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BIODEGRADATION OF OIL REFINERY EFFLUENTS USING LOCALLY BACTERIAL ISOLATES

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Abstract

There is an environmental risk developed around Al-Najef oil refinery area, which is located in southern west of Iraq, because this refinery does not contain a wastewater treatment unit and the polluted raw effluents are discharged directly to the surrounded areas. This research aims to isolate and identify some of a locally indigenous bacterial isolates which are capable to degrade of hydrocarbons. Water samples of effluents were collected and transported to laboratories of environmental researches centre - Ministry of Science &Technology –Baghdad .Water samples of effluents were cultivated on mineral salts media containing crude oil as source of carbon and energy. Five bacteria were isolated, three of them were found to have no ability to degraded hydrocarbons, and only two showed high potential to utilize crude oil (biodegradation). The two active isolates were identifying based on morphological, macroscopicallyand biochemical characterizes which were Pseudomonas aeroginosa and Alicagenesfaecalis. The pure culture of the tow isolates were cultivated on wastewater samples of the final effluents, where an increase of the biomass were observed by measuring the optical density (O.D) of the mineral salts medium. The results showed that, biodegradation of crude oil where consumed period less than consumed period of final effluents and, Alcaligenes faecalis has high potential of biodegradation than that of pseudomonas aeroginosa.

***Keywords**: Effluents, Hydrocarbons, Hydrocarbon – degrading bacteria, Optical Density*

التحلل الحيوي لنفايات مصافي النفط باستخدام عزلات بكتيرية محلية

الخلاصة

هنالك خطر بيئي على المناطق المحيطة بمصفى النفط في مدينة النجف الاشرف الواقعة في جنوب غرب العراق بسبب عدم وجود محطة معالجة لمياه الفضلات في هذا المصفى ، حيث تطرح النفايات السائلة الى المناطق المجاورة . يهدف هذا البحث الى عزل وتشخيص الانواع

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البكتيرية المحلية المتواجدة في مياه النفايات والقادرة على تفكيك الملوثات الهيدروكاربونية . جمعت عينات من مياه النفايات النهائية لمصفى النفط في النجف الاشرف ونقلت الى مختبرات مركز البحوث البيئية في وزارة العلوم والتكنولوجيا . تم زرع مياه النفايات على اوساط زرعية ملحية تحتوي على النفط الخام كمصدر وحيد للكاربون والطاقة . عزلت خمسة انواع من البكتريا ، ثلاث منها غير محللة للهيروكاربونات واثنان فقط كانت تمتلك القابلية على تحليل الهايدروكاربونات . تم تشخيص العزلات اعتمادا على الصفات الشكلية والمجهرية والكيموحيوية وتبين انهما Pseudomonas aeuroginosa , Alcaligenes faecalis . زرعت العزلات النقية المحللة للنفط الخام على مياه النفايات النهائية لمصفى النفط في النجف الاشرف . لوحظت الزيادة في النمو البكتيري بدلالة الكثافة المرئية (optical density (O.D للوسط الزرعي . أظهرت النتائج الى ان الفترة الزمنية لتحليل النفط الخام اقل من الفترة الزمنية لتحليل النفايات النهائية ، وكانت العزلة Alcaligenes faecalis اكثر كفاءة من العزلة Pseudomonas aeroginosa .

كلمات دالة *: النفايات ، الهايدروكاربونات ، البكتريا المحللة للهيدروكاربونات ،الكثافه المرئية.*

1. Introduction

The oil manufacturing industries are required for the development of communities and improve the life quality. Global need to oil has been increased as the main source of energy, global production has arrived at the beginning of the 21st century to 85.6 million barrels per day[1].Crude oil turns into liquidation include gasoline, liquefied gas products, kerosene, jet fuel, diesel fuel, fuel oil, lubricants and other products [2].Waste residual from liquid produced from crude oil in oil refineries[3], range in size from 0.4 to 1.6 times as much as the interior volume of refinery crude oil to the liquidation process [4].
Wastewater from oil refineries contains hydrocarbons (polycyclic aromatic), phenols and dissolved salts [5, 6].

