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Effect of Light on the Biodegradation of Crude Oil by the Algae *Closterium* species

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ABSTRACT

Effect of light on the ability of algal isolates to degrade crude oil was studied using standard methods. Identification tests revealed isolates of *Closterium* sp which was used in this study. Total viable counts were evaluated, which showed an increase from 6.0 x 10^4 to 3.2 x 10^6 cfu/mL in 21days in the presence of light and from 6.0 x10^4 cfu/mL to 3.56 x 10^6 cfu/mL in the dark phase. Optical densities (OD) at 520nm also increased from 0.160 to 0.500 in 21days in the presence of light and from 0.160 to 0.805 in 21days in the absence of sunlight. The gas chromatographic analysis showed a decrease in total petroleum hydrocarbon (TPH) from 12,405 to 1,470mg/L in 21days in the presence of sunlight and from 12,405 to 144mg/L in 21 days in the dark. Algae possess a greater ability to degrade crude oil in the absence of sunlight than in the presence of sunlight. We suggest that *Closterium* sp can potentially bioremediate the crude oil-polluted environment, particularly in the dark.

Keywords: Biodegradation, algae, total petroleum hydrocarbon, *Closterium* sp, gas chromatography

1. INTRODUCTION

The demand for crude oil as a source of energy and as primary raw material for chemical industries caused an increase in the crude oil production worldwide. Increasing petroleum exploration, refining and other industrial activities in the Niger Delta area of Nigeria has led to large scale contamination of most swamps, creeks, streams and rivers (Okpokwasili and Odokuma, 1990) by hydrocarbons and dispersal products. In addition, contamination by petroleum products is further compounded by the sabotage and vandalization of pipelines in restive communities, particularly in the Niger Delta region of Nigeria (Obayori *et al.,* 2008). Environmental pollution caused by petroleum hydrocarbons has become a serious problem worldwide. The driving force of petroleum biodegradation is the ability of microorganisms to use hydrocarbons for satisfying their cell growth and energy needs (Adegbola *et al.,* 2014). Microbial degradation markedly contributes to the removal of organic molecules including oil from freshwater, brackish water and marine environments (Amanchukwu *et al.,* 1989).

Among microorganisms, algae are associated with the biodegradation of crude oil. Walker *et al.* 1975c showed that a particular strain of achlorophyllous algae *Prototheca zopfi* isolated from oil-polluted sediments can degrade 10% of motor oil and 40% of crude oil. When grown on crude oil, resin and asphaltene fractions were reduced more than other components. Cerniglia *et al.* 1980 demonstrated the production of numerous metabolites from naphthalene by several species of marine microalgae. The degradation potentiality was comparable to that of bacteria (Walker *et al.,* 1975b). Notably, the ability to oxidize petroleum hydrocarbons is widely distributed not only in bacteria and fungi but also in cyanobacteria and algae. Cerniglia *et al.* 1980 demostrated that the ability to oxidize aromatic hydrocarbons is widely distributed in algae and cyanobacteria such as *Oscillatoria* sp., *Anabaena* sp., *Nostoc* sp., *Aphanocapsa* sp. and *Chlorella* sp. (Gibson *et al.,* 1980;Juhasz and Naidu, 2000).

The aim of this study was to isolate the cultures of algae from an oil-polluted environment and compare the biodegradation potential in the presence and absence of sunlight.

2. MATERIALS AND METHODS

2.1 SAMPLING SITE

The oil-polluted water sample was obtained from location 1 of Shell Petroleum Development Company, Ukwugba village in Ohaji Egbema L.G.A of Imo State Nigeria. An anthropologic study of the site showed that no human activities occurred there.

2.1 SAMPLE COLLECTION

The crude oil-polluted water sample was aseptically collected using sterile dark BOD bottles. The bottles were half-filled with samples collected against the water flow by submerging it to a depth of approximately 15cm.The bottles were then immediately closed and taken to the microbiology laboratory for isolating algae (Cerniglia *et al.,* 1980).

