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Environmental Technology

Microbial enhanced oil recovery (MEOR) by Pseudomonas sp. under laboratory conditions

Recuperação microbiana avançada de petróleo (MEOR) por Pseudomonas sp. sob condições laboratoriais

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ABSTRACT

The purpose of this work was to propose sustainable solutions for advanced oil recovery by evaluating the ability of the bacterium Pseudomonas sp. in the biotransformation of alkanes, in addition to determining strain growth patterns under extreme conditions. For this, the work was initially carried out under laboratory conditions, in which the crude oil was fractionated to obtain the saturated fraction used in the experiment. The bacterial tolerance to salinity and temperature was also tested to determine the experimental conditions and set up the experiment in regard to these parameters. Additionally, an experiment was performed to produce a biosurfactant through biostimulation. The biotransformation experiment consisted of a triplicate with treatment and a control. For treatments, Erlenmeyers flasks received 100 mL of broth containing the biosurfactant, 10 g (10%) of NaCl, 3% of the strain and 1% of the saturated fraction. Erlenmeyer flasks were incubated at 40 °C and 180 rpm for 18 days with periodic analysis. The results initially showed the bacteria exhibited better tolerance at a temperature of 40 °C, and there was no significant change for the different salinities, which was a nonlimiting parameter. For the final experiment, the bacterial growth analysed by Optical Density (OD). exhibited a low variation, in which the lowest point was in T18 with an absorbance of 0.115 and the highest point was in T6 with an absorbance of 0.149. In the qPCR analysis of the bacterial population, the pattern found was similar to the optical density results, with low variation; the lowest number of copies of the 16S rRNA gene (6.66x 10^3) was found in T0 and the highest number was found in T12 (7.86x 10^3). For biotransformation analysis, time 6 was observed to have the highest rate, with 54% oil recovery (C30), followed by 52% (C31) and 51% (C29).

Keywords: MEOR; Biotransfomation; Biosurfactant; qPCR

RESUMO

A proposta deste trabalho foi avaliar a capacidade da bactéria *Pseudomonas* sp. na biotransformação de alcanos a fim de propor soluções sustentáveis que possam ser aplicadas na recuperação avançada de petróleo, além de determinar padrões de crescimento da cepa em condições extremas. Para isso o trabalho foi realizado em condições laboratoriais, inicialmente, com o fracionamento do petróleo bruto para obtenção da fração de saturados, utilizada no experimento. Foi também realizado teste de tolerância das bactérias a salinidade e a temperatura, para determinar as condições de montagem do experimento em relação a estes parâmetros. Adicionalmente, foi montado um experimento para produzir biossurfactante, através de bioestimulação. O experimento de biotransformação foi composto por uma triplicata com tratamento e um controle. Para tratamentos, os frascos erlenmeyrs receberam 100 mL de caldo contendo o biossurfactante, 10g (10%) de NaCl, 3% da cepa e 1% da fração de saturados. Os frascos erlenmeyrs foram incubados a 40 °C e 180 rpm por 18 dias com análises periódicas. Como resultados foram observados inicialmente a tolerância das bactérias que tiveram melhor desempenho para a temperatura de 40° C e não houve alteração significativa para as diferentes salinidades, sendo um parâmetro não limitante. Para o experimento final o crescimento bacteriano analisado por O.D. teve uma baixa variação com menor ponto no T18 apresentando absorbância de 0,115 e o maior ponto no T6 com absorbância 0,149. Para a análise da população bacteriana através de qPCR o padrão encontrado se mostra similar aos resultados de densidade óptica, com baixa variação sendo encontrado o menor número de cópias do gene 16S rRNA 6,66x 10^3 no T0 e maior número foi no T12 com número de cópias 7,86x 10^3. Para análise da biotransformação o tempo 6 foi observado com maior taxa sendo de 54% de recuperação do óleo (C30), seguida de 52% (C31) e 51% (C29).

Palavras-chave: MEOR; Biotransformação, Biossurfactante, qPCR

1 INTRODUCTION

Advances in microbial enhanced oil recovery (MEOR) can improve the strategy of tertiary recovery. Therefore, the manipulation of microorganisms and their organic products are well documented, and these substances include biopolymers, biosurfactants, atmospheric substances, biomass and biogas. These substances can alter the formation water, oil and gas mixture as well as alter the properties of the storage matrix, playing a significant role in the recovery of residual oil (YOUSSEF *et al*., 2009; GOODMAN, 2017).

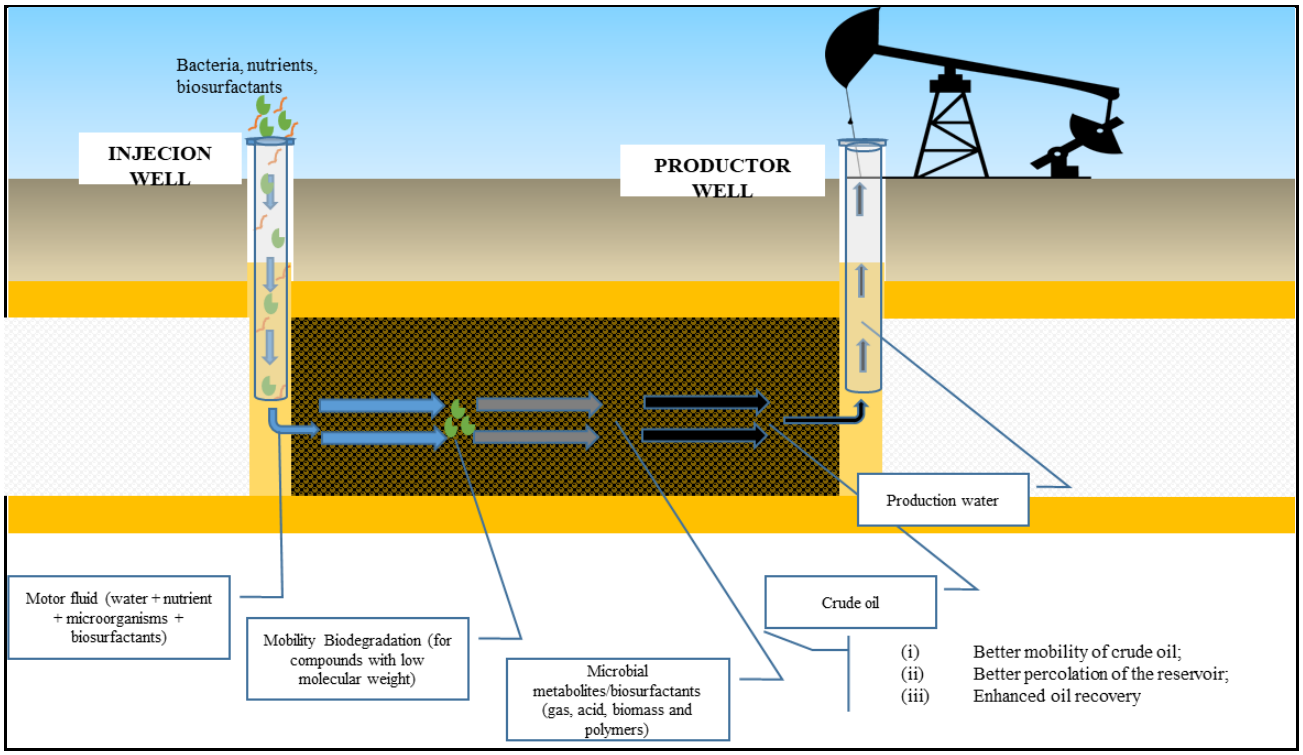
MEOR mechanisms (Figure 2) are different from the mechanisms obtained from other advanced chemical oil recovery (EOR) methods, as they involve the direct biological injection of live microorganisms. MEOR is considered an ecological method, as it does not involve the use of chemical methods, and a viable method, because it does not require water in the injection facilities during its application. In addition, MEOR

methods can metabolize different hydrocarbons at different levels (YERNAVA, 2016; SHIBUAL *et al*., 2018).

Currently, there is growing interest in the application of MEOR methods to increase oil production in low depleted reservoirs due to its capital cost and respect for the environment, as the microbial products exhibit toxicity and are biodegradable in the environment (SHIBULAL *et al*., 2014; YERNAZAROVA, 2016).

The MEOR methodology can be classified into two processes. The first is known as bioaugmentation, which involves adding bacteria that exhibit a high ability to survive in extreme conditions, such as the well conditions. A group called allochthones, for example, withstands high temperature and salinity. To support their growth in this environment (ex situ microbial growth), the bacteria are added together with the necessary nutrients (GOODMAN, 2017).

Figure 2 - Diagram of MEOR mechanisms



Source: Adapted from SHE, 2019

The second process, known as biostimulation, involves injecting nutrients to stimulate the second growth of indigenous bacteria, which are natural in this environment. The bacteria in this process must exhibit characteristics of survival in different environments (GOODMAN, 2017).

