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Biodegradation of marine fuel MF-380 by microbial consortium isolated from seawater near the petrochemical Suape Port, Brazil

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ABSTRACT

This work aimed to study the biodegradation of marine fuel MF-380 by selected microbial consortium and to determine the most favorable conditions for this process. Initially, strains isolated from seawater of the port of Suape – PE were subjected to a test to assess the occurrence of microbial interactions and then microbial consortia were mounted and the most promising consortium was selected by redox indicators. The strains of the selected consortium were acclimated to increasing levels of marine fuel MF-380 (1%, 2% and 3%, v v^{-1}) and NaCl (1%, 2% and 3%, w v^{-1}). Subsequently, it was applied a full factorial 2^3 experimental design to investigate the influence of variables (temperature, pH, C: N ratio) in the biodegradation process and finally an experiment in a bioreactor employing the best conditions found in the experimental design. Consortium named C5 was the most promising and reached as best result a total hydrocarbon biodegradation of 93.5% in the experimental design and reached levels of degradation optimized on trial in the bioreactor for the constituents of the marine fuel MF-380. Therefore, the consortium C5 is capable of degrading marine fuel MF-380 and can be used as a bioremediation agent of environments polluted by this fuel.

1. Introduction

The release of petroleum and petroleum byproducts into the environment is one of the main causes of global pollution and has become a focus of great concern both in industrialized and developing countries once oil pollution can have dramatic detrimental effects to the environment and cause significant damages to resident organisms (Luna et al., 2013; Lin et al., 2014a). The major hydrocarbon source in oceans comes from routine operations of ship washing, natural oil leakage on the sea bed, and accidents during oil exploration and transportation (Souza et al., 2014).

The media has consistently reported the leakage of thousands of tons of oil that contaminate seawater (Souza et al., 2014; Silva et al., 2014). One of the most brazilian impacting spills occurred in November 2011 on the Sedco 706 oil rig operated by Chevron Brazil in Campos Bay (Rio de Janeiro, Brazil). A total of 5943L leaked, covering 163 km^2 (Souza et al., 2014). Another of the largest oil spills in the Brazil occurred in January 2000, when more than 1.3 million L of heavy Marine Fuel MF-380 leaked from a refinery pipeline into Guanabara Bay in Rio de Janeiro, Brazil, causing extensive damage to preserved mangrove areas (Maciel-Souza et al., 2006; Silva et al., 2014). Marine fuel MF-380 is the most transported oil in Rio de Janeiro and it is related to several cases of spills along the Brazilian coast. This fuel has great impact on marine organisms due their high toxicity (Farias et al., 2008; Nudi et al., 2007; Santos et al., 2010, 2015). Therefore, it is very important the study of mitigation measures for its impacts.

Remediation of oil contaminated sites could be achieved by either physicochemical or biological methods. Conventional physical and chemical methods could rapidly remove the majority of leaked oil, but in most cases the removal just transfer contaminants from one environment to another, even produce toxic by-products.

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More importantly, oil could not be completely cleaned up by physical and chemical methods. Due to negative consequences of the physicochemical approach, more attention is now given to the exploitation of biological alternatives (Malik and Ahmed, 2012; Lin et al., 2014a).

Due to its costs and benefits, bioremediation is an attractive alternative that has been used to eliminate or minimize the effects of pollutants, employing microorganisms with potential for biodegradation and being able to transform contaminants into less toxic substances or mineralizing them (Cerqueira et al., 2012; Sathishkumar et al., 2008). For the success of bioremediation technologies, the occurrence of microorganisms with the appropriate metabolic abilities is the most important requisite on oil spill bioremediation (Al-Wasify and Hamed, 2014). However, microorganisms with the ability to degrade hydrocarbons are generally low abundance in marine environments (Harayama et al., 2004), making isolation and selection of these extremely important to further increasing the number of autochthonous microorganisms capable of degrading quickly hydrocarbons (Nikolopoulou et al., 2013; Vasconcellos et al., 2009; Teramoto et al., 2009; Sakalle and Rajkumar, 2008; Harwati et al., 2007).

The biodegradation of hydrocarbons complexes usually requires the cooperation of more than one species. This is true for pollutants that have different compounds in their composition, such as oily products. Microorganisms isolated can metabolize only a limited portion of hydrocarbons, while mixed populations with large enzyme capacity are needed to increase the rate and extent of biodegradation (Ghazali et al., 2004; Silva et al., 2015). Furthermore, the use of a consortium of microorganisms native ensures that it has high tolerance to toxicity of hydrocarbons and is resistant to environmental variations (Hadibarata and Tachibana, 2009).