2.3 SOURCE OF CRUDE OIL SAMPLE USED

The Bonny-light crude oil sample was obtained from the Bonny terminal of Shell Petroleum Development Company of Nigeria at Port Harcourt, Rivers State.

2.4 ISOLATION AND IDENTIFICATION OF ALGAE

Isolation was performed on mineral salt agar and the agar was incubated for 7 – 10 days in the presence of fluorescent light and was identified as per the study conducted by John *et al*. 2002.

2.3 CRUDE OIL UTILIZATION ADAPTATION TEST

The algae isolate (*Closterium* sp.) identified was adapted for the crude oil utilization test in 99 mL of mineral salt broth containing 1 mL of Bonny light crude oil as the carbon source (Al-Hasan *et al.,* 1998). This mixture was manually agitated and incubated at ambient temperature (30°C) for 14 days (Atlas, 1984). Subsequently, 0.1 mL of the adapted culture medium was inoculated on fresh mineral salt agar and incubated for 7 – 10 days. The colonies grown were transferred to slants and stored in the refrigerator at 4°C for future use (Chikere and Okpokwasili, 2003).

2.6 LIQUID SYSTEM BIODEGRATION SET UP

Twelve 250 mL amber bottles, each containing 99 mL of mineral salt broth supplemented with 1 mL of Bonny light crude oil, were used. Another two 250 mL amber bottles containing the same volumes of mineral salt broth and Bonny light crude oil served as controls (Al-Bader *et al.,* 1998). Twelve amber bottles were inoculated with 5 mL of the 2 weeks old crude oil adapted *Closterium* sp. Among these bottles, six were incubated in the presence of sunlight and the remaining six in the dark. The bottles were manually agitated by shaking at 100 strokes /min for 30 min every day for 3 weeks (Wang, 1984). Sampling period was at 7 days interval for 21 days, and the algae growth was monitored by evaluating the total viable count and optical density at 520nm (Ekpo and Ekpo*,* 2006). The qualitative changes in the hydrocarbon profile of the residual crude oil were monitored using gas chromatography (Onyeike and Osuji, 2003).

3. RESULTS

In this study, we isolated two algae species *Closterium* sp. and *Oscillatoria* sp. Furthermore, Table 1 describes their morphology and arrangement when viewed under the microscope using the phycological atlas.

Table 1: Characteristics of algae isolates

Isolates No.	Colony Morphology	Microscopy	Genera
1	Soft woody network of colonies that shrink when touched	Comma shaped rod-like unicellular cells	*Closterium* sp.
	Non-motile	Gram negative	
2	Blue green colonies	Thick meshwork filaments	*Oscillatoria* sp.
	Non- motile	Gram negative	

3.1 TOTAL VIABLE COUNT

The total viable count of the *Closterium* sp. was evaluated at 7 days interval. It showed an increase in the total algal population (Table 2). The viable count was greater in the dark (3.56×10^6 CFU/mL) than in the presence of sunlight (3.2×10^6 CFU/mL)

Table 2:Total viable count of hydrocarbon degrading *Closterium* sp.

Days	**L**	**D**	**C**
0	6.0×10^4	6.0×10^4	0
7	2.0×10^5	2.92×10^5	0
14	4.4×10^5	5.20×10^5	0
21	3.2×10^6	3.56×10^6	0

L = Light phase, D = Dark phase, C = Control

The optical density values increased as the incubation period progressed. In addition, the values were higher in the dark phase than in the light phase (Table 3). The increased optical density is directly related to the increased growth (turbidity) of *Closterium* sp.

Table 3:Optical densities of *Closterium* sp.

