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Accumulated and weekly CO_2 production by lubricant and soy oil biodegradation in aqueous media by aerobic respirometry

Produção de CO_2 acumulado e semanal pela biodegradação de óleos lubrificantes e de soja em meio aquoso por respirometria em aerobiose

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Abstract

Petroleum activities have caused great concern due to the impact of major contamination it and its composites have caused to nature, mainly to aquatic environments. Among the physical, chemical and biological methods available to remediate wastewater contaminated with hydrocarbons bioremediation is considered an environment-friendly and relatively cost-effective technology. That is due to the biodegradation the process is based on, using microbial populations in the contaminated environment remediation. The objective of this study was to assess biodegradation in contaminated aqueous media contamined by automotive lubricant oils and vegetable oils using respirometry technique. The Bartha and Pramer's respirometric method promotes CO_2 quantification in the respirometers. The used lubricant oil had higher CO_2 production and the used vegetable oil was more biodegradable than the still unused vegetable oil. However, these vegetable oils were less biodegraded due to the presence of TBHQ preservative in their composition, thus causing a reduction in CO_2 production. Thus, an environmental behavior analysis of lubricant and vegetable oils is important to provide new strategies in treating contaminated environments.

Keywords: Water. Bioremediation. Hydrocarbon. Microbial respiration. TBHQ.

Resumo

A preocupação ambiental em relação ao setor ligado ao petróleo tem crescido por causa do grande impacto que esse composto e seus derivados causam na natureza, principalmente em ambientes aquáticos. Dentre os métodos físicos, químicos e biológicos utilizados na remediação de efluentes contaminados com hidrocarbonetos, a biorremediação é considerada uma tecnologia ecologicamente viável e de baixo custo. Isso se deve à biodegradação na qual é baseado o processo, utilizando-se populações microbianas na remediação do ambiente contaminado. O objetivo do estudo foi avaliar a biodegradação de meio aquoso contaminado por óleos lubrificantes automotivos e óleos vegetais utilizando a respirometria. O método respirométrico de Bartha e Pramer promove a quantificação de CO_2 nos respirômetros. O óleo lubrificante usado apresentou a maior produção de CO_2 e o óleo vegetal usado foi mais biodegradável do que aquele sem uso. Entretando esses óleos vegetais foram menos biodegradados graças à presença do conservante TBHQ em sua composição, o que reduz sua produção de CO_2. Portanto, a análise do comportamento ambiental dos óleos lubrificantes e vegetais torna-se importante na busca por novas estratégias de tratamento de ambientes contaminados.

Palavras-chave*: Água. Biorremediação. Hidrocarboneto. Respiração microbiana. TBHQ.*

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Introduction

The aquatic environments are also the center of many natural biological processes and special attention was therefore given to them. The waste-lubricating oil, otherwise called spent oil or used-lubricant, obtained after servicing and subsequent draining from automobile, generators and industrial machines is disposed off indiscriminately in the world, and adequate attention has not been given to its disposal (1).

Pollution generated by discarding a ton of used oil is equivalent to a 40 thousand inhabitant's domestic sewer, and only one liter of this compound is able to deplete the oxygen of a million liters of water (2). Also, the pollutants released from oil spilled 5 years ago are still harming the ecosystem of the damage area (3).

Another environmental contamination concern by lubricant oil is due to the residual volume of these oils in commercial bottles. About 11.58mL of lubricant oil remained in bottles after its use. Therefore, a 200,000 inhabitants city has a potential to pollute 47 liters of water per day (4).

Their hazards of the used lubricant oil are harder to quantify since they may have a wide range of compositions, reflecting the potential range of service conditions and possibilities of contamination (5). Also, the use of this oil in automotive engines contributes to a higher toxicity (6).

Additionally, lubricants are emitted from leaks and significant amounts remaining in filters and containers have to be taken into account (7). For these reasons, the distribution, biodegradability and toxicity of lubricants are important factors with respect to environmental management. In spite of the large number of works on petroleum, petroleum derivatives and hydrocarbon biodegradation, little information is available on lubricant oils biodegradation (8).

