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Assessment of the bioremediation technique in the attenuation of an oil from the Campos Basin/RJ

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Abstract: Various types of operations with petroleum are carried out in the Campos Basin/RJ, which results in risks of environmental accidents as a result of a spill and / or contamination of the environment with oil. The aim of this research is to evaluate the use of the bioremediation technique in the attenuation of an oil from this region. The bioremediation of an oil from the Campos Basin by microrganisms present in local sea water was simulated in laboratory in units containing NPK fertilizer with components of the saturated and aromatic fractions of the oil. After seven days, the effect of the bioremediation on the aromatic and saturated fractions of the oil was assessed by means Gas Chromatography / Mass Spectrometry (GC / MS). When compared to a reference simulation, that did not use an exogenous stimulant, one can conclude that the NPK fertilizer does have a positive effect of stimulation. Aromatic compounds had a negative influence on the biodegradation of saturated compounds due to their complexity, toxicity and stability.

Keywords: Biostimulation. Bioremediation. Oil spills. NPK

Avaliação da técnica de biorremediação na atenuação de um óleo proveniente da Bacia de Campos/RJ

Resumo: Diversas atividades petrolíferas são realizadas na Bacia de Campos/RJ, o que traz como consequência riscos de acidentes ambientais em decorrência de vazamento e/ou contaminação de óleo no meio ambiente. A pesquisa teve por finalidade avaliar o emprego da técnica de biorremediação na atenuação de um óleo dessa região. Realizou-se, em laboratório, uma simulação da biorremediação em unidades contendo fertilizante NPK com componentes das frações saturados e aromáticos do petróleo. O monitoramento da biodegradação foi realizado após 7 dias por meio da Cromatografia Gasosa acoplada ao Espectrômetro de Massa (CG/EM). Foi possível constatar que o estimulante NPK favoreceu a biodegradação, se comparado com os dados coletados das amostras sem a utilização do fertilizante. Os compostos aromáticos exerceram uma influência negativa na biodegradação dos compostos saturados em virtude da sua complexidade, toxicidade e estabilidade.

Palavras-chave: Bioestimulação. Biorremediação. Derrames de óleo. NPK.

Evaluación de la técnica de biorremediación en la atenuación de un petróleo proveniente de la Cuenca de Campos/RJ

Resumen: Varias actividades petroleras se realizan en la ciudad de Macaé/RJ, lo que trae como consecuencia los riesgos de accidentes ambientales por fuga y / o contaminación de petróleo en el medio ambiente. La investigación tuvo como objetivo evaluar el uso de la técnica de biorremediación en la atenuación de un petróleo de esta región. Se realizó, en un laboratorio, una simulación de biorremediación en unidades que contenían fertilizante NPK con componentes de las fracciones saturadas y aromáticas del petróleo. El seguimiento de la biodegradación fue realizado, a los 7 días, mediante Cromatografía de Gases acoplada a Espectrometría de Masa (CG / EM). Se pudo constatar que el estimulante, NPK, favoreció la biodegradación, en comparación a los datos recogitos de las muestras sin el uso de fertilizante. Los compuestos aromáticos ejercieron una influencia negativa en la biodegradación de los compuestos saturados debido a su complejidad, toxicidad y estabilidad.

Palabras clave: Bioestimulación. Biorremediación. Derrames de petróleo. NPK.

1 Introduction

The various fractions of oil provide important inputs for energy, transport, agriculture and synthesis of polymers. However, the growth of the oil industry, whether through exploration and/or production, transportation and oil products, can lead to accidental spills in the marine and/or terrestrial environment. Chemical accidents involving petroleum hydrocarbons are relatively common, especially during oil distribution and transportation. As a result, the potential contamination of air, water and soils together with the toxicity of oil hydrocarbons can have a significant impact on the environment and the health of society (RUSSELL et al., 2009)

Petroleum is a complex mixture formed by thousands of compounds and separating them into simpler, pure components or mixtures of known composition is practically impossible. It is normally separated into fractions according to the boiling range of the compounds. Among these typical fractions of oil, obtained after refining, we can mention gas, Liquefied Petroleum Gas (LPG), gasoline, kerosene, light and heavy diesel, lubricants and waste (ROCHA, 2005; THOMAS, 2001). The main groups of oil components are saturated hydrocarbons, aromatic hydrocarbons, resins and asphaltenes. However, elements such as sulfur, nitrogen, oxygen and metals can also be found in oil.

