



## Motor oil wastewater treatment in a packed bed bioreactor using immobilized native microbial consortium

### Tratamiento de aguas residuales con aceite de motor empleando un biorreactor de lecho empacado usando un consorcio microbiano nativo inmovilizado

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#### Abstract

The global increase in population has led to a higher demand for motor oil from a raising number of automobiles. The inappropriate disposal of waste motor oil generates water contamination, therefore, removing motor oil from contaminated water can prevent damage to the environment and human health. In this study, a new native microbial consortium from contaminated soil was tested and exposed to different concentrations of motor oil. The kinetic analysis revealed that the maximum specific growth rate was  $0.758 \text{ d}^{-1}$ , while the saturation and inhibition constants were  $7.11$  and  $23.77 \text{ mg mL}^{-1}$ , respectively. The consortium was immobilized over a plastic packing and employed in studies of motor oil degradation using a packed bed bioreactor. The results indicated the biosurfactant production capacity was  $398.18 \text{ mg L}^{-1}$ , while the maximum emulsification capacity was  $65.69\%$  and the efficiency of motor oil degradation was  $67.76\%$ . The results obtained in the present work represent a significant advantage in oil-contaminated wastewater treatment by using a packed bed bioreactor and an immobilized native microbial consortium with the ability to produce biosurfactants.

**Keywords:** engine oil, biodegradation, hydrocarbons, bacteria, water.

#### Resumen

El incremento de la población a nivel global ha generado un aumento en el número de automóviles y en la demanda del aceite de motor. La mala disposición de este genera contaminación en el agua. Por lo tanto, es necesario remover dicha contaminación para evitar daños al ambiente y la salud humana. En este estudio el potencial de un nuevo consorcio aislado de un suelo contaminado fue evaluado y expuesto a diferentes concentraciones de aceite de motor. El análisis cinético reveló un valor de la velocidad máxima del crecimiento microbiano de  $0.758 \text{ d}^{-1}$ , de la constante de saturación de  $7.11 \text{ mg mL}^{-1}$  e inhibición de  $23.77 \text{ mg mL}^{-1}$ . El consorcio fue inmovilizado sobre un empaque plástico y usado en estudios de degradación de aceite en un biorreactor de lecho empacado. Los materiales usados para construir el biorreactor son simples y de bajo costo. Los resultados indicaron una producción de biosurfactantes de  $398.18 \text{ mg L}^{-1}$ , una capacidad de emulsificación del  $65.69\%$ , y una eficiencia de remoción de aceite del  $67.76\%$ . Los resultados obtenidos representan un avance significativo en el tratamiento de aguas residuales usando un biorreactor de lecho empacado y un consorcio inmovilizado con capacidad de producir biosurfactantes.

**Palabras clave:** aceite residual, biodegradación, hidrocarburos, bacterias, agua.

## 1 Introduction

Environmental pollution caused by hydrocarbons and petroleum byproducts has an important impact on economic development issues (Al-Hawash *et al.*, 2018; Mishra *et al.*, 2020; Sonwani *et al.*, 2020). Underground water and soil can be affected by the presence of petroleum derivatives, such as

fuel residues, mineral oil, and motor oil. This contamination is caused by leaks, accidental spills, and anthropogenic activities (Zhang *et al.*, 2019; Sonwani *et al.*, 2020; Tao *et al.*, 2020). Activities related to tourism, commercial transport, industries, and the inadequate disposal of residues are some examples of the principal sources of water contamination (Almeida *et al.*, 2019; Ostendorf *et al.*, 2019; Sandoval-Herazo *et al.*, 2020; Ray *et al.*, 2021). In some cases, the

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neglected disposal of waste oils by people can worsen the problem (Su *et al.*, 2011; Naima and Liazid, 2013; Almeida *et al.*, 2019; Ostendorf *et al.*, 2019).

One of the challenges in recent years is to provide the increasing population with an adequate quantity and quality of water in a sustainable and cost-effective way. Over the last decade, the human right to water has become central to a policy agenda addressing conflict in a situation of perceived water scarcity and competing claims (Radonic, 2017). For example, the demand for water resources in Mexico has been on a continuous upward trend due to population growth and increasing per capita demand. Wastewater contamination is a global problem; therefore, new technologies are required to removed pollutants from water. (Torjada and Biswas, 1997; Radonic, 2017).

The increase in population and urbanization around the world has led to a larger demand for motor oil. Motor oil is mixture-based, synthesized from crude oil, and enriched with chemical additives for friction reduction. Waste motor oil (WMO) is full of sludge and burnt hydrocarbons and constitutes a hazardous waste (Pimda and Bunnag, 2015). Exhausted motor oil is a combination of several contaminants such as dirt particles, soot, and combustible products, among others. If it is not disposed of properly, WMO comes into human contact and poses an environmental threat (Su *et al.*, 2011; Mishra *et al.*, 2020). Around two million gallons of waste oil, including lubricating oil, hydraulic fluids, and gear oils, are inappropriately disposed each year (Naima and Liazid, 2013; Mishra *et al.*, 2020).

