IJBPAS, October, 2014, 3(10): 2229-2239

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

BIOMASS PRODUCTION OF TWO BLUE-GREEN ALGAE USING J.K.PAPERMILL EFFLUENTS, RAYAGADA AS NUTRIENT IN CULTURE MEDIUM

SWAIN PK*, PANDA MK, BEHERA GS, MOHANTA RK AND PADHI SB

Centre for Environmental Studies (CES), Forest & Environment, Department, Government of Odisha

***Corresponding Author's E Mail: prasannaswain19@gmail.com; Mob.: +91-7873059381; Fax: 0674-2553182**

ABSTRACT

Two filamentous cyanobacteria, *Anabaena iyengarii* and *Lyngbya* sp. isolated from paddy field soils near the vicinity of J. K. Paper Mill, Rayagada were grown in varying concentrations of industrial effluent in defined culture media under laboratory condition to study the effect of the effluent on growth, chlorophyll a, nitrogen content and photosynthetic rate of the isolates. *Anabaena iyengarii* exhibited good growth in J. K. Paper Mill effluents and contained appreciable amount of chlorophyll a, nitrogen with higher photosynthetic rate. The results are discussed on a comparative basis to show the response of the two cyanobacterial isolates to different concentrations of the industrial effluents.

Keywords- PaperMill, effluents, *Anabaena iyengarii*, *Lyngbya* sp.

INTRODUCTION

Industrialization brought about prosperity comfort, health and wealth of mankind through industrial revolution and rapid utilization of natural resources, but also threatened the ecological security to the globe with consequent damage the ecosystem by generating huge waste and pollutants. Every day about 2 million tons of sewage, industrial wastes and agricultural wastes are discharged into worlds water **[1]**. Industry alone is responsible for dumping of 300-400 million tons of heavy metals, solvents, toxic sludge and other wastes into waters each year **[2]**. In India almost every water system (about 70%) now polluted to a considerable extent **[3-4]** due to the release of untreated or partially treated effluents into natural water bodies. Thermal and steel plants are the higher contributors to annual industrial waste water discharge and next to it occupied by paper and pulp industry and it is among the 20 high polluting industries **[5]**. The paper industries consumed around 905.8 million m^3 of water and discharged around 695.7 million m^3 of waste water annually **[6]**. Paper Mill liberates heavily loaded wastes to surrounding environment **[7-8]**. Mainly eight types of effluent released from paper industry. 1. Colouring substances, 2. turbidity and sediments, 3. oxygen consuming substances, 4. nutrients, 5. malodorous substances and these affecting taste, 6. acidity/alkalinity, 7. chlorate and 8. toxic substances.

The bluegreen algae (BGA) are also called Cyanobacteria and are cosmopolitan in distribution. They are found in almost every aquatic and terrestrial environment **[9]** They flourish well either in nutrient rich and warm water or at times in water with apparently low nutrient concentrations subject to higher temperature and bright light conditions **[10-15]** .Any alternation in the environment leads to the change in algal communities in terms of tolerance, abundance, diversity and dominance in their habitat. Algae countered in a water body reflect the average ecological condition of the water body and therefore can be used as an indicator for assessment and evaluation of water quality of diverse habitats **[16-19]**.

Among aquatic organisms algae are very sensitive to pollutants discharge than other organisms. They show some adverse effect of chemicals more quickly than other organisms **[20]**. Depending on toxicity of pollutants, the growth of algae can be either inhibited or stimulated .Primary production, pigments may also get affected. There was ample evidence that the response of algae to polluting chemicals varies according to degree of temperature and light stress **[21]**.

The present study deals with physico-chemical characteristics of the effluent and its effect on growth, chlorophyll a, nitrogen fixation, rate of photosynthesis of two cyanobacteria, Anabaena iyengarii and *Lyngbya* sp.

