ISSN 2250-3153

DHBA Increases Solubility of Iron With Surface Reductase And Enhances Algal Growth of Marine S. *obliquus/Nostoc*

Jalil J. Bhatnase[a*], Usha B. Naik[b], Dinesh K. Dabhadkar[c], Roshan D. Dhore[b]

[a]Water Quality Research Center, Eureka Forbes Ltd. New Delhi. 110075. India.
[b]Department of Microbiology, ACS and Biotechnology Research Centre, Wardha. 442001. India.
[b]Department of Zoology, Govt. Vidarbha Institute of Science and Humanities, Amravati. 444604. India.

Abstract- DHBA (2,3-Dihydroxybenzoic acid) is increases the solubility of iron and this phenomenon is help out for enhances the growth of marine algae for CO2 sequestration to minimize the atmospheric global warming. Stability constant of DHBA by mole ratio method at pH 7.8 is that 15 days. The growth of *S. obliquus* and *Nostoc* is analyzed by analyzing chlorophyll-a content in this medium. Chlorophyll-a in DHBA-Fe sufficient is high as compare with DHBA, Fe deficient medium. DHBA-Fe (4:1) shows maximum growth as compare to (2:1) and (1:1). Finally this growth was reduced due to lack of nutrients in the medium. The solubility of iron with DHBA is due to surface reductases enzyme present on algae.

Index Terms- Nostoc, S. obliquus, Siderophore, Stability constant, XRD

I. INTRODUCTION

Siderophore is an iron chelating compound secreted by all microorganisms. The importance of siderophores extends beyond their immediate role in microbial physiology and their applications in biotechnology (Messenger and Ratledge, 1985) as well as in antimicrobial activity (Duffy and Defago, 1999). Global warming is a predominant problem in front of world. To minimize this problem carbon capture and storage within plant and algae by photosynthesis can be done. For increase growth of algae in ocean, siderophore is important enhancer (Balley and Taub, 1980). Particulate and colloidal iron is believed to be unavailable to phytoplankton (Wells et al., 1995; Finden et al., 1984; Rich and Morel, 1990) and the solubility of iron in open ocean waters is extremely low, log [Fe(III)]b_9 (Liu and Millero, 2002). Regarding dissolved iron, it has been proposed that eukaryotic phytoplankton species utilize only inorganic iron species (Anderson and Morel, 1982). However, more recent studies have shown that some eukaryotic algae are able to utilize iron bound to organic chelators via a cell surface reductase mechanism (Jones et al., 1987; Soria-Dengg and Horstmann, 1995; Hutchins et al., 1999; Maldonado and Price, 1999; Weger, 1999). Unlike eukaryotic algae, marine bacteria acquire iron through a siderophore-mediated uptake system (Winkelmann, 1991). The stability constants of Fe (III)–siderophore complexes are in the range of log K=25–50 (Albrecht-Gary and Crumbliss, 1998). Besides increasing the solubility of iron, siderophores also accelerate iron oxide dissolution (Hersman et al., 1995; Yoshida et al., 2002; Cheah et al., 2003; Kraemer, 2004). Siderophores are biologically important and therefore in this study we used 2,3-Dihydroxybenzoic acid (DHBA).

II. MATERIALS AND METHODS

2.1 Materials

2,3-Dihydroxy benzoic acid (DHBA) catecholate type and ferric oxide (red) were obtained from Across and Fine-Chem Limited respectively. All others chemicals used in this study were of Analytical grade from E-Merck, India Ltd., Mumbai, India.

2.1.1 Algal Cultures

Axenic culture of of *Scenedesmus obliquus and Nostoc* were obtained from the Botany Department of the Nagpur University and grown in a BG-11(Rippka et al., 1979) medium in batch culture in 250ml flasks. Cultures were incubated at 25^{o} C under an intensity of 40 $\mu Em^{-2}s^{-1}$ provided by cool white lamps, with 14h/10h/light/ dark cycles. Cultures were shaken manually once every day.

2.2 Methods

2.2.1 XRD Pattern

XRD (X-ray diffraction) analysis was done by the Rigaku miniflex II X-ray diffractometer. The crystal structure of ferric oxide was observed.

2.2.2 Estimation of iron

Iron was estimated by 1,10-phenanthroline method (APHA, 1995). In which DHBA was added in reference for detection of solely iron. Then the absorbance was measured at wavelength 510 nm and the concentration of samples was calculated by plotting standard graph using ferrous sulphate.

2.2.3 Stability constant

The stability constant was performed by mole-ratio method in which the series of the solution was prepared containing constant amount of Fe but increasing the concentration of DHBA and OD was measured.

2.2.4 Incubation and cultivation

The *Scenedesmus obliquus* and *Nostoc* were incubated in both standard and modified material BG-11 medium (Rippka et

al., 1979). In the latter, iron in the form of ferrous sulphate along with chelating agent DHBA were excluded from the original medium having pH 7.8.

