The Use of Terrestrial Indigenous Bacterial Strains In The Bioremediation of Oil Contaminated Soils.

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ABSTRACT

Environmental pollution with petroleum and petrochemical products such as diesel and used oils has been recognized as one of the most serious current problem in the world, especially in developing countries. These petrochemical products devastate the soil, surface and the microbial population at the polluted sites. Thus, the present work aims to study pollutants (bioremediation). The application of bioprocess "Landfarming" in the bioremediation of samples contaminated by their concentration. This strain chosen for its short generation time is so powerful best removal of oil valuated at 98.43% in 28 days of experimentation in the bioaugumented and biostimulated sample by biosurfactant, 98.22 % in 35 days in the bioaugumented and biostimulated sample by urea and 86.1 % in 35 days in the bioaugumented and biostimulated sample by nutrient solution.

KEYWORDS: Soil, oil, Pollution, Bioremediation, Landfarming, Bacillus megaterium, Biosurfactant, Urea.

INTRODUCTION

The extensive consumption of petroleum hydrocarbons as a major energy source and an important feedstock to chemical industries has increased concerns about oil contamination and its harmful environmental consequences all over the world [27]. Accordingly, several technologies have been developed to address the problem and satisfy the strict environmental regulations. Among them, bioremediation is one of the most promising techniques which has certain inherent merits (e.g., low costs, in situ treatment capability and environmental friendly nature) over other physical or chemical based processes [25].

This technology involves the removal of petroleum hydrocarbons from the contaminated site by the act of microorganisms in a complex multi-phase system. Consequently, the efficiency of the process, it may be said, depends strongly on the ability of microorganisms in degrading hydrocarbons effectively. As such, the isolation of potentially applicable microorganisms to bioremediation of a variety of oily contaminations has received a lot of ink in the related literature[24] [22]. However, the main drawback of bioremediation processes in a majority of cases is the slow biodegradation rate of hydrocarbons[18]. The problem is even more serious, especially in economical terms, when the process would be performed in a reactor or a vessel such as that of slurry phase reactor bioremediation. The aim of this study was to study the microbial strain isolated from diesel-polluted soils for their degrading power.
MATERIALS AND METHODS

2.1. Equipment:
The soil sample contaminated with drilling discharges is taken from the surface horizon of a quagmire Haoud Alhamra at the Hassi Messaoud field (80 Km by contribution in the center of Ouargla). The age of this mess is one year.

The biosurfactant is used to biostimulate soil microflora. This biosurfactant commercial product dosed to 2% biosurfactant.

2.2. Physico-chemical analysis:
The physicochemical analyzes performed are: particle size analysis (NF ISO 1146), the water content (AFNOR X 31-103), electrical conductivity [6] and the total hydrocarbon concentration in the soil studied by distillation [5].

2.1.1. Bacteriological analysis:
Of the bacterial flora of the sample is isolated and counted on nutrient agar medium and are then purified on the same medium and pre-identify by biochemical tests.

2.1.2. Selection of isolates:
The growth rate has long been considered an essential criterion for the choice of the microorganism. For this, we have made a selection of the strains based on the calculation of the kinetic parameters after 8 hours of incubation [15].

The selection will be made from the stem holders da capacity gradient isolated sample E1 hydrocarbons.

2.1.2. Monitoring the growth kinetics of the purified strains:
From stem isolated on a mineral medium containing oil, the selection will be on the best growth of these strains and in the growth parameters, We have been tracking the growth of the strains on medium containing oil (2%) by measuring the microbial concentration every 2 hours for eight fortunes of fermentation.

2.1.3. Preparation of inoculum:
Preparation of inoculum is an embodiment of a culture isolated from the best with the time shorter generation strain. It will be prepared by removing a colony of this strain from a youth culture (24 hours) and seeded in Erlen Meyer containing 250 ml of BH medium (2%) at 30°C in an incubator shaker (150 rev / min) for 24 hours.

After centrifuging the culture at 3000 rev / min for 10 min the pellet is recovered and is subjected to successive washing with sterile saline in the pellet is diluted with 100 ml of distilled sterile water.

