Occurrence of Yeasts in an Experimental Crude Oil Contaminated Soil in Nigeria

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ABSTRACT
Effect of crude oil spillage on microorganisms had been extensively studied; in most cases, the impact had been depressive, significantly reducing microbial populations. This study therefore investigated the occurrence of yeasts in crude oil polluted soil at different concentrations (0ml, 90ml, 180ml and 270ml) of contamination of soil. Unpolluted (0ml) served as control. Standard microbiological procedures were employed for analysis of soil samples. Of the nine yeast species isolated Candida, Cryptococcus, and Saccharomyces occurred in unpolluted soil and in the three polluted soil options while Rhodotorula occurred in control soil option only; Geotrichum, Hansenula, Phaeococcomyces, Prototheca, and Sporoblomyces species occurred in control soil and one polluted soil option only. Mean counts of yeasts in the unpolluted and polluted soils (0ml, 90ml, 180ml, and 270ml concentrations of crude oil) were: $5.1\pm2.6X10^{3}CFU\ G^{-1}$, $5.0\pm3.7X10^{3}CFU\ G^{-1}$, $3.7\pm2.6X10^{3}CFU\ G^{-1}$, and $4.1\pm2.6X10^{3}CFU\ G^{-1}$ soil respectively, which showed that control sample, had the highest population of yeasts. The investigation showed that crude oil had depressive effect on yeasts population in soil, and that the yeast species isolated could be employed as bioremediation agents in the clean-up of crude oil polluted sites.

Keywords: *Effect, crude oil, yeasts, contamination, bioremediation, agents.*

INTRODUCTION

Yeasts applied to a number of microscopic fungi; although a number of fungi are sometimes called yeasts, the true yeasts are unicellular, consisting of oval or round cells, and reproducing chiefly by budding. Under certain conditions, some yeast cells secrete a thickened wall, and the cytoplasm of the single cell within divides to form four or eight cells, or spores, known as ascospores, which emerge when the wall ruptures. In a few species, two cells fuse before undergoing spore formation. Yeasts have long been found to be of commercial importance because they are the chief agents in fermentation, and in the early times, they are used in treating various ailments (Barnett, 2003; Schwab *et al*., 1994).

Crude oil is a highly complex mixture, composed of saturated hydrocarbons, aromatic hydrocarbons and polar organic compounds (Atlas, 1981; Wemedo *et al*., 2002; Kumari and Abraham, 2011). Crude oils have different physical and chemical properties (Leachy and Cowell, 1990). These properties affect the behaviour of crude oil in water and in soils, hence affecting the efficiency of clean-up operations in spillage conditions (Doeefer, 1992). Contamination of soils and sediments with petroleum hydrocarbons is a serious ecological problem, primarily in countries that produce, transport and refine crude oil (Sarma and Sarma, 2010). Oil production activities release large amount of hydrocarbons in to terrestrial and aquatic

environments. The conflict about biosphere and technosphere has sharply increased. Hence, the level of soil pollution by petroleum and oil sludge has approached millions of cubic meters (Zukauskaite and Viktorija, 2008).

The potential to detoxify hazardous organic compounds, through transformation, mineralization or polymerization, has been among microbial community. In order to develop a strategy for microbial degradation of crude oil, it is necessary to isolate specific native microbes and to test the efficacy of the microbes in degradation of various hydrocarbon compounds present in oil contaminated site prior to application to the field (Janiyani *et al*., 1993). Large amount of hydrocarbons are extracted, produced, refined and handled every year and despite improvements in careful handling, transportation and containment, there is still the possibility that some may contaminate the soil and water environments (Kumari and Abraham, 2011). In addition, oil pollution accidents have nowadays become a common phenomenon and have caused ecological and social catastrophes (Burns *et al*., 1993). Accidental release of oil in the environments has been shown to cause serious damage to natural ecosystem in both prevalence and quantity (Rahman *et al*., 2002a; Chayneus *et al*., 2005).

Hydrocarbon degrading bacteria and fungi are widely distributed in marine, fresh water and soil habitats (Atlas and Bartha, 1992). Bacteria and yeasts appear to be the prevalent hydrocarbon degraders in aquatic ecosystem (Cooney and Summer, 1976). Ability to isolate high number of crude oil degrading microorganisms from an environment is evidence that they are the most active oil degraders of that environment prior to exposure of microbial community to hydrocarbons. It has been observed that bacteria and fungi have similar patterns of hydrocarbon degradation, and that the use of fungi enhanced bioremediation (Colwell *et al*., 2001; Atlas and Bragg, 2009).

The aim of this study therefore was to isolate yeast organisms from a crude oil contaminated soils; the objective being to identify the species of yeasts that occur in the crude oil polluted soil which could in turn serve as possible agents in clean-up of crude oil contaminated environment. Furthermore, to make a comparative analysis of yeast population in unpolluted and crude oil polluted soil as to determine the effect of crude oil on soil yeasts at population level.

