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Utilization of Hydrocarbons of Two Types of Crude Oil By the Action of One Species of Bacteria and Two Species of Fungi and Synergism between them.

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Abstract

This research includes many dimensions aims to know the range of capability of *Bacillus cereus* (Bc) and each of two fungi *Aspergillus niger* (An) and *Penicillium vendicatum* (Pv) to biodegrade each of Kirkuk crude-mid oil and Al-Qayarah crude heavy-oil by the action of above bacteria and two fungi and by synergism between them.

The results developed that capability are varied between each of bacteria and two fungi used in this research, so the percent of biodegrade of Kirkuk crude-mid oil were 63% by *B. cereus* and 57% by synergism between *B. Cereus* and *A. niger* and 54% by *P. vendicatum* and 53% by synergism between *B. cereus* and *P. vendicatum* and 52% by synergism between three microorganisms and 46% by synergism between *A. niger* and *P. vendicatum*, then the action of *A. niger* at the last stage by percent 38%.

The ability for the same microorganisms to biodegrade Al-Qayarah crude heavy-oil were differ from Kirkuk crude mid-oil. The synergism between *B. cereus* and *P. vendicatum* was 69%, synergism between the same bacteria and *A. niger* was 67% but the synergism between three microorganisms used in this research was 54%. The percent of biodegradation by *B. cerceus* was 36% and 32% for *P. vendiactum*, the synergism between two fungi *P. vendicatum* and *A. niger* were 31%, the last step was 29% by *A. niger*.

Results of viable counts reflex the ability of biodegradation of microorganism and adaptation for the new contaminated ecology, the analysis of two kinds of crude oil by Gas-chromatography determine the data in this research.

Introduction

Pollution caused by petroleum and its derivatives is the prevalent problem in the environment (Millioli, *et al.*, 2009).Environmental pollution occurred on both offshore and onshore when oil and products spills (parakash *et al.*, 2014).Contamination environmental is frequently associated with hydrocarbon pollution because of the increasing global demand for petroleum hydrocarbons and its products (Akpoveta *et al.*, 2011). A critical environmental impact of the petroleum industry is the contamination of soil by oil and other related products which are highly toxic and exhibit molecular recalcitrance (Husain *et al.*, 2011).

Some fractions of crude oil are toxic for living organisms, various microorganisms are able to use some crude oil fractions as sole carbon source (Sebiomo *et al.*, 2011).

Hydrocarbon degrading bacteria and fungi are mainly responsible for the mineralization of oil pollutants (Salam *et al.*, 2011)

Every natural system is self-purifying, this is made possible by the diverse micro and macro flora in soils, bacteria and fungi constitutes the greatest population in soils implying that in the case of pollution, microbes are the major agents of purification (Isinguzo and Bello, 2005).

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In modern time variant techniques (biochemical, physical, chemical) have been applied to degrade oils spills (Das *et al.*, 2014).

The biodegradation pattern is dependent on the local environment because, the susceptibility of individual hydrocarbons to biodegradation depends on the bacterial culture present (Skaare, 2007), The microorganisms may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated site, contaminant compounds are transformed by living organisms through reactions that take place as a part of their metabolic processes biodegradation of a compound is often a result of the actions of multiple organisms (Robles-Gonzalez *et al.*, 2008).

The list of microorganisms that are in oil-containing medium can not be completed because new populations and individuals with the new ways of metabolism and enzyme specifity are formed (Giedraityte *et al.*, 2001).

The microorganisms participating in the biodegradation of hydrocarbons are bacteria, fungi, yeasts, algae, the most important groups of microorganism involved in biodegradation of hydrocarbons are bacteria and fungi (Fogarasi, 2011).

There is no single strain of bacteria with the metabolic capacity to degrade all the components found with in crude oil. In nature, biodegradation of a crude oil typically involves a succession of species with in the consortia of microbes present. (Milak and Ahmed, 2012).

So this research aimed to show the ability of bacteria and fungi to determine their roles to decrease pollution by degrade crude oil in the environment because some microorganisms play an essential role in biogeochemical cycling, interference with microbial metabolic activities by pollutants in the environment can have far reaching ecological consequences (Obire and Anyanwu, 2009).

Materials and Methods

Samples: Samples of crude oil:-

Two kinds of crude oil were used in this research Kirkuk crude mid-oil and Al-Qayarah crude heavy-oil, Kirkuk crude mid-oil brought from pump station of crude oil in Baiji Refinery, but Al-Qayarah crude heavy oil from the wells of crude oil near Al-Qayarah Refinery. Used for bring samples glasses bottles size 200ml which were sterilized dark, marked, and good closed and kept the samples in these bottles in cold place for the time of using its.

Sterilized Kirkuk crude mid-oil by **(filtration)** using filter paper with pores size 0.45 nanometer (Kind GF/F) but Al-Qayarah crude heavy-oil Sterilized by **(tyndillation)** .

Contaminated soils samples were bought from Baiji Refinery and from AL- Qayara Refinery, all soils samples were collected in triplicates.

