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Reduction of Hydrocarbon Waste Water using *Chlorella* sp

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Abstract:- Hydrocarbon and its derivatives pose serious environment problem globally, especially Sub-Sahara Africa. Thus, the aim of this study is to evaluate the treatment of hydrocarbon rich waste water using algae. Microalgae was collected from pond water containing the microalgae and it was transported to thelaboratory for immediate analysis while the waste water was collected from an oil company in Port Harcourt, Nigeria. Baseline parameter of the sample was determined. Microalgae was cultured using BG11 medium and was used for the treatment of the waste water and the rate of remediation was monitored by the change in total petroleum hydrocarbon (TPH) concentration. The mean physicochemical composition of the sample is: conductivity (162.5 µs/cm), salinity (18.2ppt), TDS(242.4mg/kg), BOD (181.1mg/L), COD (121.8mg/L) and pH(7.8). A microalga (*Chlorella* sp.) was identified from the culture. Results revealed that treatment B had the highest mean remediation of hydrocarbon 74% (4494.2947mg/kg) followed by treatment Awith 55.5% (3367.8453mg/kg) and lastly2.3% remediation (138.76mg/kg) was observed in control sample.Also, Analysis of Variance (ANOVA) results revealed that total petroleum hydrocarbon(TPH) are significantly different ($p<0.05$) between treatments. Multiple comparison test using Fisher's statistic buttressed the pair significances ($p<0.05$) between treatments. Apparently, this study showed the potential of microalgae, (*Chlorella* sp) in the treatment of waste contaminated by hydrocarbon and also, the growth of microalgae in remediation can be enhanced by organic nutrient.

Keyword:- *Hydrocarbon, Effluent, Microalgae, Remediation, Contaminate*

I. INTRODUCTION

Hydrocarbons are made up of hydrogen and carbon. They are composed of aromatic compounds, which have one or more benzene rings bound together, and aliphatic compounds, which have chains of carbon atoms strongly bound together. It is well recognized that the unintentional or intentional release of hydrocarbons and their derivatives into the environment poses issues that are getting worse all over the world (Akpor et al., 2014). Pollutants are produced in substantial quantities by petrochemical industry and petroleum refineries processing operations. (Chavan and Mukherji, 2008). A number of on-site treatment technologies are used to handle this wastewater, including American petroleum institute (API) separators, tilted plate interceptor (TPI) separators and dissolved air floatation (DAF) units. These operations are followed by biological treatment in a suspended growth processChavan and Mukherji, 2008). Studies have shown that symbiotic association between algae and bacteria yield higher treatment efficiency (Chavan and Mukherji, 2008). Under certain environmental conditions, algae can facilitate spontaneous flocculation of bacteria to improve the quality of treated effluent. In the coastal environment of the Arabian Gulf experiencing frequent oil pollution, oil degrading bacterial cultures are found to associate with cyanobacterial (blue-green algae) mats. While some cyanobacterial cultures may play a direct role in hydrocarbon degradation, others may facilitate hydrocarbon degradation indirectly by providing surfaces for adherence of oil degrading bacterial cultures (Chalian *et al*., 2006). Bioremediation is a biological treatment system to destroy, or reduce the concentration of hardous waste from a contaminated site (Biswas *et al.*, 2015). The aim of the study is to evaluate the treatment of hydrocarbon rich waste water using algae.

II. MATERIALS AND METHODS

A. Sample Collection

Microalgae was collected from fresh water containing the microalgae and it was transported to the Microbiology laboratory for immediate analysis. Hydrocarbon rich waste water was collected from an oil company in Port Harcourt Rivers state.

B. Physicochemical Assessment of the Sample

The physicochemical analysis of the waste water samples was conducted using standard methods. The pH and electrical conductivity were measured using pH meter 3015, Jenwayconductivity meter while dissolved oxygen (DO) and total dissolved solids (TDS) were measured. DO and TDS meter respectively. Turbidity was measured using direct spectrophotometer (method 8237) and then estimated against deionized water as blank at 450 nm. Nitrate (NO^{3-}), phosphate (PO_4^{3-}), and total petroleum hydrocarbon (TPH) were determined and biological oxygen demand (BOD) were determined volumetrically.

