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Assessment of degradability potential of *Penicillium oxalicum* on crude oil

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ABSTRACT

The ability of three fungal isolates (XA, XB, XC) isolated from crude oil contaminated soil to degrade crude oil was studied. The isolates were identified to be Penicillum oxalicum based on their microscopic and macroscopic features. Degradability test showed that Penicillum oxalicum XA, XB and XC were able to metabolize the crude oil constituents with evidence from increase in mycelia weight. After 5 th day of incubation the mycelia weight for the isolates; XA, XB and XC were measured to be 0.39kg, 0.35kg and 0.35kg respectively. On the 20th day of incubation the mycelia mass increased to 0.56g, 0.54g and 0.51g respectively. The percentage of the residual oil on the 5th day of incubation for XA,XB, and XC isolates were quantified to be 73.5%, 68.6% and 75% respectively, whereas on the 20th day of incubation the percentage has reduced to 33% 35% and 31.4% respectively. This study showed that Penicillium oxalicium can be implicated in the remediation of site that may be contaminated with crude oils. Further understanding of the metabolic process of this organism on the crude oil will increase possibilities of developing models and strategies for removing crude oil pollutants from oil-impacted environments.

INTRODUCTION

Crude oil is the chief source of hydrocarbons, it is found in huge underground deposits in many parts of the world. Crude oil was firstly discovered in Nigeria in 1956 and it has brought in much money to Nigeria. Large deposits of crude oil have been found in Niger Delta, Abata, Bomu Owaza, Egbema and the Ughelli-Kokori zone .Crude oil has being in existence for millions of years before man developed the technology to remove it from the soil and use it as energy source (Overton *et. al.,* 1994). Since the beginning of commercial exploration of crude oil in Nigeria over fifty years ago, the frontier oil exploration in the country has been expanding, producing medium and light crude oil (Okoh,*et a.l.,* 1996).

Optimum utilization and benefits from crude oil are derived by converting crude oil through processing in a refinery into a wide range of products such as petroleum fuels, lubricants, bitumen and waxes based on market demand (Okoh, 2002). The increasing demand for crude oil as a source of energy in Nigeria has resulted in contamination and pollution of the oceans and terrestrial environments (Ajisebutu,*et al.,* 2003). The major route of transportation of hydrocarbon in Nigeria is by road, ships and network of underground connecting pipes (Okoh *et. al.,* 2000).

The advent of offshore oil, accidental oil spillages and the increasing number and size of oil tankers have brightened concern for the impact of oil on the ocean and soil environment. The release of crude oil into human environment has impacted negatively on the health and social well being of human population (Nwachukwu *et.al.*, 2001). Researches have shown that the massive and extensive pollution of the environment constitute socioeconomic and public health hazards. Some of the hydrocarbons and related materials are known to be highly toxic to plants and animals. This is because crude oil, particularly the aromatic fractions, is acutely lethal in concentrations of a few parts per million (ppm) and chronically lethal in sublethals in parts per billion (ppb) (Odiete, 1999). Benzene which is a component of crude oil is known to have serious toxic effects on bone marrow and chronic exposure to benzene may cause Leukemia.

However, the re-use of crude oil impacted land for agricultural purpose for food production has become a necessity; especially in countries where fertile arable land is scarce to avoid poor yields for agricultural produce and this involve some bioremediation treatments (Nwachukwu, *et al.*, 2000a). Bioremediation is now considered as one of the promising oil spills counter-measures. Bioremediation is a technique enhance the natural rates of pollutants carried out by selected microorganisms (Providenti *et. al.*, 1993) Thus, the eventual focus of a bioremediation program of contaminated land is to decommission the soil for farmers use for food and livestock productions(Atlas ,1991)

Penicillium oxalicum is recognized by their dense-brush like spore bearing structures. Branching is an important feature for identifying *Penicillium spp.which* is a large and difficult genus encountered almost everywhere and usually the most abundant genus of fungi in soils. *Penicillium oxalicum* produces two isozymes of polygalacturonase (PG) and a pectase lyase (PL), the enzymes are important in biodegradation, these enzymes are the focus of intense research because of their applications in the detoxification of a broad range of environmental pollutants (Seeley, 1981). This study however is aimed at assessing the potential capability of *Penicillium oxalicum* to degrade crude oil in a polluted soil.

