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Comparative rate of biodegradation of crude oil in dark and light phases using *Oscillatoria* specie.

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ABSTRACT

Comparative study on the rate of biodegradation of crude oil by alga sp under dark and light phases were carried out using standard microbiological methods. Algal growth and biodegradation indices in an oil supplemented mineral salt medium was used. Isolation and identification tests conducted revealed the presence of *Oscillatoria sp*. The optical density (OD) measurements increased higher in the dark phase from 0.185 to 0.532 while that in the light phase was from 0.185 to 0.380. The total viable count (TVC) increased from $5.0x10^4$ to 6.80×10^6 in the dark phase and from 5.0×10^4 to 4.2×10^6 in the light phase. The degradation analysis showed quantitative reductions in the total petroleum hydrocarbon (TPH) which ranged from 11, 525mg/l to 156mg/l in the dark phase and 11, 525mg/l to 1,620mg/l in the light phase. There was greater degradation of crude oil by *Oscillatoria sp* in the dark phase than in the light phase which makes this alga potentially viable to be applied in the bioremediation of crude oil polluted environments especially in the Niger Delta-region.
Keywords: Crude oil, optical density, comparative study, total petroleum hydrocarbon, *Oscillatoria* sp

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INTRODUCTION

Petroleum remains the world's most important derived energy source (Moffat and Linden, 2005). In Nigeria, due to the growth and activities of petroleum associated industries, there have been increased incidence of pollution in our environment. The oil producing areas especially the Niger Delta have had a devastating experience of oil spills on both the aquatic and terrestrial environment due to crude oil exploration and production (Kadafa, 2012). Algae are free-living photoauthotrophic microorganisms that can derive energy from sunlight and carbon from atmospheric air. Algae species including cyanobacteria are wide spread in many ecosystems including polluted ecosystems (Gibson and Smith 1982; Fogg, 1987). Many researchers have presented evidence of a natural ability of algae and cyanobacteria to degrade organic pollutants (Abed *et al.,* 2002; Kuritz and Wolk,1995;Uzoh *et al.,*2015). Work is being done to optimize the biodegradation of crude oil (Bertrand *et al.,* 1983). The use of algae for degradation of petroleum hydrocarbon started with the isolation of *Prototheca zopfi* which exhibited the ability to degrade crude oil and a mixture of hydrocarbons (Walker *et al.,* 1975). Apart from *Oscillatoria sp.,* other organisms found to degrade hydrocarbons include *Dunaliella* sp., *Porphyridium* sp., *Microcoleus* sp., *Coccochloris* sp., *Nostoc* sp., *Chlamydomonas* sp., *Ulva* sp., *Cylindretheca* sp., *Chlorella* sp. and *Anabaena* sp. A number of microscopic algae species that occur in river Nile can grow on crude oil and degrade the oil to release carbondioxide and water (Cerniglia *et al.,* 1980a). The aim of this research work was to study the rate of biodegradation of crude oil by *Oscillatoria* sp under light and dark conditions.

MATERIALS AND METHODS

SAMPLING SITE DESCRIPTION

The crude oil polluted water sample for this study was obtained from location 1 of Shell Petroleum Development Company (SPDC), Ukwugba village, Egbema Ohaji-Egbema Local Government Area of Imo State, Nigeria. The sampling site is about 148Km east from Chukwuemeka Odumegwu Ojukwu University (COOU), Uli Campus main gate. Anthropogenic survey of the location showed that no human activities like washing and dumping of refuse took place there.

SAMPLE COLLECTION

Crude oil-polluted sample was obtained from location 1 Shell Petroleum Development Company (SPDC) Ukwugba village, in Ohaji-Egbema Local Government Area, Imo State. By adopting the method of Okpokwasili and Amanchukwu (1988), water sample was collected in sterile 1 litre plastic bottles which has been previously rinsed with 70% ethanol. The samples were collected against water flow by submerging it to a depth of about 10 – 15cm. The bottles were immediately closed and taken to the Microbiology Laboratory of Chukwuemeka Odumegwu Ojukwu University(COOU), Uli Anambra State for isolation of the algae (Cerniglia *et al.,* 1980a).

