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Role of Advanced Oxidation Process using the Ultra Violet in the Biodegradation Capacity of Egyptian Crude Oil Wax

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ABSTRACT

In a trial to reduce the wax content in one of the local Egyptian crude oils (Elsalam) collected from Khalda company (western desert), the wax biodegradation capacity of ***Bacillus liechiniformis*** was kinetically studied. Different concentrations of organic fertilizer (Rice straw extract), and the photocatalyst (FeOH) separately and in combined formula were used. The maximum biodegradation capacity was achieved at 5 ml rice straw extract / 100 ml produced water and 30 mg photocatalyst /100 ml produced water. The data of Gas chromatography showed significant reduction of wax content in the treated cultivated crude oil than those of untreated oil and barrel oil. The paraffin fractions (C21-C30) were obviously decreased in cultivated crude oil than those in both untreated oil and barrel oil. Where as the lighter fractions (C13-C20) were increased more than in cases of both untreated oil and barrel oil that may be attributed to the degradation of longer hydrocarbon chains to lighter by the bacterial action and the photooxidation process. In case of isoparaffins there was a marked increase of light hydrocarbon chains (C13- C16) & (C20-C21) and slight decrease of (C26- C30) in cultivated crude oil, that may be attributed to the degradation of polyaromatic hydrocarbon to opened chained isopraffins.

KEYWORDS

Hydrocarbons, Biodegradation, Bacteria, Photooxidation.

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INTRODUCTION

The oil industry is currently Suffer from high levels of dissolved paraffin wax in the oil which induce precipitation of crystals. These crystals lead to the formation of paraffin wax deposits because of temperature and pressure reduction, and the escape of light alkanes to the atmosphere (**Azevedo and Texeira, 2003**). Furthermore, the crystals attach to the walls of the oil wells, the casing wall, pump, and other production equipment, which reduces the mobility of oil and blocks the transport in the pipelines **(Leorpz, 2004 & Huang *et al.*, 2010).**

As traditional methods of wax removal (mechanical, thermal, and chemical) are problematic (**Towler *et al.*, 2011)**, microbial method has been considered as an alternative Microbial remediation which utilizes microbes or their metabolic **(Ayejina *et al.*, 2011).**

Etoumi (2007) described the use of *Pseudomonas* for mitigation of wax precipitation in waxy crude oils. *Pseudomonas* exhibited an ability to emulsify kerosene at 90.0% activity, and thus may serve as a good candidate for mitigating paraffin deposition.

Some byproducts (e.g. surfactants and paraffin solvents) could be applied in the oil and gas systems to prevent and eliminate the paraffin damage **(Lazar *et al.*, 1999).**

This work aimed to study the application of advanced oxidation process using UV radiation combined with the biodegradation of waxy crude petroleum oil in produced water.

MATERIALS AND METHODS

Paraffin wax

The crude oil wax used is El Salam crude oil which is collected from khalda petroleum company and characterized by the following: Specific gravity at 15/4°C = 0.92, pour point = 62°F, flash point = 363 °F, viscosity at 100°C = 4-5 Cst.

Photocatalysts

Ferric hydroxide powder with a surface area of 40 m^2 $g^{-}1$, was used as supplied. Chemicals were obtained from Sigma Aldrich in highest available purity (~99%).

Medium used for isolation and preservation of petroleum utilizing bacteria

Mineral silica-gel-oil-agar (MSOA) (Zakaria, 1998).

The medium contained NaH_2PO_4 0.042 g, KNO_3 0.9 g, and agar 20 gm added to 1000 ml of filtered clean produced water, 0.5 g of paraffin oil which was previously adsorbed on 15 gm activated silica gel was incorporated into the above constituents before autoclaving. The use of silica gel as hydrocarbon carrier agent has been shown to improve the reliability of procedures for enumerating hydrocarbon utilizers **(Zakaria, 1998) and (Elshahawy, 2007).**

Medium used for studying kinetic of Hydrocarbon degradation (Fertilized sea water) (Elshahawy, 2007).

