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Bioremediation of Crude oil Contaminated Marshland Muddy Soil By Bioaugmentation Approach Using Candida Tropicalis And Penicillium Chrysogenum

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Abstract: *Bioremediation of crude oil contaminated Marshland muddy soil by bioaugmentation approach using two fungal species Candida tropicalis and Penicillium chrysogenum were evaluated. Penicillium chrysogenum and Candida tropicalis were used to augment the indigenous microorganisms present in the muddy soil to speed up the degradation rate for a period of 28days, sampling and analysis were carried out at weekly interval (1, 7, 14, 21, and 28 days). The indigenous fungi isolates from the soil were identified to be of the following genera, Mucor sp., Penicillium sp., Aspergillus sp.,Candida sp., and Fusarium sp. while the hydrocarbon utilizing bacteria were also identify to be Bacillus, Pseudomonas, Micrococcus, Staphylococcus, Lactobacillus and Klebsiella. During the bioremediation process, it was observed that the augmenting organisms used were able to degrade the petroleum hydrocarbon in the soil. Four batches were setup; Control (contaminated mud without augmenting microbes), Contaminated mud with Penicillium Chrysogenum, Contaminated mud with Candida tropicalis, Contaminated mud with Penicillium Chrysogenum with Candida tropicalis The initial concentration of Total Hydrocarbon Content (THC) of crude oil marsh mud samples setup at day 1 was 938mg/kg; while on the day 28, the residual value were as follows: Contaminated mud with Penicillium Chrysogenum with Candida tropicalis (148mg/kg) < Contaminated mud with Candida tropicalis (247mg/kg) < Contaminated mud with Penicillium Chrysogenum (360mg/kg) < Control (646mg/kg). The percentage bioremediation rates of the fungal species were as follows: Contaminated mud with Penicillium Chrysogenum with Candida tropicalis (84.22%) > Contaminated mud with Candida tropicalis (70.79%) < Contaminated mud with Penicillium Chrysogenum (61.62%) < Control (31.13%).. Comparatively, the mixed consortium of Candida tropicalis with Penicillium Chrysogenum express higher bioremediation potential; while in relation to individual organism bioaugmenting potential Candida tropicalis is higher than Penicillium chrysogenum. This study therefore reveals that Pencillium chrysogenium and Candida tropicalis can be used in treatment of contaminated marshland muddy soil thereby minimizing the adverse environmental risks and human health hazards associated with the toxic effects of this petroleum hydrocarbon in marshland ecosystem.*

Keywords*: Bioaugmentation, crude oil, Marshland muddy soil, Candida tropicalis, Penicillium chrysogenum*
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I. Introduction

The refining of crude oil as well as their inappropriate use, improper disposal and accidental leakage has resulted in contamination of many areas. Depending on their concentrations, these substances have caused destructive consequences on ecosystems, as well as cause severe damage to humans and other organisms nearby. Soil pollution is of special importance because of its impact on surface, groundwater and air contamination and can easily spread and be consumed by humans. There are many methods for the removal of these pollutants from soils. They involve both physico-chemical and biological approaches. Although the first ones are more effective than biological methods, they are expensive and requires high energy demand and consumption of many chemical reagents (Hamali et al, 2007) with the resultant environmental hazards. This is the reason why the use of microorganisms capable of converting contaminants to harmless products by mineralization, generation of carbon(iv)oxide and water or by conversion into microbial mass by exploiting its diverse metabolic abilities known as bioremediation has become an attractive technology (Obire and Ramesh, 2009, Nrior and Echezeolom, 2016). One of the insitu bioremediation is bioaugmentation, which improve biodegradative capacities of contaminated sites by introduction of single strains or consortia of microorganisms with desired catalytic capabilities.

The term Bioaugmentation means the addition of exogenous microorganisms to polluted hazardous waste sites in order to accelerate the removal of undesired compounds (Ruberto et al., 2003). There is a growing public concern as a wide variety of toxic organic chemicals are being introduced inadvertently or deliberately into the environment. Petroleum hydrocarbons are one of the common examples of these chemicals, which enters the environment frequently and in large volume through numerous pathways (Bento et al., 2005). Oil contamination has become a global problem in industrialized and developing countries. It is one of the most dangerous pollution factors known today. It has cause a threat to our environment today by imposing a serious health hazard to human health, causes economic loss and decrease in Agricultural productivity on soil (Tebyanian et al, 2013). Some types of microorganisms are able to degrade petroleum hydrocarbon and use them as source of carbon and energy. Microbial degradation has emerged as the most significant natural mechanism for the removal of non volatile hydrocarbon pollutants from the environment. Although biodegradation occurs at distressing slow rate, it can be enhanced by inoculation with microbial species (bioaugmentation) that will degrade the oil waste more efficiently and/or by introducing air or nutrients into the environment (Obire and Ramesh, 2009).Oil degrading microorganisms are abundant and are not limited to oil producing areas, but are present in conceivable environment. Filamentous fungi, yeasts, actinomycetes and bacteria all have the ability to utilize hydrocarbon substrates, though their ability to do so vary among individual strains and in some cases depends on hydrocarbon chain length (Rowell, 1977; Walker et al, 1973). For instance Bactria and yeast showed decreasing abilities to degrade alkanes with increasing chain length. Filamentous fungi did not exhibit preferential degradation for particular chain lengths (Walker et al, 1973).