The most important hydrocarbons posed to the environment from oil refinery operations are gasoline, coloring compounds , ethyl benzene and xylenes (BTEX), all of which are carcinogenic compounds [7,8,9], Where a high concentrations of these compounds may cause leukemia[6]. Phenols and dissolved metals are toxic to aquatic life and thus can cause injuries, liver, lung, kidney, and circulatory system [10, 11]. Discharge of oil refineries waste to water bodies can cause the depletion of dissolved oxygen resulting from the transformation of organic matter to inorganic materials. The biochemical oxygen demand(BOD5) and the chemical oxygen demand(COD) considered to be of the basic metrics to determine the level of pollutants in waste water,BOD5 and COD ranging from 300-600 mg/ L and from 150 – 250 mg/L for COD and BOD5 respectively[12].

Biodegradation is one of the most important methods used to reduce levels of (BOD5) and(COD) in wastewater where the hydrocarbon pollutants are decomposed into water and carbon dioxide and biomass as well as the liberation of some inorganic contaminants [13]. The traditional wastewater treatment is characterized as unfriendly to the environment, in addition of being expensive [14].Where biological treatment methods are considered to be effective, inexpensive, and environmentally friendly [13].

The hydrocarbon - degrading bacteria are widely spread in different environments, they are effective analyzers because of plentiful, the wide diversity, easy of transformation in building and destruction processes and they can survive in different environmental. Bacteria are described as adaptive and resistance to pollutants toxicity because of continues exposure [15].

The biodegradation of hydrocarbons by bacteria is now the main way to address the persistent hydrocarbon pollution from the environment [4], and especially Pseudomonas aeuroginosa bacteria [16, 17]. The oil refinery under study is located in Najaf-Iraq, west of the city on the highway link between Najaf and the holy city of Karbala at Alhaidariya city, and it is considered as small refinery constructed in a way rapid which recently

established to meet the growing city's need of oil derivatives, and due to lack of wastewater processing unit, the final refinery waste launches into neighboring desert areas by metal tube diameter of 10 cm, and velocity of approximately 0.25 m/min. This wastewater discharged into small storage pit, predominately had color due to the accumulation quantities of products liquidations of hydrocarbons that may be implemented to the bottom surface of the soil and possibly reached to the groundwater which is considered as the main source of drinking water, irrigation in this region, which raises concern for the possibility of the arrival of pollutants in waste Najaf refinery to nearby orchards and drinking water.

This research is aimed to isolate and diagnose the bacteria isolated from effluent Najaf refinery wastewater and using these isolates for hydrocarbons.

2. Materials and Methods

2.1. Wastewater samples

Samples of the effluent wastewater were collected from the metal pipe that launches the final waste of Al-Najaf oil refinery to the neighboring desert areas, using eight sterile glass bottles with a tight lid and 1 liter volume. All bottles were kept immersed in ice insulator as they were transported to the laboratory where they were stored in the refrigerator before achieving the microbiological tests within 24 hours [18].

2.2. Isolation of Al-Najaf refinery wastewater degraded bacteria

Bushnell-Haas Broth [2] was used to isolate the bacteria from the collected sampling . The composition of the growth media in one liter of is listed in Table 1.

Table 1. The composition of Bushnell-Haas Broth [2].

