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Research Article

Biodegradation Ability of Yeast Isolate AB – 01 on Engine Oil Polluted Soils in Jos Metropolis

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Abstract

The degradation of crude oil by yeasts and bacteria has recently developed become an important beneficial effect in connection with marine pollution. This has helped in the reduction in the dangers of spraying large quantities of toxic chemical detergents and emulsifying agents on the soils. The aim of this study was to evaluate the effect of already isolated indigenous yeast AB-01 on engine oil polluted soil samples for its biodegradation.

The soil samples used were collected from two locations. Sample A was collected from Federal College of Forestry and Sample B was collected from Bauchi Junction, both from Jos metropolis. The samples were collected from 10 cm depth using soil surface sterilized auger. They were taken to the laboratory in clean polythene bags for processing. The polythene bags were labeled accordingly. Each contained about 500g of soil samples. The analysis of different ecological parameters such pH, Moisture content, Organic content and colour and Texture, Rate of water percolation and yeast isolate on formulated medium using 1% engine oil were done.

Results: The results of the ecological parameters of the soil samples revealed that the pH of samples A and B were 5.52 and 5.28 respectively indicating that the soils percentage slightly acidic. The moisture content of the soil samples A and B were found to be 9.75 and 16.80 respectively. The percentage organic content of soil samples A and B w are found to be 22.2% and 11.4% respectively.

The results of water percolation rate of the soil samples A and B revealed that the soils that were bioremedied with the yeast cells drained more water than the control. The result as shown indicates that within the same time limits, soil samples A and B drained more water, 190 ml than the control sample. The results of the inoculation of the yeast isolate on formulated medium using 1% engine oil showed that there was growth of the yeast cells grow on the medium supplemented with engine oil as the sole carbon source. The yeast cells grow faster on the medium without engine oil which served as control. The initiation of growth on the engine oil Agar took a longer time of 48 hours before there was growth. After then, the growth flourished.

Keywords: Engine Oil; Degradation; Crude Oil

Introduction

Crude oil is a complex mixture of hydrocarbons and compounds containing oxygen, sulphur, nitrogen and trace amount of metals no single type of crude oil has ever been completely defined chemically [2]. Crude oil is a naturally occurring bituminous liquid made of various organic chemicals. The oil is found in large quantities below the surface of the earth and can be used as a fuel or raw material in the chemical industry. It is often refined to kerosene, gasoline, diesel fuel, engine oil and some other fractions.

Oil is the result of changes brought about over millions of years because of the transformation of the organic matter deposited in layers of sediment or the bottoms of primeval seas and lakes. The source of oil is a marine sediment oil, primarily because of the pressure to which it is subjected migrate from the sedimentary rocks, it may reach the surface and lead to natural seepage or as is more often the case, it is trapped below a layer of impermeable rock [15].

Crude oil spill

Oil spills on land, rivers, and the ocean are mostly cursed by accidents involving tankers, burgers, pipelines, refineries and storage facilities. These accidents can be cursed by human mistakes, carelessness or sometimes by natural disasters such as hurricanes or earthquake.

Deliberate acts by terrorists, countries at war, vandals or illegal dumpers show that oil spills are not always by accidents. Oil spills are often harmful to marine birds, mammals and sometimes, fishes including shellfish. Birds are protected from the elements by their feathers which overlap like tiles on a roof. The separate strands on each feather are bound together by rows of tiny hooks, creating a tight weave. The bird's skin stays warm and dry underneath. However, oil and cloy the feather's strands a hook and allow water to penetrate to the bird's skin [11]. However, since oil production started in Oloibiri, Nigeria in 1966 by the Shell- BP and later by many other oil companies (Agip, Chevron, EIF, Gulf, Mobil, Texaco and others) oil spills in the Nigeria aquatic and terrestrial environment have been a common occurrence. Some of the spills have resulted from sea going oil tankers, accidental discharges; blow out oil from wells, operational mistakes, while some have stemmed from sabotage [14].

[16] stated that the removal of oil that has been accidentally or purposely spilled into the environment is of great concern to the petroleum industry. In the efforts to ameliorate the consequences of such oil spillage in the past, man made a serious mistake by employing artificial methods of oil clean-ups. [8,9] reports that the British authorities made things worse during Torey Canyon oil disaster in 1967. They intervened without consultancy with ecologists. The authorities ordered that spraying of large quantities of toxic chemical detergents and emulsifying agents on the soil.

