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Bioremediation of Industrial Wastes of Oil Refineries as an Environmental Solution for Water Pollution

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Abstract

Four waste water samples were taken from Cairo Oil Refinery Company (CORC) at Mostorod, representing two groups according to their total petroleum hydrocarbon (TPH) contents; the relatively highly polluted samples were taken from El-Taktir American Petroleum Institute (API) separator and the low polluted ones were taken from El-Islah API separator. Mechanical treatments show percentages removal in Total petroleum hydrocarbons (TPH) of 50% and 35% in the outlet effluent of El-Taktir and El-Islah API separators, respectively with TPH concentrations of 250 mg/l and 65 mg/l, respectively. Biotreatment of sterilized mechanically treated water samples with *Staphylococcus xylosus* for 7 days of incubation at 30°C, pH 7 and 200 rpm show the high biodegradation efficiency of the isolated bacteria and its preference to work on the high concentration than the low one with percentage removal of 90% and 72.31%, respectively. This led to final TPH concentrations in El-Taktir and El-Islah water samples of 25 mg/l and 18 mg/l, respectively with approximately complete removal of normal and iso alkanes independent of the TPH concentration. AB1 is also able to degrade Pristane (Pr) and Phytane (Ph) in the highly polluted sample with 73.41% and 54.96%, respectively while it shows complete removal of Pr and Ph in the low polluted sample. Biotreatment shows also high biodegrading efficiency in the unresolved complex mixture (UCM); aromatics, naphthenes and alicyclic hydrocarbons with percentages removal of 88.59% and 68.93% for El-Taktir and El-Islah water samples, respectively.

Introduction

Production, transportation and refining of crude oil result in large volume of oily waste water [1]. Oil pollution incidents in fresh water and their effects on living resources have received much less attention than those in marine ecosystems. Accidents in fresh water ecosystems are much more localized and hence attract much less public outcry [2].

Ismailia Canal is fed from the Nile in front of Delta barrages at a length of 16 km and it moves to the east penetrating the governorates of Cairo, Kalioubeya, Sharkeya and Ismailia supplying these regions with their needs of Nile water. Large scale industrialization may have its impact on the water quality of the canal. Some petroleum activities (especially refineries) are present near the Cairo side of the canal at Mostorod. Poor water quality leads to large capital expenditures on this water for it to be useful for drinking and agricultural activities.

The oil refining sector plays a vital role in the national economy. The production capacity of Egypt's nine refineries is approximately 714,000 barrels/day this sector is the biggest on the African continent. The government has spent over $3.5 billion since 1982 to upgrade the existing plants in order to raise and improve their product range. The Mostorod Refinery, north of Cairo, has a working capacity of 142,000 barrels/day, which represents about 20% of Egypt's production. It is operated by Cairo Oil Refining Co. (CORC), unit of the state-owned by Egyptian General Petroleum Corp. (EGPC). Industrial wastes from CORC refinery, mainly oily water, are discharged into Ismailia Canal after being treated. Approximately 400 m^3/hr, *i.e.* 6400 m^3/day (or

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approximately 2,336,000 m^3/year) are discharged into the canal which represents an environmental burden.

Due to the ever increasing tightness of environmental regulations, the waste water has to be treated to remove oil as well as other pollutants down to a level that is acceptable for discharge to a particular water body, based on the environmental laws, regulations and other requirements applicable to the case under consideration.

Treatment techniques used in CORC for treatment of oily waste water is either Mechanical Treatment; API Separators or Physico-Chemical Treatment; Dissolved Air Floatation Unit (DAF units aided with $FeCl_3$).

Petroleum components can be classified into four major groups: saturates, aromatics, resins, and asphaltenes. Bioremediation of oil contaminated wetlands, including fresh and salty water by micro flora is an expanding field that has increasingly come to the attention of the world via a series of high-profile oil spills [3,4].

