CRUDE OIL BIODEGRADATION BY MICROBIAL FORMULATION WITH REFERENCE TO *ASPERGILLUS VERSICOLOR*

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ABSTRACT

The cleaning up of petroleum hydrocarbons in the soil environment is a real world problem. Biodegradation of hydrocarbons in the environment is the natural way of cleaning the nature. The potential biodegradation of hydrocarbon contaminants by microorganisms is dependent on the environmental factors and the nutrients available. From petroleum-contaminated soils A. *versicolor* was isolated for their capacity to grow in the presence of petroleum hydrocarbons. Their growth rates and biodegradation ability were determined in mineral basic media supplemented with crude oil and the parameters like temperature 35ºC, pH 7.0 and nitrogen source were optimized conducted by one factor at a time for the maximum yield of biomass by A. *versicolor*.. After determining the crude oil utilization efficiency of A. *vesicular*, isolated pure cultures were tested for their degradation efficiency of crude oil by gravimetric analysis. Degradation of hydrocarbon fractions of crude oil was investigated by comparing with GCanalysis. The results revealed the extents to which the isolate could degrade crude oil hydrocarbon that are toxic in the environment.

Key words: Biodegradation; crude oil; A. *versicolor*; Gravimetric Analysis; Gas Chromatography Analysis.

1. Introduction:

The Spilled petroleum constitutes (Hydrocarbons) are one of the main environmental pollutants. Their abundance and persistence in several polluted environmental areas have been reported [Mohammed. et al, 2004]. Oil spillage may be caused by natural disasters like earthquakes in the sea surface or due to accidental leaks during exploration, refining, storage and transportation. The causes can be numerous but the consequences are the same. In case of crude oil, the different types such as heavy or light crude oil can affect the clean-up procedures. Crude oil spreads very rapidly on the sea surface and after a short period of time the thickness of the oil film can be at least 1mm. It is therefore necessary to prevent the spreading to reach the shoreline. Once it reaches the shoreline, it contaminates the soil and cause a great damage to the soil ecosystem as well causing the death of many Ridley turtles that breed there during that period .It hence becomes a necessity to clean up this oil in order to save the marine life.

Clean-up and recovery of hydrocarbons from an oil spill is difficult and the strategies for cleaning up an oil spill are greatly affected by a variety of factors such as the type of oil spilled, the temperature of the water body, and the types of shorelines and beaches involved . A number of approaches and technologies have been developed for spreading of oil spills in marine shorelines and freshwater environments.

Environmental factors like temperature and pH were considered as important factors as they have a significant effect on the rate of microbial growth and hence on the degradation. To enhance the bioremediation process addition of nutrients (N and P) were very essential. To identify the adequate amount of nutrients, the optimization study in laboratory has to be carried out. Nutrients are utilized by the microorganisms for the synthesis of organic compounds (Proteins) and for intracellular metabolism. In biodegradation process, microorganisms consume hydrocarbons as a carbon source, Carbon dioxide and water are released as by products.

To enhance the bioremediation process, the microbial population present in the polluted environment is to be stimulated. Bio-stimulation involves the addition of nutrients to increase threat of biodegradation process. In most shoreline ecosystems that have been highly contaminated with hydrocarbons, nutrients are likely the limiting factors in biodegradation of oil Inadequacy in nutrient concentration affects the biodegradation process. Hence, addition of appropriate nutrients in sufficient quantity is very essential to promote or enhance the microbial growth and thus the biodegradation.

1.1. Hydrocarbons of Crude oil: Oils are further categorized into three broad groups, according to their molecular weight. General statements can be made for each of the three categories namely light weight, medium weight, and heavy weight components. Crude oils are composed of various combinations of these three categories: Light Weight Components: These are carbon atoms range from C1 to C10 which are smaller molecules with few numbers of atoms. They are characterized by high volatility, readily dissolve and evaporate and leave little or no residue because of their short residence time. Many of these components (e.g., benzene, and toluene) are thought to be more bioavailable to animals by primary exposure route (respiratory system .These are carbon atoms ranging from C11 to C22 which have complex molecules. It has low rate of evaporation and dissolves very slowly that take several days with some residue remaining.

1.2. Heavy Weight Components: These are components which Carbone atoms more than C23. It has the longest residence time with very little loss due to evaporation or dissolution. It can cause chronic effect through smothering as residue in the water column and sediments (tar balls, etc). Its primary exposure route is direct topical contact. Some heavy weight components contain carcinogens that are absorbed through the skin. its risk of exposure is increased due to long residence time, probability of contact, and adsorption property of the oil components.

