Evaluation of Marine Oil Spill using Phytoremediation and Bioremediation

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Abstract: ***Oil spill pollution, a severe environmental problem which persists in marine environment has grown to an alarming magnitude. There are several oil spill cleanup technique exist for deep sea. In contrast, offshore oil spill often goes unnoticed. Thus remediation of these hydrocarbons by natural decontamination process is of utmost importance. This study focuses on evaluating the most effective oil spill remediation option for Mirkarwada boat jetty, Ratnagiri, Maharashtra using the four pilot scale studies (Phytoremediation, Bioremediation, Hybrid unit & Control Unit). In this study the degrading potential of enhanced indigenous mixed culture of microorganisms towards hydrocarbons had been investigated. Parallel to that efficiency of Phytoremediation using 'Amaranthus palmeri' in degrading hydrocarbons was evaluated. Combination of Bioremediation and Phytoremediation is studied in Hybrid setup. Parameters viz. pH, Salinity, Temperature DO, and Oil and Grease was analyzed. From the observations bioremediation using hydrocarbon degrading bacteria shows promising results compared to phytoremediation. Bioremediation using bacteria show an efficiency of 64.1% where as phytoremediation gives an efficiency of 52.3% and the Hybrid setup has an intermediate efficiency of 53.2% at a pH of 7.5,8.2 and7.8, DO of 1.2 ,1.3 and 1.6 mg/liter, Salinity of 29,28 and 29g/kg, Temperature of 28.1^{o}C,28.2^{o}C,29.8^{o}C and Oil and grease of 15.2,20.2,18.1mg/liter respectively.***

Keywords: Bioremediation, Hybrid Remediation Unit, Offshore oil spill, Phytoremediation.

1. Introduction

Petroleum hydrocarbons are important source of energy and are extensively used everyday world wide. Petroleum hydrocarbon in the form of LPG, gasoline, diesel oil etc., is used as fuel for internal combustion engines are the primary oil contaminant present in the harbor water along with the natural oil from the decayed fish [6].

Bioremediation is a non-invasive and cost effective technique for the clean-up of the petroleum hydrocarbons by the process of breaking down of complex hydrocarbons to simpler and less harmful products. Among the different techniques of Bioremediation, Bioaugmentation which enhance natural biodegradation by indigenous microorganisms have attracted the most interest. Other promising green technology for cleaning up environment contaminated with Hydrocarbon is Phytoremediation, which remove, stabilize, degrade, and/or metabolize hazardous contaminants [6].

The biodegradation of petroleum in the marine environment is carried out largely by diverse bacterial populations, including various Pseudomonas species. The hydrocarbon-biodegrading populations are widely distributed in the world's oceans; surveys of marine bacteria indicate that hydrocarbon-degrading microorganisms are ubiquitously distributed in the marine environment.

Generally, in pristine environments, the hydrocarbon-degrading bacteria comprise < 1% of the total bacterial population. [10]

Phytoremediation specifically is the use of plants to remove pollutants from the environment or render them harmless. Several species of plants have been shown to have the ability to grow in contaminated soils and actually extract the pollutant from the growth medium. These plants function in several different ways. Some plants can hyper accumulate toxic heavy metals in their tissues. Others can converts the pollutants to less toxic compounds and volatilize. Some aquatic plant roots can filter contaminants/pollutants from water [11].

If effective, phytoremediation can be an attractive alternative to current remediation methods because the treatment can be done in situ, the cost of plants is lower than most other current technologies and it is relatively environmentally safe. Phytoremediation have shown great promise in the cleanup of aquatic environment polluted with petroleum hydrocarbon.

Though in its infancy and not fully understood yet, it is one remediation strategy that holds tremendous prospects for the future. It will clean up the environment without any of those negative impacts that is associated with physical and chemical processes of oil spill remediation [5],[7].

2. Materials and Methods

2.1 Mesocosm studies or Pilot Scale Study

Mesocosm or pilot-scale systems can help to simulate actual conditions at relatively low cost, and are frequently used as bridges between microcosms and field systems. Mesocosm have been used to evaluate the effectiveness of numerous bioremediation strategies. The experimental work was carried out using four setup, one each for Bioremediation using Microbes, Phytoremediation, Hybrid Unit (Combination of Bioremediation and Phytoremediation) and a Control Unit.