Petroleum hydrocarbons and related compounds can be degraded by microorganisms (bacteria, yeasts, and hyphal fungi) which are widely spread in marine water, fresh water and soil habitats [5]. In the marine environment, bacteria are considered to be the predominant hydrocarbon-degraders with a distribution range that even covers extreme cold Antarctic and Arctic environments. In the fresh water environment, yeast and fungi may also play a significant role in degrading petroleum hydrocarbons. Some of the most important hydrocarbon degrading microorganisms in both marine and fresh water environments are for bacteria: *Achromobacter*, *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Brevibacterium*, *Corynebacterium*, *Flavobacterium*, *Nocardia*, *Pseudomonas*, *Staphylococcus* and *Vibrio*, and for fungi and yeast; *Aspergillus*, *Candida*, *Cladosporium*, *Penicillum*, *Rhodotorula*, *Sporobolomyces* and *Trichoderma* [6-8].

The main scope of this research is to apply bioremediation to oil contaminants in oily waste water resulting from the refining processes in CORC in order to minimize, or prevent if possible, the expected pollutants coming to Ismailia Canal.

Experimental Work

Materials and Methods

Luria-Bertani (LB) Medium

LB medium was prepared as previously described by Kirimura *et al.* [9]. For maintenance of isolate and preparing of LB-agar plates for bacterial count; LB solidified with 2% agar was used.

Culture Medium

To meet the nutritional requirements of the microorganism for robust growth, the wastewater samples were supplemented with mineral salts medium (MSM) containing the following constituents (g/l distilled water) 2.0 g $NaNO_3$, 1.0 g K_2HPO_4, 0.5 g $MgSO_4{\cdot}7\,H_2O$, 0.5 g KCl and 0.01 g, $FeSO_4$.

Microorganism

The polluted samples were inoculated with *Staphylococcus xylosus* AB1 bacterium isolated from hydrocarbon polluted waste water effluent of CORC discharged in Ismailia Canal according to the method reported by Chaerun *et al.* [5] and identified by Biolog system model; Biolog/Microlog 3420 program (Egyptian Plant Disease Research Institute).

Analytical Methods

Total Petroleum Hydrocarbon (TPH) Concentration

Oil was extracted from water samples using carbon tetrachloride (CCl_4) according to the method described by Moustafa [10]. The TPH concentration in the extract was determined gravimetrically [11].

Gas Chromatographic Analysis

All extracted samples were analyzed using Agilent model 6890 plus gas chromatograph. The conditions of analysis were as follows:

- Column: HP-1 (100% methyl silicon siloxane) 30 m length and 0.25 mm I.D.;
- Oven temperature: 80-300°C (3 °C/min);
- Detector: flame Ionization Detector (FID) 325°C;
- Injector: splitter injector 300°C using N_2 (2 ml/min) as carrier gas.

Locations of Samples

Four waste water samples were taken from CORC at Mostorod, representing two groups according to their TPH contents; the highly polluted samples were taken from the API separator (El-Taktir) used to treat effluents resulting from process activities and the low polluted ones were taken from the API separator (El-Islah) used to treat effluent resulting from maintenance activities. These samples were taken as pairs; specifically, one before the API inlet and one after the API outlet; each pair represents one sample to be studied. Total Petroleum hydrocarbon content (TPH) and Gas chromatographic analysis (GC) for the extracted hydrocarbons were performed for all samples before and after the mechanical treatment.

Biological Treatment

One liter samples of sterilized, mechanically-treated water were placed in 2 l Erlenmeyer conical flasks; the samples were subjected to biological treatment by inoculation with 100 ml of bacteria suspended in sufficient sterilized MSM to yield a total viable count of approximately 10^5 CFU/ml; the pH was adjusted under aseptic conditions to 7.0 using sterilized 10% NaOH; the samples were then incubated in a shaking incubator at 30°C (200 rpm) for seven days. Flasks without inoculation subjected to all the above steps were used as negative controls. Total Petroleum hydrocarbon content (TPH) and Gas chromatographic analysis (GC) for the extracted hydrocarbons were performed for all samples before and after biological treatment. pH and bacterial count were also determined for all samples before and after the biological treatment.

Enumeration of Bacteria

Bacterial growth during bioremediation was measured by the determination of viable cell counts on LB-agar plates at the beginning and end of the process. From each flask 1ml of the cell suspension was aseptically added into 10 ml of sterile saline solution (8.5 g NaCl per 1l distilled water) and vortexed vigorously then serially diluted. The cell densities of the appropriate dilutions were determined by spreading on LB-agar plates. Plates were incubated at 30°C, and colonies were enumerated after 48 hours.

