INFLUENCE OF MOISTURE CONTENT AND OXYGEN CONCENTRATION ON BIODEGRADATION OF PETROLEUM HYDROCARBONS

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ABSTRACT

The biodegradability of petroleum hydrocarbons was evaluated using petroleum oily sludge from VRL logistics ltd situated at Kengeri, Bangalore (India). The soil rich in native microorganisms was collected from Bangalore University campus and the same is used to prepare simulated contaminated soil. The initial Total petroleum hydrocarbons (TPH) concentration in the simulated contaminated soil was 83,940 mg /kg of soil. Biodegradation was examined for three different conditions i.e. four bioreactors for studying variation of moisture, four bioreactors for studying variation of oxygen and one bioreactor as control Treatability studies on TPH contaminated soil was conducted for 12 weeks to evaluate TPH mass loss rates under the most favorable conditions, for which a set of nine bioreactors each with 15 kg of fresh soil, 3 kgs of oily sludge, 1.5 kgs of inoculated soil in the ratio of (10:2:1) were thoroughly mixed and maintained under laboratory conditions. The TPH, moisture content, pH, bacterial counts and oxygen were monitored regularly along with the nutrient concentration i.e.. C: N: P ratio maintained at 100:10:1 in all the bioreactors except the control reactor. From the study it is concluded that the optimal conditions for better degradation of TPH is found to be between 50% - 60% of moisture content with biodegradation rate of 0.0128 - 0.0174 day^{1} and TPH removal efficiency of 68.5 - 79.2% and oxygen concentration of 50 - 60 mg/kg/day, with biodegradation rate of 0.0120 – 0.0153 day^{1} and TPH removal efficiency of 65.9 - 74.9 % under the C: N: P ratio of 100:10:1 in the contaminated soil.

Key Words : Biodegradation, Moisture, Oxygen, Hydrocarbons, Soil, Bioreactor

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INTRODUCTION

Petroleum or crude oil is a natural product, resulting from the anaerobic conversion of biomass under high temperature and pressure. Petroleum hydrocarbons are the most commonly used chemicals in the industrial world, manufactured from crude oil. Petroleum hydrocarbons are found in gasoline, kerosene, fuel oil, asphalt and even in some chemicals used at home or at work[1,2]. When petroleum hydrocarbons are released through a spill or leak into the environment, they migrate down through soils, becoming adsorbed to the soil particles until they reach groundwater, where they will dissolve in water, float on the water surface or sink to the bottom of aquifer. A variety of techniques have been used to cleanup soil contaminated with petroleum hydrocarbons including excavation of shallow contaminated soils and vapor extraction. However, many of these technologies are either costly or do not result in the complete destruction of contaminants. Biological treatment on the other hand has developed as one of the most promising treatment technologies for petroleum hydrocarbons. The biochemical abilities of microorganisms are the most popular strategy for the biological treatment of contaminated soils. Microorganisms and the other class of organisms have a unique ability to interact both chemically and physically with a huge range of both man-made and naturally occurring compounds leading to a structural change to or the complete degradation of the target molecule.[3,4]

A number of research works have been carried out related to Bioremediation of Petroleum Hydrocarbons including the work conducted at University of Calgary. The highest diesel degradation efficiency (about 70%) was obtained when 60 mL of 20% H_2O_2 was used. Fuel Oil Spill – Germany approximately 112,500 litres of fuel oil polluted the vadose zone of a sandy soil in Germany. A nine month cleanup program reduced the concentration of hydrocarbons from an excess of 1000 mg/kg to less than 20 mg/kg. Hydrogen peroxide was added at 100 mg/L to the infiltration water source.[5]

The study describes the performance of bioreactors on oily sludge biodegradation by native microorganisms. The microbes were acclimatized in soil containing oily sludge as the only source of carbon and energy Emphasis was given to study the influence of oxygen and moisture on the TPH reduction efficiency.[6]

The overall objective was to study the initial physico-chemical and biological characteristics of the soil (uncontaminated and simulated contaminated soil) and the influence of Moisture content and Oxygen on biodegradation of Petroleum Hydrocarbons and thus to study the efficiency of the process in reducing total petroleum hydrocarbons and the growth of microorganisms.

