International Journal of Science and Technology
(STECH)
Bahir Dar- Ethiopia
Vol. 5 (2), S/No12, October, 2016: 13-23
ISSN: 2225-8590 (Print) ISSN 2227-5452 (Online)
DOI: http://dx.doi.org/10.4314/stech.v5i2.2

Potential Use of Algae *Microcystis aeruginosa* (Chroococaceae) in Bioremediation

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Abstract

A comparison of growth response of algal species in varying concentrations of petroleum products to assess their bioremediation potentials was carried out using *Microcystis aeruginosa* as test organism. The modified Chu #10 standard medium for algal culture was used for the experimental set up which lasted for twenty-one days. The test alga was subjected to growth medium with varying concentrations of petrol

and kerosene. Algal growth was determined by measuring optical density of inoculated medium at three days' interval using a spectrophotometer at 750nm wavelength. The effect of the hydrocarbons on algal growth was either stimulatory or inhibitory depending on the concentration. The inhibitory effects of the hydrocarbons increased with increasing concentrations. Comparatively, petrol had more inhibitory effect than kerosene which did not appreciably alter growth either at lower or higher concentrations. The experiment concludes that *Microcystis aeruginosa* can be used for bioremediation of soil or water impacted by crude oil or petroleum products.

Key Words: *Algal culture, Petroleum hydrocarbons, Bioremediation, Microcystis aeruginosa*

Introduction

Algae are chlorophyll a bearing, photosynthetic non-vascular plants which have simple reproductive structures (Reynolds *et al*., 2004). They occur commonly in water, be it freshwater, marine or brackish water. They may also be found in extreme environments such as in snow or ice, desert, in hot springs and in lichen association on tree barks. They vary in size from small, microscopic, single-celled forms to complex, multicellular form (filamentous, colonial and thallose). Some have been reported in fossil record dating back to Precambrian era. They exhibit a wide range of reproductive strategies, from simple asexual cell division to complex forms of sexual reproduction (Murray, 2004).

Algae have different uses which include food for animals including man (because of its protein content), serve as thickening agent in shampoo and ice cream and are also used as drugs to fight diseases. They help also in ecological balance. They constitute the primary producers of the aquatic environment and organisms at higher trophic level in the aquatic habitat depend directly and indirectly from their productivity. Algae are also used as indicators of water quality owing to their rapid growth and short life cycle and hence respond fast to changes in environmental conditions (Wiersma, 2004). Megharaj *et al.* (2000) noted that the alternation in algal species composition serves as useful bioindicator of pollution.

Algae themselves could also cause water pollution. This usually occurs when the water contains abundant nutrients usually as a result of eutrophication which results in an excessive algal growth referred to "algal bloom" (Lee, 2009). An example of this is the poisonous and destructive "red tides" that occur frequently in the coastal areas, which are often associated with great population explosions or bloom of dinoflagellates (Raven, 2009). When these algae die, their decomposition by micro-organism depletes the oxygen in water leading to death of aquatic organisms as well as prevents light penetration for algae at lower depth preventing photosynthesis.

Literatures abound on growth response of algal species to changing environmental conditions. Petkov *et al.* (2002) studying the behavior of *Spirulina platensis*, *Chlorella vulgaris* and *Scenedesmus incrassatulus* R-83 after petroleum pollution, observed that the addition of 0.15 g/l of petrol into the medium killed *Chlorella vulgaris* and *S. platensis* while *S. incrassatulus* survived.

Goutx *et al.* (2007) exposed the marine diatom, *Phaeodactylum tricornutum* to aromatic hydrocarbon, 9 – 10 dihydroanteracene and its biodegradation products and they observed that the growth was inhibited on exposure to aromatic hydrocarbon whereas no inhibition was observed in the presence of biodegradation products alone. They also observed the synergistic effects between dihydroanthracene and its biodegradation increased the toxicity of this aromatic hydrocarbon.

