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Original Research Article

Bioremediation of Bonny light crude oil polluted soil by bioaugmentation using yeast isolates (*Candida adriatica* ZIM 2468 and *Candida taoyuanica* MYA-4700)

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The elimination of a wide range of pollutants and wastes from the environment is an absolute requirement to promote a sustainable development of our society with low environmental impact. The focus of this study is to remediate, monitor the progress of bioaugmentation in a hydrocarbon polluted soil using yeast isolates and to highlight the needs for integration of laboratory data to full scale *in situ* bioremediation. In this study, yeast isolates were used to bioaugment Bonny light crude oil polluted soil in Niger Delta. Yeast isolates used were *Candida adriatica* ZIM 2468 and *Candida taoyuanica* MYA-4700. The indigenous fungal isolates identified from the soil were species of *Alternaria*, *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus*, *Trichoderma*, *Candida*, *Rhodotorula* and *Saccharomyces*. One kilogram of fresh soil sample was polluted with crude oil (10%). Physico-chemical analysis was carried on the fresh soil before and after pollution. *Candida adriatica* ZIM 2468, *Candida taoyuanica* MYA-4700 and a consortium containing both isolates were inoculated into the different microcosms to increase the number of microorganisms. At every fourteen days, samples were analyzed for total petroleum hydrocarbon (TPH) and microbial counts were carried out. Total petroleum losses of 84.6% (A - consortium of *Candida adriatica* ZIM 2468 and *Candida taoyuanica* MYA-4700), 77.3% (B - *Candida adriatica* ZIM 2468), 73.4% (C - *Candida taoyuanica* MYA-4700) and 28.7% (Control – unamended) were recorded in the bioaugmentation set-up, respectively at day 56. On the whole, amending with both *Candida* species proved to be more effective in hydrocarbon utilization.

Keywords: Bioaugmentation, hydrocarbons, yeast species, Niger Delta.

INTRODUCTION

Soils which are contaminated by hydrocarbons have extensive damage of local ecosystems since accumulation of pollutants in animals and plants tissue may cause progeny's death or mutation. The toxic components of the petroleum convert arable land to barren soils and destroy the aesthetic quality of the environment. Other environmental consequences of oil pollution include the adverse effects on the soil microflora and ground water contamination. These spills cause an imbalance of both habitat and the organisms that live there, these organisms' growth and reproduction patterns are altered (Liu et al., 2010).

Particularly, in Nigeria and Niger Delta oil pollution problems of both terrestrial and aquatic environments are very prevalent. Once an oil spill occurs, the oil will experience a series of physical, chemical and biological change, the effect of which is overall defined as "weathering". However, this process happens slowly in the natural environment and requires a long time

for the contaminated environment to recover (Thapa et al., 2012).

Soil bioremediation is the process in which most of the organic pollutants are decomposed by soil microorganisms and converted to harmless products such as such as carbon dioxide, water and biomass. This approach makes use of indigenous oil consuming microorganisms (Ahmad et al., 2015). However, some isolated microorganisms can effectively degrade single pollutant in laboratory conditions but when introduced into actual field conditions with multiple pollutants they cease to function as anticipated, hence, the need for bioaugmentation (Alonso-Gutierrez et al., 2011).

According to Thapa et al. (2012), bioaugmentation is the introduction of highly effective oil degrading microbes from natural population or from the test tube of genetically engineered microorganism to a polluted area. Data from several field demonstrations of bioaugmentation have been reported in peer-reviewed journals and these have greatly increased confidence in reliability of bioaugmentation as a credible technology. Bioaugmentation requires low cost, small amounts of added biomass, and do not require extreme manipulation of environmental conditions to allow introduced microorganisms to be effective (Nrior and Onwuka, 2017). There are several genera of oil degrading microorganisms including bacteria, yeasts or molds. The following genera of yeasts have been described as being able to utilize hydrocarbons: *Candida*, *Clavispora*, *Trichosporon, Rhodotorula, Sporidiobolus, Sporobolomyces, Stephanoascus, Debaryomyces, Leucosporiddium, Lodderromyces, Metschnikowia, Rhodosporidium, Pichia* and *Yarrowia* (Csutak et al., 2010).

The elimination of pollutants and wastes from the environment is an important requirement to promote a sustainable development with low or no environmental impact. Biological processes play a vital role in the removal of contaminants and take advantage of the metabolic versatility of microorganisms to convert them. Due to the physical and chemical nature of petroleum, these spillages adversely affect the soil chemical and microbiological properties; modification of ecosystem through species elimination, delay in flora succession and loss of mangrove. Microorganisms in these ecosystems may lack the ability or may not be in sufficient number to degrade the oil, hence there is need to remediate such ecosystems and one of such ways is through bioaugmentation.

