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Investigation on the hydrocarbon utilization potential *of Pseudomonas putrefaciens, Bacillus stearothermophilus* and *Streptococcus faecium* isolated from crude oil contaminated soil

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ABSTRACT

The biodegradation potential of Pseudomonas putrefaciens, Bacillus stearothermophilus and Streptococcus faecium isolated from crude oil contaminated soil was investigated in this study. The 3 bacteria strains were inoculated into crude oil supplemented with Mineral Salt Medium and incubated at 37^{o} C for 25 days. The results obtained showed that P. putrefaciens, B. stearothermophilus and S. faecium were able to degrade 70%, 58% and 48% at the end of 25 days of incubation respectively. IR analysis of the residual crude oil was done, the results obtained showed that the bacteria strains have exerted microbial action on the crude oil. The results obtained from IR analysis revealed 17 bands, 16 bands and 12 bands for P. putrefaciens, B. stearothermophilus and S. faecium respectively, while control had 10 bands. This is an indication that the bacteria strains have modified the crude oil constituents. P. putrefaciens had highest percentage of degradation compare to the other two bacteria. The order of degradation was as follows P. putrefaciens > B. stearothermophilus > S. faecium. This study showed that the bacteria strains used in this study could be relevant in the bioremediation of ecosystem that may be contaminated with hydrocarbons.

Keyword: Biodegradation, MSM, Crude oil, Residual oil, Bioremediation.

INTRODUCTION

Crude oil is the chief source of hydrocarbon, it is found in huge underground deposits in many part of the world. Nigeria is a major producer of crude oil in the world and pollution of the environment has steadily increased (Korie-Siakpere, 1998; Odiete, 1999).

Oil spills are an inevitable consequence of using petroleum.Spills can occur at the oil refinery, during transportation, natural seeps or during routine maintenance of infrastructure (Ainon *et al., 2*010).These oil spills devastate the soil surface, and underground water and alter the microbial population at the polluted sites.

The risks of oil spillage, involved in many activities of petroleum industry, poses a serious environmental problem, due to the possibility of air, water and soil contamination (Trindade *et al.,* 2009).Environmental pollution with petroleum and petroleum products has been recognized as one of the most serious current problems especially when associated with accidental spills on large scale (Udeani *et al.,* 2008) if this occurs, hydrocarbons may reach the water table before becoming immobilized in the soil

Odiete (1999) reported that crude oil particularly the aromatic fraction is acutely lethal in concentration of a few part per million and chronically lethal in sublethel in parts per thousand. James *et al*, (1999) also reported that oil spills can cause great damage to sensitive environment.

Among the different technologies used during oil spills responses, a widely preferred and promising technology is bioremediation. Bioremediation has become an alternative way to remediate oil polluted sites, where the addition of specific microorganism (bacteria, cyanobacteria, algae, fungi and protozoa) or enhancement of microorganisms already present can improve biodegradation efficiency (Hagwell *et al. , 1*992). The main purpose of bioremediation is to remove contaminant from the environment (Atlas and Bartha, 1973).

Bioremediation uses relatively low cost, low technology techniques which generally have a high public acceptance and can often be carried out on site (Vidialli, 2001). However, by definition bioremediation is the use of living organisms, primarily microorganisms, to degrade environmental contaminants into less toxic forms (Muller and Cernigilia, 1996).Because bioremediation seems to be a good alternative to conventional clear–up technologies, research in this field rapidly increasing, bioremediation has been used at a number of sites world vide, including Europe with varying degree of success (Vidalli, 2001).

Among the petroleum hydrocarbon degraders reported are *Alcaligenes spp, Cyanobacterium spp, Bacillus spp Capnocytophage spp , Moraxella spp, Yokella spp* and *Flavobacterian.* This study was primarily carried out to investigate the degradability potential of *P. putrefaciens, B. Stearothermophilus and S. faecium i*solated from crude oil contaminated soil.

MATERIALS AND METHODS

Collection of soil sample

Soils contaminated with crude oil were collected from Niger Delta area, Nigeria to a depth of 15cm and transported to the Biology laboratory of Ladoke Akintola University of Technology Ogbomoso, Nigeria.