2. Methods: bioprocess "landfarming" :
In order to prove the ability of indigenous peoples to degrade hydrocarbons in soil bacterial microflora, we followed the evolution of various parameters related bacterial activity during five weeks. Soil (sample E1) will be spread over eight plastic containers 30 x15x15cm. Each tray containing 2 kg of contaminated soil (Figure ID).

The stimulation of our soil microflora will be achieved by means of the biosurfactant 2% 200ml, 250ml of nutrient solution and urea solution (6g / l) 200ml. The hydration and aeration is carried out continuously and regularly throughout the trial period.

B1: witness tray containing a sample of the contaminated soil.
B2 : bac stimulated by external nutriment.
B3 : bac stimulated by the addition of biosurfactant.
B4 : pot stimulated by the addition of urea (6g / l).
B5 : bioaugmenté tray by adding an inoculum of a bacterial strain isolated selected.
B6 : bac bioaugmenté by a bacterial strain and stimulated by nutrient intake.
B7 : bac bioaugmenté by a bacterial strain and stimulated by biosurfactant.
B8 : bioaugmenté tray by a bacterial strain and boosted by urea (6g / l).

The bins are kept at ambient temperature and airy.

To estimate the rate of hydrocarbon degradation by soil bacteria contaminated soil studies, samples will be taken weekly for 5 weeks by witness bins and bins bioaugmentés and / or biostimulés. The parameters monitored are:

• Concentration of residual hydrocarbons and hydrocarbon removal rate:
• Microbial Concentration measured by a simple count on the environment " GN " nutrient agar " :
• Soil pH
RESULTS AND DISCUSSIONS

3.1. Physico-chemical characteristics:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Texture</td>
<td></td>
</tr>
<tr>
<td>SF</td>
<td>41.3% ± 0.67</td>
</tr>
<tr>
<td>SG</td>
<td>23% ± 0.79</td>
</tr>
<tr>
<td>LF</td>
<td>11% ± 1.14</td>
</tr>
<tr>
<td>LG</td>
<td>16% ± 1.32</td>
</tr>
<tr>
<td>A</td>
<td>9% ± 0.93</td>
</tr>
<tr>
<td>Water content (%)</td>
<td>6 ± 1.32</td>
</tr>
<tr>
<td>pH</td>
<td>7.52 ± 0.45</td>
</tr>
<tr>
<td>CE (dS à 25 °C)</td>
<td>0.54 ± 0.89</td>
</tr>
<tr>
<td>[HCT]</td>
<td>123.4 ± 1.1 g/kg de sol</td>
</tr>
</tbody>
</table>

According to this table, the particle size analysis shows that the predominant fraction of the sands (over 50%). The soil sample is studied also characterized by a relative humidity of 6 % and has an alkaline pH of 7.6 (Table I). The value of the electrical conductivity of the sample is of the order of 0.54 dS/m. We note the contents of the sample oil is de 123.4g/kg.

Results of Physico-chemical analysis indicates that the studied sample has sandy and loamy nature according to the GEPPA [6] this texture facilitates fluids circulation, fluids contain nutrients and oxygen which are all accessible to the microorganisms if the medium is permeable [5]. The slightly alkaline pH is favorable for the development of microorganisms [9] this salinity is medium according to wide salinity [20]. This can reduce the number of microorganisms in soil [22]. The total hydrocarbon content of the sample studied is much greater than the value set by the Dutch standard (0.1 g/kg soil). [19] This result confirms that this soil is polluted by hydrocarbons.

3.2. Evolution of bacterial biomass:

The bacteriological diagnosis reveals that the sample having a bacterial concentration of $6.41 \times 10^8$ CFU/g soil.

![Fig. 1: Evolution of bacterial biomass in the samples treated](image)

According to the time course of biodegradation and the bioprocess according adapted, the results show an increase in the bacterial concentration for the eight samples. The witness (Tray 1) shows a very slow growth kinetics by providing other treated samples.

The kinetics of growth we obtained is divided into two separate phases by a stationary phase. It looks like a classic bacterial growth curve. At first, the increase in bacterial mass is the exponential phase, phase during which the hydrocarbons are sufficient for metabolic requirements of bacteria. On the other hand when the microbial concentration increases beyond the third week and the bins bioaugmentés 4th week for other trays, the nutritional requirements of level exceeds the rate of degradation of the substrate. Bioavailability will become limiting and that is why the growth is linear (3-4 weeks). This phase could be characteristic of the degradation.
of complex hydrocarbons, after which there has been a decline in growth translates into reductions in bacterial biomass in the bioincreased tanks beyond the fourth week [3]

3.3. Evolution of concentration of residual hydrocarbons:

![Graph](image)

Fig. 2: Evaluation of the concentration of residual hydrocarbons in the treated samples.