The focus of this study is to remediate and monitor the progress of bioremediation/bioaugmentation in a hydrocarbon polluted soil using yeast isolates, also to highlight the needs for integration of laboratory data to full scale bioremediation.

MATERIALS AND METHODS

Soil characterization

Soil samples were collected randomly with an auger at a depth of 0-15cm from an agricultural farm in the University of Port Harcourt, Rivers State. Samples were homogenized, sieved through a 2mm sieve iron mesh to remove debris and stones and stored in black polythene bags at room temperature.

The soil was characterized two weeks before and after pollution with crude oil. Parameters included particle size, pH, conductivity, nitrate content, phosphate content, total organic carbon (TOC) and gas chromatography (GC) analysis of oil constituent. Particle size was determined with the hydrometer method. Available phosphorus, pH, electrical conductivity (EC), total organic carbon (TOC), and total nitrogen were determined according to the method of APHA (2008).

Soil microbial population was estimated by the ten-fold serial dilution. Total heterotrophic fungi were estimated using potato dextrose agar (Ataikiru et al., 2017). Petroleum hydrocarbon utilizing fungi was estimated using vapour phase transfer method (Okerentugba et al., 2016) with mineral salt medium.

Enumeration of total culturable fungi

Soil heterotrophic fungi were estimated by soil dilution plate count method using sterile physiological saline, i.e. 0.85 % (w/v) sodium chloride as diluent. Ten-fold serial dilution was carried out using 1g of the soil sample. A 0.1ml of the different dilution (ie 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}) of soil sample were plated in triplicates (Ataikiru et al., 2017). Total heterotrophic fungi were estimated using potato dextrose agar after incubation at 25 ± 2^0C for 24 hours. Petroleum hydrocarbon utilizing fungi was estimated using vapour phase transfer method of Okerentugba et al. (2016), with Bushnell Haas agar ($MgSO_4$ 0.2g, $CaCl_2$ 0.02g, KH_2PO_4 1g, K_2HPO_4 1g, $FeCl_2$ 0.05g, NH_4NO_3 1g and 15g agar agar/L, pH 7.2 ± 0.2). Inoculated plates were incubated at 25 ± 2°C and counts were taken at intervals of 5 - 7 days. Colonies which developed were counted, computed and recorded as heterotrophic fungi and petroleum utilizing fungi, respectively. The colonies counted were expressed as colony forming unit (cfu) per gram soil. Discrete colonies were sub cultured onto fresh medium for the development of pure cultures for characterization and identification. Fungal isolates from the fresh soil used in the study were identified following the scheme of Barnett et al. (1990) and Malloch, (1997).

Soil contamination

A modified method of Ezekoye et al. (2017) was utilized in polluting the soil. One kilogram (1kg) of soil portions were weighed into sterile plastic bowls sanitized with sodium hypochlorite (3.5%w/v). These were moistened to 60% of their field moisture capacity and left at room temperature 25 ± 2^0C in the laboratory for one week. Samples were polluted with 100ml of crude oil (10%) and left at same temperature for another two weeks.

Table 1: Physicochemical and microbiological properties of fresh soil used in the study

Parameters	Values
Total heterotrophic fungi	5.8×10^3cfu/ml
Hydrocarbon utilizing fungi	4.0×10^2cfu/ml
pH	5.04
Electrical conductivity	217.05µs/cm
Available phosphorus	0.034%
Total nitrogen	0.13%
Total organic carbon	1.64%
Moisture content	13.2%
Total petroleum hydrocarbon (TPH)	0.001mg/kg
Nitrate	0.575ppm
Phosphate	0.011ppm
Particle size distribution	
Sand	70%
Silt	16%
Clay	14%

Scale-up of test organisms for inoculation into biodegradation set-up

The method of Odokuma and Dickson (2003) was used in the inoculum size development. Sterile wire loop was used to scrap a 48 hour culture (*Candida adriatica* ZIM 2468, *Candida taoyuanica* MYA-4700 and a consortium of *Candida adriatica* ZIM 2468 and *Candida taoyuanica* MYA-4700) into a 20ml test tube containing 9ml of normal saline (0.85% NaCl), respectively. This suspension was transferred into 190ml Bacto Bushnell Haas broth containing 1ml crude oil in a 500ml conical flask. This mixture was agitated and incubated at 25 ± 2°C for 7days. One hundred and ninety milliliter of the above medium was subsequently transferred aseptically into a 3L conical flask containing 1800ml of Bacto Bushnell Haas broth and 10ml crude oil. Incubated at 25 ± 2°C for seven days and agitated daily for aeration. Yeast cells were harvested by centrifuging aliquots of the final culture using the centrifuge 80-2 (Techmel and Techmel, U.S.A) at 4000rpm (revolution per minute) for 20 minutes. The deposits were collected and weighed. The process (centrifugation) was repeated until the weight (wet) desired was achieved. The pellet was collected and suspended in 100ml sterile normal saline. This served as the inoculum source for biodegradation. The microbial load applied per microcosm was 5grams.