Results and Discussion

Mechanical treatment of CORC waste water

Effect on TPH

Quantitative analyses of TPH extracted from water samples of inlet and outlet of the API separators El-Taktir and El-Islah (as listed in Tables 1 and 2) show relatively high values. The TPH at the outlet of El-Taktir API separator was reduced 50% from 500 mg/ml to 250 mg/ml. While the TPH content at the outlet of El-Islah API separator was reduced 35% from 100 mg/ml to 65 mg/ml. Final concentrations are far higher than the allowable level present in the Egyptian law of environmental protection, specifically 5 mg/l for effluents to be discharged in fresh waters. These high values have demonstrated that the efficiency of the separators were not quite satisfactory for the industrial effluent to reach the limits indicated by the law to be discharged safely. Further these high values are known to have lethal effect on living organisms through the interference with cellular or subcellular processes. Based on the biological response values that might accompany or follow oil pollution, lethal effect occur in the range of 1-10 mg/l [12].

Table 1
Treatment of El-Taktir sample

Measured parameters	Treatment			
	Inlet of API	Outlet of API	Biotreatment	Total removal after Biotreatment
TPH, mg/l	500	250	25	475
% removal		50	90	95
Changes in n- and iso- alkanes and UCM of extracted oil				
Initial carbon number	nC_{10}	nC_{18}	nC_{19}	
Final carbon number	nC_{35}	nC_{38}	nC_{40}	
Pr/Ph	0.85	1.46	0.86	
nC_{17}/Pr	3.85	0	0	
nC_{18}/Ph	2.77	2.89	0	
TRP/UCM	0.33	0.22	0.07	

Table 1
Continued

Measured parameters	Treatment			
	Inlet of API	Outlet of API	Biotreatment	Total removal after Biotreatment
Changes in TRP of extracted oil				
% TRP	24.72%	17.90%	6.36%	
TRP, mg/l	123.6	44.75	1.59	122.01
% removal of TRP		51.61	96.45	98.71
Changes in Pr and Ph of extracted oil				
% removal of Pr		76.65	73.41	93.80
% removal of Ph		86.43	54.96	93.89
Changes in UCM of extracted oil				
% UCM	75.28	82.10	93.64	
UCM, mg/l	376.4	205.25	23.41	352.99
% removal of UCM		46.27	88.59	93.78

Table 2
Treatment of El-Eslah sample

Measured parameters	Treatment			
	Inlet of API	Outlet of API	Biotreatment	Total removal after Biotreatment
TPH, mg/l	100	65	18	82
% removal		35	72.31	82
Changes in n- and iso- alkanes and UCM of extracted oil				
Initial carbon number	nC_{13}	nC_{14}	nC_{19}	
Final carbon number	nC_{41}	nC_{41}	nC_{41}	
Pr/Ph	0.51	0.98	0	
nC_{17}/Pr	2.62	0	0	
nC_{18}/Ph	1.97	2.07	0	
TRP/UCM	0.25	0.25	0.11	
Changes in TRP of extracted oil				
% TRP	20	19.97	9.62	
TRP, mg/l	20	12.98	1.73	18.27
% removal of TRP		35.1	86.67	91.35
Changes in Pr and Ph of extracted oil				
% removal of Pr		72.84	100	100
% removal of Ph		85.88	100	100

Table 2
Continued

Measured parameters	Treatment			
	Inlet of API	Outlet of API	Biotreatment	Total removal after Biotreatment
Changes in UCM of extracted oil				
% UCM	80	80.21	90.38	
UCM, mg/l	80	52.14	16.2	63.8
% removal of UCM		34.83	68.93	79.75

Effect on Different Oil Components

GC/FID analysis of oil extracted from the inlet and outlet of the API separators were performed to study the effect of mechanical treatment on the different oil components.

Figures 1 and 2 present the chromatographic patterns of the extracted oil before and after mechanical treatment. Mechanical treatment independent on the initial TPH concentrations express a remarkable change in the total resolvable peaks, total resolvable peaks (TRP) (linear and branched alkanes) and unresolved complex mixture, UCM (aromatics, naphthenes and alicyclic hydrocarbons) of the contaminating oil, but with different efficiencies and trends. Tables 1 and 2 summarize these changes.