Tukaj (2007) observed that various concentration of crude oil fuel oil caused a decrease in numbers of cells, dry matter and chlorophyll α distribution while the water extracts of both oil stimulated dry mass and chlorophyll content with respect to a single cell. Karydis and Fogg (2008) in an experiment to determine the physiological effects of hydrocarbons on the marine diatom, *Cyclotella cryptica*, observed that low hydrocarbon concentration (100ug/l) stimulated growth whereas higher concentration (1mg/l) inhibited growth. They also noted that aromatic hydrocarbons were more toxic while the paraffin do not seem to have any serious effect on growth or photosynthesis of the algae.

Some algal species, specifically cyanobacterial species such as *Oscillatoria salina, Plectonema terebrans*, *Aphanocapsa* sp. and *Synechococcus* sp., developed as mats in aquatic environments, have been successfully used in bioremediation of oil spills in different parts of the world (Raghukumar *et al*., 2001). During a study carried out by Chan and Chiu (2005) to determine the effect of diesel oil and oil dispersant on growth, photosynthesis and respiration of *Chlorella* species, it was revealed that inhibitory effect of the treatment was concentration-dependent. It was also reported that low concentrations of both treatments either in mixture or alone stimulated the growth rate, biomass yield, chlorophyll α level and photosynthesis of the estuarine alga, *Chlorella salina* and slightly inhibited algal respiration.

Hydrocarbons are quantitatively the most important constituents of petroleum (Clayden, 2003). Petroleum is a complex, naturally occurring mixture of organic compound that is produced by the incomplete decomposition of biomass over a geologically long period of time (Freedman, 2008). Sulphur and nitrogen are other elements that are present in crude oil with concentration of <0.1% to 5-6% and < 0.1% to 0.9% respectively. Oxygen is also present at up to 2% concentration. Petroleum components are divided into three categories - light, medium and heavy weight fractions based on their relative densities.

Hydrocarbons are of great importance because they encompass the constituent of major fuel (coal, petroleum and natural gas), biofuels as well as plastics, paraffin, waxes, solvents and oil etc. Due to the increasing usage of petroleum products, there is currently an increase in their pollution effects and those of their by-products. Pollution is the occurrence of substances or energy in a quantity than the environment assimilates causing harm to man and any deleterious side effects of the tremendous rate of energy based in modern civilization (Hill *et al*., 2007). Oil pollution usually results from oil spillage. Some fractions of spilled crude oil are soluble in water and are known as the water-soluble fraction (WSF). The solubility is determined by the chemical composition of the oil. In general, light fractions are more soluble. Freedman (2008) noted that the toxicity of a particular hydrocarbon is strongly related to its chemical structure and hydrophobicity.

Notable studies have been carried out on algal growth in petroleum or hydrocarbon medium in Nigeria and they include Eboigbodin (2005) and Osiriomo (2005). Enoma (2006) also studied the effect of water soluble fraction of petrol, diesel and kerosene on *Eudorina elegans* and *Selenastrum capicornutum* using different concentrations (0%, 5%, 10% and 20%) and observed that the concentration were more or less stimulatory with reference to control especially in diesel and kerosene. Also percentage inhibition of the WSF of the three fuel oils was higher with *E. elegans* than *S. capicornutum*. Bott and Rogenmuser (2008) investigated the effects of No. 2 fuel oil, Nigerian crude oil and used Crankcase oil on attached algal communities. Used crankcase oil and depressed biomass but Nigeria crude oil extracts did not and both of these extracts have less effect on community composition than did No 2 fuel oil. They also observed that toxicity was greater from extracts prepared in the light than those prepared in the dark.

Dunstan *et al.* (2005) in an experiment to study the stimulation and inhibition of phytoplankton (Diatoms, green algae sand cyanobacteria) growth by low molecular weight hydrocarbon (toluene, benzene and xylene) observed that the degree varied with concentration, compound and species. Using a mixture of No. 2 fuel oil, they showed a species-specific stimulation at low concentration. It was also observed that the source of growth enhancement at low concentration and a major growth inhibition at high concentration.