Bioremediation is one of the most extensively used methods due to its low cost and high efficiency (9). And, it is an attractive approach to cleaning up petroleum hydrocarbons because it is simple to maintain, applicable over large areas, cost-effective and leads to complete destruction of the contaminant (10). The prevailing mechanism that breaks down these petroleum products is biodegradation, which is carried out by natural microbial populations (11). Biodegradability assessments results are particularly important when the chemical products enter the natural environment during service, or due to inadequate use or utilization (12). Viable methods for obtaining data with the application of biotechnological procedures in those substances are a fundamental piece when elaborating original references (13).

Finally, it is known that large quantities of wastewater that contains lubricating oil were produced, which can lead to harmful effects when released into the environment (14). Therefore, water and soil pollution caused by lubricating oil present in wastewater has become an important environmental issue that must not be neglected (15). Although the biodegradation of lubricating oil has been reported in several studies (8, 10, 16), these studies focused on the bioremediation of soil contaminated by hydrocarbon mixtures. Thus, the aim of the present work is to evaluate the biodegradation in contaminated aqueous media by automotive lubricant and vegetable oils.

Materials and methods

Microorganism selection

Soil microorganisms were used in attainment instead of water due to the fact that microorganism count in soil is higher than in water. Besides, the microorganisms are expected to come from the soil moisture, so they are already adapted to a simulated wastewater medium. The whole sack surface was then perforated with small holes, in order to allow the microorganism exchange between the external soil and the inner medium containing soil and oil. Microorganisms were attained in a soil contamination simulation, using a plastic sack filled with 3 kg of soil, 1 g of Tween 80® surfactant, 100 ml of distilled water and 50 ml of a lubricant and vegetable oil mix, containing 10 ml of synthetic lubricant oil, 10 ml of semi-synthetic lubricant oil, 10 ml of mineral lubricant oil, 10 ml of used lubricant oil and 10 ml of vegetable oil. Thereafter the soil was dried at room temperature, and then buried in a pristine environment.

After 15 days, it is assumed that a reasonable pre-selection of microorganisms tolerant to the oil contaminated soil had already happened. A fraction of 1,000 g of this inoculum was then removed from the sack and 1,500 ml of water was added to it, being the homogeneous solution without the non-solubilized soil defined as "base liquid". This experiment evaluates a simulated water medium, much similar to wastewater effluents found in contaminated sites. The water proportion of the "base liquid" is indeed higher than the soil proportion, and viable to analysis.

Respirometry

The inoculum used in biodegradation assays was prepared following Lopes and Bidoia (17). Microorganisms that are able to biodegrade lubricant oils were obtained through a soil contamination simulation in a plastic bag filled with 3 kg of sandy soil, 50mL of a lubricant oil mixture, 1mL of Tween 80 and 100mL of distilled water. 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, 500 g of this inoculum was added to 500mL of distilled water. This solution was homogenized and the supernatant removed, which led to the base liquid.

During the respirometric test, carbon dioxide evolution rates were monitored by using respirometric flasks (18) according to Bartha and Pramer's respirometry (19). Carbon dioxide which was evolved during the microbial respiration process was trapped in 10.0mL of aqueous solution of KOH 0.20 M located in the side arm attached to the Bartha respirometric flask. After 1.0mL of BaCl2 0.05 M was added to KOH solution in order to precipitate the carbonate ions, the amount of carbon dioxide was then measured by titration of the residual KOH with a standard solution of HCl 0.10 M. The calculations of CO_2 evolution, biodegraded carbon and biodegradation effectiveness were accomplished in agreement with Technical Norm L6.350 (18).

