International Journal of Modern Biology and Medicine, 2015, 6(1): 22-29

Modern Scientific Press

International Journal of Modern Biology and Medicine

Journal homepage: www.ModernScientificPress.com/Journals/IJBioMed.aspx

ISSN: 2165-0136

Florida, USA

Article

Effects of pH Change on Growth of Microalga in Water Soluble Fractions of Crude Oil

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Article history: Received 20 September 2014, Received in revised form 5 January 2015, Accepted 15 January 2015, Published 19 January 2015.

Abstract: Laboratory experiments were performed to access the effects of pH change on growth of a microalga in water soluble fractions of crude oil using *Chlorella pyrenoidosa.* Growth of the alga was observed in culture by estimating chlorophyll *a* content. The effect of varying pH on growth in water soluble fraction of crude oil on a 14 day bioassay shows no significant difference at $p< 0.05$ in growth of the microalga in both pH moderated and non pH moderated culture of Water Soluble Fractions (WSF) of crude oil. Maximum growth and utilization of hydrocarbon by the microalga was obtained at pH of 8.2 and the least at pH 7.5. Biomass production (chlorophyll *a* synthesis) by *C. pyrenoidosa* in continuous culture was considerably affected by the pH at which the cultures were maintained. Biomass production experiment revealed that pH values in the range of 8.2 and 8.5 synthesized more photosynthetic materials than those in 7.5 pH level. Dry weight showed higher physiological response at pH 8.5 and the least at pH 7.5. The result obtained in this study is a vital contribution in processing and cultivating of algae.

Keywords: Water Soluble Fraction, pH change, microalga, biomass production and hydrocarbon utilization.

1. Introduction

Algae are large and diverse group of simple, typically, autotrophic organisms, ranging from unicellular to multi-cellular forms, such as the giant kelps that grow up to 65 metres in length. The largest and most complex massive forms are called seaweeds. They are photosynthetic like land plants and simple because they lack the many distinct organs found in land plant. (Patternson and Laura, 2006). Algal biomass is the total mass of a heterogeneous community of Algae. (Murray, 2004).

Algae are prominent in water bodies and could also be found in terrestrial environments and in unusual environment such as snow and ice. (Wellman *et al*., 2003). Algae are of significant economic value due to their biological role in ecosystems and as sources of commercially significant products.

Algae play significant roles in aquatic ecology. Microscopic forms that live suspended in the water column (phytoplankton) provide the food base for most marine food chains, in very high densities (algal blooms) these algae may discolor the water and outcompete, poison, or asphyxiate other life forms. (Nikolaev *et al*., 2007). They produce and maintain the concentration of oxygen in the aquatic environment due to their high rate of photosynthesis. They grow luxuriantly from active spores and actas biofilter of large amount of minerals nutrient in the water bodies were they are found. They can be used as chief food for some of the fishes and other aquatic animals. Algae like the *Prophyra tenora* is eaten throughout Japan, *P. umblicaules* is eaten in England, *P. perfrate, Ulva lactuvea* and *Chlorella* are all edible form.

Pigments from algae can be used as dye and as stabilizer in milk products. Algae can be used as soil conditioners, seaweed can be used as fertilizers, and treatment of effluents pumped through ponds (Borowitzk and Hallegraeff, 2007).

Crude oil is a naturally occurring flammable liquid consisting of a complex mixture of hydrocarbons of various molecular weights and other liquid organic compounds that are found in geologic formations beneath the earth’s surface. Crude oil is recovered mostly through drilling. It includes a lot of processes which alters the composition of the environment. It consists of a variety of hydrocarbons and large reserves of natural gas. Both crude oil and natural gas are predominantly mixtures of hydrocarbons under surface pressure and temperature, the lighter hydrocarbon such as methane, ethane, propane and butane occur as gases, while the heavy ones from pentane and above are in the form of liquids or solids (James, 1999).

2. Materials and Methods

2.1. Isolation of Pure Culture of Microalga

Unialgal culture of *Chlorella pyrenoidosa* used for this investigation was isolated from a water body collected around Oron road in Uyo local government area in 2008. It was subjected to series of sub culturing in modified Chu 10 artificial growth medium.

2.2. Standardization of pH

The pH of the experimental culture was standardized by addition of Calcium hydroxide stock solution to chu 10 modified growth medium.

2.3. Culture Medium

The microalgae species were grown in an artificial medium, Chu's modified No 10 solution medium (Chu 1942).

2.4. Culture Vessels

950ml round bottomed transparent bottles were used for both sub culturing and in the main experiment. They were washed thoroughly with detergent and further rinsed with sulphuric acid solution to remove any trace of algal spore present.

