

# Removal of Poly Aromatic Hydrocarbons and Total Petroleum Hydrocarbon by Using of Bio-Enzyme: a Batch Study

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**Abstract**—Poly aromatic hydrocarbons (PAHS) and total petroleum hydrocarbon (TPH) on receiving water were a major concern in many country of the world specially producer of oil and related petrochemical products. Such pollutants often had an effect on aquatic environment and on human health. The removal of PAHS and TPH from one of Iran's petrochemical effluent by using of a Bio-Enzyme mixture had been studied at laboratory scale. The investigation review Biochemical characterization of the sample in contact with application of bio-enzyme, by installing of batch reactor (with and without application said bio-enzyme). The batch reactor runs for 10 days and result shown that without presence of said bio-enzyme PAHS, TPH, and chemical oxygen demand (COD) did not remove and there is not any changes after 10 days, even the batch reactor by apply of a mixture of bio-enzyme removed TPH /PAH, and COD about 99.98%, and 100% after 10 days, respectively. Main factors that manage the removal process were the bio-enzyme activity in hydrolysis and oxidation of pollutants. As a result, the Bio-enzymes activity and the mineralization of pollutants due to presence of millions of net micro-Bio-enzymes were responsible for the removal TPH/PAH/COD.

**Index Terms**—Bio-enzyme, chemical oxygen demand, poly aromatic hydrocarbons (PAH), total petroleum hydrocarbon, (TPH).

## I. INTRODUCTION

Petroleum hydrocarbons shown degradable and biodegradable properties in aquatic environment (Mills et al. 1994; Prince et al. 1994). The term total petroleum hydrocarbons (TPH) was applied to explain a mixture of several hundred chemical compounds that originally obtained from crude oil. The TPH was identified as the measurable amount of petroleum-based hydrocarbon in environmental media (Research Triangle Institute 1999).

Re-refined petroleum products, including, gasoline, diesel fuel, used engine oil constitute are an important group of contaminants for environmental researchers. The carbon numbers in these petroleum products were as follows: gasoline (C6 – C12), diesel (C8 – C26), kerosene (C8 – C18), fuel oil (C17 – C26), and lubricating oils (C21 – C50) (George 1994).

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TPH divided into aromatic and aliphatic hydrocarbon fractions. The aromatic compounds tended to be more soluble in water and slightly less volatile than aliphatic compounds.

Crude oil was used to make petroleum products, which could contaminate the environment by leakage of hydrocarbons during extraction, refining, storage, and transportation activities (Balba, et al. 1998). The values based on USEPA derived reference doses (RFDs) had been defined fluorene, anthracene, fluorantene, naphthalene and pyrene. (STSC 2002). Some TPH compounds could affect our blood, immune system, lungs, skin, eyes, central nervous system (CNS), causing headaches and dizziness, and causing nerve disorder called peripheral neuropathy, (STSC 2002). The International Agency for Research on Cancer (IARC) had defined that benzene was carcinogenic to humans. Also, IARC had defined benzo[a]pyrene and gasoline were probably carcinogenic to humans (Steinsvag et al. 2008). Aesthetic issues, such as odor and visual impact, which maybe required consideration at sites affected by petroleum contamination. For example n-hexane compound considered as a potent neurotoxin. During the last decade, concerns about hydrocarbons in the environment had greatly exceeded. Between of them, TPHs. were identified as the most dangerous because the accumulation of these compounds in water resulted in considerable risks to human through different exposure pathways (Daryabeigi Zand et al. 2010).

