International Journal of Bioassays
ISSN: 2278-778X
www.ijbio.com

Research Article

TOXIC EFFECT OF DIESEL AND KEROSENE ON GROWTH AND BIOCHEMICAL COMPOSITION OF *SPIRULINA PLATENSIS*

Rajni Karoriya
Department of Biotechnology, Madhav Institute of Technology and Science, Gwalior, (M.P.) 474005, India

Received for publication: December 23, 2013; **Accepted:** January 11, 2014

Abstract: *Spirulina platensis* is a photosynthetic, filamentous, spiral shaped, multicellular and blue-green microalga. Diesel and kerosene are used in great amount for different purposes causing environmental pollution. In the present study, the effect of diesel and kerosene were tested on *Spirulina platensis* at the concentrations of 0.5,1,2,5 and 10%. Growth rate were observed for both diesel and kerosene and compared with control samples. Protein content, carbohydrate content and chlorophyll-a content were also determined. There was dramatic change in the growth and population increment. The day wise study was carried out and it was found that with increase in time span the growth decreases and concentration of diesel and kerosene also affected its growth. It was concluded that diesel is more toxic than kerosene.

Keywords: *Spirulina platensis*, Diesel, kerosene, carbohydrate, protein, chlorophyll-a.

INTRODUCTION

Diesel oil is a complex mixture of hydrocarbons with an average carbon number of C8–C26. The majority of components consist of alkanes, both straight chained and branched and aromatic compounds including mono-, di- and polyaromatic hydrocarbons. *Spirulina* is a commercially important filamentous cynobacterium that is grown in large scale and processed industrially (Dillon et al., 1995). It is one of the most essential marine fauna in terms of its commercial and economic value.

Pollution of ecosystem by different products is a real threat to environment. Pollution affects all forms of life though the degree of toxicity varies for different organisms.

MATERIALS AND METHODS

Experimental Organism

Pure culture of *Spirulina platensis* was obtained from Department of Biotechnology, Jiwaji University, Gwalior (M.P.) India. *Spirulina* culture was maintained in Zarrouk's medium (Zarrouk, 1966) in culture room at 30°C and under the light of white fluorescent tubes in 12/12 hour dark light cycle.

Culture medium

Zarrouk's medium was supplemented with diesel and kerosene at concentrations of 0.5, 1, 2, 5 and 10%.

Growth measurement

The growth rate in the culture media was monitored by measuring an increase in carbohydrates, chlorophyll-a content and protein content in the presence of different diesel and kerosene concentrations. The specific growth rate (μh^{-1}) was calculated by following formula (Guillard, 1973):

$$\mu = \log (N_2/N_1) \times (2.303/t)$$

where, $t = T_2 - T_1$.
N_1 initial optical density at time T_1.
N_2 initial optical density at time T_2.

Estimation of Protein content (Lowry, 1951)

0.5 ml of the homogenized algal culture was taken in a test tube and 0.5 ml of 1N NaOH was added to it and then placed in boiling water bath for 5 minutes. After cooling in the cold water 2.5 ml of reagent "B" was added and allowed to react for 10 minutes. 0.5 ml of 1N Folin reagent was then added and mixed thoroughly and allowed to stand for 30 minutes for development of blue colour, and then centrifuged at 5000 rpm for 10 minutes. The absorbance of the clean liquid was measured at 650nm, and the amount of algal cell protein calculated as µg /ml culture with reference to a standard-calibrated curve, obtained with BSA. The same procedure was adopted in preparing standard curve using graded concentration of BSA.

Carbohydrate content estimation

The total Carbohydrate content of the cyanobacteria cells was estimated by the method described by **(Dubois et al., 1956).**

0.5 ml of an algal culture was taken into a thick wall test tube to which 1.5 ml of distilled water and a set of glucose standard was prepared simultaneously and 1ml of 5% phenol was added to each tube. After through mixing, 5ml of sulfuric acid was added from a fast blowing auto pipette, directing the stream of acid to the agitated reaction mixture for fast mixing. Such

***Corresponding Author:**
Rajni Karoriya,
Department of Biotechnology,
Madhav Institute of Technology and Science,
Gwalior, (M.P.) -474005, India

tubes were incubated at room temperature for 10 min. for complete reaction, and thereafter, shaken and placed in a water bath (30°C, 20min). The intensity of the characteristic straw color thus developed was determined by reading absorbance at 492 nm and the carbohydrate content (µg/ml carbohydrate) calculated from the glucose standard.