Amendment of soil sample

A 100ml of the suspended yeasts population was added to the different microcosms and mixed properly using sterile spatula. The set up was left for fifty six days. Unamended control was also set up. Microcosm A was amended with yeast consortium, microcosms B and C were amended with single yeasts population. Every week, 80ml of sterile deionised water was added to the microcosms and tilled for proper aeration and adequate distribution of microbes.

Microbiological analysis

Microbiological analysis was carried out every fourteen days. Soil samples were collected in a random manner and properly mixed together. Ten-fold serial dilution was carried out using 1g of polluted soil sample and 0.85% (w/v) sodium chloride as diluent. The spread plate method using a glass spreading rod that has been sterilized in flaming alcohol was used to inoculate 0.1ml aliquot of the different dilutions (ie 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}) on appropriate media in triplicates. Sabouraud dextrose agar was used for total heterotrophic fungal counts and mineral base medium for hydrocarbon utilizers. The vapour phase transfer of Okerentugba et al. (2016) was used for hydrocarbon utilizers.

Residual TPH analysis

Gas chromatography (GC) analysis of total petroleum hydrocarbon (TPH) was carried out at day 0, day 14, day 28, day 42 and day 56 respectively using the gas chromatography Varian 3400.

Statistical analysis

Statistical analysis was done using the staistical package for social sciences (SPSS, Version 17.0). Analysis of variance (ANOVA) was done at 95% level of confidence.

RESULTS

Physicochemical and microbiological characterization of the soil sample

Table 1 shows the physicochemical and microbiological characteristics of the fresh soil used in the study before pollution. The pH of the soil samples used in this study was slightly acidic which is favourable for the growth of fungal isolates. The particle size distribution allowed for proper aeration and drainage supporting the growth of the aerobes present thus, enhancing biodegradation.

Total culturable fungal counts during the bioremediation process

The value of total heterotrophic fungi (THF) in microcosms A, B and C were significantly different from the control ($P<0.05$) during the entire period of bioremediation as seen in Table 2. However, the value of total heterotrophic fungi increased in value as the bioremediation period increased and peaked at day 14, then started reducing in A, B and C; while the reverse in trend was the case in the control where there was a reduction in value from 5.21±0.06 – 4.20±0.12 as the bioremedation period increased.

Table 2. Total heterotrophic fungi (THF)

Days	A	B	C	Control
0	6.21±0.15^{a}	6.20±0.07^{a}	6.00±0.05^{a}	5.21±0.06^{b}
14	7.70±0.12^{a}	7.36±0.09^{b}	7.12±0.10^{b}	5.70±0.06^{c}
28	7.28±0.25^{ac}	6.95±0.08^{ab}	6.53±0.27^{bc}	5.18±0.09^{d}
42	7.07±0.18^{ab}	6.70±0.12^{ab}	6.40±0.23^{b}	4.61±0.17^{c}
56	6.70±0.12^{a}	6.33±0.23^{a}	6.37±0.18^{a}	4.20±0.12^{b}

NB: All values are expressed as Mean ± S.E. Similar letters at the superscript indicates no significant difference while different letters at the superscript indicates significant difference.

DISCUSSION

Bioremediation has often been viewed as a potent tool in the cleanup of oil polluted environment. It is economical, environmentally friendly and minimally disrupts site. According to Ataikiru et al. (2017), microorganisms are the main degraders of petroleum hydrocarbons in contaminated ecosystems.

In this study, heterotrophic fungal species belonging to a total of ten known genera and one unidentified fungus were isolated from the fresh soil sample. These included species of *Alternaria*, *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus* and *Trichoderma* which were moulds and *Candida*, *Rhodotorula* and *Saccharomyces* which were yeasts isolates. Of these moulds species; *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium* and *Trichoderma* and the yeast isolates belonging to the genera - *Candida*, *Saccharomyces* and *Rhodotorula* were petroleum-utilizers. Several researchers have reported similar findings in a related study (Akpoveta et al., 2011, Benal et al., 2014; Ataikiru et al., 2017; Nrior and Onwuka, 2017).

Monitoring the microorganisms within a contaminated site is the simplest approach for cleanup of hydrocarbons (Orji et al., 2013). The total heterotrophic fungal (THF) and hydrocarbon utilizing fungal (HUF) counts in the fresh soil were 5.8×10^3 cfu/g and 4.0×10^2cfu/g, respectively. The low proportion of hydrocarbon utilizers compared to the total heterotrophic population indicates that the soil ecosystem from which the samples were obtained probably had not been exposed to heavy and consistent crude oil pollution.

