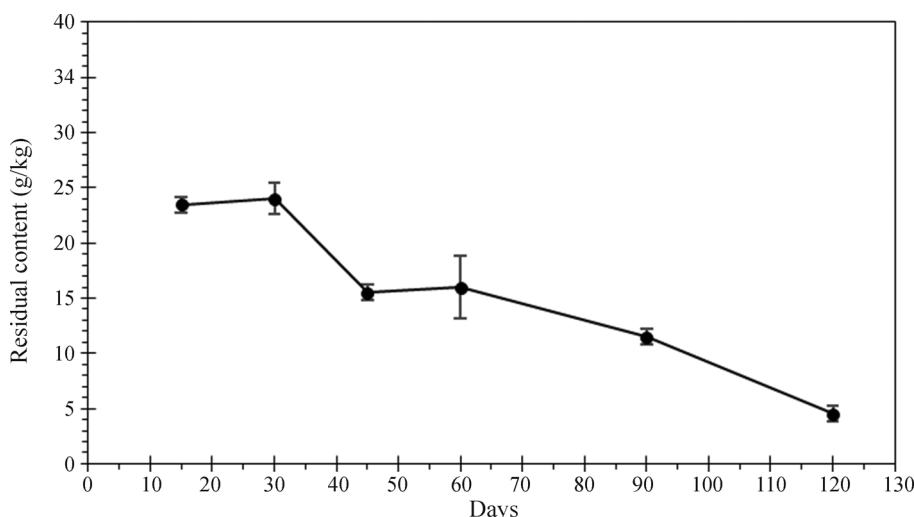
15 g/kg of biodiesel. For the sampling performed at 90 days, the residual content decreased to 11.5 g/kg, that is, a degradation of 72.5%. After 120 days of experiment, the percentage of contaminant decreased to 4.5% and reached 89% of the degradation, that is, only 11% of the value added. This reduction is due to the action of microorganisms, since biodiesel is non-volatile at a temperature of 24 °C, and no contaminant leached through the drainage system of the bioreactor.

### 3.2. Evaluation of the residual content of experiment *in situ* (field)

Figure 4 shows the residual biodiesel contents at different depths during the period of the field experiment (*in situ*). As in the experiment *ex situ*, the data presented represent a mean of the values obtained at each of the depths analyzed.

Figure 4 shows that the lowest residual pollutant results during the experiment period were observed at depth 1 (NA.D1). The contaminant content at 15 days was 32 g/kg for depth 1 and 35 g/kg for depths 2 and 3. These values are below the 40 g/kg initially used in the experiment, representing an average reduction of 16% in the initial contamination. It should be noted that the field experiment was set up in January, the hottest month in the Southern Hemisphere, with an average temperature of 22.1 °C, but average maximum of 28 °C. Another fact that should be mentioned is that during the first 15 days of the experiment, no rainfall occurred. Therefore, the environmental conditions of the first 15 days of the experiment were very similar to those that occurred in the laboratory.

Between 15 and 30 days of the experiment, a 32 mm rainfall with several cloudy days and below-average temperature was observed. This rainfall and temperature influ-



**Figure 4** - Average residual biodiesel content at different depths for the field experiment.

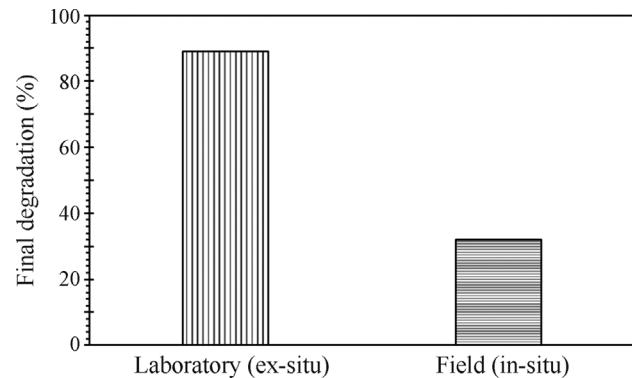
enced greatly the results of residual content. Fig. 4 shows that at depth 1, the mean value was 30 g/kg, which is very close to that obtained at 15 days. At depth 2, the mean value was 45 g/kg, and at depth 3, the mean residual value was 42 g/kg. These values above the value initially added indicate that biodiesel leaching occurred from the upper to the lower layers. This finding becomes acceptable when we observed that the residual contents at depths 2 (NA.D2) and 3 (NA.D3), up to the 60 days of experiment, increased gradually, reaching values of 65 g/kg. It is noteworthy that, in this period, there was a total rainfall of 155 mm. A factor that draws attention is that at depth 1, the residual value remained practically constant at the measured points. We concluded that this occurred because the guide tubes were placed for sample removal, and these tubes were closed with a cap to prevent the entry of animals. This may have influenced the volume of water entering these depths, not changing the residual value. A significant decrease in contaminant was observed at this depth, reducing to 14 g/kg at 90 days and to 12 g/kg at 120 days.