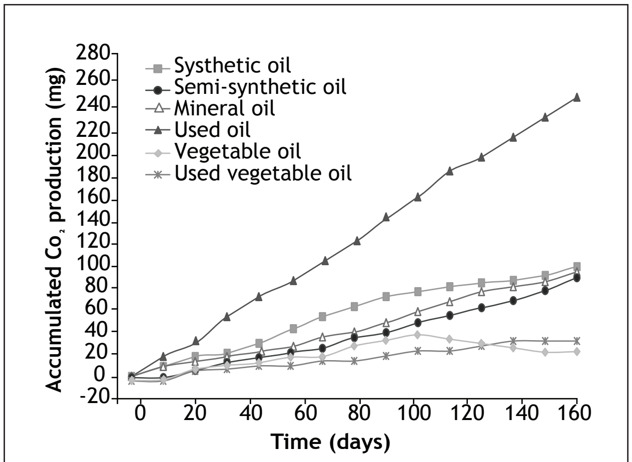
Therefore, it was prepared seven assays in triplicate: four of them containing different types of automotive lubricant oil, two containing vegetable oils and a single control assay, containing only distilled water instead of oil.

The control assay (A0) contained 1% v/v of distilled water in "base liquid". The other assays were also made of "base liquid" and the analyzed oil in 1% v/v as following: Synthetic Automotive Oil (A1), Semi-Synthetic Automotive Oil (A2), Mineral Automotive Oil (A3), Used Automotive Oil (A4), Soy Oil (A5) and Used Soy Oil (A6). Every respirometer (except the control assay) had the oil subject to biodegradation along with the "base liquid", which is the inoculum providing the pre-selected microorganisms to the process.

Results

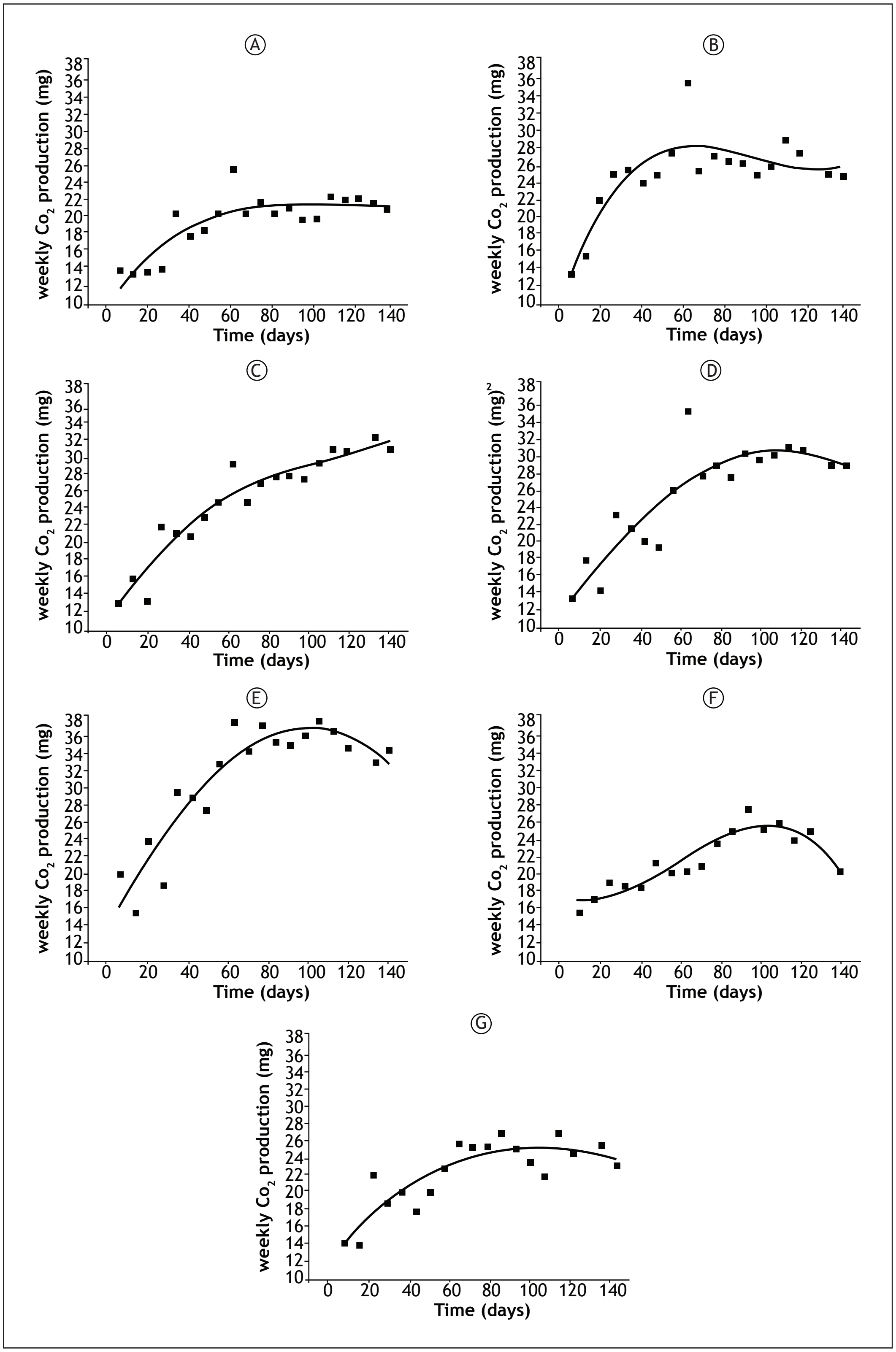
The respirometric technique allowed the calculation of CO_2 production as an indirect measure of biodegradation through time. Graphic 1 demonstrates the accumulated CO_2 production. Data of blank experimental results were not included in Graphic 1 since all of the plotted points already had subtracted from their values the control value. Graphic 2 shows the weekly CO_2 production by each type of oil, including their respective polynomial regression fit to the data plotted.

