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LIFirr with an Indicator of Microbial Activity in Paraffinic Oil

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***Abstract*—**Paraffinic oils were submitted to microbial action. The microorganisms consisted of bacteria of the genera *Pseudomonas* sp. and *Bacillus lincheniforms*. The alterations in interfacial tension were determined using a tensometer and applying the hanging drop technique at room temperature (299 K ±275 K). The alteration in the constitution of the paraffins was evaluated by means of gas chromatography. The microbial activity was observed to reduce interfacial tension by 54 to 78%, as well as consuming the paraffins C_{19} to C_{29} and producing paraffins C_{36} to C_{44}. The LIFirr technique made it possible to determine the microbial action quickly.

***Keywords*—**Paraffins, biosurfactants, LIFirr.

I. Introduction

OLD or depleted oil reservoirs usually retain the heavier fractions of the crude oil, are usually denominated mature reservoirs, their remaining oil is quite hard to extract, and their production is declining. For oil of lagoon origin, the paraffinic content is quite high due to the raw material being algae. Within mature paraffinic fields, the paraffin crystallization hinders production and transportation, being a challenge to scientific research as well as to technological development [1]-[4]. The increase of paraffins intermolecular orientation leads to their nucleation and, consequently, to the beginning of crystal formation, [5], [6] which is dependent on the paraffin molecular structure and content that strongly affect thermodynamic and fluidynamic conditions. [7]

The paraffins are usually divided in micro and macro crystalline, respectively quite branched paraffins and less branched paraffins. Although the paraffins are hard to produce, they have a well-established market and the oil refinery plants usually operate with a blend between heavier and lighter oils. Thus, if it was possible to control which paraffins are preferentially dislocated within the reservoir, the production of mature fields could be tailor made in order to achieve specific market goals, according to international prices of the industrial applications. Recently, it was found that the reactor Earth yields crude oil molecules where the polycyclic aromatic compounds have an inverse relationship between alkyl chains length and aromatic rings number, i.e., asphaltene molecules with longer alkane chains have a larger CH_2/CH_3 ratio and a larger fraction of saturated carbon [8], [9].

Microbial enhanced oil recovery (MEOR) is used to increase the production of marginal oil wells. The majority of conventional oil recovery processes are able to recover approximately 35% of the oil available in an area. MEOR can extend the productive life of a well without increasing the average cost of extraction. The method consists of injecting certain species of bacteria in water, followed by the injection of a nutrient. This process generates an 'in situ' bio-polymer, the function of which is to divert the flow of subsequently injected water to unswept areas of the reservoir [10].

Studies have been made to verify the potential utilization of different strains of bacteria in the microbial recovery of oil. The strains identified as *B. licheniformis* and *B. polymyxua* produce the most active surfactants, reducing the surface tension of the culture from 66 to between 37, and 41 dyne.cm^{-1} respectively. They are also the most anaerobic and heat resistant. The production of surfactant by *B. licheniformis* was greatest in the logarithmic phase and occurred under both aerobic and anaerobic conditions. [11]

Many microorganisms are capable of degrading crude oil. Some of them degrade alkanes, others degrade aromatics, and still others are able to metabolize both [12]. Alkanes C_{10} to C_{26} are easily degraded. Aromatics with low molecular masses, such as benzene, xylene and toluene, which are among the toxic components of oil, are easily degraded by marine organisms. Molecules with more complex structures containing ramifications and aromatic rings are degraded by a smaller number of microorganisms and at a lower rate, when compared with molecules with simpler structures.

The techniques currently used to evaluate microbes are slow and costly, as the series of biochemical tests (analytical procedures) require great skill on the part of the analyst and considerable time to carry out.

Laser induced fluorescence in function of sample irradiation (LIFirr) is a recently developed technique, which offers fast and efficient analysis. LIFirr consists of inducing fluorescence in the cromophores present in a sample, or added to it, and the detection of the total fluorescence emitted [13].