After amendment, total heterotrophic fungal and hydrocarbon utilizing fungal counts increased significantly, from 10^2 - 10^3cfu/g to 10^6 - 10^7 cfu/g within the first fourteen days. There was no lag phase thus, showing that the seeded microorganisms adapted rapidly to the hydrocarbon contamination and were able to utilize crude oil as the sole carbon source. According to Okerentugba et al. (2016), microbial communities exposed to hydrocarbons adapt to this exposure through selective enrichment and genetic changes resulting in an increase in hydrocarbon-degradation. This pre-exposure of microorganisms make them better suited to degrade the pollutant through higher growth, reproduction and more efficient metabolism thus maximizing the rate of hydrocarbon removal from the soil.

An immediate increase in the population density of the microorganisms could ensure the rapid degradation of the pollutants. The highest number of microorganisms was attained between 0-14 day of the remediation process. The total number of total heterotrophic fungi was 10^6– 10^7cfu/g (Figure 1) and hydrocarbon utilising fungi was 10^6 – 10^7cfu/g (Figure 2). Diaz-Ramirez et al (2013) in their study used native and exogenous microbial inocula from a Mexican soil during a laboratory study and reported that the number of viable counts increased with the boiaugmentation strains.

Oxygen is very essential for bioremediation of oil because most hydrocarbon degraders are aerobes. The initial steps in the catabolism of aliphatic, cyclic and aromatic hydrocarbons by bacteria and fungi involves oxidation by oxygenases, much molecular oxygen is required (Csutak et al., 2010). According to Adams et al. (2015) if there is no amendment to improve oxygen supply, then it is conceived that under microaerophilic or anoxic conditions the rate of oil removal will drop to zero, which is true from this study.

Bioaugmentation of the contaminated soil with aeration (tilling) and addition of water showed that changes in the abundance and diversity of microorganisms occurred from the first day to fourteenth day after treatment. Chikere (2012) reported the importance of agitation and homogenization during degradative studies increased rates of hydrocarbon losses. Both total culturable heterotrophic and hydrocarbon utilizing fungal counts increased appreciably during this period. Obire and Putheti (2009), reported that yeasts population in a fresh water stream increased by several orders of magnitude in five days after an oil spill.

There were decreases in both total heterotrophic fungal and hydrocarbon utilizing fungal counts as the day progressed in this study. The decline growth stage observed in the microcosms toward the end of incubation period may have been as a result of nutrient exhaustion and introduction of toxic metabolites. Obire and Nwaubeta (2002), in a related study on bacteria reported similar findings.

There has been confusion over the efficacy of bioaugmentation for remediation of polluted soil. Radwan