MATERIALS AND METHODS

The study area is located in Rayagada, about 390 Km. away from Bhubaneswar, Odisha, India. It receives effluent from the J.K.Paper Mill. Standard methods (APHA, 1976) **[22]** were followed during collection and analysis of effluent from the affected area. Bacteria free pure cultures of *Anabaena iyengarii* and *Lyngbya* sp. were employed in this investigation. The above test organisms *Anabaena iyengarii* and *Lyngbya* sp. were isolated from water logged paddy fields near J.K.Paper Mill and made pure cultured (bacteria free) in the CES laboratory. The organisms were identified by using the monograph on Cyanophyta by Desikachary (1959) **[23]** and works of **[24-25]**. They were grown in Kratz and Myer **[26]** medium with the trace elements, free from combined nitrogen and were maintained in sterilized cotton stopper conical flasks at a temperature of 24±2°C in a culture room and illuminate with day light fluorescent tubes at an intensity of 2,200 lux. The cultures were hand shaken twice daily and were transferred at 45 days intervals to fresh nitrogen free medium to keep the culture in exponential growth phase for experiments.

Experiments were conducted by inoculation of aliquots (1.0 ml) of exponentially growing algal suspension into various dilution of effluent concentrations (0.01, 0.1, 0.5, 1, 5, 10, 25 percentage) along with control. Triplicates were set up for each set of experiment and the cultures were harvested on 4th, 8th, 12th, 16th and 20th day of incubation in continuous light. Growth was estimated on dry weight basis as adopted earlier (Padhi, 1983) **[16]** and the specific growth rate constants (K) were plotted from mg dry weight per 25.0ml in logarithmic growth phase of organisms Vs difference in days as per Kratz and Myer (1955) **[26]**. Chlorophyll a was extracted with 80% acetone and estimated accordingly (Tailing and Driver, 1963 and Davies, 1976) **[27, 28]**. Total nitrogen was determined by the Nesselerisation method Herbat, 1971**[29]**. With different days of intervals and the specific nitrogen rate constants (K) were plotted from µg dry weight per 25.0 ml in logarithmic nitrogen of organism Vs different days. Photosynthetic oxygen evolution was determined with a Clark type oxygen electrode. The cells were suspended in 50mM phosphate buffer, pH 7.5 containing 5mM $NaHCO_3$ for assay of photosynthesis. The samples were illuminated with white light from a 600w halogen lamp and passed through heat-absorbing filter. The light intensity was 1.2×10^5 erg / cm^2 sec. All these readings were recorded and depicted in Figure 1,2,3,4,5,6,7 simultaneously.

RESULTS AND DISCUSSION

Physico-chemical characteristics of the effluent are depicted in **Table 1**. Effect of different concentrations of effluent on growth of *Anabaena iyengarii* in different days of incubation was studied (**Figure 1).** The result shows that the growth rate of the organism was more than control value at 0.01, 0.1, and 0.5 percent of the effluent .The growth rate declined gradually from 1 to 5 percent of the effluent. At 10 percent of the industrial effluent, the growth rate of the organism was drastically declined and the organism totally bleached. The growth of *Anabaena iyengarii* on 20th days at control (no effluent), 0.01%, 0.1%, 0.5%, 1%, 5%

& 10% effluents was 15.8, 16.5, 17, 16, 11.5, 10, & 1.0 mg.dry wt/25 ml of culture respectively. The effect of different concentrations of the effluent on growth of *Lyngbya* sp. in different days of incubation was studied (**Figure 2)** .The result indicates that the growth rate of the organism was higher than the control value at 0.01 percent of the effluent. The growth rate declined gradually from 0.1, 0.5 and 1 percent of the effluent At 5 and 10 percent of the industrial effluent, the growth rate of the organism was reduced from 8th day onwards and the organism was totally bleached. The growth of *Lyngbya* sp. on 20th days at control (no effluent), 0.01%, 0.1%, 0.5%, 1%, 5% & 10% effluents was 14.8, 15.8, 11, 10.3, 7.2, 1.6 & 0 mg.dry wt/25 ml of culture respectively.

The effect of different concentrations of the effluent on chlorophyll-a content of *Anabaena iyengarii* in different days of intervals was studied **(Figure 3)**. At 0.01 percent the chlorophyll-content was more than the control value. Then the values declined with a increase in effluent concentrations till 20th day of incubation. Maximum reduction observed at 5 percent of effluent. The chlorophyll a content of *Anabaena iyengarii* on 20th days at control (no effluent), 0.01%, 0.1%, 0.5%, 1%, 5% & 10% effluents was 87, 97, 67, 47, 15 & 9 µg. Chl. a/25 ml of culture respectively.