Fungi can possess all three traits, are found in oil-contaminated environments, and are known to degrade hydrocarbons (Leitao, 2009). Further, the ease of transportation, genetic engineering, and scaling-up makes fungi the organisms of choice in bioremediation. In a taxonomic study of fungi Bennet et al. (2002) found that hydrocarbon assimilation is most common in the orders Mucorales and Monilales, as well as in the genera Aspergillus and Penicillium. Furthermore, in comparison with eight other genera, Aspergillus and Penicillium species were the most efficient metabolizers of hydrocarbons (Obire et al., 2008). Hydrocarbon assimilation was, however, relatively rare, and was a property of individual strains, not of species or higher taxonomy (Leitao, 2009). Nevertheless, diverse fungi have been isolated from oil-contaminated environments, and are shown to degrade hydrocarbons in the lab. As one striking example, yeast populations in a fresh water stream increased by several orders of magnitude in the five days after an oil spill (Machado, et al., 2009).

In addition to degrading hydrocarbons directly, fungal mycelia can penetrate oil, thereby increasing the surface area available for biodegradation and bacterial attack. Fungi can also grow under environmentally stressed conditions such as low pH and poor nutrient status, where bacteria growth might be limited (Davis and Westlake, 1979). It was also reported that although bacteria initiated the degradation of a synthetic petroleum mixture, twice as much was degraded when both bacteria and fungi were present. This study reports the bioaugumention potential of two fungal species Candida tropicalis and Penicillium chrysogenum on crude oil contaminated marshland muddy soil.

II. Materials And Methods

Sample (Soil) Collection

The soil sample collected for this study was taken from Marshland muddy soil in Amadi-Ama, Port Harcourt. The soil sample was taken from the surface with the use of shovel into a sterile black polythene bag. The mouth of the polythene bag was not completely air tight so as to allow penetration of air for the survival of the natural occurring microorganisms.

Sources of Organisms

The desired organism for bioremediation of hydrocarbon polluted soil sample were collected in petri dishes. The organisms used were specifically Penicillium chrysogenum and yeast i.e. Candida tropicalis. The organism were cultured from the soil sample using a selective media for fungi (Sabouroud dextrose agar) followed by macroscopic and microscopic identifications.

Moisture Content analysis

This was carried out by removing 5g of contaminated soil from each of the set up and weighing it inside of a wash glass, then they were placed inside a hot air oven for drying. After drying, the soil was immediately transferred into desiccators for cooling. After cooling the soil was then reweighed and the new weight in grams gotten were then subtracted (minus) from the initial 5 grams of the soil to get the moisture content value.

Soil Preparation and Application of Organisms

The soil sample collected from the site was weighed 1kg each into four plastic containers for the set up. For a proper monitoring, there was a control which was without organism while others were augmented with organisms (Penicillium chrysogenum and Candida tropicalis) and labeled accordingly. Twenty milliliter (20ml) of crude oil was added to each of the four containers containing the soil, to contaminate it, then mixed properly using a sterile spatula. There after 50ml of the broth culture organism (Penicillium chrysogenum) to the seond set up, 50ml broth culture organism (Candida tropicalis) was added to the third set up and then 25ml of each of the two broth culture organism (ratio 1:1) was added to the fourth set up containing 1kg of polluted sample respectively.

During the setup process for spectrophotometric analysis, 10g of soil were weighed from each of the setup containers containing 1kg of contaminated muddy soil sample into a sterile conical flask and mixed with 20ml of xylene, shaken properly to extract the oil from the soil and the extracted oil was sieved with filter paper into test tube that was transferred into colorimeter curvette and placed in a chamber to determine the Total Hydrocarbon Content (THC) at 420nm wavelength. Some of the extracted soil had high concentration so they were diluted with 10^{-1} dilution ratio before analyzing.