2.2 Materials

The setup for all the four are cylindrical in shape of a diameter of 15' and height 16'. Materials are filled in two

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layers. Gravel of 8mm is filled up to 7.5'. Red Subsoil of 7.5' is filled above it leaving 1' free board. Oil degrading microbes which is isolated from the sample is introduced in one setup. *'Amaranthus palmeri'* plant in other setup, the combination of plant and microbes in the other setup. Finally the fourth setup was maintained as Control Unit, without any plant or microbes for reference.

a) Phytoremediation

Plant : *'Amaranthus palmeri'*
Common Name : Pigweed

Distinguishing Features: The stem of the pigweed is what makes this plant so distinctive. Stems are erect, and can grow anywhere from 10 cm - 2 m high, but usually 50 - 90 cm, simple or branched, lower part thick and smooth, upper part usually rough with dense short hair, greenish to slightly reddish but usually red near the roots

Pigweeds thrive in hot weather, tolerate drought, respond to high levels of available nutrients(Hydrocarbons, Phosphorus, Nitrates) and are adapted to avoid shading through rapid stem elongation. [14].

'Amaranthus palmeri' were potted and grown separately by pouring seawater to ensure the sustainability of plant in seawater environment. After two weeks of growth, it is then planted in the subsoil of other setup.

b) Bioremediation

Oil degrading microbes where isolated from the sample of Mirkarwada boat jetty, Ratnagiri, Maharashtra. The mixed culture of oil degraders where used in the Bioremediation Setup.

2.3 Sampling

Sea water samples were collected from Mirkarwada harbor in Ratnagiri city, Maharashtra by random sampling method. 20 liters of sample is collected for each setup. Separate sample of 100 ml were collected at the same day and time for field testing of initial parameters viz. DO, Salinity, pH, Temperature using following instruments respectively.
a. Lutron DO-5509 Meter
b. Taiwan Salinometer
c. Hanna pHep pH meter
d. TP3001 Digital Thermometer

2.4 Isolation of Oil Degraders

Oil degrading microbes where isolated from the Mirkarwada jetty sample. Sterile Nutrient Broth Media containing Tween 80 which is source of oil is prepared and incubated at room temperature for 48 hrs. Oil degrading bacteria where purified by streaking the grown culture on nutrient agar media. After incubation, the colonies obtained where observed and maintained of slants for future use. 50 ml of the liquid grown culture of absorbents = 1 at 530 nm was mixed in the top soil.

2.5 Experimental Work

Samples of 20 liters for each setup was collected from the Mirkarwada Boat jetty. Initial Sample characteristics were analyzed. The parameters viz. pH, DO, Temperature, Salinity were analyzed on the field using portable instruments. Oil and Grease was quantified using both soil and output water analysis using Gravimetry Method 1664 [1].

The contact period of 12hrs is kept for all the four setup. The output water sample and the topsoil of all the four setup were analyzed on a daily basis. Batch process is followed in order to effectively quantify the oil degradation efficiency of the setup.

3. Results and Discussion

3.1 Water Quality Standards For Coastal Waters Marine Outfalls

In a coastal segment, marine water is subjected to several types of uses. Depending on the types of uses and activities, water quality criteria have been set by the Environmental Protection Agency , Substituted by Rule 2(iii) of the Environment (Protection) (Second Amendment) Rules, 1999 published in the Notification G.S.R.682(E), dated 5.10.1999 is given in Table 1

Table 1: Primary Water Quality Criteria for Class SW-IV Waters (For Harbour Waters)

S. No	*Parameter*	*Standards*	*Rationale/Remarks*
1.	pH range	6.0---9.0	To minimize corrosive and scaling effect.
2.	Dissolved Oxygen	3.0 mg/l or 40 per cent saturation value whichever is higher.	Considering bio-degradation of oil and inhibition to oxygen production through photosynthesis.
3.	Colour and Odour	No visible colour or offensive odour	None from reactive chemicals which may corrode paints/ metallic surfaces.
4.	Floating materials, Oil, grease and Scum (including Petroleum products)	10 mg/l	Floating matter should be free from excessive living organisms which may clog or coat operative parts of marine vessels/equipment.
5.	Fecal Coliform	500/100 ml(MPN)	Not exceeding 1000/100 ml in 20 per cent of samples in the year and in 3 consecutive samples in monsoon months.
6.	Biochemical Oxygen Demand (3days at 27^{o}C)	5mg/l	To maintain water relatively free from pollution caused by sewage and other decomposable wastes.