Bioremediation is considered an efficient, cost-effective, and eco-friendly method to degrade hydrocarbons in petroleum-contaminated soil and water (Wu *et al.*, 2017; Xu *et al.*, 2018; Bhattacharya *et al.*, 2019; Interiano-López *et al.*, 2019; Yalaoui-Guellal *et al.*, 2020). Biodegradation by indigenous microorganisms is a major mechanism and a reliable method that operates by biologically removing toxic compounds such as hydrocarbons, petroleum, and its derivatives (Al-Hawash *et al.*, 2018; Sonwani *et al.*, 2020; Tao *et al.*, 2020). The main challenge in the bioremediation of hydrocarbons is their low water solubility (Cazals *et al.*, 2020; Tripathi *et al.*, 2020). However, many hydrocarbon-degrading bacteria can produce emulsifying agents (biosurfactants) that promote the utilization of hydrocarbon substrates as a carbon source (Su *et al.*, 2011; Tripathi *et al.*, 2020).

Biosurfactants are secondary metabolites that play a beneficial role in the biodegradation of hydrocarbons (Karlupudi *et al.*, 2018; Cazals *et al.*,

2020; Yalaoui-Guellal *et al.*, 2020; Ray *et al.*, 2021). They are surface-active compounds with emulsifying activities. Biosurfactants contain both hydrophilic and hydrophobic ends, which allow them to interact at the interface between aqueous and non-aqueous systems (Ostendorf *et al.*, 2019; Cazals *et al.*, 2020; Rani *et al.*, 2020). Crude biosurfactants can be used in wetting, emulsification, and foaming. Biosurfactants have become commercially significant due to their various applications in petrochemical, food, agricultural, and industrial sectors (Karlupudi *et al.*, 2018; Sharma *et al.*, 2018; Cazals *et al.*, 2020; Ray *et al.*, 2021).

Recent research has focused on the alternative to hydrocarbon bioremediation. For example, previous studies have shown that waste motor oil is degraded by *Nostoc hatei* TISTR 8405 (Pimda and Bunnag, 2015) and *Pseudomona aeruginosa* (Su *et al.*, 2011); oilfield wastewater is biotreated using *Rhodococcus* cultures (Kuyukina *et al.*, 2017). The biodegradation potential of crude petroleum has been assessed by hydrocarbonoclastic bacteria isolated from Soummam wadi sediments (Yalaoui-Guellal *et al.*, 2020). The hexadecane uptake has been identified using an oil-degrader consortium (Hernández-Martínez *et al.*, 2019; Sandoval-Herazo *et al.*, 2020), and the evaluation of the ability to degrade crude and fuel oil has been determined by a native microbial consortium (NMC) (Canul-Chan *et al.*, 2018; Rani *et al.*, 2020). Besides, the enhancement of microorganism potential to degrade petroleum hydrocarbons can be improved using bioreactors, such as packed-bed bioreactors (PBBRs) operated in continuous mode (Kureel *et al.*, 2018; Sonwani *et al.*, 2020), fluidized bed bioreactors (Kuyukina *et al.*, 2017), inverse fluidized bed bioreactors (Mallikarjuna and Dash, 2020), and a PBBR (Hussain *et al.*, 2015; Kumar *et al.*, 2015; Berrelleza-Valdez *et al.*, 2019).

PBBRs have been used in the biodegradation of toxic compounds with immobilized cells (Kumar *et al.*, 2015; Banerjee and Ghoshal, 2017; Sonwani *et al.*, 2020). This technology is a useful method that can efficiently improve the metabolic activity via the localization of intact cells in a defined region and the preservation of catalytic activity for further biochemical process (Hussain *et al.*, 2015; Sonwani *et al.*, 2020). Some reports indicate that a high concentration of substrate considerably reduces bacterial growth due to substrate inhibition, resulting in the deterioration of the overall bacterial efficacy toward substrate degradation (Banerjee and Ghoshal, 2017; Ray *et al.*, 2021). Methods like adsorption, covalent binding, cross-linking, entrapment, and

encapsulation are widely used for immobilization. However, there is an increasing interest in the research and development of packing materials such as coal, polyurethane foam, Ca-alginate beads, and various agro-waste materials (Kureel *et al.*, 2018; Sonwani *et al.*, 2020).

The successful biodegradation of pollutants like pesticides, mono, and polycyclic aromatic hydrocarbons, dyes, and pharmaceuticals has been previously reported (Geed *et al.*, 2017; Kureel *et al.*, 2018). Therefore, PBBRs offer several advantages such as high-yield operation, ease of fabrication, and low cost; they can treat a large amount of wastewater continuously and reuse biomass (Banerjee and Ghoshal, 2017; Sonwani *et al.*, 2020). However, the mass transport of the substrate through immobilized cells in PBBR is limited by internal (rate of transport inside the system) and external (transfer of reactants to and products from immobilized cell system) mass transfer resistance. The mass transfer limitation plays an important role and determines the rate of pollutant biodegradation when PBBRs are operated at a large scale (Hussain *et al.*, 2015; Sonwani *et al.*, 2020).

This work aims to study oil-contaminated wastewater treatment using a PBBR with a NMC and a different packing material. Due to the bad disposal of the motor oil at automobile repair shops, it is necessary to study and establish an efficient, cost-effective, and eco-friendly treatment option. Additionally, the capacity of the microbial consortium to produce biosurfactants and emulsified is measured. The treatment presented in this work can be an alternative to generate a value-added product along with wastewater treatment.