Samples of Microorganisms:

Used *Bacillus cereus* which isolated from the soil polluted with crude oil by API technique but samples of fungi received from College of Agriculture, Tikrit University.

Then repeated culturing of each of fungi on Malt Extract Agar (MEA) and culture at 30°C for 5 days after that transported the cultures on slants from the same media and kept the two samples of fungi in the refrigerator temperate for the time of used.

Print ISSN: 2073-8854 & Online ISSN: 2311-6544

Cultured or cultivated of microorganisms on two kinds of crude oil:

Used mineral salt media which prepared with (Sebiomo *et al.*, 2011). For cultivated biodegraded microorganisms, then separated 50ml of media in flasks 250 ml after sterilized the media and cooled, added two types of crude oil which sterilized by filtration and tyndillation by percent 3% (v/v) then inculcated some of flasks by *Bacillus cereus*. Another by *A. niger* and *P. vendicatum* and left another to control without inculcated.

The pH of the medium was adjusted to 7.2 and 5.6 respectively for bacterial and fungal estimation. All flasks incubated at 30°C for 28 days (Kastner *et al.*, 1994).

Determine viable count of microorganisms on two kinds of crude oil (Abioye *et al.*, 2009). Added 1.0 ml from of each of suspension of bacterial and two fungi which used in this research to the mineral salt media which contain 3% (v/v) of two kinds of oil each of them alone and synergism, the number of bacteria was determined by the plate method on nutrient agar (Milic *et al.*, 2009). Which contain each milliliter of 1×10^{-7} colony forming unit (cfu) and incubated, at 30°C for 28 days after that determine the viable count for three microorganisms weekly at zerotime, after (1, 2, 3, 4) weeks from the beginning of incubation by spread 0.1 ml of dilute solution 10^{-7} on plates of nutrient agar and incubated at 30°C for 24 hours for *B. cereus* and on Malt Extract Agar (MEA) and incubated of 30°C for 3-5 days for two fungi used in this research and determine the viable count for three microorganisms by cell counter.

Determine the percentage of loss quantity of two kinds of crude oils:

Gravimetric method: (Abioye *et al*, 2013). Determine the rate of biodegrade two kinds of crude oils by Gravimetric method by calculate the difference in quantity of two kinds of crude oil added to media (Mineral salt media). Which used before and after cultivated each of bacteria and two fungi in media contain crude oils were determind by weight method following extraction with diethlylether.

By use specific chemical method:- Gas liquid chromatography:

To sure of biodegradation of hydrocarbons of two kinds of crude oils by using gas liquid. Chromatography provided from American Varian Company, analysis as column OV-17 chromosorb W;

Length 30m, 2ml; detector, FID

Det. temp. 275°C; carrier gas: N_2;

Flow rate: 45ml/min

Oven program temperature:

Primary initial temp: 100°C

Initial time: 4 mint.

Ratio /c/ mint. 10°C/mint.

Final column temp I: 200°C

Canal column temp II: 250°C

Final time I and 2: 10 mint (Pathak *et al.*, 2011).

Statistical analysis: Analyzed by Excel and analyzed by statistical method (SPSS Inc).

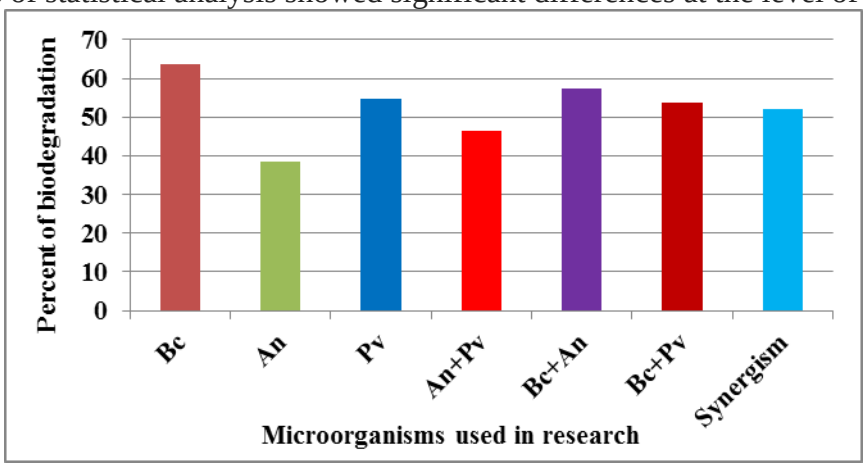
Print ISSN: 2073-8854 & Online ISSN: 2311-6544

Results and Discussion

Results of Kirkuk crude mid-oil :-

Results of current research showed that abilities of each of *B. cereus* and *A. niger* and *P. vendicatum* were differe of biodegradation Kirkuk crude mid-oil, *B. cereus* showed that ability to utilize Kirkuk crude mid-oil upper from each of *A. niger* and *P. vendicatum* was 63%, the lower value to utilize Kirkuk crude mid-oil was by *A. niger* 38%, synergism between *B. cereus* and *A. niger* 57%, utilize of the same crude oil by *P. vendicatum* was 54%, the synergism between *B. cereus* and *P. vendicatum* to utilize Kirkuk crude mid-oil was 53%, the percent of utilize this crude oil by synergism of all microorganisms used in this research was 52%, according to the results of utilization of Kirkuk crude mid-oil by two fungi was 46%. **Figure (1)** showed percentage utilization of Kirkuk crude mid oil by the action of three microorganisms used in current research and synergism.