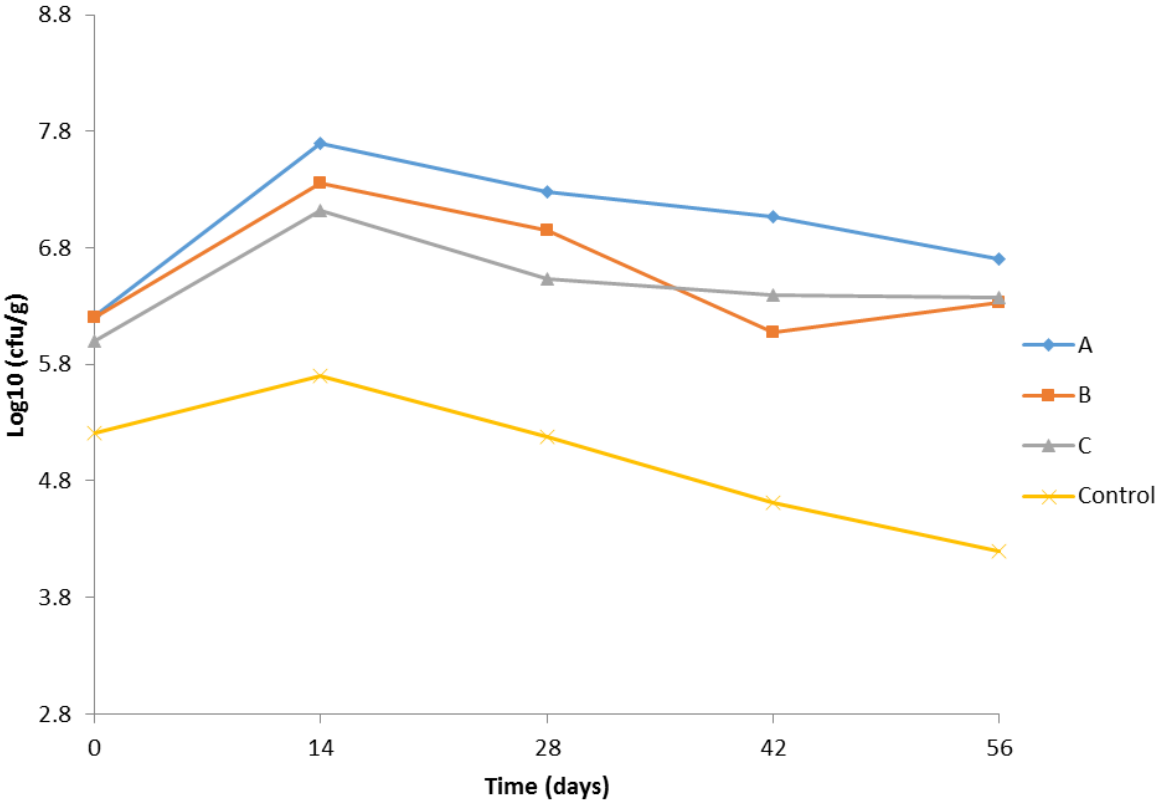

Figure 1: Total heterotrophic fungal counts during the bioremediation process

A: Microcosm amended with *Candida adriatica* and *Candida taoyuanica*
B: Microcosm amended with *Candida adriatica*
C: Microcosm amended with *Candida taoyuanica*
Control: Unamended soil

et al. (2007), described a feasibility study in which both exogenous and indigenous cultures were introduced into sand ores artificially contaminated with weathered crude oil. They concluded that in the case of terrestrial oil spill, management of environmental conditions to stimulate the natural indigenous microbial population was likely to produce better results than bioaugmentation, especially after the spill. They suggested that inability of introduced cultures to compete effectively with indigenous population was the reason for the failure. Also, Cosgrove et al. (2010), in a related study reported that bioaugmentation using fungi species to remediate environments contaminated with polyurethane waste was effective. An effective degradation of crude oil would require simultaneous action of several metabolically versatile microorganisms with favourable environmental conditions such as pH, temperature and availability of nutrients.

This study has shown that bioaugmentation using these yeast isolates was effective in the bioremediation of crude oil polluted soil. In addition to degrading hydrocarbons directly, fungal mycelia can penetrate oil, thus increasing the surface area available for biodegradation and bacterial attack (Obire and Putheti, 2009).

Results of the Gas chromatography (GC) analysis of the microcosms showed the degradative ability of *Candida adriatica* ZIM 2468 and *Candida taoyuanica* MYA 4700 (Figure 3). An increase in crude oil degradation corresponded to an increase in cell number during the degradation process demonstrating the isolates' ability of utilising crude oil. It has been reported by Joo et al. (2008), Obire et al. (2008), Obire and Putheti (2009), Csutak et al. (2010), Nrior and Onwuka (2017) in several studies that *Candida* species are potent hydrocarbon degraders.

Microcosm A (amended with the yeast consortium) showed the greatest decrease of the contaminants at day 14 (55.2% loss of TPH), at the early stages of the experiment. At the end of this study, microcosm A had the highest decrease of 84.6% while microcosms B (amended with *C. adriatica*) and C (*C. taoyuanica*) had decreases of 77.3% and 73.4%, respectively (Figure 4). Highest loss observed in the microcosm amended with the consortium could be attributed to the synergistic catabolic effect (cometabolism) of both isolates. Research has shown that there were decreases in total petroleum hydrocarbons and higher

