Biodegradation of Hydrocarbons (Oil Fuels) by *Pseudomonas aeroginosa, Candida sp* and *Aspergillus terreus* by Isolated from the Coast Line of Arzew – Oran-Algeria

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Abstract

Among many refineries are located along the coast of Algeria, the refinery of Arzew in the north-west of Algeria is the subject of our study. Since always the sea was the universal receptacle of pollution by hydrocarbons negatively modified the natural balance of the aquatic environment and can give many problems for the environment. Our study aimed on the biodegradation as a natural elimination of these pollutants and used as control of this pollution. The aim of this work is the study of marine pollution by physical; chemical and biological methods. The species of *Pseudomonas aeruginosa, :Candida petrolium* and *Aspergillus terreus* isolated from the sea water of three stations port from Hyproc, fishing port and Marsa el Hajadj showed their capacity of adaptation and assimilation of strong concentration of the hydrocarbons oil Arabian light and crude oil of Hassi Messoud 10% in a natural environment and 3% in a synthetic medium , their roles of transformation and degradation of the crude oil of Hassi Messoud and the petrol of the Arabian light.

Keywords: Bioterioration, crude oil;light arab oil; Pseudomonas aeruginosa; Candida sp, Aspergillus terreus

1. Introduction

The rejection of hydrocarbons (HC) of oil-bearing origin in the environment constitutes one of the most alarming phenomena of pollution in the sense that these HC are toxic for the man, fauna and flora (Belhaj and *al.*, 2000).

The elimination of the oil in marine environment requires the intervention of the various biotic and abiotic factors. Among these factors; the biological breakdown by the micro-organisms and in particular the bacteria is the natural process most important in depletion of maritime environment. Consequently, mechanisms of the biological breakdown of the substances tankers (linear alkanes, phénylalcanes, cycloalcanes, hydrocarbons polycyclic and polyaromatic) by the marine bacteria (Soltani, 2010).

Metabolic reactions of the bacteria and other micro-organisms which are naturally present in the seamen circles are usually called mechanisms of biological breakdown.

According to several authors, metabolic ways of degradation by stocks of Pseudomonas sp. were the first studied ways and are very known (Sutherland and *al.*, 1995).

The objective of this study is the insulation of the micro-organisms marinades able to eliminate the oil substances or in the event of waste from an industrial complex (Andrade, 2001; Cardoso da Silva. and *al.*, 2003).

2. Material and methods

To appreciate the phenomenon of the biodegradation of hydrocarbons in sea water we prepared a culture medium by natural sea water for that a source of carbon was selected as well as a microbial population.

2.1. Sampling sources

It is natural sea water taken in a not polluted zone. A quantity of one liter is filtered on Whatman paper. At summer then added of ammonium chloride (2 g/l) as source of nitrogen and sodium phosphate (0.1 g/l) as source of phosphorus. To agitate this medium magnetically. To preserve at 4° C with the darkness for one month. The pH is adjusted to 8 (Boutefnoucht and *al.*, 2009).

According to Boutefnoucht and *al.* (2009) the source of carbon added in the middle of culture is a derivative of the crude oil (Arabian light) of Arzew "Oran".

3. Determination the microbial biodegradable

The source of carbon is a light fraction oil, a bacterial;yeast and fungi species would be able with it to only degrade this source of carbon in the Oil "Arabian Light", it is what directed us with the insulation and the purification of various stocks starting from our studies microbiological of the 3 stations and to test them on the oil crude.

The bacteria;yeast and fungi used for the inoculation of our test come from our insulation and identification with tests bacteriological first part of our experimental of 3 stations.

4. Experimental device

Technique used in our experimental and based known the manometer technique of the apparatus of Warburg . For each culture to be tested one needs 14 bottle of Warburg for pipe side, clean and dry: for each of the 12 substrates, like endogenous witness and the last like barometric witness thermo. Each bottle of Warburg with pipe has three compartments: the principal compartment, a compartment with pipe and a central tank.

To measure the reagents and to introduce them into the compartments of each bottle, as follows:

Bottles of test	**Principal compartment**	**Central flask**
	1.4 ml of oil where oil and 1.4 ml of calibrated cellular suspension (microorganisms)	**0.1 ml of solution of KOH with 20%α**
Endogenous pilot bottle	1.9 ml of oil plug where oil and 1.0 ml of calibrated cellular suspension (bacteria)	0.1 ml of solution of KOH with 20%α
Pilot bottle thermo barometric	3.0 ml of plug of oil where oil	20%α (p/v) of potassium hydroxide in distilled water -

5. Analytical method

5.1. Potential of degradation

The potential of degradation is given thanks to the analyzes the rate of CO2 release in the medium by the relationship between the quantity of substrate consumed in the tests and that presents in the abiotic witnesses in each 4 days of the incubation period 20 days.

5.2. Determination the rate of mineralization (CO_2)

The output of mineralization is the relationship between the numbers of moles of carbons released in the form of CO2.