Aim and Objectives

The aim of this study is to ascertain the Potential Use of Algae *Microcystis aeruginosa* (Chroococaceae) in Bioremediation. Other objectives include:

- To investigate algal growth response in nutrient culture
- Estimate effect of growth of the blue-green alga *Microcystic aeruginosa* in medium with varying concentrations of hydrocarbons – petrol and kerosene.

Materials and Methods

Collection of Algal Samples

Pure culture of *Microcystis aeruginosa* was obtained from a collection of algal cultures deposited at the Phycology/phytoplankton laboratory at the Department of Plant Science and Biotechnology, University of Benin, Benin City.

Preparation of Phytoplankton Culture Medium (Chu #10 modified)

Step 1: Using a 1liter volumetric flask 1ml of each of the already prepared stock solutions was added aseptically. The stock solutions were made from $CaCl_2.H_2O$, $MgSO_4$. $7H_2O$, $NaHCO_3$, K_2HPO_4, $NaNO_3$ and $NaSiO_4$.

Step 2: Next 1 ml of iron solution prepared by dissolving 3.35g citric acid ($C_6H_8O_7.H_2O$) in 100ml distilled water and adding 3.35g Ferric citrate ($FeC_6H_5O_3$. $5H_2O$) was added. The above mixture was autoclaved and refrigerated in the dark (wrapped in aluminium foil) to prevent photooxidation of the ferric ion.

Step 3: 1 ml of trace element solution was added. Trace solution was prepared with $CuSO_4$. $5H_2O$, $ZnSO_4$. $7H_2O$, $CoCl_2$. $6H_2O$, $MnCl_2$. $4H_2O$, NaMO4 .$2H_2O$ and H_3BO_3.

Step 4: 1ml of vitamin stock solution was added and the volumetric flask made up to mark

Identification and Sub-Culturing of Algal Species

A little drop on each of the collected samples was placed on the slide, covered with cover slip, viewed under the microscope. Water samples from the pond and river were inoculated into Chu no. 10 nutrient medium in 250ml conical flasks and placed on laboratory window pane for adequate light for photosynthesis. The cultures were further sub-cultured to get pure cultures in preparation for the experiment. Identification to species level and taxonomic description of algal species were as reported by Prescott (1975).

Taxonomic Description of *Microcystis aeruginosa*

Cells very numerous and crowded within the colonial mucilage (in some species showing the false vacuole which refracted light so that the cell appears brownish, black or purple). The marble-like cell of this genus are closely compacted and irregularly arranged in definitely shaped but mostly irregular colonies, enclosed in mucilage.

Isolation and Culturing of Algae

Initial algal samples were collected from temporary water bodies within and around Benson Idahosa University campus. 50ml of culture solution was measured differently with a pipette into four (4) conical flasks and later mixed with of water from fish pond in the proportion of 0% (Control), 25%, 50% and 75% respectively. The culture medium was used as the blank during spectrophotometric measurements.

Algal Growth Measurement

Algal growth was determined by measuring the optical density of the inoculated medium at three days' intervals using a spectrophotometer at a wavelength of 750 nm. The spectrophotometric reading is directly proportional to the algal biomass and a reflection of the phytoplankton density in each culture.

Petroleum Products

The test microalga *Microcystis aeruginosa* was added to 50ml Chu no. 10 culture medium. Triplicate cultures were mounted for each experimental set up with varying concentrations of petrol and kerosene. The conical flasks were plugged with cotton wool and kept on a rack by the window pane in the laboratory.

Results

The results obtained from the present study of growth response of phytoplankton samples cultured in Chu No. 10 medium as well as monitoring the growth rate of *Microcystis aeruginosa* in culture medium with varying concentrations of petrol and kerosene are shown below.