Chlorophyll-a estimation (Mackinney, 1941)

Known volumes (3ml) of *Spirulina platensis* culture was harvested by centrifugation (5000 rpm, 10 minutes) and the resulting pellet was resuspanded in the equal volume amount (3ml) of methanol. After thorough mixing, the suspension was kept at 40C for 24 hours. Thereafter, it was centrifuged, and the absorbance of the cell – free metabolic extract was recorded at 663 nm against methanol as a blank.

RESULTS AND DISCUSSION

The growth conditions for *Spirulina platensis* were optimized in Zarrouk's medium. Growth medium play a critical role in the development of cells, biosynthesis and metabolic activity. In the present study diesel and kerosene toxicity is checked on *Spirulina platensis*. At the 10% concentration of both diesel and kerosene inhibit the growth of *Spirulina*. Diesel is more toxic than kerosene as shown in fig.1.

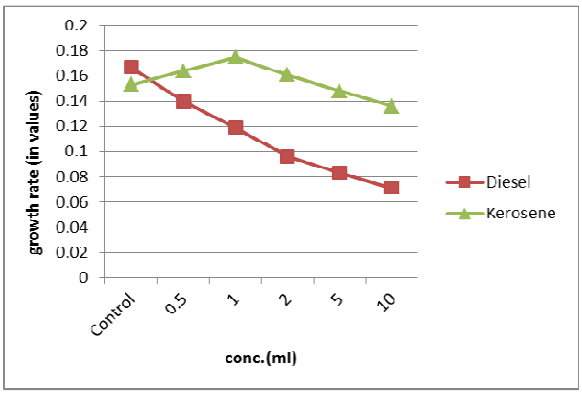

Figure 1: specific growth rate of *Spirulina platensis* in the presence of diesel and kerosene.

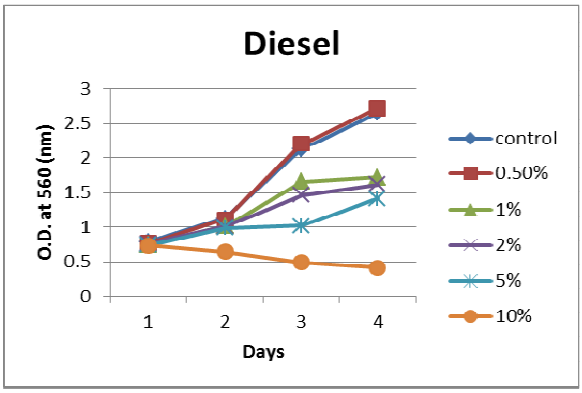

Figure 2: Effect of diesel on the growth of *Spirulina platensis.*

In both figure 2 and 3 growth of *Spirulina* was effected by different concentrations of diesel and kerosene. Increment in the concentrations will directly influence the growth of *Spirulina platensis*.

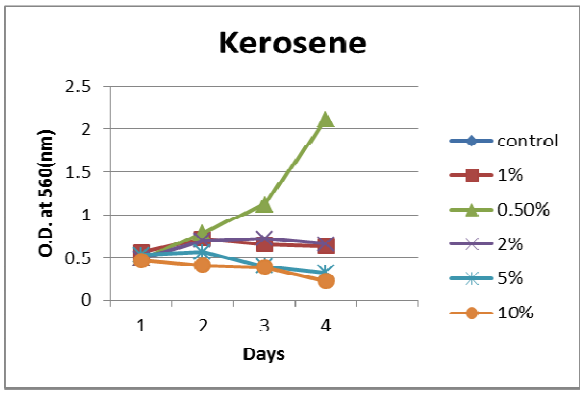

Figure 3: Effect of kerosene on the growth of Figure 1: Effect of diesel on the growth of *Spirulina platensis.*

Effect of diesel and kerosene on the protein content

As shown in fig. 4 diesel concentrations influence protein content in *Spirulina* in a dose and time dependent manner. Kerosene concentrations were not as much affected as diesel. In 2%, 5% and 10% of the protein content of diesel was very less in comparison to kerosene.

