

## GROWTH RESPONSE OF THE BLUE-GREEN ALGA, *Westiellopsis prolifica* IN SEWAGE ENRICHED PAPER MILL WASTE WATER

Aditya K. DASH and Pramod C. MISHRA\*

Department of Environmental Sciences, Sambalpur University, Jyoti-Vihar. 768019. Orissa, India

(Recibido mayo 1997, aceptado febrero 1999)

Keywords: growth, blue-green alga, cyanobacteria, paper mill waste water, domestic sewage, *Westiellopsis prolifica*.

### ABSTRACT

Protein, pigments (chlorophyll *a* and carotenoids) and biomass of *Westiellopsis prolifica*, a blue-green alga, were measured when grown in different concentrations of paper mill waste water enriched with domestic sewage and basal nutrient medium. The average percentage increase in protein, chlorophyll and carotenoid content was 10.0, 43.0 and 60.7, respectively over the culture without sewage enrichment on the day of optimum growth. There was a 4 day postponement of the declining phase due to the availability of additional nutrients from the sewage. The biomass increase ranged from 7 to 25 %. Thus sewage could be utilised as a supplement to inorganic nutrient media for the growth of blue-green algae during biological treatment of waste water, thereby reducing costs.

### RESUMEN

Se midieron proteínas, pigmentos (clorofila *a* y carotenoides) y biomasa del alga verde-azul *Westiellopsis prolifica* que se desarrolló en diferentes concentraciones de desperdicio de las fábricas de papel enriquecido con agua del drenaje doméstico y medio basal nutritivo. El incremento porcentual promedio en el contenido de proteínas, clorofila y carotenoides el día de crecimiento óptimo fue de 10.0, 43.0 y 60.7, respectivamente, al compararlo con el cultivo sin enriquecimiento. La fase de declinación se pospuso cuatro días debido a la accesibilidad a nutrientes adicionales del agua de drenaje. El intervalo de crecimiento de la biomasa fue de 7 a 25 %. Así el agua del drenaje se utilizó como suplemento a los medios nutritivos inorgánicos para el crecimiento de las algas verde-azules durante el tratamiento del agua de desecho y por lo tanto los costos se redujeron.

### INTRODUCTION

The use of cyanobacteria (blue-green algae) in waste treatment systems has proved to be cost-effective and beneficial because they bring about oxidation and mineralization in waste waters and serve as excellent indicators of water pollution (Elnabarawy and Welter 1984). The use of sewage for enhancing cyanobacterial production is also an attractive proposition in view of economic implications and environmental contingencies (Saxena *et al.* 1974, Subramanian and Shanmugasundaram 1986). Controlled photosynthesis involving algal-bacterial symbiosis is an inexpensive method in the reclamation of waste water

containing high organic matter and nutrients (Oswald *et al.* 1953, Gotaas and Oswald 1955, Oswald and Gotaas 1957, El-Baroudi and Moaward 1967, Amin 1969, Ganapathi and Amin 1972).

*Westiellopsis prolifica*, a blue-green alga, is capable of growing in different types of waste water and can utilize various organic substances under varied growth conditions (Adhikary 1982, Shaw *et al.* 1989, Routray *et al.* 1991, Adhikary *et al.* 1992). It is a nitrogen-fixing, oxygenic, photoautotrophic and heterocystous blue-green alga. The growth of *W. prolifica* in paper mill waste water enriched with basal nutrient medium has shown better growth in comparison to the culture in paper mill waste water without basal nutrient medium (Dash and Mishra

\* For all correspondence

TABLE I. CHEMICAL CHARACTERISTICS OF BASAL NUTRIENT MEDIUM, SEWAGE AND PAPER MILL WASTE WATER (IN mg/10 ml VOLUME, EXCEPT pH)

A. Basal nutrient medium

Magnesium sulphate	61.0	Sodium molybdate	2.5
Calcium chloride	18.0	Copper sulphate	0.25
Sodium chloride	58.0	Zinc sulphate	0.25
Dipotassium hydrogen phosphate	94.0	EDTA	1.3
Manganous chloride	2.5	Ferrous sulphate	1.24
Boric acid	2.5	Potassium hydroxide	0.54

B. Paper mills waste water and sewage

Parameters	Sewage	Paper mill waste water
pH	7.4	9.5
TSS	0.66	8.5
TDS	1.92	7.0
Total alkalinity	0.25	1.05
Sodium	0.136	1.65
Potassium	0.148	0.1
Calcium	0.48	1.0
Magnesium	0.1	0.336
Nitrogen	12.54	0.0093
COD	12	8
Phosphate	0.0031	-
BOD	-	2
DO	negligible	0.01
Sulphate	-	1

1996a). High pigment and protein content in cyanobacterial growth in paper mill waste water with basal nutrient medium indicate that the alga is not only tolerant but can even grow well. On the other hand, decreased chlorophyll and protein content of the alga when grown in waste water without basal nutrient medium showed that the paper mill waste water lacks one or more essential nutrients for sustained algal growth.