The role of microorganisms in different reservoirs is still a well-studied field, even in the context of discovering different solutions and their actions. It is inferred that these organisms are caused by an effect of rocks acting to obtain the oil (HALIM, 2015).

The main processes carried out by microorganisms during MEOR are the formation of bioacids capable of dissolving minerals; increasing solvents and auxiliary biogases of oil from oil transformation; forming biosurfactants, biopolymers and other nonchemical compounds to cause emulsion; and producing biomass influencing rock wettability (YERNAZAROVA, 2016; CHAFALE, 2022).

Biotransformation was not observed later the methods but was a more promising technique, which suggested that the MEOR supply process reduced the oil and the freezing suggestion point, which in turn increased the oil flow. This brought new perspectives on the characteristics that directly imply the recovery of vehicles, such as freezing point recovery, flow capacity, recovery difficulty and high recovery cost (SHIBUL, 2014).

The transforming action of microorganisms does not lead to the reduction of longer hydrocarbon chains, but rather generates a more fluid and lighter oil (WENTZEL *et al*., 2007). This is because the transforming action causes fundamental changes in the physical properties of the oil. Biodegradation has the following main pathways: aerobic and anaerobic pathways, in which there is oxygen and absence of the element, respectively. Biodegradation that occurs in hydrocarbons in reservoirs is anaerobic (AITKEN *et al*., 2004; NIU *et al*., 2020).

Studies of the anaerobic biotransformation process aim to increase the level of knowledge about the biochemistry of microorganisms through identifying metabolites and describing the main genes involved in this process. Under these conditions, the types of transformation are hydrocarbons, including faltene, phenanthrene, alkane and

mixed hydrocarbons, and the transforming types are hydrocarbons, such as benzene, naphthalene, alkane and mixed hydrocarbons, with 2 promissory classifications of alkanes.

The aerobic condition is less prevalent. Its purpose is to allow the oil to be installed and to apply the transport solution in the form of fluid release.

2 MATERIALS AND METHODS

2.1 Fractionation of paraffinic oil

For vacuum fractionation, an apparatus was assembled, which consists of a system composed of an open extractor in glass with a Ø 1 mm perforated plate and conical ground joint with a boron vacuum outlet coupled to a round-bottomed flask and a vacuum pump. A wad of glass wool was deposited at its lower end to seal the holes and prevent stationary phase particles (silica gel 0.063-0.200 mm) from passing. A fibreglass membrane with a diameter of 47 mm was placed in the system. Approximately 50 g of silica gel was added. Subsequently, 0.2 g of sample (the sample is mixed with a small amount of silica before being transferred to the system) was transferred to the system so that it did not create preferential paths during the elution of the solvent, and a cotton swab was placed over the sample.

Gradually and continuously, 120 mL of n-hexane was added to the system to elute the saturated compounds (F1), and initially, a small amount of the solvent was placed in the system. The vacuum pump was activated so that the compounds descended uniformly, and then the remainder of the solvent was added. Between the first fraction and the second fraction, 100 mL of n-hexane was eluted through the column to remove possible remnants of saturated compounds that could be retained in the silica, and this is called the “dead” phase.

Subsequently, the aromatic compounds (F2) were eluted with a mixture of two solvents, 120 mL of hexane + dichloromethane (DCM), in a 4:1 ratio. The pattern of the

fractionation process was the same as the saturated fraction (F1) pattern. Between F2 and F3, 60 mL of the mixture of n-hexane and DCM were be eluted through the column to remove possible remains of aromatic compounds that may have been retained in the silica, thus preventing the next fraction form being contaminated.

To elute the polar compounds (F3) (NSO), a mixture of 120 ml of DCM + methanol with a ratio of 4:1 was used using the same procedure described above.

2.3 Biosurfactant production

Four Erlenmeyer flasks were prepared, which each contained 100 mL of NaCl solution, 3% BH (Bushell Haas) culture medium, 1% glucose (dextrose) and Pseudomonas sp. Subsequently, the flasks were incubated in a shaker incubator (Lab Companion™) at 180 rpm and 37 °C for 4 days. After this period, the cell mass of the microorganism was removed through centrifugation followed by vacuum filtration with membrane filtration, and supernatant broth containing the biosurfactant was used to set up the experiment.

2.4 Tolerance test under salinity and temperature conditions

The strain was grown in LB medium and aliquoted into 12 Erlenmeyer flasks, which contained 100 mL of saline solution at concentrations of 3%, 5%, 8% and 10%, and the temperatures were 25 °C, 40 °C and 60 °C. Thus, during the period of 15 days, each incubator contained 4 flasks with different salinities plus the strain and 4 flasks with only with saline solution (control), totalling 8 Erlenmeyer flasks for each temperature; growth analysis was performed through membrane filtration and cell counting of colonies on Days 0, 7 and 15.

2.5 Bacterial culture preparation

Four Erlenmeyer flasks were prepared that contained 200 mL of 3% BH mineral medium and Pseudomonas sp. The flasks were incubated in a shaker (Lab Companion™) at 150 rpm at 37 °C for a period of 3 days. After this period, the microbial

mass was obtained from centrifugation and later quantified and added to the final experiment.

2.6 Experiment setup

Five Elernmeyers flasks (B1, B2, A1, A2 and A3) were assembled for 4 evaluation times (T0, T6, T12 and T18), totalling 20 vials. The flasks identified as A were triplicate samples that contained 100 mL of broth containing biosurfactant, 10% NaCl, 3% Pseudomonas sp. (1.5 x 108 CFU/mL) and 1% saturates fraction (F1). For B1, the same pattern was followed, but without the addition of the strain, and the sample was used as a blank to evaluate the fraction throughout the experiment. For B2, the same pattern was also followed, but without the addition of the saturates fraction; the sample was used as a blank in the evaluation of bacterial growth.

All flasks were submitted to Shaker (Lab Companion™) under conditions of 180 rpm and 40 °C of temperature, and collections were performed on the first day and then on Days 6, 12 and 18.

2.7 Analysis of bacterial growth and genetic quantification

The growth study was carried out from the withdrawal of an aliquot containing 200 µL from the experiment in a multiwell plate and analysed by spectrophotometer, model LMR 96-4 (Loccus) in a microplate at an absorbance of 590 nm.

For genetic analysis, the DNA extraction process was initially performed using the Norgen Biotek Corp extraction kit™ following all the steps described in the manufacturer's protocol. To determine the concentration and purity of the extracted DNA, a Nanodrop™ ND-2000 UV–Vis spectrophotometer (Nanodrop, Wilmington, DE, USA) was used.

Then, using the primer for 16S rRNA (Table 1), the standard curve was generated. The gene was amplified by conventional PCR, and cleaning of the PCR product was performed using 20% PEG.

The number of copies of 16S rRNA genes in each sample was quantified by the quantitative PCR (qPCR) method. The qPCR process was performed using AriaMx Real-Time PCR (qPCR) Agilent™ and a SYBR Green I fluorophore protocol. Reaction volumes of 20 µL contained 10 µL of GoTaq® Qpcr Master Mix, 0.35 µL of each primer (10 µM) (Table 1), 1 µl of template DNA and 8.3 µl of PCR grade water. All amplifications were performed in triplicate. The addition of 2 µL of PCR grade water instead of DNA template was used as a negative control.

Thermal cycling consisted of 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at 55 °C for 30 s and elongation at 72 °C for 35 s. After amplification, a melting curve was obtained by heating the products to 95 °C, cooling them to 65 °C and then gradually heating them to 95 °C for 30 s, then 55 °C for 30 s at a rate of 0.2 °C/s and finally 95 °C for 30 s. Triplicate results from real-time PCR measurements were averaged. The number of genes in an aliquot was calculated using the mass (m) of the fragments in a given volume and the molecular weight (Wm) using the following equation:

Copies µL-1 = (m x 10-9 x 6.02 x 1023)/Wm

The concentration of gene copies was calculated in the AriaMx Real-Time PCR System V.1.7 Software (Agilent™).

Table 1 - 16S rRNA primers used for absolute quantification by qPCR

Gene	Primer	Sequence	Fragment	Temperature of alignment °C	References
16S rRNA	515F	GTGCCAGCMGCCGCGGTAA	411	55	Caproso, *et al*. 2011
	926R	CCGYCAATTYMTTTRAGTTT			Parada, *et al*. 2016

Source: Authorship

2.8 Extraction and quantitative analysis of alkanes

The extraction of saturates throughout the experiment was carried out by vigorously mixing the contents with 30 mL divided into 3 steps with the addition of 10 mL of DCM in a separatory funnel, allowing the mixture to separate into different fractions. The DCM fraction with the alkanes was carefully collected in a round bottom flask passing through a funnel containing silica gel and cotton. The three flasks of each sample were rotaevaporated, and the analyte was transferred using DCM to one vial per sample. Subsequently, the samples were injected into a gas chromatograph with a flame ionization detector (CG/FID, Model 7890B, Agilent Technologies, California, USA).