2.5. Crude Oil Source

The crude oil used for this study is Honey sweet light oil. It was obtained from QIT Exxon Mobil in Ibeno local government area of Akwa Ibom. Nigeria

2.6. Preparation of Water Soluble Fractions

The WSF was prepared according to the method of Anderson *et al* (1974) and Phatarpekar and Ansari (2000).

2.7. Inoculation

250mls of the water soluble fractions of crude oil maintained at three different pH (7.2, 8.2 and 8.5) were measured separately into the experimental vessels. These were then inoculated separately with 2mls of *Chlorella prenoidosa* using 5ml syringe. Each experiment was set up in triplicate. The experimental bottles were plug with sizeable cotton wool to limit evaporation and prevent contamination. Twenty five mill aliquot each was taken from each triplicate for analysis.

2.8. Chlorophyll a Determination

Growth measurement was done by chlorophyll *a* determination. Absorbance at three different wavelengths (680, 645 and 630nm) was used to determine the amount of chlorophyll *a* synthesized using DR 2000 HACH spectrophotometer.

3. Results and Discussion

The growth responses of *Chlorella pyrenoidosa* in different concentrations of water soluble fractions of crude oil at three different pH level (7.5, 8.2 and 8.5) is shown in figures 1 – 3. An exponential increase in growth was recorded by the alga in 10% -75% concentrations from day 0 through day 14 while 100% concentration showed a lag phase of growth in the first six days of the study. Complete inhibition of alga growth was observed from day six through day 14 of the study in the water soluble fractions of crude oil at pH of 7.5(figure 1). Maximum growth stimulation was recorded in 10% concentration by the microalga.

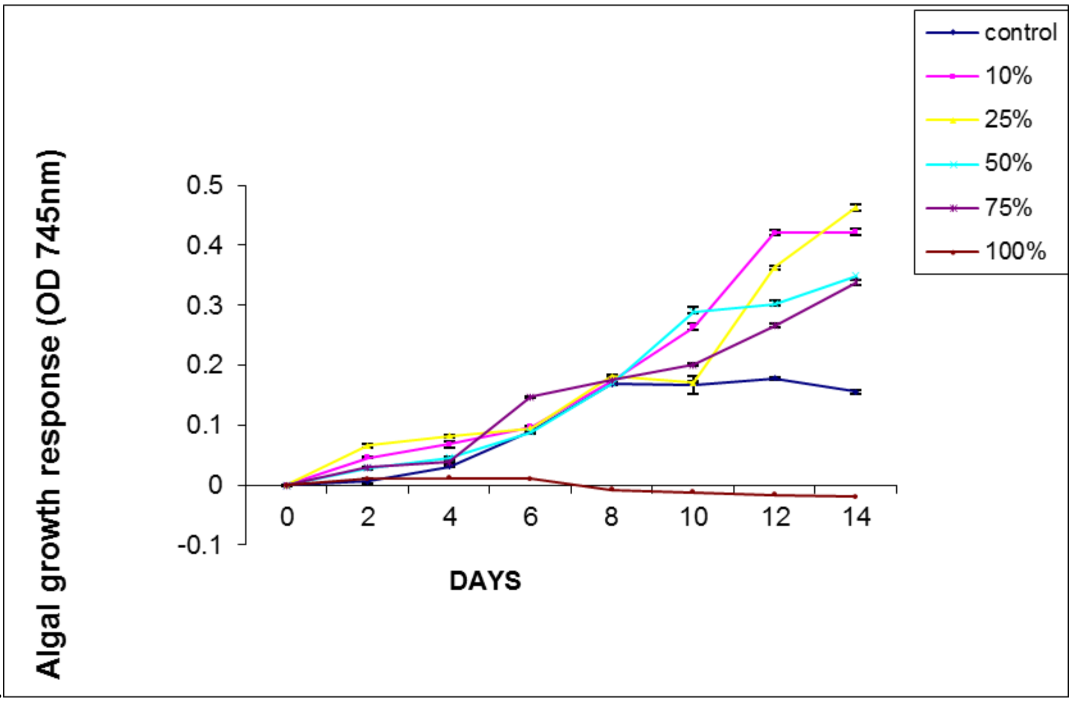

Fig. 1: Response of *Chlorella pyrenoidosa* to different concentrations of WSF of crude oil at 7.5 pH level

The growth response of *Chlorella pyrenoidosa* in different concentrations of water soluble fractions of crude oil at pH of 8.2 is illustrated in figures 2. There was no significant difference ($p<0.05$) in growth response of the microalga in culture stabilized at 7.5 and 8.2 pH level. Exponential increase in growth was also observed in 10% -75% concentrations while 100% concentration was completely inhibitory to the alga growth. Maximum growth stimulation was recorded in 25% concentration.