MATERIALS AND METHODS

Crude oil contaminated soil sample was collected from Niger-delta area. The soil was collected to the depth of 15cm at four random sampling sites. Each of the four soil samples was kept in separate sterile plastic bag and transported immediately to Biological research laboratory of Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

In other to meet with the nutritional requirement of the microorganisms for proper growth the method described by Akhavan *et. al.*, (2008) was adopted for the preparation of mineral salt

medium in this method, the following salts were dissolved in one litre of water, $NaNO_3$(2.0 g/l), NaCl (0.8 g/l), KCI (0.8 g/l), KH_2PO_4(2.0 g/l), Na_2HPO_4 .$12H_2O$ (2.0 g/l), $MgSO_4$(0.2 g/l), $FeSO_4$.$7H_2O$ (0.001g/l);

Isolation and identification of fungal isolates

The method described by Olutiola *et. al.,* (1991) for isolation of fungi from soil sample was employed. The fungal isolates were identified based on their colonial morphology. Microscopic examination of fungal isolates was carried at both vegetative and sporulating stages. A clean slide with a drop of lactophenol in cotton blue in its centre was used. A portion of the mycelium at the edge of the colony at the desired stage was picked and dropped in the lactophenol in the cotton blue. This was teased and covered carefully with a cover slip. The slide was mounted on the microscope, focused and observed.

The three fungal isolates were tested for their ability to degrade crude oil. This was carried out by employing the method described by Nwachukwu *et. al.,* (2000). In this method 8.5ml and 1.5ml of MSM and crude oil were transferred into a sterile conical flask respectively in triplicate. The flasks containing Oil-MSM was inoculated with the fungal isolates and incubated at 35^oC, the flasks were agitated every 24hours for proper utilization of the crude oil. Another flask that contained 8.5ml of MSM and 1.5ml of crude oil was used as control and it was not inoculated with fungal isolates. The residual crude oil left undegraded in the entire flasks was extracted by using solvent extraction method (liquid – liquid).

RESULTS

The fungi isolates were identified to be *Penicillium oxalicum* based on their microscopic and macroscopic features. The results presented in (Table 1 and 2) showed that the organism was able to degrade the component of crude oil.

However, with change in time, from 5^{th} to 10^{th} day of incubation, the organisms were able to degrade the crude oil component further (Table 2). Likewise, on the 10^{th} day of incubation, at 35^0C, the amount of crude oil not degraded by *Penicillium oxalicum* isolates were quantified to be 0.70ml, 0.86ml and 0.80ml respectively. Also on the 15^{th} day of incubation at 35^0C, the amount of the crude oil extracted was very small compare to those that have been previously extracted and this showed that the organisms have been able to degrade crude oil further. The results showed that 0.87ml, 0.85ml and 0.87ml of crude oil have been degraded by *Penicillium oxalicum* isolates respectively (Table 1).

Therefore, with the results obtained on the 20^{th} day of incubation. It was obvious that the organisms were actually degrading the crude oil, because the amount of crude oil recovered on the 20^{th} day of incubation was extremely lower compare to those previously harvested (Table 1). The result increase in mycelia dry weight is presented in Table 2 and fig 1-3.