Results of statistical analysis showed significant differences at the level of probability p≤o.o5.



(BC) B. cereus – (An) A. niger –(Pv) P. vendicatum

Figure (1) Percent of biodegrading of Kirkuk crude mid-oil by microorganisms used is this research

The results in this research exposed that *B. cereus* was the higher degrader of hydrocarbons of crude oil. Abioye *et al.*, (2009) observed that *Bacillus sp*. utilize crude oil at higher rate than other isolates.

So this research agreement with the findings of other researcher, e.g.,Milak and Ahmed (2012) indicated that *Bacillus sp*. was the dominant bacteria than the other genera of bacteria to utilize crude oil.

Bacillus strains were identified as efficient crude oil degraders (Ijah and Ukpe, 1992).

The results of present research was similar to findings of Sulayman (2009) which showed that *Kluyvera ascorbata* was capable to degrade Kirkuk crude mid-oil by percent 56% while *E. coli* degrade the same crude oil by percent 40% in concentration 2% v/v for the two bacteria. The **figures (2,3,4)** showed the viable counts of microorganisms used in this research, so, the **figure (2)** showed the relation between of viable counts of microorganisms and time of incubation and **figure (3)** showed the rate of viable counts of microorganisms, and **figure (4)** showed the rate of viable count and the time of incubation.

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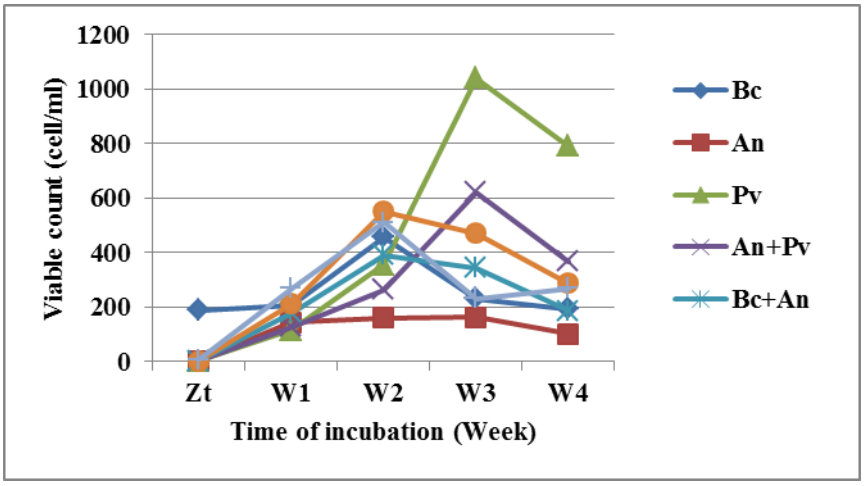
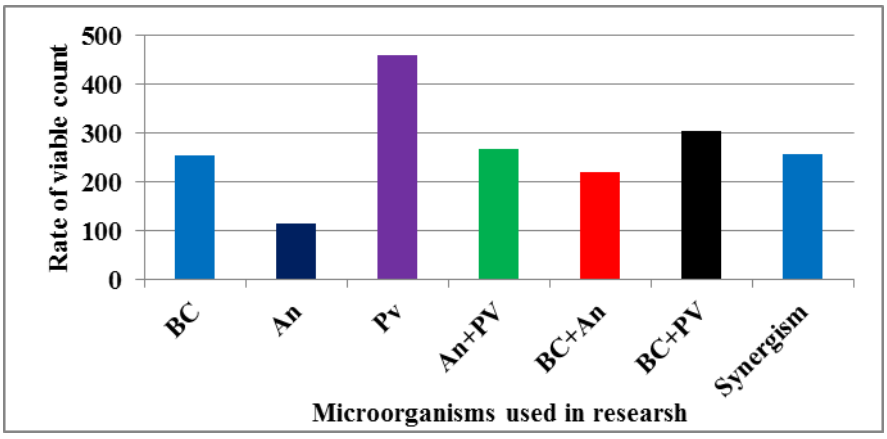


(BC) B. cereus – (An) A. niger –(Pv) P. vendicatum

Figure (2) Viable count (cell/ml) at time of incubation (week)

The viable counts of all microorganisms used in this research, reflex the degree of ability to utilize the hydrocarbons of crude oil, and had increased from the beginning of experiment, from zero time and increased at the first week of incubation and continuous increasing since the final of 3th week for *B. cerceus* but decrease for fungi.

So Nasir, (2000) solved that the ability of isolates of *Pseudomonas strains* degrade Basrah light crude oil by percent (82.1, 77.8, 75.7)% for three isolates of *Pseudomonas strains*, light crude oil degraded relatively faster by higher percent from mid and heavy crude oil.