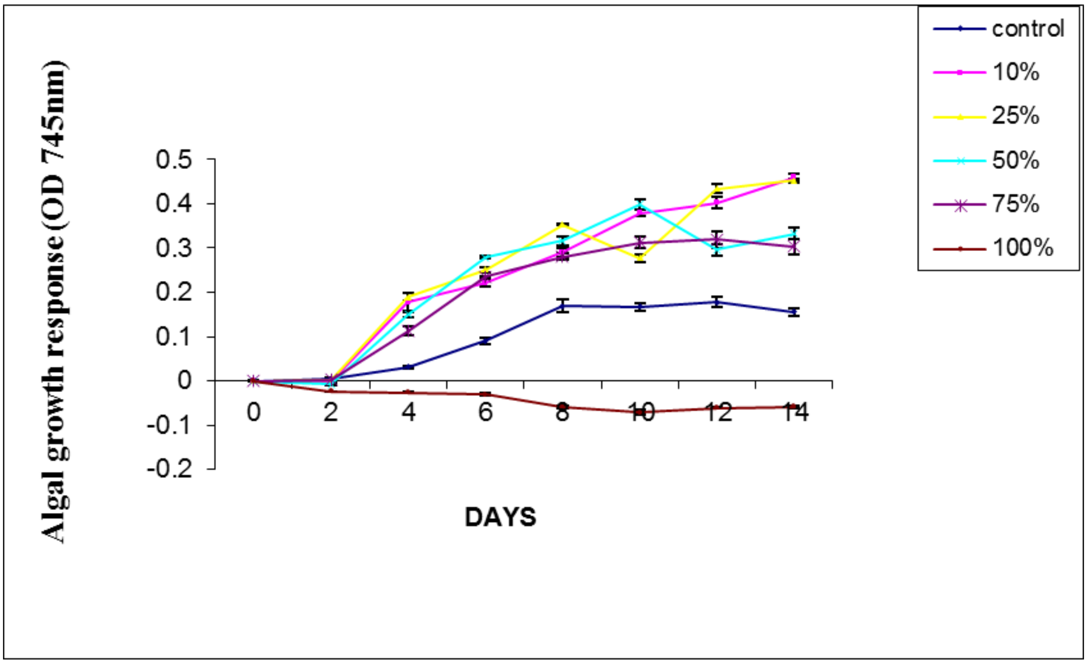

Fig. 2: Response of *Chlorella pyrenoidosa* to different concentrations of WSF of crude oil at 8.2 pH level

The growth responds *C. pyrenoidosa* in different concentrations of water soluble fractions of crude oil at pH of 8.5 is depicted in figures 3. Growth stimulation was observed at all concentrations investigated 10% - 100% concentration. A gradual increase in growth was observed within the first 4 days of the study in all concentrations, this was followed by an exponential increase from days 4 to day 14 of the study. The result obtained reveals higher growth stimulation in treatment culture than control experiment. No significant difference at $p<0.05$ was observed in the growth of the alga in lower concentrations (10% and 25%) and mid concentration (50%), and between 25% and 75% when compared with that in control experiment. Growth suppression was observed between day 0 and 4 thereafter a gradual and consistent increase in growth of the microalga was observed till the end of the study.

Tables 1-3 show effect of pH change on algal biomass. Biomass production (chlorophyll *a* synthesis) by *C. pyrenoidosa* in the continuous culture was considerably affected by the pH at which the cultures were maintained. Biomass production experiment revealed that pH values at the range of 8.2 and 8.5 synthesized more photosynthetic product such as chlorophyll a dry weight matter than those in 7.5 pH level. Dry weight and Chlorophyll *a* showed higher physiological response at pH 8.5 than at pH 7.5 were the least physiological response were recorded. Total petroleum utilization by the microalga showed a higher utilization at the mid age of the culture than the early age and the terminal age of the culture. This appears to be proportional to the hydrogen ion concentration of the culture medium.

