Isolation of naphthalene degrading bacteria from oily contaminated soils

Farah Tariq Abdul-Ridha **Naghem Adil Ghani** **Sura Ihasn Abed**
Babylon University, College of Science

Abstract

Twenty bacterial isolates capable of utilizing naphthalene , as the sole source of carbon and energy for growth were isolated from three oily polluted soil from different sites in Hilla city . By standard bacteriological methods , these bacteria were identified as belonging to the species *Pseudomonas aeruginosa Burkholderia cepacia* and *Aeromonas media*. The first two genera demonstrated the highest growth during incubation period as indicated by an increase in their optical density (OD_{600}) . There were also changes in the medium pH during seven days of incubation .

الخلاصة

تم عزل 20 عزلة بكتيرية لها القابلية على استهلاك النفثالين كمصدر وحيد للكربون والطاقة ، عزلت من ثلاث مواقع مختلفة من الترب الملوثة بالمشتقات النفطية في مدينة الحلة. وقد شخصت هذه العزلات باستعمال طرق التشخيص التقليدية وتبين انها تعود الى الاجناس .*Pseudomonas aeruginosa* و *Burkholderia cepacia.* و *Aeromonas media.* وقد اظهر النوعان الأولان افضل كفاءة . كما تبين النتائج زيادة معنوية في نمو البكتريا على مستوى ($p \leq 0.05$) بدلالة قيم الكثافة الضوئية (OD_{600}) وانخفاض قيم دالة الحموضة pH خلال فترة الحضانة البالغة (7) ايام .

Introduction

PHAs are hydrophobic organic pollutantes that are abundantly present in contaminated soil and give raise to envirommental concern because of their toxicity and mutagenticity. Polyaromatic hydrocarbons (PAH)are unique contaminates in the environment because they are generated continuously by the inadvertently incomplete combustion of organic matter , for instance in forest fires ,home heating , traffic , and waste incineration. PAH contaminated sites are mostly found in or near cities, thus representing a considerable public health hazard.

Aromatic hydrocarbons e.g. benzene, toluene, ethylbenzene and xylenes (BETX) compounds and naphthalene are widely used as fuels and industry.

Polycyclic aromatic hydrocarbons are ubiquitous contaminants of aquatic and terrestrial ecosystems whose presence is attributable to a number of petrogenic and pyrogenic sources, which had increased since the end of the second world war (Laflomme and Hite , 1978 and Jonsen *et al*., 2005) .

Lower molecular weight aromatic hydrocarbon, such as naphthalene (containing two benzene rings) , anthracene and phenarthene (both of which contain three benzene rings) are known to have health effects could be potentially hazards (Klaason, 2001) .

Some of PAHs are degradable by microorganisms and the important biochemical aspects of the PAH-degredation have been releaved. PAHs are nevertheless considered persistent pollutants in soil.Biodegredation using microorganism is usually the preferred not a major route of PAH removal from contaminated environments because of some inherent advantages such as its cost effectiveness and more complete cleanup (Pothuluri and Cerniglia , 1994). Moreover , the physical processes are often limited to aquatic environments only. Although some physical processes such as volatilization , leaching , chemical and photooxidation are often effective in reducing the environmental level of PA Hs (Bossert and Bartha , 1984 and Heitkamp *et al*.,1988).The microorganlams should possess all the necessary enzymes needed to degrade PAHs. It is known that selection or adaptation of PAHs degrading microorganisnis as with other chemicals occur as a result of their previous exposure to

this substances in the environment (Lewis *et al*., 1984 and Spain *et al*., 1980).However , these adaptations occur slowly, and usually depend on the recalcitrance or biodegradability of the partlcular substance involved (Spain *et al*., 1980). This is especially so considering that PAHs usually have low aqueous solubility and thus, are poorly available (low bloavailability) for microbial utilization. (Jonsen *et al*., 2005).

Material and Method

Collection of soil samples

About 5 g soil samples were asceptically collected from soils of safyaldeen fuels station , Ishtar fuels station and Hamorabi fuels station in Hilla city . All samples were placed into sterile polythene bags and stored at 4°c and they were brought to the laboratory immediately.