The GC chromatogram of the oil extracted from the outlet of El-Taktir API separator (Fig. 1) shows the complete removal of the relatively light fraction up to nC_{18} with a total removal in TRP of 51.61% and in UCM of 46.27%. The percentage of UCM after the API separator was raised to 82.1% despite being reduced in quantity from 376.4 mg/l in the inlet to 205.25 mg/l in the outlet sample. This percent increase is due to the removal of large quantities of TRP which decreased from 123.6 mg/l at inlet to 44.75 mg/l at the outlet of El-Taktir API separator. Hence, the relative percentage of UCM increased in the water coming out of the API separator while the total hydrocarbon content was decreased as a total concentration (Table 1). Hydrocarbons higher than nC_{35} cannot be detected in the GC chromatogram of the oil extracted from the inlet sample and were detected in the chromatogram of the outlet sample. This difference may be due to their relatively low concentrations in the inlet sample; alternatively, these hydrocarbons were already present in the API separator and were not removed during cleaning processes.

The GC chromatogram of the oil extracted from the outlet of El-Islah API separator (Fig. 2) shows slight removal of the relatively light fractions up to nC_{16} with a complete removal of nC_{17} and total removal in TRP of 35.1% with UCM removal of 34.83%. The percentage of UCM after the API separator was slightly increased to 80.21% despite of being reduced in quantity from 80 mg/l at the inlet to 52.14 mg/l at the outlet sample. This difference may be due to the removal of smaller quantities of TRP compared to the first sample of El-Taktir API separator. TRP were decreased from 20 mg/l at the inlet to 12.98 mg/l at the outlet of El-Islah API separator. Hence the percentage of UCM was slightly increased in the effluent water from the API separator while the total hydrocarbon content was decreased as a total concentration (Table 2).

Effect on Pristane and Phytane

The two isoprenoids, pristane (Pr [2, 6, 10, 14-tetramethylpentadecane]) and phytane (Ph [2, 6, 10, 14-tetramethylhexadecane]) are commonly found in petroleum and its derivatives. High concentrations of pristane alone can be derived from zooplankton and phytoplankton, while the presence of phytane is used as a marker compound for petroleum. Several investigators have used changes in the ratios Pr/Ph, nC_{17}/Pr, nC_{18}/Ph, and TRP/UCM to estimate the effect of biological treatment on oil [13,14].

After mechanical treatments, there was generally an increase of Pr/Ph indicating that the removal of phytane was much higher than that of pristane, where the percentage removal of Pr after El-Taktir and El-Islah API separators was 76.65% and 72.84%, respectively while for Ph it was 86.43% and 85.88%, respectively. nC_{17}/Pr ratio is of zero value indicating the complete removal of nC_{17} after mechanical treat-

