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Biological Reduction of Chemical Oxygen Demand (COD) & Biological Oxygen Demand (BOD) of AL-Najaf Oil Refinery Wastewater Using Locally Bacterial Isolates of Pseumonasaeruginosa , Alcaligenesfaecalis

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ABSTRACT

The chemical and biological oxygen demands, COD and BOD considered as an important indicator for organic pollution of water and soil, This research aims to reduce the biological and chemical demands for oxygen (COD and BOD) of Al Najaf oil refinery final wastewater which located in medium western of Iraq before discharging it to the environment , by biodegradation process using two isolates of gram negative bacteria, *Pseudomonasaeuroginosa* and *Alcaligenesfaecalis*, which isolated from refinery wastewater . The percentage of poly-aromatic hydrocarbons (PAH) removal were 85.84 % by *Ps.aeuroginosa* and 76.13 % by *A. faecalis.* The percentage of COD reduction were 60%, 40 % and BOD reduction 53.13 %, 42.34 % for two isolates respectively. This proves the efficiency of both bacterial isolates in treatment of petroleum contaminants found in the wastewater.

Keywords

Ps.Aeuroginosa; *A. faecalis*; oil refinery; hydrocarbon; pollutants. COD, BOD

1. Introduction

The global need for crude oil is increasing dramatically, in 2010 it was reached to about 94.3 million barrels per day increasing to 101.6 barrels per day by 2015 [1]. Waste water for oil refineries can be defined as those that are produced from industries that rely mainly on crude Oil, manufactured fuels, oils, and middle Petrochemicals materials [2]. The discharged water containing oil includes, in addition to oil refinery waste, crude oil production wastes, garage waste, petrochemical industries, metal process oils and car wash stations. These sources are a major contributor to pollution problems, especially soil and water pollution [3]. Both waste and untreated compounds from the above-mentioned sources are called oil residues that are usually too hard to process or recycle them [4]. The discharged water mainly contains crude oil, cyclone hydrocarbons, phenols, metallic salts, sulfides and naphthalene acids [5-7]. Many of these pollutants are toxic, mutagenic or carcinogenic [8]. The cyclic aromatic compounds are the most importantpollutants, which enter the environment in different ways, including man-made, combustion, un-wanted unloading of oil tankers, spills in the vicinity of oil refineries [9].

Frequently, the water discharged from oil refineries contains high concentrations of hydrocarbon contaminants and an unsuitable acidic function, which make its processing very difficult and expensive [10]. Large quantities of water are used in filtering process of crude oil, whether in cooling of the equipment, removing salts, cooling systems, distillation, desalination, reddening of equipment and drainage tanks [11]. Therefore, the volume of waste water is also large, where the discharged water quantities from oil refineries are about 0.4-1.6 times more than the amount of crude oil entering the liquidation process [12]. It is possible to imagine the seriousness of the water discharged from oil refineries to the ecosystems of the world through the amount of crude oil consumed globally, Million barrels / day (mbpd), and there are 33.6 million barrels / day of water bank [13]. And that the global need for crude oil will reach 107 million barrels / day, and it is noted that the water discharged from the oil industry will continue to increase, which requires more work to find appropriate solutions to their negative effects on the ecosystem.

Some human activities, such as oil filtration, produce large quantities of organic matter (hydrocarbons) with the water discharged to the aquatic environment, which leads to excess consumption of oxygen by the bacteria in an attempt to oxidize the organic matter, so depleted oxygen is faster than oxygen dissolved in water from the air, this problem leads to non-preservation of high-end forms of life.

The biological treatment of water discharged from oil refineries is more efficient and widespread than any chemical or mechanical method, and is characterized by its relatively low cost and a simple technological approach[14,15] , and environmentally friendly [16]. There are about 100 bacterial species belonging to 30 species of bacteria that have the potential for hydrolysis of hydrocarbonic pollutants and their resistance to the toxicity of these pollutants, including the species *Pseudomonas, Sphingomonas, Aeromonas, AlcaligenesAcinetobacter, Arthobacter, Brevibacterium, Xanthomonas, Mycobacterium, Rhodococcus, Bacillus* [17,18,19]. The most commonly used are the Gram-negative bacteria of Coliform or spherical shape, especially the sex-species Pseudomonas [20,21,22,23], and (Alcaligenes) [24,25,26].