II. LIF irr

LIFirr measures the quantum fluorescent production of a sample in function of the temperature. Since the temperature increases as the laser irradiation continues, the greater the variation in temperature, the greater the variation in heat, thus:

$$\uparrow \Delta Q = mc\Delta T \uparrow \quad (1)$$

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where ΔQ is the amount of heat, m is mass, c is the specific heat (the function of the chemical composition of each oil) and ΔT is the variation in temperature. As the irradiation absorbed is constant, the LIFirr is lower,

$$I_{ABS} = \downarrow LIFirr + \uparrow \Delta Q \quad (2)$$

where I_{ABS} is the intensity of energy absorbed; LIFirr is the intensity of fluorescence and ΔQ is the quantum variation in the amount of heat.

$$I_{ABS} = cte = I_{LIF_{irr}} \downarrow + mc\Delta T \uparrow \quad (3)$$

The aim of this paper is to evaluate LIFirr as a fast and non-invasive method to determine MEOR activity in the field. Furthermore, this study aims to determine which was the microorganisms' effect on the crude oil paraffins content and if the method was sensitive to microorganisms action as a function of time. For this purpose, crude paraffinic oil was submitted to four different microorganisms, two with good surfactant yields and two efficient to degradate paraffins, for 30 days and for two years of exposition. The crude oil was analyzed after the microorganisms' activity by chromatography and by LIFirr.

As far as we are aware, this is the first time that LIFirr is reported as a method to evaluate MEOR activity.

III. Experimental

A. Materials and Methods

The oil used was from the Recôncavo Basin in Bahia, Brazil and had a paraffinic content of 14.7 % w/w, 0.20 % v/v of emulsionated water, subtrace sediments, a density at 288.75 K of 0.8727, 30.64 °API, and fluid point of 312.15 K and a flash point of 322.37 K.

The microorganisms were chosen for their capacity to generate biosurfactants and consume crude oil, having previously been submitted to a series of biochemical tests [14] to verify their metabolisms, measure their potential to produce polymers and biosurfactants, and their incapacity to reduce compounds of sulphur and hydrogen sulphide. The microorganisms were then identified by the amplification and sequencing of fragments of the genes rRNA 16S (DNA 16 S). The microbial strains named ANZPABR and PDPG were identified as being of the genus *Pseudomonas* and the strain EC3 as *Bacillus lincheniforms*.

B. Microbial Activation

Approximately 200 g of oil N were submitted to microbial activity, 10 g per strain of bacteria. Fig. 1 shows the procedure of microbial activation on the oil.

The culture mediums selected were TSB [15], which is rich in carbonates and proteins, and Mineral Medium Minimum (MMM), which is poor in organic substrates but rich in inorganic substrates such as sulphates and phosphates (Table I).

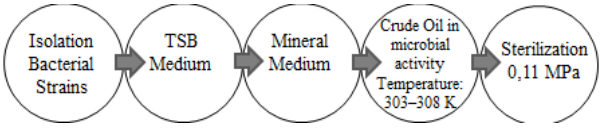

Fig. 1 Microbial activation procedure

TABLE I
Mineral Medium Minimum Composition [9]

Chemical	Concentration (g/L)
Potassium Phosphate (KH_2PO_4)	2
Potassium Phosphate Dibasic (K_2HPO_4	2
Ammonium Sulphate $(NH_3)_2SO_4$	3
Sodium Chloride (NaCl)	0,1
Ferrous Sulphate ($FeSO_4$)	0,01
Manganese Sulfate ($MnSO_4$)	0,2
Calcium Chloride ($CaCl_2$)	0,002
Yeast Extract	0,01
Glucose	0,03

To prepare the solutions, 100 cm³ of de-ionized water was added to each 3 g of medium.

30 test tubes containing 10 cm³ of the TSB medium were prepared and sterilized in an autoclave at a of pressure 0.11 MPa and a temperature of 394.15 K for 900 seconds. After cooling the test tubes were shaken and 0.001 cm³ of each culture was added to 10 cm³ of the TSB medium.

After 48 hours of bacterial growth 10 cm³ of crude oil was added to each test tube, and they were kept in an incubator at 308.15 K for periods of 30 days and 730 days.

After the period of interaction between the microorganisms and the oil, the samples were again sterilized in an autoclave at a of pressure 0.11 MPa and a temperature of 394.15 K for 900 seconds and transferred to LABLASER to measure surface tension and carry out gas chromatography and LIFirr.

C. LIFirr System

The LIFirr system consists of a linear optical array, as shown in Fig. 2. The stimulus is provided by an argon laser (COHERENT INNOVA 70C). The strength of the radiation emitted was in the range of 100 to 300 mW at wavelengths (λ) of 4.88×10^{-5} cm and 5.35×10^{-5} (488 and 535 nm). The laser beam is diverted by a mirror (Newport) with 98% reflectivity at 5.32×10^{-5} (532 nm).