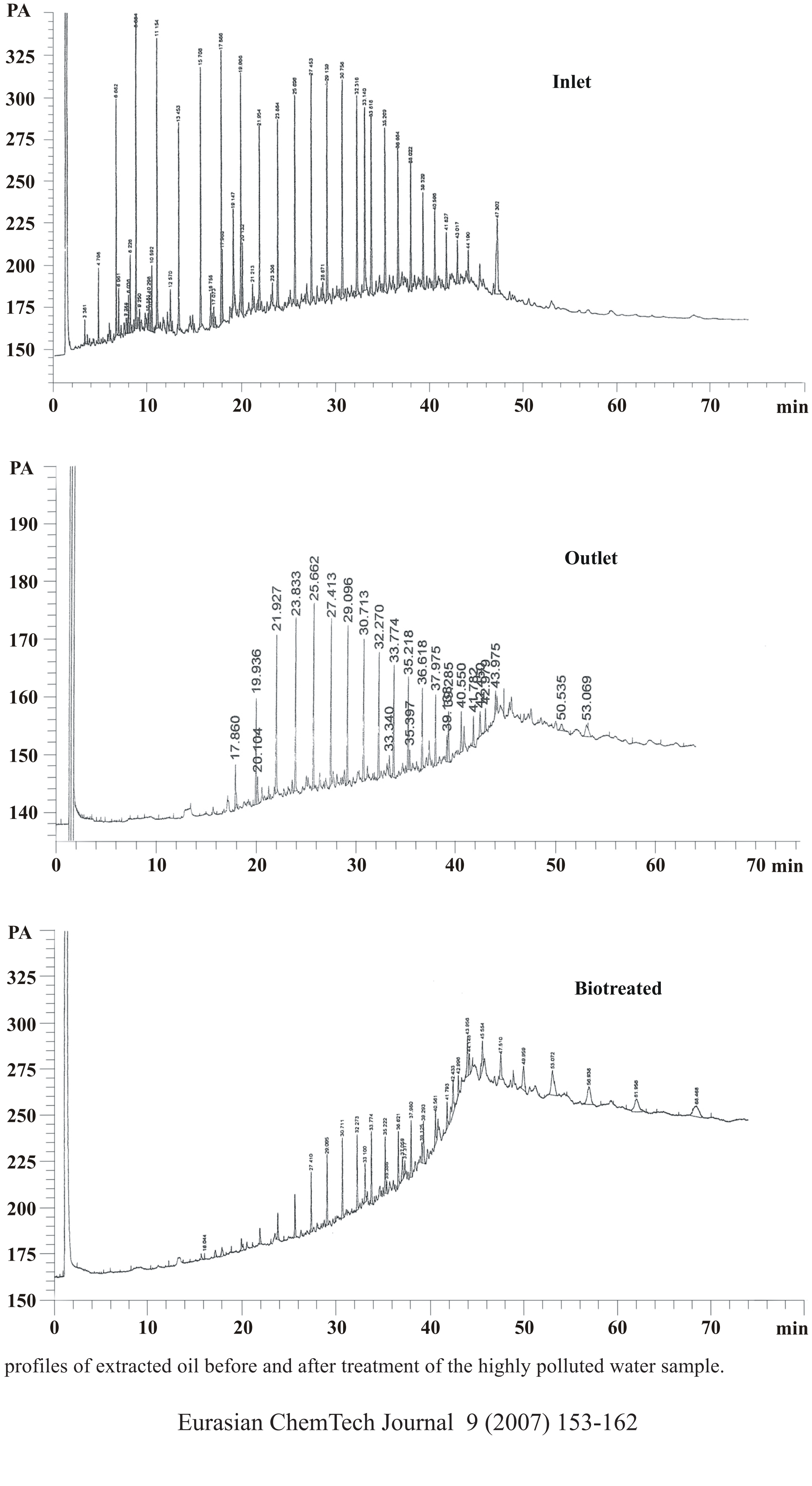

Fig. 1. GC profiles of extracted oil before and after treatment of the highly polluted water sample.