MATERIALS AND METHODS

Sample collection

Composite surface soil samples from the top soil (0 - 15cm depth) were collected from a soil in Nkpolu-Oroworukwo area of Port Harcourt metropolis in Rivers State, Southern Nigeria. The area is a plain land characterized by high humid atmosphere particularly during wet season. Soil samples were collected using a clean sterile auger borer and bulked soil samples, from the center of the borer, were put into fresh unused black polythene bags capable of holding the required quantity of soil. The soil samples were taken to the green house for treatment and crude oil application. Crude oil was obtained from Agip Oil Company located in Brass, Bayelsa State.

Treatment of Soil Sample

For the purpose of soil treatment, four sets of soil samples were distributed in 3kg weight in triplicates labeled A, B, C and D; the polythene bags were perforated at the bottom to allow excess water drain off during the incubation. Each sets of four samples were designated A1, A2, A3 which was left uncontaminated to serve as control; B1, B2, B3; C1, C2, C3 and D1, D2, D3 contaminated with 90ml, 180ml and 270ml crude oil respectively. Ten grammes (10g) portion of the soil samples was collected immediately (2 hours) after contamination with crude oil from

each treatment option for analysis to represent day 1. All the options were then allowed to stand and samples were taken for analysis at intervals of 7 days, 14 days and 21 days. The soil samples were moistened weekly immediately after each sampling. Contamination with 90ml, 180ml and 270ml crude oil correspond to 3%, 6% and 9% respectively volume/weight crude oil to soil.

Microbiology of Soil Samples

Microbiological analysis of soil samples involved enumeration, isolation and identification of heterotrophic yeast organisms from control (unpolluted) and crude oil polluted soils. One milliliter (1ml) of mixture of 1g soil and 10ml sterile normal saline was diluted serially in one-tenth stepwise to give the required dilutions. Aliquots (0.1ml) of appropriate dilutions were spread plated onto the surface of sterile dried potato dextrose agar medium in Petri dishes. The inoculated plates were incubated at 37 ± 2^{0}C for 24 – 48 hours. After incubation, colonies were counted and recorded, and studied based on their morphology such as colour, texture, shape and surface appearance. Purified colonies were obtained and microscopic identification of the yeast organisms was carried out using gram staining, wet preparation and slide culture techniques described by Barnett and Hunter, (1983); Abbey (1995).

Table 1: Densities of Yeasts in Uncontaminated (Control) and Crude Oil Contaminated Soils

Days of Analysis	Densities of yeasts ($X10^{3}CFU\ G^{-1}$) in Soil Options			
	Unpolluted soil	Polluted soils		
	0ml	90ml	180ml	270ml
Day 1	3.8	2.4	2.1	2.8
Day 7	4.5	4.6	2.9	2.6
Day 14	8.9	10.3	7.6	7.9
Day 21	3.1	2.6	2.3	3.0
Mean	5.1±2.613	5.0±3.686	3.7±2.606	4.1±2.555

RESULTS

Counts of yeasts are shown in Table 1.0. Ranges of yeast densities in the different soil options were: **Unpolluted soil** (0ml): 3.1 to $8.9X10^{3}CFU\ GL^{-1}$; **Polluted soils** (90ml): 2.4 to $10.3X10^{3}CFU\ GL^{-1}$, (180ml): 2.1 to $7.6X10^{3}CFU\ GL^{-1}$, and (270ml): 2.6 to $7.9X10^{3}CFU\ GL^{-1}$. Population of yeasts in control and crude oil contaminated soils is shown in Figure 1. Mean ±SD yeast counts for each soil option were: 0ml: $5.1\pm2.6X10^{3}CFU\ GL^{-1}$; 90ml: $5.0\pm3.7X10^{3}CFU\ GL^{-1}$, 180ml: $3.7\pm2.6X10^{3}CFU\ GL^{-1}$ and 270ml: $4.1\pm2.6X10^{3}CFU\ GL^{-1}$. Yeast population decreased with increasing concentration of crude oil but increased again at 270ml crude oil concentration. Yeast species isolated, characterized and identified are shown in Table 2.0 and include: *Candida, Cryptococcus, Geotrichum, Hansenula, Phaeococcomyces, Prototheca, Rhodotorula, Saccharomyces,* and *Sporoblomyces.*

Table 3: Yeast Species Isolated from Uncontaminated (Control) and Crude Oil Contaminated Soils

Yeast Organisms	Control	90ml	180ml	270ml
Candida species	+	+	+	+
Cryptococcus species	+	+	+	+
Geotrichum species	+	+	-	-
Hansenula species	+	+	-	-
Phaecoccomyces species	+	-	+	-
Prototheca species	+	-	-	+
Rhodotorula species	+	-	-	-
Saccharomyces species	+	+	+	+
Sporoblomyces species	+	-	+	-