The medium contained NaH_2PO_4 0.042 g and KNO_3 0.9 g added to 1000 ml of filtered clean produced water, waxy oil was added as sole carbon source at concentration of 400 ppm.

The Microbial isolate used for the wax oil degradation

Bacillus licheniformis isolate previously isolated from Suez Gulf sea water was identified according to API system and Bergey's manual of systematic bacteriology in faculty of agriculture- El Fayoum university **(Elshahawy, 2014).**

Identification of bacterial isolates by 16S rRNA gene

The bacterial isolate were identified by 16S rRNA gene according to the following steps:

DNA extraction using protocol of Gene Jet genomic DNA purification Kit.

The bacterial cells (up to $2x10^9$) were harvested in a 1.5 or 2 ml micro centrifuge tube by centrifugation for 10 min at 5000 x g. The supernatant was discarded and the pellet was resuspended in 180µl of digestion solution then 20µl of Proteinase K Solution were added and mixed thoroughly by using the vortex to obtain a uniform suspension. The genomic DNA was isolated using Gene Jet genomic DNA purification Kit.

PCR Amplification

The 16S rRNA gene was amplified by polymerase chain reaction (PCR) using Maxima Hot Start PCR Master Mix (Thermo K1051). PCR performed using the recommended thermal cycling conditions outlined below:

Step	Temp °C	Time	Number of Cycles
Initial denaturation / enzyme activation	95	10 min	1
Denaturation	95	30 s	35
Annealing	65	1min	
Extension	72	1 min30s	
Final Extension	72	10 min	1

Finally sequencing to the PCR product was made using ABI 3730xl DNA sequencer using forward and reverse primers.

Forward primer (5′ to 3′): AAGCAACGCGAAGAACCTTA

Reverse primer (3′ to 5′): AGAGTGCCCAACTGAATGCT

Preparation of concentrated rice straw extract (Bioferitilizer)

Fifty grams of grinded rice straw was soaked in 1 liter distilled water and sterilized by the autoclaving at 121 ^{O}C for 20 minutes. The autoclaved mixture was filtered using whattman filter paper 42.

Determination of the photocatalyst different concentrations effect on the degradation of the waxy oil

The Ferric hydroxide was added to the sterile cultivation media at concentrations 10, 15, 20, 25, 30 mg/100ml in triplicate microcosms for each concentration, the microcosms exposed to UV lamp (Medium Bi-pin (2 Pin), Double Ends - G13 Base - 30 Watts - 35.22 Inch Length Lamp) for 6 hours, and incubated for 168 hours at sterile conditions. The residual oil concentration was determined kinetically along 168 hrs.

Determination of the rice straw extract optimum concentration for the maximum microbial isolate biodegradation capacity

The biodegradation capacities of the hydrocarbons degrading isolate were determined by cultivation of the tested isolate in (250 ml microcosm) containing 100 ml of produced water with 4 g/100 ml wax oil, fertilized by the rice straw extract (instead of above mentioned inorganic nitorogen and phosphrous sources) at concentrations 1, 2, 3, 4, 5 ml / 100ml of produced water. The biodegradation kinetics was determined by monitoring kinetically the residual hydrocarbons by solvent extraction method using 25 ml Chloroform along 168 hours **(Elshahawy, 2014)**

Untreated sterile control was used to follow up the abiotic degradation.

The treated and untreated sterile control flasks were incubated on shaker at room temperature about 25-30 °C and 150 rpm with day and night periods.

Determination of the photocatalyst different concentration effect on the biodegradation of the waxy oil

The biodegradation capacities of the hydrocarbons degrading isolate were determined by cultivation of the tested isolate in 250 ml microcosm containing 100 ml of produced water with 4 g/100 ml wax oil, fertilized by the rice straw extract at concentrations 5 ml / 100 ml of produced water with different concentrations of the photocatalyst 10, 15, 20, 25, 30 mg/ 100 ml culturing broth medium.