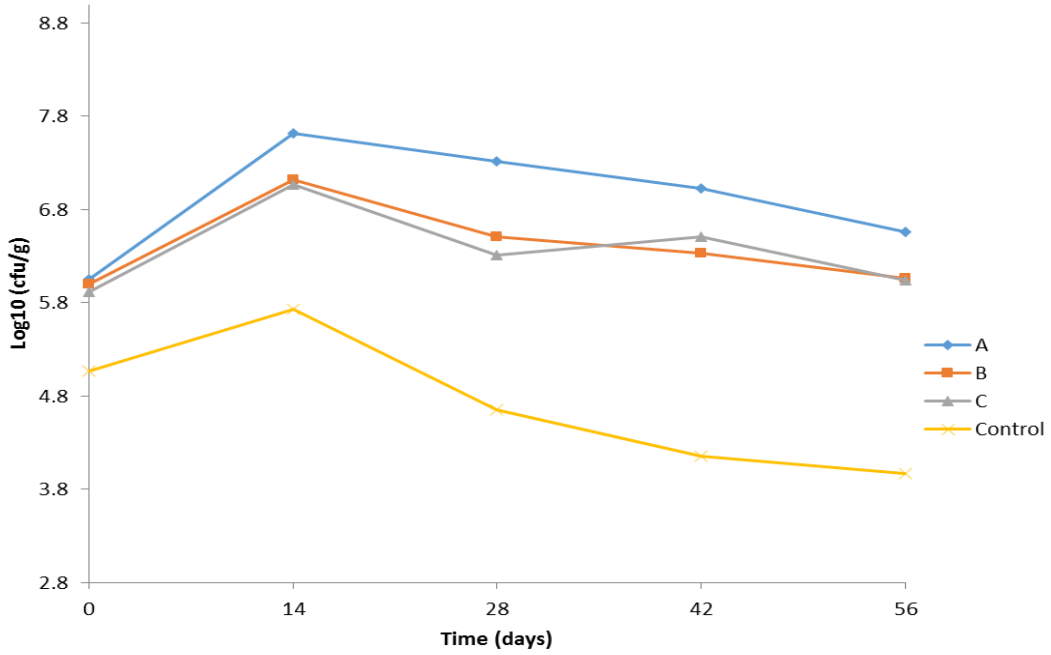

Figure 2: Total hydrocarbon utilising fungal counts during the bioremediation process

A: Microcosm amended with *Candida adriatica* and *Candida taoyuanica*
B: Microcosm amended with *Candida adriatica*
C: Microcosm amended with *Candida taoyuanica*
Control: Unamended soil

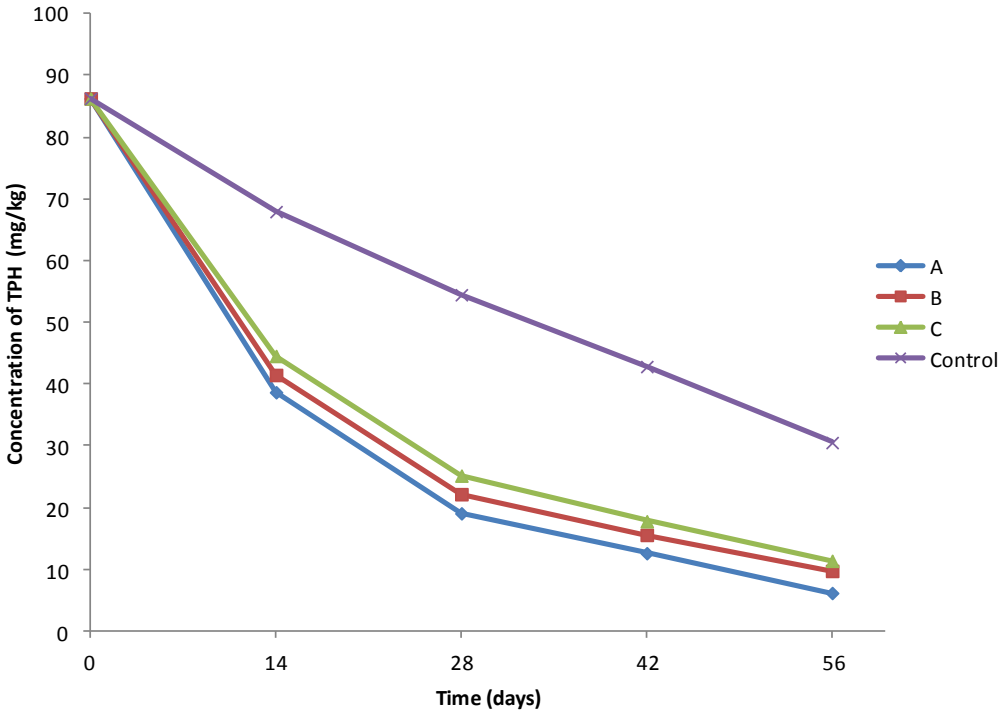

Figure 3: Hydrocarbon levels in different microcosms during the bioremediation process

A: Microcosm amended with *Candida adriatica* and *Candida taoyuanica*
B: Microcosm amended with *Candida adriatica*
C: Microcosm amended with *Candida taoyuanica*
Control: Unamended soil