Many studies had been performed to find cost-effective methods to remove petroleum contamination in water. Technologies such as physical separation, dissolved air flotation (DAF), membrane processes, adsorption onto activated carbon, chemical separation, advanced oxidation processes and chemical oxidation, had been suggested (Health Risk Technical Support Center 2002, Ayotamuno et al. 2006, Ghidossi et al. 2009). However, these technologies led to incomplete decomposition of these contaminants. Biodegradation was an environmentally friendly process. Among a variety of biological processes, bio-enzyme was one of the most famous applied approach for PAH/TPH removal, and thus it was considered as the most powerful microbial oxidizing agents for applying to mineral bio-refractory organic compounds in aquatic environment, owing to its high oxidative potential and its simplicity operation and maintenance. The equations happened in Bio-enzyme reactions were as follows:

Hydrolysis of organic material

Total petroleum hydrocarbon  $\rightarrow$  CO<sub>2</sub> + H<sub>2</sub>O + cell constituents product

Microbial consortium

This process, regarded as the use of microbial consortium to mineral product due to their diverse metabolic potential was an promising method for the mineralization the products of petroleum industry (Medina-Bellver et al. 2005). In total, bio-enzyme technology was considered to be noninvasive and relatively cost-effective (April et al. 2000; Ulrici 2000). The success of process depended on many factors that increased the rate of oil bio-degradation (Nilanjana & Chandran 2011).

One important requirement was the presence of favor microorganisms with the suitable metabolic abilities. The presence of these microorganisms led to sustain optimum rates of growth and hydrocarbon biodegradation. Biodegradation phenomenon depended on many factors, such as suitable concentrations of nutrients and oxygen, and pH value. Most executed studies had concentrated on estimating the parameters effecting on oil biodegradation or testing favored products and methods through laboratory studies (Mearns 1997). Only limited numbers of pilot scale and field studies had performed (Venosa et al. 2002). These field studies emphasized on the evaluation of bioremediation technology for dealing with large-scale oil spills on marine shorelines. This study provided a new approach on microbial degradation of petroleum hydrocarbon contaminants (PAH/TPH) in effluent of petrochemical plant. The effects of the operational factors on the removal were examined by performing batch bioreactor and considering reaction duration- time, mechanisms of petroleum hydrocarbon degradation, pH and temperature of sample.

## II. MATERIAL AND METHODS

### A. Description of the Studying Point (BuAli Sina Petrochemical Complex)

The location of BuAli sina petrochemical complex (BASPC) is in petrochemical special economic zone. The products of Buali sina petrochemical complex are Benzene(180000 t/y) , Paraxylene (40000t/y) ,Orthoxylene (30000t/y), Light end(350000 t/y), Raffinate (199000 t/y), LPG(39000 t/y), C5 cuts(21000 t/y), Heavy cuts naphtha(488000 t/y), Heavy cuts pyrolysis gasoline(11000t/y), Heavy aromatics(23000t/y).

### B. Specification of Bio-Enzyme BIOVITA (Trade Mark)

Table 1 showed specification of BIOVITA. A mixture of of Bio-Enzyme BIOVITA GRS and ZIPAK (BVGZ) were supplied via R&D section of Biotechnology application in WWT , Pandab Tadbir engineering Co. (Iran).

## III. PREPARATION OF SAMPLES

The sample had been taken from the oily water collection basin which currently collected the effluent from different

process units of BASPC. 400 ml of sample (oily water of BASPC) mixed with 1600 ml of tap water (without residual chlorine). The solution was aerated with an aerated jet pump (RS. Model 610, China) for 120 min. The pH of solution was measured after pouring into batch bioreactor. Chloridric acid and sodium hydroxide (0.1 N) were used for pH adjustment. After these steps 10 grams of BIOVITA GRS and 10 grams BIOVITA ZIPAK was added into bioreactor and mixed with a magnetic stirrer (Aika, Germany).