Therefore, in the present study, *Westiellopsis prolifica* was chosen and its growth response in sewage enriched paper mill waste water in terms of protein, pigment and biomass was studied over time.

## MATERIALS AND METHODS

A pure strain of bacteria-free *Westiellopsis prolifica* Janet was obtained from the Department of Botany, Berhampur University, Orissa, India. The alga was grown in nitrogen free medium as described by Allen and Arnon (1955), with the modification (Table I A) that trace elements were replaced by manganese, boron, molybdenum, zinc and copper at the concentration used by Fogg (1949). The nutrient medium contains major nutrients such as  $\text{MgSO}_4$ ,  $\text{NaCl}$ ,  $\text{CaCl}_2$ ,  $\text{K}_2\text{HPO}_4$  and  $\text{Fe-EDTA}$  and micronutrients such as  $\text{MnCl}_2$ ,  $\text{H}_3\text{BO}_3$ ,

$\text{Na}_2\text{MoO}_4$ ,  $\text{CuSO}_4$  and  $\text{ZnSO}_4$ . The cultures were maintained in Erlenmeyer flasks in triplicate at a temperature of  $28 \pm 2^\circ\text{C}$  and illuminated with daylight fluorescent tubes at an intensity of 2200 lux. Prior to inoculation of the alga the medium was adjusted to pH 7.5-7.8 and then sterilized. The cultures were hand shaken twice daily to get a uniform suspension. Axenic cultures of the alga were used as the experimental material.

Waste water was collected from the Orient Paper Mill located at Brajaraj Nagar, Orissa, India, approximately 100 Km distance from Sambalpur University Campus. Liquid sewage was collected from the domestic sewage drain of Sambalpur University Campus, Orissa, India. Standard methods (APHA 1976) were followed for analysis of the paper mill waste water and domestic sewage water (Table I B).

The experiment was conducted in the laboratory by inoculation of the alga into different concentrations of paper mill waste water with (10% v/v) or without the basal nutrient medium (Table II). The paper mill waste water was diluted with double distilled water during the preparation of different concentrations and finally 10 ml of sewage water (unsterilized) were added to each of the concentrations. Growth in terms of biomass, protein and pigments (chlorophyll *a* and carotenoid) content were studied over a period of 24 days or until the declining phase.

### Chlorophyll content

Algal suspensions (10 ml) were centrifuged for 10 min at 5,000 rpm. Residues containing the homogenised algal filaments were extracted in 10 ml of 80 % chilled acetone and retained for 24 h at low temperature in dim light. The absorbance of the clear extract was measured at 663.2 nm and 646.8 nm for total chlorophyll measurement in a spectrophotometer, using the formula of Lichtenthaler (1987) i.e. total chlorophyll ( $\mu\text{g/ml}$ ) =  $12.25 A_{663.2} - 2.79 A_{646.8}$ .

### Carotenoid content

The carotenoid content was measured at 470 nm and calculated using the formula of Lichtenthaler (1987) i.e. total carotenoid content ( $\mu\text{g/ml}$ ) =  $[1000 \times A_{470} - 1.82 \times \text{total chlorophyll} (\mu\text{g/ml})] / 198$ .

TABLE II. DIFFERENT CONCENTRATIONS OF PAPER MILL WASTE WATER WITH OR WITHOUT THE BASAL NUTRIENT MEDIUM

Concentration of waste water (%)	Basal nutrient medium 10 ml
Control (DDH)	+
25	+
50	+
75	+
100	+
100	-

+ nutrients added

- nutrients not added

DDH - Double distilled water



**TABLE III.** INCREASE (%) IN PIGMENTS AND PROTEIN CONTENT AT THE RESPECTIVE OPTIMAL GROWTH PHASE AND BIOMASS ON THE HARVEST DAY OF *Westiellopsis prolifica* IN SEWAGE ENRICHED WASTE WATER OVER WASTE WATER WITHOUT SEWAGE AND WITH AND WITHOUT BNM