The Campos Basin is a Brazilian sedimentary basin that is located along the north coast of Rio de Janeiro and on the south coast of the State of Espírito Santo, approximately southern of the Brazilian east side, occupying a marine area of approximately 100 thousand square kilometers. The limits of the basin are the Vitória arch, to the north, the Cabo Frio arch, to the south, and the Precambrian rocks of Serra do Mar, to the west (CARVALHO; ROS, 2015). Figure 1 illustrates the current configuration of the Campos Basin.

Figure 1: Campos Basin

Source: CPRM (2014)

The basin currently contains about 57 fields, 52 of which are in the production phase and five in the development phase. Four exploratory blocks under concession are recorded. According to the Geological Summary of the ANP (Brazilian Petroleum Agency), 2017, in March 2017, the daily oil production in the Campos Basin was around 1.37 million barrels and the daily production of natural gas in the order of 25 thousand mm^3 (ANP, 2017).

In this region, the production of hydrocarbons is carried out in a marine environment (*offshore*) and the technology of exploration in deep waters has allowed to explore wells at ever greater depths. The pre-salt layers are responsible for producing approximately 300,000 boe/day (Barrels of oil/day) in the Campos Basin. This production comes from the fields of Jubarte, Baleia Azul, Baleia Franca, Marlim Leste, Caratinga, Barracuda, Marlim, Voador, Albacora Leste, Linguado, Badejo, Pampo and Trilha (ANP, 2017).

During exploration and production activities, oil is occasionally thrown into the sea as a result of line failures or through transfer and transport operations to the terminals. Spills that occur at sea are directly related to the intense transfer between pipelines and storage facilities that operate daily around the world (IVSHINA et al., 2015).

Intense production also contributes to the recurrent occurrence of oil spills in the marine environment, such as the recent oil spill at the FPSO Rio de Janeiro in Campo Espadarte in the Campos Basin, Figure 2, under operation by the company Modec do Brasil, due to the existence of cracks in the ship's hull, after inspection of the vessel's external tanks, as communicated by the service provider Modec. The Espadarte field is located more than 130km off the coast of the municipality of Campos dos Goytacazes. The initial volume of the slick was estimated at 1.4 m^3 of oil (1,400 liters) (SINDIPETRO-NF, 2019).

Figure 2: FPSO Rio de Janeiro

Source: https://sindipetrosp.org.br (2019)

The extraction of oil at sea, even if they are happening on a smaller scale, still has considerable impacts on the environment. The frequency and intensity with which these impacts occur contributes significantly to the development of scientific research to understand the biochemical processes that involve the degradation of hydrocarbons by microorganisms in the sea. This contributes to the development of new methodologies for the remediation of oil spill accidents (IVSHINA et al., 2015).

1.1 Biodegradation of hydrocarbons

Biodegradation is a technique used in order to treat contaminated environments through the use of microorganisms capable of altering or decomposing certain components of the oil, favoring the transformation of the components more into less complex products (GOMEZ; SARTAJ, 2014). The technique can occur naturally without the interference of any source of remediation technology, but it can be biostimulated with the addition of nutrients, such as NPK, oxygen and others (AGNELLO et al., 2016). The chemical and physical changes induced in the composition of a petroleum hydrocarbon mixture are, in general, known as weathering. Biodegradation is its main cause. It involves the reduction of the complexity of chemical compounds by microorganisms. The resistance of hydrocarbon mixture to biodegradation depends on the characteristics and quantity of the oil, the nature of the microbial community and a variety of at environmental factors that influence microbiological activity. In one environment, oil hydrocarbons can persist indefinitely, while in others they can be fully biodegraded within relatively few hours or days (KHALIFA, 2017; ATLAS, 1981).