The final contents observed in the experiment were 12 g/kg for depth 1, 31 g/kg for depth 2 and 55.4 g/kg for depth 3. Therefore, it can be concluded that, in a place where natural attenuation is being monitored *in situ*, precautions should be taken to prevent water from entering the soil. This may mask degradation results and, depending on where the sample was taken, it can be concluded that biodegradation has occurred. However, in fact, what occurs is contaminant leaching from upper to lower layers.

To calculate the amount of biodiesel degraded in the *in situ* experiment, the difference in volume of soil at the different depths should be considered. For depth 1 the total residual biodiesel mass was 10 kg ( $12 \text{ g/kg} \times \text{dry soil in layer 1}$ ), for depth 2 the biodiesel mass was 24 kg ( $33 \text{ g/kg} \times \text{dry soil in layer 2}$ ) and for depth 3 was 33 kg ( $55.4 \text{ g/kg} \times \text{dry soil in layer 3}$ ). The total amount of the biodiesel mass at the end of the experiment was 67 kg, *i.e.* 68.7% of the value initially added (97.4 kg). Therefore, it can be stated that the amount of contaminant degraded in the *in situ* experiment was 32%. The amount of contaminant obtained in the drainage liquid was negligible. Probably the geotextile placed as a separator served as a filter element for the biodiesel.

### 3.3. Comparison of degradation in laboratory and in field

Figure 5 shows the percentages of degradation of biodiesel after 120 days of experiment *ex situ* and *in situ*. By analyzing Fig. 5, the percent degradation in the experiment *ex situ* was 89%, while for the experiment *in situ* was 32%. The degradation in the field was 3 times lower than the laboratory degradation. This finding leads to conclude that the abiotic effects of climate directly affect the efficacy of bioremediation, making the results obtained in the field (*in situ*) worse when compared with laboratory data (*ex*



**Figure 5** - Percentage degradation after 120 days of experiment *ex situ* and *in situ*.

*situ*). The control of the entry of water into the soil must occur in *in situ* experiments to avoid problems of erroneous analysis of results. This is because, in the case of installing a monitoring well at a fixed depth, reducing the amount of contaminant in the site can be attributed to biodegradation, whereas in fact it is due to leaching of the contaminant to deeper layers.

## 4. Conclusions

This study evaluated the difference in behavior of biodegradation in laboratory and in field experiments, considering the moisture and density characteristics of the residual clayey soil. From the results obtained, it was possible to reach the following conclusions:

- The conduction of the experiment in laboratory provided better results of biodegradation, since it is possible to perform an effective control of abiotic factors, such as temperature, humidity and pH. However, these results are not representative of the actual behavior in the field.

- The field experiment allowed identifying the migration and leaching of the contaminant to lower layers of the reactor, since there was a considerable reduction in the volume of contaminant in the top soil and a progressive increase in the contaminant at lower soil depths.

- At the end of the experiment, the total contaminant reduction in the field was 3 times lower than the contaminant reduction in laboratory. This leads to the conclusion that abiotic factors directly influence the efficiency of bioremediation, especially the natural attenuation.

- Waterproofing the soil surface is suggested in places where natural attenuation monitoring is carried out *in situ* to avoid leaching, thus preventing an erroneous analysis of biodegradation.