TABLE I: SPECIFICATION OF BIOVITA (GRS AND ZIPAK)TRADE MARK

Odor	pH	particle size ( $\mu$ )	Bulk density (g cm <sup>-3</sup> )	Total microbial count (CFU/GM)
Yeast like	7.6	30	0.7	$4.5 \times 10^{-9}$
Microbial diversity	<i>Salmonella</i> ss., <i>Shigella</i> ss., and Total <i>Coliform</i> were absent			

## IV. BATCH BIOREACTOR

Fig. 1 showed a batch bioreactor made of a 3000 ml glass vessel. An Aerated jet pump equipped with a fine bubble diffuser with rate of oxygen injection equal to 1 lit min<sup>-1</sup> was used for aerating of bioreactor. To evaluate the effect of bio-enzyme reaction for removal of the chemical oxygen demand (COD), PAH<sub>s</sub>, and TPH, bioreactor runs for a duration (1-10 days). Because there were many different chemicals in crude oil and in other petroleum products, here our team made decision to limit the monitoring the pollutant parameter for few items. Therefore, it had been decided to analysis the Percentage of COD removal , and reduce of PAH<sub>s</sub>, and TPH , the result calculated according to the following equation:

$$\text{®Removal (\%)}: (1-A/B)$$

where R was the percentage of COD, PAHS, and TPH reduction; B and A were the average of concentration of COD, PAHS, and TPH reduction before and after treatment (mg l-1 or  $\mu$ g l-1). All batch tests were performed in triplicate, and the average data values were reported. The TPH were measured by ASTM method D 7066-04 gas chromatography / Mass spectrometry (GC / MS) (ASTM method D 7066-04). The specification of GC / MS were as follows: detector, MS; injection technique, split 2:1; injector temperature, 160 °C; injection volume, 3  $\mu$ l; carrier gas, helium; flow rate, 1 ml min-1; aux temperature, 290 °C. The specification of used column were as follows: type of column, capillary; length, 30 m; diameter, 0.25 mm; film thickness, 0.5  $\mu$ m; phase, DB5-MS. The specification of temperature program were as follows: initial temperature, 36 °C; isothermal, 6 min; first rate, 6 °C min-1 to 200 °C min-1; isothermal, 5 min.

The gas chromatography-mass spectrometry (GC / MS) ASTM method D 7363-07 was used for analysis of PAHS such as naphthalene in samples. The specification of GC / MS were as follows: detector, MS; injection technique, splitless; injector temperature, 290 °C; injection volume, 3  $\mu$ l; carrier gas, helium; flow rate, 1 ml min-1; aux temperature, 290 °C. The specification of column used were as follows: type of column, capillary; length, 30 m; diameter, 0.25 mm;

film thickness, 0.5  $\mu$ ; phase, DB5-MS. The specification of temperature program were as follows: initial temperature, 60  $^{\circ}$ C; isothermal, 1 min; first rate, 10  $^{\circ}$ C min<sup>-1</sup> to 100  $^{\circ}$ C min<sup>-1</sup>; second rate, 4  $^{\circ}$ C min<sup>-1</sup> to 285  $^{\circ}$ C min<sup>-1</sup>; isothermal, 15 min. The COD were measured by standard method 5220C. Reactor without Bio-Enzyme and without aeration was used as the test control. The pH was measured using a pH-meter (Hach, USA).



Fig. 1. The batch bioreactor (experimental conditions: 25  $^{\circ}$ C, pH: 6-9, reaction time: 1-10 days)

## V. RESULT AND DISCUSSION

### A. Effect of Reaction Time

This study using of bio-enzyme on oily water effluent shown reduction of COD, PAHS, and TPH. Bio-enzyme BVGZ reduce the rate of COD, PAHS, and TPH. Figures 3-5 shown the effect of the reaction's duration on removal efficiency. The COD, PAHS, and TPH reduction was increased by an increase in the reaction's duration from 1 day to 10 days. The bio-enzyme batch reactor test result shown percentage removal of COD in 100% in 10 days, with the initial concentration of 440 mg ml<sup>-1</sup>. and 99.98% for PAHS with the initial concentration of, 16  $\mu$ g l<sup>-1</sup>, 99.98% for TPH with the initial concentration of 770 mg l<sup>-1</sup>. As expected, by increasing the reaction's duration, accordingly the bio-enzyme activity were increased. This phenomena is the same as microbial consortium which consisting of two isolates of *Pseudomonas aeruginosa* and one isolate *Rhodococcus erythropolis* (Cameotra and Singh, 2008). They were investigated the effect of bioremediation on oil sludge. Even that Microbial consortium was able to degrade 90% of hydrocarbons in 6 weeks in liquid culture, here there is better result, on oily effluent of petrochemical plants in 10 days.