The effect different concentrations of the effluent on chlorophyll-a content of *Lyngbya* sp. in different days of incubation was studied **(Figure 4).** The result shows that the chlorophyll-a content of the organism at 0.01, 0.1 percent of effluent was more as compared to the control value. But on 20th day, the chlorophyll a content at 0.1 percent of the effluent decreased from the control value. The chlorophyll-a content of the organism declined gradually from 0.5 of the industrial effluent. At 5 percent of effluent concentration, the chlorophyll a content of the organism showed maximum reduction and became colorless. The chlorophyll a content of *Lyngbya* sp. on 20th days at control (no effluent), 0.01%, 0.1%, 0.5%, 1%, 5% & 10% effluents was 46,70, 37, 30, 7.5 & 2 µg. Chl. a/25 ml of culture respectively. The effect of different concentration of effluent on nitrogen content of *Anabaena iyengarii* in different days of incubation was studied (**Figure 5)**. At concentration of 0.01 percent the nitrogen content increased as compared to control value. The nitrogen content declined gradually at 0.1 0.5 1 5 percent of the effluent. At 10 percent of the industrial effluent, the growth rate of the organism was declined significantly. The nitrogen content of *Anabaena iyengarii* on 20th days at control (no effluent), 0.01%, 0.1%, 0.5%, 1%, 5% &10% effluents was 3.5, 3.7, 3.4, 1.7,1, 0.8 & 0.7 mg. Nitrogen /25 ml of culture respectively.

The effect of different concentration of effluent on nitrogen content of *Lyngbya* sp. in different days of incubation was observed (**Figure 6**). The result shows that the nitrogen content of the organism declined with increase in effluent concentration from 0.01 percent onwards. At 5 & 10 percent of effluent, the nitrogen content of the organism totally declined from 8th day onwards. The nitrogen content of *Lyngbya* sp. on 20th days at control (no effluent), 0.01%, 0.1%, 0.5%, 1%, 5% & 10% effluents was 3.15, 2.1, 1.75, 1.5, 1.2, 0.14 & 0.13mg. Nitrogen /25 ml of culture respectively. **Figure 7** depicts the photosynthetic rate at different effluent concentration on 20th day of incubation. The result shows the decrease of oxygen evolution with increase of concentration from 0.01 to 10 percent of effluent. The photosynthetic rate of *Anabaena iyengarii* shows high photosynthetic rate than *Lyngbya* sp. The photosynthetic rate was more at 0.01 percent over the control value. The above observations have similarities with the earlier observations **[30]**.

The water having conductivity more than 2.00mhos/cm is unsuitable for plant growth was suggested **[31, 32]**. In the present study conductivity was less than prescribed limit hence it would not affect plant growth. Negative impact of higher concentration of various industrial effluents on algae was noticed **[30]**. Higher concentration inhibit pigment production due to absorption of bio-available contaminants by algal cells from the effluents .Similar observations of enhance- ment of pigment content in lower concentration of effluents and decrease in higher concentration was recorded **[30, 33]**.

Table 1: Physicochemical characteristics of effluent of J.K.PaperMill effluents of Rayagada

Characteristics	Mean±SD
Temperature°C	31±1.2
pH Near Industry	8.9±0.114
Far from Industry	7.5±0.03
Total Dissolved Solids(TDS)mg/L	1470±29.31
Total Suspended Solids(TSS)mg/L	4.04±0.031
Total Solids(TS)mg/L	1745±40.51
Conductivity(mmho/cm)	1.35±0.024
Turbidity(NTU)	134±4.04
Dissolved Oxygen(DO)mg/L	0.76±0.015
Biochemical Oxygen Demand(BOD) mg/L	398.34±6.45
Chemical Oxygen Demand(COD) mg/L	594±22.34
Alkalinity, mg/L	317±6.19
Total Nitrogen(TN) in ppm	4.69±0.03
Total Phopsphorous(TP) in ppm	1.03±0.04
Sulphate in ppm	159±4.82
Total Hardness in ppm	587±39.31