The mixture of emulsified oil and detergent was far more toxic than the oil itself at least in coastal water. All or nearly all detergents are toxic themselves, but once the oil is emulsified, it ceases to float as a surface layer and becomes instead an actual part of the pelagic environment. This may be taken into the gills of fish or ingested by filter-feeding organisms. When oil slicks are sprayed with detergents, pelagic fish may be killed. In addition, the emulsified mixture could do considerable harm to plankton which ascends to the surface at night. The use of microorganisms to degrade such oil spill has proved more rewarding.

The biodegradation of accidentally spilled or waste oil in aquatic and terrestrial environments has been the subject of several recent reviews [1,4].

[10] defined Bioremediation as the use of microorganisms to breakdown environmental pollutants in soil and water. Pollutants are persistent chemicals compounds that are poisonous to plants and animals. They may have the potentials to cause birth defects or carcinogenicity in humans. Such pollutants can be broken down to carbon dioxide and water during bioremediation. Microorganisms decompose organic compounds using enzymes and protein based molecules that control metabolism in all living cells. The microorganisms utilize the hydrocarbons for the supply of energy and carbon for growth with the production of waste like CO_2 and water. Petroleum is a rich source of organic matter and the hydrocarbons within it are readily attacked aerobically by a variety of microorganisms, it is not surprising that when petroleum is brought into contact with air and moisture, it is subject to microbial attack [16]. It has been reports that in situations as oil spills, microbial utilization of oil is desirable and may even be promoted by the addition of organic nutrients [6]. The authors reported that microorganisms participate in oil spill clean-ups by oxidizng some of the oil to CO_2.

[6] reported that hydrocarbon-oxidizing bacteria and fungi are the main agents responsible for decomposition of oil and oil products. Bacteria and yeasts, however, appear to be prevalent hydrocarbon degraders in aquatic ecosystems.

Hydrocarbon-oxidizing microorganisms develop rapidly within oil films and slicks. Brock and Madigan [6] reported that significant aliphatic hydrocarbon oxidation occurs only in the presence of oxygen. However, if the oil gets carried into anaerobic button sediments, it will not be decomposed and may remain in place for many years.

Materials and Methods

Sample collection

The soil samples used were collected from two locations. Sample A was collected from Federal College of Forestry. Sample B was collected from Bauchi Junction, both from Jos metropolis. The samples were collected from 10cm depth using soil surface sterilized auger. They were taken to the laboratory in clean polythene bags for processing. The polythene bags were labeled accordingly. Each contained about 500g of soil samples.

pH determination of the soil

The pH of the soil samples were assessed by suspending 20g of the soil sample in 100 ml of sterile distilled water. The pH meter model 151R was first calibrated with buffer solution before it was used to determine the pH of the experimental soil samples. The pH measurement was done in replicate and the average of the replicate was recorded.

Moisture content of the soil samples

The moisture content of the experimental soil samples was determined by heating about 30g of each of the soil samples to a constant weight in the oven at temperature of 160^0C for 2hours. The experiments were done in replicates. The moisture content was determined using the following formula.

% moisture content of soil sample = $\frac{W-X}{W} \times 100$

The original weight of soil samples = Wg

The weight of soil sample after heating to constant weight = Xg.

Organic content of the soil sample

The soil samples that were already dried to constant weight above were used to assess the organic content. The soil samples dried to constant weight were burnt to ash in an electric furnace for 5hours and reweighed. The organic content was determined using the following.

% organic matter content = $\frac{Y-Z}{Y} \times 100$

Weight of dried soil = Yg

Weight of burnt soil = Zg.

Colour and texture of the soil samples

The colour of the soil sample A was dark brown while soil sample B was light brown in colour. The texture of soil sample A was coarse while that of sample B was fine.

Rate of water percolation

A weight of 100g of a sterilized soil samples A and B were put into two beakers. 10 ml of engine oil obtained from Total filling station was used to amend the soil samples.

A volume of 100 ml of 10^{-5} suspension of the yeast cells was added to the soil samples each, mixed thoroughly with the aid of a metal rod.

The soil samples were incubated at 37^0C for a period of one week in other to stimulate the degradation of the hydrocarbons by the yeast cells.

The contents of the beakers were then poured into a funnel and place on top of a cylinder.

A volume of 200 ml of sterile water was poured into the polluted soil in the funnel and was allowed to stand for a period of one week to determine the rate of water percolation on the soil samples.

The time water was poured and time the water stopped dropping, average volume of water drained and retained were all recorded.

The experiment was replicated three times. The water percolation rate was calculated using the time it will take the water to drain in 1 second.