The efficiency of bacteria depends on bio-hydrolysis of hydrocarbonates on the effectiveness of bacteria and environmental conditions that lead to the final output of the process of biodegradation, namely water and carbon dioxide [27], in addition to the nature of the chemical composition of the hydrocarbon pollutant.

This research focuses on the possibility of reducing concentrations of hydrocarbons, especially polyaromatic hydrocarbons (PAHs) in the discharged water from Najaf oil refinery, in terms of the biological reduction of the value of both the chemical and the biological demands of oxygen by using the isolates Pseudomonas *aeuroginosa* and *Alcaligenesfaecalis* as a method of biological treatment of hydrocarbons pollutants to the environment, especially as this refinery is located near agricultural areas, which may lead to increase the possibility of indirect environmental impact through crops that mainly irrigated from groundwater in this region.

2. Materials and Methods

The water samples were collected from the nozzle of the prepared pipe to drain discharged water outside the Najaf Refinery using 1 liter of glass bottles. The samples were stored in a refrigerated sample box using ice till reaching the laboratory and doing the necessary tests. The values of each of the following variables were measured for waste water:

a- PAH-prepared aromatic compounds using (SYKMA: Germany HPLC),
b- Acid function using (WTW 82362 Weilheim , Germany),
c- Oxygen chemical demand using (AL 200 COD Vario),
d- Oxygen biological demand using (OXITOP BOX WTW),
e- Conductivity and soluble matter using (MI 170 Bench meter MARTINI),

f- Oil content using (Horiba, oil content analyzer),

g- Adenosine tri-phosphate energy complex ATP using (Glomax, USA),

h- Finally, measuring heavy element concentrations using (Atomic Absorption Flam Emission Spectrometer SHIMADZU, 6200, JAPAN).

Biological reduction for COD and BOD of the Najaf Oil Refinery Refuse

In this contribution, biodegradation was carried out as a vital method that ultimately aimed to reduce the value of both the COD and the biological demand of water discharged from the Najaf oil refinery using the bacterial isolates *Pseudomonas aeuroginosa*, *Alcaligenesfaecalis* that have been isolated from previous study [28], which proved good susceptibility to the hydrocarbons hydrolysis.

The two isolates were activated on Nutrient Agar for 24 hours at 30° C. The bacterial suspension was prepared for the purpose of fecundation by taking a colony of isolation *Pseudomonas aeuroginosa* and *faecalisalcaligenes* isolations individually and suspended in 100 ml of the regulated solution of potassium phosphate $KH2PO_4$ (0.1 molar, acid function PH 7). The discharged water was treated with bacterial isolates individually by fecundation about 500 ml of the discharged water with about 10 ml of bacterial suspension in a 500 ml vial. The control vial containing 500 ml of drained water was left without fecundation by bacterial isolates. Three replicates were performed for each isolate and all bottles were incubated at a temperature of 30° C in a vibratory incubator (Lab Copanion, Korea) at 150 rpm speed. Bacterial growth was monitored daily on the basis of the marked change in the turbidity of the contents of each vial.

3. Results

Chemical and biological demands of Oxygen are the most important variables that indicate the organic pollution level of water. The minimum quantity of dissolved oxygen required for life in the aquatic environment is about 2 mg/l [29]. The results shown in Table (1-a), shows the HPLC instrument analysis for (PAHs) of discharged waste water from the Najaf refinery before processing with *Pseudomonas aeuroginosa and Alcaligenesfaecalis* isolates while tables (1-b) and (1-c) show the results after processing with *Pseudomonas aeuroginosa and Alcaligenesfaecalis* isolates respectively.