The radiation is focused by a convergent lens (L1) on the sample positioned at a distance of 42.5 cm. A Glan-Thompson type P polarizer (Newport) is placed between the lens and the sample to guarantee 100% vertical polarization of the photon field.

In preparation for LIFirr, each sample was heated to a temperature of 328 K, homogenized and deposited on the surface of a glass slide with a thickness of between 0.1 and 0.12 cm, a length of 7.62 cm and a width of 2.54 cm. The films of oil were then compacted to a thickness between 0.005 a 0.006 cm by the longitudinal movement of a second slide [16].

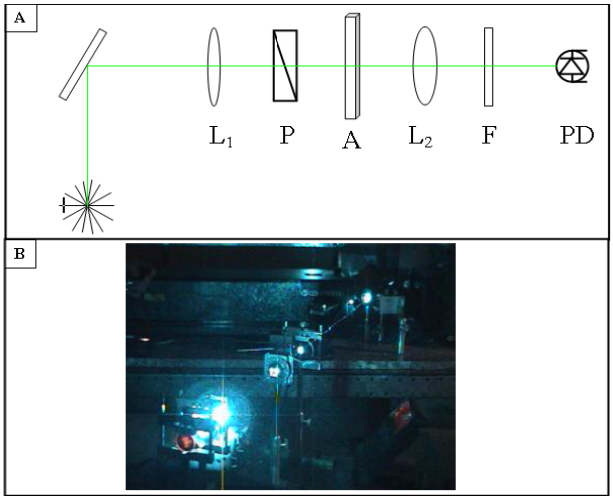

Fig. 2 Experimental System as for LIFirr: (A) constituted by L1 – Lens 1, P – Polarizer, A - Sampe, L2 – Lens 2, F – Cut-Off Filter, PD - Photodiode and a photo of the system (B)

The molecules of the sample absorb the laser radiation, suffer radioactive decay and emit fluorescence, as shown in Figs. 2 (A) and (B) [17], [18]. The fluorescence is collected by the lens (L2) at a focal distance of 4.8 cm from the sample and focused on the photodiode (PD) after passing through a $5.5x10^{-5}$(550 nm) cut-off filter (F) with 99.99 % efficiency.

The signal is captured remotely by an interface composed of two 74S374 chips (LATCHs), two 74LS244 chips (buffers) and a 74LS138 chip (addresser).

The data acquired is processed using Origin® software to generate graphs representing the variation in the LIFirr analytical signal.

D.Chromatography

One sample of oil N without microbial activity and four samples without microbial activity were analyzed using chromatography. About 0.05 g of oil dissolved in hexane were heated to 333.15 K for 600 seconds and centrifuged for 300 seconds to remove the mineral component of the oil. The organic portion was again heated to 333.15 K for 300 seconds in order to inject it into a column.

The samples were analysed in a gas chromatograph (Shimazdzu, GC-17A) with a capillary column of fused cilica (J&W Scientific). Eight paraffins were chosen, C_{19}, C_{20}, C_{22}, C_{28}, C_{36}, C_{40}, C_{42} e C_{44} retention time, respectively: 705.42; 748.98; 829.44; 1034.34; 1250.28; 1341.54; 1383.84 e 1424.16 seconds.

The peak areas for each of the paraffins were calculated for each chromatogram. The areas of all of the peaks of each chromatogram were then added together and the area of each peak of each chromatogram was divided by the sum of the areas of the peaks of that chromatogram and expressed as a percentage (Fig. 3).

Fig. 3 shows the sequence of procedures for the gas chromatography analysis.

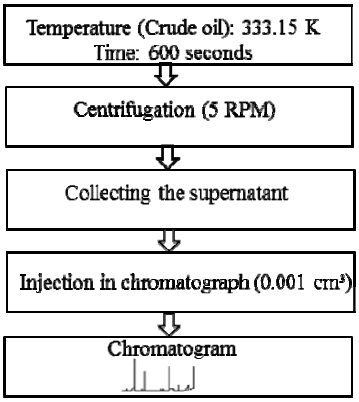

Fig. 3 Gas chromatography procedure

E. Surface Tension

Interfacial tension was measured using a tensometer (Data Physics®, Oca 20 Plus) and applying the hanging drop technique at room temperature (299 K ±275 K). The evaluation of the drop was made by the automatic video image system using the Oca 10/ Oca 20 software of the equipment itself. The instrument was calibrated with distilled water and air for a reading of 71.62 ± 1.0 dyne.cm^{-1} [19]. For some of the analyses it was necessary to use the inverted needle technique.