Isolation of bacteria from the soil samples

Bacteria were isolated from the soil samples using a mineral salts medium containing naphthalene . (Bushnell and Hass , 1941) the naphthalene was added after autoclaving the medium . The medium was first dispened in 100 ml volumes into 150 ml Erlenmeyer flasks and autoclaved at 121°c and 15 psi for 15 min.. Thereafter , 1.0g of each soil samples suspension was inoculated into each flask of the medium and incubated at 120 rpm at 30c° for one week in shaker incubater . After wards , 1.0 ml samples was taken from each culture and transferred into fresh mineral salts medium , followed by incubation described above for one week . The enrichment procedure was repeated for the third time, before their bacterial contents were isolated using a solid medium containing the mineral salts medium and 15.0 g/L of pure agar. Inoculated plates were purified by repeated by subculturing. Pure cultures obtained by this procedure were stored in slants of mineral salts medium with 15.0 g/L pure agar, and also in nutrient agar , and stored at 4°c.

Identification of isolates.

Twenty bacterial isolates which degrade naphthalene were identified to their species level using conventional microbiological and biochemical procedures. The tests were carried out according to the procedures described by Cowan and Steele (1974) , Cheesebrough (1998) and Bergey's manual of determinative bacteriology (1994) .

Determination of the bacterial efficiency to degradation of naphthalane.

The three isolates were inoculated into 250 ml flasks containing 100 ml of sterile mineral salts medium with different concentrations of naphthalane (50 ppm , 100ppm , 150 ppm , 200 ppm, 250 ppm , 300 ppm). The flasks were then incubated in shaker at 120 ppm at 30c° for 3 days . After 3 days , 5 ml sample was collected from each fask and assayed for OD at 600 nm using a spectronic (20 Baush and Homb company) (Nnamchi *et al*., 2006).

Effect of different concentrations of naphthalene on the growth of the isolates

The mineral salts medium (1800ml) was prepared in 2L flask and dispensed in 100ml volumes into eighteen 250ml flasks before autoclaving . The flasks were then divided into three sets of six flasks of each, the following concentrations of naphthalene were added to each of the three sets of flasks : 50 ppm, 100 ppm, 150 ppm, 200 ppm, 250 ppm 300 ppm . Each set of flasks were inoculated with one isolate . Inoculated flasks were then incubated for one week in rotary shaker at 121 rpm at 30 c°.

At 24 hours intervals, 5.0 ml sample aseptically was collected from each flask and assayed for the level of microbial growth by measuring the O.D at 600 nm in spectronic (20 Baush and Homb company) and pH using digital Bechman model-3500 / England.

Results

Identification of the isolates: The result showed that the isotates belonged to three bacterial species which were *Burkholderia cepacia* , *Pseudomonas aeruginosa* and *Aeromonas media*.

Determination of the bacterial efficiency to degradation of naphthalene.

The efficiency of naphthalene utilization by the three isolates as their sole source of carbon and energy when using the different concentrations (50 ppm, 100ppm, 150ppm, 200ppm, 250ppm, 300ppm) after 3 days of incubation is shown in fig-1-from the fig 1. it is seen that isolate , *Burkholderia cepacia* the better growing organism in naphthalene due to OD value which 0.61 at 600 nm. wavelength, whereas *Pseudomonas aeruginosa* peaked at an OD value of 0.42 .

Effect of naphthalene concentration on the growth of the isolates.

There was acorrespondingly higher growth of all isolates as the concentrations of naphthalene were increased from 50 ppm to 300 ppm (fig 2, 3,4) . It seen the lowest optimum growth of (OD 600) was observed when the concentration of naphthalene was lowest (50 ppm), while the highest optimum growth of was recorded when the concentration of naphthalene was highest (300 ppm). For all isolates.

Effect of different concentration of naphthalene on pH value .