Bioreactors have played an important role in the detoxification of hazardous organic contaminants such as hydrocarbons and have been successfully applied in ex-situ bioremediation strategies (van Hamme et al., 2003; Gargouri et al., 2011; Kuyukina et al., 2009). Bioreactors are basically tanks in which living organisms carry out biological reactions (Chikere et al., 2012). These types systems display as main advantage a larger control of environmental and physico-chemical conditions affecting rates and extents of microbial growth and oil transformation such as oxygen supply, optimal pH, temperature and specific quantity nutrient providing an increase on biodegradation (Hughes et al., 2000; van Hamme et al., 2003; Chikere et al., 2012). On the other hand, in environments where the *ex-situ* bioremediation can hardly be used, such as seawater, bioreactors can be also efficiently applied to provide the biostimulation of autochthonous microorganisms growing in the presence of oil for subsequent reintroduction in the affected environment as strategy of bioremediation of polluted seawater (Maliji et al., 2013).

Experimental design is another important approach that have been successfully applied in the world to optimize biodegradation process of crude oil and petrol-derivatives (Mohajeri et al., 2010; Martins et al., 2012; Farag and Soliman, 2011; Agarry et al., 2012; Mohan et al., 2009; Ramírez et al., 2009; Pala et al., 2006; Xia et al., 2012; Lin et al., 2014b). This statistical technique allows build models and investigates the effects of the factors and the interaction of these on enhancing of the biodegradation, providing information about the best balanced proportions of the constituents of the culture medium and of the culture conditions more favorable for the process (Silva et al., 2015).

The installation of the Abreu e Lima refinery terminal in Suape port in the state of Pernambuco, Brazil, will likely contribute to an increase in accidents involving oil and its derivatives. Thus, the study of the biodegradation of fuels by native microorganisms is of extreme ecological importance, as the nearby beaches are subject to contamination by pollutants coming from the port complex (Miranda et al., 2007; Silva et al., 2015).

Therefore, this work aimed to study marine fuel MF-380 degradation by microorganisms in consortium, isolated from seawater near the petrochemical Suape, under different conditions of temperature, pH and C:N ratio aiming its application as a bioremediation agent.

2. Materials and methods

2.1. Marine fuel MF-380 characterization

Marine fuel MF-380 sample used in this work was gently provided by PETROBRAS Transport S.A. – TRANSPETRO. Due to marine fuel MF-380's complexity and unknown chemical formula, an elemental analysis of carbon, hydrogen, nitrogen and sulfur were performed in an elemental analyzer (CE Instruments, model EA 1110, UK).

2.2. Microorganism

The microorganisms used throughout this study consisted on five bacteria (P11B1, P11B2, P11B3, P11B4 and P11B5), one yeast (P12) and seven filamentous fungi (P13F2A, P13F2B, P132, P12F4B, P22F2A, P22F2B and P23F4) isolated from seawater near the Termopernambuco Company S.A. – TERMOPE located in the Petrochemical Suape port – PE, Brazil.

2.3. Media composition

The Bushnell-Haas mineral medium (BH) contained (per liter): KH_2PO_4 1.00 g, K_2HPO_4 1.00 g, NH_4NO_3 1.00 g, $MgSO_4 \cdot 7H_2O$ 0.20 g, $CaCl_2$ 0.02 g and $FeCl_3 \cdot 6H_2O$ 0.05 g, pH 7.2 ± 0.2. The medium was sterilized by autoclaving (121 °C for 15 min). The marine fuel MF-380 was used as carbon source.

2.4. Microbial consortia composition

The strains were subjected to an antagonism test based on the method of Bauer et al. (1966) and reported by Silva et al. (2015) to verify the occurrence of antagonistic interactions. Subsequently, microbial consortia were mounted so as not to contain strains that showed antagonism. A second criterion used in the composition of the consortia required the presence of bacteria, yeast and filamentous fungi in order to increase the potential degradation of marine fuel as a function of microbial diversity (Ghazali et al., 2004).

2.5. Microbial consortium screening test

The 2,3,5 triphenyl tetrazolium chloride (TTC) and 2,6 dichlorophenol indophenol (DCPIP) redox indicators, purchased from Sigma Chemical Co (St. Louis, MO, USA), were used to selecting the most promising microbial consortium for their ability to utilize marine fuel MF-380 as substrate. These indicators act as electron artificial acceptors in the process of biological oxidation (Braddock and Catterall, 1999; Cerqueira et al., 2012; Hanson et al., 1993; Gomes et al., 2009), being possible to predict if consortia to be tested are able to oxidize some of the constituents present in the marine fuel MF-380 by color change of the culture medium. Microbial consortia were allowed to grow in Büshnell-Haas mineral medium (BH) 50 mL containing 0.25 mL of DCPIP or TTC and 1% ($v\ v^{-1}$) of marine fuel MF-380 as carbon source, in 250 mL Erlenmeyer flasks at 30 °C and 150 rpm. Abiotic control was utilized in all experiments. The required time that promotes the changes in