F. Results and Discussion

Variations in the paraffins due to microbial activity over a period of one month can be seen in the gas chromatograms of the paraffins summarized in Fig. 4.

Studies carried out by [19] have shown that microorganisms in contact with oil in both aerobic and anaerobic conditions were able to degrade approximately 90-95% of the n-alkanes (nC_{10}-C_{35}). The strain identified as BS2201 reduced the quantity of carbon molecules of nC_{11}-C_{14} and nC_{35} by 25-30%, and the quantity of carbon molecules of nC_{15}-C_{18} by 20-25%. The strains BS2202 and BS2203 reduced the quantity of carbon molecules of nC_{18}-C_{30} by 15-20%.

The results obtained in the present study show a reduction in the number of carbons nC_{19}-C_{23} in the samples submitted to the microbial activity by the strains used here.

It can be seen in Fig. 4 that irrespective of the strains, the greatest alterations in the concentration of paraffins occurred between C_{11} and C_{28}. Generally speaking, paraffins C_{11} and C_{12} increased while paraffins C_{13} and C_{28} were reduced. It should also be noted that C_{18}, C_{23} and C_{24} showed a slight tendency to increase, especially with the action of the EC1 and ANZPABR strains.

This effect was expected for the strains ANZPABR and PDPG, which were selected for their capacity to degrade paraffin chains in oil, but the strains EC1 and EC3, chosen because they produce biosurfactants, also demonstrated the ability to degrade paraffinic chains. This should not come as a surprise however, since these two effects are usually linked in microbial action, as their metabolisms are intrinsically connected.

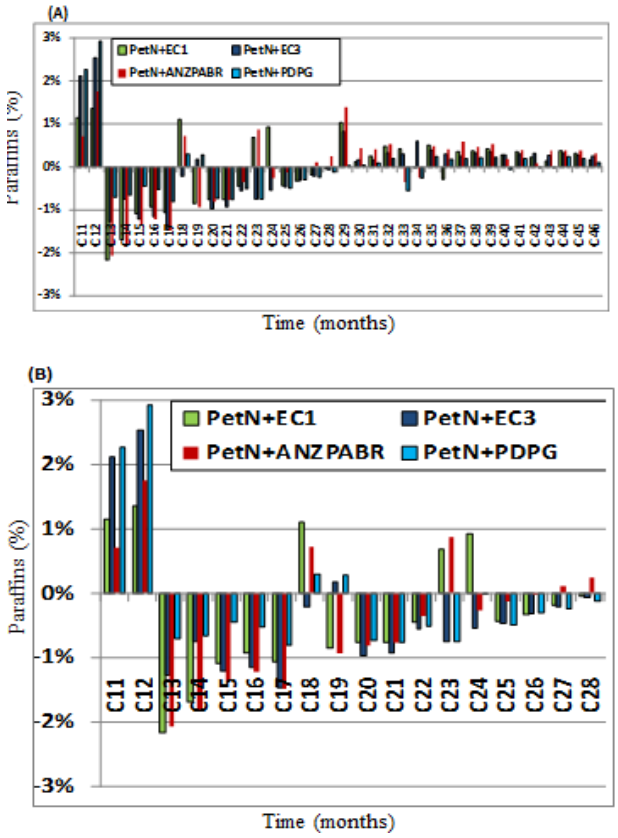

Fig. 4 Variations in the paraffins due to microbial activity over a period of one month

Fig. 5 shows the surface tension of oil N with and without microbial activity for two years.

It can be observed that the microorganisms identified as EC1, EC3 and PDPG reduced the interfacial tension by 60, 54 and 78% respectively. This was due to the production of polymers and surfactants which reduce surface tension provoking an increase in the LIFirr signal as shown in Fig. 6.