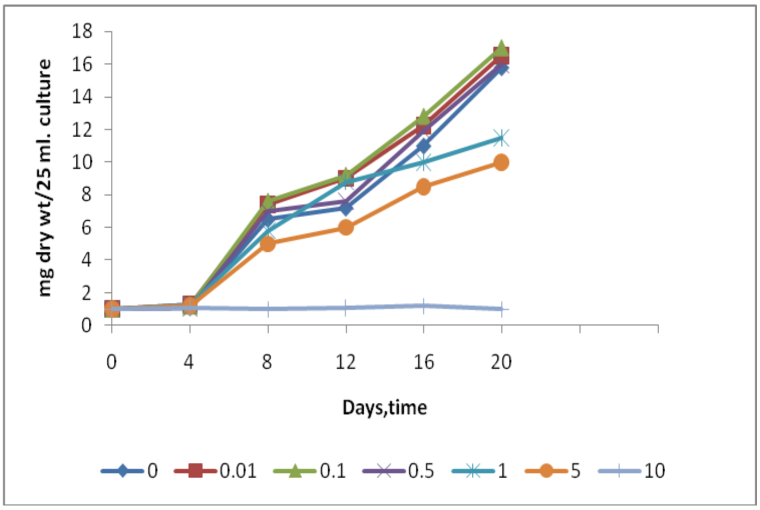

Figure 1: Effect of different concentration of the industrial effluent on growth of *Anabaena iyengarii* in different days of incubation

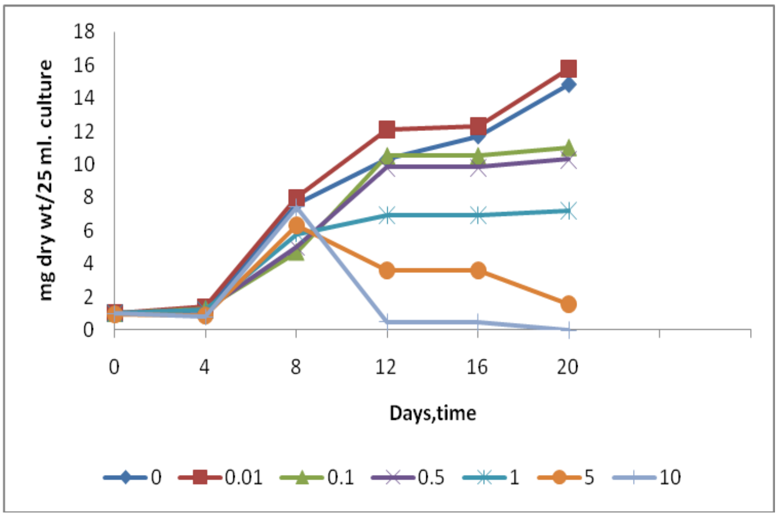

Figure 2: Effect of different concentration of the industrial effluent on growth of *Lyngbya* sp. in different days of incubation

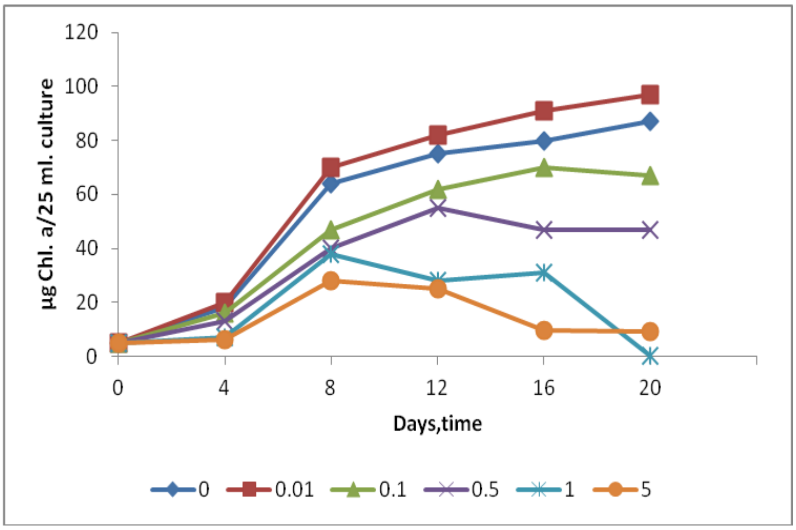

Figure 3: Effect of different concentration of the industrial effluent on chlorophyll –a growth of *Anabaena iyengarii* in different days of incubation