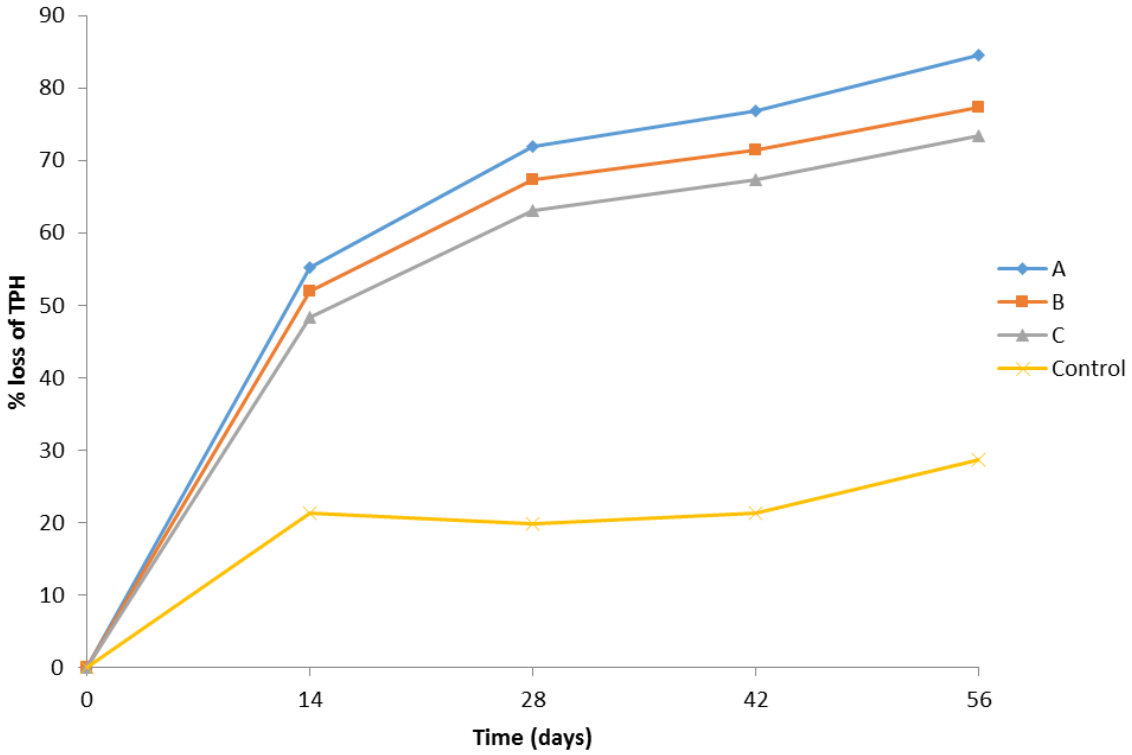

Figure 4: Percentage loss of total petroleum hydrocarbon during the bioremediation process

A: Microcosm amended with *Candida adriatica* and *Candida taoyuanica*
B: Microcosm amended with *Candida adriatica*
C: Microcosm amended with *Candida taoyuanica*
Control: Unamended soil

decreases when a consortium was used in bioremediation. In similar laboratory study, Capelli et al. (2001), reported a decrease in total petroleum hydrocarbon (TPH) of almost 70% in microcosms when pre-selected microorganisms were used. Bento et al. (2005), also said that the addition of pre-selected microbial consortium degraded 73-75% light (C_{12}-C_{23}) and heavy (C_{23}-C_{40}) fractions of the total petroleum hydrocarbon (TPH) present in the long beach soil contaminated with hydrocarbons. Sharma and Rehman (2009), using bioreactors for their biodegradation studies reported that microbial consortium was able to remove hydrocarbons present in the soil and concluded that it was a better option for bioremediation of contaminated soil from results of GC-MS (gas chromatography - mass spectrometry). Farag and Soliman (2011) reported that yeast strains degraded different aliphatic and aromatic hydrocarbons and some derivatives as sole carbon sources. Hesham et al. (2012), in their bioaugmentation studies used a yeast mixture of five different strains and recorded 80.7% – 98.5% loss of high molecular weight polycyclic aromatic hydrocarbons (PAHs). Fan et al. (2013) in their studies used *Candida tropicalis* in their bioaugmentation studies and reported 96% (saturated) and 42% (aromatic) hydrocarbon losses at optimum pH. Nrior and Onwuka (2017) investigated the biodegradation of crude oil impacted marshland soil by bioaugmentation using *Candida* and *Penicillium* species singly and as a consortium. They reported that the consortium containing *Candida* and *Penicillium* species exhibited higher bioremediation potentials than the individual species. Also, they stated that *Candida* species had better potentials than the *Penicillium* species in bioremediation. The ability of *Candida* species to degrade long and short chains is due to having a very efficient degradative enzyme system.

By day 56, the heights of the residual hydrocarbon peaks were all reduced in the microcosms in the order, A>B>C>Control. Reduction of the heights of hydrocarbons as displayed in chromatograms is used qualitatively to score the progress of hydrocarbon degradation (Chikere et al., 2012; Orji et al., 2013, Ezekoye et al., 2017).

Bioremediation studies have demonstrated that this technique is potent for cleaning up contaminated areas and has been established that areas damaged by oil spill could be returned to its natural state within a shorter period. According to Adams et al. (2015) the microorganisms which play the major roles in the process and their nutrient requirements (carbon, nitrogen, phosphorus, etc.) as well as environmental conditions (oxygen or other electron acceptor, temperature, pH, etc.) should be determined. This knowledge would help in the manipulation of

Table 3: Hydrocarbon utilizing fungi

Days	A	B	C	Control
0	6.05±0.03[a]	6.00±0.05[a]	5.92±0.11[a]	5.07±0.09[b]
14	7.62±0.06[a]	7.12±0.09[a]	7.07±0.07[a]	5.73±0.12[b]
28	7.32±0.11[a]	6.51±0.10[b]	6.31±0.04[b]	4.65±0.13[c]
42	7.03±0.09[a]	6.33±0.06[b]	6.51±0.10[b]	4.16±0.07[c]
56	6.57±0.18[a]	6.06±0.17[ab]	6.04±0.11[b]	3.97±0.09[c]

NB: All values are expressed as Mean ± S.E. Similar letters at the superscript indicates no significant difference and different letters at the superscript indicates significant difference.

environmental factors that may prevent the process of biodegradation of petroleum hydrocarbons. Thus, bioaugmentation or seeding would be useful to enhance the cleanup process of oil contaminated soils. Seeding with bacteria or fungi stimulates the synergistic effect between populations during the biodegradation process.