MATERIAL AND METHODS

Oily sludge is characterized as heavy oil, since its carbon fraction ranges over C_{21}[7] Scheme of experimental methodology was formulated to investigate the influence of moisture content and oxygen on the rate of reduction of TPH in the simulated contaminated soil for a period of 12 weeks in bioreactors kept at the Environmental

Engineering laboratory, Department of Civil Engineering, U.V.C.E, JB Campus, Bangalore University, Bangalore(India).

Bioreactor Description

The bioreactors with inner dimensions of 0.3m x 0.3m x 0.23m with volume of 0.0207 Cubic metre made up of glass sheet were fabricated. The soil sample was prepared by mixing contaminated soil borrowed from polluted site, fresh soil from unpolluted site and oily sludge from VRL logistics ltd. To get simulated contaminated soil and the same is filled in the bioreactors.

Preparation of samples

Fresh soil and contaminated soil was sieved using the I S sieve 4.75mm and mixed with the oily sludge to ensure the ratio of 10(fresh soil): 2(oily sludge): 1(contaminated soil) for preparing simulated contaminated soil. the initial TPH concentrations of 83,940 mg/kg with oil loading of 8.5% w/w. Each bioreactor was filled with 15 kgs sieved fresh soil, 3 kgs of oily sludge and 1.5 kgs of contaminated soil. Analysis for the different parameters was carried out once in a week.

Laboratory analysis

The following are the parameters which were analysed in the laboratory to determine the characteristics of the TPH contaminated soil.

Table 1 : Characteristics of the fresh soil as well as TPH contaminated soil were determined for the following parameters and the Methods/Instruments adopted to determine them.

Sl.No	Parameters	Unit of Measurement	Methods and Instrument Adopted
A	**Physico-Chemical**		
1	pH	-	Potentiometric Method
2	Temperature	^{o}C	Digital Thermometer
3	Moisture content	%	Oven Drying Method
4	Total Carbon	mg per gm of the soil	Colorimetric Method
5	TPH	mg per kg of the soil	Soxhlet Extraction Method
6	Nitrogen	mg per gm of the soil	Kjeldal Mehod
7	Phosphorous	mg per gm of the soil	Olsen Extractant Method
B	**Biological**		
1	Bacteria	Cells per gm of the soil	Plate Count Method
C	**Heavy Metals**		
1	Cadmium	mg per kg of the soil	Atomic Absorption Spectrophotometer
2	Copper	mg per kg of the soil	Atomic Absorption Spectrophotometer
3	Nickel	mg per kg of the soil	Atomic Absorption Spectrophotometer
4	Zinc	mg per kg of the soil	Atomic Absorption Spectrophotometer
5	Chromium	mg per kg of the soil	Atomic Absorption Spectrophotometer
6	Lead	mg per kg of the soil	Atomic Absorption Spectrophotometer

Nutrient requirement

The nutritional requirement of Carbon to Nitrogen ratio is 10:1 and Carbon to Phosphorus is 30:1 by the microorganisms. Moreover these nutrients in the optimal range allow microbes to create necessary enzymes to break down the contaminants with the

C:N:P ratio of 100:10:1 (optimal conditions)

In the present study actual C:N:P ratio was 194:1.5:1 or 100:0.77:0.51

Hence, C:N:P = 1045:104.5:10.5 to be maintained to get C:N:P ratio of 100:10:1.

In order to maintain the above ratio the simulated contaminated soil was supplimented with Nitrogen and Phosphorus.

Source of Nitrogen

Urea - a dry, prilled, synthetic, organic material containing 45 - 46% nitrogen. Very soluble. Moves freely in the soil solution as urea but later combines with water to form ammonium ions.

Source of Phosphorus

Superphosphate is a fertilizer produced by the action of concentrated sulfuric acid on powdered phosphate rock.