Untreated sterile control was used to follow up the abiotic degradation.

The treated and untreated sterile control flasks were incubated on shaker at room temperature about 25-30 °C and 150 rpm with day and night periods.

Effect of the optimum photo-catalyst and Rice straw extract concentrations on the microbial isolate biodegradation capacity in crude oil

The biodegradation capacities of the hydrocarbons degrading isolates were determined by cultivation of the tested isolate in 250 ml microcosm containing 100 ml of produced water with 4 g/100 ml crude oil, fertilized by 5 ml of prepared rice straw extract and 300 mg of photocatalyst per one liter of produced water.

Chromatographic analysis

The residual hydrocarbons in the extracted crude oil hydrocarbons cultivated with 50 ml/ rice straw extract and 300 mg of photocatalyst per one liter of produced water, untreated control oil, and barrel oil were analyzed to determine its fractions ratios (paraffins, isoparaffins and unresolved complex mixture) via Gas Chromatography (GC) using nitrogen as carrier with a rate of (6 psi) (pound/square inch). Temperature program was 80- 320 ^{o}C at a rate of 3^{o} C/min., The injection port and flame ionization detector (FID) temperature were 300 ^{o}C and 325 ^{o}C, respectively.

RESULTS and DISCUSSION

The Microbial isolates used for the wax oil degradation

Bacillus licheniformis isolate were previously isolated from Suez Gulf sea water was identified according to API system and Bergey's manual of systematic bacteriology at faculty of agriculture- El Fayoum university **(Elshahawy, 2014).**

Identification of bacterial isolates by 16S rRNA gene

The gene sequence of that isolate were used for phylogenetic tree construction and it can inferred from that phylogenetic tree that the bacterium isolated from oil contaminated soil contains ***Bacillus licheniformis***. The DNA which was isolated from the culture was amplified using 16S rDNA universal primers and sequenced for the identification of bacterial strain at molecular level. ***Bacillus licheniformis.***

The isolated thermophillic bacteria ***Bacillus licheniformis A10*** was grown on diesel oil. It can be inferered that the growth of bacteria can be directly related to the degradation of diesel oil **(Malik and Ahmed, 2012).**

Liu *et al.* (2012) reported a ***Bacillus cereus Y-1*** exhibiting excellent paraffin removal capacity in three test wells in Daqing Oilfield, China. Strain Y-1 efficiently reduced the freezing point of crude oil and prolonged the period of thermal cleaning.

Determination photocatalyst different concentration effect on the degradation of the wax oil.

The data illustrated in Table (1) revealed that the

hydrocarbon degradation capacity increased by the increase the photocatalyst concentration. The maximum capacity was achieved at 30 mg/100ml of photocatalyst after 72 hrs incubation.

Photocatalytic decay profiles of polycyclic aromatic hydrocarbon (PAH) benzo[a]pyrene (B[a]P) have been investigated on various synthesized iron oxides (haematite, magnetite, akaganeite and maghemite) and on soil surfaces under a set of diverse conditions **(Himanshu and Bina, 2015)**.

Table (1) *Effect of photocatalyst different concentrations on the degradation of the waxy oil.*

Time (hrs)	Residual Waxy Oil (gm)					
	Photocatalyst Concentration (mg/100ml)					
	Control	10	15	20	25	30
0	3.967	3.86	3.855	3.858	3.862	3.857
12	3.876	3.386	3.183	3.121	3.015	2.714
24	3.865	3.386	3.015	2.935	2.573	2.449
48	3.833	3.351	3.024	2.82	2.44	2.369
72	3.769	3.236	2.935	2.776	2.4	2.3
144	3.743	3.218	2.926	2.776	2.405	2.334
168	3.621	3.28	2.909	2.714	2.414	2.369

Determination of the Rice straw extract optimum concentration for the maximum microbial isolate biodegradation capacity.