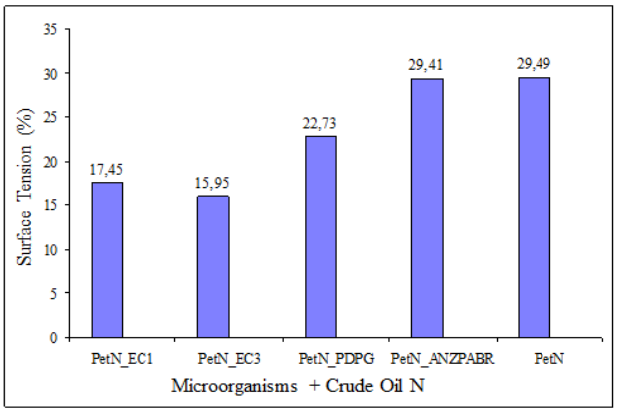

Fig. 5 Surface tension of the oils submitted to microbial activity

Fig. 6 shows the decay in fluorescence in the oil samples with and without microbial activity. There was an increase in the LIFirr signal in the oils submitted to microbial activity at all laser strengths applied when compared to the same oil without microbial activity. It can also be seen that the LIFirr signal increased in all of the samples irrespective of the time for which the oil was exposed to the microorganisms.

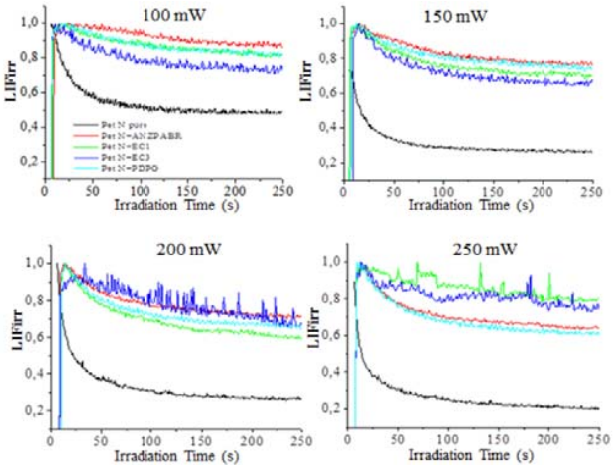

Fig. 6 LIFirr of oil N in relation to the time and strength of irradiation of the sample after 30 days of microbial activity

IV. Conclusion

The microorganisms altered the distribution of paraffins. The oils submitted to microbial activity showed a reduction in the areas of the chromatographic peaks corresponding to the number of carbons C_{19}-C_{36} in the paraffinic chain and an increase in carbons C_{40}-C_{44}, suggesting a break in the links or the formation of new links.

The chromatographic profile of a sample of oil is like its fingerprint and is one of the first qualitative indicators of the occurrance of biodegradation, since linear compounds are more abundant in unbiodegraded oils and for this reason are the first to be consumed by the microorganisms. Thus, when the profile of the sample shows a reduction in the concentration of these compounds, it is said that the oil has suffered biodegradation, becoming gradually heavier and more acidic [20].

The microorganisms identified as EC1, EC3 and PDPG reduced the interfacial tension by 60, 54 and 78% respectively. This was attributed to the production of polymers and surfactants which reduce the surface tension provoking an increase in the LIFirr signal.

The LIFirr signal increased irrespective of the type of microorganism and the strength of the laser within the range studied.

The LIFirr technique showed itself to be sufficiently sensitive for the verification of chemical alterations in oil submitted to microbial activity. This alteration can be attributed to two processes: the degradation of the oil by microbial metabolism generating more of the fluorescent compounds (fluorophores) that absorb the wavelength used, and the surfactant effect which unlinks the fluorophores, reducing technical decay and increasing fluorescent decay.

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References

[1] P. F. Almeida, R. C. Moreira, A. K. Guimarães, A. S. Carvalho, C. M. Quintella, M. C. A. Esperidião, C. A, Taft, "Selection and application of microorganisms to implovise oil recovery", *Eng. Life Sci.*, vol. 4, no. 4, pp. 319-324, 2004.

[2] G. Andreatta, C. C. Gonçalves, G. Buffin, N. Bostrom, C. M. Quintella, F. Arteagalarios, A. Pérez, O. C. Mullins, "Nanoaggregates and structure-function relations in asphaltenes," *Energ. Fuel.*, v. 19, no. 4, pp. 1282-1289, 2005.

[3] R. M. Atlas et al., "Response of microbial-populations to environmental disturbance", *Microbiology Reviews*, vol. 45, pp. 249-256, 1981.

[4] F. S. Brito, M. S. Teixeira, A. P. Musse, C. M. Quintella, *Anais da 25ª Reunião da Sociedade Brasileira de Química*, Brazil, 2002.