C. Determination of Ph

A portable pH meter with the following code was used to calculate the pH of the soil sample: HI9811-5 Hanna Instruments (Romania). The meter was turned on and left running for a while. It was then calibrated by dipping the electrode into buffer solutions with a higher pH range between 8 and 9 as well as a lower pH range between 1 and 6. A 100 ml beaker containing 10 g of soil was weighed; 25 mls of distilled water was then added to allow the electrode to be submerged; the mixture was then mixed for a few minutes by vigorously stirring. Beaker was left to stand for a

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further 15 minutes. Each sample's pH value was recorded after the electrode was submerged in the slurry.

D. Determination of Temperature

The Temperature for each sample was determined using a mercury-in-glass thermometer; code: G00127766-5 Hanna Instruments (Romania). The thermometer was immersed in the samples such that the mercury bulb was well covered by the samples. The final readings were considered the actual reading and were taken after it was allowed to stabilize.

E. Determination of Phosphate

The phosphate levels for the samples were determined using an ultraviolet (UV) spectrophotometer. The sulfuric acid - nitric acid Digestion method as described by APHA was adopted. Twenty-five millilitres (25 mls) of 2.5% Acetic acid was added to 1 g of sample andshaken for 30minutes. The suspension was filtered through a filter paper, 10 ml of the extract was transferred into 50ml volumetric flask. The extract was diluted with distilled water until the flask was about two-thirds full. 2 ml of ammonium molybdate reagent was added and mixed with extract, 2 ml of stannous chloride was also added and mixed and the solution was diluted to 50 ml mark with distilled water. The flask was allowed to stand for 30minutes and the absorbance was measured at the wavelength of 690 nm

F. Determination of Total Petroleum Hydrocarbon

Total Petroleum Hydrocarbon (TPH) analyses were carried out on all the six setups using Gas Chromatography (GC) for Day 1 and 56. Total Petroleum Hydrocarbon (TPH) in each of the set-ups was determined by a modified Environmental Protection Agency 8015 technique. The samples were extracted using a gas chromatograph, equipped with a Flame Ionization Detector (FID). The residual Total Petroleum Hydrocarbon (TPH) in the different treatment set up was extracted with 40 ul of n-pentane (HPLC grade) by sonicating the sample 5min at each extraction for 3 times. The pentane extract was centrifuged at 3000 g for 5 min, the three organic phases were oven-dried over sodium sulphate (Na_2SO_4).

G. Isolation of Hydrocarbon Utilizing Microalgae

The growth of the algae was enhanced using BG 11 medium supplemented with 1% manure and 1% (v/v) crude oil in 1L Erlenmeyer flask. This mixture was agitated and incubated at 25 ± 2°C for 7days with sunlight as the source of light. This` served as the inoculum source for biodegradation. Isolates of microalgae was identified by microscopy and in comparison, with those documented in the Identification Guide to freshwater and terrestrial algae. These isolates were used for remediation.

H. Treatment Formulation

A 100ml of the microalgae population was added to the different treatment of the contaminated hydrocarbon rich waste water (as shown in table 2.1) and mixed properly in 1L Erlenmeyer conical flask using sterile spatula. Atreatment was amended with biostimulants only, and another treatment was formulated with biostimulants and microalgae and poultry manure, while control treatment did not contain biostimulant and microalgae. Before the amendment of the contaminated waste-water, the total petroleum hydrocarbon content was determined. (the waster water above the intervention level of 5000mg/kg was considered for the study).

Table 1: Treatment (s) Formulation

Treatment (s)	Volume of the contaminated waste water (ml)	Microalgae (ml)	Poultry Manure (g)
Control	1000	-	-
A	1000	100	-
B	1000	100	50

Key: A= Microalgae(*Chlorella*sp);B = Microalgae (*Chlorella*sp) and Organic Nutrient (Poultry Manure)

I. Determination of Amount and Percentage (%) of Crude Oil Bioremediation

Adopting the method of Awari *et al*. (2020), the amount remediated (AR) and bioremediation (%) was determined based on equation (1) and equation (2) respectively.

$$AR = I_c - F_c \quad (1)$$

$$Bioremediation(\%) = \frac{AR}{I_c} \times \frac{100}{1} \quad (2)$$

where:

AR = Amount of pollutant remediated
I_c = Initial concentration of pollutant (day 1)
F_c = Final concentration of pollutant (day 28)

J. Method of Data Analysis

This study applied charts, mean, standard deviation, multiple comparison (Fisher's statistic) and analysis of variance (ANOVA) at 95% level of confidence. These statistical analyses were aided using statistical package for social sciences (SPSS, version 21).