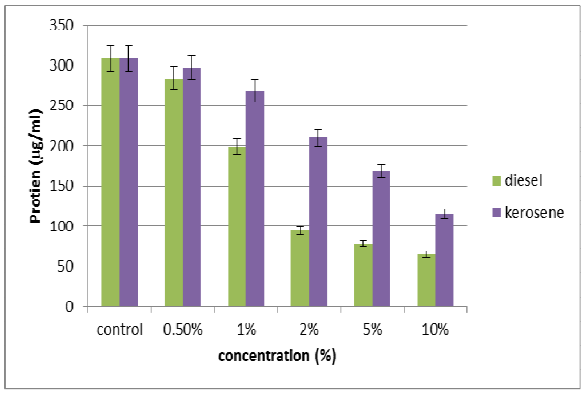

Figure 4: Effects of 0.5, 1, 2, 5 and 10 % diesel and kerosene on protein content of *Spirulina platensis*.

Effect of diesel and kerosene on the carbohydrate content

The carbohydrate content of *Spirulina platensis* in diesel and kerosene concentrations is same. From the figure 5 we can see there is no more difference in the value of carbohydrate content.

Effect of diesel and kerosene on the chlorophyll-a content

Chlorophyll-a content is indicated the growth and development in *Spirulina platensis*. By the figure 6 we can say that diesel is highly toxic comparative to kerosene. The value of different concentrations of diesel is less than kerosene concentrations comparison with control.

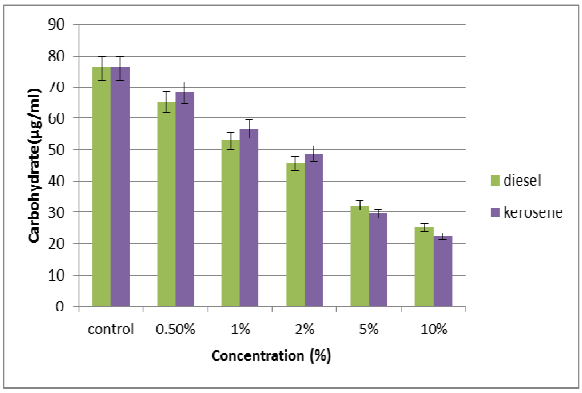

Figure 5: Effect of Diesel and Kerosene on carbohydrate content of *Spirulina platensis.*

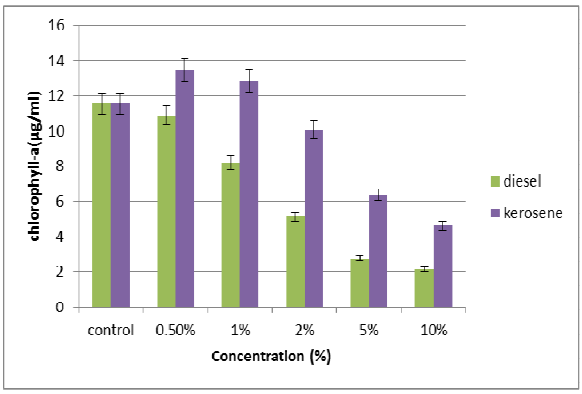

Figure 6: Effect of Diesel and Kerosene on chlorophyll-a content of *Spirulina platensis.*

CONCLUSION

From the result of this study we can say that diesel is more toxic than kerosene in *Spirulina platensis.* The resulted value of protein, carbohydrate and chlorophyll-a is demonstrated that the composition of diesel is highly toxic to the microalga.

REFERENCES

1. Dillon JC, Phan PA, Dubacq JP (1995) Nutritional value of the alga Spirulina. World Rev. Nutr. Diet. 77:32-46.

2. Dubios, M, Gilles KA, Hamilton JK, Rebers PA and Smith F. (1956). Colorimetric method for determination of sugar & related substances. *Anal. Chem*; Vol.28, 350-356.

3. Zarrouk C, 1966. Contribution à l'étude d'une cyanophycée. Influence de divers' facteurs physiques et chimiques sur la croissance et la photosynthèse de Spirulina maxima. Ph.D. Thesis, Université de Paris, Pari.

4. Guillard RRL, (1973). Divison rates in handbook of phycological methods (Ed.:).R.Etein). Culture Methods and Growth Measurements". Chambridge University Pres., Cambridge.289-311.

5. Lowry OH, Rosebrough NL, Farr AL and Radall RJ, Protein measurement with the folinphenol reagent. J. Bio. Chem. 193:195. 265-275.

6. Mackinney G, Absorption of light by chlorophyll solution, J. Bio. Chem., 140,: 1941. 466-46.

Source of support: Nil
Conflict of interest: None Declared