The biodegradation process will depend on the biotic control parameters and the physical properties of the oil (COOKSON, 1995; BAKER; HERSON, 1994). The temperature varies from environment to environment and according to seasonality and, therefore, significantly changes the biochemical reactions related to the physiology of the microorganisms involved (SINGH; WARD, 2004). Under aerobic conditions, the efficiency of degradation by microorganisms will be much higher as compared to a degradation processed in an oxygen-free medium, that is, anaerobic (PETERS et al., 2007). The most favorable pH range, for most of microorganisms, is between 6.0 and 8.0 with an optimal value around 7.0 (VIDALI, 2001). Water is important because it is used directly as an appropriate means for the occurrence of biochemical reactions (PETERS et al., 2005). Biodegradation is severely affected by the high salt concentration, observed in hypersaline environments such as lagoons (WANG; STOUT, 2007). Degradation of oil in seawater is faster and more efficient when nitrogen and phosphorus-based compounds are added (VIDALI, 2001). The higher the solubility of the organic compound in water, the higher will be its biodegradability (PERRY, 1984).

In order to mitigate the impacts caused by an accidental spill of oil in the environment, the bioremediation technique has been widely used in numerous researches for the remediation of environments contaminated by hydrocarbons, highlighting the bioremediation technique, as they are considered safer, economical and environmentally friendly (PACWA-PLOCINICZAK et al., 2016; BALDAN et al., 2015).

1.2 Bioremediation

Bioremediation is a process of natural mitigation of oil pollutants and consequently a "clean" solution (GAUR et al., 2014; ATLAS, 1995). Organic waste is degraded biologically under controlled conditions to a harmless state or to levels below the concentration limits established by the authorities (MUELLER et al., 1996). It consists of the optimization of the environmental conditions for the development of the natural occurrence of degrading microorganisms through the addition of nutrients and the occasional maintenance of aerobic conditions (CLAUDE-HENRI, 2003). The bioremediation technique makes use of bacteria and fungi, either from the contamination site itself or introduced from other places to degrade or detoxify substances toxic to human health and the environment. The degradation of a compound is the result of the action of multiple organisms. Contaminants are transformed, into less complex structures, by living organisms through reactions that are part of their metabolic processes (VIDALI, 2001). During bioremediation, petroleum hydrocarbons are converted by microorganisms into carbon dioxide, water, bacterial cells (biomass) and organic matter through the biodegradation process (SINGH; WARD, 2004). Bioremediation has advantages, such as: low-cost, minor environmental disturbances and ability to completely recover areas contaminated by oil. But disadvantages, such as: its efficiency is limited by the complexity of petroleum compounds. Therefore, not all compounds are easily biodegraded. This, leads to intense scientific research aiming to find mechanisms that make the technique more efficient, such as seen in Chart 1. For this reason, it is necessary to analyze very well the environment where the technique will be used (LOGESHWARAN et al., 2018; ROY et al., 2018; COOKSON, 1995).

Chart 1. Advantages and Disadvantages of using bioremediation

ADVANTAGES	DISADVANTAGES
Fast and low cost technique	Pollutant toxicity
Minimal environmental disturbance	Intense bioremediation research
Eliminates transportation costs	The technique may not be appropriate
Does not remove water from aquifer	Not all compounds are degraded
Public acceptance	Intensive monitoring
Recovery of the contaminated area	Production of little-known compounds

Source: Modified by Cookson (1995)

The bioremediation technique can be conducted through the processes of bio-augmentation, biostimulation, bioventilation, among other existing bioremediation techniques (XU et al., 2018; LINDSTROM et al., 1991). Bio-augmentation aims to accelerate the degradation process by introducing non-indigenous microorganisms (alien), oxygen, water and nutrients to the contaminated environment (VARJANI, 2017; ATLAS; BARTHA, 1992; HOFF, 1993; MERLIN et al., 1994; SANTAS et al., 1999). Biostimulation consists of adding nutrients to increase the rate of biodegradation. Therefore, to make use of the biostimulation process, it is necessary that there is a natural community of microorganisms, in the contaminated site, capable of biodegrading the present contaminants and that the environmental conditions are insufficient to obtain high rates of microbiological activity in this population (LOGESHWARAN et al., 2018; HOFF, 1993; VENOSA et al., 1991).

2 Material and Method

The experiment was carried out using sea water collected at Praia dos Cavaleiros, in Macaé/RJ, with native microorganisms and NPK fertilizer (10:10:10) as a stimulator of bioremediation. During the experiments, the average temperature was 27ºC without any sudden changes.