[5] E. Buenrostro-Gonzalez, H. Groenzin, C. Lira-Galeana, and O. C. Mullins, "The overriding chemical principles that define asphaltenes," *Energ. Fuel.*,v.15, pp. 972-978, Jul. 2001.

[6] A. Cosulchi, E. Garciafigueroa, A. García-Bórquez, E. Reguera, Yee-H. Madeira, V. H. Lara, P. Bosch, "Petroleum solid adherence on tubing surface," *Fuel*, v. 80, pp. 1963-1968, Feb. 2001.

[7] D. L. Dorset, J. Hanlon, G. Karet, "Epitaxy and structure of paraffin-diluent eutectics," *J. Phys. Chem. B*, v. 22, no. 5, pp2169-2176, May. 1989.

[8] G. D. Floodgate, "The preservation of marine bacteria," *Aquatic Microbial Ecology*, vol. 24, no. 1, pp. 87-93, Nov. 1961.

[9] D. S. Francy, R. L. Raymund, C. H. Ward, "Emulsification of hydrocarbons by subsurface bacteria," Journal of Industrial Microbiology, v. 8, pp. 237-246, 1991.

[10] Y. H. Jang, M. Blanco, J. Creek, Y. C. Tang, W. A. J. Goddard, "Wax inhibition by comb-like polymers: Support of the incorporation-perturbation mechanism from molecular dynamics simulations," *Phys. Chem. B*, v. 111, no. 46, pp. 12173-13179, 2007.

[11] J. G. Leahy, R. R. Cowell, "Microbial-degradation of hydrocarbons in the environment," *Microbiology Review*, vol. 54, pp. 305-355, 1990.

[12] C.M Quintella, A. P. S. Musse, M. T. P. O. Castro, J. C. Scaiano, L. Mikelsons, Y. N. Watanabe, "Polymeric surfaces for heavy oil pipelines to inhibit wax deposition: PP, EVA28, and HDPE," *Energ. Fuel.*, v. 20, pp. 620-624, 2006.

[13] E. B. Silva, C. M. Quintella, A. P. Musse, H. Fraga, L. Friedriech, "Determinação das regiões de oxidação do PEBD através da alteração da tensão interfacial dinâmica por PLF-FI," *Anais da 25ª Reunião da Sociedade Brasileira de Química* Brazil, 2005.

[14] C. M. Quintella, L. A. Friedrich, A. P. S. Musse, A. M. V. Lima, M. A. Macedo, R. M. Silva, I. M. Pepe, E. B. Silva, L. C. S. Soares, "Wettability under imposed flow as a function of the baking temperatures of a DGEBA epoxy resin used in the crude oil industry," *Energ. Fuel.*, v. 21, no. 4, pp. 2311-2316, 2007.

[15] C. M. Quintella, A. M. V. Lima, E. B. Silva, "Selective inhibition of paraffin deposition under high flow rate as a function of the crude oil paraffin type and content by fluorescence depolarization: PP and HDPE," *J. Phys. Chem.* v. 110, no. 14, pp. 7587-7591, 2006.

[16] C. M. Quintella, C. C. Gonçalves, I. Pepe, A. M. V. Lima, A. P. Musse, "Intermolecular alignment dependence of ethylene glycol flow on the chemical nature of the solid surface (Borosilicate and SnO_2)," *J. Braz. Chem. Soc.*, v. 12, no. 6, 2001.

[17] C. M. Quintella, C. C. Gonçalves, I. Pepe, A. M. V. Lima, A. P. Musse, "Automated system to acquire fluorescence, polarization and anisotropy maps within liquid flows,"*Journal of Automated Methodos & Management in Chemistry*, v. 24, no. 2, pp. 31-39, Jan. 2002.

[18] A. J. Rosa, R. Carvalho, J. A. D. Xavier, "Engenharia de Reservatórios de Petróleo", 2006.

[19] V. G. Grishchenkov, R. T. Townsed, T. J. McDonald, R. L. Autenrieth, J. S. Bonner, A. M. Boronin, "Degradation of petroleum hydrocarbons by facultative anaerobic bacteria under aerobic and anaerobic conditions," *Process Biochem.*, v. 35, pp. 889-896, Nov. 2000.

[20] G. F. Cruz, A. J. Marsaioli, "Processos naturais de biodegradação do petróleo em reservatórios", *Quim. Nova*, vol. 35, no. 8, pp. 1628-1634, 2012.