Media Preparation

Nutrient Agar: It is a general purpose medium supporting the growth of wide range of non-fastidous organisms. Nutrient agar was used for the isolation of total heterotrophic bacteria with the manufacturer's description of 28grams into 1000ml of distilled water. Sabouroud Dextrose Agar:It is used for the isolation of fungi. The preparation of this media is 65g into 1000ml of distilled water, so you the manufacturers specification to calculate depending on the number of plate to be prepared. Oil Agar:This medium is prepared for the isolation of hydrocarbon utilizing bacteria. The medium was prepared with composition of K_2HPO_4 (0.5g), $MgSO_4$ (0.03g), $ZnCl_2$ (0.3g) and agar (15g) into 1 litre of distilled water. 1% of pure crude oil was added to the mixture and then autoclave at 121°C for 15 minutes.

Stock Solution

Ten percent glycerol solution was prepared dispensed in McCartney bottles and autoclaved at 121°C for l5minutes, allowed to cool, then the pure cultures were inoculated into each McCartney bottle, until the clear colourless solution turns turbid and were stored in the refrigerator. This served as storage medium for pure cultures for subsequent characterization.

Culturing and Isolation of Hydrocarbon Utilizing Bacteria

It was done using Oil Agar. Spread plate method were used and incubated for 5to 7 days and colonies were counted and were later subcultured into another plate to obtain pure cultures to be used for biochemical tests. The cultural, morphological and biochemical characteristics of the discrete bacterial isolates were compared with the recommendation in Bergey's manual of determinative bacteriology (1994). The morphological and biochemical test include; gram staining, motility, catalase, oxidase, citrate utilization, hydrogen sulphide production, indole production, methyl red and voges proskauer tests.

Culturing and, Isolation and Identification of Total Heterotrophic Bacteria

It was done using Nutrient Agar. Same spread plate method were used and incubated for 24hrs and colonies were counted and were later subcultured into plate to obtain pure culture to be used for biochemical tests. The cultural, morphological and biochemical characteristics of the discrete bacterial isolates were compared with the recommendation in Bergey's manual of determinative bacteriology (1994). The morphological and biochemical test include; gram staining, motility, catalase, oxidase, citrate utilization, hydrogen sulphide production, indole production, methyl red and voges proskauer tests.

Culturing, Characterization, Isolation and Identification of Total Fungi

It was done using Sabouroud Dextrose Agar, same spread plate method were used, pure cultures of fungi were obtained by subculturing discrete colonies into fleshy prepared Sabouroud Dextrose Agar plates and incubated at 28°C for 2-3days. The Isolates which developed were stored in the refrigerator for subsequent characterization tests. The presence or absence of septa in the mycelium, type of spore, presence of primary or secondary sterigmata, and other microscopic characteristics as well as cultural characteristics were used in the identification of the fungal isolates of the biodegradation flask set up (Cheesbrough, 2006).

Percentage (%) Bioremediation analysis

The percentage (%) bioremediation rate is calculated from the la used by Nrior and Echezolom (2016) as follows; **Step 1**: Amount of pollutant remediated equals to Initial concentration of pollutant (Day 0 or 1) minus

Final concentration of pollutant at end of experiment (Last day). **Step2**: Percentage (%) Bioremediation equals to Amount of pollutant remediated divided by Initial concentration of pollutant (Day 0 or 1) multiplied by 100.

$\mathbf{B_C = I_C - F_C}$
Where;
B_C = Amount of pollutant remediated
I_C = Initial concentration of pollutant (Day 0 or 1)
F_C = Final concentration of pollutant at end of experiment (Last day)

$$\textbf{\% Bioremediation} = \frac{B_C \times 100}{I_C}$$

III. Results And Discussion

The moisture content result in Fig. 1, shows the differences in the moisture content of the different experimental set-up, indicating the mixed consortium; Contaminated soil + Penicillium chrysogenum + Candida tropicalis (CS+PC+CT)(1.5g/5g) having the highest moisture content, followed by single organism application; Contaminated soil + Penicillium chrysogenum (CS+CT)(1.3g/5g) = Contaminated soil + Candida tropicalis (1.3g/5g), while Control (contaminated soil without added organism CTRL)(0.07g/5g) has the lowest. Nrior and Echezolom, (2016) report similar observation on the effect of moisture content on bioremediation potential of bio-stimulating agents. Alternatively, this study revealed the effects of different types of augmenting organisms and crude oil on the moisture content of the affected soil. The high moisture content observed in the mixed consortium Contaminated soil + Penicillium chrysogenum + Candida tropicalis (CS+PC+CT) could be due to its inherent moisture retention ability of the augmenting organisms while the control devoid of added organisms has least moisture content. These attributes (high moisture content) enhances the growth of microorganisms up to day 28 which was evident in their higher percentage bioremediation Fig. 2-5