The obtained data is represented in Table (2). The data showed that the capacity of oil biodegradation increase kinetically with time and with increase of Rice straw extract concentration, where the high significant capacity was occurred at 5 ml/100ml after 48 hrs and slightly increase with prolonged time up to 168 hrs.

Initially, rice straw substrates contained 25.9% of cellulose, 26.9% of hemicellulose, and 13.9% of lignin + cutin, **(Malek *et al.*, 1994)**

Rice straw biochar on soil contaminant biodegradation and microbial community compositions were investigated in the laboratory during a 180-day period. he results of soil microcosm experiments showed that contaminant degradation efficiency was significantly higher in soils amended with biochar than in soils without **(Qin *et al.*, 2013).**

Table (2) *Effect of Rice straw extract at different concentration on the degradation of the wax oil.*

Time (hrs)	Residual Waxy Oil (gm)					
	Rice Straw Extract Concentration (ml/100ml)					
	Control	1	2	3	4	5
0	3.986	3.997	3.855	3.858	3.862	3.857
12	3.885	3.884	3.528	3.271	3.059	2.741
24	3.876	3.865	3.4	3.068	2.776	2.193
48	3.883	3.833	3.395	3.024	2.6	2.139
72	3.799	3.769	3.3	2.891	2.5	2.033
144	3.765	3.743	3.183	2.723	2.378	1.989
168	3.689	3.621	3.147	2.7	2.369	1.812

Several studies showed positive effects of biochar on microbial degradation. For example, **Bushnaf *et al.* (2011)** found that a 2.0% biochar amendment could accelerate polycyclic aromatic hydrocarbon (PAHs) biodegradation rate, while **Vasilyeva *et al.* (2006)** reported that AC amendment accelerated biodegradation of 3,4-dichloroaniline (DCA) in sandy soils.

Determination of the different concentrations photo-catalyst effect on the maximum microbial isolate biodegradation capacity.

The obtained data illustrated in Figure (1) showed the obvious synergic effect of photocatalyst and rice straw extract on the biodegradation capacity of ***Bacillus licheniformis.*** The biodegradation capacities showed a marked increase by prolonged cultivation time period among all the applied concentrations. Biostimulation using organic substances such the poultry manure, cow dung, biochar and food waste were more effective in optimizing the process of bioremediation. Aerobic degradation process is the most viable technique for field application of bioremediation of soils **(Clarkson and AbuBakar, 2015).**

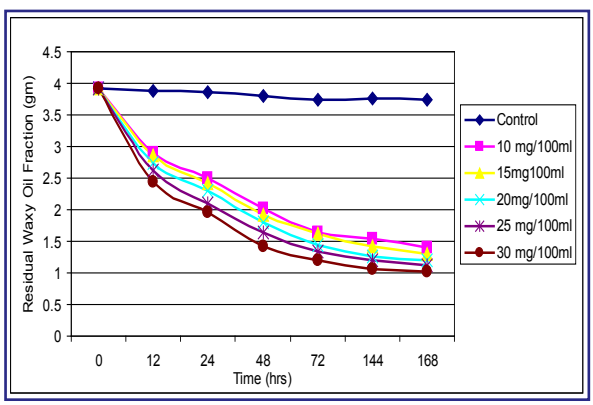

Fig. (1): Effect of the different concentrations photo-catalyst for the maximum microbial isolate biodegradation capacity.