A sample of 4.997 g of an oil produced in the Campos Basin with the following physical characteristics: ºAPI = 26.9; Density = 0.8892 g/L and Dynamic viscosity = 54.2 cp was used. The oil sample was subjected to a disasphaltenization process, by gravitational segregation, using a volume of 80 mL of the n-Hexane apolar solvent. After 24 hours, the asphaltene had precipitated at the bottom of the beaker and the maltene (name given to the mixture of the fractions of saturated, aromatic and resin of the petroleum) remained solubilized in the solvent. The separation of maltene and asphaltene was carried out by a filter, using a total volume of 240 mL of the n-Hexane solvent. The maltene mass totaled 4.0154 g and the asphaltene mass was 0.2016 g. This indicates that there was a loss of mass throughout the process, possibly due to the evaporation of the lighter fractions of oil and also during the filtration stage.

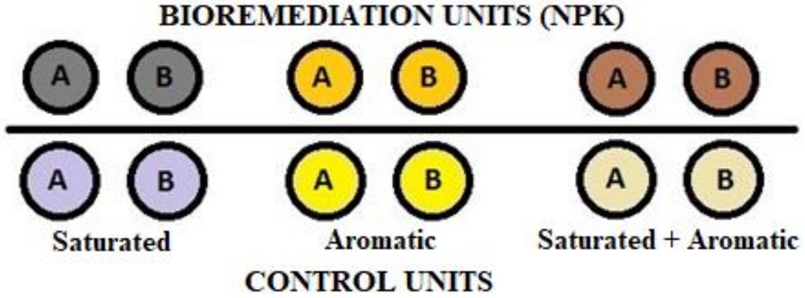
The maltene fraction was subjected to the chromatographic fractionation process on a silica gel column to obtain three distinct fractions: F1 (Saturated hydrocarbons), F2 (Aromatic hydrocarbons) and F3 (Resins).

After separating the fractions, using specific eluents, they were stored in amber glasses, properly identified, and placed inside the chapel for further analysis by gas chromatography.

The NPK fertilizer used, sprayed, throughout the experiment, is composed of a mixture of three main nutrients (Nitrogen, Phosphorus and Potassium), also called macronutrient. In its chemical composition it contains: Ammonium Phosphate $[(NH_4)^3PO_4]$, Ammonium Sulfate $[(NH_4)^3SO_4]$ and Potassium Chloride [KCl], in the proportion of (N:P:K) 10:10:10.

The simulation was carried out in 12 glass containers, 100 mm in diameter and 15 mm deep. The isolated fractions of saturated hydrocarbons, aromatic hydrocarbons were dissolved with n-Hexane and Dichloromethane. The solventes were evaporated and a film was formed at the bottom of the beaker. In the following, the film and 80 mL of sea water was poored in a container. Using the same procedure, a film of a mixture of saturated and aromatic hydrocarbons was prepared. At a whole, four containers of each petroleum fraction were prepared. In six of the containers NPK fertilizer was added in the proportion of 10% of the volume of the corresponding oil fraction used to make the film. No fertilizer was added to the remaining six containers. These are the group of control. Figure 3 illustrates the containers used in the simulation.

Figure 3: Bioremediation simulation units of the Saturated, Aromatic and Saturated + Aromatic Fractions, using the NPK Fertilizer, and the Control units, without adding the fertilizer.



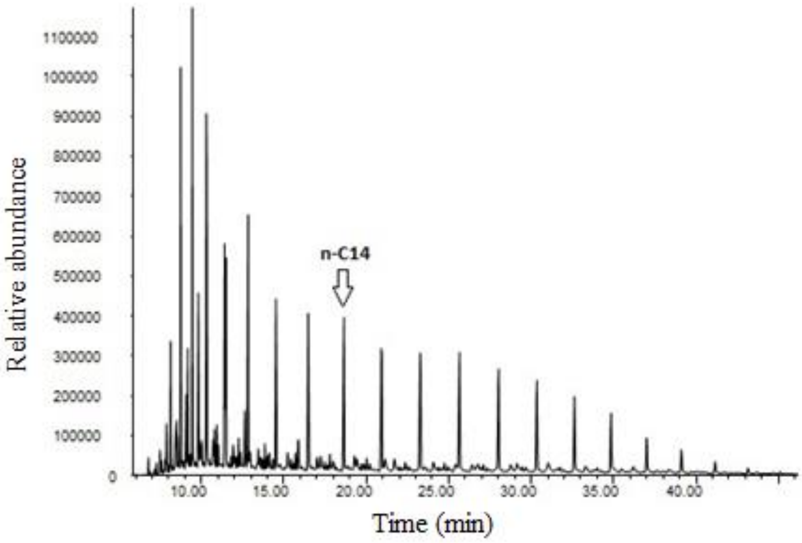
Throughout the bioremediation simulation, the containers were shaken 2 times a day in order to oxygenate the sea water. Weekly, an aliquot of the saturated, aromatic fraction and the saturated + aromatic blend was collected from the floating portion present in the glass containers of the Control and Bioremediation units. The samples were analysed by Gas Chromatography / Mass Spectrometry (GC / MS), performed in a gas chromatograph.