## 2 Materials and methods

### 2.1 Isolation of native microbial consortium

#### 2.1.1 Collection of samples

A soil sample was collected in a local automobile repair shop located (18°51'35.2"N 97°04'31.2"W) in Orizaba, Mexico, where soil contamination with waste motor oil was visible. A simple sampling was employed where one kilogram of soil was collected with a clean shovel to remove the superficial layer (two inches) of the soil surface, placed in a sterile bag, and

taken to the laboratory. The sample was homogenized manually, and rocks and other foreign particles were eliminated.

#### 2.1.2 Enrichment and adaptation of the NMC

The enrichment and adaptation of the NMC were carried out in 250-mL Erlenmeyer flasks. Bushnell-Haas medium (Bushnell and Haas, 1941), one gram soil sample, and 500  $\mu\text{L}$  of (0.5% v v<sup>-1</sup>) WMO as carbon source were added. The flasks were incubated at a controlled temperature (35°C) and agitation (150 rpm) for 15 days. The NMC was cultured with fresh medium (Bushnell-Haas) and 500  $\mu\text{L}$  of WMO in batch mode for two months.

### 2.2 Potential of native microbial consortium

After the enrichment and adaptation period, the NMC was tested to evaluate its degradation and growth capacities and its ability to produce biosurfactants at different WMO concentrations (0.2, 0.5, 1.0, and 2.5 % v v<sup>-1</sup>). The experiments were conducted in Erlenmeyer flasks with Bushnell-Haas medium, and the NMC was adjusted initially to 0.20 (OD<sub>600nm</sub>) for 14 days.

#### 2.2.1 Evaluation of the kinetic microbial growth

The microbial growth was evaluated using the change in optical density. The biomass in the liquid culture was harvested every two days by centrifugation (10,000 x g for 10 min at room temperature). The cell pellet was washed twice using 1 mL distilled water and resuspended in 1 mL distilled water. The change in absorbance was measured at 600 nm. The specific velocity of the microbial growth was calculated in the exponential phase and compared between different WMO concentrations. The Haldane-Andrews model was used to fit the experimental data and assess the kinetic parameters:

$$\mu = \mu_{\max} \frac{S}{K_s + S} \exp(-S/K_I) \quad (1)$$

where  $\mu$  is the specific growth rate (d<sup>-1</sup>),  $\mu_{\max}$  is the maximum specific growth rate (d<sup>-1</sup>),  $K_s$  is the half saturation constant (mg mL<sup>-1</sup>),  $K_I$  is the inhibition constant (mg mL<sup>-1</sup>), and  $S$  is the substrate concentration (mg mL<sup>-1</sup>) (Ray *et al.*, 2021).

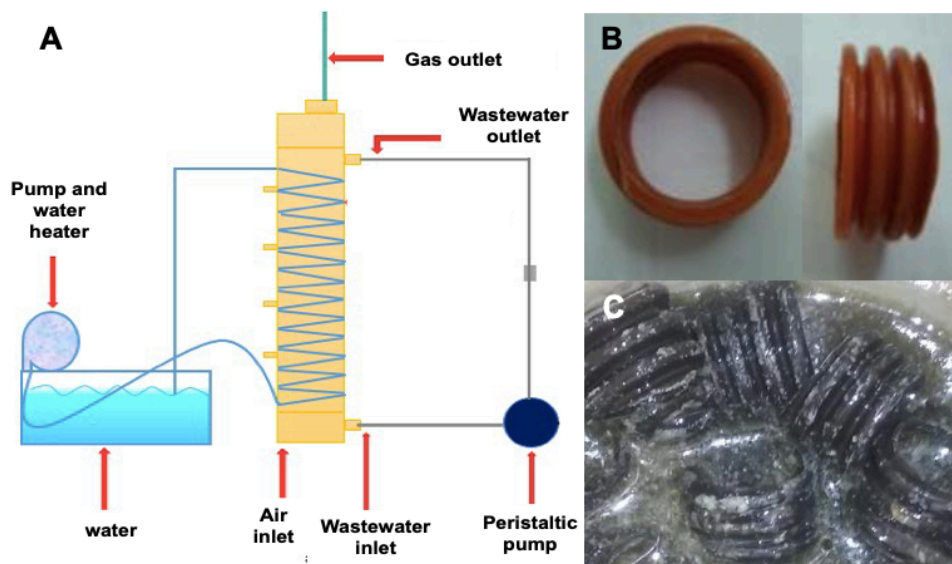


Fig. 1. Schematic diagram of the experimental set up for the biodegradation of waste motor oil. A) Scheme of the packed bed bioreactor, B) plastic rings used as packed material, and C) biofilm formed over plastic rings used as packed material.

### 2.2.2 Biosurfactants production and degradation capacity

The biosurfactant production during the hydrocarbon biodegradation process was determined every two days using the oil displacement method. Fifty milliliters of distilled water were added to a Petri dish (15 cm) along with 250 L of crude oil. One drop (10  $\mu\text{L}$ ) of cell-free culture supernatant was added to the center and the clear zone diameter was measured (Morikawa *et al.*, 2000). Tween 20 (Meyer<sup>®</sup>) was used as model surfactant to compare biosurfactant production and distilled water as a control. The remaining amount of hydrocarbons from the media culture was evaluated 14 days after the biodegradation process. The residual hydrocarbons were extracted using a gravimetric method with 100 mL of culture media and 100 mL hexane in a Soxhlet apparatus (Ganesh and Lin, 2009; Sonwani *et al.*, 2020).