Isolation and identification of bacteria

One gram of the soil contaminated with crude oil was serially diluted up to 10^{-8} dilution . An aliquot (0.2m1) of the 10^{-8} dilution of the contaminated soil was plated out in duplicates unto nutrient agar using pour plate method. The plates were incubated at 37^{o}C for 48 hours. After incubation, the plates that were between 30 to 200 colonies were selected and used. Each bacteria colony type was subcultured repeatedly into nutrient agar plate to obtain a pure culture. The isolates were characterized based on cultural characteristics.Morphological characteristics of the isolates were identified by gram stain and biochemical reactions as well as motility test.

Preparation of mineral salt medium

Mineral salt medium (MSM) was prepared with the following salts $NaNo_3$ (2.0g/L), Nacl (0.8g/L), KH_2PO_4 (2.0g/L), $Na_2HPO_4.12H_2O$(2.0), MgSO4 (2.0g/l), FeSO4.7H20 (0.001g/L), $NH4NO_3$ (1.0g/L), KH_2PO4 (1.0g/L) and $Cacl_2$ (0.02g/L) all the salts were dissolved in one litre (1L) of water and sterlize in the autoclave at 121^{o}C for 15 minutes.

Biodegradation experiments

The biodegradation experiments were carried out in 30ml sterile bottles. Into each bottle 15ml of MSM and 5ml of crude oil that has been sterilized by tyndallization was added aseptically. The bottles were inoculated with the bacteria isolates entitled as K1, K2 and K3 representing *P. putrefaciens , S. faecium and B.stearothermophilus* respectively. Bottles that were not inoculated with the bacteria isolates were used as control for the experiment. All the bottles were transferred into incubator for incubation at 37^{o} C for 25 days.

Extraction of residual oil

The residual crude oil in the experimental bottles and control bottles was extracted by using liquid – liquid solvent extraction method. The organic solvent used was chloroform. This was done by measuring 20ml of chloroform into the bottles containing OIL- MSM, the contents were later transferred into separating funnel. The funnel was allowed to stand for 30 minutes , the layer containing the organic solvent and residual oil was emptied into a pre-cleaned container, the organic solvent was allowed to evaporate, after the evaporation the residual oil was measured by using syringe and recorded at 5, 10, 15, 20 and 25 days of incubation.

Infrared analysis

The residual oil recovered from both control and experimental bottles was subjected to IR analysis after 25 days of incubation at the Central research Laboratory of Ladoke Akintola University of Technology,Ogbomoso.

RESULTS AND DISCUSSION

Table 1: Decrease in residual oil concentration

P.putrefaciens	5ml	4.5ml	4.2ml	3.45ml	2.5ml	1.5ml
S.faecium	5ml	4.8ml	4.6ml	4.0ml	3.5ml	2.60
B.stearothermophilus	5ml	4.6ml	4.30ml	3.80ml	3.00ml	2.10ml
Control	5ml	5ml	5ml	5ml	5ml	5ml

Table 2: Percentage of crude oil degraded and undedgraded

P.putrefaciens	10,90*	16,84*	31,69*	50,50*	70,30*
S.faecium	4,96*	8.92*	12,88*	20.80*	48,52*
B.stearothermophilus	8,91*	14,86*	24,76*	40,60*	58,42*
Control	100*	100*	100*	100*	100*