The ion selective monitoring method was used to evaluate the following classes of compounds: n-alkanes and phytane (m/z 85), hopans (m/z 191) and methyl phenanthrenes (m/z 192). The qualitative results were processed by the Agilent Chemstation operating system. The relative abundances of each ion, individually, were obtained by integrating the peak areas recorded in the mass chromatogram.

3 Results

One of the most used parameters in the characterization of oils is the distribution of n-alkanes. This distribution is called oil “fingerprint” and differs from oil to oil. From this analysis it was possible to obtain a chromatographic profile of the distribution of the saturated compounds of the oil used in the experiment, as seen in Figure 4.

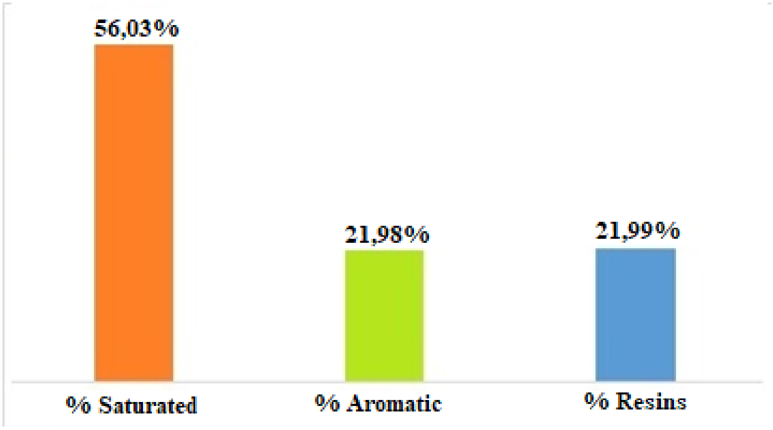
Figure 4: Chromatogram of the original oil from the Campos Basin



It is noted, in the chromatographic profile, that the oil sample used in the spill simulation experiment has a high abundance of n-paraffins. Therefore, it does not show considerable biodegradation rates, since saturated compounds are the first to be affected by this process, as they are less resistant (WANG et al., 2005; WANG; STOUT, 2007).

After the fractionation of the oil, by liquid chromatography, to obtain the saturated fractions and aromatic compounds used in the experiment, it was possible to determine the percentage of saturated, aromatic and resin hydrocarbons, as shown in Figure 5.

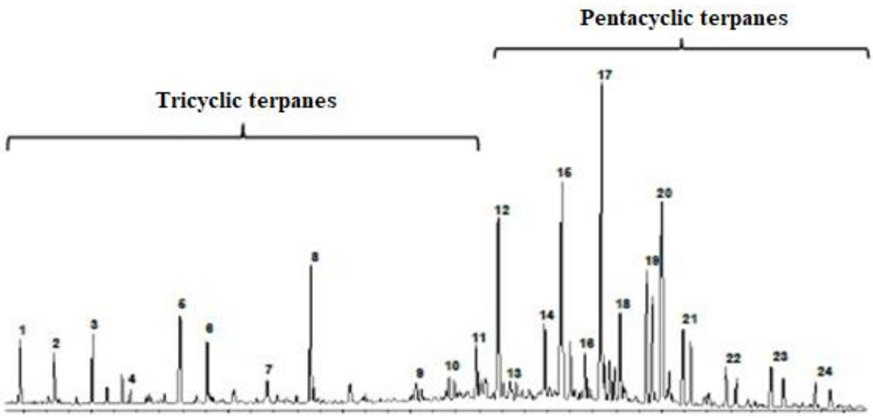
Figure 5: Result of analysis of oil from Campos Basin by liquid chromatography



From the results, it can be concluded that it is a paraffinic oil, that is, it does not present considerable levels of biodegradation.