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## References

- Agnello, A.C.; Bagard, M.; Van Hullebusch, E.D.; Espo-  
sito, G. & Huguenot, D. (2016). Comparative biore-  
mediation of heavy metals and petroleum hydrocarbons  
co-contaminated soil by natural attenuation, phytore-  
mediation, bioaugmentation and bioaugmentation-as-  
sisted phytoremediation". *Science of the Total  
Environment*, 563-564(1):693-703.
- Aktas, D.F.; Lee, J.S.; Little, B.J.; Ray, R.I.; Davidova,  
I.A.; Lyles, C.N. & Suflita, J.M. (2010). Anaerobic me-  
tabolism of biodiesel and its impact on metal corrosion.  
*Energy and Fuels*, 24 (5) 2924-2928.
- ASTM (2014). Standard Test Methods for Moisture, Ash,  
and Organic Matter of Peat and Other Organic Soils -  
D2974-14. ASTM International, West Conshohocken,  
PA, USA, 4 p.
- ASTM (2017a). Standard Test Methods for Particle-Size  
Distribution (Gradation) of Soils Using Sieve Analysis  
- D6913/D6913M-17. ASTM International, West Con-  
shohocken, PA, USA, 34 p.
- ASTM (2017b). Standard Test Methods for Determining  
the Amount of Material Finer than 75- $\mu$ m (No. 200)  
Sieve in Soils by Washing - D1140-17. ASTM Interna-  
tional, West Conshohocken, PA, USA, 6 p.
- ASTM (2017c). Standard Test Method for Particle-Size  
Distribution (Gradation) of Fine-Grained Soils Using  
the Sedimentation (Hydrometer) Analysis - D7928-17.  
ASTM International, West Conshohocken, PA, USA,  
25 p.
- ASTM (2017d). Standard Test Methods for Liquid Limit,  
Plastic Limit, and Plasticity Index of Soils -  
D4318-17e1. ASTM International, West  
Conshohocken, PA, USA, 20 p.
- ASTM (2017e). Standard Test Method for Density of Soil  
in Place by the Drive-Cylinder Method - D2937-17e2.  
ASTM International, West Conshohocken, PA, USA, 8  
p.
- ASTM (2017f). Standard Practice for Classification of  
Soils for Engineering Purposes (Unified Soil Classifi-  
cation System) - D2487-17. ASTM International, West  
Conshohocken, PA, USA, 10 p.
- ASTM (2018a). Standard Test Methods for Laboratory De-  
termination of Density (Unit Weight) of Soil Specimens  
- D7263-09(2018). ASTM International, West Con-  
shohocken, PA, USA, 7 p.
- ASTM (2018b). Standard Test Methods for pH of Soils -  
D4972-18. ASTM International, West Conshohocken,  
PA, USA, 6 p.
- ASTM (2018c). Standard Test Method for Measuring the  
Exchange Complex and Cation Exchange Capacity of  
Inorganic Fine-Grained Soils - D7503-18. ASTM Interna-  
tional, West Conshohocken, PA, USA, 5 p.
- ASTM (2019). Standard Test Methods for Laboratory De-  
termination of Water (Moisture) Content of Soil and  
Rock by Mass - D2216-19. ASTM International, West  
Conshohocken, PA, USA, 7 p.
- Azubuike, C.C.; Chikere, C.B. & Okpokwasili, G.C.  
(2016). Bioremediation techniques-classification based  
on site of application: principles, advantages, limita-  
tions and prospects. *World Journal of Microbiology and  
Biotechnology* 32(180):1-18.
- Bento, F.M.; Camargo, F.A.O.; Okeke, B.C. & Franken-  
berger, W.T. (2005). Comparative bioremediation of  
soils contaminated with diesel oil by natural attenua-  
tion, biostimulation and bioaugmentation. *Bioresource  
Technology*, 96:1049-1055.
- Borges, J.M.; Dias, J.M. & Danko, A.S. (2014). Influence  
of the anaerobic biodegradation of different types of  
biodiesel on the natural attenuation of benzene. *Water,  
Air and Soil Pollution*, 2146:1-10.
- Cecchin, I.; Reginatto, C.; Thomé, A.; Colla, L.M. & Red-  
dy, K.R. (2016). Influence of physicochemical factors  
on biodiesel retention in clayey residual soil. *Journal of  
Environmental Engineering (New York)*, 142(4):  
04015093.
- Corseuil, H.X.; Monier, A.L.; Gomes, A.P.N.; Chiaranda,  
H.S.; Rosario, M.D. & Alvarez, P.J.J. (2011). Biode-  
gradation of soybean and castor oil biodiesel: implica-  
tions on the natural attenuation of monoaromatic  
hydrocarbons in groundwater. *Ground Water Moni-  
toring and Remediation*, 31:111-118.
- Das, N. & Chandran P. (2011). Microbial degradation of  
petroleum hydrocarbon contaminants: An overview.  
*Biotechnol. Res. Int.*, 2011:941810.
- Decesaro, A.; Rampel, A.; Machado, T.S.; Thomé A.; Red-  
dy, K.R.; Margarites, A.C. & Colla, L.M. (2017).  
Bioremediation of soil contaminated with diesel and  
biodiesel fuel using biostimulation with microalgae  
biomass. *Journal of Environmental Engineering*,  
143(4):04016091.
- Declercq, I.; Cappuyns, V. & Duclos, Y. (2012) Monitored  
natural attenuation (MNA) of contaminated soils: State  
of the art in Europe - A critical evaluation. *Science of  
the Total Environment* 426:393-405.
- DeMello, J.A.; Carmichael, C.A.; Peacock, E.E.; Nelson,  
R.K.; Arey, J.S. & Reddy, C.M. (2007). Biodegradation  
and environmental behavior of biodiesel mixtures in the  
sea: an initial study. *Marine Pollution Bulletin*, 54:894-  
904.
- Dott, W.; Feidicker, D.; Steiof, M.; Becker, P.M. & Kämp-  
fer, P. (1995). Comparison of ex situ and in situ tech-  
niques for bioremediation of hydrocarbon-polluted  
soils. *International Biodeterioration & Biodegradation*,  
16:301-313.
- Empresa Brasileira de Pesquisa Agropecuária (EMBRA-  
PA). (2018) Laboratório de Agrometeorologia.  
Disponível em: [http://www.cnpt.embrapa.br/pesquisa/  
agromet/app/principal/agromet.php](http://www.cnpt.embrapa.br/pesquisa/agromet/app/principal/agromet.php).