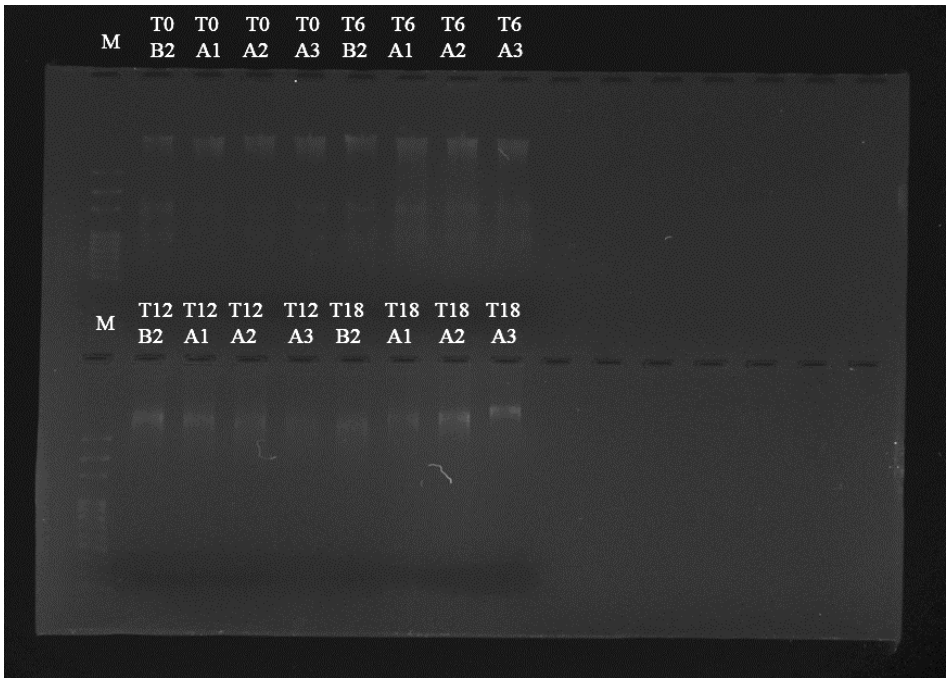
3 RESULTS AND DISCUSSION

3.1 DNA isolation and purity

For DNA isolation, the pellet was used after the contents of each sample were centrifuged, and the protocol of the commercial Norgen™ kit was followed. For quantitative and qualitative evaluation of the extracted material, we proceeded by using the spectrophotometric method in the NanoDrop™ equipment, as well as analysis by electrophoresis in agarose gel (Figure 3). The values obtained are described below (Table 2). In the table, it is possible to observe the times T0 and T6 corresponding to high amounts of DNA; in contrast, the times T12 and T18 represent a drop of these values applying a temporal approach of this parameter.

The relationship presented between the absorbance ratio 260/280 measures an indicative calculation of the purity of the samples, in which the maximum absorbance for DNA corresponds to the wavelength of 260 nm, while the proteins will show absorbance to a UV ray in the wavelength of 280 nm. The minimum value for a sample to be considered pure is 1.8 as a result of the ratio of absorbances obtained (KE *et al*., 2018; SHE *et al*., 2019; OKORO *et al*. 2022).

Figure 3 - Electrophoresis gel of extracted DNA samples



Source: Authorship

Table 2 - Quantification values obtained in a NanoDrop™ spectrophotometer

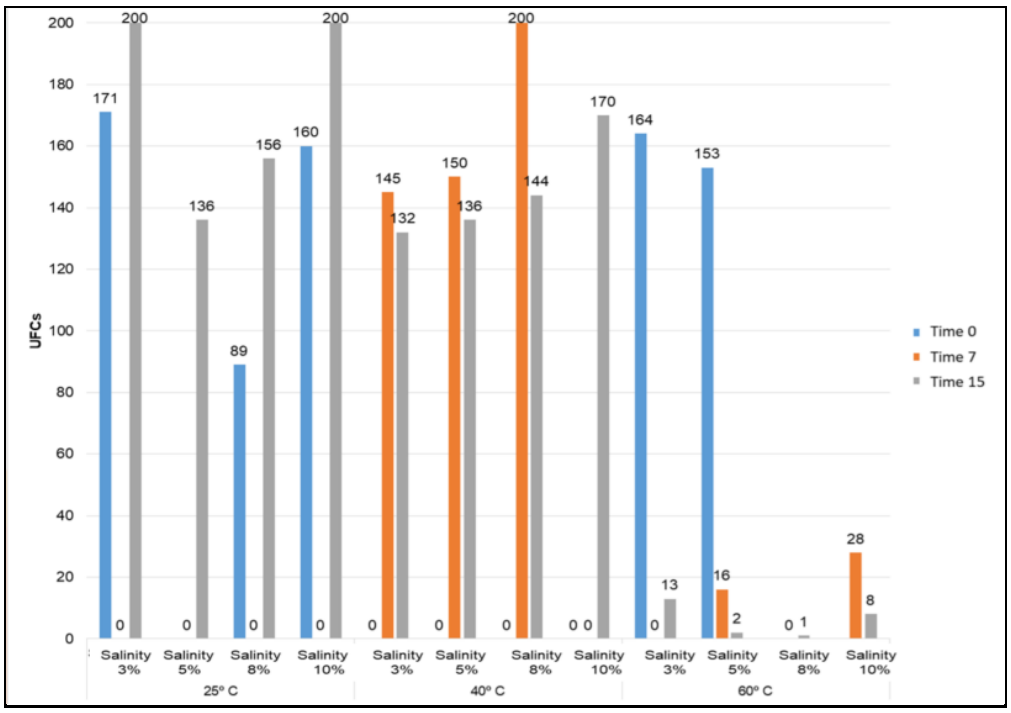
DNA Quantification (NanoDrop™)				
		ng/µl	A260/280	A260/230
T0	Branco	289,3	2,09	2,17
	A1	311,6	2,06	2,07
	A2	280	2,05	2,12
	A3	377,5	2,07	2,21
T6	Branco	298,9	2,05	2,14
	A1	344,2	2,08	2,17
	A2	200,3	2,07	2,14
	A3	403,7	2,08	2,16
T12	Branco	73,6	2,01	1,93
	A1	30	1,99	1,7
	A2	42,1	1,97	1,87
	A3	31	1,94	1,82
T18	Branco	98,8	1,98	2,02
	A1	153,7	1,98	2,05
	A2	27,5	1,87	1,85
	A3	27,7	1,8	1,37

Source: Authorship

3.2 Effect of salinity and temperature on strain growth

The results obtained at times 7 and 15 (Figure 4) were favourable at a temperature of 40 °C combined with a salinity of 8% with plate growth above 200 CFUs at time 7 and 144 UFCs at time 15. Overall, it was possible to conclude that temperature rather than salinity caused a greater influence on the growth of *Pseudomonas* sp.

Figure 4 - Result of bacterial growth assay. The data represent the CFU/mL values for growth in the experiments at 0, 15 and 7 days with salinity and temperature variation



Source: Authorship

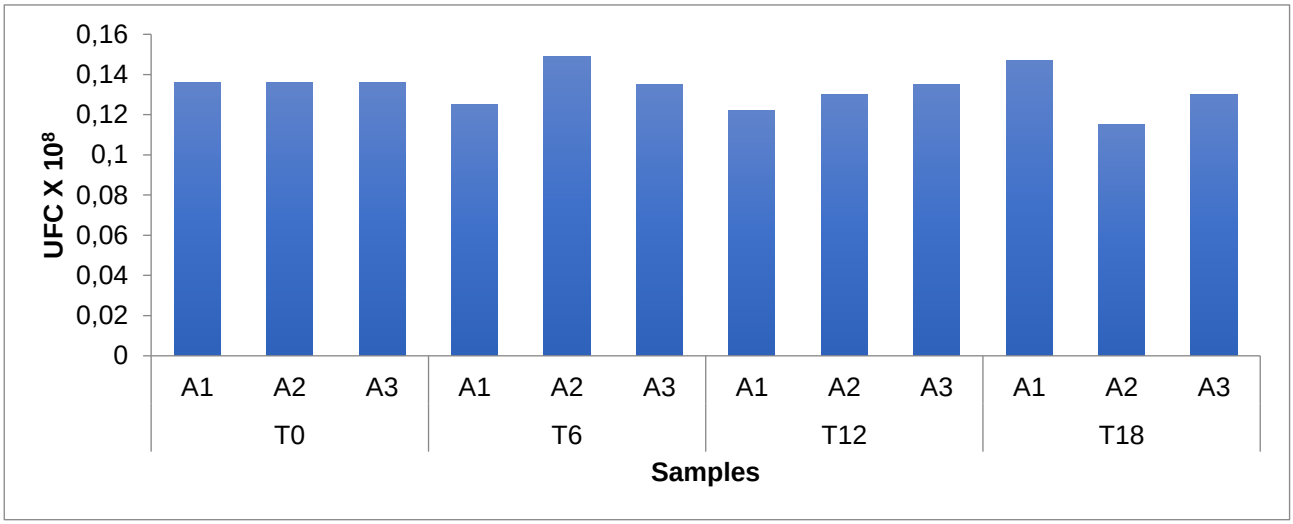
In a test to evaluate the effects of temperature on bacterial growth, KE *et al*., 2018 used microorganisms from different strains, including the genus Pseudomonas, and their results showed that at the temperatures tested (20, 30, 40, 50 and 60 °C), the temperature of 40 °C presented the highest value for optical density (OD), which was its evaluation method. The test was applied with the objective of evaluating the resistance of the strain, considering that the studies preliminarily suggest the application of the bacteria in an oil reservoir environment, in which the conditions are considered extreme for several parameters, including temperature and salinity.

