ABSTRACT

The Bartha and Pramer’s respirometric method was used to monitor the biodegradation of automotive lubricant oil in aqueous medium and to evaluate the effect of adding the surfactant Tween 80®. It was prepared a base and an aqueous inoculums with and without the surfactant, and the microbiota metabolism was measured by chemical analysis in quantifying the CO₂ production in respirometers. Three treatments were carried out: T1 (without Tween 80®); T2 (with Tween 80®) and T3 (with Tween 80® and used lubricant oil). The results presented the following crescent order of accumulated CO₂: T1 < T2 < T3. It was concluded that the used lubricant oil was degraded by microorganisms and Tween 80® maximized the oil biodegradability due to the micellisation process with the lubricant oil. Also, its use promoted a reduction of the superficial tension and permitted the oil-water contact that facilitated the microorganisms’ action in the aqueous medium contaminated with lubricant oil.

Keywords: Biodegradation. Tween 80®. Respirometry. Liquid medium.
RESUMO

O método respirométrico de Bartha e Pramer foi utilizado para acompanhar a biodegradação de óleo lubrificante automotivo em meio aquoso e para avaliar a influência da adição do surfactante Tween 80®. Para realização do experimento, foram preparados um inóculo base e um inóculo aquoso, e a ação microbiana foi monitorada por análise química na quantificação da produção de CO₂ nos respirômetros. Três tratamentos foram realizados: T1 (sem Tween 80®); T2 (com Tween 80®); e T3 (com Tween 80® e óleo lubrificante usado). Os resultados apresentaram a seguinte sequência crescente de CO₂ acumulado: T1 < T2 < T3. Concluiu-se, portanto, que o óleo lubrificante usado foi decomposto pelos microrganismos e que o Tween 80® melhorou a biodegradabilidade do óleo devido ao processo de micelização realizado junto ao óleo lubrificante. Também, seu uso promoveu uma redução na tensão superficial e permitiu o contato óleo-água, facilitando a ação dos microrganismos no meio aquoso contaminado com o óleo lubrificante.


INTRODUCTION

Lubricant oils are petroleum derivates and they are widely used in automobile engines, hydraulic systems and industrial machines (AMUND, 1996). The lubricant oil discarded in nature causes continuous concern due to its impact in the environment. However, its hazard has not being quantified yet. It was observed that the lubricant oil persisted in the environment for more than six years in some ecosystems and it was able to cause chronic problems for biota (BURNS et al., 1994).

Hamblin (1995) showed that the percentage of used lubricant oil that was poured in ecosystems without any treatment were about 13% for European community and 32% for U.S.A. In Brazil, Lopes et al. (2008) found that a city with 200000 inhabitants discarded daily about 47 L of lubricant oil remained in the plastic bottles after their use and this volume was able to pollute 47 million liters of water by depleting oxygen.

Rosato (1997) emphasized that the bioremediation is the best treatment to attenuate the environmental impact caused by hydrocarbons derived from petroleum because it is less aggressive and

more adjusted to maintain the ecological balance. This is due to the biodegradation residues of a microorganism are used by other microorganisms’ metabolism as energy source. Microbiota uses organic compounds as substratum and converts the contaminants to less dangerous products, CO₂, biomass, inorganic salts and water (LANDIS and YU, 1995; BOOPATHY, 2003).

Some parameters about lubricants behavior are important to promote a sustainable environmental development as distribution, biodegradability and toxicity. Thus, a characterization of the physical chemical properties and environmental behavior of lubricants in production and use are the basis for the development of new lubricants (EISENTRAEGER et al., 2002).

In aquatic ecosystems polluted with petroleum derivates, microorganisms generally live in the oil-water interface where they can attack oil molecules (BROCK and MADINGAN, 1991). The biodegradation was enhanced when the hydrocarbon molecules were in close contact with the water due to the use of tensoactives, as surfactants (ZOBELL, 1969).

The surfactants produce the micellization process when they are in contact with water and a non polar solute. The surfactant molecules seek to arrange in minimize the repulsion between hydrophobic groups and water (ALLINGER et al., 1990). In this process, the surfactant molecules group around the oil by their non polar extremities, while their polar extremities interact with the water. Therefore, the oil becomes more susceptible to biodegradation in facilitating the interaction oil-microorganisms (SINGER et al., 1994).

Consequently, remediation *in situ* using surfactants stimulates hydrocarbons biodegradation. The tensoactives promotes an emulsion oil-water and allow the solubilization of diverse petroleum components. Their use increases the surface area to bigger contact of the microorganisms with the substratum and the emulsification also permits a bigger aeration and nutrients dispersion (ROSATO, 1997).

The purpose of this work was to evaluate the effect of the Tween 80® in automotive lubricant oil biodegradation in aqueous medium.

**MATERIAL AND METHOD**

Different treatments were prepared to evaluate the biodegradability of automotive lubricant oil with and without surfactant. The respirometric method was used in assays for accomplishment of the biodegradation and it was necessary the elaboration of three inocu-
lums: a base inoculum and two aqueous inoculums (with and without surfactant).