KEY: + = Yeast isolated, - = yeast not isolated

DISCUSSION

The main focus of this study was enumeration and isolation of yeast organisms from crude oil polluted soil and compared to unpolluted soil which served as control. Results of characterization of yeast species from the unpolluted and polluted soils revealed that nine (9) yeast organisms were observed in this study. All the nine (9) yeast species isolated occurred in the unpolluted (control) soil; *Candida*, *Cryptococcus* and *Saccharomyces* occurred in both unpolluted and crude oil polluted soil samples; *Geotrichum* and *Hansenula* occurred in the 0ml and 90ml concentrations only and not in 180ml and 270ml concentrations while *Phaecoccomyces* and *Sporoblomyces* occurred in 0ml and 180ml, and *Prototheca* occurred in 0ml and 270ml concentrations. *Rhodotorula* occurred in unpolluted soil only. Previous workers isolated similar genera encountered in this work such as *Geotrichum* and *Rhodotorula* which are not commonly found in soil (Obire and Anyanwu, 2009; Romeo et al., 2010; Al-Nasrawi, 2012). Occurrence of yeast species in one or two concentrations and not in the other concentration could probably be due to the ability of the microorganisms to utilize different hydrocarbon components when crude oil is introduced into the environment. Some microbial species utilize long chain hydrocarbon components, others utilize short chain components, and still some utilize straight or branched chain hydrocarbons (Atlas and Bartha, 1977; Bartha, 1984; Rahman *et al*., 2002a). Hence some microbial species start degradation of hydrocarbon and other species become active whenever the preferred intermediate components appeared (Atlas and Bartha, 1977; Bartha, 1984; Rahman *et al*., 2002a). This could be the case for yeast organisms in this study. It has been reported that degradation of crude oil is best achieved by mixed microbial cultures in a process called co-oxidation or co-metabolism (Herbes and Schwall, 1978). The occurrence of yeast species in the contaminated soils showed that yeast can survive in crude oil polluted soils even at higher (270ml) concentration, and can utilize hydrocarbon as sole source of carbon and energy

Counts of heterotrophic yeasts obtained in this study showed variations within different crude oil concentrations and between the days of analysis. Generally speaking, yeast populations were highest in unpolluted soil when compared to those of crude oil polluted soil options except for day 7 and day 14 at 90ml crude oil concentration where the counts were slightly higher than control. However, yeast population decreased with increasing concentrations of crude oil. Fluctuations in the yeast densities were observed in all the soil options as the day of experiment increased. In the unpolluted soil, yeast population increased in day 1 to day 14 and decreased at the end of experiment (day 21). At 90ml crude oil concentration, yeast population increased from

day 1 to day 14 and decreased in day 21. At 180ml concentration, yeast population increased from day 1 to day 14 and decreased again towards the end of the experiment (day 21) while at 270ml crude oil concentration, the population increased in day 1, decreased in day 7, increased again in day 14 and then decreased in day 21. Mean counts of the different concentrations showed that yeast population were highest in control soil when compared to those of the crude oil polluted soils but decreased with increasing concentration of crude oil and slightly increased again at the end of experiment. The study revealed that contamination of the soil with crude oil depressed yeast population when their counts in control soil were compared with those of polluted soils. Hence, yeasts responded negatively to presence of crude oil in the soil, and that the difference between the yeast counts of the unpolluted soil (0ml) and those of the polluted soil (90ml, 180ml and 270ml) were seen as the effect of the addition of crude oil on the yeast organisms at population level. Statistical analysis showed that there was no significant difference between yeast counts of control and 90ml concentration of crude oil but significant difference ($P<0,005$) exist between yeast counts of control soil and 180 – 270ml concentrations of crude oil.

The observed decrease in counts of yeasts in the contaminated soil could be attributed to toxic effect of crude oil. Odu (1972) reported that contamination of soil by crude oil or its products resulted in initial depression in microbial numbers and activity even in relatively mild contamination. The yeast counts observed in this study is significant to predict the effect of crude oil on yeast in the soil. There was general decrease in the yeast counts in the polluted sample than the unpolluted samples. This suggested that yeast species responded negatively to pollution of the soil by crude oil, and that crude oil altered the growth and activity of soil yeasts which could in turn influence the general performance of soil environment in terms of food production.

CONCLUSION

Of the nine (9) yeast organisms isolated during this study, all occurred in unpolluted soil, some species occurred in two options of polluted soil while some occurred in one soil option only. A comparative analysis of the yeast counts between control and those of the crude oil polluted soils showed that yeast populations were generally higher in the unpolluted soil than those of polluted soils. Generally, unpolluted soil had highest population of yeast which decreased with increasing concentrations of crude oil; and different species are shown to utilize different hydrocarbon intermediates, and probably occurred in the presence of such utilizable intermediate components. This study showed that yeasts responded negatively to the presence of crude oil in contaminated soils being that petroleum contamination of soil depressed their populations, and could in turn alter soil productivity. This study further revealed that yeast species survived and utilized hydrocarbon as their source of carbon and energy, and that these yeast species can be isolated and used for clean-up of crude oil contaminated soil.

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