**Percentage not degraded*

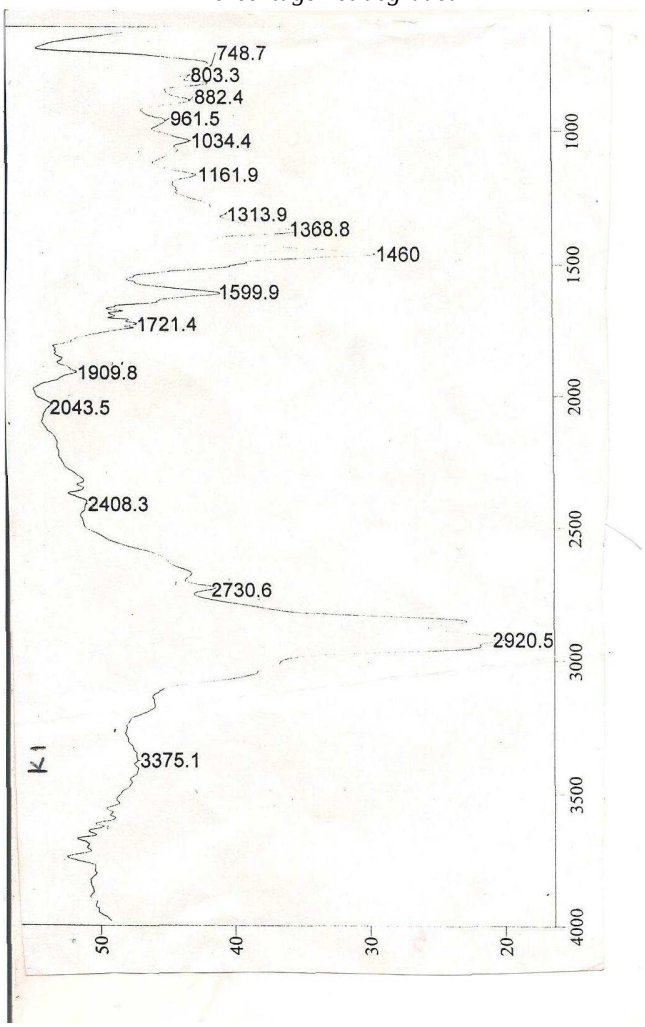

Fig. 1: IR analysis of the crude oil acted upon by *P. putrefaciens*

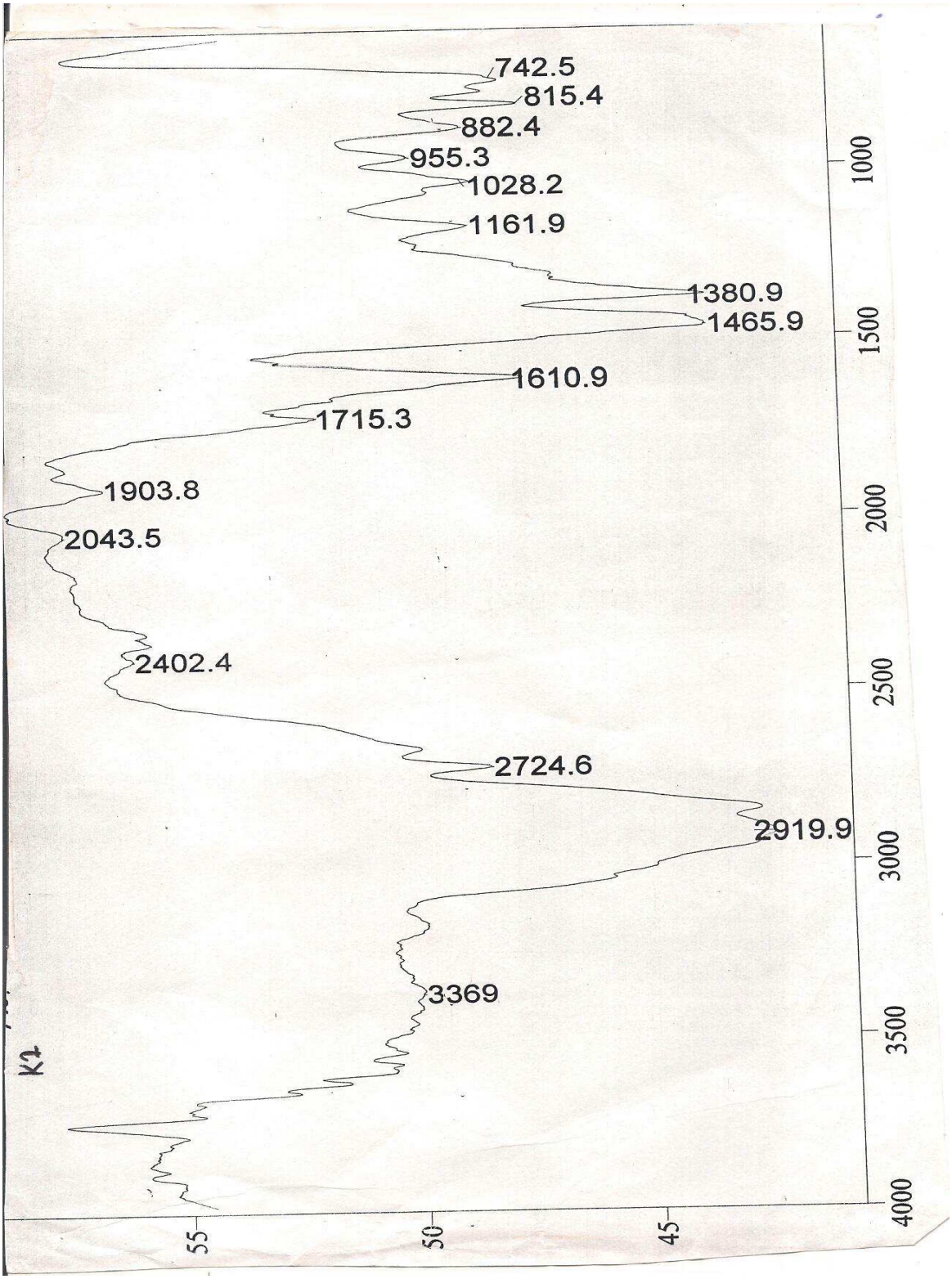

Fig. 2: IR analysis of the crude oil acted upon by *S. faecium*

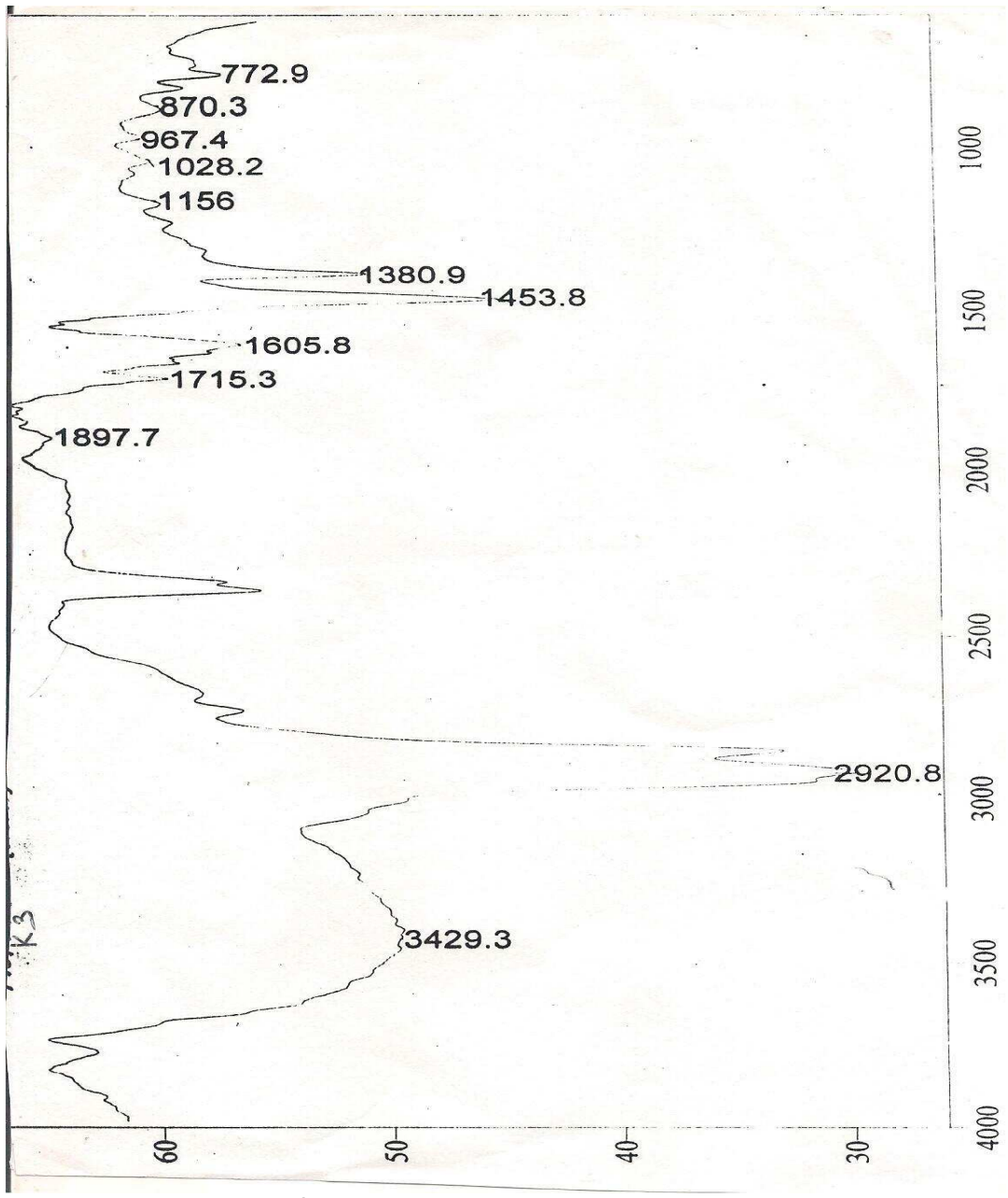

Fig3; IR analysis of the crude oil acted upon by *B. stearothermophilus*

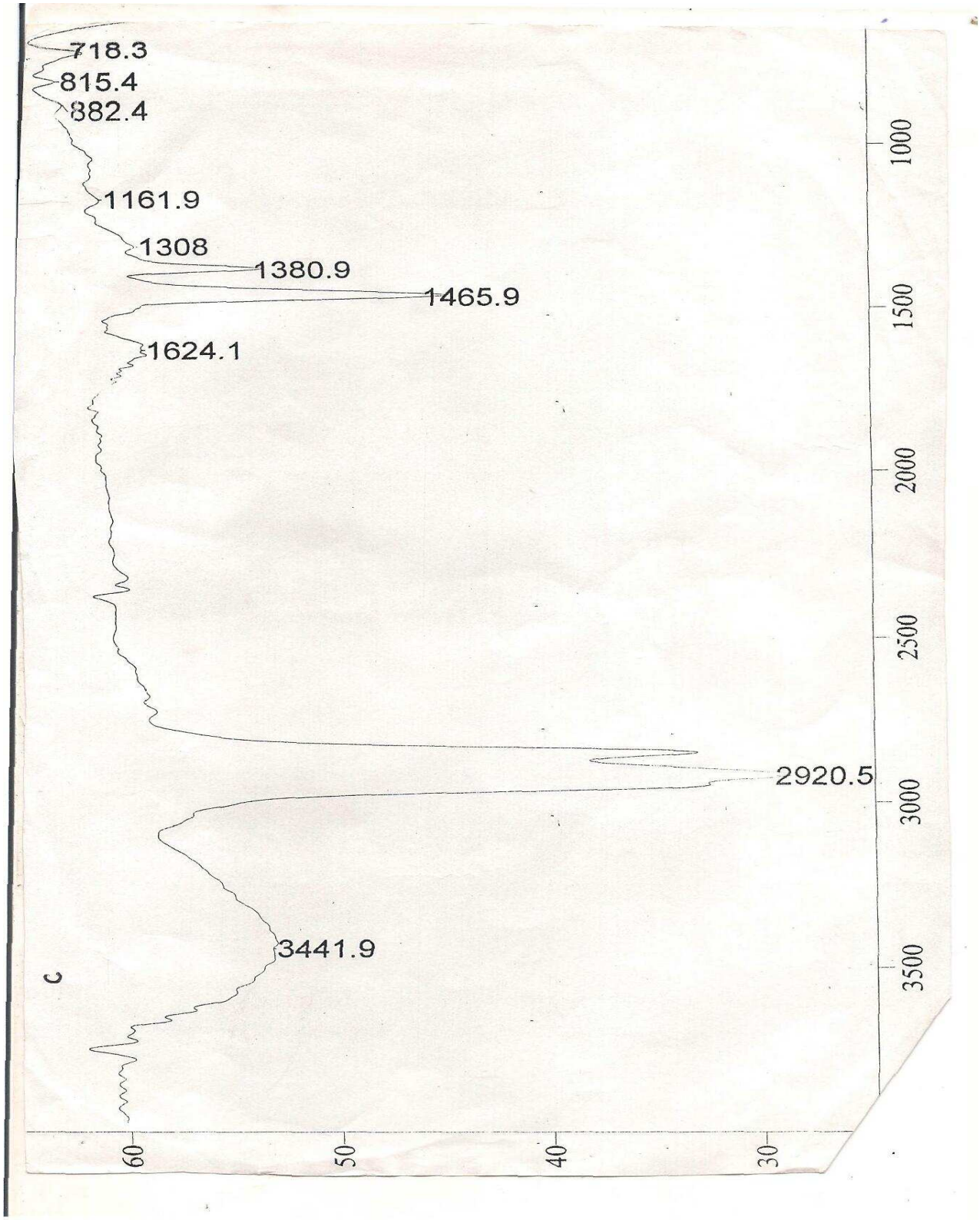

Fig. 4: control for IR analysis

Table 1 showed the pattern of reduction of crude oil in the mineral salt medium. The pattern of reduction differs from organism to organism. After 25 days of incubation the three bacteria isolates were able to degrade crude oil up to 70%, 58% for *P. putrefaciens , B. stearothermoplilus and S. faecium* respectively (Table 2). The results showed that the three bacteria isolates have varying ability to utilize crude oil as source of carbon in the MSM. This observation is in consonance with the report of Nwaogu *et al.*, (2008) that many microorganisms have different rate at which they utilize hydrocarbons in soil and water.