The analysis of the components of the biomarkers of the Terpanes Family points out the presence of tricyclic terpanes and pentacyclic terpanes, as shown in Figure 6.

Figure 6: Chromatogram showing the distribution of Tricyclic and Pentacyclic terpanes from the Campos Basin oil used to obtain fractions of saturated and aromatic compounds



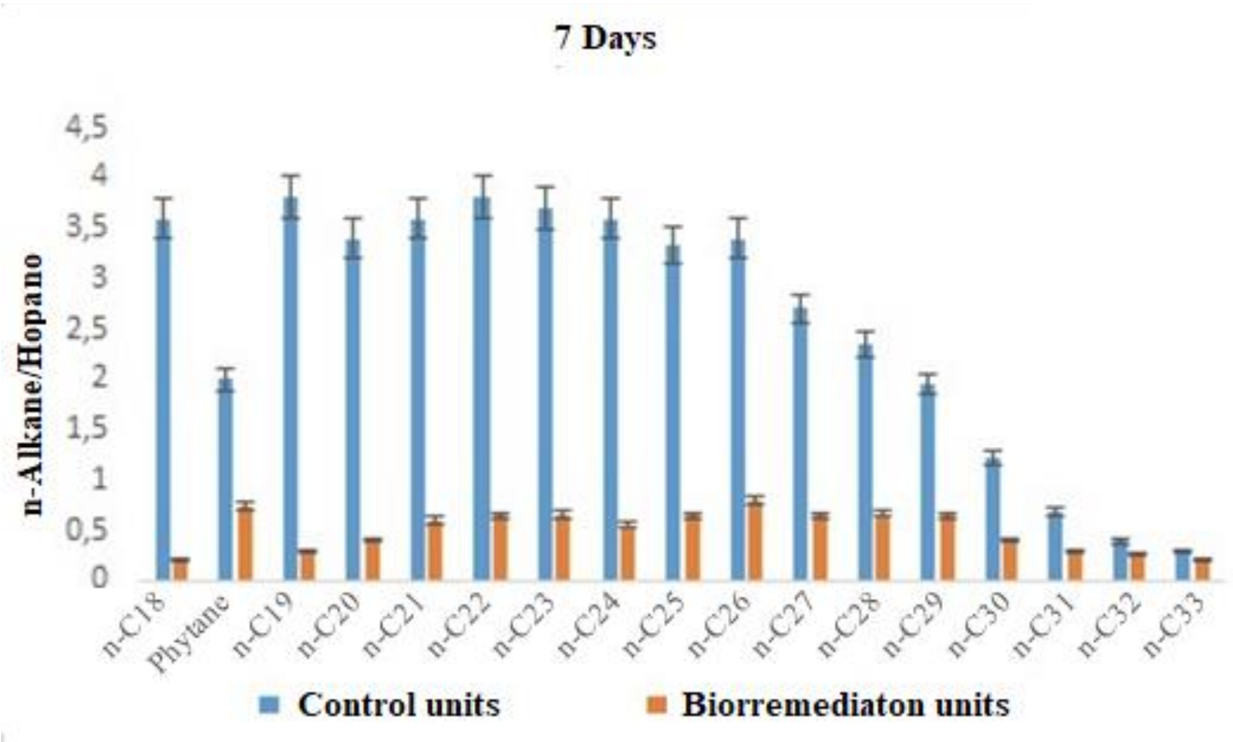
From the analysis of the chromatographic profile, it can be concluded that the distribution of Terpanes is dominated by Pentacyclics, with a high abundance of Hopane (30C). Both Tricyclic and Pentacyclic terpanes are characteristic of all types of oil, in addition they are very resistant to biodegradation (WENGER et al, 2001).

3.1 Biodegradation Assessment of n-Alkanes

Samples collected from the Bioremediation and Control containers containing the fractions of saturated compounds and the saturated + aromatic blend were analyzed after 7 days of the experiment. The results were expressed in the form of ratios between the relative abundances of n-Alkanes from n-18C to n-33C and Phytane in relation to Hopane. By comparing the results of the control group samples to those of the bioremediation group, one can see the effectiveness of the biorematiation.

Figure 7 shows the n-Alkanes to Hopane and Phytane to Hopane ratios of the bioremediation and control group samples after seven days of experiment for the saturated fraction of the oil.

