

### Preparation of biokit to treatment the Petrol spots in water.

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#### Abstract

Microorganism isolates used in plant protection were obtained from the College of Agriculture / University of Baghdad. Its purity was assured. The microbial biokit was prepared through using several unsatisfactory and environmentally friendly isolate. The bioactivity of biokit were studied in laboratory and showed susceptibility in removing contaminants. The results showed that the biomass of the isolates prior to the loading process on a medium containing crude oil as a single carbon source of 5 g/ L, which indicates growth ability and efficiency in the decomposition of pollutants when increase the isolates biomass more than 3 g / L, as well as the appearance of turbidity in the media compared to the control sample (media without oil), and obtained bioemulsifier with a concentration of 2.3 g / L. The chemical analysis of the samples under study was carried out using Gas Chromatography (GC) and GC-MS compared with a non-treatment (control).

The results showed the appearance of 43 topographic peaks each indicating a chemical compound in the control sample, while a number of these peaks disappeared and a space was reduced in other peaks that refers to the concentration of compounds, in the biomass sample, and reached the number of peaks in 26, indicating the efficiency of the biokit in the oil decomposition and dismantling of hydrocarbons. The increase in the number of live microorganisms and the incubation period is more than 28 days by increasing the growth intensity in the third week may be due to decomposing oil compounds and their exploitation in nutrition, where crude oil provides a source of carbon and energy for microorganism's isolates. In other results showed that the mass of crude oil was converted to a mass of gel and emulsification compared to the control sample after seven days period at a temperature of  $28 \pm 2^\circ\text{C}$ , Which is characterized by low toxicity and easy decomposition and reduces the surface tension of hydrocarbons and makes them more solubility and preparation for the dismantling of life and increase the rate of disintegration. The biokit was tested for retention in storage conditions for a period of one year.

The results showed that the viability of the biokit was effectively compared to the isolates

at alone prior to the production of the biokit, which showed poor storage capacity. We conclude that biomass has the potential to treat hydrocarbons (oil contaminants) in the environment.

**Keywords** Preparation, biokit, treatment, petrol spots, water.

### **Introduction**

Oil contains many organic substances that are toxic to organisms. The most dangerous of these compounds is benzopyrene, where it is a carcinogenic that can cause the death of aquatic organisms. The toxicity of crude oil depends on its chemical and physical properties, quantity, seasons of the year and other environmental factors. Crude oil contains many other toxic compounds such as gasoline, which are the most carcinogens, Ether and gasoline are toxic compounds at high concentrations, while phenol and its derivatives cause allergy when inhaled [1]. Oil floats on the surface of water forming a thin insulating layer between the water and the air. This layer spreads over a large area of the surface water (one liter of leaking oil in the river covers an area of more than 4,000 meters square of surface water) where this layer prevents the gas exchange between air and water and prevents dissolving of oxygen in the water, which affects the gas balance. The spread of oil on the surface water can be affected by several factors, including the nature of oil, prevailing winds, waves and river currents and its strength. Oil compounds take a long time to get rid of them, where they are stored in the liver and fats of marine animals and these have long-term effects that appear on the human body after several years [2].

Usually a large mortality recorded among fish as a result of occurrence of heavy crude oil, the damage remains for a long time and affects the eggs and larvae of many commercial fish, as well as the damage reaches to the phytoplankton and algae, which is the primary responsible for the stabilization of energy in the marine environment (through the process of photosynthesis) which is considered a food source to marine animals directly or indirectly [3].

There are several ways to combat oil pollution, include mechanical and chemical methods, but this solution is not substantially a treatment for the problem, because the arrival of these materials to the bottom can cause the destruction of fish, snails and sandworms, Thus, this method will increase the complexity of the pollution problem. Therefore, biological treatment has emerged where it can treat the oil pollution in water by using biological decomposition. Where scientists found a number of microorganisms that have the ability to analyze oil materials and convert it to very fine drops in water. Some oil companies and specialized chemical

laboratories in western countries have used these microorganisms extensively in treating oil stains in the seas and oceans that's caused by leaching of oil tankers [4].

A number of microorganisms Isolated and they have proved a highly efficient in the degradation of different types of hydrocarbons; their species are returned to bacteria, fungi and yeast. Biodegradation has many benefits, where it is an inexpensive method and does not require complex equipment; in addition it is a cheap method compared to other treatment methods. Microorganism are able to exploit carbon compounds in oil as a source of energy [5].

