MICROBIAL SURVEY FOR SPECIFIC PLACES OF AL-DAURA REFINERY

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ABSTRACT

16th oily samples were collected during 10th October 2009 to the 10th July 2010 from different specific places at Al-Daura refinery which included: tanks, soils, refinery operation stages, and waste accumulation area. Each sample was well studied and its microbial flora were isolated on agar medium and identified by biochemical tests and Remel electronic RAPID system. Different microbes were isolated included bacteria (both Gram –ve and +ve), fungi yeast and algae. The results show that their isolation percentages were 100%, 20%, 5%, 1%, respectively. The heavy metals concentrations were also measured in the sludge waste samples that were: Zn, Fe, Mg, Sn, Cr, Cu, Ni, and Pb, the results show 17, 400, 38, 4, 3, 9, 6, 1 ppm, respectively and for the sludge from the tanks was 420, 64, 2, 2, 16, 7, 1 ppm, respectively.

KEYWORDS: Microbial, Survey, Al-Daura Refinery

INTRODUCTION

PAHs are found in high concentrations at industrial sites especially sites that use or process petroleum products. They are considered carcinogens and mutagens, and are very recalcitrant, pervading for many years in the natural environment. Other contaminants include residuals from flares (perchlorate) and explosives (TNT, RDX); metals (chromium, lead); plutonium and uranium; potassium and nitrogen. Much of the high levels of these contaminants found in nature is a result of human activity [(1)].

Bioremediation refers to the use of microorganisms to degrade contaminants that pose environmental and especially human risks. Due to its safety and convenience, it has become an accepted remedy for cleaning polluted soil and water [(2)]. Bioremediation processes typically involve many different microbes acting in parallel or sequence to complete the degradation process. The ability of microbes to degrade a vast array of pollutants makes bioremediation a technology that can applied in different soil conditions[(3)].

A widely used approach to bioremediation involves stimulating a group of organisms in order to shift the microbial ecology toward the desired process. This is termed "Biostimulation." Biostimulation can be achieved through changes in pH, moisture, aeration, or nutrient additions. The other widely used approach is termed "Bioaugmentation" where organisms selected for high degradation abilities are used to inoculate the contaminated site[(3)]. These two approaches are not mutually exclusive- they can be used simultaneously.

Bioremediation is accomplished either in situ or ex situ. In situ remediation efforts focus on treating the contaminant at the polluted site. Ex situ remediation refers to the treatment of contaminated water or soil at an offsite location. In such cases, soil and groundwater from the contaminated site are transported to a place (like a bioreactor), where conditions favorable for biological degradation can be controlled and enhanced [(1)].

Species of *Desulfovibrio* and anaerobic *Clostridium* species especially *Clostridium acetobutylicum* were successfully used as a microbial treatment for oily sludge waste. Many species of bacteria actually consume the oil as a source of carbon, in the presence of other nutrients producing methane and carbon dioxide [(4)].

MATERIALS AND METHODS

Materials included:

1- Sterile environment sample collecting containers.
2- Aseptic Hygiene when dealing with samples (gloves, masks, and aseptic lab environmental techniques and instruments).
3- Electronic Rapid One System Biochemical analysis for Identification from Remel/USA. Other biochemical tests were also applied like urease test, Citrate utilization test, Motility, IMVIC and Triple sugar fermentation test.

4- Agars were previously prepared according to the manufacturing company, it included the types as listed below:

No.	Agar media	Manufacturing Company
1	Mineral Salt Agar media	Prepared According to Mahjub R. [5]
2	Nutrient agar media	Himedia
3	Brain-Heart Infusion agar media	Himedia
4	Blood Agar media	Himedia
5	MacConkey agar media	Himedia
6	Sabrauod Agar media	Himedia
7	PCAH* Agar media	Prepared According to Mahjub R. [5]

- **PCAH – Polycyclic Aromatic Hydrocarbones.**

METHODS

1. After preparing isolation agar media Petri dishes, a twice inoculums of 0.1 ml of each sample were placed on each type of the agar media then incubated overnight in the incubator at 37°C.
2. The isolated microbes were identified by direct examination of the colony special characteristics and with microscope after applying the suitable procedure for preparing each examined slide.
3. The biochemical tests were applied after isolation of each type colony that was grown on the agar media.
4. Applying Electronic Rapid One system biochemical analysis for highly specific identification of the bacteria.
5. Applying each isolated colony for PCAH decomposing on PCAH media for further qualification.
6. After overnight incubation the qualified strain were selected after measuring the clear zones around each active colony.
7. The selected strains were preserved for further experiments.

The total aromatic hydrocarbons rate was determined in the broth media before and after 24 hours of growth and chemical analysis of heavy metals for the decomposed sludge were done in ISSC Laboratory (Ibn Sina State company Laboratory/Ministry of Industry and Minerals).

RESULTS AND DISCUSSION

All the collected samples showed a heavy growth off different microbes that included Bacteria (both Gram negative and positive), fungi, yeast and algae. Table 1 shows the isolated microbes of each sample.

All the isolated microorganisms had been identified according to the microscopic examination, biochemical test results and growth on different agar media which were listed in table (2).