3.3 Characteristics of bacterial growth

The analysis of bacterial growth was performed by reading the samples in a spectrophotometer, model LMR 96-4 (Loccus) in a microplate, and the absorbance was given using a wavelength of 590 nm to obtain the degree of absorbance of the samples. What was observed throughout the experiment is that the number of cells exhibited a low variation between the initial and final periods (Figure 5), leading to the inference that there was no significant cell growth in the treatments.

Through the analytical tool, it was possible to quickly analyse the bacterial growth monitored, and highly reliable data were generated because the method is considered classic, especially when evaluating the growth of pure cultures (BROWN, 2022).

Figure 5 - Arrangement of the UFC numbers of each sample during the experiment



Source: Authorship

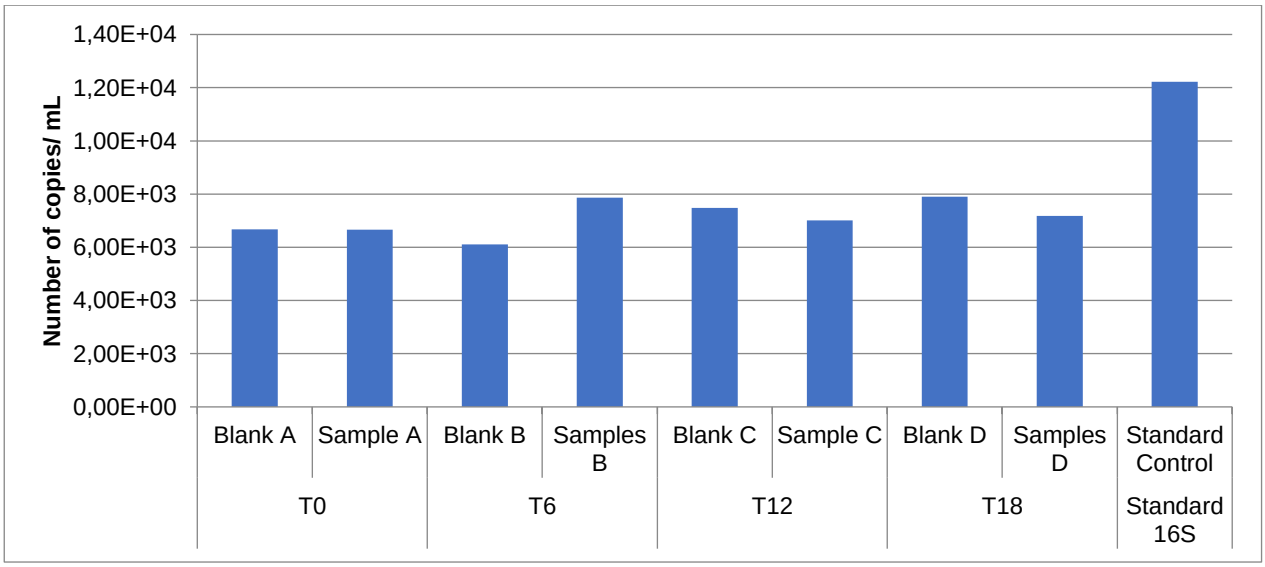
3.4 Absolute quantification of the 16S rRNA gene by qPCR

Quantitative qPCR was performed to estimate the total population of *Pseudomonas* sp. for each treatment throughout the experiment. As a standard curve, the PCR product treated with PEG was used at concentrations of 10-1, 10-2, 10-3, 10-4 and 10-5. In the graph of Figure 6, it is possible to verify that the quantification does not increase over time, which is a probable inference that indicated no increase in the

bacterial population occurred. These data corroborate what was observed through bacterial quantification by OD.

It is also possible to highlight that the averages of the treatments that contained the strain only show a slight increase in T6, as shown in the graphs of Figure 6, which revealed a number of copies of 7.86 x 10^3; this result indicated that at the same point, an equivalent increase relationship occurred with the analysis by OD, which was considered a point of positive of growth response in the second point of analysis.

Figure 6 - Number of copies of the 16S rRNA gene from *Pseudomonas* sp. The results are presented as averages



Source: Authorship

The analysis tool shows the efficiency of that method, demonstrating that the method offers an alternative for evaluating the quantity of bacterial strains. The technique has been widely used to measure the number of bacterial strains through the marking of genetic information, as the technique can detect individual genes of bacteria even at low concentrations; furthermore, the technique can be applied from a general objective to a more specific objective based on the primers used. (COTTO, 2015; BROWN, 2022).

3.5 Biotransformation of alkanes

For this stage, comparative graphs were prepared between the blank and the mean of the triplicates of each treatment and between the beginning and end of the experiment (Figure 7). For all times, is a loss in the lighter compounds between C8 and C16 was observed.

Compared with the blank, in which the sample did not contain bacteria, the solution used was the same for all treatments; specifically, the solution contained biosurfactant from the initial biostimulation process. The presence of this compound, as well as the possible presence of bioemulsifiers, may explain why a loss in lighter compounds occurred at the end of the experiment. In general, these bioactive compounds are produced by various microorganisms that are applied in the advanced oil recovery process (CHAFALE, 2022).

The activity observed in the most pronounced presence of these emulsifying compounds is the change in the properties of the fluid to solubilize the mixture between oil and water, leading to the formation of emulsions that hold the oil in its micellar core (CHAFALE, 2022).

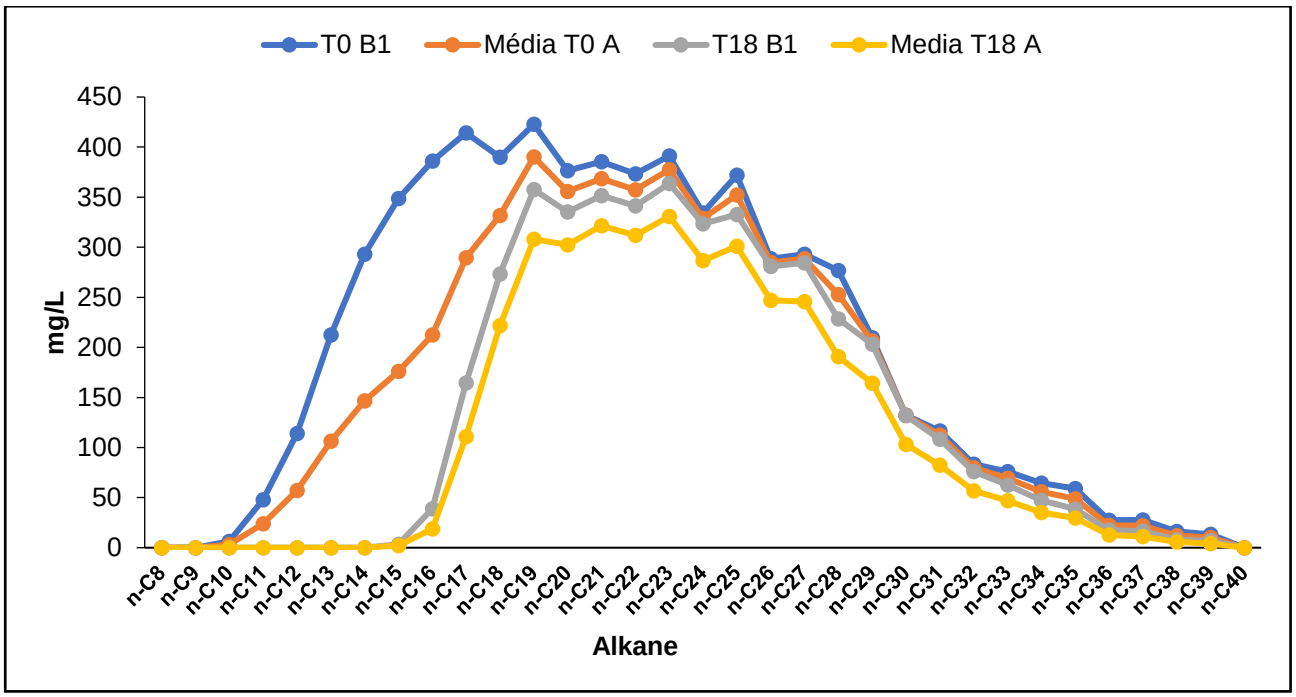
When some oil droplets were trapped, this bridge between organic compounds, water and oil in which the micelle is formed minimizes the free energy, leading to an increase in the solubility of hydrocarbons in the hydrophobic medium, favouring the maintenance of the hydrophilic-lipophilic balance (ASTUTI). *et al*., 2019).