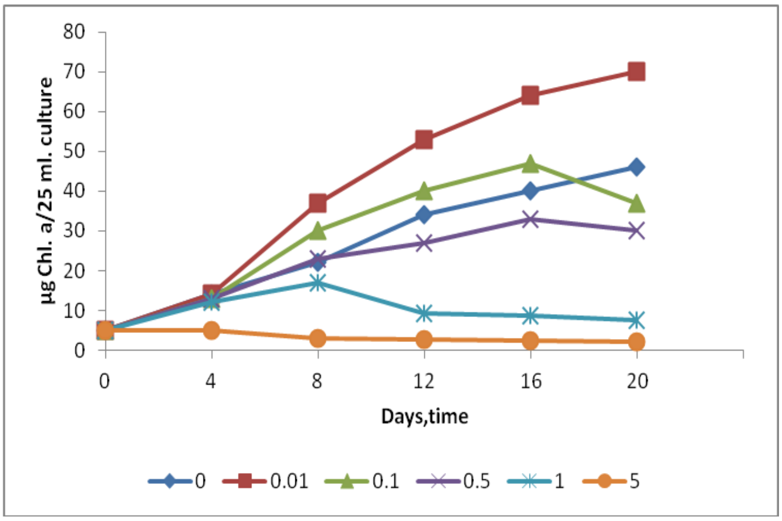

Figure 4: Effect of different concentration of the industrial effluent on chlorophyll –a growth of *Lyngbya* sp.in different days of incubation

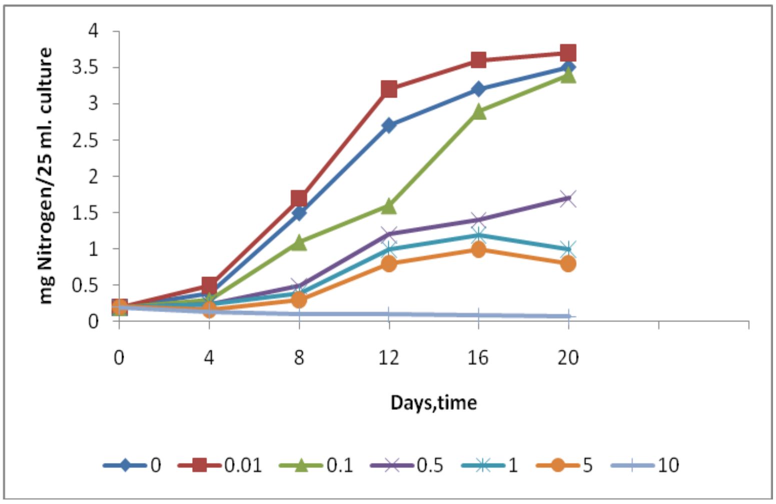

Figure 5: Effect of different concentration of the industrial effluent on Nitrogen content of *Anabaena iyengarii* in different days of incubation

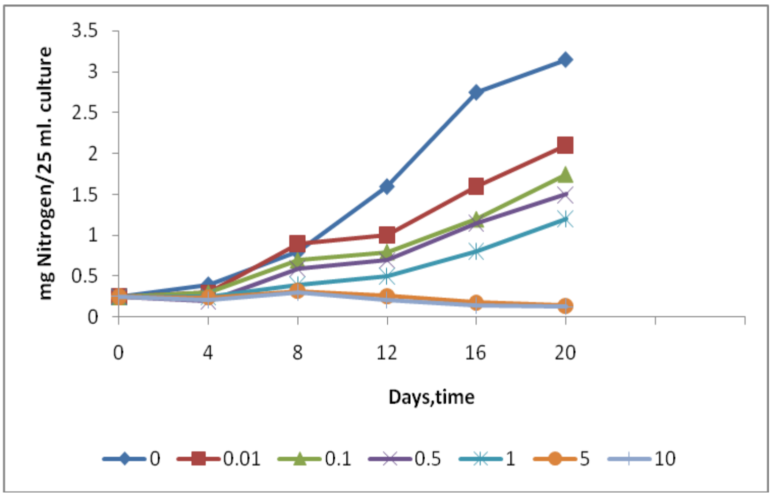

Figure 6: Effect of different concentration of the industrial effluent on Nitrogen content of *Lyngbya* sp.in different days of incubation