According to Graphic 1, the used lubricant oil biodegradation is noticeably higher when compared to other types of oils, due to its greater accumulated CO_2 production levels in the end of biodegradation process after 150 days (666.27 mg). A general pattern concerning the studied oils biodegradation assume that the CO_2 production values reach the highest levels in used lubricant oils, while the other types of oils are in very similar levels of total biodegradation below the used lubricant oils and above the vegetable oils, which showed the lowest biodegradation performance of all evaluated oils, due to their very low accumulated CO_2 production (452.58 mg in soy oil and 470.29 mg in used soy oil) during the 150 days of measurement (Table 1). Also, the vegetable oils presented the lowest mean weekly CO_2 production values (22.32 mg in soy oil and 23.08 mg in used soy oil).



Graphic 1 - Accumulated CO_2 production after 150 days of biodegradation

Source: Research data.



Graphic 2 - Weekly CO_2 production after 150 days of biodegradation in (a)inoculums ($9,98795 + 0,3294\ x + 0,00297\ x^2 + 0.000008\ x^3$);(b)synthetic oil ($9,4312 + 0,68773\ x + 0,00776\ x^2 + 0.000002\ x^3$); (c)semi-synthetic oil ($9,64249 + 0,4155\ x + 0,00314\ x^2 + 0.000009\ x^3$); (d)mineral oil ($11,56351 + 0,31175\ x + 0,00083\ x^2 + 0.000003\ x^3$); (e) used oil ($13,74738 + 0,44797\ x + 0,00183\ x^2 + 0.000002\ x^3$); (f)soy oil ($11,56351 + 0,31175\ x + 0,00083\ x^2 + 0.000003\ x^3$); and (g)used soy oil($12,64514 + 0,27559\ x + 0,00168\ x^2 + 0.000002\ x^3$)

Source: Research data.

Table 1 - Accumulated CO_2 production and mean weekly CO_2 production after 150 days of biodegradation

Assay	Accumulated CO_2 production (mg)	Accumulated CO_2 production from source (mg)	Mean weekly CO_2 production (mg)
A0 (inoculum)	414.92	-	19.84
A1 (synthetic oil)	531.41	116.49	25.87
A2 (semi-synthetic oil)	530.13	115.21	25.73
A3 (mineral oil)	536.84	121.92	26.41
A4 (used oil)	666.27	251.35	31.81
A5 (soy oil)	452.58	37.66	22.32
A6 (used soy oil)	470.29	55.37	23.08

Source: Research data.

Statistical analysis

The F-test provides a bicaudal probability that the variances in two databases are not significantly different. This statistical tool is used to determinate if two samples have different variances. For example, from the respirometric tests results in different oils, it is possible to verify if each type of oil present a different level of biodegradation evaluated through CO_2 production parameters.