In this work, initial sample was kept constant for all the setup except for hybrid and batch process was followed to calculate the performance efficiency of both the setup. The initial characteristics of the sample water for three setup is

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shown in the table 2 and the initial characteristics for Hybrid setup is illustrated in table 3

Table 2: Initial Sample Characteristics

Sr. No:	*Parameter*	*Initial Sample Values*
1	pH	8.3
2	Salinity	35g/kg
3	Temperature	29.4^{o}C
4	DO	2.2mg/l
5	Oil and Grease	42.4g/l

Table 3: Initial Sample for Hybrid Setup (Bioremediation and Phytoremediation)

Sr. No:	*Parameter*	*Initial Sample Values*
1	pH	8.6
2	Salinity	33g/kg
3	Temperature	28^{o}C
4	DO	2.3 mg/l
5	Oil and Grease	38.7mg/l

3.2 Effect of pH in the experimental setup

The pH of seawater is generally stable and slightly alkaline. From the experimental results it is seen that degradation of oil increases with decreasing pH, and that optimum degradation occurs under slightly acidic conditions. For Microbial setup, the pH reduces from pH 8.3 to 7.5. Maximum efficiency is attained at pH = 7.5 Whereas plant shows maximum efficiency at pH = 8.2. For hybrid setup, pH shows a constant decrease from initial value 8.6 to 7.8 There is no noticeable variation in pH for the control unit and Plant setup. Daily Analysis of the all four setup and the corresponding values of pH was observed , which is illustrated in Fig 1a and Fig 1b

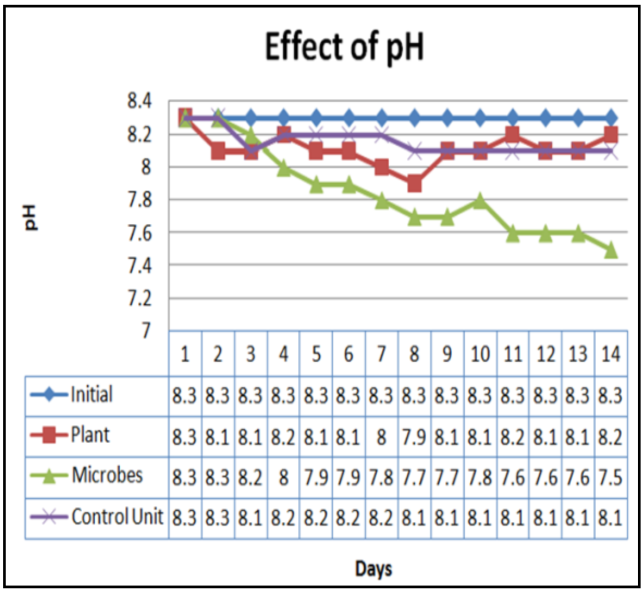

Figure 1a: Effect of pH

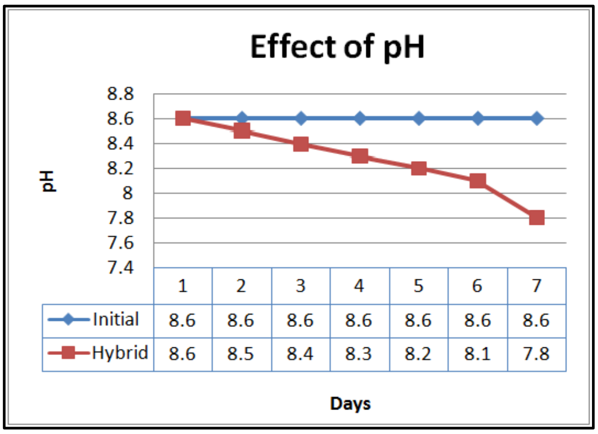

Figure 1b: Effect of pH in Hybrid Setup

3.3 Effect of Salinity and Temperature

Hydrocarbon Biodegradation can occur over a wide range of temperatures, the rate of biodegradation generally decreases with decreasing temperature. Results have shown that, temperature and salinity have least influence on all the setup. Initially there is a salinity drop in all the setup from a salinity of 35g/kg to 30g/kg and 31g/kg for plant and Microbes setup respectively.

Whereas 36g/kg to 30g/kg for hybrid setup. Salinity is used up as an alternative for minerals for the enhancement of the process. Anyhow the maximum efficiency were attained at temperature 28.2^{o}C, 28.1^{o}C, 29.8^{o}C and Salinity of 28g/kg, 29g/kg and 29g/kg for Plant, Microbes and Hybrid Setup respectively. Control Unit shows less variation of temperature. It remains equal to the room temperature. Graphical representation of the effect on salinity and temperature on the four setups is illustrated in Fig 2a, Fig 2b, Fig 3a, Fig 3b respectively.