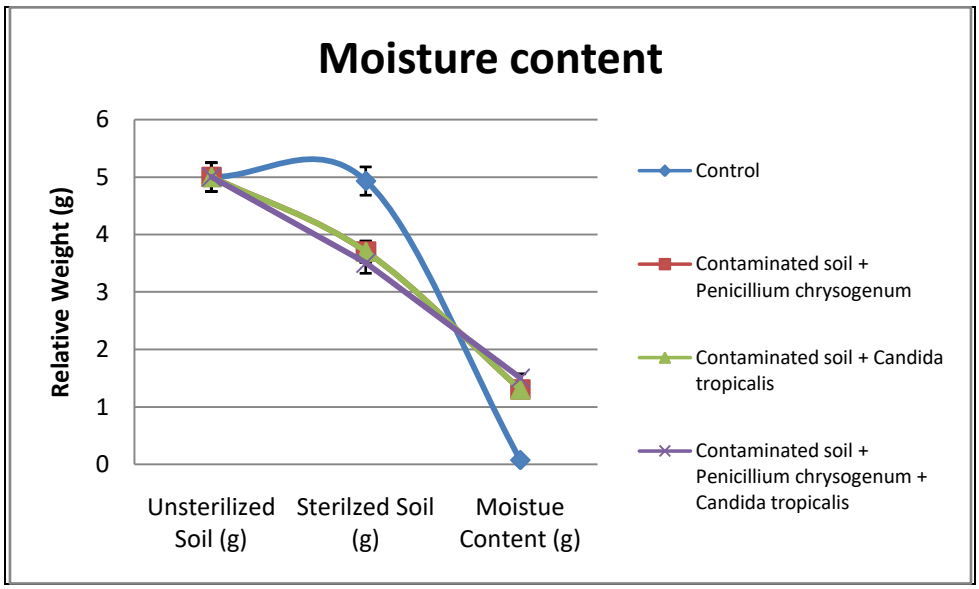

Fig. 1: Moisture Content Of Experimental Setup Batches

The analysis carried out to perform bioaugmentation potential of Marshland Crude Oil Spill Site using Penicillium chrysogerum and Candidia tropicalis to augment indigenous microorganism found in the soil, revealed that this organisms helped in bioremediation rate as well as reducing the contaminant caused by crude oil in the soil with time. The analyses were done on weekly interval; Day 1, 7, 14, 21and 28 as shown in Fig. 2 revealing the potentiality of how the organisms were able to degrade the petroleum hydrocarbon in the pollution soil. The total hydrocarbon content (THC) degradation was determined by the decrease in amount from 938mg/kg day 1 to Contaminated soil + Penicillium chrysogenum CS+PC (360mg/kg), Contaminated soil + Candida tropicalis CS+CT (274mg/kg), Contaminated soil + Penicillium chrysogenum + Candida tropicalis CS+PC+CT (148mg/kg) and Control[Contaminated soil without added organisms]CTRL (646mg/kg) in day 28 (Fig. 2).

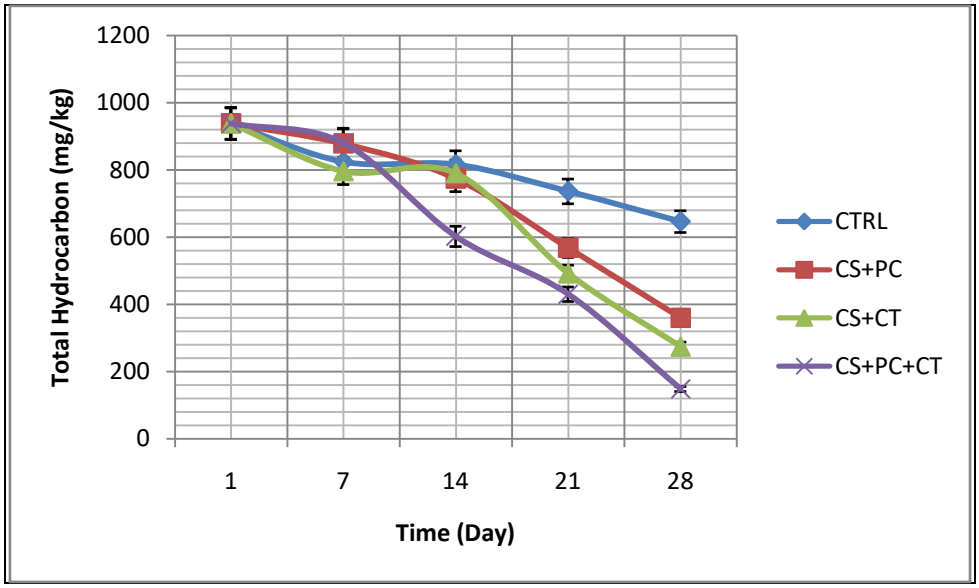

Fig. 2: Total Hydrocarbon Content (THC – mg/kg) of bioremediation of marshland crude oil spill site using Penicillium chrysogenum and Candida tropicalis.