Moisture Content requirement For Bioreactor (W1 to W4)

The desired moisrure content was maintained by considering the Bioreactor

volume (i.e.= 0.3m x 0.3m x 0.18m = 0.0162 m^{3}) Porosity = n = 19%, and Initial moisture content of the contaminated soil(i.e. 1.28%)

Oxygen source

In order to optimize the bioremediation process with respect to the oxygen at different concentrations in each of the four different bioreactors (O1 to O4) keeping all other parameters constant. The oxygen is supplied in the form of hydrogen peroxide.

RESULTS AND DISCUSSION

Simulated contaminated soil was analysed for initial physical, chemical and biological characteristics. The characteristics are shown in **Table 2**.

Analytical results of different bioreactors for various parameters like physical, chemical and biological characteristics of the soil samples of different bioreactors including the concentration of TPH remained after 12th week is shown in **Table 3**.

Change in pH: It has been stated that, in the presence of excess nutrients and oxygen, the pH value of an oily sludge under microbial degradation would drop[3,4] and due to microbial action organic acids are produced which in turn leads to pH depletion. The initial pH of the simulated contaminated soil was 7.908 and the final pH varied from 7.002 to 7.158 except in the control reactor which was 7.614 at the end of 12th week.

Change in temperature

Temperature exerts a major control on the metabolic activity of micro-organisms because the entire microbiological organic breakdown occurs through the activity of enzymes. However, microbial activities increase until approximately 45°C and beyond this temperature, microbiological

activities decrease and eventually ceases. The overall temperature obtained inside the laboratory during the study period, which ranged from 27.1°C to 30.4°C during day time.[8]

Table. 2 : Initial physical, chemical and biological characteristics of the simulated contaminated soil

Sl No	Parameters	Unit of Measurement	Concentration
1	**Type of Soil**		Sandy (well graded)
2	**Porosity**	%	19.0
3	**pH**		7.908
4	**Temperature**	^oC	31.2
5	**Moisture content**	%	1.28
6	**TOC**	mg/ gm of the soil	69.670
7	**TPH**	mg/ kg of the soil	83,940
8	**Nitrogen**	mg/ gm of the soil	0.54
9	**Phosphorous**	mg/ gm of the soil	0.36
10	**Bacteria**	No. of Cells per gm of the soil	$0.62x10^5$
11	**Cadmium**	mg/ kg of the soil	0.048
12	**Lead**	mg/ kg of the soil	1.447
13	**Zinc**	mg/ kg of the soil	0.163
14	**Chromium**	mg/ kg of the soil	0.516

Table. 3 : Parameters analyzed for different Bioreactors

Parameters analyzed	Bioreactors								
	W1	**W2**	**W3**	**W4**	**O1**	**O2**	**O3**	**O4**	**C.C**
pH	7.158	7.141	7.129	7.126	7.158	77.115	77.086	7.002	7.614
Moisture	40	50	60	70	40	40	40	40	0.10
TOC	26.38	24.05	16.05	14.07	26.38	18.10	17.10	14.26	38.00
Nitrogen	1.966	1.778	1.584	0.961	1.966	1.407	1.602	1.426	0.197
Phosphorus	0.265	0.241	0.188	0.178	0.265	0.178	0.213	0.193	0.195
TPH	28,586	26,439	17,456	15,996	28,586	21,056	18,193	16,391	62,315
Bacterial Count	2.81	3.11	4.99	5.62	2.81	4.42	4.54	4.73	0.007

Moisture %, TOC mg/gm, Nitrogen mg/gm, Phosphorus mg/gm, TPH mg/kg, Bacterial count $x10^5$ CFU/ gm of soil.