It has been documented in many studies that most potential bacteria for petroleum hydrocarbon degradation have been isolated from areas contaminated with crude oil (Chaerun *et al .*, 2004). Since all the bacteria isolates used in this study were isolated from crude oil contamimated soil, the innate potential of degradation exhibited by these bacteria isolates is in conformity with the reports of Chaerun *et al .*, (2004) and Vidalii (2001).

A large number of *Pseudomonas spp* capable of degrading polycyclic aromatic hydrocarbons have been reported in many studies (Johnson *et al.*, 1996, Kiyohara *et al*., 1992, Zhang *et a,l.q*, 2005). *Bacillus spp* capable of degrading hydrocarbon have also been reported (Nwagwu *et al*., 2008). In this study the ability of the bacteria isolates to degrade crude oil as revealed by the degradability test was in this order *P putrefaciencs > B stearothermophilus >S. faecium* with 70% > 58%> 48% on the 25th day of incubation respectively (Table 2).

High degradability potential recorded for *P. putrefaciens* is in agreement with the reports of Das and Mukhererjee, (2003); Antai and Mgbomo (1993).This may be due to the fact that *Pseudomonas spp* is a common bacterium capable of degrading hydrocarbons. Many studies have reported the efficiency of *Pseudomonas spp* in degradation of hydrocarbons such as benzenes (Munoz *et al.*, 2007), toluene, P- xylene (Yu *et al* , 2001), biphenyl (Ohta *et al.*, 2001) and phenol (Yuang and Tsai, 2006). Therefore encountering *P. putrefaciens* and its high degradability potential exhibited in this study is not rare.

However, degradation of crude oil by the 3 bacteria isolates was possible because microorganism have been reported to have enzymatic systems which empower them to degrade and utilize petroleum hydrocarbon as source of carbon and energy (Ijah and Antai, 1988; Antai and Mgbomo, 1993; Ezeji *et al*., 2005).Biodegradation of crude oil by microorganisms have been reported to be a natural process by which the bulk of the polluting oil is used as an organic carbon sources , causing breakdown of petroleum components to lower molecular compounds or transferred into the other organic compounds or into energy, cells mass and biological products (Chattre *et al*., 1996). Based on this assertion infrared analysis of the residual oil after 25 days of incubation was carried out.

The results of IR analysis showed that the residual oil from the bottles acted upon by *P. putrefaciens, B. stearothermophilus and S.faecium h*ave 17 bands, 16 bands and 12 bands respectively, meanwhile control has just 10 bands. This indicated that the bacteria isolates have modified the crude oil component (fig .1-4).

CONCLUSION

This study showed that *P. putrefaciens , B. stearothermophilus and S. faecium* isolated from crude oil contaminated soil have ability to degrade crude oil. *P putrefaciens* was able to degrade crude oil at highest percentage compare to other two isolates. Therfore, the three bacteria isolates used in this study could be relevant in the abatement of ecosystems that may be contaminated with hydrocarbons.

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