Substance	Concentration (mg/L)
Magnesium sulfate $MgSO_4.7H_2O$	200
Calcium chloride $CaCl_2$	20
Monopotasium phosphate KPO	1000
Diammonium hydrogen phosphate$(NH_4)_2HPO_4$	1000
Potassium nitrate KNO_3	1000
Ferric chloride $FeCl_3$	50

Bushnell-Haas Broth media was prepared by dissolving the above ingredients in distilled water and heating on low temperature. The pH was adjusted to 7.0 using 50% NaOH solution. The prepared media was kept in glass bottles of 150 ml with a tight lid and then sterilize at 121°C for 15 min. In order to isolate the bacterial species for hydrocarbons degradation, the broth media in each bottle was provided with 0.1 ml of crude oil as the source of carbon and energy, then 10 ml of the final waste of Al-Najaf refinery was added.(one bottle without adding was kept wastewater for the purposes of control). The bottles were kept in shaker incubator (Lab Companion, Jeo tech, model SI-600R) at a temperature of 30oC and mixing velocity of 150 rpm for 60 hours.

Turbidometry was used to determine bacterial growth. Turbidity have been identified in terms of visual density (OD) using spectrophotometer (Thermo, Model) that took readings at the wavelength of 595 nm from time zero hour to the time 60 hours at a rate of reading every 24 hours for mixed Al-Najaf waste samples and the broth media and for the blank broth media. 0.1ml of bacterial growth was planted for each bottle on the nutrient agar and all the dishes were incubated in an incubator (Mikro-mag, Model M 7040 R) at a temperature of 30 °C for 48-24 hours. Each distinct colony was planned on the solid nutrient agardish individually. A tinge of each dish was planted in the bottle containing the Bushnell-Haas Broth fresh media.10 ml of the final waste samples of Najaf refinery to be the sole source of carbon and energy was added to each bottle, except one of the bottles were left without the addition of a sample of waste water to represent the control and incubated all bottles in the shaking incubator at a temperature of 30 °C and shaking at 150 rpm for 60 hours.

2.3. Diagnosis of the bacteria in Al-Najaf refinery wastewater degraded

For the purpose of diagnosing bacterial isolates, 0.1 mL of bacterial growth for each of the bacterial isolates was planting on agar nutritious dish individually. Several dishes were work for each isolation, while one isolate kept for subsequent tests by planting in bottle containing liquid growth media with glycerol 20%.Allbottles were preserved in freeze. Phenotypic characteristics, microscopic and biochemical adopted in the diagnosis of refinery wastewater analyze bacterial [17]. The phenotypic specifications for developing isolates on a plate nutrient agar were identified and Gram dye interaction of each isolation was achieved for determining the cellular characteristics.

Biochemical characteristics were determined using the broth media for each tests for consumption tests; jackets, lactose, maltose, sucrose, glucose, fermentation glucose enzyme amine secretary group of tryptophan, the production of hydrogen sulfide were detected oxidative enzyme using a 15% solution of dimethyl p-phenylenediamine hydrochloride, was detected using an enzyme Alkatales 3.0% solution of hydrogen peroxide. It was an examination of the movement hanging-drop technique.

3. Results and Discussion

3.1 Diagnosis of hydrocarbons bacterial isolates

Five bacterial species was isolated from Al-Najaf wastewater effluents, three of which were found not analyzed for crude oil and the final refinery wastewater and two of the bacterial isolates showed a good possibility in the final dismantling of crude oil and refinery wastewater. The two bacterial isolates were diagnosed according to phenotypic specifications, microscopic, and biochemical. After comparing the readings shown in (Table 2), with manual of determinative bacteriology, it was found that first isolation shown in Fig. 1, belonging to the Pseudomonas aeruginosa bacteria, and isolation in Fig. 2 return of the Alcaligenes faecalis bacteria. Both types have been isolated from refinery wastewater in many previous studies [18, 19].

Table 2. Bacteria isolated according to phenotypic specifications, microscopic, and biochemical.