K. Macroscopic and Microscopic Identification of the Isolates

The macroscopic and microscopic identification of the isolate depicted a solitary, non-motile and spherical unicellular cells were observed in addition to the greenish colonies and the algae was identified as *Chlorella* sp. The physical morphology of the isolated algae is also shown in Plate 1.

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L. Baseline Physicochemical Parameters of Waste Water Samples

Table 1 shows the baseline physicochemical parameters of waste water samples. These parameters are conductivity (162.5 **µs/cm),** salinity (18.2ppt), TDS (242.4mg/kg), BOD (181.1mg/L), COD (121.8mg/L) and pH(7.8); which are all above the department of Petroleum resources (PDR) speculation of waste water effluent.

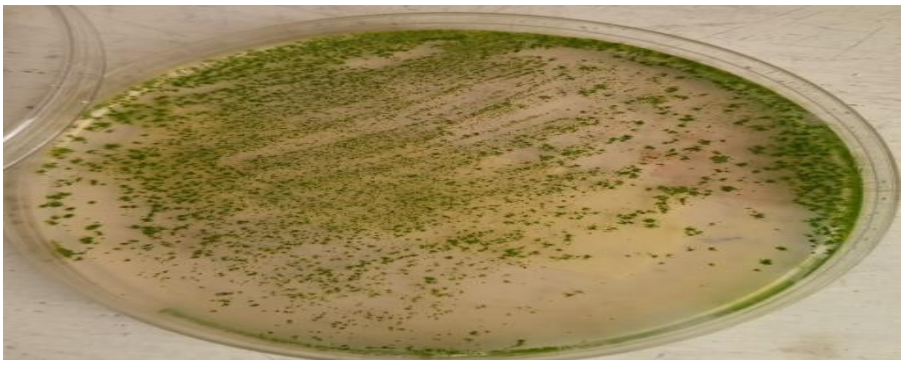

Plate 1: Culture of the algae

Table 1: Baseline Parameter of Contaminated Waste Water

Parameters	Contaminated Waste Water	Standard (DPR Specification)
Conductivity (µS/cm)	162.5	140
Salinity (ppt)	18.2	NA
TDS (mg/kg)	242.4	<200
BOD (mg/L)	181.1	10
COD (mg/L)	121.8	40
pH	7.8	6.5-8.5
Phosphate (mg/kg)	1.8	0.2

III. RESULTS

A. Improvement in Physicochemical Parameters based on Treatment (s)

Results revealed improvement in physicochemical parametersas shown in Figure 1 to Figure 4. The highest reduction in pH was observed in treatment B followed by treatment A and control respectively. Also, there was slight change in the temperature between the day 1 and day 28 as treatment B increase from 29.4^{o}C to 30.2^{o}C followed by treatment A which increased from 28.9 to 30^{o}C and slight decrease observed in control which decreased from 29.3 to 28.1^{o}C. The highest reduction in phosphate was observed in treatment B followed by treatment A and control respectively. Also,nitrate reduction was observed in treatment B from 6.5 mg/kg to 4.9 mg/kg followed by treatment A from 5.9 mg/kg -5.2 mg/kg and control from 6.4 mg/kg -6.3mg/kg respectively.