ISOLATION AND IDENTIFICATION OF ALGAE

Isolation was carried out using Mineral Salt Medium (MSM) and incubated for about 8 – 10 days under the presence of continuous illumination of fluorescent lamp light at 25±2^{0}C. The identification guide of John *et al.,* 2002 was used for identification.

SOURCE OF CRUDE OIL SAMPLE:

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

CRUDE OIL UTILIZATION ADAPTATION TEST

By adopting the method of (Al-Hasan *et al.,* 1998), the alga isolate was adapted for crude oil utilization in 99ml mineral salt medium containing 1ml of Bonny-light crude oil as the carbon source. The mixture was agitated and aerated manually by shaking at 100 strokes per minute as described by (Chikere and Okpokwasili, 2003) for 30minutes per day for 14 days. Aliquots (0.1ml) of the adapted culture medium was

transferred onto mineral salt medium and incubated for 7-8 days. Discrete colonies of the isolate was transferred onto slants, incubated and stored at 4°C in the refrigerator for further use.

BIODEGRADATION SYSTEM SET UP

Mineral salt medium broth was autoclaved in two 1000ml conical flask. 99ml of the liquid medium was dispensed into ten (10) sterile 250ml dark amber bottles into which 1ml of sterile crude oil was added (Ekpo and Ekpo, 2006). Five milliliters of the adapted culture of *Oscillatoria* sp was inoculated into eight (8) dark amber bottles in two sets each for the light and dark phases of incubation. The ninth and tenth bottles served as controls as they were uninoculated. The bottles were incubated at ambient temperature (30^0C) in the dark and light conditions. Adopting the method of (Wang, 1984), the culture bottles were manually shaken at 100 strokes per minute for 30 minutes each day for 21 days. Samples were collected from the bottles for analysis at 7 days interval for 21 days. The growth of the *Oscillatoria sp* was monitored by optical density at 520nm and total viable count. The total petroleum hydrocarbon (TPH) was measured and changes in hydrocarbon profile of crude oil were monitored by gas chromatography.

RESULTS

Table 1: Characteristics of alga isolated

Isolate No	Colony Morphology	Microscopy	Genera
1	Bluish green colonies that are non-motile	Thick meshwork network of filaments	*Oscillatoria* sp.

In table 1 above, the characteristics of the alga was highlighted while in table 2, there was a noticeable increase in the optical density (OD) value during the incubation period. The OD values were higher in dark phase incubation period than that of the light phase counterpart. The increase in optical density with time was due to the multiplication of the cells as they utilize the hydrocarbon components of the crude oil as a sole source of carbon. The OD value of 0.532 recorded in the dark phase was higher than 0.380 in the light phase.

Table 2: Optical densities (OD) of *Oscillatoria* sp during biodegradation

Days	D	L	C
0	0.185	0.185	0.00
7	0.210	0.260	0.01
14	0.830	0.410	0.01
21	0.532	0.380	0.01

L = Light phase incubation, D = Dark phase incubation, C = control

The Total Viable Count (TVC) conducted at 7 days interval showed an increase in the population of *Oscillatoria* sp. This value increased more in the dark phase than in the light phase during incubation. The low TVC values recorded at day zero (Table 3) may be as a result of the adaptation of the organism to new environmental conditions and the non-commencement of the hydrocarbon utilization by the organism for their cell multiplication. There was significant degradation in the total hydrocarbon (Figures 1 and 2) in the dark phase than observed under light phase as in the chromatograph on figures 1 and 3. However, there was no hydrocarbon degradation in the control (figure 4).