So, this study aimed to produce local kit that are safe and friendly to environmental, composed from several microorganism isolates that are capable of degraded crude oil by loading them on chemical salts to increase their efficiency in degradation and reducing oil pollution in the water, as well as increase the storage period for a relatively long time compared to the global product with low cost and ease production.

## **Materials and Methods**

### **1- Micro-organism that analyze hydrocarbons:**

Microorganism isolates were obtained from the Department of Plant Protection, Faculty of Agriculture/ University of Baghdad which identified by the same Department. Their purity was verified using some preliminary and biochemical tests and save until being use.

### **2- Preparation of Culture media:**

Mineral Salt Medium (MSM) which used for growth of microorganism isolate according to [6], Modified Mineral Salt Medium (MMSM) used to encourage oil emulsion process which leads to oil degradation this media prepared according to [7], Lactose- broth used to activate the preserved isolate and check its purity [8], also Nutrient Agar was used for growth of microorganism isolate, this Nutrient Agar was made by HIMEDIA Indian company and prepared according to manufacturer instructions.

### **3- The effectiveness of biokit:**

#### **A. preparation of biokit.**

Take the 100 g of  $\text{CaCO}_3$  and sterilized by oven under  $160^\circ$  for one hour and left to cool, and then distributed in a shallow pot sterile and 100 ml from fermentation media that prepared previously, which contain the mixed microorganism at 48 age were put. The fermentation media was prepared according to [9]. Pots that are containing  $\text{CaCO}_3$  and fermentation media was transferred to the oven at  $35^\circ$  for 5 days to dry well then crashed under sterilize conditions.

### B. Estimation of the microorganism density in 1 ml from the biokit.

The Microorganism density was calculated in 1 ml according to [10].

### C. Estimation of the amount of bioemulsions produced by Microorganism

The sample of crude oil was obtained from Kirkuk oil field and has the following characteristic in table (1).

**Table (1) characteristics of Crude oil from Kirkuk oil field**

API 60 F°	34.1
Density 15 °C	0.8540
Salt content	0.0012
Water and sediment Vol %	0.05

50ml of sterile liquid MMSM had been vaccinated by biokit, 2 ml of crude oil were added (two replicate) , the bottles were incubated in vibration incubator in 30°C at 150r/min for 27days, then the amount of bioemulsions was calculated according to [11].

### D. The a viability of the biokit for storage.

After the completion of the production process for biokit it was store in sterilize container under lab conditions, after one year the density of microorganisms was estimated in 1g of the kit, in addition to study the morphological changes in crude oil degraded by the action of biokit through the incubation of the kit for one week at room temperature and recording the morphological changes.

#### 4- Estimation of the quantitative loss of crude oil as a result of use the biokit

##### a. by using the Non-specialized chemical methods.

Test the viability of the kit to degrade the crude oil through:

##### 1. Account of Biomass:

50 ml of sterile liquid MSM was vaccinated by microorganism isolate that's consisting the biokit after it's grow on activated media, then added 2 ml of crude oil (Three replicate) in 250 ml conical Flask (two replicates) . Flasks were placed in vibration incubator at 150 r / min in 30° c for 27 days, the same procedure was done for control sample. The dry way method was used to estimate the weight of biomass. The broth put in a special tube and then expelled by Centrifuge at 12000 r / min for 30 minutes to precipitate the cells. Cells were extracted and dried at 105 °C for 24 hours in a thermal oven [12].

## 2. Account the quantitative loss of crude oil: as a result of the growth of microorganism isolate that's consisting the biokit.

The pure isolate that's consisting the biokit that's wanted to testing their ability to degradation the crude oil was planted as mentioned in pervious section (Account of Biomass) and compared with the control sample (media without microorganism), the filtrate was collected with hexane extract after cell sedimentation then the pH was adjutancy to reach 2 by adding a few drops of hydrochloric acid. Hydrocarbons were extracted by adding Chloromethane solvent by using separating funnel to separate the water layer from dissolving hydrocarbons. The last layer was filtrated through filter paper that contains anhydrous sodium sulfate ( $\text{Na}_2\text{SO}_4$ ), the filtrate was collected in a clean flask, and then the filtrate was concentrated by rotary evaporator, the sample was weighted to calculate the remaining weight of hydrocarbons, the percentage of degradation rate was determined according to [13] through the equation below:

$$\text{PRD CO} = \left( \frac{\text{remainder weight of the ceude oil}}{\text{Weight of crude oil of control sample}} \right) \times 100$$