(BC) B. cereus – (An) A. niger –(Pv) P. vendicatum

Figure (3) Rate of viable count (cell/ml) according to interaction between time and type of microorganisms

Some researchers indicate that bacteria and fungi are known to be principle a gents of hydrocarbons biodegradation (Saadoun *et al.*, 2008).

Hydrocarbons in the environment were biodegraded primarily by bacteria, yeast, and fungi (Das and Chanaran, 2011).Results of statistical analysis showed significant differences at the level of probability p≤o.o5

Damisa *et al.*, (2013), clear that *A. niger* can degraded crude oil after (16) days period of incubation by percent 53.7%.

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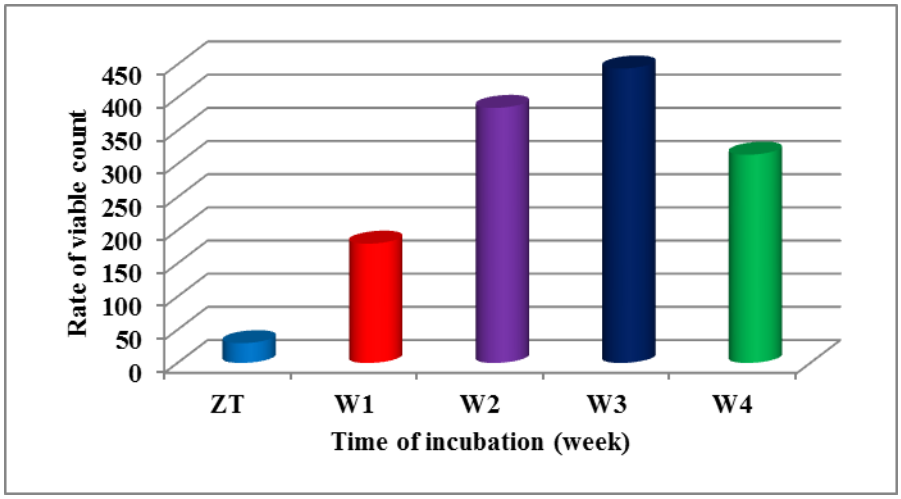

Figure (4) Rate of viable count (cell/ml) according to time of incubation

White rot fungi are increasingly been investigated and used in bioremediation because of their abilities to degrade an extremely diverse range of very persistent or toxic environmental pollutants (Isikhuemhen *et al.*, 2003).

The Results of statistical analysis showed significant differences at the level of probability p≤o.o5.

The results of analyzed by Gas Chromatography used as a modern technique to measure biodegradation of crude oil in soil, after (28) days growing of fungal strains in crude oil contaminated soil, revealed disappear many peaks when compared with untreated crude oil (Al-Jawhari, 2014).

We can recognize the amount of biodegradation in the sample which tested in this research **figure (6)** when compared with the controlled samples **Figures (5)**.

Gas Chromatography used to improve the biodegradation of crude oil, many components converted to an intermediate and other component converted to complex compounds as a result of uncontrolled reactions (Aboiye *et al.*, 2013).

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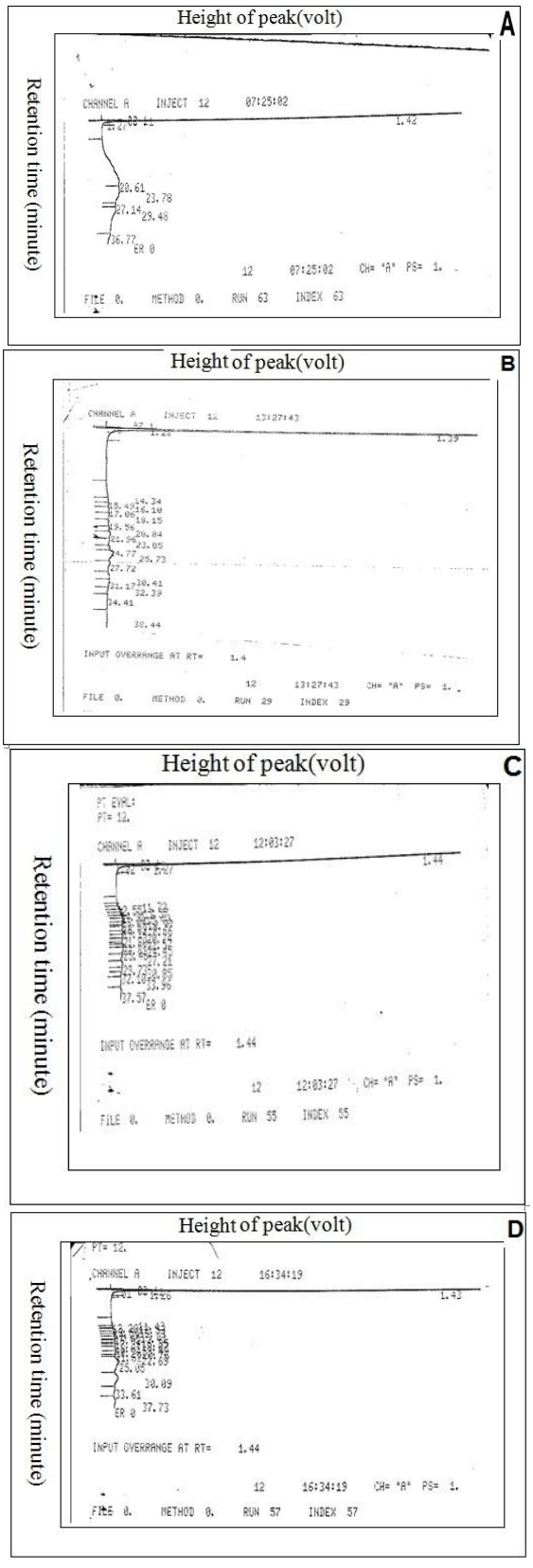