Figure 7: Comparison between the mean values of the n-Alkane / Hopane and Phytane / Hopane ratios of the saturated samples collected from the Bioremediation and Control units, containing only the saturated hydrocarbon fraction, 7 days after the start of the simulation

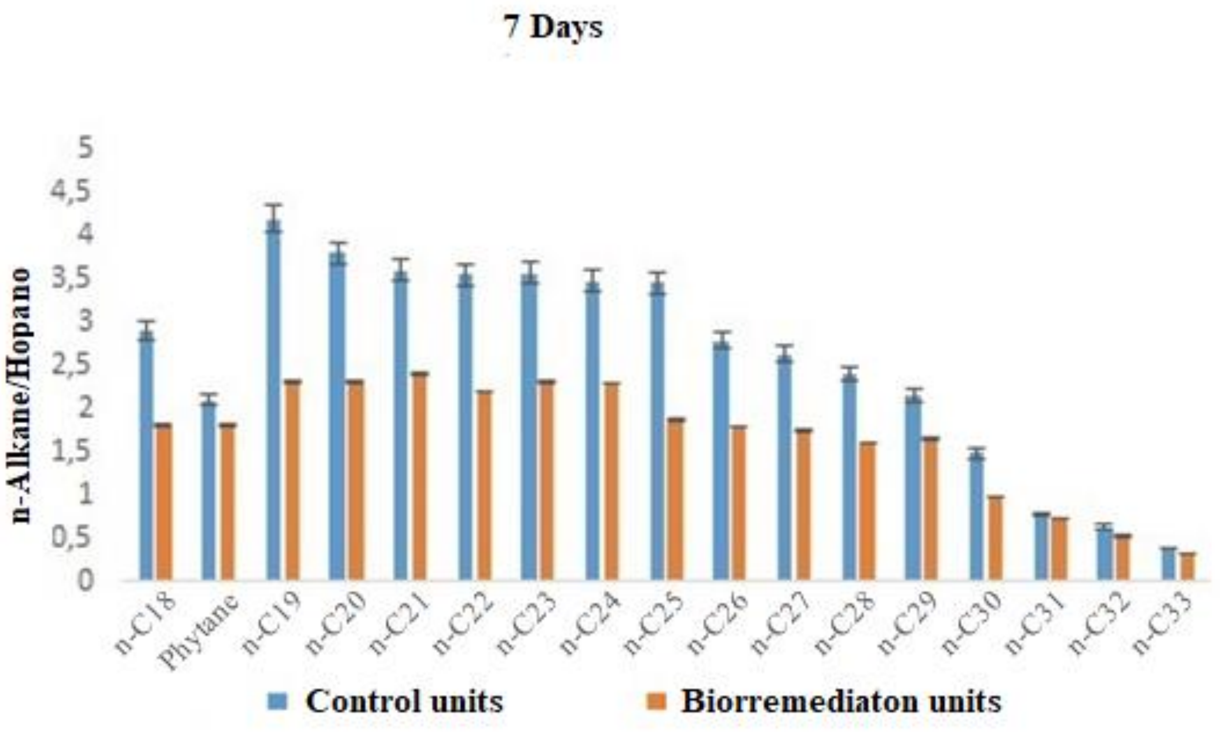


The ratios n-Alkane to Hopane and Phytane to Hopane for the bioremediation group are notably lower than those of the control group. It demonstrates that the addition of the NPK fertilizer was effective to stimulate the microorganisms to degrade the saturated fraction of the petroleum.

According to SEO; KEYN; LI, (2009), when biostimulation is used, the hydrocarbon metabolization becomes more efficient, also showing a higher speed of microbial action due to the appropriate C / N / P ratio.

Figure 8 shows the n-Alkanes to Hopane and Phytane to Hopane ratios of the bioremediation and control group samples after seven days of experiment for the saturated and aromatic blend.