Table 2: Mean ± Standard Deviation of Physicochemical Parameters based on Treatment(s)

Treatment (s)	pH		Temperature (^{0}C)		Phosphate (g/kg)		Nitrate (mg/kg)	
	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28
Control	6.72±0.63	6.77±0.36	29.3±0.19	28.1±0.35	2.81±0.23	2.25±0.37	6.4±0.41	6.3±0.54
Treatment A	6.8±0.31	5.73±0.25	28.9±0.27	30±0.26	3.21±0.18	1.2±0.18	5.9±0.23	5.2±0.16
Treatment B	6.1±0.28	5.28±0.17	29.4±0.16	30.2±0.31	2.4±0.15	0.3±0.13	6.5±0.16	4.9±0.23

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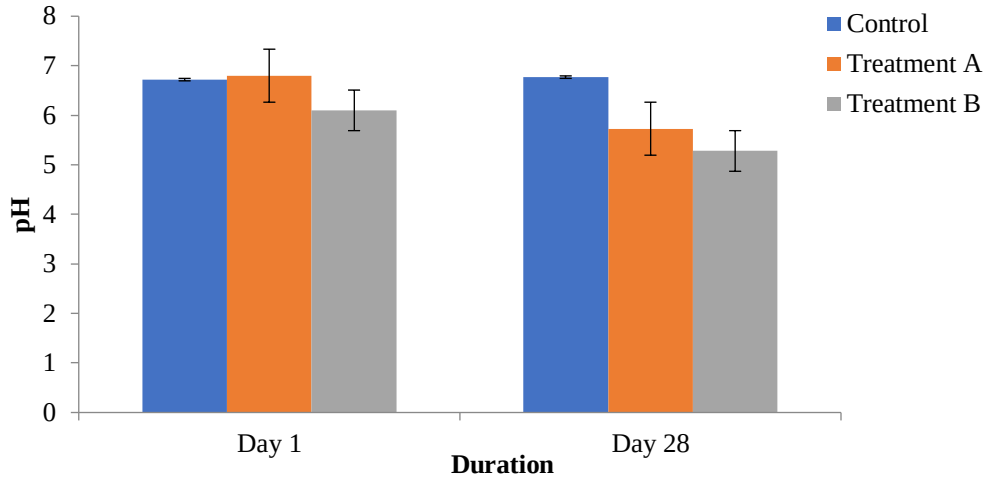

Fig. 1: Distribution of pH

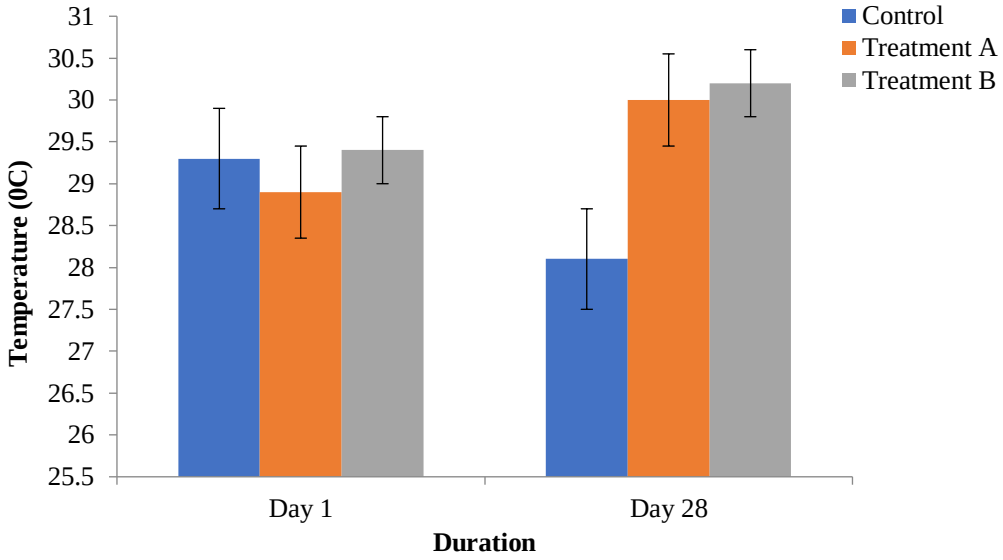

Fig. 2: Distribution of temperature (^{0}C)