Measurement was taken each 4 days for one 20 days period, and this technique based on the method of Warburg and calculates the carbonization gas rate of them according to Waes (1971) the formula was used for calculations.

$$KCO_2 = \frac{\frac{Vg273}{T} + Vfa}{Po}$$

X	Representing the quantity of gas in µl (0° C, 760 m Hg)
h	Representing the modification in mm of the open arms of the pressure gauge
KCO_2	Representing the constant of the bottle
Vf	Representing the quantity in µl, of liquid in the bottle
Vg	Representing the difference, in µl, between the total volume of the pressure gauge and the bottle and the number of µl of liquid of the bottle
T	Representing 273 + the temperature of operation (27° C)
α	Representing the solubility of CO2 in the solutions, in ul CO2/ul solution
P_0	Representing the standard pressure expressed according to the manometer solution

The value used was the value of CO_2 in the water, which is of 0.759 with 25° C; the manometer solution was the known solution of Brodie with density 1.033, so that P_0:

$$Po = 760 \frac{13,6}{1,033} = 10000$$

6. Results

The produced CO_2 rate is deferred in the Graph .On notices after the incubation period, that the production of CO_2 is increasing according to time for each pure population of the microorganisms, the results showed an increase in the CO_2 rate which is in direct contribution with the reduction in the rate of the oil crude and light arab oil.

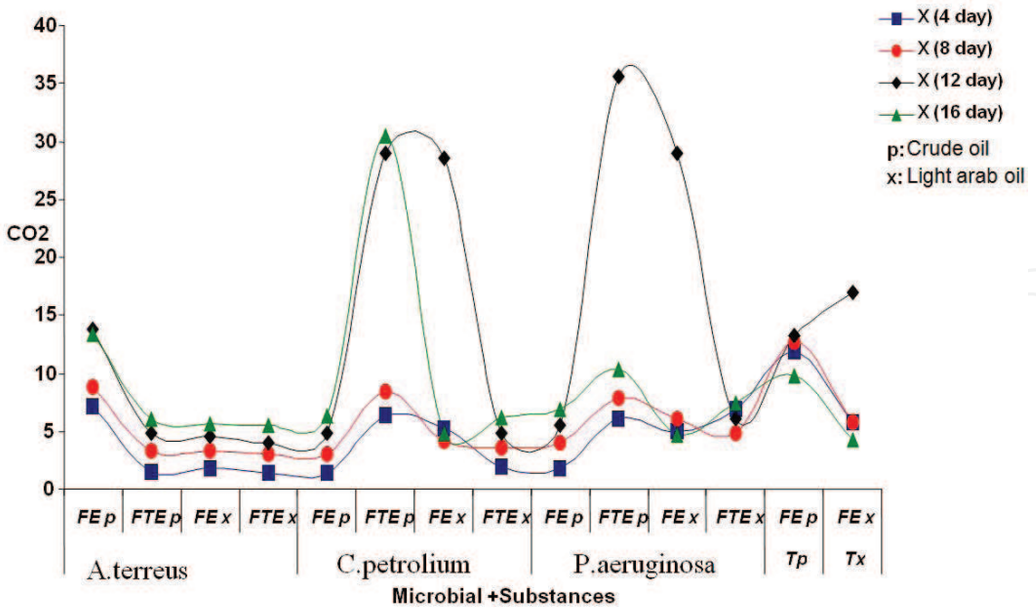

Fig 1. The concentration of CO2 (µl) released by *P.aeruginosa*; *C.petrolium* and *A.terreus*

7. The biodegradability test of DCPIP indicator

The biodegradability of the microorganisms was verified using the technique based on the rodox indicator 2,6-dichlorophenol indophenol (DCPIP) (Hanson et al 1993).

The principal of this technique is that during the microbial oxidation of the carbon source ,electrons are transferred to electron acceptors by incorporating an electron acceptor such DCPIP to the culture medium; the ability of the microorganisms to utilize the substrate by observing the color change of DCPIP from blue (oxidized) to colorless (reduced) This technique was used by pirollo et al 2008

The time to decolorization of the DCPIP indicator was registered of each microorganism Pseudomonas aerugenosa was 8hours .Candida petrolium 12 hours and Aspergillus terreus in 17 hours. we have noticed during the experiment no decolorization of the substrate controles (Without inoculums) or of the inoculums controls(without oils)was observed, similar results were found by junior et al 2009.

8. Discussion

From the graph we noticed a difference in rate of mineralization both have substance by the rate of CO_2 to release by *Pseudomonas aerugenosa* .*Candida petrolium* and *Aspergillus terreus*, it reaches 35.62 µl for *Aspergillus terreus*. after 12 days of incubation; according to Cerniglia (1992) that the metabolic way of degradation of Naphthalene by *Aspergillus terreus* and utilizes a dioxygenase which oxidizes one of the benzene cycles to form a cis-dihydrodiol. Clear mineralization is regular positive in the suspensions after one 12 days and 16 days period; the metabolic ways of degradation by stocks of *Aspergillus terreus* . Were the first studied ways and are much known. The contribution of oil biological breakdown causes significant increase in the rate of mineralization of carbon by report the, which rises with (28.99 µl crude oil; 28.6 µl for

Arabian light oil /12 days) for *Candida petrolium*, and (35.62 µl Oil; 28.99 µl Arabian light oil /12 days) for , and weak for *Pseudomonas aerugenosa* with (13.78 µl crude oil; 5.72 µl arabiant light/12 days) and this quantity CO_2 to release decreases with time before 12 days for *Aspergillus terreus* The oil carbon very quickly mineralizes by report Arabian light according to our results for the different microorganisms.In the 16 days have observed one followed by increase in the rate of CO_2 release by to 30.55 µl for (*Pseudomonas aeurogenosa*), and an absence of the release of CO2 for (*Aspergillus terreus* and *Candida petrolium*).

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