The gram negative bacteria, the gram positive spore forming bacteria and fungi were highly frequent according to their ability to tolerate extreme conditions and enzymes they possess which utilize complex compounds to use them as a source of energy.

The chemical analysis results of the heavy metals and the total hydrocarbons are shown in table (3), which give concentration before and after treatment with microorganisms.

Figure 1 A show the rapid one system biochemical test results for the three bacterial strains selected, while B shows the qualified B_1 bacterial strain grown on different agar media, that included Mineral salt agar, MacConkey agar with pale non lactose fermenting colonies, hemolysis on Blood agar, and PCAH agar that showed the highly decomposing area by this bacterial strain.

Many studies in the same field showed similar results when treating oil spills and even using microbes especially bacteria[6,7]. In USA a report recorded that microbial organisms play an important role as a biodegradative agent[8]. While in Italy an event promoted researchers to find a way to activate bacterial population to treat an oil polluted soil[9].

The chemical analysis of the total hydrocarbons were measured in the used samples as an inoculums in order to detect the decomposing ability of the bacteria before and after treatment and it showed that it decreased from 16.4% to Nil in the broth media, and the clear zone showed in figure (1) that referred to the decomposing ability of bacteria on PAHs-agar media gives a clear image to the ability of the bacteria and role of microorganisms to degrade the petroleum

derivatives which also was proved after the Leak disaster in the Gulf of Mexico in the pipeline of the British petroleum company that a group of scientist in Georgia University proved the ability of the microbes to that degraded more than 10% of the total discharge of the raw petroleum leaked and authorized at the science conference in Washington[(10)].

Microorganisms use a wide range of metabolic pathways to harvest energy from their environment. In some cases, pollutants serve as the carbon and energy source for microbial growth, while in other cases; pollutants serve as the terminal electron acceptor (ex. perchlorate degradation). This manifests itself in the diverse ability of microbes to transform and degrade toxic molecules [(11)]. From the obtained results it can conclude that microorganisms from isolated from the Al-Daura refinery can consume the oily sludge wastes as a carbon source for its growth.

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Table (1) The isolated microbial flora from each collected samples on 10th. February 2010

No.	Sample No.	Depth and place	Isolated Microbes			
			Bacteria	Fungi	Yeast	Algae
1	Solid (Soil)	10cm depth – Soil around Refining Tanks	+++	+	+	-
2	Solid (Soil)	=	+++	+	+	-
3	Solid (Sludge)	Sludge Tank before Refining Process	++++	-	-	-
4	Solid (Soil)	10cm depth – Soil around Refining Tanks	++++	-	-	-
5	Liquid	Sludge Tank through Refining process	+++	++	+	-
6	Liquid	=	+++	-	-	-
7	Liquid	=	+++	-	-	-
8	Liquid	=	+++	-	-	-
9	Liquid	=	++++	-	-	-
10	Liquid	=	+++	-	+	-
11	Solid (Soil)	10cm depth – Soil around Refining Tanks	++++	-	-	-
12	Solid (Sludge)	Accumulated Sludge waste	++++	-	-	-
13	Solid (Sludge)	Tank walls – After Refining Process	+	+	+	+
14	Liquid	Accumulated Sludge waste	++++	-	-	-
15	Semi-Solid (Soil)	10cm depth around accumulated sludge waste area	+++	-	-	+
16	Solid (Sludge)	10cm depth	++	++	-	-

+ Positive for growth (+++-T.M.C.) - Negative for growth.

Table (2) The identified species isolated from the collected samples.

Type of the Microbe	Species	
Bacteria	Gram -ve	Gram +ve
	Pseudomonas aeruginosa	*Micrococcus*
	Pseudomonas fluorescence	*Methylococcus*
	E. coli	*Bacillus*
	Providencia	*Peptococcus*
	Xanthomonas	
	Proteus	
Fungi	*Penicillium, Aspergillus, Mucor , Rhizopus*	
Yeast	*Saccharomyces*	
Algae	*Spirogyra*	

Table (3) Concentration of Heavy metals in sludge before and after treatment with microorganisms

Before Treatment, ppm								
Sample	**Zn**	**Fe**	**Mg**	**Sn**	**Cr**	**Cu**	**Ni**	**Pb**
Sludge waste	17	400	38	4	3	9	6	1
Sludge tanks	20	420	64	2	2	16	7	1
After Treatment, ppm								
Sample	**Zn**	**Fe**	**Mg**	**Sn**	**Cr**	**Cu**	**Ni**	**Pb**
Sludge waste	1	4	15	1	1	8	1	1
Sludge tanks	1	4	37	2	1	4	1	1