Scenedesmus obliquus and *Nostoc* cells were cultivated on Fe and chelating agent deficient as well as Fe and chelating agent (DHBA) sufficient growth media. Five different experiments were carried out for study of DHBA and Fe complexation on growth of *S. obliquus* and Nostoc which included DHBA and Fe deficient, DHBA (0.005 M) and Fe (0.005 M) i.e. in 1:1 ratio, DHBA (0.01 M) and Fe (0.005 M) i.e. in 2:1 ratio, DHBA (0.02 M) and Fe (0.005 M) i.e. in 4:1 ratio. The culture were incubated for 21 days at 25^0C.

2.2.5 Measurement of chlorophyll-a concentration

The chlorophyll-a concentration was used to track algal growth. Chlorophyll-a were measured by standard methods (APHA, 1995) extraction with acetone, and absorbance measurements at 664 nm and 750 nm (Jeffrey and Humphrey, 1975; Lorenzen, 1967; Srickland and Parsons, 1968).

III. RESULTS AND DISCUSSION

3.1 XRD Analysis

X-ray differaction spectra are observed that shows ferric oxide has been recorded using Rigaku miniflex II X-ray diffractometer (Fig. 1). It is observed that the ferric oxide shows rhombohedral symmetry. The major XRD peaks were observed at 2θ values 33.260^0, 24.200^0, 35.700^0, 43.620^0, 49.580^0, etc. compared with (JCPDS-85-0987).

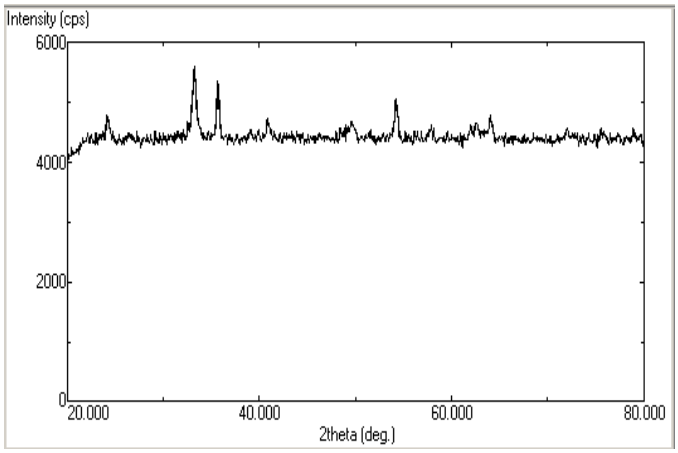
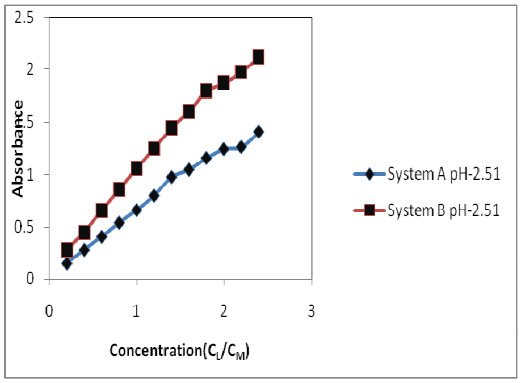


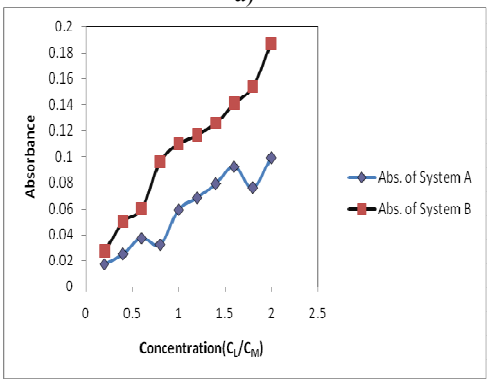
Figure 1: The XRD spectrum of Ferric Oxide

3.2 Stability Analysis

The stability constant of Fe-DHBA for a pH range of 2.51 and 7.8 as calculated by mole-ratio method is shown in (Fig. 2). An absorption maxima of Fe-DHBA complex is showed 310 nm and 547 nm at pH 2.51 and 7.8 respectively. On the basis of assumption the Fe^{3+} in the Fe^{3+} -DHBA complex is more stable at pH 2.51 than 7.8. The stability constant of Fe-DHBA is 19d and 15d at pH 2.51 and 7.8 respectively.



a)

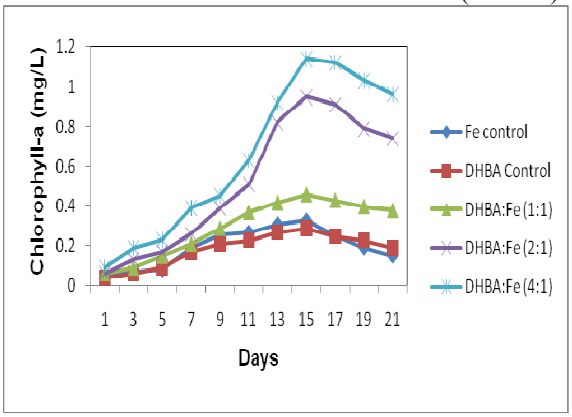


b)