Table 1: Mean of quantity of extracted residual oil for twenty days of incubation

	Incubation Period			
	5th Day	10th Day	15th Day	20th Day
CONTROL	1.40	1.40	1.40	1.40
P. oxalicum XA	1.03	0.70	0.63	0.49
P. oxalicum XB	0.96	0.86	0.65	0.50
P. oxalicum XC	1.05	0.80	0.63	0.44

Table 2: Mean of mycelia weight for 20 days of incubation

	5th Day	10th Day	15th Day	20th Day
CONTROL	0.01	0.01	0.01	0.01
P. oxalicum XA	0.39	0.46	0.51	0.56
P. oxalicum XB	0.35	0.40	0.48	0.54
P. oxalicum XC	0.35	0.41	0.46	0.51

Fig 1: Graph of increase in mycelia dry weight for P. oxalicum XA

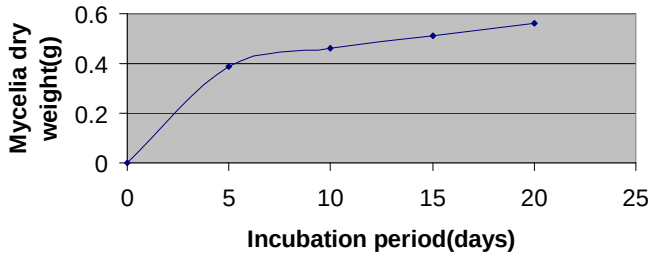
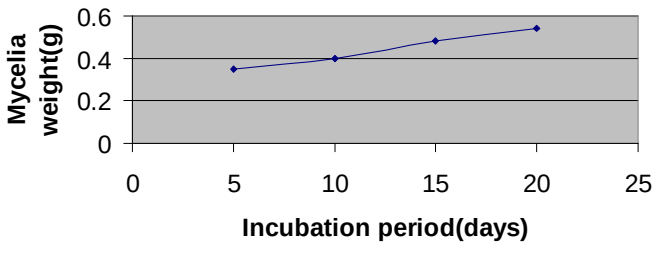


Fig. 2: Graph of increase in myclia dry weight of P. oxalicum XB



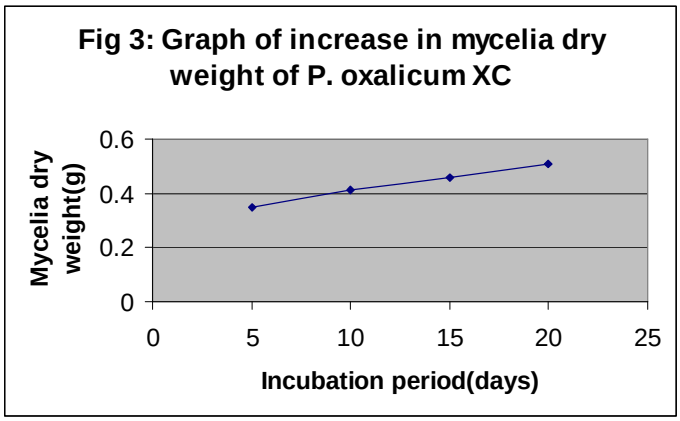


Fig 3: Graph of increase in mycelia dry weight of P. oxalicum XC
Mycelia dry weight(g)
0.6
0.4
0.2
0
0
5
10
15
20
25
Incubation period(days)

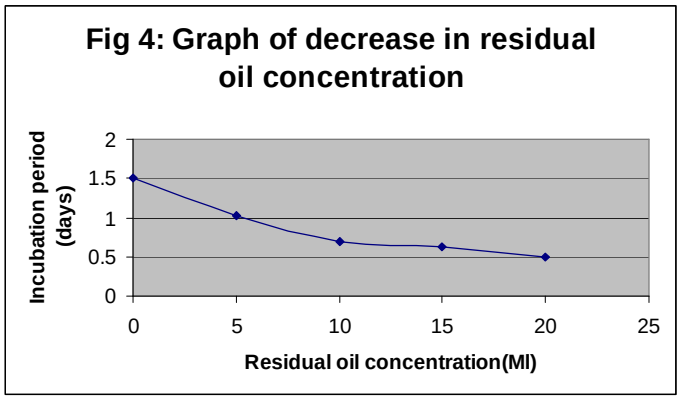


Fig 4: Graph of decrease in residual oil concentration
Incubation period (days)
2
1.5
1
0.5
0
0
5
10
15
20
25
Residual oil concentration(Ml)