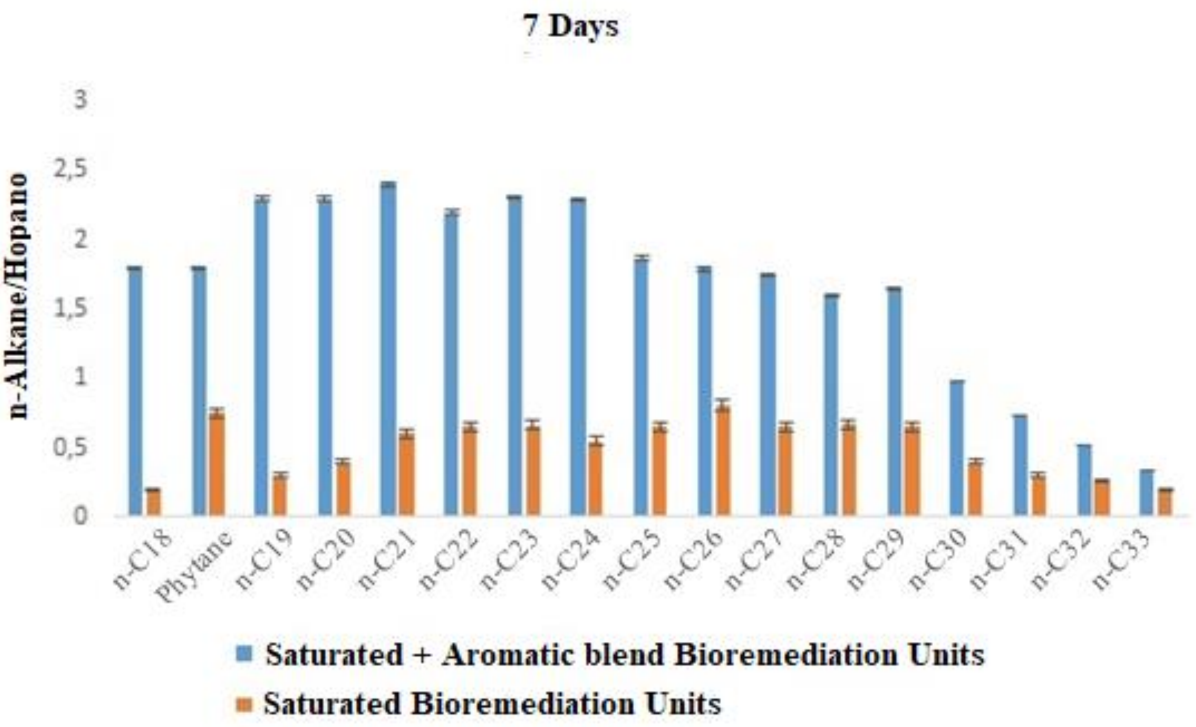
Figure 8: Comparison between the mean values of the n-Alkane / Hopane and Phytane / Hopane ratios of the Saturated + Aromatic samples collected from the Bioremediation and Control units of the experiment, 7 days after the start of the simulation



Comparing the results of the bioremediated and control group samples, one can see that again the addition of fertilizer stimulated the microorganism to degrade the blend. One can also see that degradation of the n-31C to n-33C was limited, in comparison to the other n-Alkanes in both samples, the blen and the saturated samples.

In Figure 9, the ratios n-Alkene/Hopane and Phytane/Hopane of the bioremadiated saturated and blend are plotted together. This allows a direct comparison between them. The degradation of the saturated sample was rather higher than that of the blend. The presence of aromatic fraction in the sample in some way hindered the biodegradation process.

Figure 9: Comparison between the mean values of the n-Alkanes / Hopane and Phytane / Hopane ratios of the analysis of the samples of the Bioremediation units for the Saturated and Saturated + Aromatic fractions, for 7 days of experiment.



Several hypothesis can be used to explain this result. Aromatic compounds can be toxic to the microorganisms presente in the sea water; the microorganisms present in the sea water are not able to degrade the aromatic compounds; the superior chemical stability of the aromatic compounds avoids its degradation and the combination of aromatic and saturated compounds somehow hinder the action of the microorganisms responsible for the biodegradation.

According to Jonhsen et al. (2004), aromatic compounds are not degraded by most microorganisms, due to stability, due to the resonance of Aromatic rings, the complex chemical structure and low water solubility, thus contributing to contamination of the medium.

4 Final considerations

The use of the fertilizer, NPK (10:10:10), contributed significantly to the biodegradation of the Saturated fractions, mainly the n-Alkanes, by the microorganisms, in the first 7 days of the experiment.

Saturated compounds from n-18C to n-30C showed a reduced biodegradability rate when mixed with aromatic compounds. Probably because the aromatic compounds showed high toxicity.

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