2. MATERIALS AND METHODS

2.1. Sampling: Soil samples extending from the ground surface to a depth of 10–20 cm were collected from petroleum-contaminated areas near petroleum storage, distribution and refining areas. Samples were taken from areas near storage, areas near distribution facilities and areas near a refinery. Samples were then kept sterile and on ice and were transferred immediately to the laboratory where petroleum-degrading bacteria were isolated from them. Aseptic crude oil was used in the assays for ability of the isolated bacteria to degrade crude oil petroleum. Screening of crude oil degrading Bacteria: 5gms of soil sample was inoculated in R2B broth and was incubated at 37°C for 2 days. After incubation 0.1ml of broth culture was plated in mineral salt medium using spread plate technique. An ethereal solution of crude oil (10% w/v) was uniformly sprayed over the surface ofthe agar plate. The ether immediately vaporized and thin layer of oil remained on the entire surface. The plates were incubated at 25°C for 2 days. The organisms that formed clear zones around the colonies were considered as crude oil degraders.

2.2. Isolation of pure microbial cultures: Microbial isolation experiments performed in solid Minimal salts media (MSM) containing1.5% agar and 1% of the n-Octane as a sole carbon source. The medium was autoclaved before inoculation. Rushikulya beach sand samples and broth samples from microbial enriched jar was serially diluted and 1ml aliquot of 10-4 dilution samples was added to sterile Petri plate by spread plate technique. Agar plates were incubated for four days at room temperature (28 - 30ºC) at a pH of 7.4 ± 0.2. The parameters temperature, pH and nutrients were optimized based on one-factor at a time approach. Maximum growth of the microorganisms was obtained and the growth was analyzed in terms of biomass.

2.3. Biodegradation Assays: The isolated pure cultures were transferred to conical flasks, each containing 100 mL of sterile mineral salts medium with (0.2% v/v) crude oil. The experiment was carried out in duplicate and uninoculated flasks constituted the controls, accounting for abiotic losses. All flasks were incubated at 22°C for determined intervals of time i.e; 15, and 26 days .Residual concentrations of crude oil were determined gravimetrically and by gas chromatography.

2.4. Gravimetric Analysis: At the end of each incubation period to assess residual concentrations of crude oil, Sample with chloroform was placed in a separating funnel with continuous shaking, after which the contents were allowed to settle; two layers were formed: watery layer and chloroform layer containing the residual hydrocarbons. The last layer was decanted and air dried. After chloroform evaporation, the residual oil was quantified gravimetrically .The consumed oil was calculated by subtracting the residual hydrocarbon from the original weight of hydrocarbons. Bacterial biomass was estimated after the culture medium was centrifuged at 1500 rpm for 20 minutes in order to separate the biomass (bacterial cells) for each flask at the end of each incubation period. This biomass was washed several times with water then with chloroform to remove residual hydrocarbons and dried at 100°C till constant weight.

2.5. Gas Chromatography Analysis: Residual crude oil at the end of each incubation period was quantified chromatographically via capillary gas chromatography (CGC) using Agilent 6890 plus gas chromatograph equipped with flame ionization detector (FID), split/split less injector, and fused silica capillary column HP-1 of 30 m length, 0.35 mm internal diameter, and 0.5 μm film thickness. The detector and injector temperatures were maintained at 300°C and 250°C, respectively. The column temperature was programmed to rise from 80°C to 300°C with a rate of 3°C/min and final time 15 min. Nitrogen (free oxygen) was used as a carrier gas at flow rate 2 mL/min.

3. RESULTS AND DISCUSSION

Maximum Clearing of crude oil in the mineral salt medium was observed due to the bacterial growth. It indicates the degradation efficiency of crude oil by Aspergillus *versicolor*. In this study, the efficiency of crude oil degradation by Aspergillus *versicolor* was determined qualitatively by estimating the consumed hydrocarbons after biodegradation and by estimating the dry weight of the Aspergillus *versicolor* .The results demonstrated that the consumed hydrocarbons after crude oil biodegradation was 1.62 to 1.80 g/L, 0 while the dry weight was 0. 59 to 0.68 .The residual oil at the end of each incubation time was analyzed quantitatively using capillary gas chromatography (CGC). The chromatograms appear as a

number of peaks which represent the residual hydrocarbons over a hump which represent the unresolved complex mixture (UCM) of high molecular weight hydrocarbons.

The results clearly showed that the values of consumed hydrocarbons and dry weight increase gradually with increasing incubation period (as a result for the biodegradation of crude oil) and their values after biodegradation by the were the highest .

4. CONCLUSION

The rate of crude oil biodegradation in the soil seems to be rapid. This may be due to the fact that the microorganisms in the soil have efficiency ability in utilizing the residual crude oil as a source of carbon and energy. Crude oil contains hydrocarbon and does not resist attack by microorganisms. This study proposes degradation of petroleum hydrocarbons using indigenous microorganisms isolated from the hydrocarbon contaminated sites .The isolate Aspergillus *versicolor* have shown very good growth on crude oil hydrocarbons. This investigation had achieved its primary objective of designing microbial formulation that could be employed in the bioremediation of soil polluted by crude oil.

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