A factor that is also associated with the bioavailability of hydrocarbons in this type of treatment is salinity. A study by Khademolhosseini *et al*. (2019) pointed out that the presence of salt ions attracts water molecules, causing the biosurfactant to appear at the interface. A high amount of salt in the medium can decrease the emulsifying activity, as the bioavailability of hydrocarbons presents a tolerance between 10-15% of salinity in the medium that is accessed by biosurfactants (HENTATI *et al*., 2021).

When comparing the blanks of the initial and final times with the initial and final averages of the samples (Figure 8), it is possible to identify that the samples without

microorganisms maintain a higher general quantity of alkanes compared to that of the treatments, which suggests that there is a possible action of degradation by bacteria present in the medium.

Figure 8 - Comparison of the quantity of alkanes between the samples and the blank

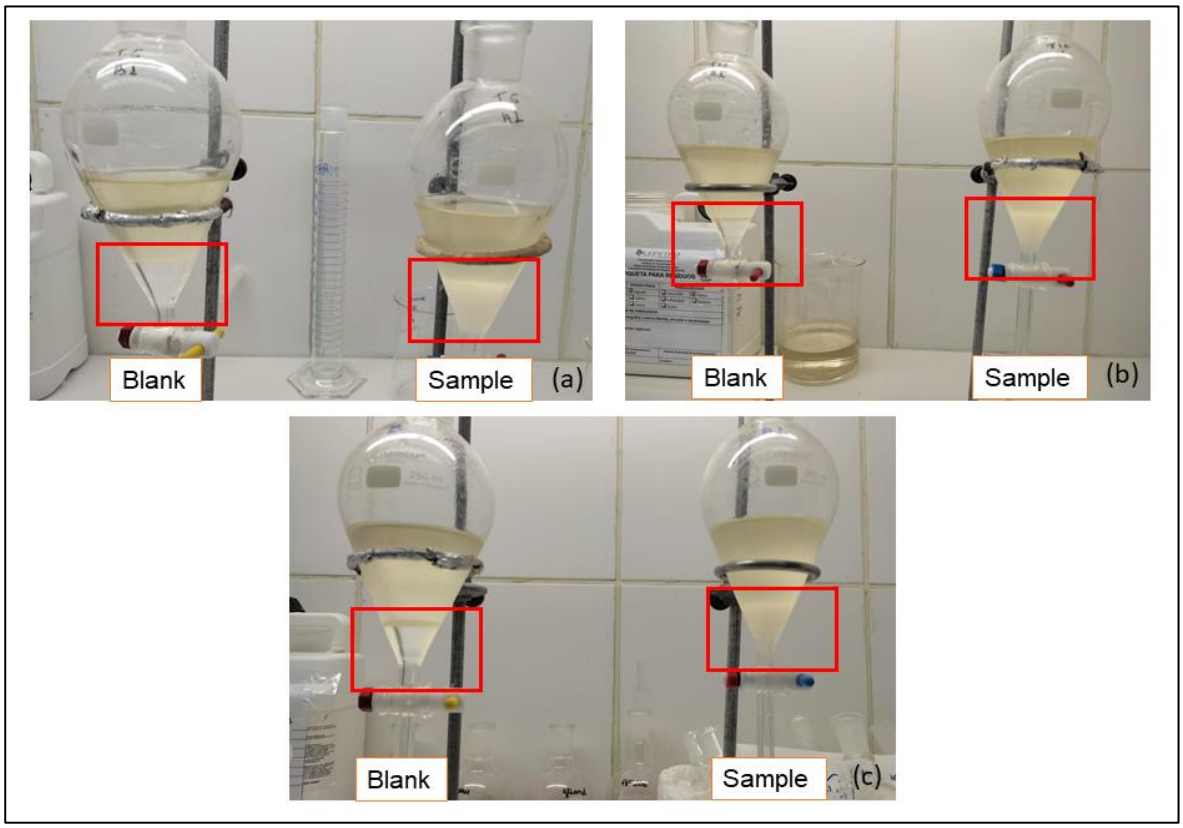


Source: Authorship

Through Figure 8 it is possible to demonstrate where the action of microorganisms was greater. The degradation rate of the compounds infers that the heavier chains from C31 to C39 have high degradation activity; however, the intermediate compounds between C19 and C30 exhibited the lowest percentage of degradation. This behaviour evidenced in the results is expected for the application of the *Pseudomonas* bacterium, since this microorganism has a high capacity to metabolize longer chains of hydrocarbons, such as *Pseudomonas* aeruginosa, with degradation reported in chains between C36-C40, and *Pseudomonas* fluorescens degrades between C12 and C32 (NIU *et al*. 2020; CHAFALE, *et al*. 2022).

The recovery factor obtained after the end of the treatment is shown in Figure 9. When the values corresponded to the triplicate means, we observed the highest recovery of the intermediate fractions between C18 and C30, and there was no significant recovery between C8 and C14. This result may be related to the formation of micelles by the biosurfactant.

Figure (10) - Photographic records showing the formation of emulsion in the extraction process, highlighting the difference between the blank and the treatment on days: T6 (a), T12 (b) and T18 (c)



Source: Authorship

It is possible to observe that the recovery in the blank samples, absent of bacteria, but with the presence of biosurfactant, the recovery rates are higher compared to that of the treatments. During the experimental process in the collection of samples, it was observed that the control samples showed a low formation of emulsion, which formed structures to stabilize the reaction between water and oil, during the agitation process

for liquid–liquid extraction; this procedure was carried out to obtain the fraction of saturates to be analysed (Figure 10). This indicates a possible explanation for the higher recovery rates observed in the blank samples.

Figure 7 - Quantification of alkanes between the initial and final time of the experiment in the samples. White (a), Triple 1 (b), Triple 2 (c) Triple 3 (d)

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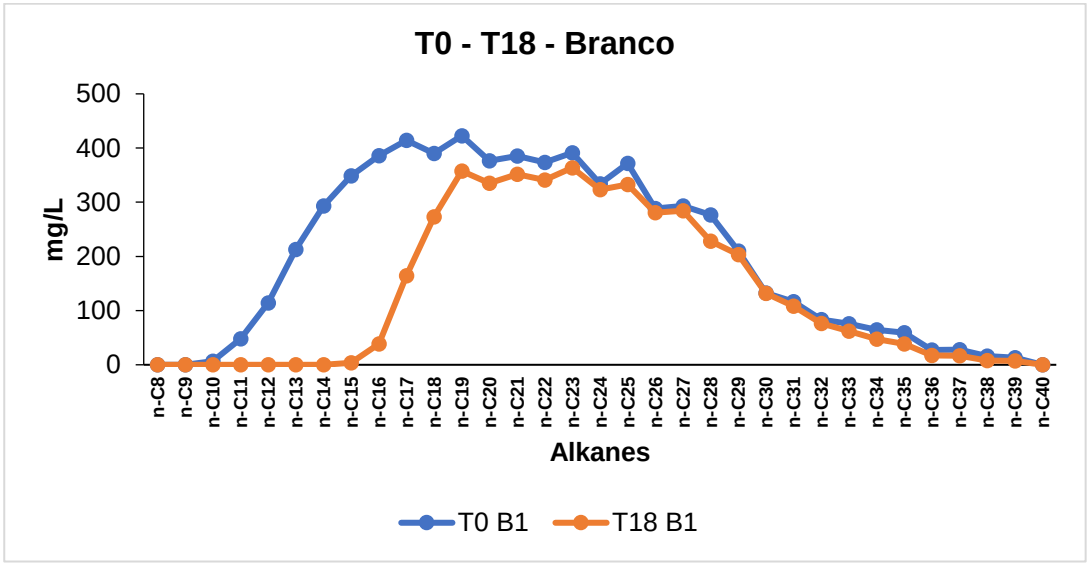