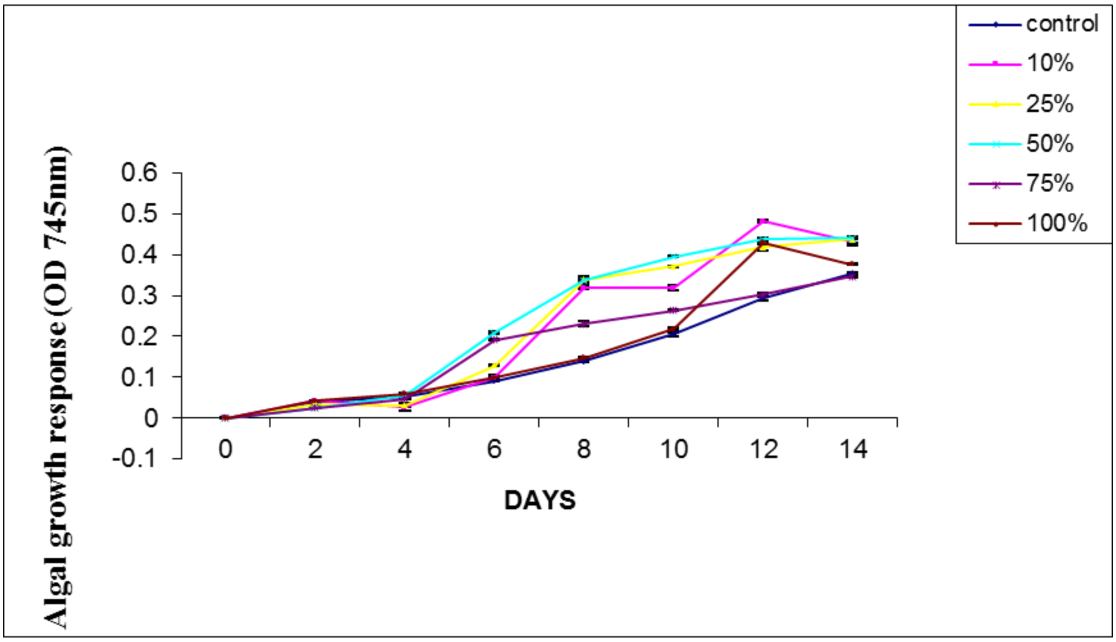

Fig. 3: Response of *Chlorella pyrenoidosa* to different concentrations of WSF of crude oil at pH 8:5 level

Table 1: Effects of pH change on dry weight of microalga

pH	DAY 0	DAY 7	DAY 14
7.2	0.00	0.24	0.67
8.2	0.00	0.62	1.12
8.5	0.00	0.85	2.05

Table 2: Effects of pH change on hydrocarbon utilization by microalga

pH	DAY 0	DAY 7	DAY 14
7.2	**0.00**	**0.183**	**0.106**
8.2	**0.00**	**0.194**	**0.178**
8.5	**0.00**	**0.451**	**0.343**

Table 3: Effects of pH change on chlorophyll a synthesis in microalga

pH	DAY 0	DAY 7	DAY 14	SEM
7.2	0.167	0.267	0.314	±0.044
8.2	0.167	0.306	0.395	±0.066
8.5	0.167	0.322	0.416	±0.073

Ecotoxicology experiments are frequently conducted to establish the dose-response relationship for a certain compound and organism. The result of these experiments are often analyzed by a logistic model of the concentration that causes a 50% reduction in the variable under study. The logistic model can be applied data, such as survival or death and data such as weight or biomass change (Van and Hoekstra, 1993).

In this study, the growth stimulation recorded for the microalga in 10% - 75% concentration of WSF crude oil at of pH 7.5 and 8.2 (fig 1 and 2) and in all concentrations at pH of 8.5 could be due to the availability of different amounts of growth nutrient at different pH levels. There was no significant variation in growth of the alga in the different concentration at the three pH levels. This invariably reveals that the slight difference observed in the growth form of the microalga was however due to hydrocarbon utilization and not necessary due to change in pH status of the growth medium.

Exposure to high concentration of WSF of crude oil resulted in reduced photosynthesis in the microalga whereas, exposure to low concentration enhance the photosynthetic rate of the microalgae. This finding is in line with the work of O'Brain and Dixon (1970), who reported several effects such as total suppression of growth to stimulation in algal culture with hydrocarbon content. They reported modification of physiological function such as photosynthesis and respiration in phytoplankton. Vandermeulen and Aherm (1976) and Corner (1978) noted that regulation of physiological function depends on the biochemical composition of micro algae.

Biomass production (chlorophyll *a*) by *C. pyrenoidosa* in the laboratory continuous culture was considerably affected by the pH of which the cultures were maintained. Biomass production experiment revealed that cultures with pH values in the range of 8.2 and 8.5 have higher photosynthesis rate than those in pH level of 7.5. This could be due to free O_2 concentration in the growth medium with higher pH values, addition pH effects could have set in to decrease the relative amount of CO_2 to free O_2. Both absorbance and biomass estimation indicate that the growth of the microalga was sustainable in the growth medium and the water soluble fractions at maintained at the various experimented pH level. The maximum growth and biomass production recorded in culture maintained at 8.5 pH level could be due to physiological variation in microalga due to changes in environmental condition from neutral to basic environmental condition.

4. Conclusion

There is no significant difference in growth of *Chlorella pyrenoidosa* in water soluble fractions of crude oil maintained at pH of 7.5, 8.2 and 8.5 respectively. The microalga (*Chlorella pyrenoidosa*) proved efficient in growth in culture maintained at the three investigated pH levels. Higher photosynthesis was observed by the alga at pH of 8.5 and the least photosynthetic activity occurred at

7.5 levels. The microalga could be considered a possible indicator of crude oil pollution and could thrive and grow considerably in crude oil polluted environment.

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