Results of the effect of naphthalene on the growth of the isolates on pH illustrated in fig. (5,6,7) showed the decreasing of pH from a nearly neutral initial medium pH in almost all the concentralion to weakly acidic levels .The highest decreasing of pH medium naphthalene concentration 300ppm.

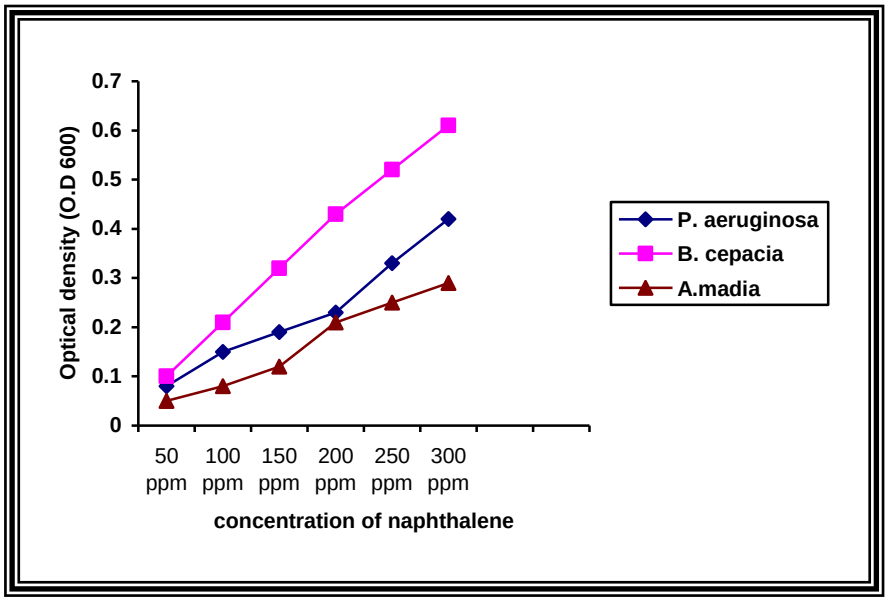

Fig. (1): Optical density of different concentration of naphthalene consuming by the bacterial isolates after three days of incubation

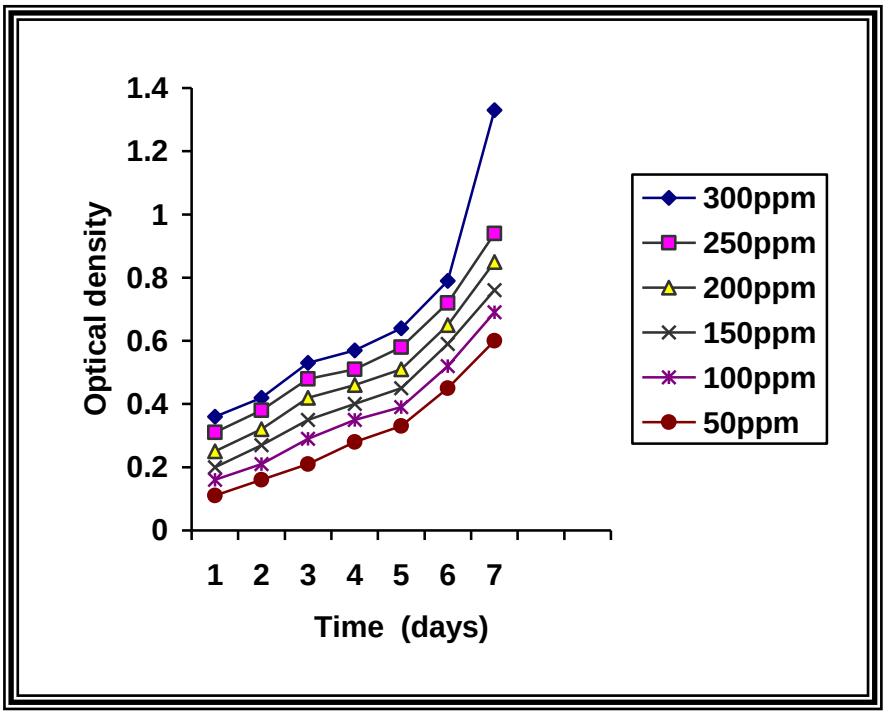

Fig .(2): Growth of *B. cepacia* on different concentration of Naphthalene during seven days of incubation