### b. Detection of crude oil degradation using specialized chemical methods.

#### 1. Detection the degradation of crude oil by using of Gas-Chromatography GC

The final product from the previous paragraph (Account the quantitative loss of crude oil) was taken to measure the degradation level of the crude oil components that degraded by using Gas-Chromatography device that's providing by Shimadzu Japanese company model 2014 Using a chromatographic separation column type Capillary column CPSIL5-CB, 0.4 ml from each sample was injected in the device; the condition that's used for operate the device was as the following; the nitrogen was utilizes as Carrier gas with 25 ml/min Flow rate, temperature of the detector (FID Detector) was adjusted at 270 °, separated column was prepared at 80° as initial temperature and then programmed to raise the temperature at a rate of 5 c°/min until the temperature reach to 260° after 60 min [14]. The peak of used compounds were compared with peaks of standard material that appear at the same Retention Time, the area was calculate to estimate the Quantity of remaining hydrocarbons after microbial degradation according to following equation:

$$\text{area} = \frac{1}{2} \text{Base} \times \text{height}$$

Remaining Percentage of hydrocarbons =  $(\text{sample area})/(\text{total area}) \times 100$  [11].

## **2- Quantitative and Qualitative analysis of the effectiveness of biokit.**

The Quantitative, Qualitative and the level of the degradation of crude oil compound before and after the activity of the biokit with 28 days of incubation was detected by utilize of Gas-Mass Chromatography device that's providing by Shimadzu Japanese company model 2014 type Agilent GC-MSD (6890N-5973) [15] at 80° in 4 min with raising rate 5° per minute until the temperature reach 250° and maintained this temperature for 20 minutes.

## **Results and Discussion**

### **1- Certainty tests for the microbiological isolates that constitute biokit.**

The morphological tests showed microorganism isolates that's constituting the biokit and represented by bacillary shape are; Gram positive, spores former, producing crystals protein urea, in addition to their resistance to antibiotic (Ampicillin and carbancillin) and positive to catalase test. Other test results biokit that the biokit; it are; Gram negative, motile, non-hydrogen sulfide production, glucose fermented, positive for oxidase and catalase tests and other characteristic.

### **2- Estimation of the density of microorganism per 1g of biokit.**

The density of microorganism was calculated in 1 g and reached  $6 \times 10^7$  cell/g, which considered a very good density and indicate to; they are not affected by antibiotics (Amoxilin) ,great compatibility and affinity between the microorganism isolates in the biokit, which eventually refer to the possibility of using them in experiments of crude oil degradation.

### **3- Estimation the amount of bioemulsions producing**

After Estimation the density of microorganism, the next step will be estimation the amount of bioemulsions produced microorganism isolates in the biokit which reach to 2.3g/l Which indicates a high production efficiency biokit for bioemulsions Compared to microorganism before loading which reach to 1 g/l.

### **4- The availability of the biokit for storage.**

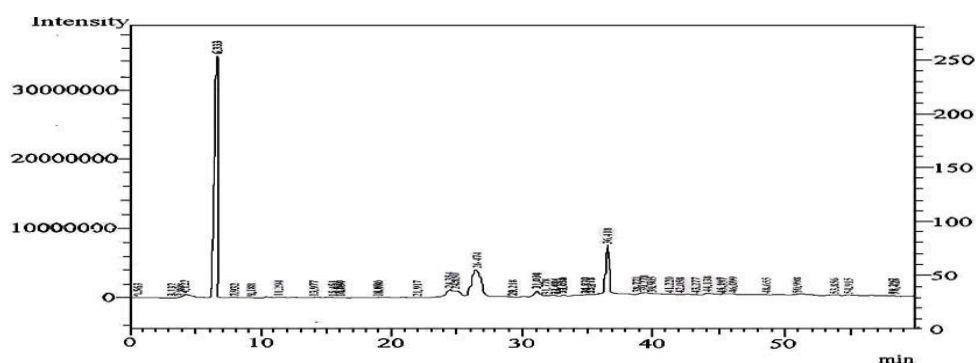
After one year of storage the density of biokit are calculated and reached to  $4 \times 10^6$  cell/g in 1 g, and estimate the amount of bioemulsions produced by the biokit that reached to 2.10g/l which indicate to presence of high density of microorganism and high efficiency in production of bioemulsions after the storage and the biokit are not significantly affected by the storage process.