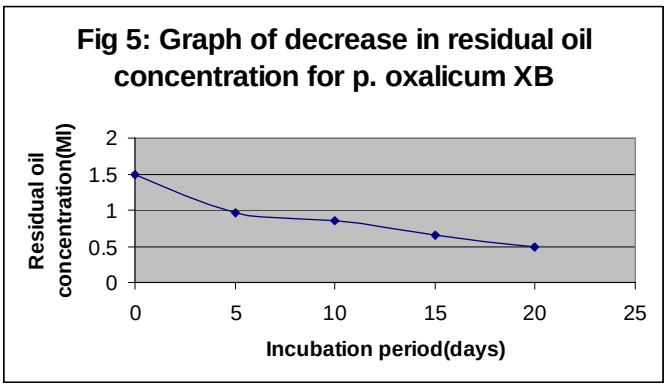
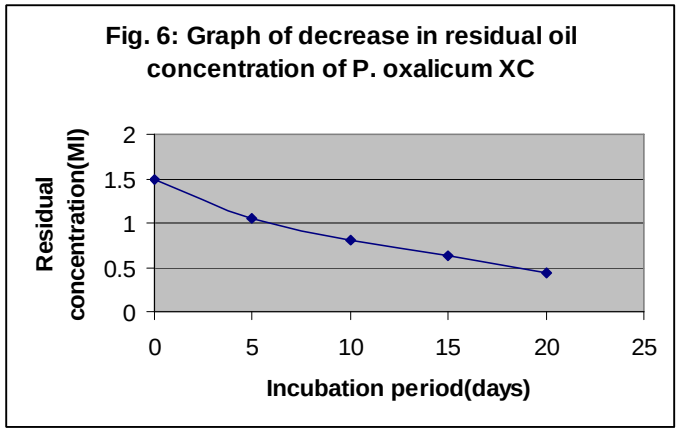


Fig 5: Graph of decrease in residual oil concentration for p. oxalicum XB
Residual oil concentration(Ml)
2
1.5
1
0.5
0
0
5
10
15
20
25
Incubation period(days)



DISCUSSION AND CONCLUSION

The ability of indigenous bacteria including among others *Serratia spp, Pseudomonas spp* and *Corynebactarium spp.* to degrade oil pollutant has been demonstrated in many studies *in vitro (*Kampfer *et al.,* 1991; Atlas,1991;Rocha *et al.,1992;Dyke et al* 1993; Mercade *et al.*,1992*).* Crude oil is highly toxic to biotic factors and persists in soils for many years causing sterilization and loss of soil fertility (Atlas, 1991; Ted and Udall, 1991; Teal et al 1992).

Today the use of microorganisms for removing crude oil pollution from contaminated sites as bioremediation was considered by scientists, because other methods such as surfactant washing and incineration lead to production of more toxic compounds and they are non-economical (Margesin, 2000; Barathi *et al*., 2002).There are so many bacteria and fungi with this ability and these organisms are widely distributed in soil, marine and freshwater habitats (Head and Swanell, 1999).

The rate of biodegradation of oil pollutant depend on different factors such as microbial composition, contaminant type, geology of polluted sites and chemical conditions at the contaminated sites (Akhavan *et. al* ., 2008)It is evident from this study that isolated *P. oxalicum* can degrade crude oil and comparison of obtained results and existing statistics of similar studies by Nwanchukwu *et. al.,* (2001) and Akhavan *et. al.,* (2008) that used *Pseudomonas putida* and *Baccillus spp* which are known to be pathogenic unlike *P. oxalicum* which can be used for bioremediation and elimination of crude oil pollutants from the environment, because they are environmental friendly and non pathogenic, this makes them better agent of bioremediation.

Further understanding of the metabolic process of this organism on the crude oil will increase possibilities of developing models and strategies for removing crude oil pollutants from oil-impacted environments.

The immense potential of *P. oxalicum* to degrade crude oil does not depend solely upon the wealth of catabolic enzymes that this organism posses but also upon its capacity for adaptive change. Therefore further scientific investigation should be carried out on this organism for the optimization of its potential for use in ex-situ bioremediation of oil impacted soil and water.

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