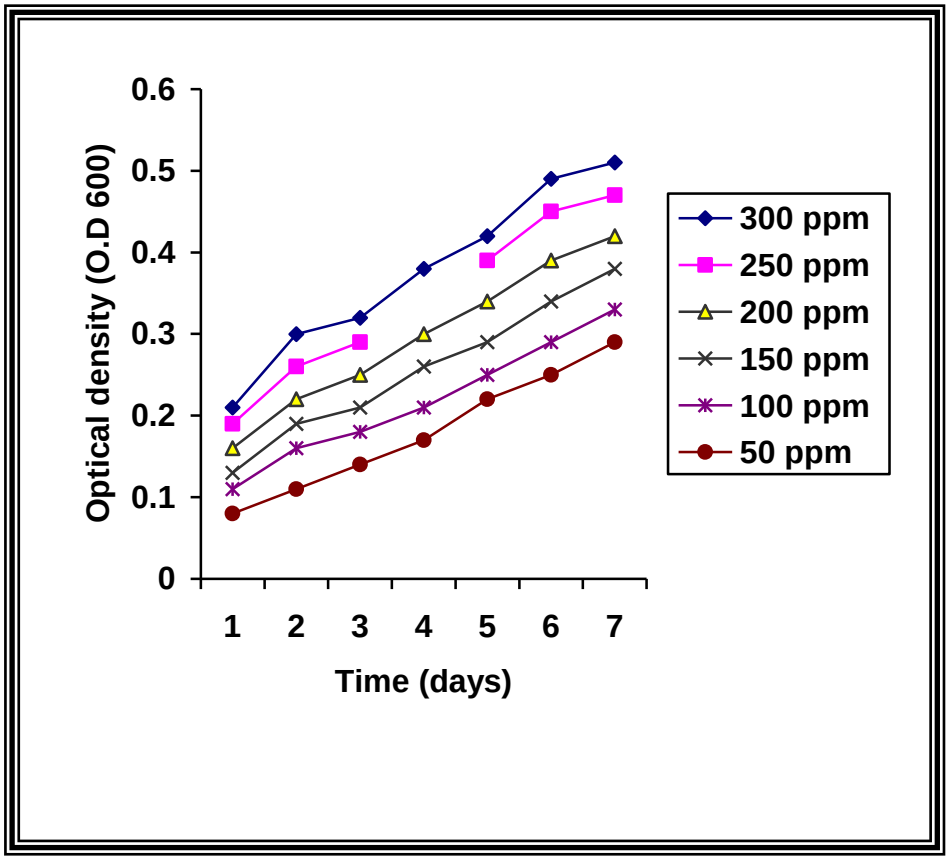

Fig.(3): Growth of *Ps. aeruginosa* on different concentration of Naphthalene during seven days of incubation