Fig. 3 shown the plots of the kinetics first, and second order reaction models fitted with the COD removal experimental data in batch bioreactor. These kinetics reaction models were calculated according to the following equations:

$$\ln Ct = \ln C_0 - K_1 t$$

$$1/C_t = K_2 t + 1/C_0$$

where  $C_0$  and  $C_t$  are the concentration of COD at the beginning and after duration "t" of the reaction, respectively.  $K_1$  and  $K_2$  are the first, and second order reaction constants, respectively. Values of  $K_1$  and  $K_2$  could be calculated from the slope of the plots  $\ln Ct$  versus  $t$ , and  $1/C_t$  versus  $t$ , respectively. The experimental data fitted better to the second order reaction. The first order reaction rate constants for COD reduction in the batch bioreactor was 0.1241 L.g.min<sup>-1</sup>

Since the adjusting of first and second order equation for 10 days result cause the reduction of regression factor, then

it was considered 8 days for drawing of graphs

The first order reaction rate constants for PAHS (as Naphtalen) reduction in the batch bioreactor was 0.1463 L.g.min<sup>-1</sup>.

Fig. 5 showed the plots of TPH removal. The first order reaction rate constants for TPH reduction in the batch bioreactor was 0.144 L.g.min<sup>-1</sup>.

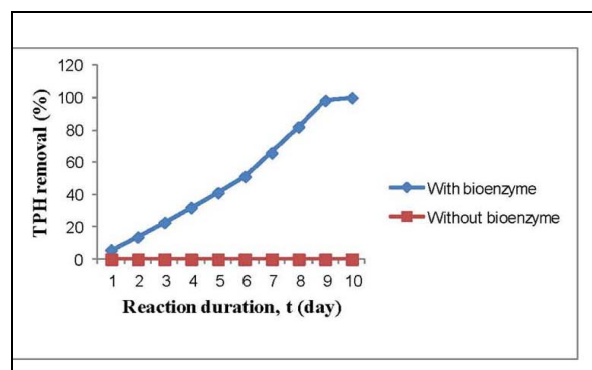
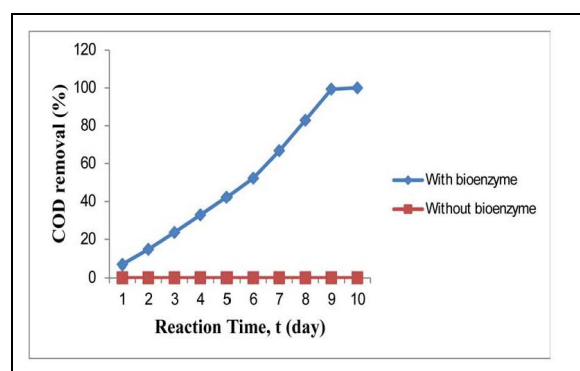
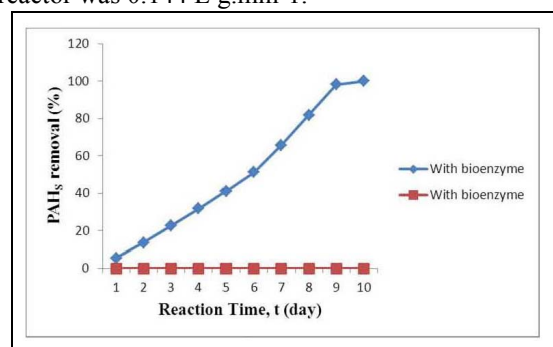