## 2.3 Design and operation of a packed-bed bioreactor for wastewater

### 2.3.1 Design and operational conditions

The fluidized bed bioreactor was constructed using PVC pipe with a height of 105 cm and an internal diameter of 11.1 cm, for a total capacity of 10.16 L. To control the temperature, a warm-up jacket

was constructed using a plastic pipe. The schematic diagram of the experimental set-up for the motor oil biodegradation is shown in Figure 1A. The packing material was made by cutting a section of rings with a length of one cm and an internal diameter of 1.27 cm (Figure 1B) out of a flexible high-density polyethylene pipe (Tupssaflex<sup>®</sup>). The consortium was immobilized over the plastic packing material rings. An external pump (Elite 799) supplied air at a flow rate of 0.6 L  $\text{min}^{-1}$  to maintain the aerobic conditions in the PBBR. A recirculation system was operated with a peristaltic pump (Masterflex<sup>®</sup>) to control the bioreactor feed. The inlet flow rate was estimated according to:

$$HRT = \frac{1}{\mu_{\max}} \quad (2)$$

$$HRT = \frac{Vol}{Q} \quad (3)$$

where  $\mu_{\max}$  is the maximum specific growth rate ( $\text{d}^{-1}$ ), HRT is the hydraulic retention time ( $\text{h}^{-1}$ ), Vol is the operational volume of the bioreactor, and  $Q$  is the volumetric flow of the feed ( $\text{L h}^{-1}$ ) (García-Peña *et al.*, 2013).

### 2.3.2 Immobilization of the native microbial consortium

The NMC was placed in Erlenmeyer flasks with an operational volume of 100 mL. Bushnell-Haas

medium was used with 1.0% v v<sup>-1</sup> WMO as a carbon source. The flasks were inoculated with NMC and initially adjusted to 0.20 (OD<sub>600nm</sub>), and eight plastic rings were previously cleaned and sterilized. The flasks were incubated for seven days in an orbital environmental shaker adjusted to 37 °C and 100 rpm. The microbial growth over the plastic rings was measured by the weight-dry method (data not shown) seven days after the incubation time. The initial biofilm formed (Figure 1C) over the ring surface was adjusted to 124.3 ± 13.5 mg biomass per support (plastic ring). The PBBR was inoculated using the plastic rings with the immobilized consortium and operated with synthetic wastewater (1% v v<sup>-1</sup> WMO and Bushnell-Haas medium). The inlet temperature of the PBBR (37 ± 2°C) was controlled using a heating jacket, and the time of exposure to WMO was 14 days; the wastewater was removed and replaced afterwards. The biofilm formation process over the plastic rings was completed after 42 days (three cycles of 14 days with synthetic wastewater).

### 2.3.3 Waste motor oil degradation and biosurfactants production

The PBBR was operated in batch mode using synthetic wastewater (8 L), and it was recycled for 21 days. The experiments were done by triplicate using the same bioreactor and initial conditions. During the operation of the PBBR, the remaining amount of WMO from the wastewater was evaluated every seven days using a gravimetric method with 100 mL of synthetic wastewater and 100 mL hexane in a Soxhlet apparatus (Ganesh and Lin, 2009; Sonwani *et al.*, 2020).

The presence of biosurfactants was analyzed with samples obtained every day. The methodologies employed were oil displacement (Morikawa *et al.*, 2000) and emulsification activity (E<sub>24</sub>) (Cooper and Goldenberg, 1987). In the evaluation of the emulsification activity, equal amounts of cell-free supernatant and mineral oil were taken in a test tube and vortexed for 2 min. The test tubes were left undisturbed at room temperature for 24 h. After that, the stability of the emulsion layer was observed. The values were calculated using the formula below (Ray *et al.*, 2021):

$$\text{Emulsification Activity}(E_{24}) = \frac{\text{Height of emulsified layer}}{\text{Total Height of liquid column}} \times 100 \quad (4)$$

### 2.3.4 Biodegradation study of the packed-bed bioreactor

The biodegradation performance of motor oil in PBBR was evaluated using the following set of equations (Yadav *et al.*, 2014; Sonwani *et al.*, 2020):

$$\text{Biodegradation efficiency (\%)} = \frac{C_{in} - C_{out}}{C_{in}} \times 100 \quad (5)$$

$$\text{WMO elimination capacity (EC)} = \frac{(C_{in} - C_{out}) \times Q}{V} \quad (6)$$

$$\text{Inlet loading rate (ILR)} = \frac{C_{in} \times Q}{V} \quad (7)$$

where  $C_{in}$  and  $C_{out}$  are the inlet and outlet concentration of WMO (mg L<sup>-1</sup>), respectively,  $V$  is the working volume of PBBR (L), and  $Q$  is the volumetric flow rate (mL h<sup>-1</sup>).

### 2.3.5 Statistical analysis

Error bars in the plots represent the standard error for triplicate samples. The data was evaluated by analysis of variance (one-way ANOVA) with  $p < 0.05$ . The analyses were carried out using Microsoft Excel.