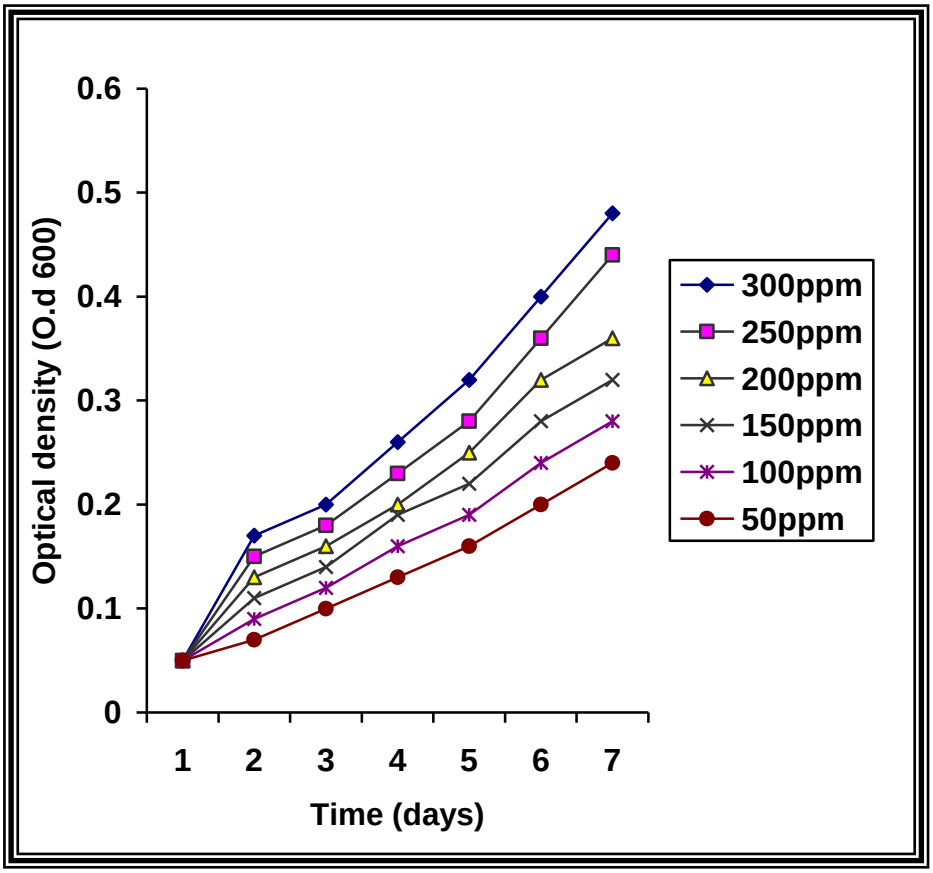

Fig. (4): Growth of *A. medis* on different concentration of Naphthalene during seven days of incubation

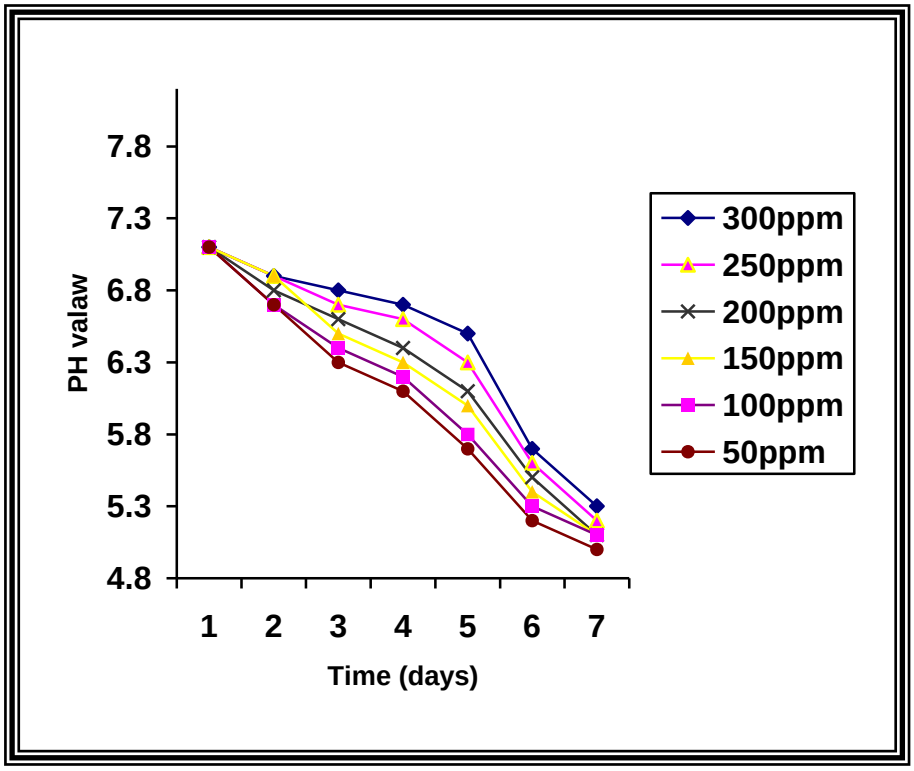

Fig. (5) Effect of different concentrations of naphthalene on pH value of the medium caused by *B. cepacia*