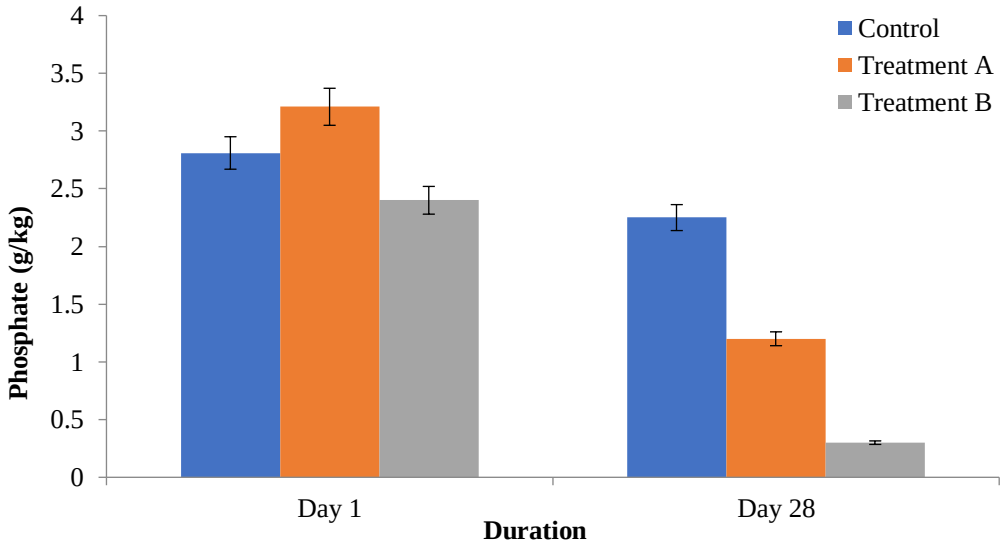

Fig. 3: Distribution of Phosphate (g/kg)

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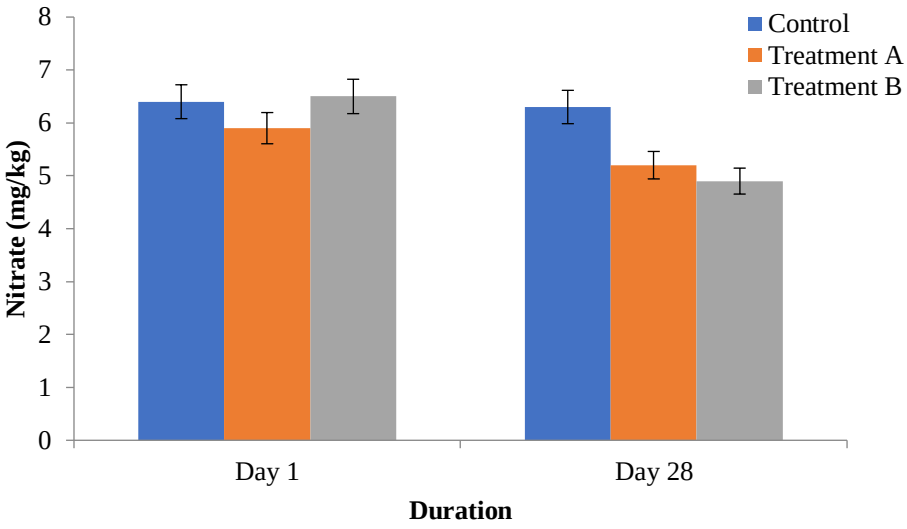

Fig. 4: Distribution of Nitrate (mg/kg)

B. Reduction in Total Petroleum Hydrocarbon (TPH) based on Treatment

Reduction in total petroleum hydrocarbon content of the different setups after 28 days of study is shown in Figure 5 and the percentage of amount remediated is shown in Figure 6. Result revealed that treatment B recorded the highest percentage remediation of hydrocarbon 74% (4494.29mg/kg) followed by treatment A with 55.5% (3367.84mg/kg) hydrocarbon remediation and control had 2.3% (138.76mg/kg) of hydrocarbon remediation. Analysis of Variance (ANOVA) results revealed that treatments are significantly different ($p<0.05$) total petroleum hydrocarbon(TPH). Also, multiple comparison test using Fisher's statistic buttressed the pair significances ($p<0.05$).