Table 3: Total Viable Count (TVC) during crude oil biodegradation

Days	D	L	C
0	5.0×10^4	5.0×10^4	0
7	3.60×10^5	3.0×10^5	0
14	6.2×10^5	5.5×10^5	0
21	6.80×10^6	4.2×10^6	0

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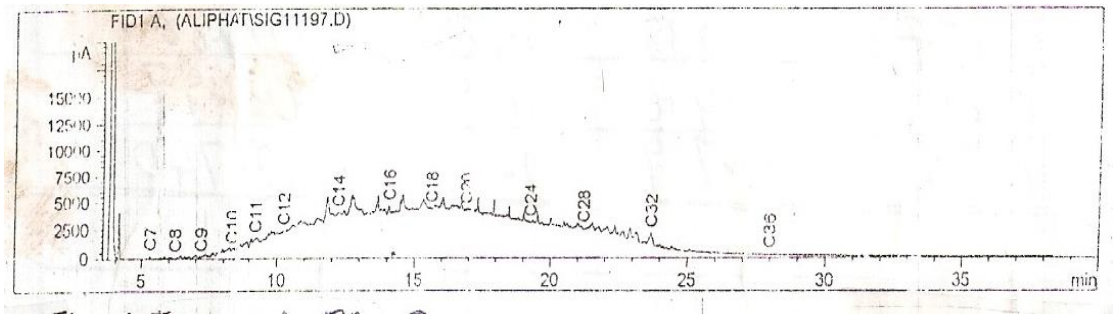

Figure 1: Chromatographic profile of TPH for Oscillatoria sp at day 0

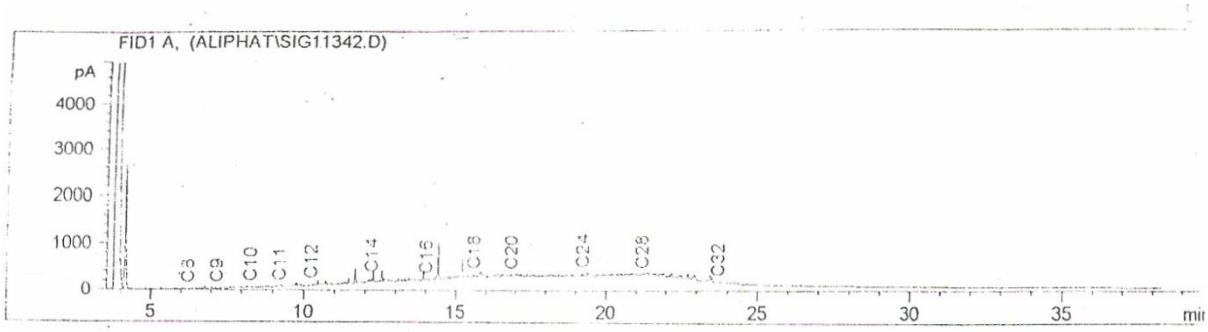

Figure 2: Chromatographic profile of TPH for *Oscillatoria* sp at day 21 (dark phase)

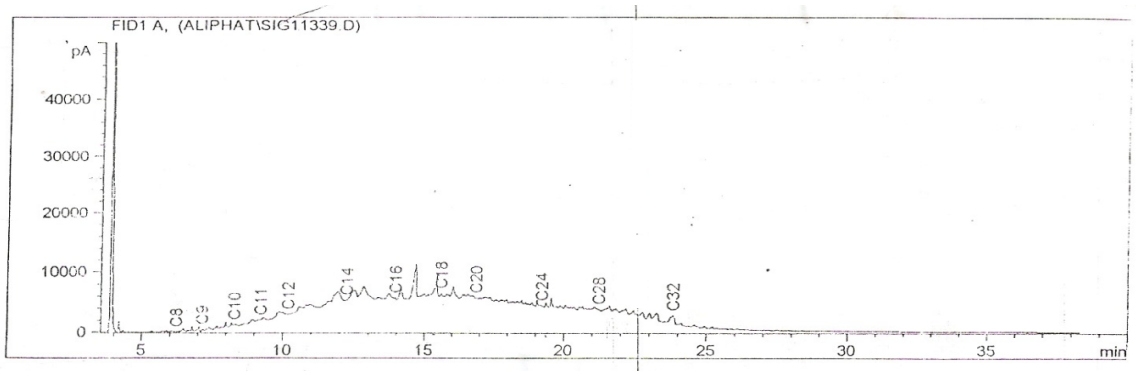

Figure 3: Chromatographic profile of TPH of *Oscillatoria* sp at day 21 (Light phase)