## 3 Results and discussion

### 3.1 Adaptation and evaluation of the NMC

The NMC was isolated from a soil sample contaminated with WMO. It was cultured using a selective medium for hydrocarbon-degrader bacteria and WMO as a carbon source after the adaptation process (60 days). The capability to degrade different WMO concentrations was evaluated.

#### 3.1.1 Kinetic evaluation of the microbial growth

The results of the analysis of the microbial growth can be observed in Figure 2. Different concentrations (0.2, 0.5, 1.0, and 2.5% v v<sup>-1</sup>) of WMO were tested. The results show a shortened lag phase in the microbial growth analysis. This can be related to the previous adaptation period of the NMC to the WMO. The maximum values of microbial growth were 0.841 and 0.716 (OD<sub>600nm</sub>) for oil concentrations of 2.5 and 1.0% v v<sup>-1</sup>, respectively (Table 1). The minimum values of microbial growth were 0.646 and 0.649

Table 1. Comparison of the kinetic parameters obtained during the biodegradation of different concentrations of waste motor oil using a native microbial consortium.

WMO (%)	WMO (mg mL <sup>-1</sup> )	Maximum growth (OD <sub>600nm</sub> )*	$\mu$ (d <sup>-1</sup> )	Maximum WMO degradation (%)**	Maximum biosurfactant production (mg L <sup>-1</sup> )***
0.2	1.822	0.646 ± 0.008	0.1825 ± 0.011	17.41 ± 1.56	181.10 ± 38.80
0.5	4.557	0.649 ± 0.071	0.2979 ± 0.039	32.05 ± 3.18	116.43 ± 38.08
1.0	9.114	0.716 ± 0.025	0.2622 ± 0.056	31.11 ± 4.05	194.07 ± 103.48
2.5	22.785	0.841 ± 0.048	0.2218 ± 0.043	12.98 ± 2.60	194.07 ± 25.87

\*, \*\*, \*\*\* maximum values obtained during the evaluation of the effect of the oil concentration over microbial growth, oil degradation and biosurfactants production.

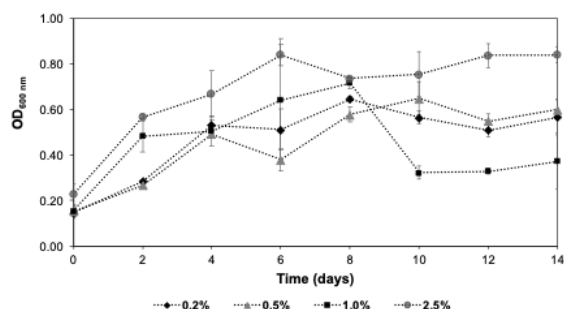


Fig. 2. Kinetic analysis of microbial growth in the native consortium exposed to different concentrations of waste motor oil.

(OD<sub>600nm</sub>) for oil concentrations of 0.2 and 0.5% v v<sup>-1</sup>, respectively.

The results were used to calculate the specific microbial growth ( $\mu$ ) at different oil concentrations (Table 1). According to the results, the maximum values obtained for the specific microbial growth rate were 0.2979 and 0.2622 d<sup>-1</sup> when the microbial consortium was exposed to oil concentrations of 0.5 and 1.0% v v<sup>-1</sup>, respectively. In contrast, the minimum value (0.2218 d<sup>-1</sup>) for the specific microbial growth rate was obtained using the concentration of 2.5 % v v<sup>-1</sup>. These results indicate that the consortium took WMO as the only carbon source. According to the values of the specific microbial growth, the consortium can grow at a major specific growth rate under 0.5% v v<sup>-1</sup> WMO.

The analysis of the growth kinetic parameters was obtained with the Haldane-Andrews model (Equation 1). The experimental data were fitted to the model and the results can be observed in Figure 3. The maximum specific growth rate was 0.785 d<sup>-1</sup>, the value for the half inhibition constant ( $K_I$ ) was 0.781% v v<sup>-1</sup> (7.11 mg mL<sup>-1</sup>), and the value for the half-saturation constant ( $K_s$ ) was 2.6% v v<sup>-1</sup> (23.77 mg mL<sup>-1</sup>). These results indicate that a concentration higher than 2.6% v v<sup>-1</sup> of oil inhibits microbial growth.

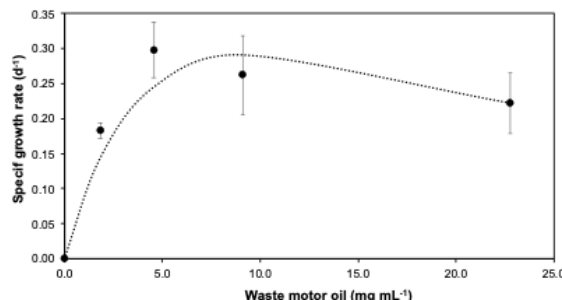


Fig. 3. Kinetic evaluation using the Haldane-Andrews model to fit experimental data, (·) experimental data, and (—) data calculated by Haldane-Andrews model.