Formulation of engine oil medium

A volume of 99 ml of distil water was poured into a conical flask and 2g of Agar powder was added to the distil water in the conical flask. This was followed by the Calcium Chloride 0.01g, Ammonium Sulphate 0.05g, Magnesium Sulphate 0.02g, Potassium Chloride 0.05g and Potassium dihydrogen phosphate of 0.1g. 1 ml of engine oil and 2g of purified Agar powder. The content of the conical flask was mixed thoroughly by heating on a hot plate. The flask was plugged properly and was placed inside the autoclave for a proper sterilization at 121^0C for 15psi at 30^0C. The media was brought out after sterilization and allow to cool to 44^0C before pouring into the sterilized Petri dishes. Plates were allowed to solidify, after the

solidification of the media, the yeast isolates were inoculated into the media with the aid of sterile wire loop and were incubated at 37°C for 48 hours. The yeast isolates were also inoculated on conventional Sabouraud Dextrose Agar medium for comparison. The experiment was done in triplicates.

Results

The results of the ecological parameters of the soil samples revealed that the pH of samples A and B were 5.52 and 5.28 respectively indicating that the soils percentage slightly acidic. The moisture content of the soil samples A and B were found to be 9.75 and 16.80 respectively. The percentage organic content of soil samples A and B w are found to be 22.2% and 11.4% respectively. The results of ecological parameters of the soil samples are summarized on table 1.

The results of water peculation rate of the soil samples A and B revealed that the soils that were bioremedied with the yeast cells drained more water than the control. The result as shown in table 2 indicates that within the same time limits, soil samples A and B drained more water, 190 ml than the control sample. The water peculation experimental set up is shown on plate 3.

The results of the inoculation of the yeast isolate on formulated medium using 1% engine oil showed that the yeast cells grow on the medium supplemented with engine oil as the sole carbon source. The yeast cells grow faster on the medium without engine oil which served as control. The initiation of growth on the engine oil Agar took a longer time of 48 hours before there was growth. After then, the growth flourished. The results are presented on plate 2. Plate 1; shows the photomicrograph of the yeast species on conventional Potato Dextrose Agar medium.

Soil	pH	% Moisture Content	% Organic Content	Colour	Texture
Sample A	5.22	9.75	22.2	Dark brown	Coarse
Sample B	5.28	16.80	11.4	Light brown	Fine

Table 1: Ecological Parameters of the Soil Samples.

Soil	Vol. of Water Provided (ml)	Average Amount of water Drained (ml)	Average Amount of water retained (ml)	Average time for water to Drain (min)	Water Percolation Rate (sec)
Sample A	200	185.45	14.55	25	7.5
Sample B	200	190	10	26	7.8
Control	200	170	30	40	12

Table 2: Water Percolation Rate of the Soil Samples.

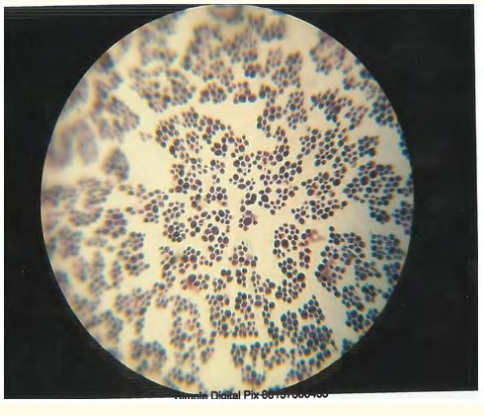

Plate 1: Photomicrograph of Yeast Cells on Potato Dextrose Agar.

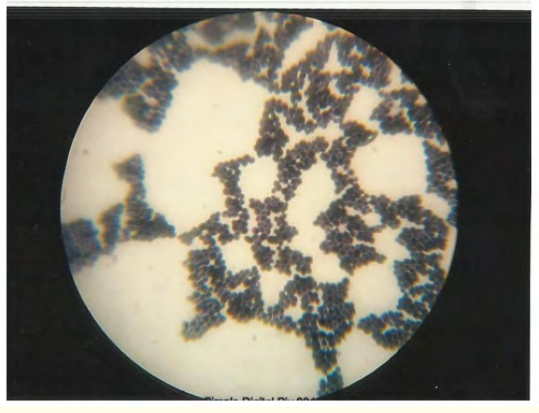

Plate 2: Photomicrograph of Yeast on Engine Oil Agar.