Chromatographic analysis

Figure (2) illustrate the fraction percentage of the residual hydrocarbons in the extracted cultivated crude oil (containing 100 ml of produced water with 4 g/100 ml crude oil, fertilized by 5 ml of prepared rice straw extract and 300 mg of photocatalyst per one liter of produced water) , untreated control, and barrel crude oil the data show obvious decrease of unresolved complex mixture (UCM) which includes the aromatics, and cyclic hydrocarbons in case of extracted cultivated crude oil, decrease of fraction percentage of both paraffins and iso-paraffins that may be attributed to the effect of microbial degradation of praffinic content compounds these results agree with **El-Motaium (2006) .**

The distribution curves of paraffins and isparaffins for extracted cultivated crude oil, untreated control, and barrel crude oil were illustrated in Figures (3) and (4) respectively. The data (Figure 3) revealed that the paraffin fractions (C21-C30) was obviously decreased in cultivated crude oil than those in both untreated oil and barrel oil, where as the lighter fractions (C13-C20) were increased more than in cases of both untreated oil and barrel oil that may be attributed to the degradation of longer hydrocarbon chains to lighter by the bacterial action and the photooxidation process. In case of isoparaffins the data (Figure 4) showed that a marked increase of light hydrocarbon chains (C13- C16) & (C20-C21) and slight decrease of (C26- C30) in cultivated crude oil, that may be attributed to the degradation of polyaromatic hydrocarbon to opened chained ispraffins.

The Advanced Oxidation Processes (AOP) based on radical reactions have found in recent decades increasing applications as supplemental technologies to conventional physico-chemical, chemical and biological methods for the remediation of wastes and treatment of waters for municipal and industrial use (**Kimura *et al.*, 2012).** In comparison to photocatalytic, Fenton or ozonation processes, the use of ionizing radiation rays from ^{60}Co sources or high energy electrons is an especially effective and fast method, which does not require the use of additional reagents, and can be based both on oxidative and reductive radical processes. It was shown

already that in spite of a large investment needed; these methods can be more cost-effective compared to other commonly employed AOP **(Kurucz *et al.*, 2002).**

Ojeyemi *et al.* (2013) studied the effect of ultra-violet radiation on aliphatic and polycyclic aromatic hydrocarbon fractions using Gas chromatograph (GC). Results of GC analysis showed that the total aliphatic and aromatic hydrocarbon contents in the ultraviolet irradiated samples were found to be less than the total aliphatic and aromatic hydrocarbon contents of the unirradiated sample. The results indicates that there was a decrease in the amount of both the total aliphatic hydrocarbon content and polycyclic aromatic hydrocarbon content of the Agbabu Natural Bitumen as a result of exposure to ultraviolet radiation.

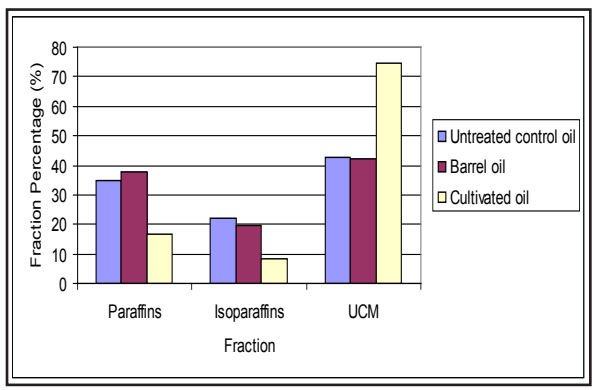

Fig. (2): Fractions percentage in the, Untreated control oil, Barrel oil and cultivated treated oil.

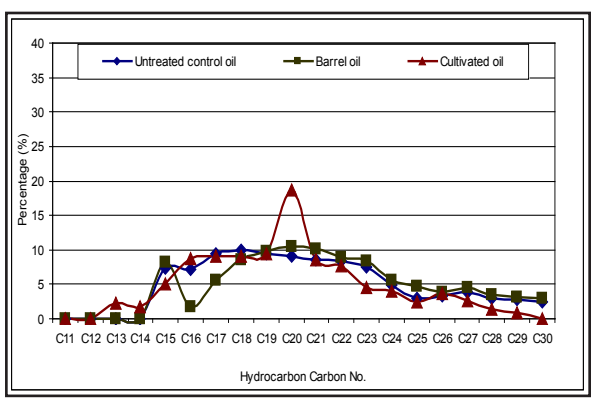

Fig. (3): Paraffins distribution Curve for Untreated control oil, Barrel oil and cultivated treated oil.