Figure (6) : Analysis of Kirkuk crude-mid oil by Gas Chromatography which incubated at (30 °C) for 28 days by action of :

A: *Bacillus cereus* , B: *Penicillium vendicatum*

C: *Aspergillums niger* , D: Synergism between three Microorganisms

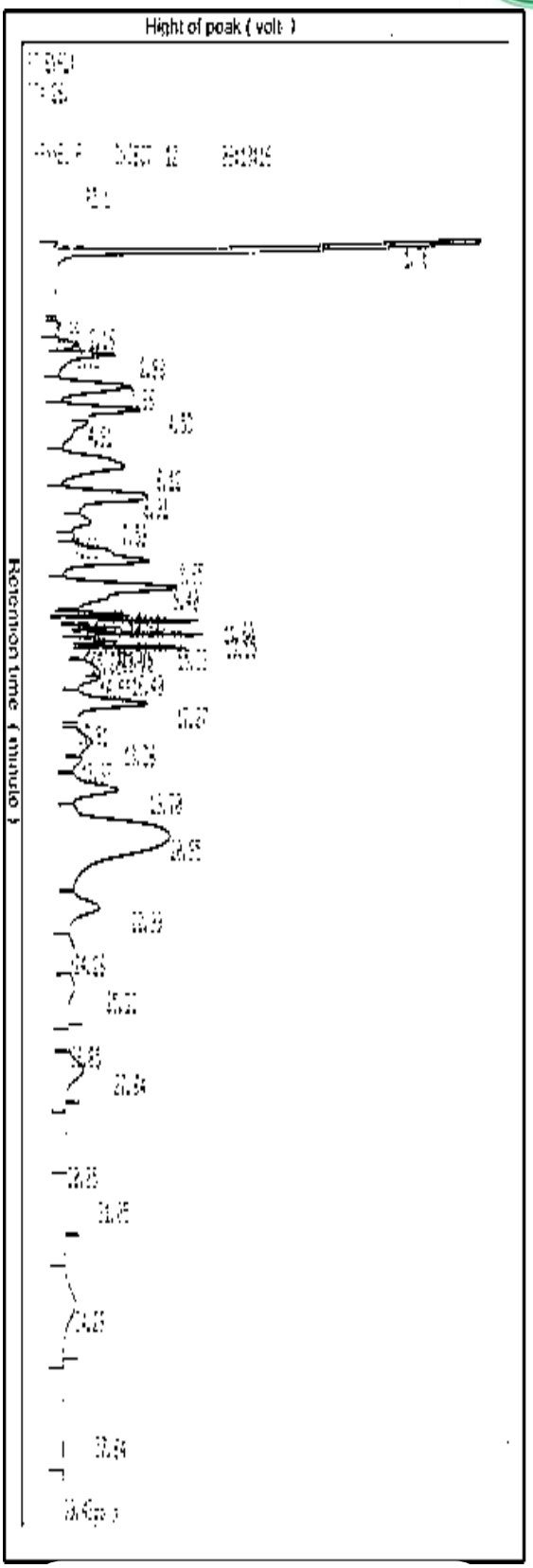

Figure (5) : Analysis of Kirkuk crude-mid oil (control) by Gas Chromatography which incubated at (30 °C) for 28 days.

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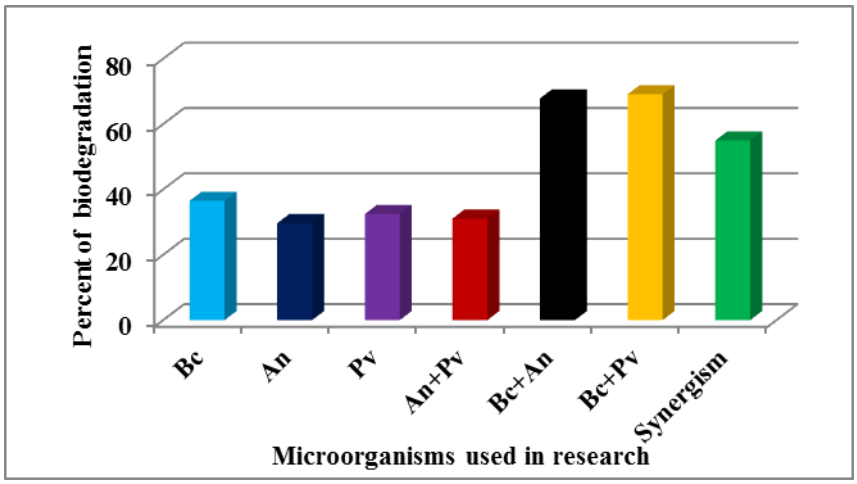
Results of Al-Qayarah Crude Heavy Oil :-