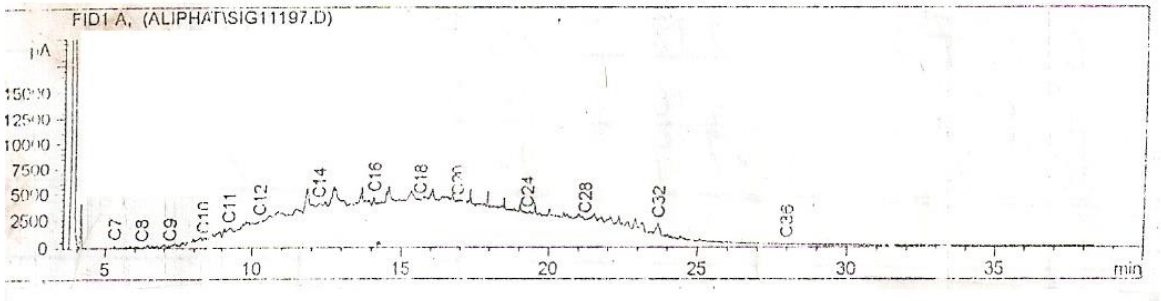

Figure 4: Chromatographic profile of TPH for control at day 21

DISCUSSION

The increase in the population of colonies of *Oscillatoria sp* suggests a quick and successful adaptation which led to the utilization of the crude oil for carbon and energy sources. This corroborates the work of Ogbulie and Ifeanyi (2006) who stated that some species of micro-algae grew on mineral salt medium supplemented with crude oil as the only source of carbon and energy. The low total viable counts and optical density recorded on day zero may be due to the fact that the organisms had not started utilizing the

hydrocarbon components in the crude oil, hence in their lag phase. Thereafter, there was a continuous increase in these values which was associated with subsequent decrease in the total petroleum hydrocarbon (TPH) values. This agrees with the report of Walker *et al.,* (1975) who first demonstrated the degradation of oil by algal species *Prototheca zopfii.* The hydrocarbon reduction equally agrees with Semple and Cain (1996). In the dark phase, there was a greater increase in both the total viable count (TVC) and optical density (OD) than in the light phase. This biodegradation studies showed reduction in the size, number and heights of the peak in the chromatogram after 21 days. Comparatively, the dark phase of the biodegradation showed more remarkable decrease in the total petroleum hydrocarbon. This could be attributed to increased metabolic activities by the algal cells. It could be that the major component of the *Oscillatoria sp* cell which majorly utilize crude oil are more active under dark conditions. However, this remarkable decrease in the TPH in the dark phase than in the light phase could be as a result of the non availability of alternative sources of carbon. These microbes were constrained to utilizing carbon in the crude oil unlike the light phase which could make use of either the carbon in the crude oil or in the atmospheric carbondioxide for photosynthetic activities. This observation corroborates the work of Uzoh *et al.,* (2015) who reported that there was more biodegradation of crude oil by *Closterium* sp in the absence of sunlight.

CONCLUSION

This research showed that the sampling site harboured alga that are hydrocarbon utilizers which were monitored by their potentials to utilize Bonny-light crude oil as their sole source of carbon. This investigation threw more light on the greater biodegradation ability of crude oil by *Oscillatoria* sp in the dark phase. This research and other researches previously conducted will help in the bioremediation of crude oil polluted environments especially in areas where sunlight is difficult to penetrate.

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