- EPA, Environmental Protection Agency (1999). Use of monitored natural attenuation at superfund, RCRA corrective action, and underground storage tank sites. OSWER Dir. 1-32.
- Foon, C.S.; Liang, Y.C.; Dian, N.L.H.M.; May, C.Y.; Hock, C.C. & Ngan, M.A. (2006) Crystallisation and melting behavior of methyl esters of palm oil. *American Journal of Applied Sciences*, 3(5):1859-1863.
- Frutos F.J.G.; Pérez, R.; Escolano, O.; Rubio, A.; Gimeno, A.; Fernandez, M.D.; Carbonell, G.; Perucha, C. & Laguna, J. (2012). Remediation trials for hydrocarbon-contaminated sludge from a soil washing process: evaluation of bioremediation technologies. *Journal of Hazardous Materials*, 199:262-271.
- Guarino, C.; Spada, V. & Sciarrillo, R. (2017). Assessment of three approaches of bioremediation (natural attenuation, landfarming and bioaugmentation - assisted land-farming) for a petroleum hydrocarbons contaminated soil. *Chemosphere* 170:10-16.
- Knothe, G.; Gerpen, J.V. & Krah, J. (2005). The Biodiesel Handbook. 1<sup>st</sup> Edition Champaign, Illinois, Ed. AOCS, 286 p.
- Krop, H.B.; Velzen, M.J.M.V.; Parsons, J.R. & Govers, H.A.J. (1997). n-Octanol-water partition coefficients, aqueous solubilities and Henry's Law constants of fatty acid esters. *Chemosphere*, 34(1):107-119.
- Mariano, A.P.; Tomasella, R.C.; Oliveira, L.M.; Contiero, J. & Angelis, D.F. (2008). Biodegradability of diesel and biodiesel blends. *African Journal of Biotechnology*, 7(9):1323-1328.
- Meneghetti, L.R.R.; Thomé, A.; Schnaid, F.; Prietto, P.D.M. & Cavelhão, G. (2012). Natural attenuation and biostimulation of biodiesel contaminated soils from Southern Brazil with different particle sizes. *Journal of Environmental Science and Engineering B*, 1:155-162.
- Mulligan, C.N. & Yong, R.N. (2004). Natural attenuation of contaminated soils. *Environ. Int.*, 30:587-601.
- NIST, National Institute of Standards and Technology (2018). 9,12-Octadecadienoic acid (Z,Z)-, methyl ester. Available in: <http://web-book.nist.gov/cgi/cbook.cgi?ID=112-63-0&Units=SI&cMS=on>.
- Owsiania, M.; Chrzanowski, L.; Szulc, A.; Staniewski, J.; Olszanowski, A.; Olejnik-Schmidt, A.K. & Heipieper, H.J. (2009). Biodegradation of diesel/biodiesel blends by a consortium of hydrocarbon degraders: effect of the type of blend and the addition of biosurfactants. *Bioresource Technology*, 100:1497-1500.
- Pasqualino, J.C.; Montané, D. & Salvadó, J. (2006). Synergic effects of biodiesel in the biodegradability of fossil-derived fuels. *Biomass and Bioenergy*, 30(10):874-879.