Then the figure (5) exposed the percent of biodegradation of Al-Qayarah crude heavy oil, so the synergism between *B. cereus* and *A. niger* and between the same bacteria and *P. vendicatum* and synergism between three microorganism appeared 69%, 67%, 54%, dramatically so that the signal ability to biodegrade heavy crude oil showed low percent 36% by *B. cereus* and 32% by *P. vendicatum* and 31% by synergism between two fungi used in this research so the last by percent 29% by *A. niger*. Results of statistical analysis showed significant differences at the level of probability p≤o.o5.



(BC) B. cereus – (An) A. niger –(Pv) P. vendicatum

Figure (7) Percent of biodegradation of Al-Qayarah crude heavy oil in this research

The rates of biodegradation vary greatly among the various components of crude oils and its products (Zehad *et al.*, 2010). In this research the results improved similarity with results of each of (Ston *et al.*, 1940) and (Jobson *et al.*, 1972) which they improved that the biodegradation of crude oil depend greatly on its density, so the light crude oil degrade by high percent compared with heavy crude oil which contain high percent of aromatic and asphalted compounds and degrade by low percent. In some studies the researchers found that *A. versicolor* and *A. niger* were which exhibited the fastest onset and highest extent of biodegradation of hydrocarbons (George-Okafor, *et al.*, 2009). (Chen, *et al.*, 2009) showed that inoculating fungal bacterial consortium removed 92.6% of hydrocarbons of crude oil. In present research improved that consortium between *B. cereus* and each of two fungi and between *B. cereus* and alone fungi removed the highest percent of hydrocarbons and remarked 69%, 67%, 54%, while the utilization of each alone microorganisms in this research showed low percent of utilization.

Sebiomo *et al.*, (2011) observed that all of bacterial and fungal isolates obtained in them research were *Pseudomonas sp*., *Bacillus sp*. and *Apergillus sp*. Were found to be more predominant in the polluted mechanic soils.

Bacteria play major role in hydrocarbon degradation. The reason for petroleum degradation is the ability of microorganisms to utilize hydrocarbons to satisfy their cell growth and energy needs (Helmalatha and Veeramanikandan, 2011).

Sathishkumar *et al.*, (2008) reported that increase of crude oil concentration decreased the percent degradation but an increase in the quantity of crude oil degradation was noticed.

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(90%) of crude oil concentration was biodegradable by the action of mixed *A. Niger* and *A. Fumigatus* after (28) days of incubation (Al-Jawhari, 2014).

The viable counts of all microorganisms used in this research, reflex the degree of ability to biodegrade the hydrocarbons of crude oil, and had increased from the first of experiment, zero time and increased at the first week of incubation and continuous increasing since the final of 2th week for *B. cerceus* but decrease for fungi, which increased viable counts at all the period of incubation for Al-Qayarah crude heavy-oil **(figures 8, 9, 10)**. In unpolluted ecosystems hydrocarbon utilizers generally constitute less than 0.1% of the microbial community while in oil polluted ecosystems they can constitute up to 100% of the viable microorganisms, the degree of elevation above unpolluted compared reference sites appears to quantitatively reflect the degree of extent of exposure of that ecosystem to hydrocarbon contaminants (Atlas, 1981).