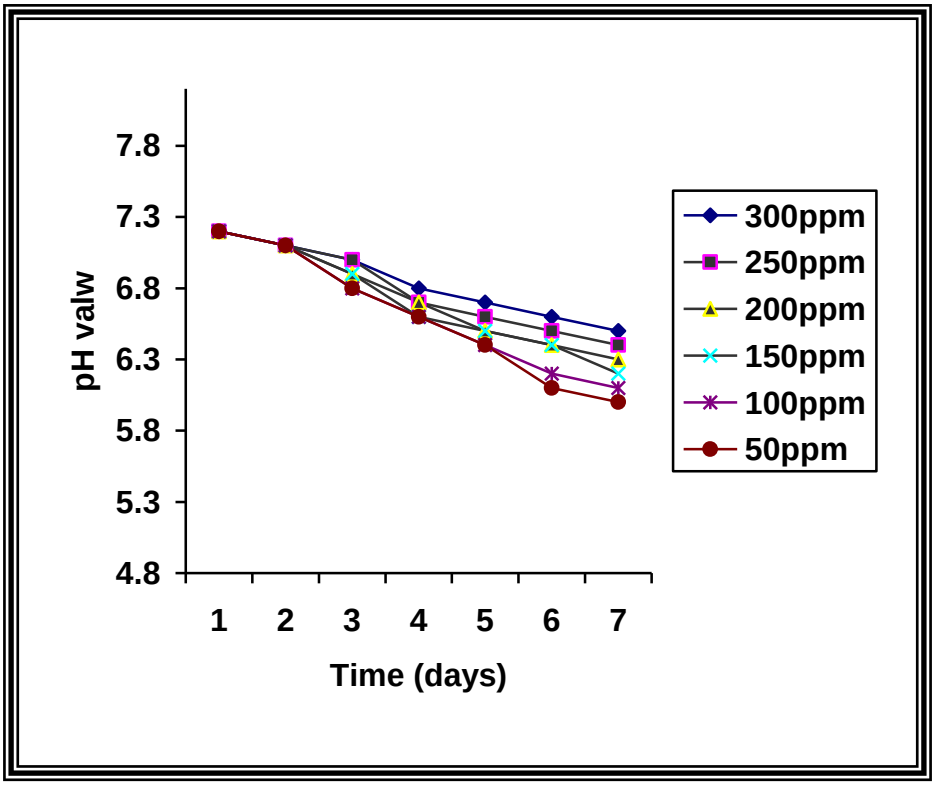

Fig. (6) Effect of different concentrations of naphthalene on pH value of the medium caused by *Ps. aeruginosa*