The soil sample used has an initial hydrocarbon content of 123.4g / kg soil. This content has gradually diminished in court time for the different treatments (Figure 2). After five weeks of treatment, the soil having neither stimulated nor bioaugmenté (Tray 1) has the highest oil content is 58.8g / kg soil and 43.5g/kg. Floor when it was stimulated by nutrient solution (B2).

The bioaugmentés samples and biostimulés by nutrient solutions (Bac6) bioaugmenté (Tray 5) biostimulé by biosurfactant (Tray 3) and biostimulé by urea solution (Tray 4) reached levels comparable hydrocarbons after 5 weeks of treatment. These levels are 23.22 g / kg soil 23.1g/kg soil 21.74g/kg and 21.64 g / kg soil soil respectively.

3.4. Evolution of biodegradability:

![Graph](image)

Fig. 3: Rates of hydrocarbon biodegradation yields in the eight bins according to time.

Figure 3 shows that the values of degradation yields of hydrocarbons in bioaugmentés trays (5,6,7 and 8) are always greater than those just biostimulés bins (2,3,4), the latter present after 35 days processing rate of removal of hydrocarbons Respectively
The sample tray 7 (bioaugmenté and biostimulé by biosurfactant) was expecting a maximum rate of degradation of near 100% at the end of the 4th week. This value is reached a week later for the sample and bioaugmenté biostimulé by urea (Tray 8). For the same period, 87.6% of HCT are removed from the sample tray 6 (bioaugmenté is biostimulé the nutrient solution), 76.4% of Tray 5 (only bioaugmenté) and only 51.2% of control (Tray 1).

The rate of degradation of hydrocarbons soil seems to be influenced by the further addition of a bacterial strain selected (Bacillus megateri um) for good growth rate on the one hand and on the other hand by the nature of stimulant brought. Despite the degree of contamination of the sample studied, a significant bacterial biomass is recorded. Typically bacteria capable of degrading hydrocarbons are significant quantities in the soil, that is to say above 10^4 UFC / g soil [26].

The effectiveness of the physical process (ventilation and humidification) achieved is evidenced by the rate of removal of hydrocarbons in the control sample where the percentage is 51.4%. Moisture is a very important parameter in the process of biodegradation. Dry soil has only a very low microbial activity. When the humidity rises, this activity gradually increases [22]. The initial step of the catabolism of aliphatic, cyclic and aromatic hydrocarbons by bacteria includes, in fact the oxidation of such substrates through oxygenases and hydroxylases, for which the molecular oxygen is essential [21]

3.5. Evolution of pH:

![Fig. 4: Evolution of pH of eight soil samples](image)

the change in pH with time is represented in FIG NO. At the initial time, the pH was 7.52. For eight bins changing the pH curve as a function of time. During the first 3 weeks of the experiment, the pH bioaugmentés bins (5, 6, 7 and 8) is reduced to values of between 7.52 and 6.6. However, the pH of the control tray remains at least slightly variable oscillating during the first 3 weeks between 7.5 and 7.4. We see the following stability will be followed by a slight decrease (pH 7.3 at week 5).

This lower pH tends toward neutrality indicate the presence of increased microbial activity in the soil treated with the control. The reason might be due to different biochemical reactions that result in the absorption of oil compounds on the one hand, with the synthesis of carriers of groups "COOH" fatty acids and various acids (formic, acetic, butyric, lactic) are produced by oxidation or fermentation of the organic matter in the soil acidity causing another side. These groups are intermediate metabolites of hydrocarbon biodegradation before complete mineralization by microorganisms [25]

**Conclusion:**

The Bacillus enhanced the degradation efficiency of crude oil contaminated soil. Indeed, multifunction of bio surfactants is known with different chemical structures and surface properties which have important roles in the uptake and mechanisms of mineralization in hydrocarbon polluted soil. However, biosurfactant and urea show high potential to remediate of hydrocarbons from contaminated soil.

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