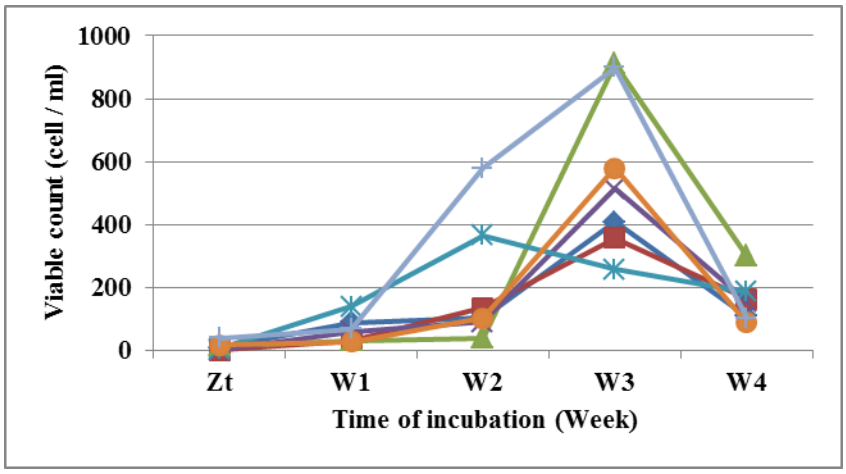
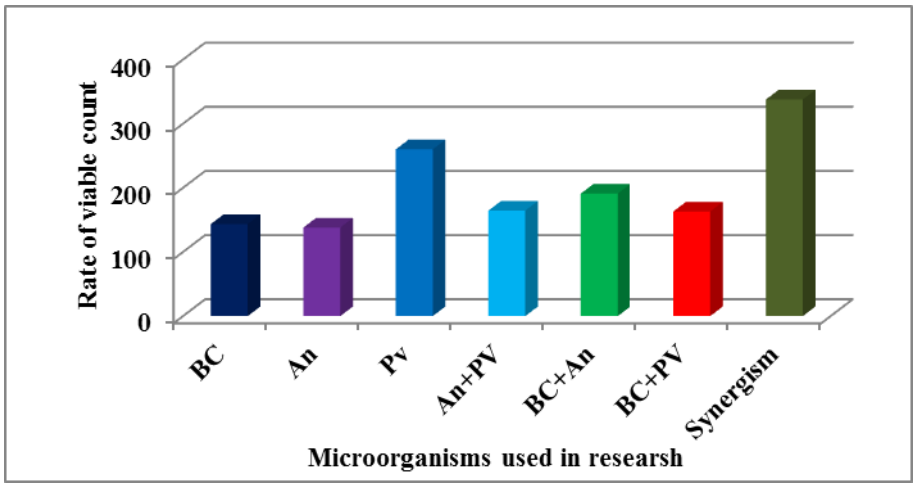


Figure (8) Viable count (cell/ml) at time of incubation (week)



(BC) B. cereus – (An) A. niger –(Pv) P. vendicatum

Figure (9) Rate of viable count (cell/ml) according to interaction between time and type of microorganisms

The growth profiles showed that none of strains of the bacterial and fungal used in this research exhibited the lag phase of growth in all the time of period of incubation at 30°C. This result was similar to the result of (Sebiomo *et al.*, 2011). Results of statistical analysis showed significant differences at the level of probability p≤o.o5.

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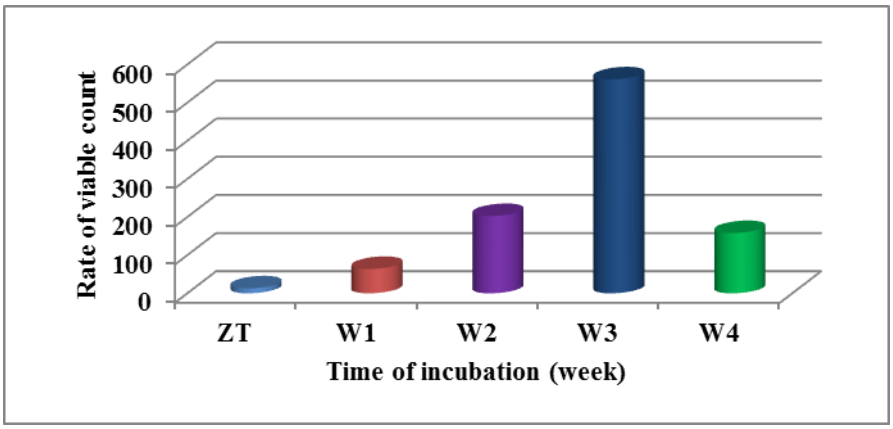

Figure (10) Rate of viable count (cell/ml) according to time of incubation

The total average counts of oil utilizing fungi were generally higher in polluted soils than in natural soil (0% or control) there was progressive increase in oil –utilizing fungi for 0.5% and 1% polluted soils to certain period, after which the oil –utilizing fungi entered the decline growth phase, some microorganisms are killed or inhibited by toxic fractions in the oil, while other heterotrophic organisms degrading oil are increasing in number, the toxicity of crude oil or petroleum products varies widely, depending on their composition and concentration (Obire and Anyanwu, 2009). Results of statistical analysis showed significant differences at the level of probability p≤o.o5.

All microorganisms used in this research improved their abilities to biodegrade crude heavy oil, Major peaks component of total hydrocarbons were biodegrade, this major peaks component reflects the biodegradation and improved the biodegradation by loss of weight of crude oil. Current research improved the disappearance of some fractions of crude heavy oil which analyzed by Gas chromatography **(figures 11, 12).**

Many microbial species have been implicated in crude oil degradation leading to a successful bioremediation of contaminated environment (Regina *et al.*, 2006).

Many scientist used this technicality to improve the range of biodegradation of crude oil by action of microorganisms.

The response of microorganisms to biodegrade heavy crude oil is slow when compared with light crude oil and oil sludge (Garapati, 2012).

Wasify and Hamed (2014), used (G-C) to improve the biodegradation as modern technique.