Table 3: Reduction in Total Petroleum Hydrocarbon (TPH) based on Treatment

Treatment (s)	**Initial (mean ±Std.)**	**Final (mean ±Std.)**	**Remediation (%)**
Control	6061.431±1.76	5922.32±4.140c	138.76 (2.3)
Treatment A	6053.161±1.23	2685.32±2.86b	3367.84 (55.6)
Treatment B	6067.238±0.95	1592.94±1.53a	4474.29 (73.7)
ANOVA (p-value)	1322989.24 (0.000)		
Decision	Significant ($p<0.05$)		

Row mean ±std. with different alphabet is significant

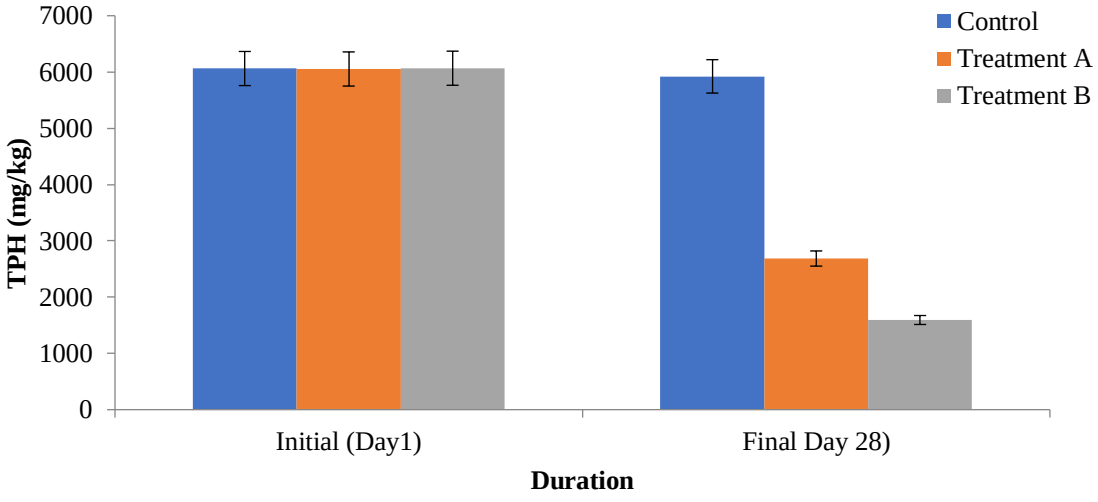

Fig. 5: Distribution of Total Petroleum Hydrocarbon (TPH)

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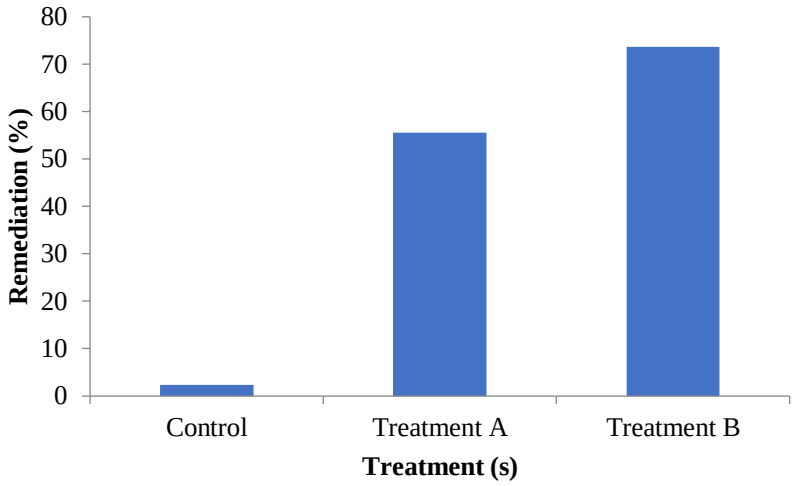

Fig. 6: Distribution of bioremediation (%)