The inoculums procedures followed the methodology of Lopes and Bidoia (2009).

The base inoculum was prepared with 100 mL of distilled water, 1.0 mL of Tween 80® and 50 mL of mineral automotive lubricant oil (without use). This solution was transferred to a plastic bag with 3.0 kg of sandy soil.

The plastic bag was homogenized and perforated with small holes of approximately 1 mm diameter and spaced at 1 cm from each other. It was then buried at 15 cm depth to allow microorganism exchange between the soil containing oil and the substrate from outside. After 30 days a previous selection of microorganisms acclimatized to the environment with oil, 1.0 kg of this inoculum was added to 1.5 L of distilled water. This solution was homogenized and the supernatant removed, which led to the aqueous inoculum (without surfactant).

On the other hand, the aqueous inoculum with the Tween 80® surfactant follows the same methodology above instead of the addition of 75 mL of Tween 80®.

The composition of each treatment (T) in the experiments describes in Table 1.

<table>
<thead>
<tr>
<th>T</th>
<th>Composition</th>
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<tr>
<td>T1</td>
<td>95 mL of aqueous inoculums without Tween 80® and 5 mL of distilled water</td>
</tr>
<tr>
<td>T2</td>
<td>95 mL aqueous inoculums with Tween 80® and 5 mL of distilled water</td>
</tr>
<tr>
<td>T3</td>
<td>95 mL of aqueous inoculums with Tween 80® and 5 mL of used lubricant oil</td>
</tr>
</tbody>
</table>

To monitor the microorganisms’ respiration it was used the Bartha e Pramer’s respirometric method following the Technical Standard L6.350 issued by Cetesb (1990) adapted to aqueous media (MONTAGNOLLI et al., 2009). This methodology evaluates the biodegradation measuring the CO₂ produced by microbota in the Bartha and Pramer respirimeters (BARTHA and PRAMER, 1965). Thus, the respirometric assay consisted on the determination of the CO₂ generated by the degradation process of organic substances.

The Bartha’s respirometer flasks consists in a closed system featuring two connected chambers, one where biodegradation occurs and the other with an alkaline solution that was able to quantify the CO₂ produced by microbial respiration (Figure 1). After each CO₂ quantification in the respirometers, the flasks were incubated at 28°C.
Therefore, the carbon dioxide accumulated can be calculated and represented as a function of incubation time (BALBA et al., 1998). The incorporated carbon dioxide permitted the evaluation surfactant effect in the lubricant oil biodegradation in watery systems.

RESULTS AND DISCUSSION

The CO₂ accumulation curves for the four treatments carried out after 185 days of incubation were shown in Figure 2.
In Figure 2, the results demonstrated that the microorganisms had been able to promote the biodegradation of automotive lubricant oil contained in the assays.

The T1 curve revealed the mineralization of lubricant oil presented in base inoculum. The T2 treatment, that represents the influence of Tween 80® in inoculum composition, had a greater biodegradation compare to T1 due to the surfactant effect in the lubricant oil and because Tween 80® also was a carbon source to microbiota.

Major CO₂ production was found in T3 and it was caused by the presence of the lubricant oil. The microorganisms metabolized the initial oil presented in base inoculum and the used lubricant added in T3 treatment.

Therefore, the lubricant oil was assimilated by the microorganisms selected in the inoculums and they were able to consume the oil as carbon source to produce biomass and CO₂ that was liberated in the respirometers.

However, in the first days of biodegradation, T3 presented less CO₂ production compared with T2. This can be explained by the adaptation period for the microorganisms to act on the emulsion formed by the lubricant oil and the surfactant. A greater adaptation period when lubricant oil was added in biodegradation assays was also found by Lopes and Bidoia (2009).
The difference in accumulated CO₂ between T1 and T2 treatments was due to the influence of the Tween 80® represented by the oil emulsification and the biodegradation of the surfactant. Surfactants induce micellization process when they are in contact with water and a nonpolar solute. The surfactant molecules seek to minimize the repulsion between hydrophobic groups and water (ALLINGER et al., 1990). Thus, Tween 80® allowed the emulsification and promoted the interface microorganisms/substrate and the oxygen captured in the emulsion improved the biodegradation. Also, the surfactant was assimilated as a substratum by micro-biota. The Tween 80® molecular structure was less complex than the carbonic chain found in hydrocarbons like automotive lubricant oils. This caused a CO₂ production by the surfactant biodegradation. The primary and partial Tween 80® biodegradation observed in this work corroborate with Al-Hadhrami et al. (1996). The authors studied the Tween 80® in contact with raw oil and observed that a fraction of the surfactant was decomposed by the microorganisms.

CONCLUSION

It was concluded that the lubricant oil was degraded by microorganisms and that the Tween 80® promoted a micellization process with the lubricant oil leading to a reduction of the superficial tension allowing the oil-water contact what facilitated the biodegradation of the aqueous medium contaminated with lubricant oil.

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