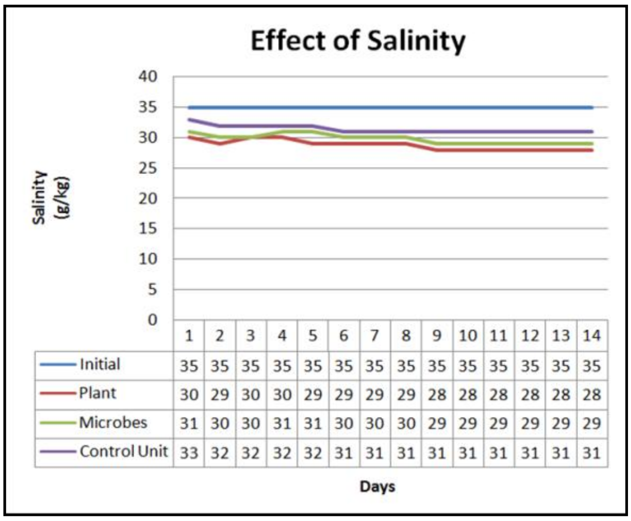

Figure 2a: Effect of Salinity

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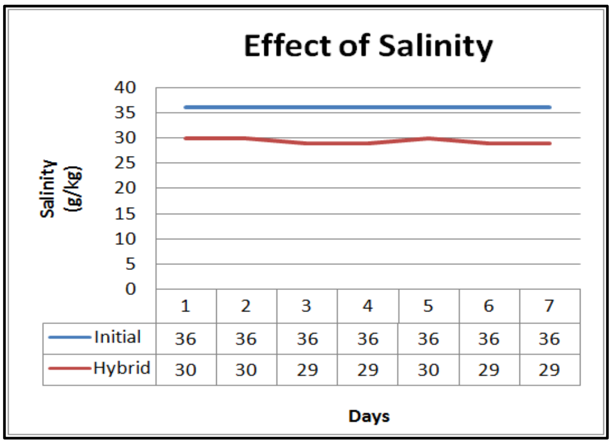