IV. DISCUSSION

The presence of hydrocarbon in receiving water bodies is known to be carcinogenic, mutagenic and neurotoxic to living organism including plants and animals. (Akpor*et al*., 2014). From the result of the baseline parameter as recorded in this study, the physicochemical parameters of the waste water samples were above the DPR standard and specification which potent environmental standard to the water body and the environment at large. This is similar to the report of Osin *et al.* (2017) which reported similar physicochemical parameter of waste water.The microalgae used in this study was *Chlorella* sp which have been reported for several studies in the environment as a result of their availability and increased growth. Successful degradation of *n*-alkanes by cyanobacteria and algal-bacterial association in batch systems was also reported by various researchers (Chavan and Murkherji, 2019). Microalgae, *Chlorella* sp, have been reported for their great potential for remediation and renewable power because they possess rapid growth rate and the ability to store high-quality lipids and carbohydrates inside their cells for biofuel production (Klinthong *et al.*, 2015).The result of TPH showed that the treatment B which was made up of the waste water, microalgae (*Chlorella* sp) and organic nutrient (poultry manure) recorded the highest percentage remediation of hydrocarbon 74% (4494.29mg/kg amount remediated) with reduction in TPH followed by treatment A (microalgae) in which 55.6% (3367.84mg/kg) hydrocarbon was remediated with TPH reduction from 6053.1610mg/kg to2685.3157mg/kg and the least percentage remediation was observed in the control in which 2.3% (138.7mg/kg) of hydrocarbon was remediated with TPH reduction from 6061.4312 to 5922.6745mg/kg. Similar reported was recorded in the study of Sawayama *et al.* (2016) which reported very significant reduction of total petroleum hydrocarbon in waste water treated with microalgae, *Botrycoccus braunii*. In this study, it was observed that stimulation of the growth of *Chlorella* with organic nutrient resulted in corresponding better and increased growth of the microalgae which resulted in the higher reduction of hydrocarbon. Successful degradation of *n*-alkanes by cyanobacteria and algal-bacterial association in batch systems is reported by various researchers (Chavan and Murkherji, 2019) and the stimulation by nutrients leads to considerable increase in the growth of the degrading microorganisms (Adam *et al.*, 2018).The significant reduction in the concentration of TPH in the contaminated sample amended with the organic nutrient (poultry dropping) compared to the waste water treated only with algae,and unamended sample at the end of 28 days remediation study, can be attributed to the additional nutrient (N, P, and K) contained in the organic nutrient (Nwogu *et al.*, 2015). These nutrients are the basic building blocks of life, which prompts or enhance microbial growth and enable microorganisms to synthesize the appropriate enzymes that break down the petroleum hydrocarbon contaminants into the smaller compound such as CO_2 (Dados *et al.*, 2015).The change in the physicochemical parameter showed that treatment setup of the microalgae (*Chlorella* sp) and organic nutrient resulted in higher reduction of phosphate and nitrate content after the 28 days of the study in comparison to the setup with only microalgae and the control setup. This is in line with the study of Sayawama *et al.*, (2016) in which nitrate and phosphate concentrations decreased considerably at the end of the study with microalgae which correlated with the remediation result of hydrocarbon in the waste water. The change in pH can be attributed to the change in the concentration of phosphate and nitrate and recorded in this study. It has also been reported that assimilation of nitrate ions by actively growing phototrophic microorganisms also tends to changes the pH of the system (Chavan and Murkherji, 2019).

V. CONCLUSION

This study revealed the potential of microalgae (*Chlorella*sp) in remediation of waste contaminated by hydrocarbon. The growth of microalgae can also be enhanced with the use of organic nutrient such as poultry manure. The increased growth of microalgae resulted in better remediation of hydrocarbon as shown in the total petroleum hydrocarbon content.Microalgae, *Chlorella* sp, being more available and ecofriendly is recommended for

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use for remediation of polluted waste water based on its potential.

DISCLOSURE OF CONFLICT OF INTEREST

Authors have declared that no conflict of interest exist

REFERENCES

[1.] Akpor, O., Ukolonike, Olaolu, T. D. and Aderiye, B. I. (2014). Remediation of Polluted Waste water effleuntl Hydrocarbon Removal. *Trends in Applied Science*, 9(4), 160-173

[2.] Akpor, O. B., Okolomike, U. F., Olaolu, T. D and Aderiye, B. I. (2014). Remediation of Polluted Wastewater Effluent: Hydrocarbon Removal. Trends in Applied Science Research, 9(4),160-173

[3.] Arun N. and Singh D. P. (2012). Microalgae: The Future Fuel (Review). *Journal of Algal Biomass ultn*, 3(1),46-54

[4.] Bakke, T., Klungsøyr, J. and Sanni, S. (2013). Environmental impacts of produced water and drilling waste dischargesfrom the Norwegian o_shore petroleum industry. *Marine and Environmental Research*, 92,154–169.