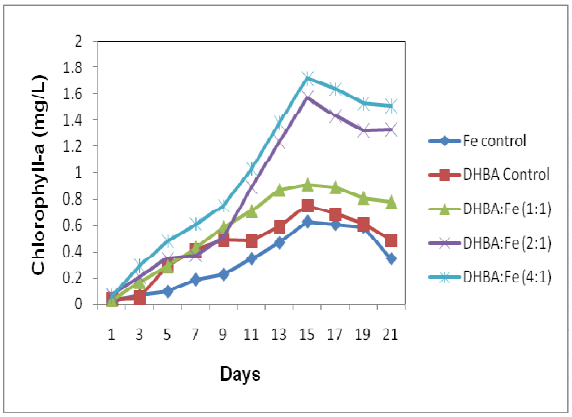
Figure 2: Graphical representation of stability constant a) at pH-2.51b) at pH-7.8

3.3 Effect of DHBA on Growth of *S. Obliquus* and *Nostoc*

The effect of DHBA and Fe complexation on growth of algae; DHBA-Fe 1:1 ratio, DHBA-Fe in 2:1 ratio, DHBA-Fe in 4:1 ratio, DHBA deficient and Fe deficient shows in figure (3). The population of *S.obliquus* and *Nostoc* are tended to increase gradually with the addition of DHBA complex with Fe. DHBA was added in media having pH 7.8 and at this pH; Fe (III) solubility was observed (Liu and Millero, 1999). The complexation between iron and DHBA allowed potentially bio-available iron and contributed to the growth of algae (Boyer & Groffman, 1996). As compare with Fe and DHBA deficient the growth of algae increases 3.45 times and 3.93 times in *S. obliquus* and 2.29 tmes and 2.73 times in *Nostoc* (Table 1).



a)



b)

Figure 3 : Effect of DHBA on growth of a) *S. obliquus* b) *Nostoc*

Table : 1 Effect of DHBA-Fe compex on times of growth of algae.

a) On growth of *S. obliquus*

S. obliquus	**Times of growth**	**Increases in *S. obliquus***	
	DHBA-Fe(1:1)	**DHBA-Fe(2:1)**	**DHBA-Fe(4:1)**
Compare with Fe	1.39	2.87	3.45
Compare with DHBA	1.58	3.27	3.93

b) On growth of *Nostoc*

Nostoc	**Times of growth**	**Increases in *Nostoc***	
	DHBA-Fe(1:1)	**DHBA-Fe(2:1)**	**DHBA-Fe(4:1)**
Compare with Fe	1.21	2.09	2.29
Compare with DHBA	1.44	2.49	2.73

DHBA= 2,3-dihydroxy benzoic acid; Fe=iron

IV. CONCLUSION

Ferric-oxide shows absorptinon maxima at 475.61 nm indicating the formation of complex with DHBA is in vissible range. The stability of DHBA at pH 2.51 and 7.8 is upto 19 and 15 days respectively. This is due to conformational changes in DHBA by increasing the pH upto 7.8. The growth of *S. obliquus* and *Nostoc* showed pronounced effect in presence of DHBA (1:1), DHBA (2:1) and DHBA (4:1). The chrophyll-a content at 15th day were 1.14 mg/L and 1.72 mg/L for *S. obliquus* and *Nostoc* at DHBA-Fe (4:1) which is indictive that potentially bioaviablity of soluble iron. The iron can be solublised with the help of DHBA due to presence of specific reductases on plasmamembrane of algae (Cristoph Volker and Wolf-Gladrow, 1999). In DHBA-Fe (4:1) the growth increases but not consistance. This is due to more concentration of DHBA itself inhibit the complexation process therefore low solubility of iron. After 15 day the growth of algae decraeses, this is due to the deficiency of micronutrient and macronutrient in the medium.

ACKNOWLEDGMENT

We would like say thanks to Director of ACS and Biotechnology Research Centre for his economical support for funding this project.

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AUTHORS

First Author –Jalil J. Bhatnase (Asst. Officer), Aquachek Water research Center, Eureka Forbes Ltd. New Delhi 110075. India, Email – jalilbhatnase@gmail.com
Second Author –Usha B. Naik (Asst. Professor), Department of Microbiology, ACS and Biotechnology Research Centre, Wardha. 442001. India., Email – naik.mohini@gmail.com
Third Author –Dinesh K. Dabhadkar (Inspired Fellow DST, JRF, Govt. India), Department of Zoology, Govt. Vidarbha Institute of Science and Humanities, Amravati. 444604. India., Email – dineshdabhadkar@yahoo.com
Fourth Author – Roshan D. Dhore (Principal), Department of Microbiology, ACS and Biotechnology Research Centre, Wardha. 442001. India., Email – rddhore@yahoo.com

Correspondence Author –Jalil J. Bhatnase, Email – jalilbhatnase@gmail.com , Contact - +91 8287958854