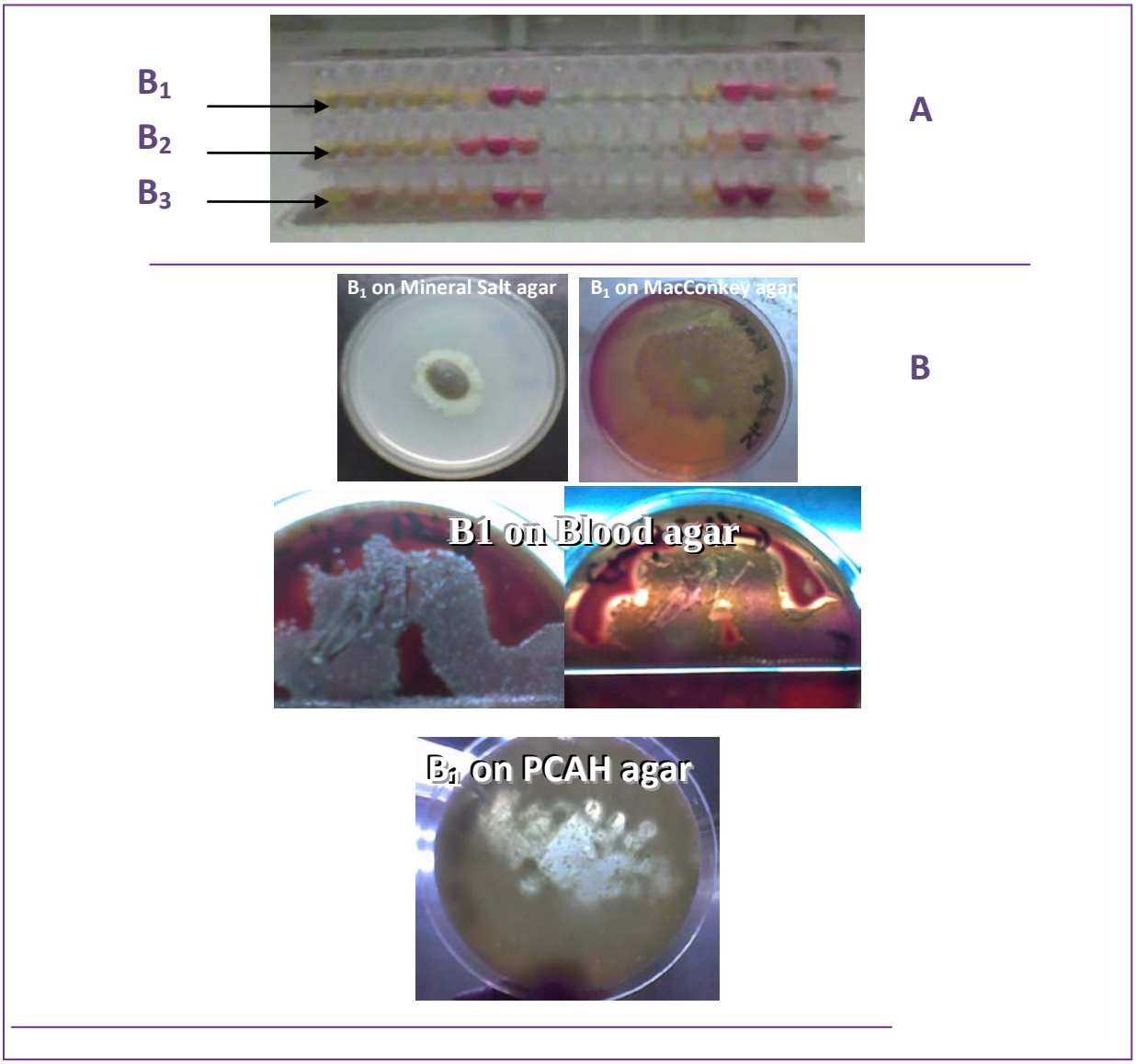

Figure (1) A- Rapid one system biochemical results for the selected bacterial strains for qualification. B- Growth of the B_1 qualified strain on different agar media.

مسح مايكروبي لأماكن معينة في مصفى الدورة

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الخلاصة:

تم جمع 16 عينة نفطية للفترة العاشر من تشرين الاول 2009 ولغاية العاشر من تموز 2010 من أماكن محددة مختلفة من مصفى الدورة شملت الخزانات ، التربة ، من مراحل مختلفة من عملية التكرير و منطقة تراكم النفايات .درست كل عينة بشكل جيد و تم عزل الاحياء المجهرية على وسط صلب ثم تشخيصها بوساطة الاختبارات البيوكيميائية و نظام Remel الالكتروني للتشخيص. وتم عزل احياء مجهرية مختلفة شملت عزل البكتيريا سواء الموجبة منها لصبغة غرام او السالبة منها والفطريات الخميرة والطحالب الذين كانت نسب عزلهم كانت 100٪ ، 20٪ ، 5٪ ، 1٪ على التوالي. كما تم قياس تراكيز المعادن الثقيلة في عينات النفايات والحمأة هي : الزنكppm 17، الحديد 400 ppm، والمغنيسيوم 38 ppm، القصدير 4 ppm، الكروم 3 ppm ، النحاس 9 ppm، النيكل 6 ppm، الرصاص 1 ppmعلى التوالي والحمأة من الخزانات 20، 420، 64، 2، 2، 16، 7 جزء من المليون وعلى التوالي.

الكلمات الدالة : المايكروبات ، المسح المايكروبي ، مصفى الدورة.