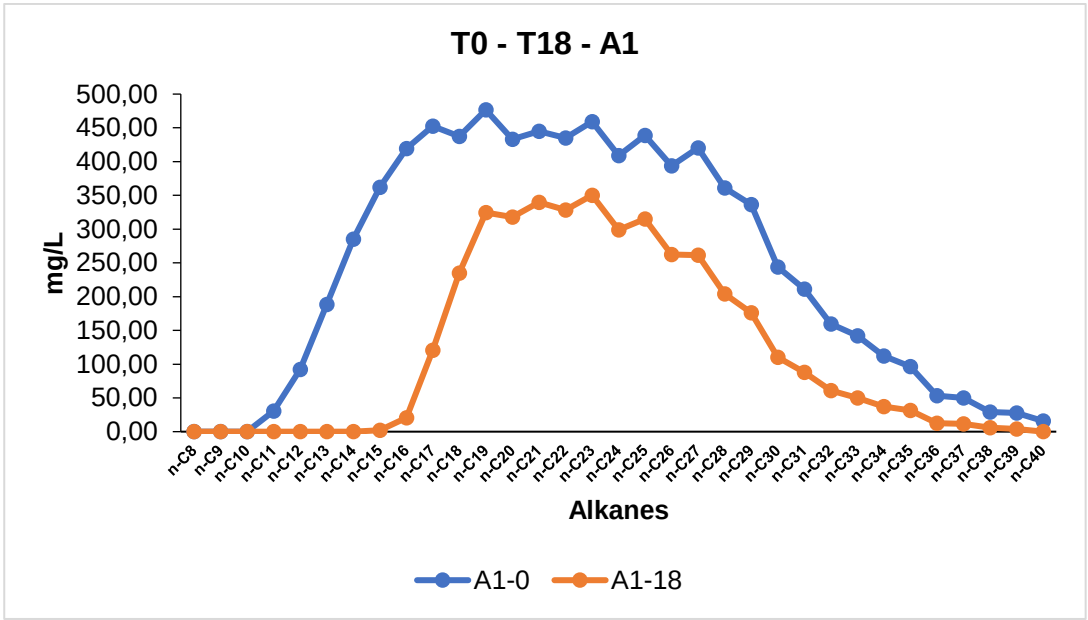

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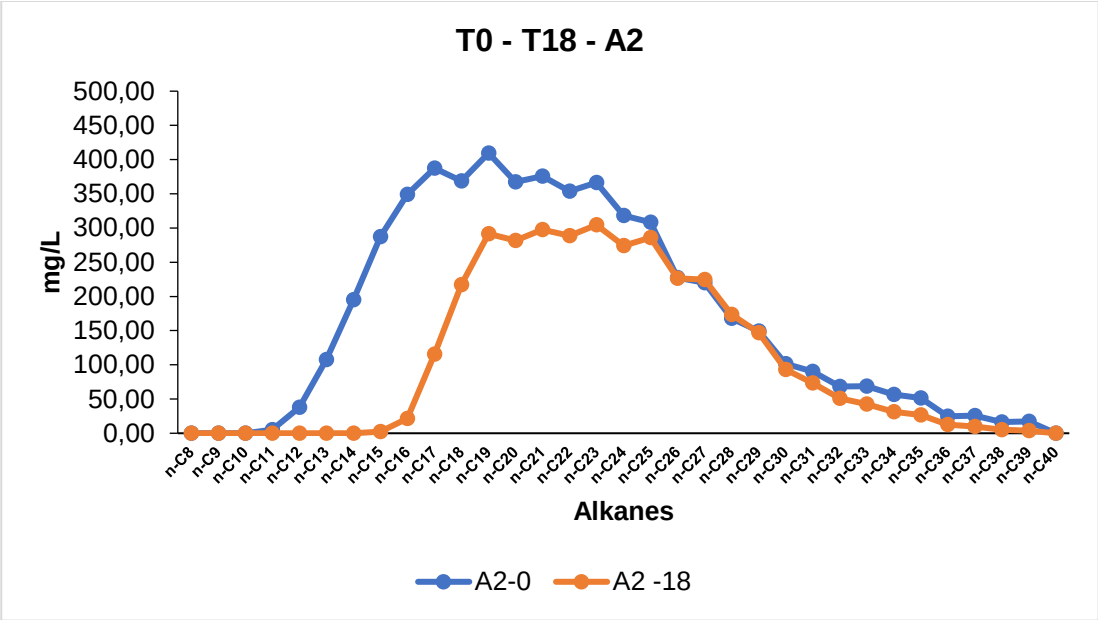
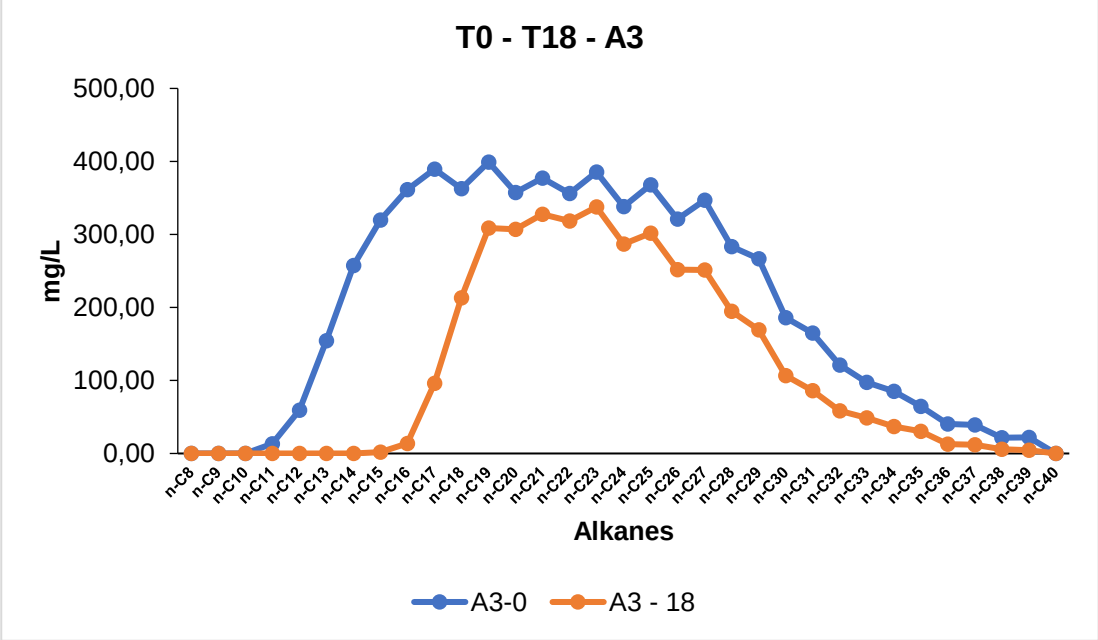
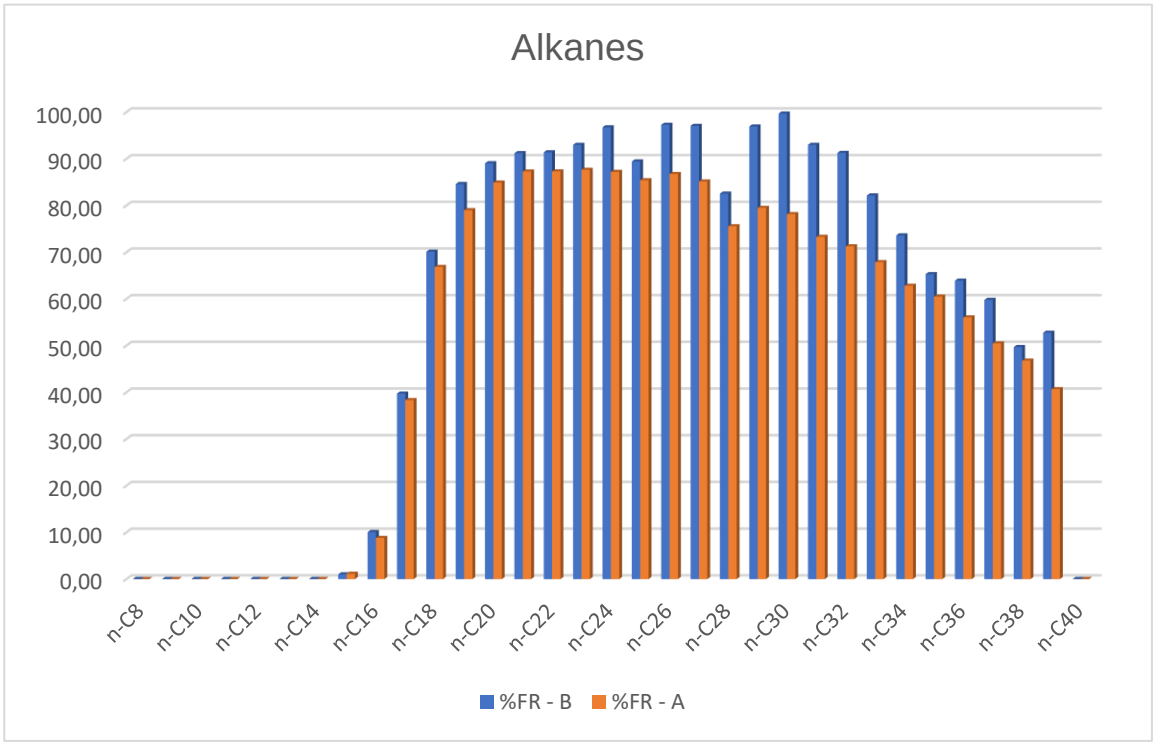
Conclusion