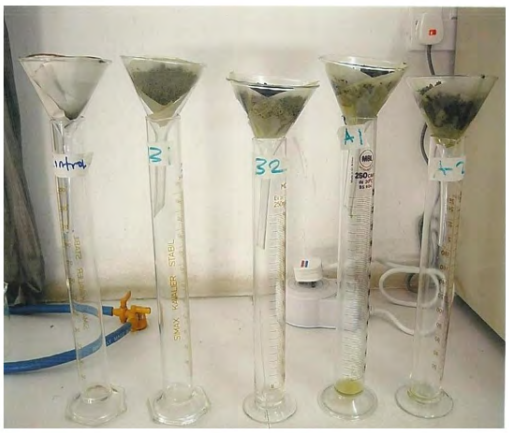

Plate 3: Rate of Water Percolation.

Discussion

The results obtained from the study showed that the yeast isolates AB – 01 survived in the soils with engine oil and was capable of degrading petroleum hydrocarbons oil treatment. This was seen from the result of the water peculation rate of the soils as the soils treated with oil was found to drain water (190 ml) more than the control soils (170 ml). The soils after treatment with the engine oil were incubated for one week to allow the yeast cells to hydrolyze the hydrocarbons of the engine oil if possible. This could be seen as had helped in the bioremediation of the polluted soil samples. The air spaces in the soils that could have ordinarily been clogged by the oil were somehow open and thus must have helped in the percolation of the water when it was introduced.

Results presented in this study showed that there was utilization of hydrocarbon of the unused engine oil by the yeast isolate AB -01, when inoculated on the medium formulated with 1% engine oil. However, the rate of growth varied with that of the yeast when grown on a conventional potato Dextrose Agar within 24 hours but the growth on the newly formulated medium with 1% engine oil was slow. In fact growth initiated after 24 hours but by 48 hours there was increase in growth which with time was luxuriant. This might be due to the fact that the yeast isolate AB-01 was able to use the hydrocarbons as substrates for growth by probably synthesizing and releasing extra cellular enzymes (lipases) and acid which are capable of breaking down and dismantling the long chains of hydrogen and carbon, thereby converting petroleum into simpler forms that were adsorbed by the yeast cells. This probably could have caused the delay in the initiation of growth of the yeast cells.

[12,13] reported that organisms' breakdown hydrocarbons and use the energy to synthesize cellular components. The pH, moisture content and percentage organic content of the soil samples used in the study were found to be within the range that supports microbial growth in culture. The results of ecological parameters as shown in table 1 recorded the pH of the soil samples to be acidic (5.52 and 5.28) which supports the growth of yeast cells in culture.

The aim of this work was to use yeast isolates to remedy oil pollution of soils. The yeast isolates, AB – 01 from the present study could be very useful in the management of crude oil spill. Such species could be made readily available and quickly applied on environments that are polluted with crude oil for ease of remediation. There were considerable changes in the experimental soil colour after the crude oil treatments. It was observed that after 2 days, the soil sample treated with engine oil appeared very dark in colour, but after one week the dark colour reduced in opacity, showing that some activities have taken place in the soil during the incubation period. [10] reported that oil spills are extremely harmful to the environment and threaten human and animal health. They affect water fowl, fisheries, the marine food web, marine; animals and can seep into ground water and make them undrinkable for years. Exposure to low concentrations of petroleum hydrocarbons can cause headaches, skin irritations, itch eyes and burning sensations in internal organs. Prolonged exposure to high concentrations of oil can cause liver and kidney diseases, increased risk of cancer and can damage bone marrow. Bioremediation is the use of living organisms to breakdown environmental pollutants in soil and water. These pollutants are persistent chemical compound that are poisonous to life, which can be broken down to carbon dioxide and water by bioremediation. Microorganisms decompose organic compounds using enzymes and protein based molecules that control metabolism in all living cells. Sources of bioremediation depends on the microorganisms used, supply utilization rate and the rate of oxygen transfer. Moisture, temperature also affects the rate of bioremediation. It has been establishes that bioremediation is an effective process for the degradation of oil into harmless products [10].

The significance of the result obtained in this study is that the yeast isolates that survival the crude oil must be those that are

equipped with special features for survival in extreme environment.

This means that yeast isolates are able to survive water stress. It has been suggested that under certain condition of water stress, energy is diverted from growth to osmo-regulation [5]. It has also been hypothesized that intracellular biochemical activities and enzyme reactions are inhabited by low internal water potentials [7].

The ability of the yeast isolates to utilize carbon source means that such yeast are equipped with the necessary enzymes needed for the breakdown of crude oil spill.

Conclusion

Further genetic manipulations can be carried out on such yeast in order to increase their crude oil spill remediation abilities. Such yeast can be massively produced and their propagules stored in the forms that can easily be used. Such yeast propagules could then be sprayed on environments (terrestrial or aquatic) that are polluted with crude oil for remediation purposes.

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