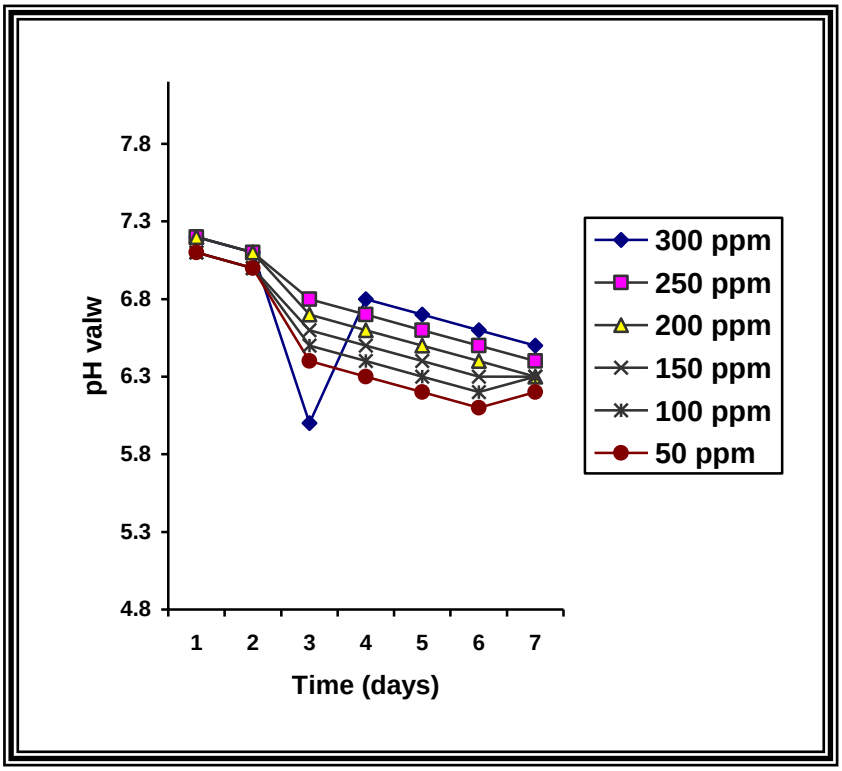

Fig. (7) Effect of different concentrations of naphthalene on pH value of the medium caused by *A. media*

Discussion

Atotal of twenty bacterial isolates were obtained and screened for their ability to utilize naphthalene as a sole source of carbon and energy .

These isolates were identified as *Pseudomonas aerguinosa* , *Burkholderia cepacia* and *Aeromones media* . This conforms to the high degradative ability and ubiquity associated with these bacterial types as it concerns biodegradation of both soil and water environments polluted with petroleum and its many derivative products (Atlas , 1995 ; Chaudhry , 1994 and Jonsen, *et al*., 2005).

This trait of *Pseudomonas* and *Burkholoderia* species both of which formely belonged to the genus *Pseudomonas* to grow on highly xenobiotic compounds(man – made organic compounds) is made possible by the weallh of catabolic enzymes they possess , and more importantly , by their immense capacity for adaptive change . It is belived that this adaptive capacity is promoted by their inherent patterns of regulation , which allows for the coincidental induction of different catabolic pathways , resulting in noval patterns of biodegradation (Ornston and Yeh, 1982).This probably explains why the two isolates not only grew on and hence metabolized naphthalene , which is considered the simplest and hence the easiest of all polycyclic aromatic hydrocarbons to degrade . The ability of *Pseudmonas* and *Burkholderia* species to degrade naphthalene and other PAHS had been reported by several workers (Catterall *et al*., 1971; Cenigilia , 1984 ; Heitkamp *et al*., 1987 ; Mueller *et al* ., 1990 and Kastner *et al*., 1994). The three isolated showed significant differences ($P \leq 0.05$) in concentration of naphthalene . This probably accounts for the slight fall in the pH of the medium during the growth course suggesting the possible production of acidic metabolites such as organic acids (Fig. 5,6,7) Alquali *et al*., 2005 found six different communities of microorganisms from soil samples of a petroleum contaminated site were isolated for their ability to degrade naphthalene of the thirteen selected naphthalene degrading bacteria belonging to *Rhodococcus* , *Arthrobacter* , *Nocardia* and *Pseudomonas* .

The results also showed that increases in the used naphthalene concentration proportionately increased the growth of microorganisms (Fig. 2,3,4) .This agrees with the findings of Bauer and Capone (1985) and Nnamcti *et al*., (2006) that PAH degradation generally increases with increases in the concentration of PAHs. Incidentally , it was observed that *Burkholderia cepacia* usually reached maximum growth at 300 ppm of naphthalene suggesting that higher concentrations of naphthalene usually gave higher bacterial growth this a grees with results of Nnamchi *et al*., (2006) who showed that though higher concentrations of naphthalene usually gave higher bacterial growth, this cessed when a threashold concentration was reached.

Many investigations have indicated that genes that encoded for naphthalene oxidation in *Aeromonas* are found on plasmids . There are three known plasmids that determine the degradation of naphthalene (Kiyohara and Nagao, 1978) , and this may demonstrate the ability of this genus to degrade naphthalene.

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