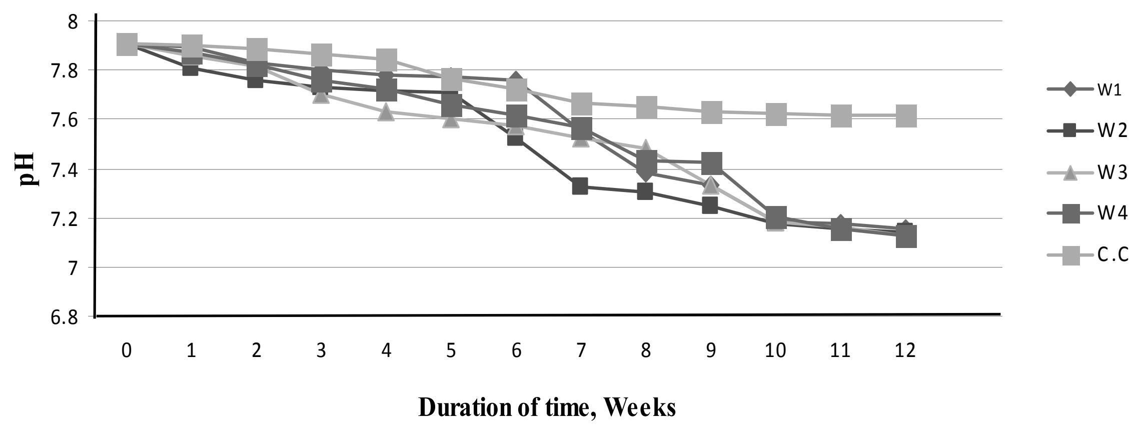


Fig. 1: Graph showing the variation of pH during the study period for bioreactors W1 to W4 and the control Bioreactor

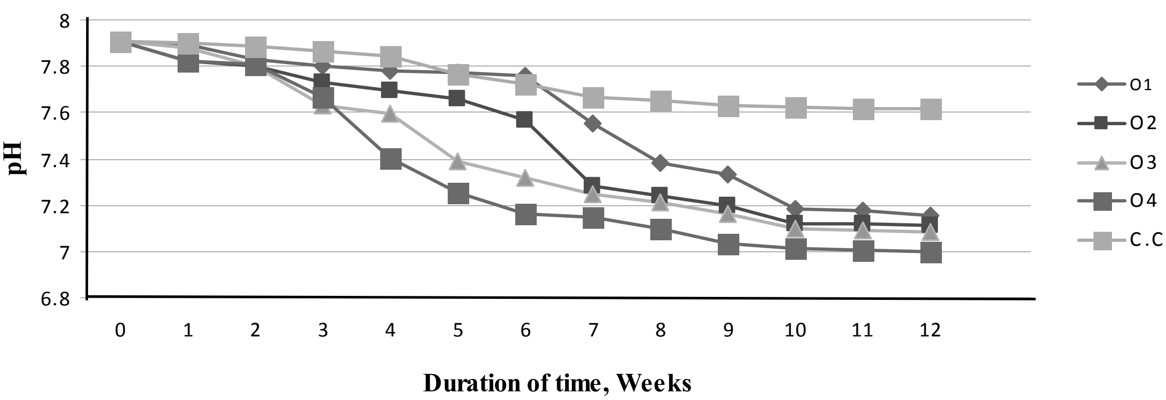


Fig. 2 : Graph showing the variation of pH during the study period for bioreactors O1 to O4 and the control Bioreactor

Soil Moisture

Historically, it has been recommended that soil moisture to be maintained at 40 to 70 percent of field capacity. The soil moisture at its field capacity means the soil macropores are filled with water and soil micropores are filled with air. This condition allows soil microorganisms to get air and water, both of which are necessary for aerobic biodegradation to occur[9,10]. Soil moisture was monitored regularly to maintain the optimum conditions for moisture i.e.. 40%, 50%, 60% and 70% of Field capacity for bioreactors W1-W4 and 40% for bioreactors O1-O4 except for control bioreactor which was 0.10 % at the end of 12th week.

Total Organic Carbon

Organic compounds serve as sources of carbon and can be estimated based on concentrations of total organic carbon (TOC). All organic chemicals present in a sample, including compounds that are unavailable or not readily metabolized, are included in measures of TOC. Thus, TOC tends to over estimate the carbon available to microorganisms. In general, the total number of organisms present is proportional to the amount of carbon available (assuming other nutrients are not limiting). The initial TOC in the simulated contaminated was 69.67 mg/gm and the final TOC varied from 26.38mg/gm to 14.07 mg/gm except in the control reactor which was 38.00 mg/gm at the end of 12th week.