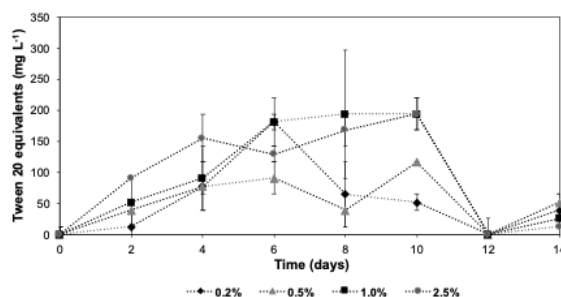


Fig. 4. Comparison of the biosurfactants production expressed as Tween 20 equivalents by native microbial consortium at different concentrations of waste motor oil.

### 3.1.2 Biosurfactants production and oil degradation

The analysis of the biosurfactant production (expressed as Tween 20 equivalents) was evaluated during the biodegradation of the WMO (Figure 4). The maximum values (194.07 mg L<sup>-1</sup>) of biosurfactant production were obtained in the presence of a higher concentration of WMO (1.0 and 2.5% v v<sup>-1</sup>). The results observed in the concentration of 0.2% v v<sup>-1</sup> WMO showed that the concentration of biosurfactant production was 181.10 mg L<sup>-1</sup>. The minimum value (116.43 mg L<sup>-1</sup>) of biosurfactant production was

obtained in the presence of  $0.50\% \text{ v v}^{-1}$  motor oil. During biosurfactant production, no lag phase of growth was observed while using the concentrations 0.5, 1.0, and  $2.5\% \text{ v v}^{-1}$  WMO. This effect can be related to the stimulation by high concentrations and the lower solubility of motor oil. Biosurfactants are growth-associated metabolites produced during the utilization of oil (Ray *et al.*, 2021). Some authors indicate that a high concentration of hydrocarbons can contribute to enhancing the production of surfactant molecules (Dutta *et al.*, 2018). However, according to the results obtained in this work, the high concentration of WMO ( $2.5\% \text{ v v}^{-1}$ ) inhibited microbial growth and biosurfactant production.

The number of biosurfactants obtained in this work was similar to that in a previous report. Some hydrocarbon-degrading bacteria produced biosurfactants in the presence of crude oil as a carbon source (Tripathi *et al.*, 2020). Another work (Ray *et al.*, 2021) reported a biosurfactant production six times ( $1240 \text{ mg L}^{-1}$ ) larger in comparison with the results obtained in the present work, likely due to the low solubility of the anthracene and fluorene employed. Therefore, the microorganism has to produce biosurfactants to use hydrocarbons as a carbon source. Biosurfactant production is an adaptative approach of microorganisms to survive under nutrient-stressed conditions. The production of these metabolites is linked to growth given that they are produced during the utilization of hydrocarbons (Vigneshwaran *et al.*, 2018). In this work, biosurfactant production was observed at the beginning of the exponential phase of microbial growth. The indigenous species have adapted to the environmental stress caused by toxic compounds and are thus better suited for biodegradation (Vigneshwaran *et al.*, 2018; Ray *et al.*, 2021).

Motor oil degradation was analyzed by the gravimetric method and the results are presented in Figure 5. The maximum percentage of WMO degradation was 32.05 and 31.11% for the concentrations of 0.5 and 1.0%, respectively. The statistical analysis revealed that there was no significant difference between these results. In addition, the high values of oil degradation were directly related to microbial growth, as demonstrated by the results obtained in the analysis of the kinetic parameters with the Haldane-Andrews model. The minimum value was observed with a higher concentration ( $2.5\% \text{ v v}^{-1}$ ) of WMO. The lower values of biodegradation could be related to WMO composition, because the motor oil included some oxidative byproducts and thermally

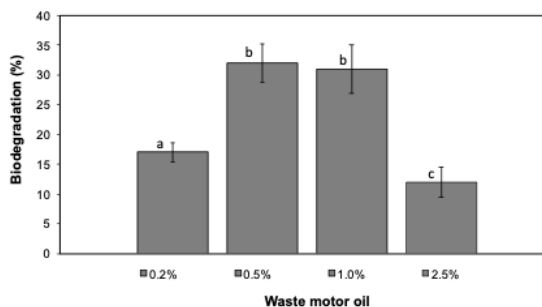


Fig. 5. Biodegradation capacity at different concentrations of waste motor oil by native microbial consortium. Different letters indicate significant differences between each evaluated concentration of waste motor oil.  $p$ -value  $< 0.05$ .

decomposed hydrocarbon fractions can interfere with microorganisms' metabolism (Su *et al.*, 2011). The results obtained in this work were superior to a previous report where three indigenous bacteria strains were tested to degrade anthracene and fluorene (Ray *et al.*, 2021). Still, waste motor oil is more complex and toxic than anthracene and fluorene.

### 3.2 Packed-bed bioreactor for waste motor oil bioremediation

The study of motor oil biodegradation in wastewater was carried out in the PBBR. The isolated consortium was immobilized over a plastic ring support and used as packing material in the PBBR. The results obtained during the wastewater treatment contaminated with WMO using PBBR are described in the following sections.

#### 3.2.1 Waste motor oil degradation and biosurfactants production

The concentration of  $1.0\% \text{ v v}^{-1}$  WMO was used in the study of oil biodegradation in the PBBR as the higher values of the biosurfactant production and oil degradation were obtained with this concentration. The determination of HRT and Q was carried out using equations (2) and (3). Based on the maximum growth rate,  $0.785 \text{ d}^{-1}$  ( $0.0327 \text{ h}^{-1}$ ), and the operational volume (8 L) of the bioreactor, the values for HRT and Q were estimated at 30.581 h and  $0.2616 \text{ L h}^{-1}$ , respectively. The Q value was used to adjust the peristaltic pump and the inlet flow of wastewater to the bioreactor.