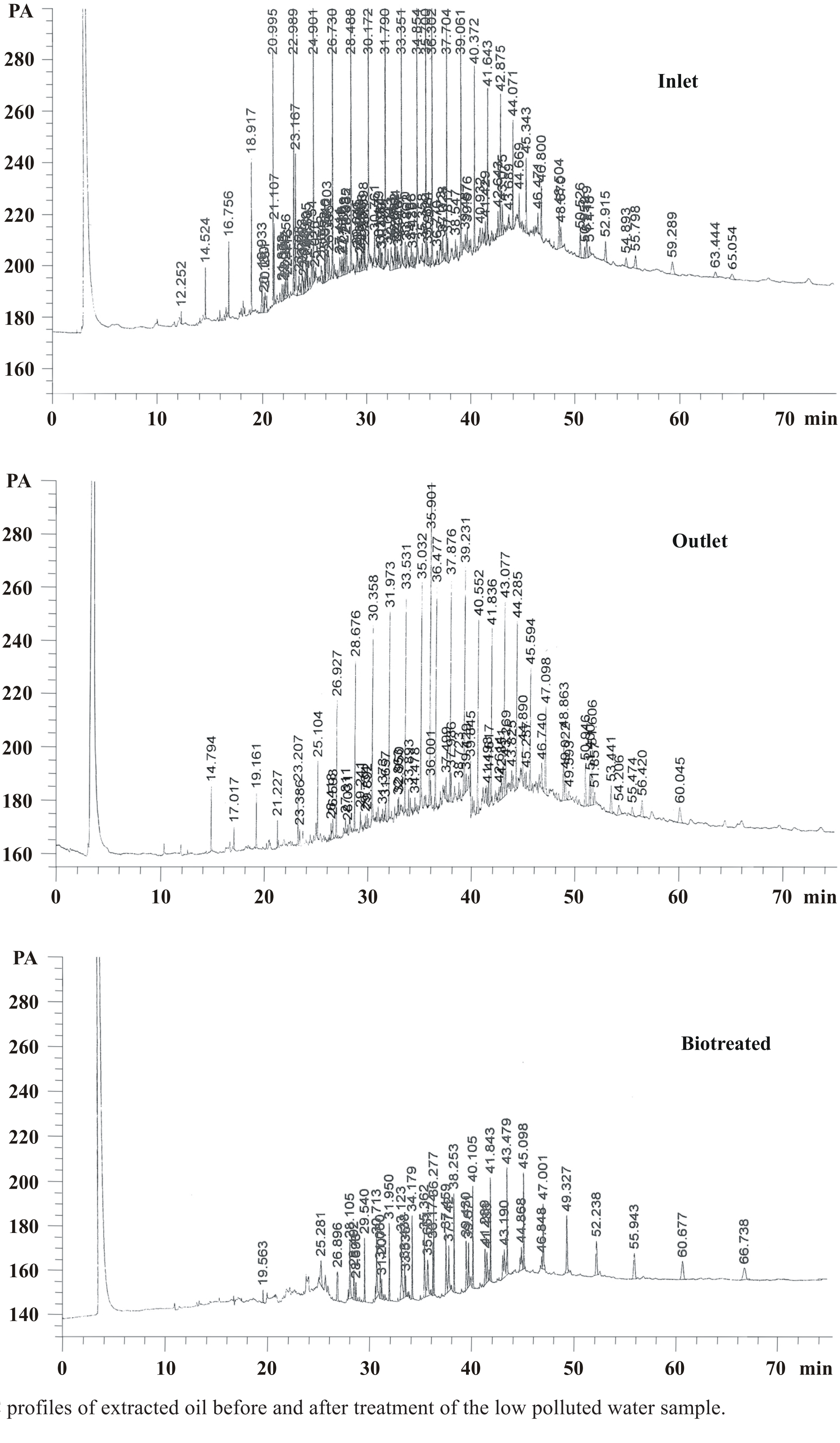

Fig. 2. GC profiles of extracted oil before and after treatment of the low polluted water sample.

ment. While there was an increase in nC_{18}/Ph ratio indicating that the removal of nC_{18} was lower than that of Ph.

Biological Treatment of CORC API Treated Waste Water

Turbidity Changes

Based on the monitoring of turbidity changes in MSM with oily waste water collected from the API outlet separators, it can be observed that at the end of incubation period, in the control flasks, oil phase was located on top of the culture medium while in flasks inoculated with *S. xylosus* AB1 the oil was emulsified in the aqueous phase. The increase in turbidity could be related to the bacterial growth and oil emulsification in the water phase as a result of the effect of biosurfactants that might be released during the microbial degrading activity [15].

Changes in pH

After 7 days of incubation, there was a decrease in pH from 7.0 to 5.90 and 6.0 in the highly polluted sample (El-Taktir) and low polluted sample (El-Islah), respectively. The change in pH could be ascribed to the production of acidic metabolites since aerobic biodegradation of aliphatic and aromatic hydrocarbons can lead to the production of organic acids [16].

Bacterial Growth

There was moderate bacterial growth after 7 days of incubation, the total viable count on LB-agar plates for El-Taktir sample was 4×10^7 and for El-Islah sample was 3×10^6. This indicates that *Staphylococcus xylosus* AB1 used in this bioremediation process has the ability to use crude oil as a sole carbon and energy source for growth. *Staphylococcus* sp. has been reported to produce heavy growth in mineral salt-base oil broth and agar media [16].

Effect on TPH

Tables 1 and 2 present the results of biotreatment of water samples taken from the outlet of El-Taktir and El-Islah API separators, respectively.

At the outlet of the El-Taktir API separator, TPH decreased 95% from 250 mg/l to 25 mg/ml after biological treatment. While it was decreased from 65 mg/l at outlet of El-Islah API separator to 18 mg/l after bioremediation with 72.31% removal and complete removal from the start of 82%. These results show the high biodegradation efficiency of the isolated bacteria *S. xylosus* AB1 for the TPH found in oily waste water and its preference to work on the high concentration than the low one. According to Brown *et al.* [17] this could be attributed to the substrate concentration which might be low to be adequate as energy and C-source for the bacteria and consequently insufficient for activation of the hydrocarbon degrading enzymes of the bacteria.