The value of total heterotrophic fungi (THF) in microcosms A, B and C were significantly different from the control ($P<0.05$) during the entire period of bioremediation as seen in Table 3. However, the value of total heterotrophic fungi increased in value as the bioremediation period increased and peaked at day 14, then started reducing in A, B and C; while the reverse in trend was the case in the control where there was a reduction in value from 5.21±0.06 – 4.20±0.12 as the bioremedation period increased.

There are several reports that fungi can grow under environmentally stressed conditions of low pH and poor nutrient status where bacterial growth might not be favoured. Bacteria are known to initiate the biodegradation process but the rate at which the pollutants are removed from the environment is twice when both bacteria and fungi are present in that environment (Nrior and Onwuka, 2017).

Biodegradative capacities of contaminated site can be improved by adding single strains or consortia of microorganisms with the desired catabolic capabilities (Adams et al., 2015). This study was carried out to assess the bioaugmentation potential of the crude oil polluted soil using two different species of *Candida* to augment the autochthonous microorganisms present in the soil. The results showed that these organisms increased the bioremediation rates thereby reducing the contaminants with time.

Several researchers have reported that some yeasts have great potentials in the field of bioremediation. Once these organisms are exposed to these hydrocarbons, a series of biochemical and morphological modifications are triggered within the yeast cell especially when alkanes are the sole carbon source. There are modifications on the cell surface due to hydrocarbon transport in cell; induction of cytochrome P450 active in alkane and NADPH-cytochrome (P450) reductase hydroxylation; induction of enzymes involved in oxidation of fatty alcohols and their aldehydes; peroxisomes proliferation, induction of the characteristic beta-oxidative pathway and of the enzymes involved in glyoxylic acid and gluconeogenesis (Csutak et al., 2010).

There are reports that yeast cells produce a variety of enzymes involved in their degradation of polycyclic aromatic hydrocarbons, complex organic compounds with fused and highly stable polycyclic aromatic rings using the cytochrome P450 (Deshmukh et al., 2016).

Moreso, other compounds such as phenols, polychlorinated biphenyls (PCBs) and polyurethane can be degraded by fungi (Stella et al., 2017).

Table 3 shows the values of hydrocarbon utilizing fungi (HUF) during bioremediation of crude oil. It showed that the value of hydrocarbon utilizing fungi in microcosms A, B and C were significantly different from the control at $P< 0.05$. Furthermore, the HUF increased in value as the bioremediation period increased and peaked at day 14. There was a decrease in number from day 28 in the microcosms A, B and C. The reverse was the trend in the control where there was a reduction in value from 5.07±0.09 – 3.97±0.09 during the study.

CONCLUSION AND RECOMMENDATION

This study raises very strong hope for cleanup of oil spill with reduced cost. From this study, high biodegradative efficiency was exhibited by *Candida adriatica* ZIM 2468 and *Candida taoyuanica* MYA-4700 showing they can be better hydrocarbon degraders. Thus, they can be used effectively for bioremediation. This report shows that exogenous microorganisms could be effective in bioremediation of polluted soil as *Candida adriatica* ZIM 2468 and *Candida taoyuanica* MYA-4700 were effective in augmenting the resident microorganisms in utilizing the Bonny light crude oil. It was concluded that fungal population changes occurred as a result of bioaugmentation. Addition of the yeasts, aeration and watering; enhanced biodegradation capabilities of indigenous microbial populations. Furthermore, fungal enzymes are non-specific and are able to breakdown many kinds of substances. Their use is one of the cheaper solution to remediation and usually require inexpensive equipment.

Although, bioaugmentation has greatly advanced over the years, there are a lot of challenges when it comes to applying laboratory isolated oil-degrading microorganisms

to contaminated areas, especially competition from other microbial consortia in such areas.

Further work is still ongoing on the suitability of these isolated strains including their genetic modifications and optimizing the processes for use in cleanup of other waste materials. A better understanding of the gene organization, structure and function can provide a model for evaluating the effectiveness and sustainability of these microorganisms in bioremediation of hydrocarbon polluted site. Thus, bioremediation should be encouraged to have a sustainable environment.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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