(CTRL = Control[Contaminated soil without added organisms, CS+PC = Contaminated soil + Penicillium chrysogenum, CS+CT = Contaminated soil + Candida tropicalis, CS+PC+CT = Contaminated soil + Penicillium chrysogenum + Candida tropicalis)

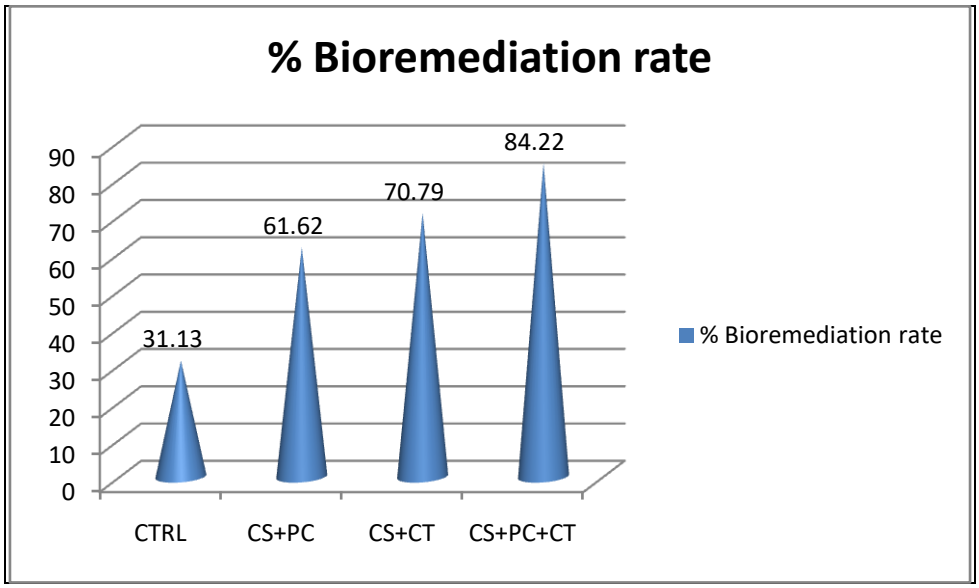

Fig. 3: Percentage (%) Bioremediation rate of Petroleum hydrocarbon on muddy marshland crude oil spill site using Penicillium chrysogenum and Candida tropicalis.

(CTRL = Control[Contaminated soil without added organisms, CS+PC = Contaminated soil + Penicillium chrysogenum, CS+CT = Contaminated soil + Candida tropicalis, CS+PC+CT = Contaminated soil + Penicillium chrysogenum + Candida tropicalis)

The degradation rates for the percentage of control contaminated soil were as follows: Contaminated soil + Pencillium chysogenium + Candida tropicalis CS+PC+CT (84.22%) > Contaminated soil + Candida tropicalis CS+PC (70.79%) > Contaminated soil + Pencillium chysogenium CS+PC (61.62%) > Control CTRL (31.13%). It was observed as contaminated soil plus Pencillium chysogenium with Candida tropicalis degraded faster with the rate of 148mg/kg. It was also observed that the hydrocarbon utilizing bacteria count decreases faster than the heterotrophic bacteria due to the competition by the bacteria population in the soil. This reduction in hydrocarbon utilizing bacteria count may be due to depreciation in nutrient quality in soil hence, petroleum

hydrocarbon degradation, so its count was reduced from 10^8 to 10^4. Petroleum utilizing fungi is been observed to be more potentially useful for bioaugmentation than bacteria because they can grow in stress conditions that bacteria cannot resist due to low disposition to nutrients (Nrior and Mene, 2017), so its count was observed to be higher 10^{-8} to 10^{-4} than hydrocarbon utilizing bacteria 10^{-7} – 10^{-3}(Fig. 4-5).