### 5- Detection of crude oil degradation by Non-specialized chemical methods.

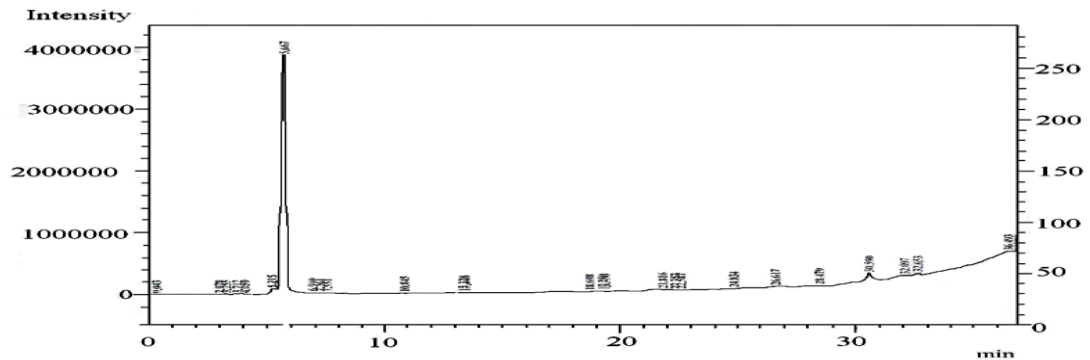
It can be test the ability of the kit for the degradation of crude oil through calculation of quantitative loss of crude oil, which reached after the use of the biokit (after loading) 80% Compared to the quantitative loss of crude oil before the loading process 49%. In addition to calculate the biomass which is a non-specialized chemical methods where the total biomass of the kit before loading 1.25 g, while the biomass of the kit reached after loading process 5 grams, which demonstrate the high efficiency of the effectiveness of the biokit in the degradation of crude oil and by doubling the effectiveness of the microorganism isolates before loading process.

### 6- The effectiveness of the biokit in the degradation of oil contaminants:

A chemical test was done for the studied samples by using the technology of Gas-Mass Chromatography (GCMS), samples where compared to un-treated a sample with biokit (control), The results showed the appearance of 43 peaks, where every peak indicating a chemical compound in the control sample (untreated crude oil). On other hand, when examining a sample treated with biokit a large number of these peaks disappeared where the number of peaks reached 26 and the area of other peaks are reduced (indicating the concentration of compounds) indicating to the efficiency of the biokit in the analysis and degradation of hydrocarbon compounds, Where many studies indicate the use of this technique to detect the degradation of oil compounds and to infer the type and concentration of these decaying compounds using chromatography technique-mass (GCMS) as mentioned in table (2, 3) and figures (1, 2, 3).



**Fig. (1) Results of gas-mass chromatography (GCMS) for the control sample (untreated crude oil) where show the retention time and the peaks of the chemical compounds that's constituent it.**



**Fig. (2) The results of a gas-mass chromatography (GCMS) for a crude oil sample that's treated with biokit which show decreasing in the peak number of chemical compounds and their area.**

**Table (2) Results of the Gas-Mass Chromatography (GCMS) for Crude Oil Samples before and after Treatment the samples with biokit.**

Crude oil sample before treatment with biokit			Crude oil sample after treatment with biokit		
Peak	Ret. Time	Area	Peak	Ret. Time	Area
1	0.563	18808	1	0.043	1212
2	3.132	5835	2	2.978	1203
3	3.808	134580	3	3.255	10853
4	4.122	12371678	4	3.712	7180
5	6.333	310450093	5	4.059	4244
6	7.932	5118	6	5.215	1922879
7	9.188	1517	7	5.667	34695432
8	11.294	10668	8	6.910	2038
9	13.977	2213	9	7.261	53400
10	15.451	1803	10	7.551	12919
11	15.825	2133	11	10.845	1012
12	15.946	3016	12	13.328	8683
13	16.057	2285	13	13.440	2330
14	18.886	97014	14	18.698	1780
15	18.980	4639	15	19.250	30285



16	21.917	156114	16	19.356	4202
17	24.354	48849407	17	21.816	1159549
18	24.850	35435168	18	22.353	38286
19	26.474	198956651	19	22.587	1934
20	29.218	545744	20	24.834	104246
21	31.034	26164157	21	26.617	659437
22	31.778	523092	22	28.479	67382
23	32.484	6081651	23	30.590	2062876
24	33.058	11223506	24	32.097	985026
25	34.830	19583428	25	32.653	689069
26	35.218	4718906	26	36.493	1084836
27	36.418	175276423			
28	38.771	13703335			
29	39.278	23285230			
30	39.905	13834088			
31	41.220	10681237			
32	42.058	8476367			
33	43.277	4271413			
34	44.138	15004804			
35	45.197	405091			
36	45.357	2050375			
37	46.099	6056145			
38	48.655	1066996			
39	50.998	4780445			
40	53.856	44450			
41	54.915	1959886			
42	58.297	1569			
43	58.428	3726			