Fig. 2. The effect of reaction's duration on the removal of PAH/TPH/COD in batch bioreactor (experimental conditions: 25  $^{\circ}$ C, pH: 7.5, reaction's duration: 1-10 days)

### B. Mechanism of Biological Stabilization of Petroleum Hydrocarbon Degradation

The most complete degradation of the mainly of organic material was occurred under aerobic conditions. Figure 6 illustrated the main principle of aerobic degradation of hydrocarbons. The initial intracellular attack of organic materials was an oxidative process and oxygen consumption. It was expected that enzymatic degradation of pollutants might affect the oxygenases and peroxidases production during bio-enzymatic oxidation. The degradation of method was highly dependent on enzymatic oxidation. For example, the tricarboxylic acid cycle was called minor degradation pathway. Biosynthesis of new cell happened from the metabolites, including, acetyl-CoA, and pyruvate. The degradation mechanisms of PAHS and TPH could be

attributed to oxygenases enzyme, attachment of bioenzyme nanoparticle to the oil droplets (W. Fritsche and M. Hofrichter, 2000).

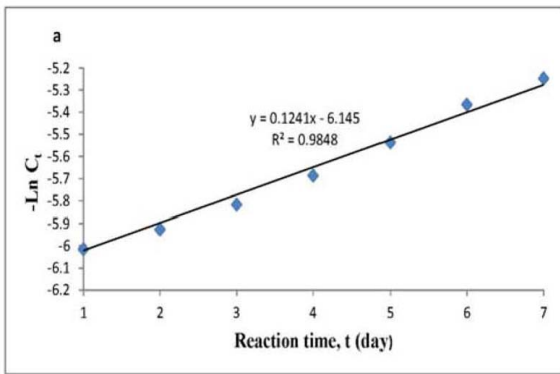


Fig. 3. The plots of first, and second order reaction models fitted with the COD removal experimental data in batch bioreactor (experimental conditions: 25 °C, pH: 7.5, reaction duration -time: 1-8 days)

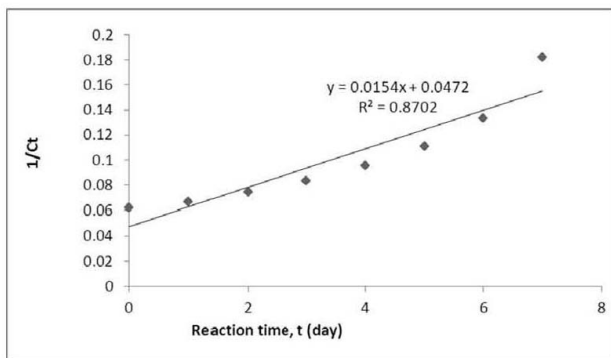
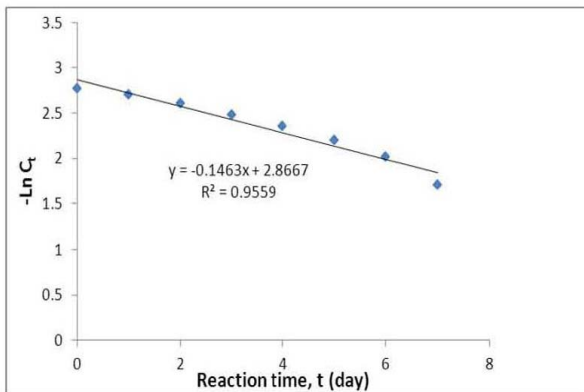


Fig. 4. The plots of first, and second order reaction models fitted with the PAHS (as Naphtalen) removal experimental data in batch bioreactor (conditions: 25 °C, pH: 7.5, reaction's duration-time: 1-8 days)