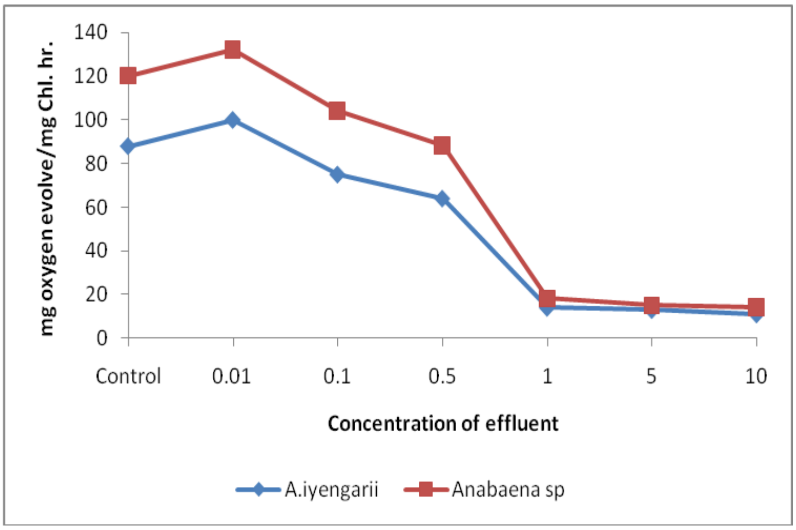

Figure 7: Photosynthetic rate at different concentration of industrial effluent

CONCLUSION

From the above observation it was concluded that the Cyanobacteria (*Anabaena iyengarii* & *Lyngbya* sp.) can be utilized for higher biomass production using paper mill effluent at 0.01 to 0.1 % concentration. This shows their relative adaptability & resistance towards effluent and these species could be utilized for biological treatment of paper mill effluents (phycoremediation).

ACKNOWLEDGEMENT

We would like to thank profoundly to Ministry of Forest and Environment, Government of Odisha for financial support. We also thank the Director, Centre for Environmental Studies (CES) for providing Laboratory facilities.

REFERENCES

[1] UN –WWAP, United nations World Water Assessment Programme.The world water development Report-1: Water for people, water for life, UNESCO: Paris, France, 2003.

[2] UNEP, UNEP Year book 2010: New Science and developments in our changing environment, 2010.

[3] Mahajan KK, Deterioration of Nations river, In ecology and pollution of Indian rivers (Ed.R.K.Trivedy) pp.1-38, Asian Publishing House, New Delhi, 1988.

[4] Martin P, River pollution in India: An Overview, Employment News. 22 (52), 1998, 1-2.

[5] Bajpai P and Bajpai PK, Reduction of organo chlorine compounds in bleatch plant effluents, Adv. Biochem. Engg. Biotechnol., 57, 1997, pp 213.

[6] Devika R, Rajendran A and Selvapathy P, Variation studied on the physic-chemical and biological characteristics at different depths in model waste stabilization tank, Pollut. Res., 24, 2006, 771-774.

[7] Anonymous, Concern over pollution caused by Nagaon Paper Mill (NPM), The Assam Tribune, 3rd Jan/1999.

[8] Baruah BK and Das M, Study on plankton as indicator and index of pollution in aquatic ecosystem receiving paper mill effluent, Ind. J. Env. Sci., 5 (1), 2001, 41-46.

[9] Castenholz RW, Water bury JB, Oxygenic photosynthetic bacteria, Group E,Cyanobacteria, In Bergey's Manual of Systematic bacteriology (Eds Staley, J.N. *et al*.) Williams and Wikins Co., Baltimore, 1989, 1710-1728.

[10] Philipose MT, Freshwater phytoplankton of Inland fisheries. Proc. Symp. Algology. ICAR, New Delhi, 1960, 272-291.