In other words, the F-test is used in this experiment data analysis to demonstrate that two data sets, when compared, are statistically different. The F-test was applied to determinate if the accumulated CO_2 production in Graphics 1 and 2 are different at a significance level $p = 0.05$. The comparative data were based in linear regressions fit (with r>0.999) to accumulated CO_2 production plotted in function of biodegradation time. The Table 2 shows the output of the F-test.

The F-test results pointed to no significant difference between the non used automotive lubricant oils and the vegetable oils biodegradation concerning the accumulated CO_2 production, when compared within their oil type group. In other words, all types of non used lubricant oils present similar biodegradation profile to other non used lubricant oils. The same applies to vegetable oils group. In addition to the F-test, the polynomial regression fit to weekly CO_2 production indicated a declining tendency in CO_2 production after 110 days of biodegradation process in Graphic 2(d, e and f). In a specific case (Graphic 2f), there was a return to starting production of weekly CO_2 production after a peak of 27.50 mg of CO_2 production. In addition to this, the CO_2 production peaks observed in vegetable oils are higher than the ones from automotive lubricant oils.

Discussion and conclusion

According to these results, Eisentraeger et al. (20) demonstrated that used oils were more biodegraded than new ones, and proposed that it is caused by changes in the chemical structure of the hydrocarbons during the mechanical interactions between the oil and the medium. The interaction of the used lubricant oil with high pressures and temperatures may also interfere with the long hydrocarbon chains, breaking them and helping with the microorganism activity.

The lower vegetable oil biodegradability showed at Graphics 1 and 2 is possibly associated with the preservatives present in the analyzed samples added during the fabrication processes according to product's specification, like citric acid and 2-(1,1-Dimethylethyl)-1,4-benzenediol (TBHQ). Respirometry results indicated

Table 2 - F-test comparison between different types of oils accumulated CO_2 production. The bold F values represent that at significance level $p = 0.05$, the data are not significantly different

	A0	A1	A2	A3	A4	A5	A6
A0	-	894.248	142.9288	226.29862	861.78693	120.63847	131.12007
A1	894.248	-	2.48856	4.70908	214.0903	264.74891	225.69452
A2	142.928	2.48856	-	3.18763	201.61227	29.11876	23.84744
A3	226.298	4.70908	3.18763	-	168.95449	61.85297	53.15645
A4	861.786	214.0903	201.6123	168.9545	-	511.67604	483.91623
A5	120.638	264.7489	29.11876	61.85297	511.676	-	0.53943
A6	131.120	225.6945	23.84744	53.15645	483.9162	0.53943	-

Source: Research data.

that vegetable oils containing preservatives is less biodegradable than automotive lubricating oils.

Thus, it is possible to consider the preservative influence in microorganisms' metabolism. The TBHQ preservative is commonly used in commercial vegetable oils at certain concentrations in order to keep its original properties after production by inhibit oxidation of some soy oil radicals (21).

When TBHQ is combined with citric acid, as found in vegetable oils used in this study, Moulton et al. (22) observed a significant increase in oxidative stability of oil and the changing reduce of the its chemical structure. Asap and Augustin (23) determined that TBHQ can also reduce the oil oxidation rate and changes in their properties even when occurs heating preparation as frying.

As used lubricating oils, used vegetable oils have their physical and chemical properties modified (20, 24). High temperatures processing can break the oil carbon chains, which facilitate the microbial activity. Thus, factors such as stability, viscosity and composition show the significance of knowing the answer of the samples contaminated with lubricant and vegetable oils in aqueous media. Therefore, the environmental performance and their chemical characteristics are important to provide strategies to treat contaminated environments.

Thus, the present results show that biodegradation is an effective method in different types of oil removal. Oil contaminated wastewater has become an important environmental issue that must not be neglected due to their enormous potential in harming aquatic environments. As a general pattern used oils, such as vegetable and lubricant are more biodegraded. Processing in high temperatures can break their carbon chains, in which the microbial activity increases. However, the vegetable based oils had a worst CO_2 production compared to automotive lubricant oils caused by the TBHQ preservative presence. Respirometric assays results provide environmental performance to better comprehension and handling of oil polluted environments.

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