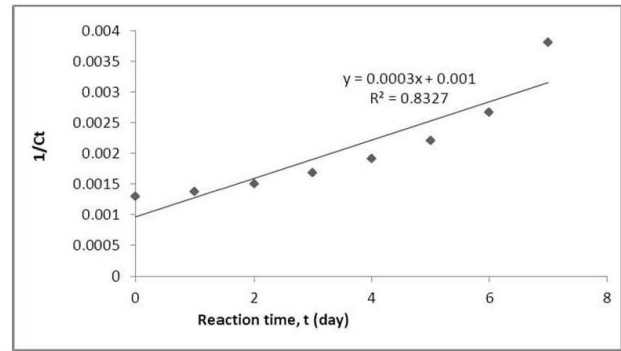
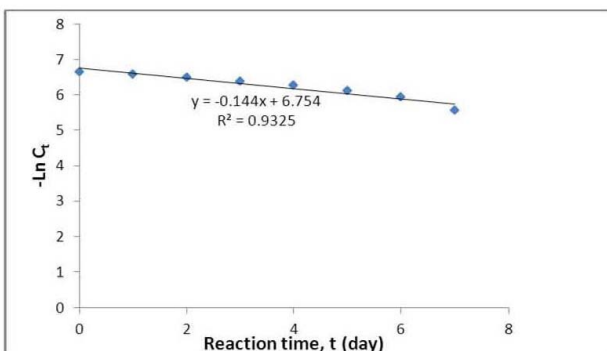


Fig. 5. The plots of first, and second order reaction models fitted with the TPH removal experimental data in batch bioreactor (experimental conditions: 25 °C, pH: 7.5, reaction's duration - time: 1-8 days)

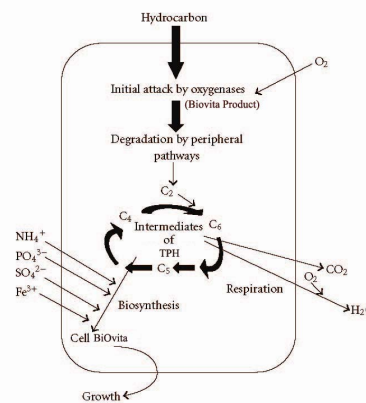


Fig. 9. The main principle of aerobic degradation of hydrocarbons with bio-enzyme



Fig. 10. The effluent visual properties before ( right bottle )and after bio-enzyme batch reactor (left bottle)

## VI. EFFECT OF PH

Table III shown the effect of the pH on reduction efficiency. Bio-enzyme batch reactor analysis of efficiency of COD, PAHS, and TPH reduction for pH 6-9 demonstrated that the activity effect of the method was highly dependent on pH, and was increased by an increase in pH. This enhancing effect could be attributed in part to a more efficient formation of oxygenases and peroxidases. The efficiency of COD, PAHS, and TPH reduction increased with increasing pH value. However, a limiting value can be observed at high pHs due to decrease in oxygenases and peroxidases production leading to decrease in bio-enzymatic oxidation. Optimum amount of pH for reaching to complete mineralization was 7.5. Experimental results shown that the bio-enzyme had a negative charge in neutral pH. The negative charge of

bio-enzyme led to increasing in biodegradation rate constants of removal COD, PAHS, and TPH reduction. This finding the same as bio-augmentation for remediation of oily sludge (Ouyang et al., 2005). They were reported that pH of the environment effected on the activity of the microorganisms. The monitoring of the sample's pH shown a low fluctuations tend to increase after adding bio-enzyme.