Other investigators have reported that *Staphylococcus* species have the ability to degrade drilling fluid base oils and oil waste attached to drill cuttings [16,18-20].

Effect on Different Oil Components

To confirm biodegradation capabilities of the bacterial strain on the different oil components, GC/FID analysis of extracted oil was performed. Figures 1 and 2 present the chromatographic patterns of the extracted oil before and after biotreatment of the outlet of El-Taktir and El-Islah API separators.

Effect on TRP

Treatments independent on the initial TPH concentration express a great change in TRP and UCM of the contaminating oil, but with different efficiencies and trends. Tables 1 and 2 summarize these changes.

The GC chromatograms of the oil extracted from the biotreatment flasks reveal that *Staphylococcus xylosus* AB1 shows approximately complete removal of the TRP independent of the TPH concentration, indicating the high efficiency of AB1 for degrading the aliphatic hydrocarbons. The TRP of outlet of El-Taktir API separator were reduced from 44.75 mg/l to 1.59 mg/l with 96.45% removal and total removal from the start of 98.71%. While the TRP of outlet of El-Islah API separator were reduced from 12.98 mg/l to 1.73 mg/l with 86.67% removal and total removal from the start of 91.35%. These changes are evident in the decrease of TRP/UCM and Pr/Ph ratios and nC_{17}/Pr and nC_{18}/Ph values of zero after biotreatment process as listed in Tables 1 and 2. The high molecular weight TRP continues to be present after biotreatment indicating their relatively lower biodegradability. Bacteria degrade normal alkanes readily whereas the isoprenoidal alkanes are relatively resistant to microbial degradation [1,21].

Pr and Ph as Biomarkers

Although the branched alkanes, pristane and phytane, have been used as conservative biomarkers in oil bioremediation studies; their recalcitrance to biodegradation has been questioned in a lot of reports due to their susceptibility to biodegradation [22,23]. *S. xylosus* AB1 was also able to degrade Pr and Ph in the highly polluted sample 73.41% and 54.96%, respectively while it showed complete removal of Pr and Ph in the low polluted sample. These reductions indicates that *S. xylosus* AB1 has good efficiency in biodegradation of such branched alkanes. It also confirms the observations reported by Xu *et al.* [24]; that Pr and Ph are not reliable biomarkers for monitoring oil biodegradation.

Effect on UCM

It is evident from the GC chromatograms of the biotreated samples Figs. 1 and 2 that *S. xylosus* AB1 has good biodegrading effect on the UCM (aromatics, naphthenes and alicyclic hydrocarbons) especially on the lower molecular weight ones where there is a general decrease in the UCM signals in the low molecular weight region and increase in the UCM signals at the high molecular weight region. UCM of outlet of El-Taktir API separator was decreased 88.59% from 205.25 mg/l to 23.41 mg/l after biological treatment with total removal from the start of 93.78%. While the UCM of outlet of El-Islah API separator were decreased 68.2% from 52.14 mg/l to 16.93 mg/l after biological treatment with total removal from the start of 79.75%.

Conclusions

Samples taken from the API separators (El-Taktir) and (El-Islah) have demonstrated that the efficiency of these separators were not quite satisfactory for the industrial effluent to reach the limits indicated by Egyptian law of environmental protection for fresh water which is only 5 mg/l in condition that the oil concentration in the receiving fresh water should not exceed 0.1 mg/l. This low efficiency implies that maintenance works should be carried out for these API separators or a reassessment should be carried out for their performance to decide if this treatment should be replaced with a more effective regimen of secondary treatment.

The bacterial strain Staphylococcus xylosus AB1 used in the research proved high effectiveness in oil removal from the polluted samples taken after the API separators. However this removal percentage of the total hydrocarbon content was not sufficient to reach the permissible limit indicated by Egyptian law of environmental protection. Further work is carried out in Egyptian Petroleum Research Institute (EPRI) and National Research Center to reach the requirements needed.

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