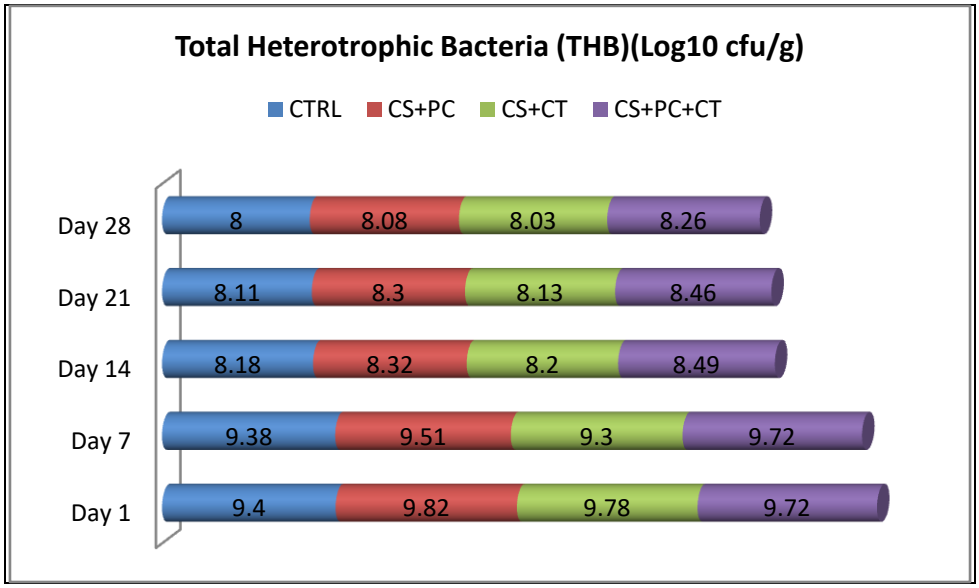

Fig. 3: Total Heterotrophic Bacteria (THB – log10 cfu/g) during bioremediation of marshland crude oil spill site using Penicillium chrysogenum and Candida tropicalis.

(CTRL = Control[Contaminated soil without added organisms, CS+PC = Contaminated soil + Penicillium chrysogenum, CS+CT = Contaminated soil + Candida tropicalis, CS+PC+CT = Contaminated soil + Penicillium chrysogenum + Candida tropicalis)

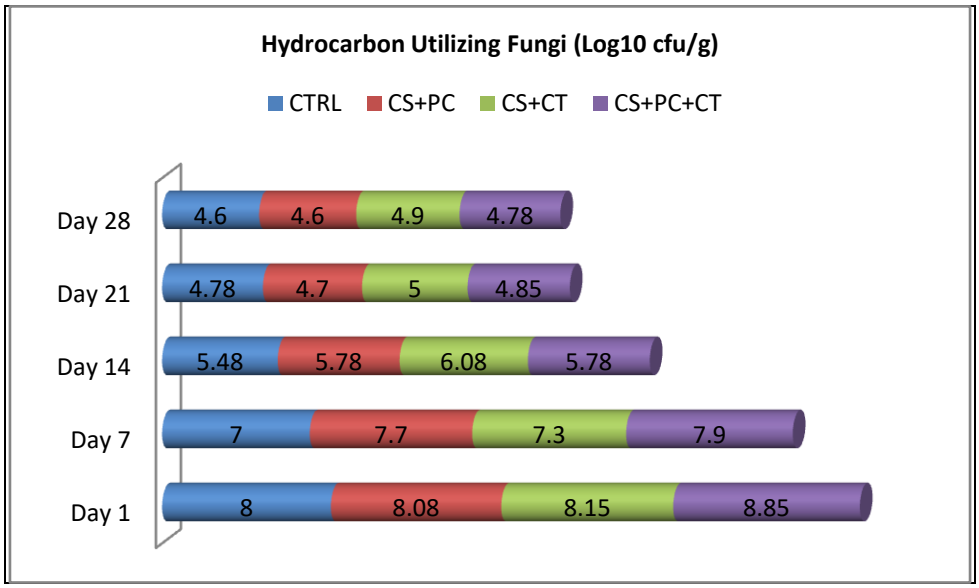

Fig. 4: Hydrocarbon Utilizing Fungi (HUF – log10 cfu/g) during bioremediation of marshland crude oil spill site using Penicillium chrysogenum and Candida tropicalis.