- Ramos, D.T.; Silva, M.L.B.D.; Chiaranda, H.S.; Alvarez, P.J.J. & Corseuil, H.X. (2013). Biostimulation of anaerobic BTEX biodegradation under fermentative methanogenic conditions at source-zone groundwater contaminated with a biodiesel blend (B20). *Biodegradation*, 24:333-341.
- Science Lab. Com - Chemicals and Laboratory Equipment (2018). Material Safety Data Sheet, available in [http://www.sciencelab.com/xMSDS-Methyl\\_linolenate-e-8363](http://www.sciencelab.com/xMSDS-Methyl_linolenate-e-8363).
- Sendzikiene, E.; Makareviciene, V.; Janulis, P. & Makareviciute, D. (2007). Biodegradability of biodiesel fuel of animal and vegetable origin. *European Journal of Lipid Science and Technology*, 109:493-497.
- Sihag, S.; Pathak, H. & Jaroli, D.P. (2014). Factors affecting the rate of biodegradation of polycyclic aromatic hydrocarbons. *Int. J. Pure App. Biosci.* 2:185-202.
- Silva, M.L.B. & Corseuil, H.X. (2012). Groundwater microbial analysis to assess enhanced BTEX biodegradation by nitrate at a gasohol-contaminated site. *International Biodeterioration & Biodegradation*, 67:21-27.
- Smith, E.; Thavamani, P.; Ramadass, K.; Naidu, R.; Srivastava, P. & Megharaj, M. (2015). Remediation trials for hydrocarbon-contaminated soils in arid environments: Evaluation of bioslurry and biopiling techniques. *Int. Biodeterior. Biodegradation* 101:56-65.
- Sorensen, G.; Pedersen, D.V.; Norgaard, A.K.; Sorensen, K.B. & Nygaard, S.D. (2011). Microbial growth studies in biodiesel blends. *Bioresource Technology*, 102:5259-5264.
- Streck, E.V.; Kämpf, N.; Dalmolin, R.S.D.; Klamt, E.; Nascimento, P.C.; Schneider, P.; Giasson, E. & Pinto L.F.S. (2008). Soils From RS-Brazil. 2<sup>nd</sup> ed. Emater, Porto Alegre, 222 p. (in Portuguese)
- TGSC, The Good Scents Company (2018). Available in <http://www.thegoodscentcompany.com/data/rw1477771.html>.
- Thomé, A.; Cecchin, I.; Reginatto, C.; Colla, L.M. & Reddy, K.R. (2017). Biostimulation and rainfall infiltration: influence on retention of biodiesel in residual clayey soil. *Environmental Science and Pollution Research International*, 24:9594-9604.
- Thomé, A.; Reginatto, C.; Cecchin, I. & Colla, L.M. (2014). Bioventing in a residual clayey soil contaminated with a blend of biodiesel and diesel oil. *Journal of Environmental Engineering*, 140(11):06014005.
- TOXNET, Toxicology Data Network (2018). Hazardous Substances Data Bank, available in <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>.
- USEPA, United States Environmental Protection Agency (2016). Method 3550 C: Ultrasonic Extraction, available in <https://www.epa.gov/sites/production/files/2015-12/documents/3550c.pdf>.
- Von Wedel, R. (1999). Technical Handbook for Marine Biodiesel. 2<sup>nd</sup> ed. National Renewable Energy Laboratory, U.S. Department of Energy.