The results in (table-2) indicate that the (COD) and (BOD) values were 235 and 111 mg/l respectively, which are higher than the global limits of 125 mg/l and the Iraqi limits of 100 mg/l. The (COD) value was reduced to 94 mg/l by using *PS. ayurigenosa* isolate and became less of these determinants, although it was reduced to 141 mg/l using *A.faecalis* isolate but it still higher than these determinants. Biochemical demand (BOD) were 111 mg /l, higher than the global limits (15 mg/l) and Iraqi (40 mg/l), indicating depletion or diminishing of natural oxygen sources of discharged water leading to the development of destructive environmental conditions for life, and also reflects high levels of biodegradation.

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Table 1a. The results of HPLC instrument analysis for (PAHs) of discharged waste water from the Najaf refinerybefore the treatment by A. *feacalis* and P. *euroginosa* isolates. Result Table (Uncal-HPLC-21_Jul_ 2015 10_28_38 AM –FLD)

	Reten. Time(min)	Area(mV.s)	Height(mV)	Area(%)	Height(%)	W05(min)
1	1.944	750.512	53.946	0.5	2.9	**0.10**
2	2.676	225.965	5.552	0.2	0.3	**0.42**
3	8.176	557.6150	33.906	3.7	1.8	**3.15**
4	14.680	40412.578	269.562	27.2	14.4	**2.16**
5	16.048	9738.357	213.141	6.6	11.4	**0.99**
6	17.036	6418.424	143.840	4.3	7.7	**0.76**
7	17.792	6281.565	147.019	4.2	7.9	**0.75**
8	19.304	79194.602	1005.653	53.3	53.7	**0.97**
	Total	**14.6189**	**1872.618**	**100.0**	**100.0**	

Table 1b. The results of HPLC instrument analysis for (PAHs) of discharged wastewater from the Najaf refinery after the treatment by P. *euroginosa* isolate. Result Table(Uncal-HPLC-21_Jul_ 2015 12_26_16 PM –FLD)

	Reten. Time(min)	Area(mV.s)	Height(mV)	Area(%)	Height(%)	W05(min)
1	2.028	581.992	52.992	2.8	21.6	**0.06**
2	2.900	45.619	2.830	0.2	1.2	**0.30**
3	3.320	37.375	1.344	0.2	0.5	**0.53**
4	4.532	50.030	1.945	0.2	0.8	**0.28**
5	6.592	258.387	3.762	1.2	1.5	**1.08**
6	7.572	560.865	4.958	2.7	2.0	**2.36**
7	21.432	19498.159	177.175	92.7	72.3	**0.06**
	Total	**21032.427**	**245.006**	**100.0**	**100.0**	

Table 1c. The results of HPLC instrument analysis for (PAHs) of discharged waste water from the Najaf refinery before the treatment by A. *feacalis* isolate. Result Table (Uncal-HPLC-21_Jul_ 2015 11_46_13 AM –FLD)

	Reten. Time(min)	Area(mV.s)	Height(mV)	Area(%)	Height(%)	W05(min)
1	1.956	659.757	56.231	1.9	16.0	**0.09**
2	2.672	130.564	3.565	0.4	1.0	**0.58**
3	3.684	54.394	2.204	0.2	0.6	**0. 30**
4	8.152	1566.008	10.436	4.4	3.0	**2.77**
5	13.904	31943.605	65.698	90.1	18.7	**9.42**
6	21.404	1111.326	214.078	3.1	60.8	**0.06**
	Total	**35465.654**	**352.212**	**100.0**	**100.0**	

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Table 2. Physio-chemical specifications of discharged waste water from the Najaf refinery after 7 days of treatment with *Paeuroginosa, A.faecalis* bacterial isolates.