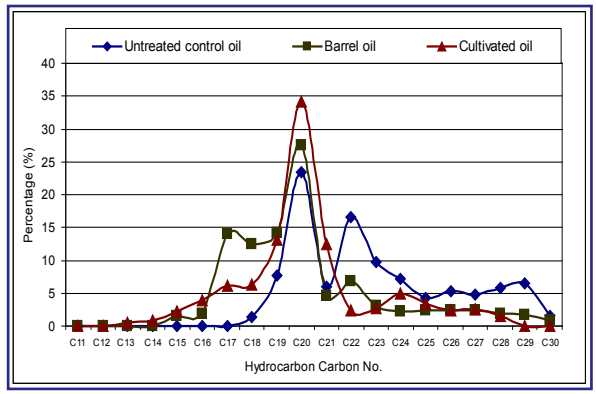

Fig. (4): Isoparaffins distribution Curve for Untreated control oil, Barrel oil and cultivated treated oil.

CONCLUSION

The maximum degradation was achieved by the triple treatment which minimizes the residual hydrocarbons using photocatalyst at concentration of 30 mg/100 ml and 5 ml/100ml of rice straw extract and ***Bacillus licheniformis***.

The treated oil with lower amount of wax could be considered as refined oil which contains more valuable lighter fractions and eliminate the problems attached by wax presence.

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مجلة
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فى العلوم التطبيقية
مجلد 5 ، عدد 4 ، ص 241 : 249 ، (2017)

دور عمليات الاكسدة المتقدمة باستخدام الاشعة فوق البنفسجية فى عملية التكسير الحيوى لشموع زيت بترول مصرى

محمد رفعت الشهاوى[1] ومحمود رمزى فرج[2]

فى محاولة لتخفيض نسب الشموع فى زيت بترول شمعى من شركة خالدة للبترول تم دراسة قدرات عزلة بكتيرية باسيلس ليتشينيفورميس على عملية التكسير الحيوى للشموع باستخدام مخصب عضوى (مستخلص مائى لقش الارز) ومحفز ضوئى (FeOH) واظهرت النتائج اعلى نسب تكسير حيوى عند ٥ مل مستخلص قش الارز /١٠٠ مل من المياه المنتجة مع ٣٠ مجم من المحفز الضوئى/ ١٠٠ مل من المياه المنتجة و قد اظهرت نتائج كروماتوجرافيا الغاز انخفاض نسب الشموع فى عينات زيت خام خضعت للمعالجة المركبة مقارنة بزيت غير معالج و زيت من مخزون الشركة. وقد ظهر بكل وضوح انخفاض نسبة الشموع المستقيمة التى تحوى (C21-C30) فى عينة زيت الخام المعالج مقارنة بزيت غير معالج و زيت من مخزون الشركة بينما ازدادت نسبة الشموع المستقيمة التى تحوى (C13-C20) وذلك يمكن تفسيره بان عملية التكسير الحيوى والاكسدة الضوئية قد شملت السلاسل الهيدروكربونية الطويلة الى سلاسل اقصر واخف. أما فى حالة السلاسل المتفرعة اظهرت عينة الزيت المعالج نسبة كبيرة من السلاسل التى تحوي (C13- C16) &(C20-C21) ونقص طفيف فى (C26- C30) مما يمكن تفسيره بتكسير مركبات متعددة الحلقات فى العينة المعالجة لسلاسل متفرعة مفتوحة.

1. قسم الميكروبيولوجيا الاشعاعية المركز القومى لبحوث وتكنولوجيا الاشعاع - هيئة الطاقة الذرية – القاهرة - مصر.
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