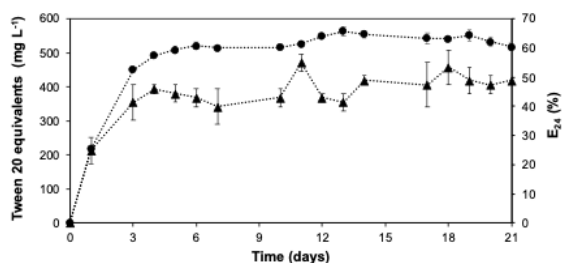


Fig. 6. Evaluation of biosurfactant production and emulsification capacity during the operation of the packed-bed bioreactor with immobilized native microbial consortium and 1.0% v v<sup>-1</sup> waste motor oil. Biosurfactant production expressed as Tween 20 equivalents (▲) and emulsification capacity (E<sub>24</sub>) (●).

The analysis of biosurfactant production and WMO degradation was carried out for 21 days of PBBR operation with the NMC immobilized over the plastic rings. The results of biosurfactant production and emulsification capacity are shown in Figure 6. A fast increase in the concentration of biosurfactant production was achieved in the first three days. After that, the average concentration was about 398.18 mg L<sup>-1</sup>. The maximum concentration obtained was 470.61 mg L<sup>-1</sup> on day eleven. The biosurfactant production capacity increased 2.42 times compared with the assays in Erlenmeyer flasks (section 3.2.1.). The biosurfactant production agrees with that from a previous report (Tripathi *et al.*, 2020) where the bacteria were cultured with crude oil and supplemented with glucose. The addition of glucose is likely responsible for the better results. The behavior of the emulsification capacity was similar to that obtained in biosurfactant production. An increase in the emulsification capacity was observed in the first three days (Figure 6). Emulsification reached an average value of 62.25 % between days 4 and 21. The maximum percentage of emulsification was 65.69 %, obtained at day 13. These results are indicators of the surfactant and emulsifying molecules synthesized by the microbial consortium. In a previous work, Angeles *et al.* (2017) demonstrated that the analysis of emulsification capacity allows to detect molecules with surfactant characteristics. These molecules can cover oil droplets and prevent their coalescence.

Some parameters like pH, temperature, aeration, and salinity also influence biosurfactant production (Ray *et al.*, 2021). Although the effect of those parameters was not evaluated in this work, they are an area of opportunity for future works to enhance biosurfactant production using wastewater

and a PBBR. The demand for biosurfactants is increasing because unpurified biosurfactants can be used in oil industries (Ray *et al.*, 2021). Biosurfactants are molecules with complex structures and different functionalities. Our research group will focus on the purification and identification of the characteristics of such biosurfactants produced when motor oil is used as the substrate.

In a similar work, Dutta *et al.* (2018) demonstrated the feasibility of biosurfactant production at higher diesel concentrations (130 g L<sup>-1</sup>). The authors indicate that a high concentration of hydrocarbons is not a limiting factor in the production of molecules with emulsifying properties. The contact between the microorganism and the hydrocarbons can affect the biodegradation and biosurfactant production. Our results suggest motor oil biodegradation is a consequence of the production of molecules with surfactant characteristics. Biosurfactant production is a microbial response to the disturbance generated by emulsified motor oil. The emulsification contributes to the formation of micelles composed of oil and biosurfactants. This is a positive effect that allows an increase in oil bioavailability for the microorganisms. The presence of biosurfactant molecules partially prevents the coalescence of the oil droplets (Angeles *et al.*, 2017). This phenomenon positively contributes to the mass transfer from the oil to the biofilm formed over the packing material.

The results of the biodegradation can be observed in Figure 7. The highest degradation percentage was 67.76%, calculated using equation (4). This result is better in comparison with the WMO degradation capacity obtained in Erlenmeyer flask experiments. The degradation capacity was 2.18 times higher after 21 days of PBBR operation. The result obtained for WMO elimination capacity (Equation 5) was 0.195 g L<sup>-1</sup> h<sup>-1</sup>, and the value obtained for the inlet loading rate (Equation 7) was 0.287 g L<sup>-1</sup> h<sup>-1</sup>. Mnif *et al.* (2015) reported 38.42 - 49.65% diesel oil degradation in experiments carried out using 0.1% lipopeptide biosurfactant, *B. subtilis* SPB1 strain, and an isolated microbial consortium. Our results show an increase in biosurfactant production as a result of the hydraulic retention time in the PBBR. The time of contact between liquid and gas phases enhanced the uptake of hydrocarbons by the microorganism. Previous works report a similar percentage (64.4%) of motor oil biodegradation by yeast extract and Tween 80 addition (Su *et al.*, 2011). Our results are comparable with the earlier report by Kuyukina *et al.* (2017) where a fluidized-bed bioreactor was used to remove 70% of