Nutrient Concentration

Sufficient concentrations of nutrients (nitrogen, phosphorous) must be available to maintain the health of the microbial population. In TPH contaminated soils the large amounts of organic carbon available tend to result in rapid depletion of other nutrients, with the limiting nutrients generally being nitrogen and phosphorus. Hence, in order to maintain C: N: P ratio the bioreactors were supplemented with urea as the source of Nitrogen and superphosphate as the source of Phosphorus.

TPH Reduction

The TPH reductions in the bioreactors (W1-W4 and O1-O4)l were ranged between 65.9%to 80.9% at the end of 12th week of treatment . The indigenous micro-organisms (as in Reactor C) could reduce the TPH by an amount 25.76 %.

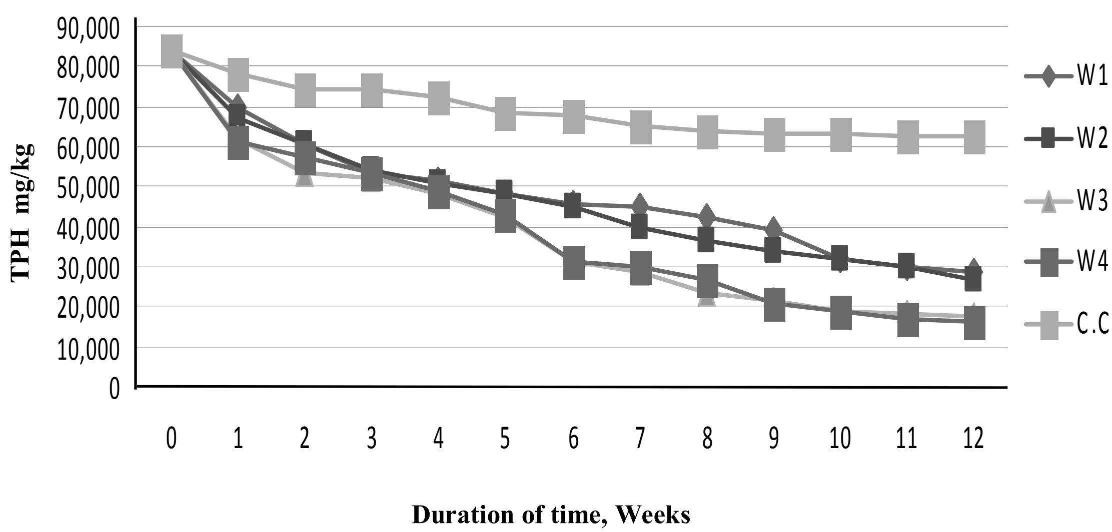


Fig. 3 : Graph showing the variation of TPH v/s time during the study period for bioreactors W1 to W4 and the control Bioreactor