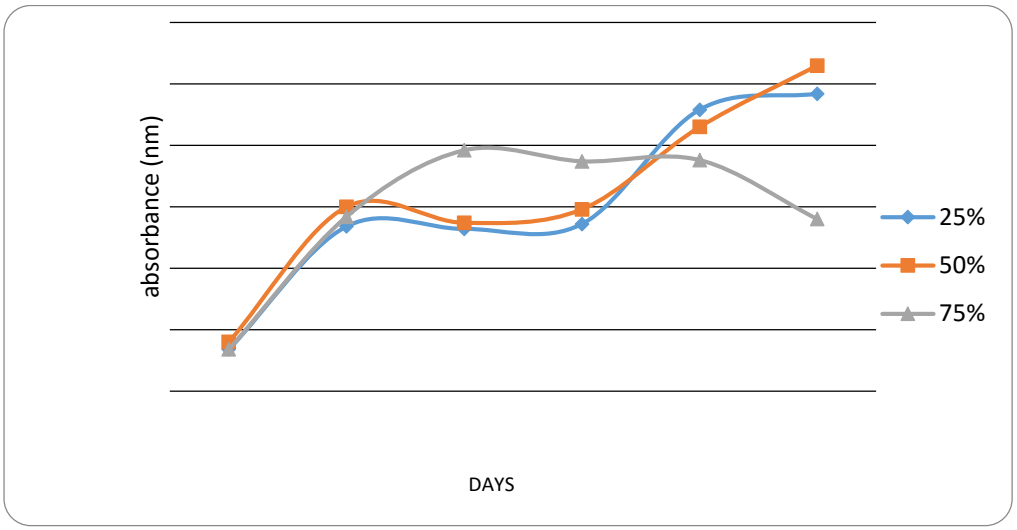

Fig 4 Growth response of *Microcystis aeruginosa* in various concentrations of Petrol

The growth rate of *Microcystis aeroginosa* in culture medium with varying concentations of petrol is shown in figure 4. All concentrations showed initial increase in the first three days of culturing. Lower concentrations (25% and 50%) recorded depressed growth in the succeeding week while the culture with 75% continued to show growth increase with slight depression. Subsequently, the 25% and 50% concentration cultures had astronomical increase while the algal biomass decreased over the same period till theend of the experiment.

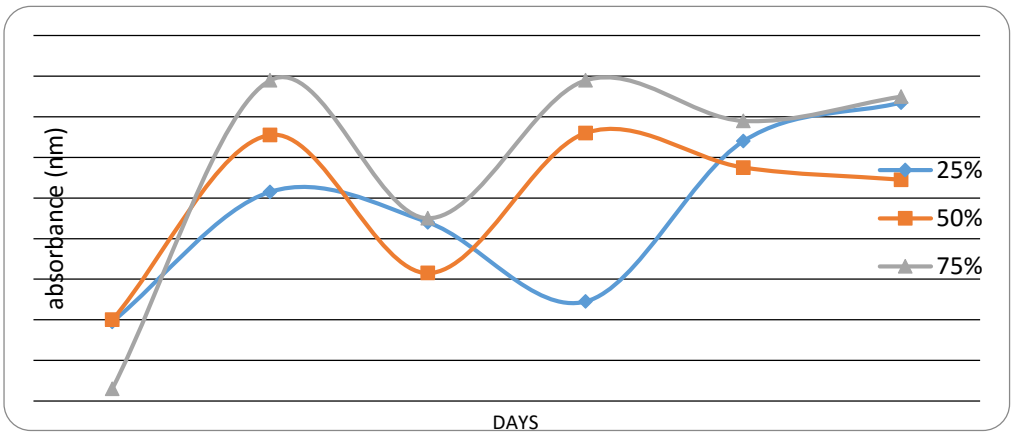

Fig 5 Growth response of *Microcystis aeruginosa* in various concentrations of Kerosene

In figure 5 the algal biomass in culture containing varying concentrations of kerosene recorded wide fluctuations in all with no definite trend of consistent increase or decrease.