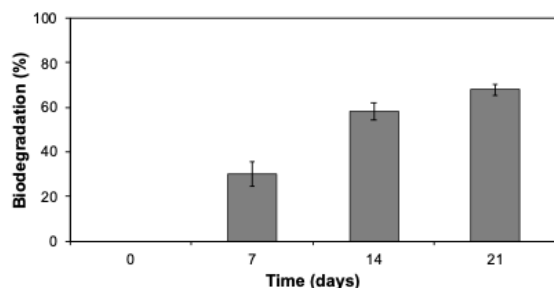


Fig. 7. Evaluation of oil biodegradation during the operation of the packed-bed bioreactor with immobilized native microbial consortium and 1.0% v v<sup>-1</sup> waste motor oil.

petroleum hydrocarbons. However, Sonwani *et al.* (2020) reported 94% of naphthalene biodegradation using a PBBR. In this report, high airflow values were employed to degrade a less complex contaminant as compared with the WMO used in the present work.

There is a large number of possible shapes for packing particles in PBBR. The particle shape selection is determined by the active surface area per volume unit of the material, structural strength, ease of construction, manufacturing cost, and transport properties (Afandizadeh and Foumeny, 2001). In earlier reports, the microbial immobilization was carried on spherical particles of alginate, chitosan, or some jellifying agents (Hussain *et al.*, 2015; Kumar *et al.*, 2015). In this work, we used the plastic ring as packing material since it is cheaper, and its shape is similar to that of Raschig rings (also known as Hollow cylinders). The superficial area of the packing material used in this work was superior compared with that of the jelly spheres used in other works. A better superficial area can be directly related to an increase amount of biofilm available for WMO degradation. The superficial area of the biofilm and the oil droplets are two key factors in motor oil degradation in the PBBR. In this work, the oil-droplet formation explained the presence of emulsifier molecules. The corrugated shape of the packing material increased the contact between oil-surfactant droplets and the biofilm.

Adequate mass transfer is necessary for the PBBR to maximize cell growth. The absence of harmful effects, such as damage from hydrodynamic forces, is also desirable (Warnock *et al.*, 2005). As we observed an increase in biosurfactant production during the motor oil degradation process, there are no substrate or oxygen mass transfer limitations with the biofilm formed over the packing material. The airflow

provided (0.6 L min<sup>-1</sup>) to the PBBR maintained the aerobic condition in the bioreactor and generated a liquid-gas mixture. During the operation of the PBBR, the biomass in the liquid phase was analyzed (data not shown). A major value of the free biomass in the liquid phase was identified in the early days when compared to that at the end of the process. This is likely due to the airflow rate and the maturation of the biofilm. However, Sonwani *et al.* (2020) employed an airflow between 0.5 and 2.0 L min<sup>-1</sup> to remove 94% of naphthalene in a PBBR. The airflow used in the PBBR experiments of current work was enough to degrade the motor oil, which agrees with that used in earlier reports (Sonwani *et al.*, 2020).

The packing material played a key role in this work. The texture of the plastic rings contributed to improving the mix of the bioreactor content and the contact between the liquid and gas phases. These characteristics of the packing material can give an advantage over the Raschig rings commonly employed. The immobilization and operation contributed to increasing the efficiency of WMO biodegradation. Benerjee and Ghoshal (2017) indicate that a high concentration of substrate markedly reduces bacterial growth due to substrate inhibition, leading to the deterioration of the overall bacterial efficacy toward substrate degradation. The results obtained in the present study are similar to those in other reports and present a significant advantage in the bioremediation of oil-contaminated wastewater. In addition, this treatment could be an option to produce value-added byproducts like biosurfactants.

## Conclusions

In this study, a NMC was isolated from contaminated soil with WMO. Its capacity to grow, produce biosurfactants, and degrade motor oil was evaluated. Our results reveal the consortium can grow with waste motor oil as a carbon source. The growth-inhibition kinetics indicate the substrate inhibition at concentrations above 2.6% v v<sup>-1</sup> of oil. The NMC produces biosurfactants during the biodegradation process of WMO.

The biotreatment of wastewater contaminated with WMO using a PBBR demonstrates a biodegradation capacity of 67.76%. This result is 2.4 times superior compared to that obtained during the enrichment of the consortium in Erlenmeyer flasks. Molecules with surfactant and emulsification characteristics

were observed during motor oil degradation in the bioreactor. The findings in this study can be used to upscale the process in real applications for wastewater.

## Nomenclature

WMO	waste motor oil
PBBR	packed-bed bioreactor
NMC	native microbial consortium
OD <sub>600nm</sub>	optic density measurement at 600 nanometers
$\mu$	specific growth rate (d <sup>-1</sup> )
$\mu_{max}$	maximum growth rate (d <sup>-1</sup> )
S	substrate concentration (mg mL <sup>-1</sup> )
K <sub>s</sub>	saturation concentration (mg mL <sup>-1</sup> )
K <sub>I</sub>	inhibition constant (mg mL <sup>-1</sup> )
HRT	hydraulic retention time (days)
Q	volumetric flow (L h <sup>-1</sup> )
C <sub>in</sub>	inlet concentration of waste motor oil (mg L <sup>-1</sup> )
C <sub>out</sub>	outlet concentration of waste motor oil (mg L <sup>-1</sup> )
ILR	inlet loading rate (g L <sup>-1</sup> h <sup>-1</sup> )

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