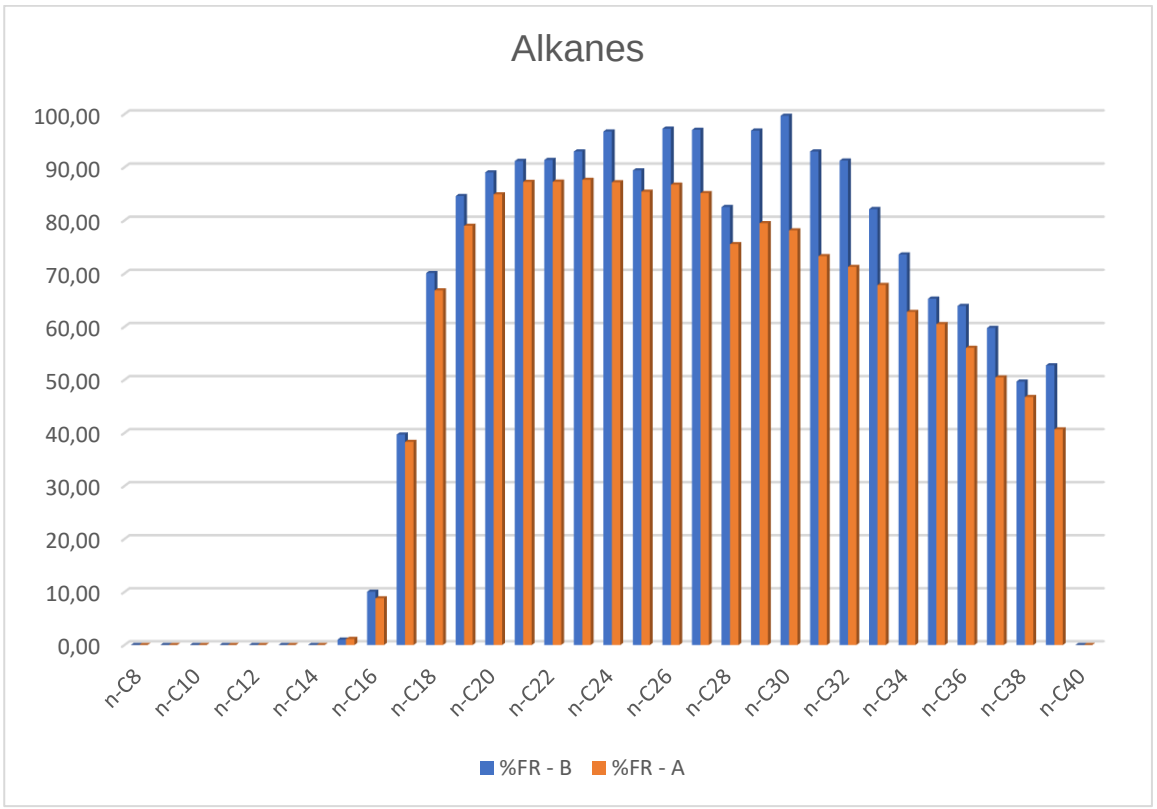




Source: Own Authorship

Figure 9 - Graphical demonstration between the rates of degradation and recovery of alkanes





Source: Own Authorship

In relation to what can be observed T0 – T6 (Figure 11), treatment A1 was disregarded, as it presented a different behaviour, which may have been caused by an analytical error. Treatment A2 was indicative of a formation process of intermediate compounds (C23 - C32), as these rates are negative in relation to the degradation process, with the most expressive value in C30 being -54%. For sample A3, the graph shows low values in intermediate compounds in relation to negative degradation; however, these values mostly do not indicate degradation.

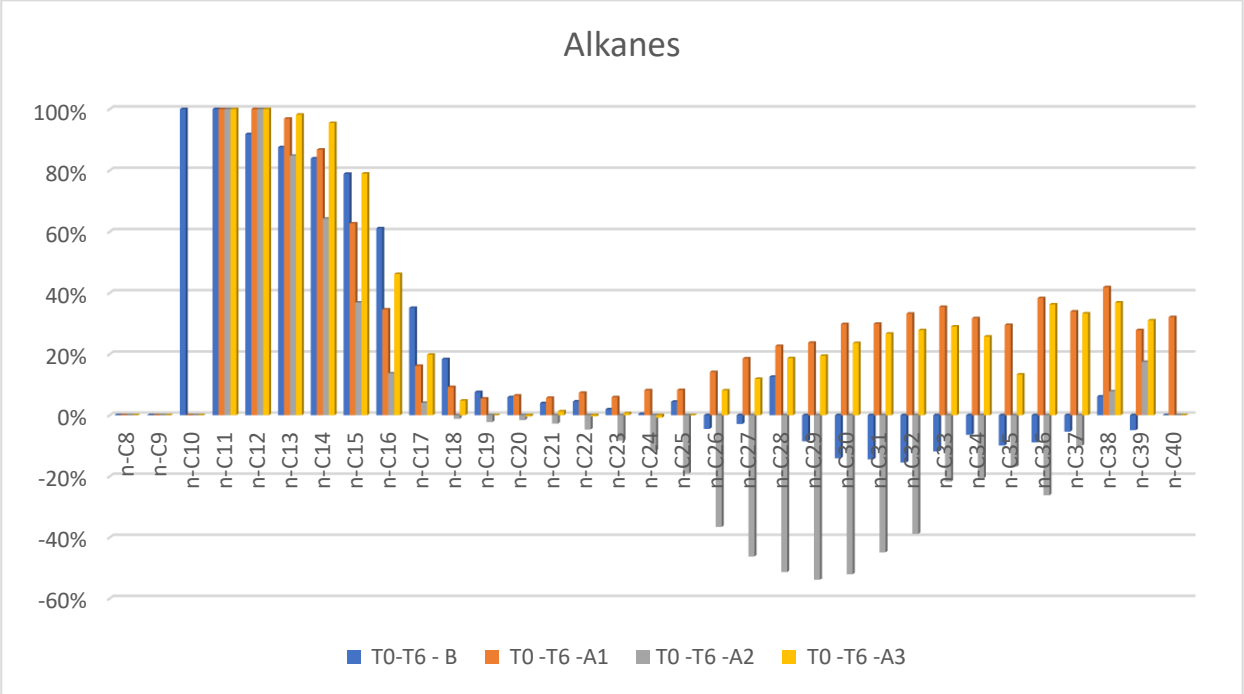
For T6 - T12, the results were positive for degradation in all treatments; that is, a great loss of compounds occurred in this phase, relating to the bacterial population, and a reduction in their number was observed in the analyses performed. What happens in relation to times T12 and T18 is a continuation of what happened in the first analysis, with the chains between C19 and C26 presenting negative values in relation to the rate of degradation, while the rates of the heavier chains grow in degradation; however, in this period for bacterial quantification, stability was observed in the population. For the general analysis of the experiment time T0 – T18, the degradation is positive. This indicator is an important parameter that serves as a basis for establishing the appropriate time in which the bacterium acts in a biotransforming manner without consuming the hydrocarbons in the degradation process.

Therefore, time 6 exhibited the most expected behaviour for the application of advanced microbial oil recovery technology. This method contains positive indicators that are related to what is possible to recover with bacteria and biosurfactant, and the short time favours a quick response.

An important method when improving performance in recovery from the emulsification process is the demulsification technique, which can have a chemical or biological origin. Demulsification is applicable due to the aggregation of oil that occurs in the micelles and is a sequential step in which the demulsifier breaks the aggregation of droplets followed by coalescence for the final recovery of the oil (MOHEBALI *et al*., 2012; CHEFALI *et al*., 2022).

Figure 11 - Evaluation of the degradation of samples A2 and A3 between times T0 and T6