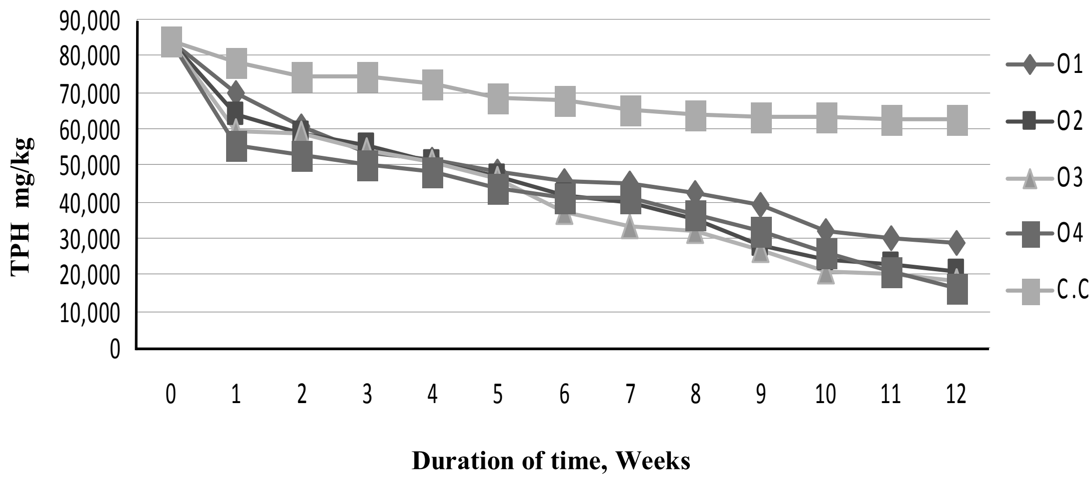


Fig. 4 : Graph showing the variation of TPH v/s time during the study period for bioreactors O1 to O4 and the control Bioreactor

Microbial Activity

The bacterial counts varied from 2.81 x 10^5 to 5.62 x 10^5 CFU/g of soil at the end of the 12^{th} week. In the control reactor, bacterial counts decreased constantly and was 0.007 x 10^5 CFU/g of soil at the end of the 12^{th} week. Thus, increase in bacterial counts had a profound influence on the rate of TPH reduction.

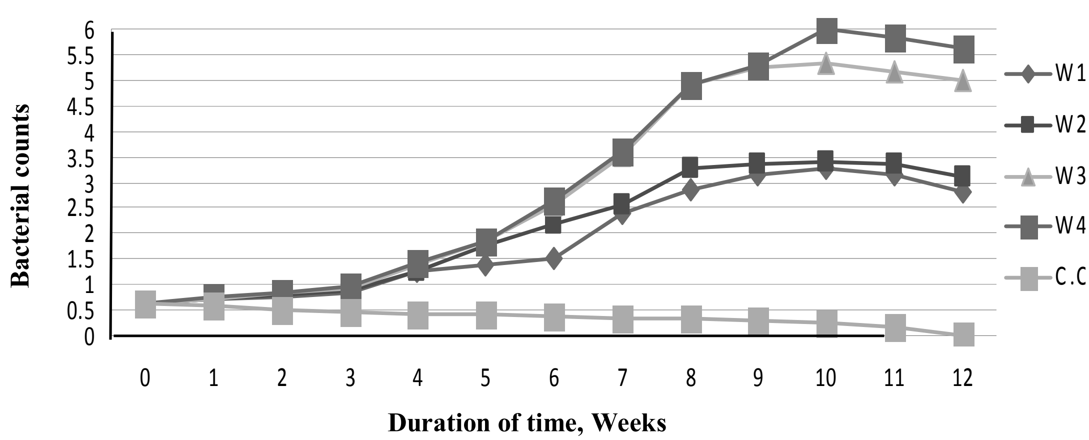


Fig. 5 : Graph showing the variation of Bacterial counts during the study period, X 10^5 CFU/g of soil for bioreactors W1 to W4 and the control Bioreactor

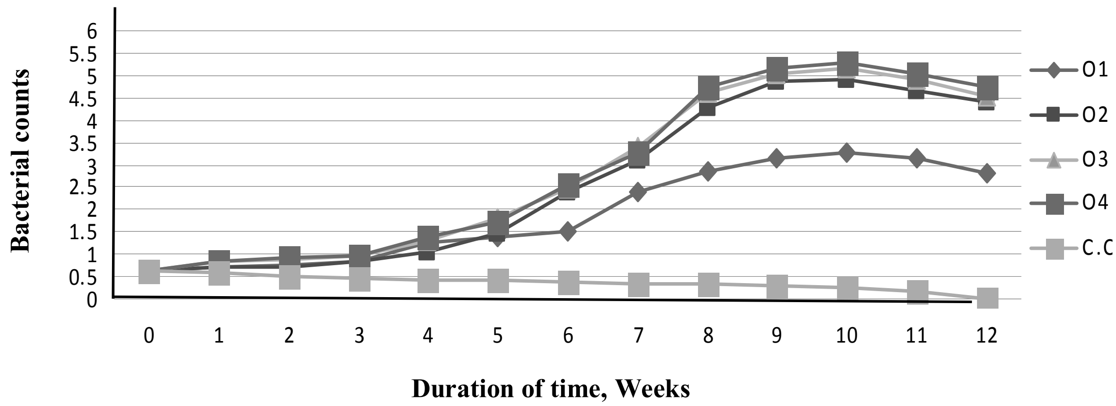
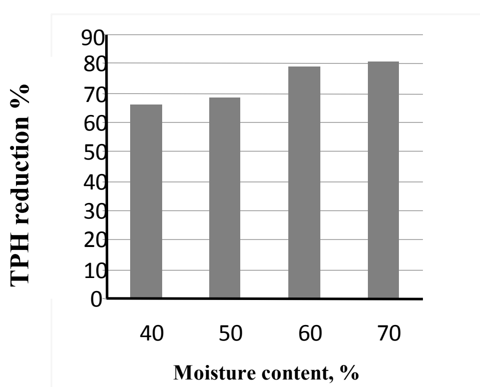