Days	**L**	**D**	**C**
0	0.160	0.160	0.00
7	0.250	0.225	0.01
14	0.381	0.725	0.01
21	0.500	0.805	0.01

L = Light phase
D = Dark phase
C = Control

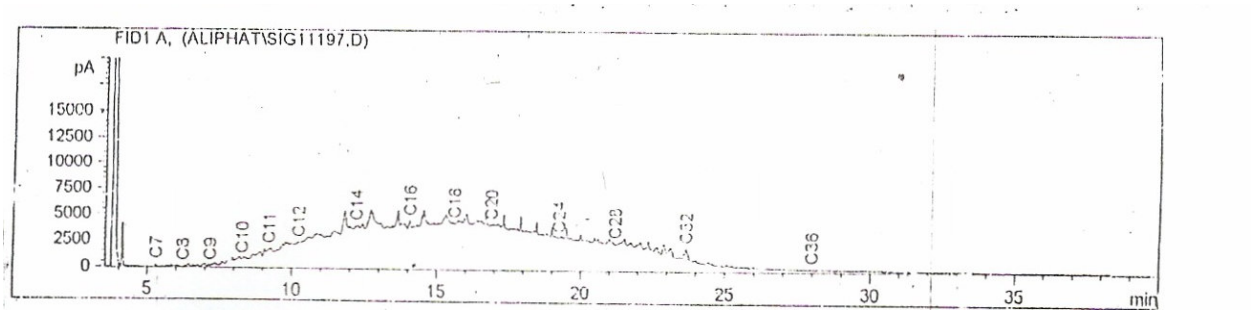

Figure 1: Chromatographic profile of TPH for *Closterium* sp. on day 0

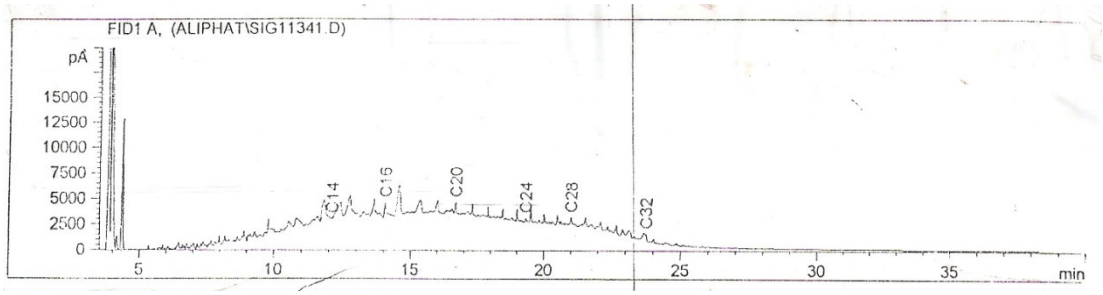

Figure 2: Chromatographic profile of TPH for *Closterium* sp. on day 7 under light

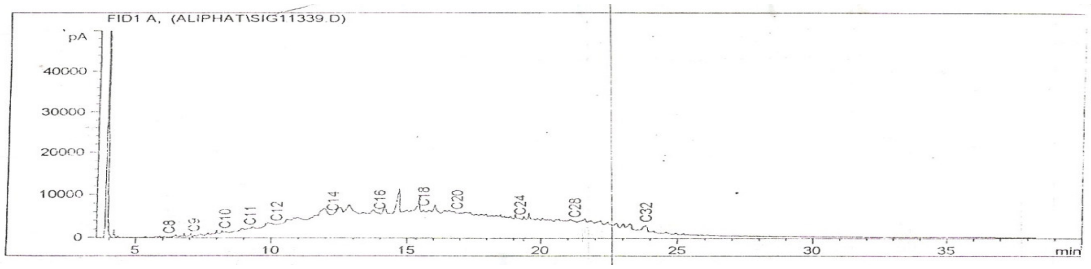

Figure 3: Chromatographic profile of TPH for *Closterium* sp. on day 14 under light