(CTRL = Control[Contaminated soil without added organisms, CS+PC = Contaminated soil + Penicillium chrysogenum, CS+CT = Contaminated soil + Candida tropicalis, CS+PC+CT = Contaminated soil + Penicillium chrysogenum + Candida tropicalis)

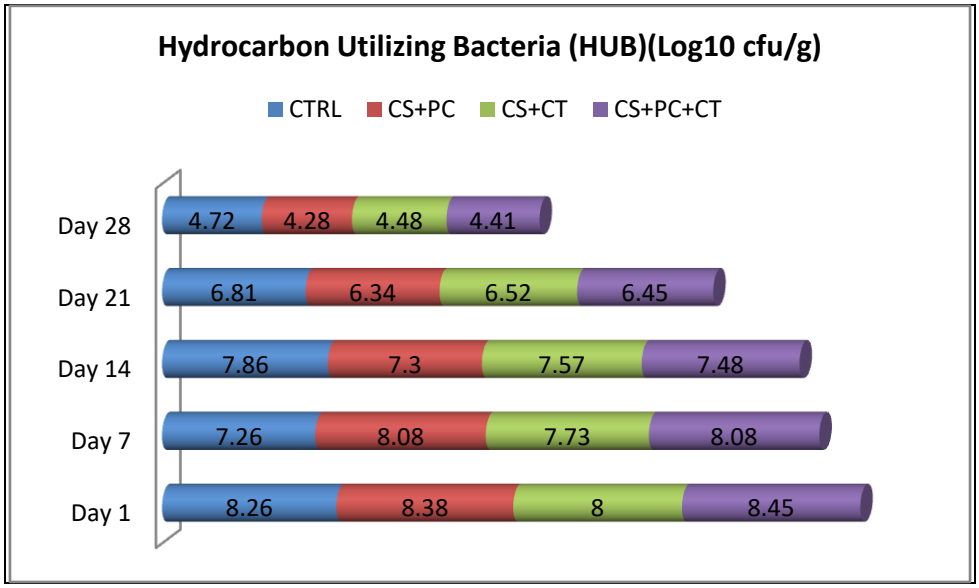

Fig. 5: Hydrocarbon Utilizing Bacteria (HUB – log10 cfu/g) during bioremediation of marshland crude oil spill site using Penicillium chrysogenum and Candida tropicalis.

(CTRL = Control[Contaminated soil without added organisms, CS+PC = Contaminated soil + Penicillium chrysogenum, CS+CT = Contaminated soil + Candida tropicalis, CS+PC+CT = Contaminated soil + Penicillium chrysogenum + Candida tropicalis)

Total heterotrophic bacteria were highly populated in the soil with the count of 10^{-9} to 10^{-8} cfu/g throughout. This is because bacteria have the ability to take part in remediation by so doing utilize hydrocarbons as a sole source of carbon and energy and hence they survive since fungi are the primary degraders and has already started the degradation process. The THC content at the first day was relatively constant as mentioned earlier. The contaminated soil + Candida tropicalis degradation rate is said to be 274mg/kg and it is said to degrade faster than contaminated soil + Pencillium chysogenium which is about 360mg/kg. This indicates that Candida tropicalis are potential organism for bioremediation hydrocarbon polluted soil than Pencillium chysogenium. The hydrocarbon utilizing bacteria in Fig. 5 was identified to be of the following genera; Bacillus, Klebsiella, Micrococcus, Pseudomonas, Athrobacter, Lactobacter, Staphylococcus which were somewhat similar to report from other researchers such as Seojs, et al., (2009).

The microbial characterization + fungi isolates found during the bioremediation process was identified based on their morphological and microscopical appearances. They include: Penicillium, Candida, Mucor, Aspergillus (table 4.7). Result of this research showed that there is a decrease in the amount of crude oil with the presence of fungal strains considerably. It therefore means that fungal strains were able to degrade crude oil and mixed two fungal isolated exhibited decreases in biodegradation of crude oil. This work might help in bioremediation studies to clean up Marshland crude oil spill site in an affected environment by the use of yeast strain and fungi with desirable degradative activities. Bioremediation is expected to play an important role as environmentally safe and cost-effective response to Marshland oil spill.

IV. Conclusion And Recommendation

The most important step in successful bioaugmentation is the selection of proper microbial strains. The most effective elimination of contaminants may be achieved by using microbial inoculants isolated from environments where contamination had occurred. The success of bioaugmentation strongly depends on the ability of the inoculants to survive in contaminated soil, cause the activities to microorganisms are very important for the renewal of our environment and maintenance of the ecosystem.

However, in most cases, this statement concerns potential degradabilities which were estimated in the laboratory by using selected cultures and under ideal growth conditions. Due to a whole range of factors, composition with microorganism, insufficient supply with essential substrates and unfavarouable external conditions (aeration, moisture, pH, temperature). So, the environmental microbiologist came up with a way to solving this problem by supporting the activities of indigenous microorganism in polluted biotopes by enhancing their degradative abilities by bioaugmentation or biostimulation, so this will help to make a difference to reduce waste, eliminate industrial pollution for us to enjoy a more sustainable future.