Fig. 6 : Graph showing the variation of Bacterial counts during the study period, X 10^5 CFU/g of soil for bioreactors W1 to W4 and the control Bioreactor

Table 4 : Percentage reduction of Total Petroleum Hydrocarbons

Sl No	Bioreactors	Initial TPH mg/kg	Final TPH mg/kg	Percentage Reduction,%	Degradation rate, /day
1	W1	83,940	28,586	65.9	0.0120
2	W2	83,940	26,439	68.5	0.0128
3	W3	83,940	17,456	79.2	0.0174
4	W4	83,940	15,996	80.9	0.0184
5	O1	83,940	28,586	65.9	0.0120
6	O2	83,940	21,056	74.9	0.0153
7	O3	83,940	18,193	78.3	0.0169
8	O4	83,940	16,391	80.4	0.0181
9	Control specimen	83,940	62,315	25.7	---



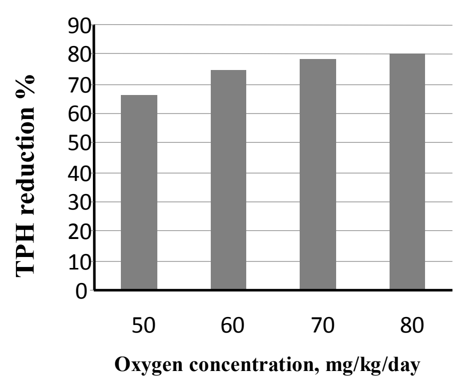


Fig. 7 : Efficiency of TPH reduction for Bioreactors Efficiency of TPH reduction for Bioreactors

Statistical analysis of the data: Regression analysis was carried out for the following data on the efficiency of TPH reduction having different Moisture content (%) and Oxygen concentration (mg/kg/day)

Table 5 : Statistical data for TPH reduction

Moisture content (%) X1	Oxygen concentration (mg/kg/day) X2	Efficiency of TPH reduction (%) Y
40	50	65.9
50	50	68.5
60	50	79.2
70	50	80.9
40	50	65.9
40	60	74.9
40	70	78.3
40	80	80.4

$\Sigma X_1 = 380 \quad \Sigma X_2 = 460 \quad \Sigma X_1^2 = 19000$

$\Sigma X_2^2 = 27400 \quad \Sigma X_1 X_2 = 21400 \quad \Sigma Y = 594$

$\Sigma X_1Y = 28456 \quad \Sigma X_2Y = 34427$

The normal Equations are given by,

$\Sigma Y = nb_0 + b_1\Sigma X_1 + b_2\Sigma X_2$ (1)

$\Sigma X_1Y = b_0\Sigma X_1 + b_1\Sigma X_1^2 + b_2\Sigma X_1X_2$ (2)