Figure 2b: Effect of Salinity in Hybrid Setup

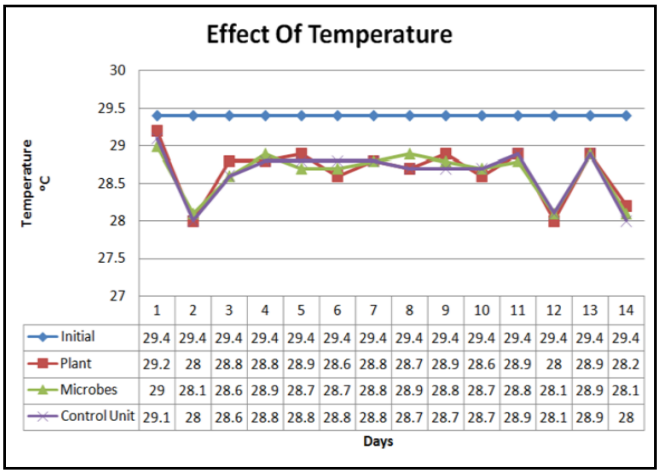

Figure 3a: Effect of Temperature

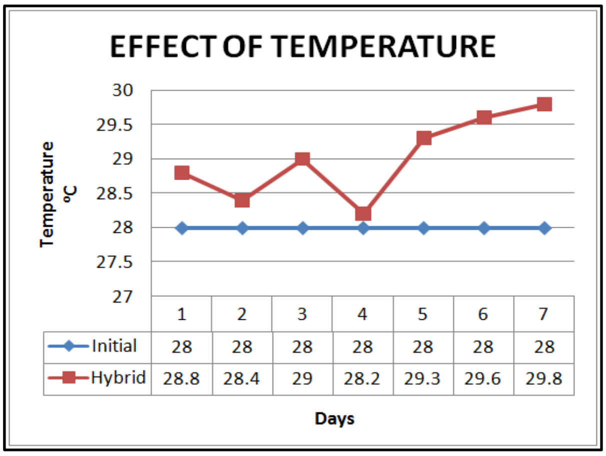

Figure 3b : Effect of Temperature in Hybrid Setup

3.4 Effect of Dissolved Oxygen

From the experimental results it is seen that the efficiency is irrespective to DO. In general it shows a trend of decrease in DO from 2.2mg/l to 1.3mg/l and 1.2mg/l for Plant, Microbes setup and 2.3mg/l to 1.6mg/l for Hybrid Setup. The dissolved oxygen concentration is used up for the degradation process. Whereas Plant is using up DO from the atmosphere hence there is no sudden drop of DO in the plant setup. Control unit maintains the initial DO level of the sample ie 2.2mg/l. The occasional increase in DO is due to the diffused dissolved oxygen from atmosphere. Fig 4a and Fig 4b shows the variation of the DO in the outlet sample on daily basis.

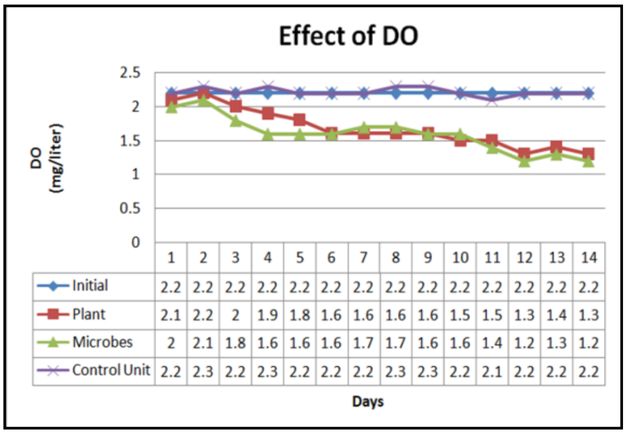

Figure 3a : Effect of DO

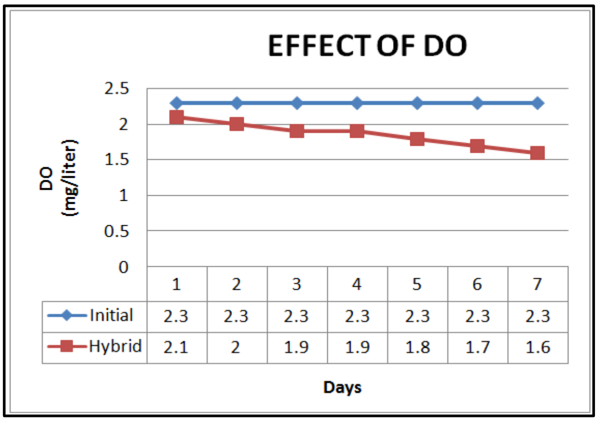

Figure 3b: Effect of DO in Hybrid Setup

3.5 Analysis of Oil and grease

The top soil sample and the outlet water sample were analyzed on a regular basis to quantify amount of degradation occurred, Thereby to find out the efficiency of the setup by adding up the oil and grease from soil analysis and outlet water analysis. Table 4, Table 5, Table 6, Table 7 shows the oil and grease removal of phytoremediation, Bioremediation using Microbes, Control Unit and Hybrid Setup and its corresponding efficiency.

Table 4: Oil and Grease Removal for Phytoremediation setup and its corresponding efficiency

Days	*Oil& Grease by Water Analysis (mg/l)*	*Oil& Grease by Soil Analysis (mg/l)*	*Total Oil & Grease (mg/l)*	*Efficiency (%)*
1	7.7	14.3	22	48.1
2	7.6	14.3	21.9	48.3
3	7.5	14.1	21.6	49
4	7.5	14	21.5	49.2
5	7.5	14	21.5	49.2
6	7.2	14.1	21.3	49.7
7	7.2	14.1	21.3	49.7
8	7.2	13.9	21.1	50.2
9	7.1	13.9	21	50.7
10	7.1	13.9	21	50.7
11	6.9	13.8	20.7	51.1
12	6.8	13.8	20.6	51.4
13	6.8	13.8	20.6	51.4
14	6.6	13.6	20.2	52.3

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Efficient removal of oil and grease is achieved from 42.4mg/l to 20.2mg/l on 14th day, with a removal efficiency of 52.3% and a average two weeks efficiency of 50% for the Phytoremediation setup using 'Amaranthus palmeri'

Table 5: Oil and Grease Removal for Bioremediation setup using Microbes and its corresponding efficiency