Continue



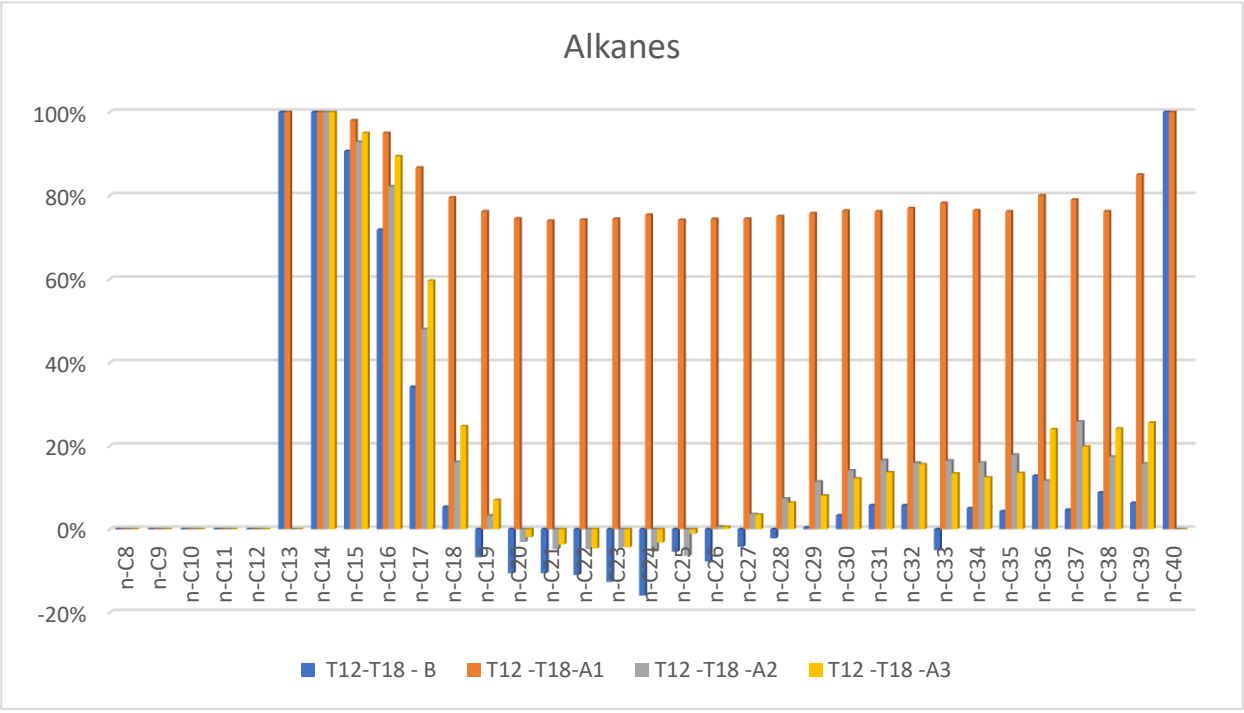
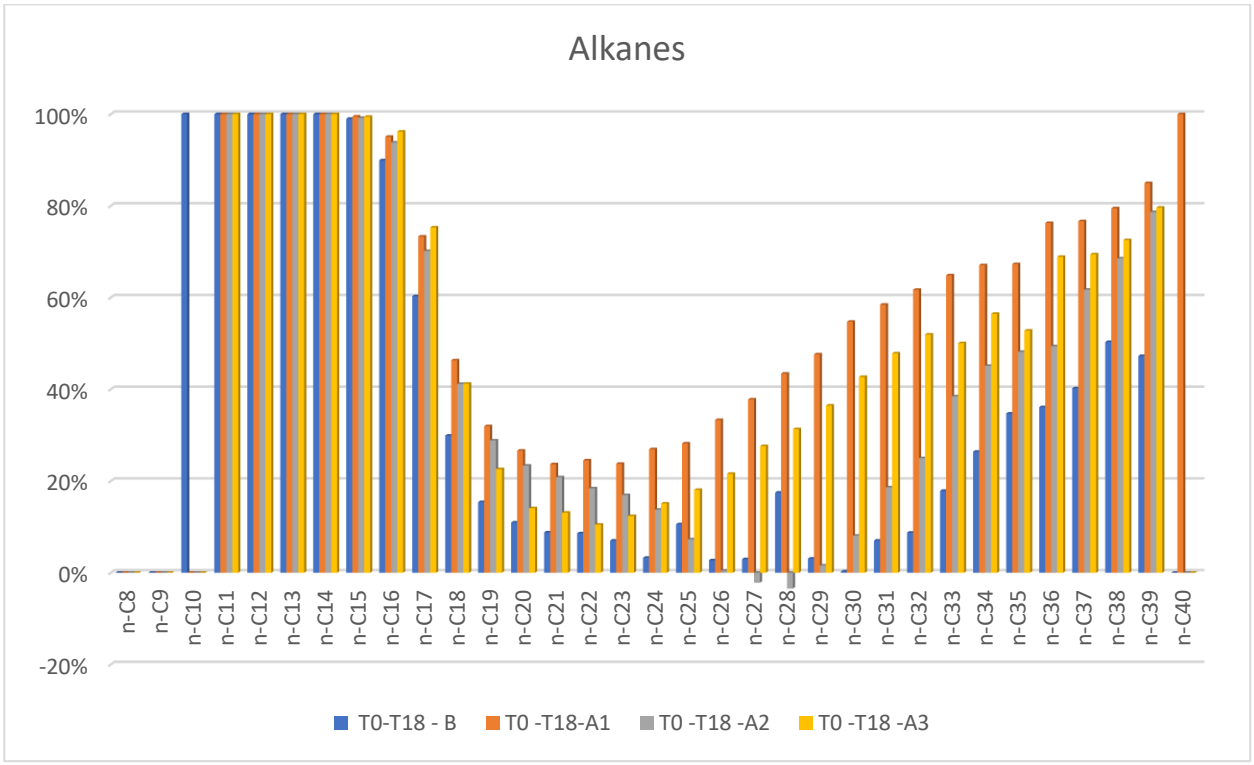
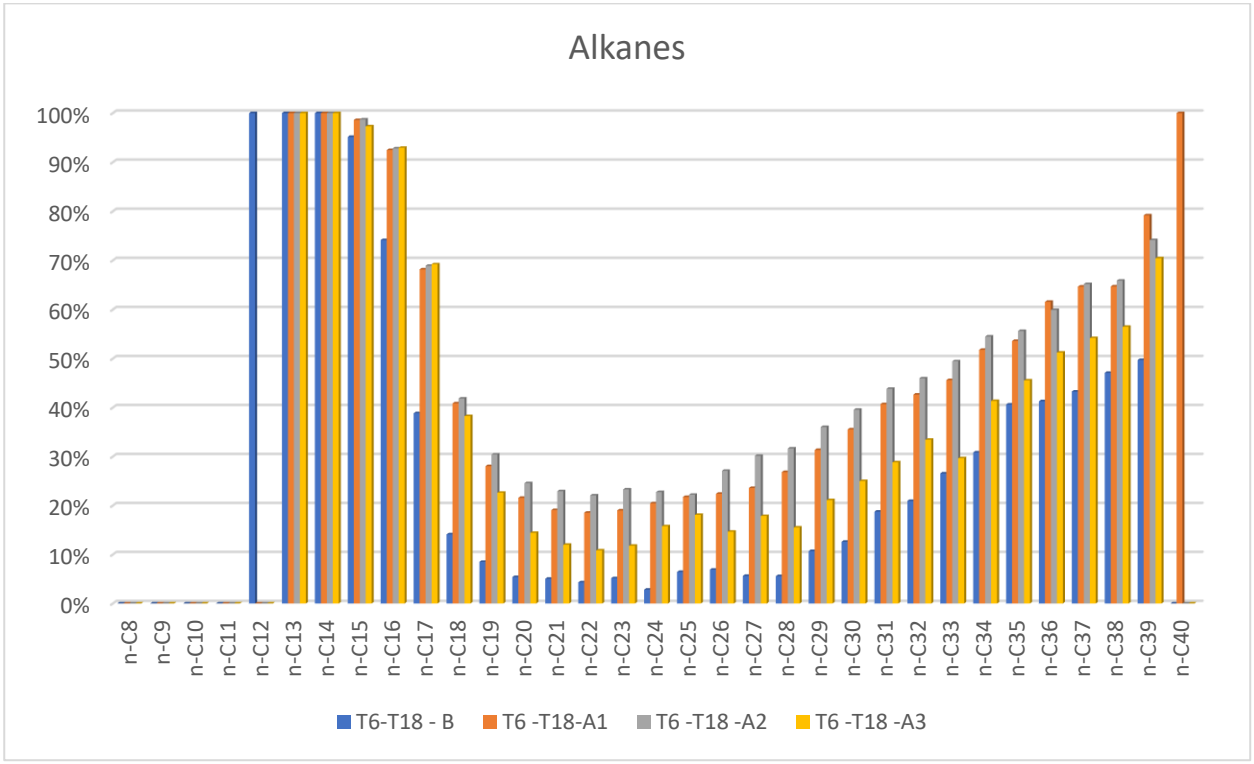


Figure 11 - Evaluation of the degradation of samples A2 and A3 between times T0 and T6

Conclusion





Source: Authourship

4 CONCLUSION

The preliminary evaluation of the use of *Pseudomonas* sp. in the paraffinic fraction of petroleum showed potential for application in the biotransformation process of these compounds. With the results of gas chromatography, it was possible to observe the behaviour of the single chain hydrocarbons throughout the experiment and to verify the microbial action on the fraction. At this stage, it was also important to observe the formation of emulsions in the extraction of the compounds due to the production of biological surfactant acting as an adjunct to biotransformation, which is a positive point for the proposed objective.

To monitor bacterial growth, two methodologies were applied (OD and qPCR), which were demonstrated to be efficient and presented similar values. With this, we were able to infer that no growth occurred throughout the overall experiment, with slight growth until the sixth day (T6), indicating the need to biostimulate and/or bioenlarge the medium from the sixth day onwards. Taking into account the dynamism of the process of oil production/refining, having a strain that acts in the biotransformation of paraffinic compounds in a short time becomes a positive point for research, requiring further study between times 0 and 6.

However, to better predict the MEOR process using the *Pseudomonas* sp strain, several parameters still need to be explored and better interpreted, such as the biosurfactant produced and its action on paraffins; in addition, the need for a sample demulsification step, measurement of the viscosity and interfacial tension of the saturated fraction, and the biotransformation of other paraffinic compounds, such as cycloalkanes, that are present in the saturated fraction of petroleum need to be studied.

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