TABLE III: THE EFFECT OF REACTION'S DURATION -TIME ON THE REMOVAL OF TPH IN BATCH BIOREACTOR (EXPERIMENTAL CONDITIONS: 25 °C, PH: 6-9, REACTION TIME: 1-10 DAYS)

Time (day)	Removal (%) at			Removal (%) at			Removal (%) at		
	Parameter			Parameter			Parameter		
	COD	PAH	TP	COD	PAH	TPH	COD	PA	TP
1	0	0	0	6.8	5.6	5.7	0	0	0
2	0	0	0	14.7	13.6	13.7	7.3	6.1	6.2
3	5.8	4.4	4.5	23.6	22.5	22.6	13	12	12
4	11.7	10.6	10.7	32.9	31.8	31.9	20.2	19	19
5	22.2	21.4	21.5	42.2	41.1	41.2	30.6	30	30
6	26.1	25	25.1	52.3	51.2	51.3	36.9	36	36
7	34.3	33.2	33.3	66.8	65.7	65.8	43.2	42	42
8	42.9	41.8	41.9	82.9	81.8	81.9	67.3	66	66
9	51.3	50.1	50.2	99.3	98.2	98.3	73.4	72	72
10	55.2	54.1	54.1	100	100	100	77.7	77	77

TABLE IV: THE EFFECT OF REACTION'S DURATION -TIME ON CHARACTERISTICS OF THE SAMPLE IN BATCH BIOREACTOR (EXPERIMENTAL CONDITIONS: 25 °C, PH: 7.5, REACTION TIME: 10 DAYS)

Parameter	Unit	Value		
		Before treatment- Start of test	After treatment- 10 days	
COD	mg/L	440	0	
PAH <sub>s</sub>	Acenaphthene	µg/L	5	<0.5
	Acenaphthylene	µg/L	3	<0.5
	Flourene	µg/L	3	<0.5
	Naphthalene	µg/L	16	<0.5
	Phenetrene	µg/L	4.5	<0.5
pH	-	4.5	8	
Total Dissolved Solids	mg/L	1900	200	
TPH	mg/L	770	0.09	

The mean values of oily effluent sample characteristics , after and before treatment by using bio-enzyme, presented in Table IV.

## VII. EFFECT OF TEMPERATURE

Temperature had a significant role in biodegradation of hydrocarbons with direct affecting on the chemistry of the pollutants, and affecting the diversity of the microbial flora. Temperature also effected on the solubility of hydrocarbons and the activity of the bio-enzyme. The biodegradation constants of removal COD, PAHS, and TPH reduction mainly reduced with decreasing temperature. The highest biodegradation rates that happened in the range 15–20°C in marine environments. At 20°C temperature the viscosity of the oil decreased, the volatility of the low molecular weight hydrocarbons were increased, bio-enzymatic oxidation were increased, and accelerating the onset of biodegradation. This

finding was the same as biodegradation of crude oil contaminating marine shorelines and freshwater wetlands (Venosa and Zhu, 2003). They were reported that ambient temperature of the environment affected the properties of spilled oil and the activity of the microorganisms. The monitoring of the sample temperature shown a low fluctuations tend to increase after adding bio-enzyme.

## A. Conclusion

The biodegradation of COD, PAHS, and TPH was investigated in an aerated bio-enzymatic reactor (BIOVITA GRS and ZIPAK) in batch mode. Several operational variables were examined to check the effects on process reduction efficiency. The following conclusions were obtained from the experiments:

- 1) The reaction time significantly effected on COD, PAHS, and TPH reduction during the bio-enzymatic process; reduction of COD, PAHS, and TPH enhanced with increasing reaction's duration- time, for given experimental conditions.
- 2) The pH had a significant effect on biodegradation of COD, PAHS, and TPH reduction, also the highest reduction obtained at neutral pH (7.5).
- 3) The 20°C temperature achieved the highest efficiency in biodegradation of COD, PAHS, and TPH during the bio-enzymatic batch reactor.
- 4) COD, PAHS, and TPH reduction followed a second-order rate equation during bio-enzymatic reactor.
- 5) The bio-enzymatic reactor, in batch mode, was shown to be an efficient and viable process for meeting a high degree removal of COD, PAHS, and TPH from petroleum-contaminated effluent.

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