parameter	Control (untreated) / zero days	Treated with *Ps.aeuroginosa* / 7 days	Treated with/*A.faecalis*/ 7 days	Iraqi standards limits (2009)	EPA / Standard limits (2008)
PH	7.8	7.2	7.3	6.5 - 9.5	**6.0 - 8.5**
Conduc.(ms/cm)	1167	1013	1038	-	**-**
TDS (ppm)	747.52	739.8	742.8	-	**500**
PAH removal	0 %	85%	76.13%	-	**-**
Oils & Grease	34.8 ppm	32.4 ppm	29.9 ppm	10	**5**
BOD	111	62	78	40	**15**
COD	235	94	141	100	**125**
ATP(RLU/ml)	$2.401x10^4$	$4.802x10^5$	$4.107x10^5$	-	**-**
Pb	0.006	0.001	0.003	0.1	**0.1**
Cd	0.198	0.082	0.099	0.01	**0.005**
Co	0.140	0.048	0.087	-	**-**
Mg	1.230	1.220	1.281	-	**-**
Cr	0.021	0.017	0.010	-	**0.2**
Cu	0.732	0.600	0.480	-	**1.0**

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4. Discussion

The efficiency of the above two isolates in the reduction of ring aromatic compounds (PAH) ranged from 85.84% using isolation Ps. *aeuroginosa* and 76.13% using isolation A.*faecalis* , Table (3), which is consistent with the other study [30,31]. The efficiency of Ps.*aeuroginosa* bacteria in the decomposition of hydrocarbons by biodegradation process is due to its production of the oxygenases group enzymes and their susceptibility as other *Pseudomonas* species to produce some of the biological surface materials in the form of glycolipids of Rhamnolipids type, which increase the oil surface area, and therefore will make this oil quantity actually available to be used. [32,33].

This conclusion is supported by a clear rise in the value of Adenosine Tri- Phosphate (ATP) from 2.401 x 10^4 RLU / ml before treatment to 4.802 x 10^5 RLU / ml using P. *aeuroginosa* isolate, and 4.107 x 10^5 RLU / ml using A. *faecalis* bacteria. The increase in the amount of ATP reflects the activity of bacterial cells, including the increase in the number of bacteria in the medium [34]. The exploitation of hydrocarbons compounds is an attempt by the bacterial cells to obtain carbon and energy.

The conductivity values and soluble solids (TDS) are belong to the concentrations of the dissolved ions in the discharged water [35]. Table 1 shows that the conductivity value and soluble solids is less than the global determinants. The two bacterial isolates were reduced the conductivity and the (TDS) slightly. In this study, the change in acidic function was inconsiderable from 7.8 (for discharged water) to 7.2 to 7.3 after the treatment with bacterium isolates and this result was expected. Biodegradation process usually leads to a change in the value of the acidic function [36], but they are still within the limits allowed in the international and Iraqi specification. The oil and grease is a mixture of organic substances that vary in molecular weight and include fatty acids, oil, and fat. Part of them dissolves in water and the other part settles in the bottom after the decrease of the volatile part. The main part of it being in the form of floating natants on water surface, which is the impact on different aquatic life.

The discharged water content of the oil and grease was about 34.2 ppm, which is higher than the permissible global limits of 5.0 ppm and that of Iraqi limit 10.0. The decrease in oil and grease value due to the bacterial isolates is ranged from 32.4 ppm to 29.9 ppm. This can simply be explained by the fact that we have been careful to treat the discharged water as it is in the field and have not exposed to any mechanical reduction of the oil inhomogeneous droplets with the waste water which are so difficult to dismantle using biological methods and therefore the mechanical methods still mainly used in most of the treatment plants for the oil refineries field.

5. Statistics

Simply, the percentage of reduction PAH by isolates: *P. aeroginosa* and *A. faecalis* can be calculated from the following equation [37]:

The percentage of reduction = [Concentration of pollutants before treatment – (concentration of pollutants after treatment / concentration of pollutants before treatment)] X 100%.