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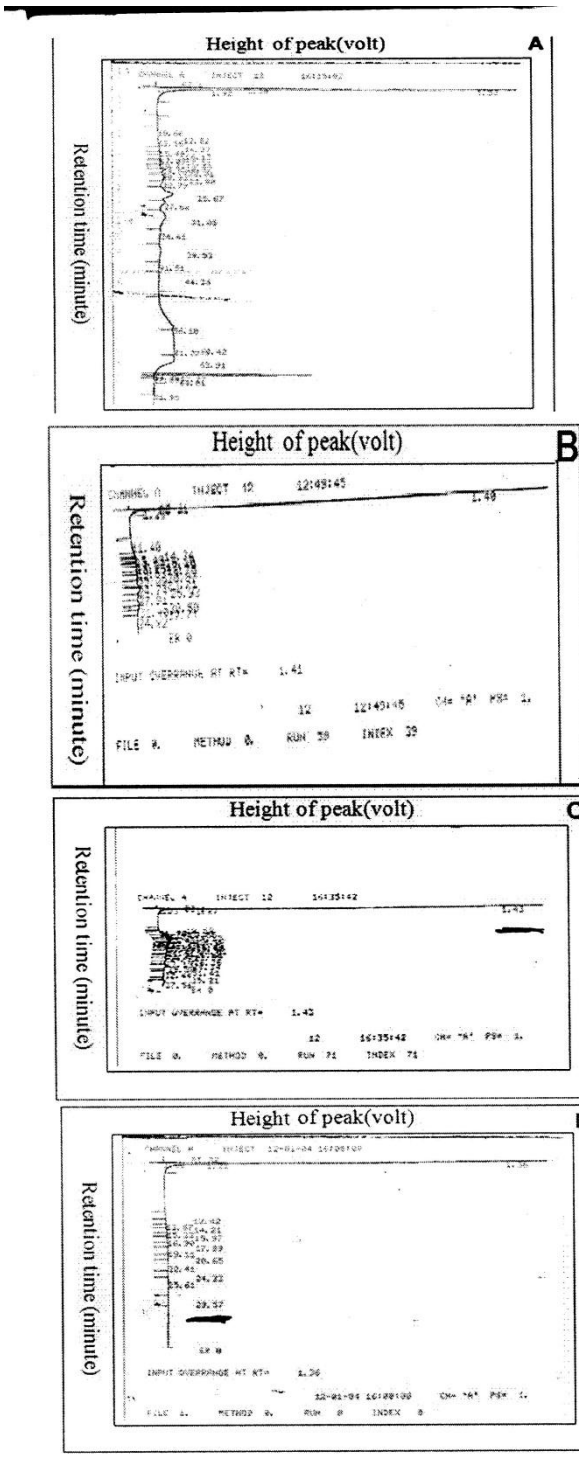

Figure (12) : Analysis of Al-Qayarah crude heavy-oil by Gas Chromatography which incubated at (30 °C) for 28 days by action:

A: *Bacillus cereus*, B: *Penicillium vendicatum*

C: *Aspergillums niger*, D: Synergism between three microorganisms.

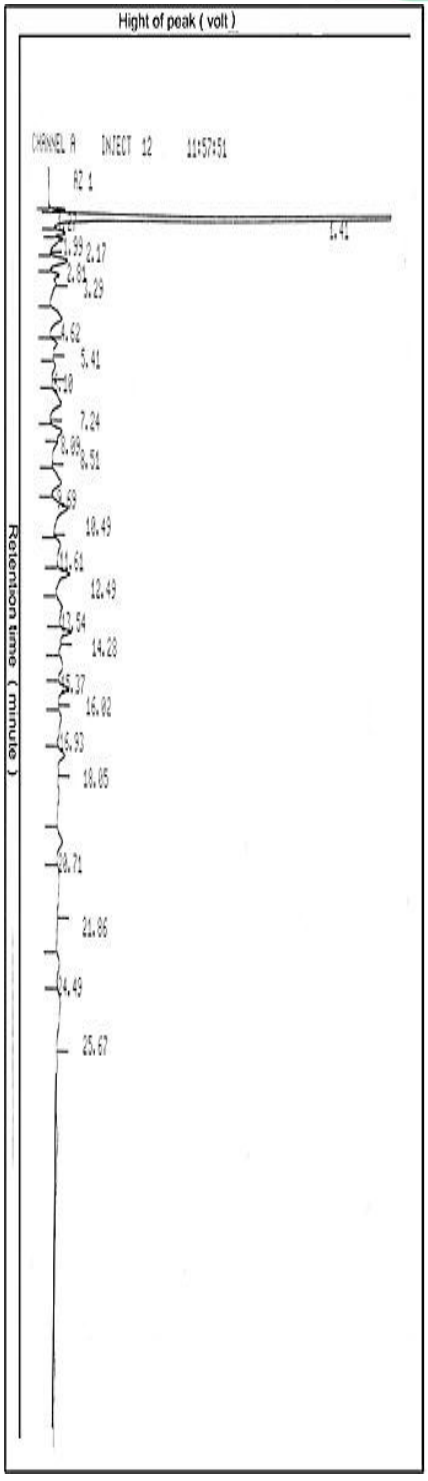

Figure (11) : Analysis of Al-Qayarah crude heavy-oil (control) by Gas Chromatography which incubated at(30 °C) for 28 days.

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