Discussion

Current trends in micro biotechnology and alternative energy options have made the culturing of algae an emerging and relevant field of phycology as an aspect of applied microbiology. Algae can serve as very good source of energy and can be used to produce microbial cell fuels (MCF). A normal algal culture growth curve is characterized by five phases; lag phase, which involves a period of no growth usually due to acclimatization by the algae, exponential phase, or the period of growth and decline phase, marked by decrease in growth due to nutrient exhaustion, death and degeneration of cells. Others are Stationary phase: this is characterized by constancy in growth of algae. This could be due to competition for space available and death phase characterized by decrease in biomass as a result of death of algal cells (Lee, 2009).

In this study, no lag phase was observed and the result obtained was at variance with that of Bhadauria *et al.* (2002) who observed in their study a lengthened lag phase as well as a depressed slope of the exponential phase. The absence of a lag phase could be as a result of the microalgae being able to acclimatize in the culture medium. The general gradual increase in growth in freshwater algal culture may be attributed to the presence of a more diversified algal assemblage than the pond sample and possible presence of non-growth depressant and the fact that the inherent conditions in the habitats where they were collected from differed. According to Freedman (2008), changes in physico-chemical conditions greatly influence phytoplankton growth both *in-vivo* and *in-vitro*.

Some interesting observations on the possible bioremediation potential of *Microcystis aeruginosa* have been made in course of this study. By adjusting the concentration of petroleum products – petrol and kerosene in the culture, stimulatory and inhibitory effects were observed. With petrol, there was initial stimulatory effect and as the experiment progressed, it became inhibitory at higher concentration (75%). This finding is corroborated by the work of El-Dib *et al.* (2001); by subjecting freshwater algae to different concentrations of aqueous extract of reference fuel oil, they observed that as the concentration of fuel oil was increased, there was a significant decrease in chlorophyll α content. Hutchinson *et al*. (2009) observed that microbial biomass, enzyme activity and microalgae growth declined in medium to high level whereas low level stimulated growth.

Decrease in algal growth with increasing concentration of hydrocarbon as observed in the present study could be due to increasing level of the toxic component of the hydrocarbon which could interfere with the photosynthesis, respiration or other activities of the algal population. The inhibition of *Microcystis aeruginosa* in the

hydrocarbons (petrol and kerosene) could be due to the fact that *M. aeruginosa* is a prokaryote without nuclear membrane

Also the stimulatory effect of low concentrations of hydrocarbons attributed to the ability of the algal species to take up the carbon and hydrogen atoms to build up the protein and carbohydrate content of their cells and thereby leading to increase in biomass (Dunstan *et al.* (2005).

Solubility of hydrocarbons also plays an important role in determining their effects on algal culture. Generally, aromatic hydrocarbons are more soluble than paraffin; petrol (an aromatic hydrocarbon) is therefore more soluble than kerosene (an alkane or paraffin) (Megharaj *et al.*2000; Freedman, 2008).

The nature rather than quantity of the petroleum hydrocarbon present may also be a major factor determining the effect of hydrocarbons on algal biomass. Shailaja (2008) reported that petrol was the most toxic and produced inhibitory effects even at low concentrations while kerosene did not appreciably alter the growth either at lower or higher concentrations. The increase in toxicity was attributed to the presence of aromatic hydrocarbons of medium to higher molecular weight and their chemical modifications. The total absence of any trend of inhibition or stimulation by kerosene can be attributed to this factor.

Conclusion

Algae are abundant in the environment and can be successfully cultured for the numerous benefits derivable from them. With emphasis on alternative sources of energy and global emphasis on limiting burning of fossil fuel, algae culture can yield clean and sustainable energy. The ponds, temporary water bodies, rivers and lakes are sources of algal species that can be cultured for this purpose. From the study, it was obvious that the effects of hydrocarbon in algal culture varied with concentration and lower concentrations of petrol stimulated growth while that of kerosene did not both at low and high concentrations.

The result of the experiment could be used in managing the environment through pollution control, pollutant standardization and bioremediation. Further research should be carried out on the potential use of several algal species for bioremediation and non-competitive feed stock for alternative energy sources.

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