Days	*Oil& Grease by Water Analysis (mg/l)*	*Oil& Grease by Soil Analysis (mg/l)*	*Total Oil & Grease (mg/l)*	*Efficiency (%)*
1	6.3	10.7	17	59.9
2	6	10.8	16.8	60.3
3	7.8	8.9	16.7	60.6
4	7.7	8.8	16.5	61
5	7.7	8.8	16.5	61
6	7.7	8.8	16.5	61
7	7.5	8.7	16.2	61.7
8	7.3	8.7	16	62.2
9	7.3	8.7	16	62.2
10	7.2	8.7	15.9	62.5
11	7.1	8.6	15.7	62.9
12	7	8.5	15.5	63.4
13	6.8	8.5	15.3	63.9
14	6.8	8.4	15.2	64.1

Efficient removal of oil and grease is achieved from 42.4mg/l to 15.2mg/l on 14th day, with a removal efficiency of 64.1% and a average two weeks efficiency of 61.9% for the Bioremediation with Microbes.

Table 6: Oil and Grease Removal for Control Unit and its corresponding efficiency

Days	*Oil& Grease by Water Analysis (mg/l)*	*Oil& Grease by Soil Analysis (mg/l)*	*Total Oil & Grease (mg/l)*	*Efficiency (%)*
1	17.8	20.5	38.3	9.6
2	17.7	20.5	38.2	9.9
3	17.5	20	37.5	11.5
4	17.5	20	37.5	11.5
5	17.3	19.8	37.1	12.5
6	17	19.8	36.8	13.2
7	17.5	20	37.5	11.5
8	17.7	20.5	38.2	9.9
9	17.6	20.7	38.3	9.6
10	18	20.4	38.4	9.4
11	18.5	19.9	38.4	9.4
12	18.8	19.7	38.5	9.1
13	188	20	38.8	8.3
14	18.6	20	38.6	8.8

Control unit doesn't shows any noticeable reduction in oil and grease hence its efficiency is nonlinear.

Table 7: Oil and Grease Removal for Hybrid Unit and its corresponding efficiency

Days	*Oil& Grease by Water Analysis (mg/l)*	*Oil& Grease by Soil Analysis (mg/l)*	*Total Oil & Grease (mg/l)*	*Efficiency (%)*
1	8.2	10.2	18.4	52.4
2	8.2	10.2	18.4	52.4
3	8.1	10.2	18.3	52.7
4	8.1	10.1	18.2	52.9
5	8.1	10.1	18.2	52.9
6	8	10.1	18.1	53.2
7	8.1	10	18.1	53.2

Efficient removal of oil and grease is achieved from 38.7mg/l to 18.1 mg/l on 7th day, with a removal efficiency of 53.2% and the one weeks efficiency is 52.8% for the Bioremediation with Microbes. The efficiency of all the four setup is illustrated graphically in Fig 4

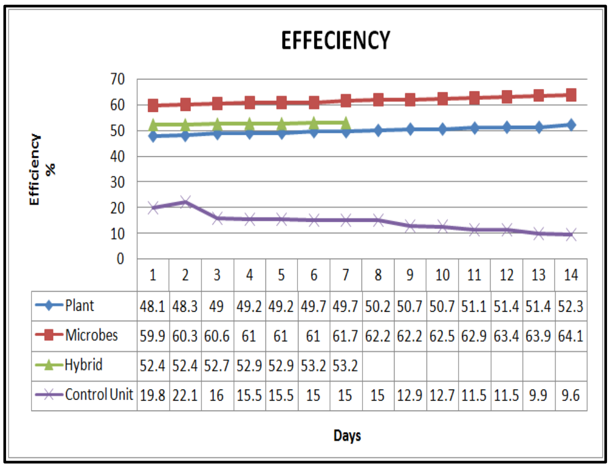

Figure 4: Efficiency of four setups

4. Conclusion

From the observations bioremediation using hydrocarbon degrading bacteria shows promising results compared to phytoremediation as it possess diverse metabolic pathway. Hybrid Bioremediation using combination of Microbes and Plants gives an efficiency more than that of phytoremediation setup and less than that of Bioremediation using Microbes. Bioremediation using bacteria show an efficiency of 64.1% where as phytoremediation gives an efficiency of 52.3% and Hybrid setup shows an efficiency of 53.2% at a pH of 7.5,8.2 and7.8, DO of 1.2 ,1.3 and 1.6 mg/liter, Salinity of 29,28 and 29g/kg, Temperature of 28.1°C,28.2°C,29.8°C and Oil and grease of 15.2,20.2,18.1mg/liter respectively.

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