Characterizes	Isolate 1	Isolate 2
Gram stain	-	-
Coloney size and apperance	Medium to large , mucoid	Small , smooth
Pigment production	Yellow - green	(-)
Cell shape	Rod	Thin rod
motility	+	+
Ureas	-	-
Utilization of citrates (simmon's)	+	+
Lactose	-	-
Maltose	-	+
Sucrose	-	-
Glucose fermentation	-	-
Tryptophan deaminase	-	-
Catalease activity	+	+
Oxidease activity	+	+
H_2s production	-	-
Probable organism	*Pseudomonas aeroginosa*	*Alcaligenesfaecalis*

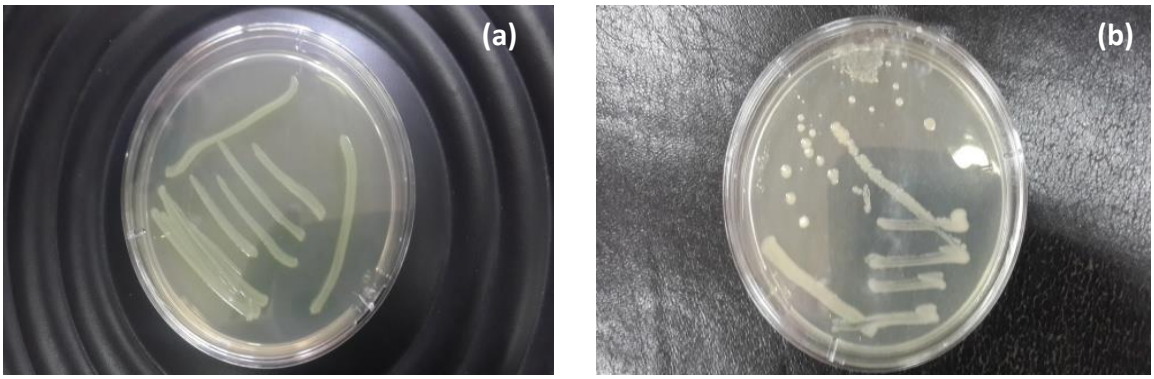

Figure 1. (a) Pseudomonas aeuroginose bacteria on nutrient agar,
(b) Alcaligenes faecalis bacteriaon nutrient agar.

3.2 *Biodegradation of Hydrocarbons*

The visual density values for both isolates as listed inTable3 and shown in Fig.2. Increased with time. This increase in OD evidence is an increase in biomass growth [6]. It suggests exploiting hydrocarbons in the broth media by pseudomonas aeroginosa and caligenes faecalis where the crude oil or final waste being the sole source of carbon compared with the broth media bottle that had not been inoculated as shown in Fig. 3. These findings are consistent with many previous studies that indicate efficiency of up to 92% for refineries wastewater by the analysis of bacterial isolates to our present [20].

Table 3. Optical density (OD) of the broth media at wavelength 595 nm for 60 hours

Isolate name	O.D for crude oil sample				O.D for Al-Najaf refinery effluents sample			
Isolate 1:as *Pseudomonas aeroginosa*	0 hr	24 hr	48 hr	60 hr	0 hr	24 hr	48 hr	60 hr
	0.00	0.010	0.041	0.66	0.00	0.006	0.020	0.54
Isolate 2: as *Alcaligenesfaecalis*	0 hr	24 hr	48 hr	60 hr	0 hr	24 hr	48 hr	60 hr
	0.00	0.19	0.46	0.67	0.00	0.023	0. 66	0. 88