Using the results of the HPLC analysis shown in Tables (1a, 1b and 1c), the percentage of elimination can be calculated using the above equation as follows:

In the case of *P. aeuroginosa* bacteria:

PAH reduction l% by *Ps. aeroginosa* = 14859206 -21032 / 14859206 x 100 = 85.84%

In the case of *A. faecalis* bacteria:

PAH% reduction by *A. faecalis* = 14859206 -35465.6 / 14859206 x 100 = 76.13%

Table 3. The percentage of reduction for each BOD, COD, PAH of discharged water discharged wastewater from the Najaf refinery

Isolate	PAH removal %	COD reduction%	BOD reduction %
Ps. Aeroginosa	85.84%	60.00%	**53.15%**
A. faecalis	76.13%	40.00%	**42.34%**

The same equation can be used to calculate the percentage of reduction of both the chemical and the biological demands for oxygen of waste water for the Najaf refinery by the two isolates. *aeroginosa*, *A. faecalis*, as follows:

COD reduction by *Ps. Aeroginosa* = 235 - 94 / 235x 100 = 60%

COD reduction by *A. Faecalis* = 235 - 141/235 x 100 = 40%

BOD reduction by *Ps. Aeroginosa* = 111-53 / 111x100 = 53.15%

BOD reduction by *A. faecalis* = 111 - 64/111 x 100 = 42.34%

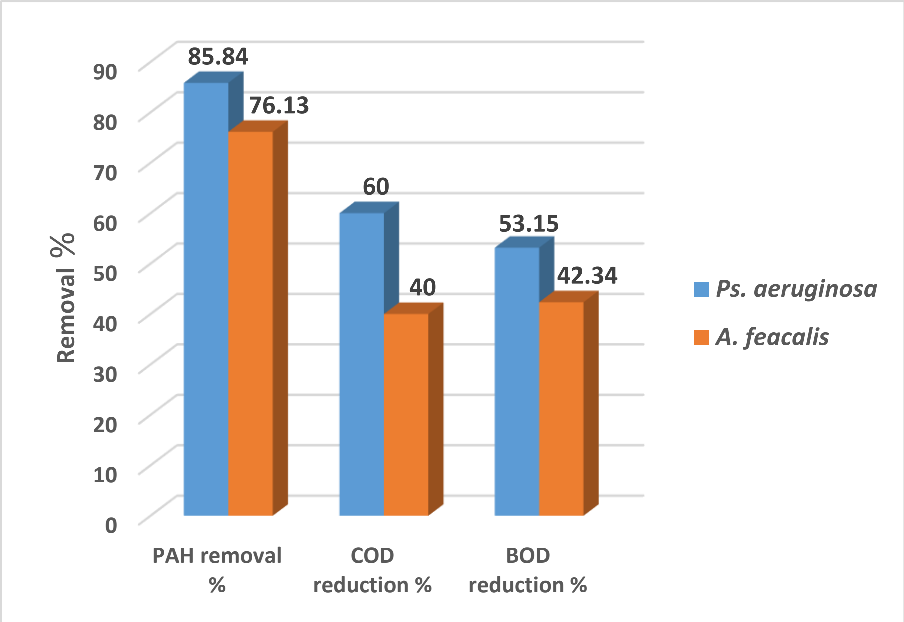

Fig. 1. The percentage of reduction for each BOD, COD, PAH of discharged water discharged wastewater using A. *faecalis* and Ps. *Aeruginosa* isolates after four days of treatment

6. Conclusion

This study results proved that the discharged water from the Najaf refinery contains varying concentrations of some heavy elements, including lead Pb, cadmium Cd, cobalt Co, Magnesium Mg, Chrome Cr and Copper Cu, ranged between 0.006, 0.198, 0.140, 1.230, 0.021 and 0.732 respectively. And all of them were less than the global and Iraqi determinants except the cobalt which was higher, also the magnesium was somewhat was with considerable concentrations There was a decrease in the concentration of these heavy metals after treatment with bacterial isolates, this result is consistent with another study [38-42]. However, cadmium concentrations exceeded the permissible limits.

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Conflicts of Interest: The authors declare no conflict of interest

Authors' Contributions:I read and approved the final manuscript.

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