(A) Control Sample

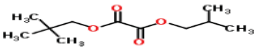

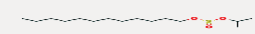
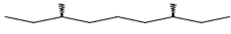

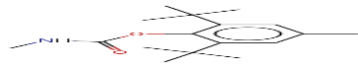
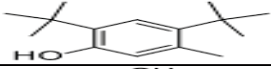
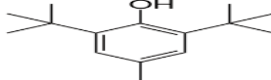



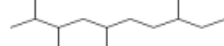
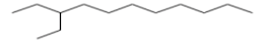
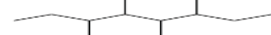

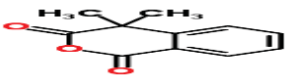
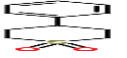

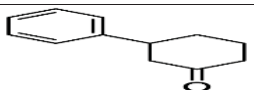


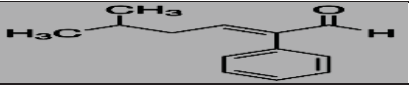
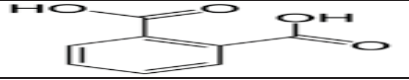
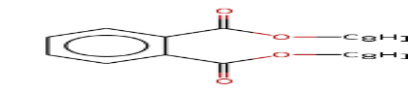
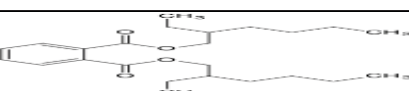
(B) Sample treated with biokit

Fig. (3) Showed the morphological changes in the degraded crude oil by the action of biokit showed clear degradation.

Table (3) Illustrate the hydrocarbons compound degraded by the action of biokit through the results of the gas-mass chromatography.

Compound name	Chemical structure
Butanoic acid, 1-1-dimethylethyl ester	
Benzene, (2-methyloctyl)	
Boron, (3,3-dimethylbutyl)(n-methylenemethanamine)	
Benzeneacetic acid, heptyl ester	
Oxalic acid, butyl propyl ester	
Propane, 1-bromo-2,2-dimethyl	
Acetic acid, butoxyhydroxy, butyl ester	 unknown chirality
Hexane, 2,-dimethyl	
Tert-butyl acrylate	
1-cyclopentyl-1-2,2-dimethyl-1-propanol	
Sulfurous acid, octyl 2-propyl ester	
Nonane, 3,7-dimethyl	

Oxalic acid , isohexylneopentyl ester	
Sulfurous acid ,nonyl 2-propyl ester	
Sulfurous acid ,decyl 2-propyl ester	
Sulfurous acid , octyl 2-propyl ester	
Nonane ,3,7-dimethyl	
Oxalic acid , isohexylneopentyl ester	
Phenol,2,6-bis(1,1-dimethylethyl)-4-methyl,methylcarbamate	
4,6-di-tert-butyl-m-cresol	
Butylated hydroxytoluene	
Cyclopropanecarboxylic acid,1-hydroxy	
Phenol,4-6di(1,1dimethylethyl)2-methyl	
Decane,3,7-dimethyl	
Decane,2,3,5,8-tetramethyl	
Undecane ,3-ethyl	
Octane,3,4,5,6-tetramethyl	
Undecane,3,8-dimethyl	
Benz[c]pyran-1,3-dione, 4,4-dimethyl	
3-phenylthiane,s,s-dioxide	
1,2,4-metheno-1H-cyclobuta[cd]pentalene-3,5-diol,octahydro	
Cyclohexanone, 3-phenyl	

5-methyl-2-phenyl-2-hexenal	
1,2-Benzenedicarboxylic acid	
1,2-Benzenedicarboxylic acid, diisooctyl ester	
Bis(2-ethylhexyl)phthalate	

### Conclusion

- 1- Production of a new microbial biokit that are introduced for first time as a bio factor in the analysis and degradation of hydrocarbon waste.
- 2- The ease of production of biokit at relatively low costs and high productivity and relatively with short periods of time.
- 3- The new microbial kit has effective bioactivity compared to the foreign product.
- 4- Production materials are locally available, friendly for environmental and public health and relatively inexpensive.
- 5- Storage capability is relatively high up to more than a year.
- 6- The potential of self-purification for the biokit that grow on oil contaminants.

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