[5.] Bérard, A., Rimet, F., Capowiez, Y., and Leboulanger, C. (2004). Procedures for determining the pesticide sensitivity of indigenous soil algae: a possible bioindicator of soil contamination. *Archives of environmental contamination and toxicology*, 46(1),24-31.

[6.] Biswas, K., Paul, D. Sinha and N. S. (2003). Biological agent of bioremediation: A concise review. *Frontier in Environmental Microbiology*, 1(3),39-43

[7.] Chavan, A. and Mukherji, S. S. (2019). Treatment of hydrocarbon rich wastewater using oil-degrading bacteria and phototrophic microorganism in rotating biological contactor; Effect of N:P. *International Journal of microbiology*, 5,81-89

[8.] Chavan. A. and Mukheji, S. (2008). Treatment of hydrocarbon-rich waste water using oil degrading bacteria and phototrophic microorganisms in rotating biological container: Effect of N:P ratio. *Journal of Hazardous material*, 154,63-72

[9.] Dados, A. Omirou, M., Demetriou, K. Papastephanou, C. and Ioannides, I. M. (2015). Rapid remediation of soil heavily contaminated with hydrocarbons: a comparison of different approaches," *Annals of Microbiology*, 65(1), 241–251

[10.] Fu P. and Secundu F. (2016). Algae and Their Bacterial Consortia for Soil Bioremediation. *Chemical Engineering Transactions*, 49,427-432

[11.] Fu, P. and Secundo, F. (2016). Algae and their bacterial consortium for soil bioremediation. *Chemical Engineering Transaction*, 49,427-432

[12.] Hosmani, S. (2014). Freshwater plankton ecology: a review. *Journal of Research and Management Technology*, 3:1-10

[13.] Klinthog, Y., Yang, Y., Huang C., and Tan, C. (2015). A review: Microalgae and their Applications in CO_2 capture and Renewal Energy. *Aerosol and Air Quality Research*, 15,712-742

[14.] Ngwogu, T. P., Azubuike, C. C. and Ogugbue, C. J. (2015). Enhanced Bioremediation of Soil Artificially Contaminated with Petroleum Hydrocarbons After Amendment with *Capra aegagrus hircus* (Goat) Manure. *Biotechnology Research International*, 657349,7

[15.] Osin, O. A., Yu, A. and Lin. S. (2017). Oil Refinery Waste Treatment in Niger Delta, Nigeria, Current Practices, Challenges and Recommendation. *Environment Science and Pollution Research*, 24,22730-22740

[16.] Ottaviano, J. G., Cai, J., Murphy and R. S. (2014). Assessing the decontamination efficiency of a three-componentflocculating system in the treatment of oilfield-produced water. *Water Research*, 52:122–130.

[17.] Sen, B., Alp, M. T., Sonmez, F. Kocer, M. A. T. and Canpolat (2013). Relationship of Algae to water pollution and waste pollution and waste water treatment. *Intech Open Science*, 4,335-342

[18.] Stephens, E., Ross, I. L., Mussgnug, J. H., Wagner, L.D., Borowitzka, M.A., Posten, C. and Kruse, O., (2010).Future prospects of microalgal biofuel production systems. *Triends in Plant Science*. 15(10):554–564.

[19.] Veil, J.A. (2015*). U.S. Produced Water Volumes and Management Practices in 2012*; Veil Environmental, LLC: Annapolis, MD, USA, pp119

[20.] Vidali, M. (2001).Bioremediation. An overview. *Pure and Applied chemistry*, 73(7):1163-1172.

[21.] Wang, S., Ghimire, N., Xin, G., Janka, E. and Bakke, R. (2017). Efficient high strength petrochemical wastewater treatment in a hybrid vertical anaerobic biofilm (HyVAB) reactor: A pilot study. *Water Practical Technology*, 12,501–513.

[22.] Wu, P., Jiang, L.Y., He, Z. and Song, Y. (2017). Treatment of metallurgical industry wastewater for organic contaminant removal in China: Status, challenges, and perspectives. *Environmental Sciience andWater Research Technology*,1015–1031.