It is therefore recommended that since microorganisms takes a proactive activity in the degradation rate of hydrocarbon pollutant, it should be use for the cleaning up of our polluted environment or soil so as to avoid the toxic effect of this hydrocarbons on our crops, fishes and aquatic environment.

References

[1]. Barahi, S. and N. Vasudevan (2001). Utilization of Petroleum Hydrocarbons by Pseudomonas Fluorescence Isolated from a Petroleum-Contaminated Soil. (26); 413-416.

[2]. Bennet, S.W.; Wunch, K.G. and Fashion, B.D. (2002). Use of Fungi Biodegradation Manual of Environmental Microbiology (2nd Edition), ASIN Press, Washington DC; 960-971.

[3]. Chaineau, C.H.; G. Rougeux; C. Yepremian and S. Outdot (2005). Effects of Nutrient Concentration on Biodegradation of Crude Oil and Associated Microbial Population in the Soil. (37): 1490-1497.

[4]. F.M. Bento, F.A. Ocamargo, B.C. Okeke, W.T. Frankenburger (2005). Comparative Bioremediation of Soils Contaminated with Diesel Oil by Natural Attenuation, Biostimulation and Bioaugmentation.

[5]. Ghazali, F.M.; R.N.Z.A. Rahman, A.B. Salleh and M. Basri (2004). Biodegradation of Hydrocarbons in Soil by Microbial Consortium. (54): 61-67.

[6]. H. Hamdi, S. Benzarti, L. Manusadzianas, I. Aoyama, N. Jedidi (2007). Bioaugmentation and Biostimulation Effect on PAH Dissipation and Soil Ecotoxicity under Controlled Conditions. (39): 1926-1935.

[7]. Jagaderan, S. and Mukhergi, S. (2004). Successful In Situ Oil Bioremediation Programmes. Journal of Biotechnology. 3:495-501.

[8]. Kim, S.; D.H. Choi; D.J. Sim and Y. Oh (2005). Evaluation of Bioremediation Effectiveness on Crude Oil Contaminated Sand. (59); 845-852.

[9]. L. Ruberto, S.C. Vazquez, W.P.M. Cormack (2003). Effectiveness of the Natural Bacterial Flora, Biostimulation and Bioaugmentation on the Bioremediation of Hydrocarbon Contaminated Soil. (52): 115-125.

[10]. Leitao, A.L. (2009). Potential of Penicillium Species in Bioremediation Field. International Journal of Environmental Research. (6):278-309.

[11]. Mrozik, A.; Piotrowska-Seget, Z. and Labuzek, S. (2003). Bacterial Degradation and Bioremediation of Polycyclic Aromatic Hydrocarbons. Journal of Environmental Studies. (12):15-25.

[12]. Obire, O. and Ramesh, R.P. (2009). Fungi in Bioremediation of Oil Polluted Environments.

[13]. Odu, C.T.I. (1978). The Effect of Nutrient Application and Aeration in Oil Degradation in Soils. Environmental Pollution. 15:235-240.

[14]. Okoh, A.I. (2002). Assessment of the Potentials of some Bacterial Isolates for Application in the Bioremediation of Petroleum Hydrocarbon Polluted Soil. Ph.D Thesis. Obafemi Awolowo University, Ile-Ife, Nigeria.

[15]. Pathak, H.; P.K. Jain; D.P. Jaroli and M. Lowry (2008). Degradation of Phenanthene and Anthracene by Pseudomonas Strain, Isolated from Coastal Area. (12):111-116.

[16]. R.I. Dams, G. Paton, K. Killham (2007). Bioaugmentation of Pentachlorophenol in Soil and Hydroponic System. (60); 171-177.

[17]. Seo, J.S.; Keum, Y.S. and Li Qx (2009). Bacterial Degradation of Aromatic Compounds. International Journal of Environmental Research. Public Health. (6); 278-309.

[18]. Walker, J.D.; Cofone and Cooney, J.I. (1973). Microbial Petroleum Degradation. The Role of Cladosponium Resinae on Prevention and Control of Oil Spills.

[19]. Walker, J.D.; Petrakis, L.; Colwell, R.R. (1978). Degradation of Petroleum by Pure Cultures of Bacteria, Algae, Yeast and Filamentous Fungi. (30); 79-81.

[20]. Wolfing, K.O. (2017). Biodegradation: Aerobic and Anaerobic. Journal of Applied Microbiology (22): pp.188-210.

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