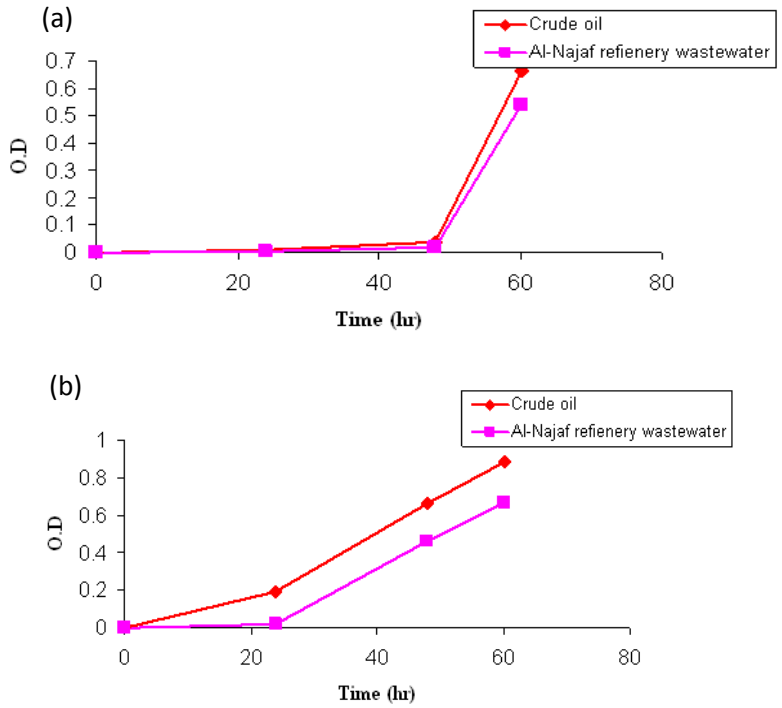

Figure 2. (a) Optical density for hydrocarbons degraded by Pseudomonas aeruginosa, (b) Optical density for hydrocarbons degraded by Alcaligenesfaecalis

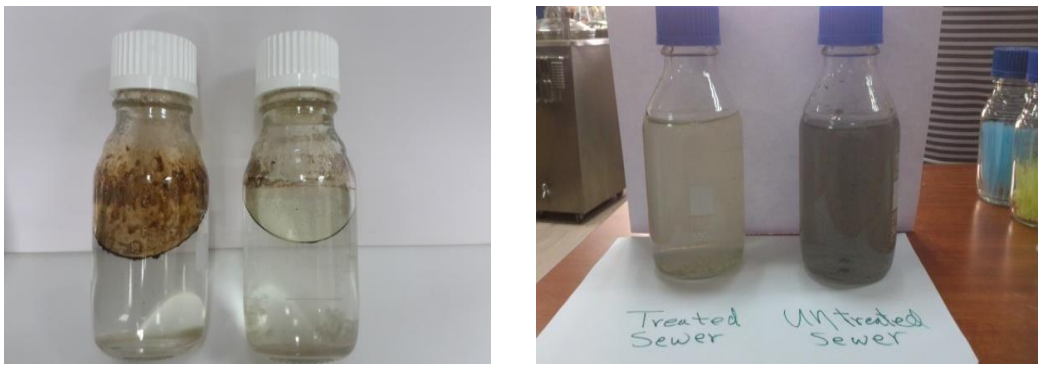

Figure 3. (a) Biodegradation of crude oil, (b) Biodegradation of wastewater refinery

From the above Table, and Figs., it could be seen that, the optical density for crude oil is more than that Al-Najaf refinery wastewater which means that, the hydrocarbons degraded by isolated bacteria in crude is more than that for refinery wastewater due to possibility of present of toxics materials in the refinery wastewater. However, in the two cases the degraded capacity seems to be very high.

4. Conclusions

It can be concluded from this research that Al-Najaf refinery wastewater pose an environmental risk, but over time hydrocarbons pollutants can be self-decays due to the presence of *Pseudomonas aeruginosa* and *Alcaligenesfaecalis* found originally in the waste water, but this decay being a partial and little due to fact that, the waste water environment is not suited ideally for the growth of bacteria because they contain toxic pollutants at high concentrations. In the case of the provision of appropriate and different environmental conditions, which is located in Al-Najaf refinery environment, such as the provision of appropriate temperatures and nutrients required for bacterial growth, such as installing biological treatment unit commensurate with the production capacity of the refinery by using these isolates.

Through what has been observed during the modeling process, Al-Najaf refinery wastewater is directly discharged into the nearby desert areas, so it was recommend turning off these random way which is incompatible with the most basic considerations to preserve the environment from the spread of toxic hydrocarbon contaminants.

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