$\Sigma X_2Y = b_0\Sigma X_2 + b_1\Sigma X_1X_2 + b_2\Sigma X_2$ (3)

therefore fitted equation is

$\hat{y} = b_0 + b_1 X_1 + b_2 X_2$

$\hat{y} = 20.29 + 0.5017 X_1 + 0.524 X_2$

$$\hat{a} = \begin{matrix} b_0 \\ b_1 \\ b_2 \end{matrix} = \begin{matrix} 20.29 \\ 0.5017 \\ 0.5240 \end{matrix}$$

$$X = \begin{pmatrix} 1_{(1)} & X1_{(1)} & X2_{(1)} \\ 1_{(2)} & X1_{(2)} & X2_{(2)} \\ | & | & | \\ | & | & | \\ 1_{(8)} & X1_{(8)} & X2_{(8)} \end{pmatrix} \qquad Y = \begin{pmatrix} Y_{(1)} \\ Y_{(2)} \\ | \\ | \\ Y_{(8)} \end{pmatrix}$$

$$X^IY = \begin{pmatrix} 1_{(1)} & 1_{(2)} & \text{----------} & 1_{(8)} \\ X1_{(1)} & X1_{(2)} & \text{---------} & X1_{(8)} \\ X2_{(1)} & X2_{(2)} & \text{---------} & X2_{(8)} \end{pmatrix} \begin{pmatrix} Y_{(1)} \\ Y_{(2)} \\ | \\ | \\ Y_{(8)} \end{pmatrix} = \begin{bmatrix} 594 \\ 28,456 \\ 34,427 \end{bmatrix}$$

$$\hat{a}^1 = (b_0 \ b_1 \ b_2)$$

$$\hat{a}^1X^IY = (b_0 \ b_1 \ b_2) \begin{bmatrix} 594 \\ 28,456 \\ 34,427 \end{bmatrix} = (20.29 \ \ 0.5017 \ \ 0.524) \begin{bmatrix} 594 \\ 28,456 \\ 34,427 \end{bmatrix}$$

Substituting the calculated values in the above equation

$594 = 8\ b_0 + 380\ b_1 + 460\ b_2$

$28456 = 380\ b_0 + 19000\ b_1 + 21400\ b_2$

$34427 = 460\ b_0 + 21400\ b_1 + 27400\ b_2$

Solving the above equations for b_0 b_1 b_2 we get,

$b_0 = 20.29 \quad b_1 = 0.5017 \quad b_2 = 0.524$

$\hat{a}^1X^I Y = 44,367$

Coefficient of determination is given by R^2

$R^2 = (\hat{a}^1X^I Y - n\tilde{y}^2) *100 / (\Sigma Y^2 - n\tilde{y}^2)$

$R^2 = (44,367 – 8 * 5,513) * 100 / (44,400 – 8 * 5,513)$

$R^2 = 88.85$ %

Multiple correlation coefficient = R = $\sqrt{0.8885} = 0.94$

Test for significance = $\dfrac{(\hat{a}^1X^I Y - n\tilde{y}^2) /2}{(\Sigma Y^2 - \hat{a}^1X^I Y) / (n-3)}$

$F_{cal} = \dfrac{(44,367 – 44,104) /2}{(44,400 – 44,367) /5}$

Table value for F- distribution = 5.79 at confidence level of 95% or level of significance of 5%, Calculated value F_{cal} = 19.92

Since $F_{cal} > F_{Table}$ we can conclude that X1 and X2 have significant influence on Y, that means the Moisture content and Oxygen concentration have significant influence on TPH reduction.

CONCLUSION

1) Decrease in the TPH concentration in different bioreactors with different Moisture content was between 15,996 to 28,586 mg/kg. Higher decrease of TPH was found in the higher moisture content, but the rate of decrease of TPH was found to be higher when the moisture content ranged between 50% to 60%.
2) Decrease in the TPH concentration in different bioreactors with different Oxygen concentrations was between 16,391 to 28,586 mg/kg. Higher decrease was found in the higher oxygen concentration of 80 mg/kg/day, but the rate of decrease was found to be higher when the oxygen concentration ranged between 60 mg/kg/day to 70 mg/kg/day.
3) The different microorganisms which were present were Pseudomonas, Mycobacterium and bacillus and the overall concentration of Pseudomonas group of bacteria in the beginning was in the range of $0.62x10^5$. The concentration of microorganisms gradually increased upto the end of 10^{th} week but later it was found to decrease the reason being the exhaustion of substrates.

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