[11] Sawyer CN, Causes, Effects and control of Aquatic growths, J. WPCF, 34, 1962, 279-288.

[12] Munawar M, Limnological studies on fresh water ponds of Hyderabad, India II, The Biotype, Hydrobiologia, 35 (1), 1970 a, 127-162.

[13] Munawar M, Limnological studies on fresh water ponds of Hyderabad, India II, The biocoenose-distribution of Unicellular and colonial phytoplankton in polluted and unpolluted environments, Hydrobiologia, 36 (1), 11970 b, 105-128.

[14] Fogg GE, Algal culture and phytoplankton ecology, The University of Wisconsin Press.U.S.A, 1975.

[15] Bhatnagar A, Makandar MB, Garg MK and Bhatanagar B, Community structure and diversity of Cyanobacteria and green algae in the soils of Thar Desert (India, .J. Arid Environment ,72, 2008,73-83.

[16] Padhi SB, Studies on the antibiotic tolerance of some Blue-Green Algae, Ph.D. thesis, Berhampur University, India, 1983.

[17] Diwedi S, Pollution Induced Structural and Physico-Chemical Changes in Algal Community: A case study of River Pandu of North India, World Academy of Sci. Engg. and Technol., 71, 2010, 735-739.

[18] Saikia MK, Kalita S and Sarma GC, Algal indices to predict pulp and paper Mill Pollution Load of Elenga Beel (Wetland) Assam, India, Asian J. Exp. Bio. Sci., 1 (4), 2010, 812-821.

[19] Saikia MK, Kalita S and Sarma GC, Growth Stimulation (+) and

Inhibition(-)of algae (*Oscillatoria chlorine*, and *Scenedesmus quadricauda*) treated to pulp and paper mill Effluents, Int. J. Appl. Biol. and Pharmaceut. Technol., IJABPT, 2 (4), 2011, 87-94.

[20] Barbour MT, Gerritsen J, Synder BD, Stribling JB, Rapid bio assessment protocol for use in streams and Wadeable Rivers: Periphyton, Benthic Macroinvertibrates and Fish, 2nd Ed., U. S. Environmental, 1999.

[21] Round FE, The ecology of Algae, Cambridge University Press, 1979, 653,

[22] APHA, Standard Methods for the examination of water, sewage and industrial wastes, 14th Ed., APHA in New York, 1976.

[23] Desikachary TV, Cyanophyta, ICAR, New Delhi, 1959.

[24] Tiwari GL, A study of the blue-green algae of paddy field soils of India, Hydrobiologia, 39 (3), 1972, 335-340.

[25] Tiwari GL, A study of the blue-green algae of paddy field soils of India, Nova Hedwigia, 26, 1975, 765-798.

[26] Kratz WA and Myer J, Nutrition and growth of several blue-green algae, Am. J. Bot., 42, 1955, 282-287.

[27] Tailing JR and Driver D, Some problems of estimation of chlorophyll a in phytoplankton. In: M. S. Dohy (ed), Proc. Conf. Prim, product, measurement in marine and fresh water, Univ. Hawaii, 1963, 142-146.

[28] Davies BH, Caretenoids In: Chemistry and Biochemistry of plant pigments, 11, Eds. T. W. Goodwin, 1976, 38-165.

[29] Herbat H, In: Methods in Microbiol., 53, Eds., J.R. Norris and D. W. Ribbons, Academic Press, New York and London, 1971, 210.

[30] Padhi SB, Behura SK, Swain PK, Behera G and Panigrahi H, Effect of chloro-alkali industrial effluent on two filamentous cyanobacteria:A laboratory bioassay, Indian Hydrobiol.,12 (1), 2009, 48-52.

[31] Trivedy RK and Goel PK, Chemical and biological methods for water pollution Studies, 2nd Ed., Environmental pollution, Karad, India, 1986, 1-210.

[32] Dutta SK and Boissya CL, Effect of Solid waste disposal from paper mill on productivity of Rice (Oryza sativa L.var.Mahsuri), Ecol. Env. & Cons., 11(2), 2005, 267-271.

[33] Reemol SA, Studies on toxicity of industrial effluents on phytoplankton, PhD Thesis M. G. University, Kottayam, 2004, 92-93.