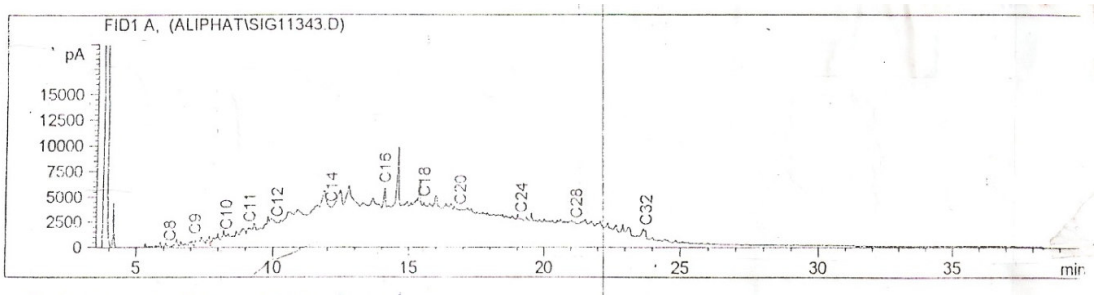

Figure 4: Chromatographic profile of TPH for *Closterium* sp. on day 21 under light

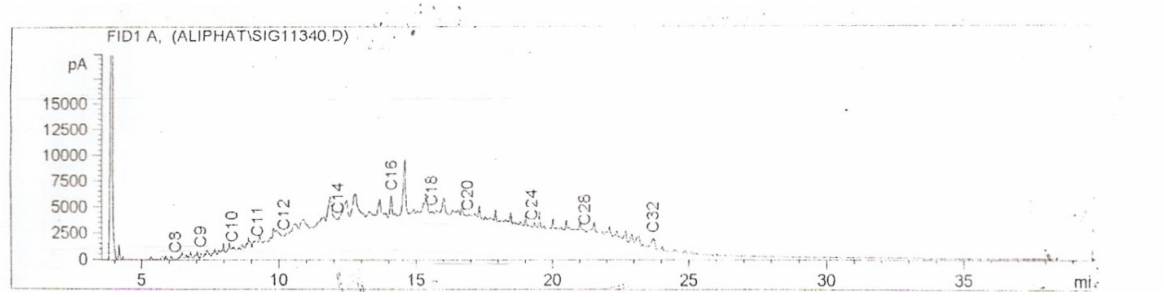

Figure 5: Chromatographic profile of TPH for *Closterium* sp. on day 7 in dark

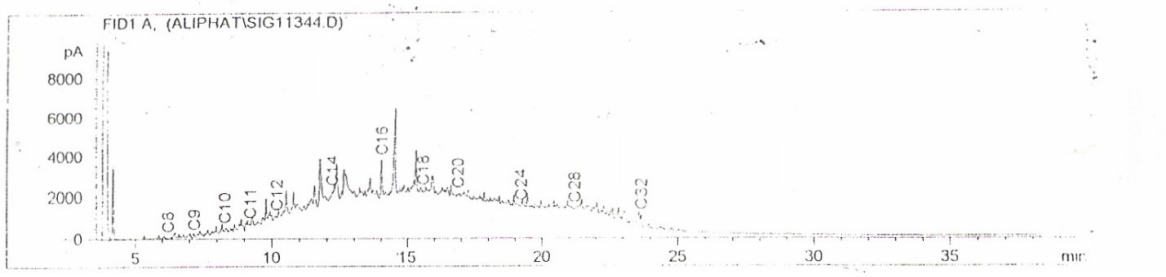

Figure 6: Chromatographic profile of TPH for *Closterium* sp. on day 14 in dark

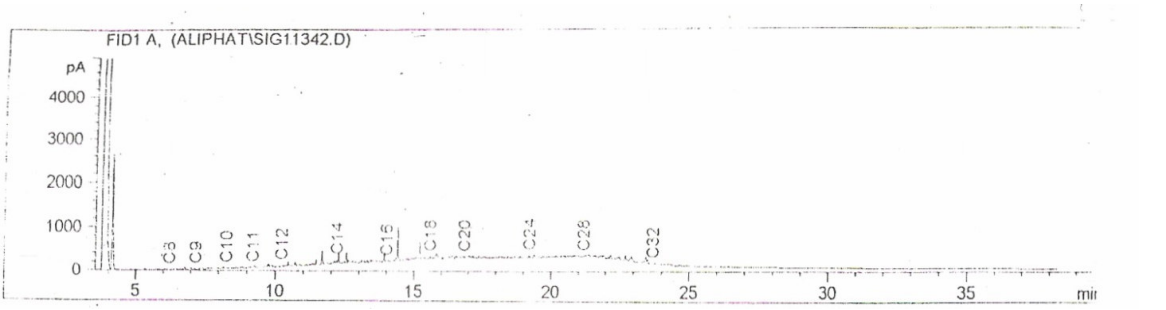

Figure 7: Chromatographic profile of TPH for *Closterium* sp. on day 21 in dark

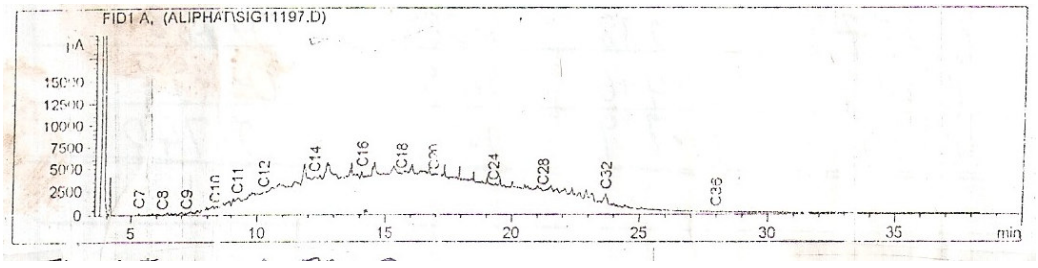

Figure 8: Chromatographic profile of TPH for *Closterium* sp. for controls on day 21

4. DISCUSSION

The increased microbial counts with the progress of the experiment indicated the enrichment of the hydrocarbon-utilizing algae *Closterium* sp. from the crude oil. The algal degradation of crude oil corroborates the study of Ibrahim and Gamila (2004). The increased microbial count was associated with the decrease in the total petroleum hydrocarbon (TPH). This result was consistent with that of Walker et al.(1975) who demonstrated the degradation of oil by *P. zopfii.* The chromatographic profile of TPH showed a decrease in the petroleum hydrocarbon (Figure 2) which was indicated by the reduced number and sizes of peaks (Figure 3). In addition, this reduction in TPH was observed by Uba and Ifeanyi, 2013. TPH of the crude oil decreased more in the absence of sunlight Figures 6 and 7 than in the presence of sunlight Figures 4 and 5. Therefore, the algae

Closterium sp. undergoes several metabolic processes faster in the dark than in the light phase, thus utilizing the crude oil as its sole source of carbon and energy. This was because in the dark phase, only one source of carbon was available, that is, carbon in the crude oil whereas in the presence of sunlight, an alternative source of carbon from atmospheric carbon dioxide was available during photosynthesis process of algae. This is similar to the studies of Jacobsin and Alexander 1981; and Cerniglia 1992.

5. CONCLUSION

This study showed that the oil-polluted water at location 1 of Shell Petroleum Development Company at Ukwugba village in Ohaji Egbema L.G.A of Imo State harbors hydrocarbon- utilizing algae which were monitored by their ability to utilize Bonny light crude oil as its sole source of carbon. The chromatographic analysis of TPH showed higher biodegradation in the dark phase than in the light phase. This study with other previous studies showed the biodegradation potentials of the algae *Closterium* sp. and suggested its efficient utilization in the bioremediation of crude oil spills particularly in areas such as subsurfaces and creeks where there is slight or no light penetration.

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