	Chlorophyll <i>a</i> (mg/ml)			Carotenoids (mg/ml)			Protein (mg/ml)			Biomass (mg/ml)		
	WWWS	SEWW	% increase	WWWS	SEWW	% increase	WWWS	SEWW	% increase	WWWS	SEWW	% increase
Control	2.384	2.460	3.187	0.584	0.662	13.356	223.582	234.045	4.679	1012.666	1101.333	8.755
25% WW with BNM	2.702	2.833	4.848	0.612	0.793	29.575	242.181	265.721	9.720	1053.333	1134.333	7.689
50% WW with BNM	1.952	2.945	50.870	0.762	0.953	25.065	223.582	278.508	24.566	1077.666	1206.666	11.970
75% WW with BNM	1.733	3.056	76.341	0.943	0.990	4.984	257.021	286.654	4.226	1114.333	1246.333	11.845
100% WW with BNM	3.120	3.485	11.698	1.001	1.154	15.284	281.124	293.991	4.576	1158.666	1293.666	11.651
100% WW without BNM	0.737	1.562	111.940	0.218	0.820	276.146	268.628	302.629	12.657	518.000	649.666	25.904
Average	2.104	2.723	43.147	0.686	0.895	60.737	249.353	276.923	10.070	989.110	1105.332	12.969

WW - Waste water

WWWS - Waste water without sewage

SEWW - Sewage enriched waste water

BNP - Basal nutrient medium

### Protein content

The residue left after extraction of chlorophyll was dissolved in 0.1N NaOH and the protein content was estimated according to Lowary *et al.* (1951) and it was expressed as  $\mu\text{g}$  of protein per ml of algal suspension.

### Biomass measurement

For biomass study the cultures were harvested on the last day (24 th day) and oven dried at 105°C until constant weight was obtained.

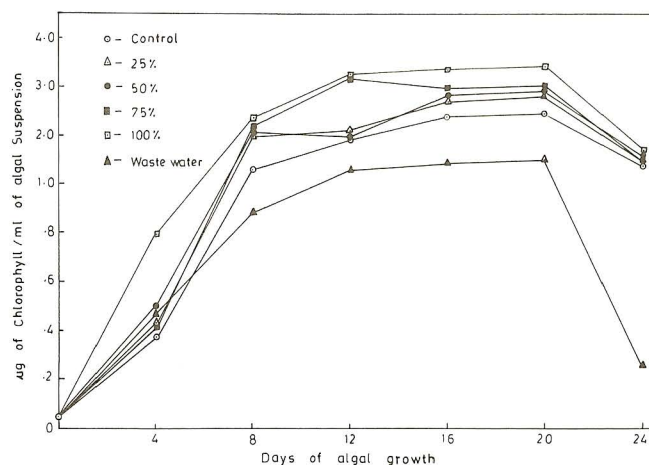
## RESULTS AND DISCUSSION

**Table I** shows a comparative quality analysis of paper mill waste water, sewage and basal nutrient medium (BNM). The addition of sewage to the paper mill waste water containing algal culture medium enhances the total nutrient and organic matter content including inorganic phosphate of the culture medium.

Changes in total chlorophyll content of *Westiellopsis prolifica* (**Fig. 1**) grown in paper mill waste water with BNM and sewage revealed an upward trend with an increase in days of incubation as well as concentrations reaching the highest value in 20 day old cultures at all the concentrations, after which the declining phase began. The lowest value was recorded in 100% waste water enriched with sewage but without basal nutrient medium. However, the absolute values are always less in 100% waste water with BNM than in 100% waste water with BNM with sewage which may be due to nutrient saturation in the medium from both sewage and BNM.

Total carotenoid content of *W. prolifica* (**Fig. 2**) showed an almost similar trend with that of chlorophyll content. After 20

days of incubation the carotenoid content decreased at all the concentrations of waste water.



**Fig. 1.** Chlorophyll *a* content during the growth of *W. prolifica* in sewage enriched paper mill waste water

The protein content of *W. prolifica* (**Fig. 3**) showed a rise with increase in days of incubation reaching the highest value during 16-20 days at all the concentrations with exception of 25% where the trend was irregular. The highest value for the protein content of the blue-green alga was observed on the 16th day of growth in 100 % waste water with sewage but without basal nutrient medium.

A comparison of this growth trend in terms of pigment, protein and biomass content, with the trend found when sewage was not added shows an average increase of 43 % in chlorophyll,

60.7 % carotenoid and 10 % protein in sewage enriched waste water (Table III).

In general, the pigment content was lowest in 100% waste water without basal nutrient medium. However, when 100% waste water was enriched with sewage, the growth rate showed significant increase even without BNM over the rate of increase with BNM in higher dilutions. Perhaps the nutrient saturation due to both BNM and sewage may be growth limiting for the organism in other dilutions.

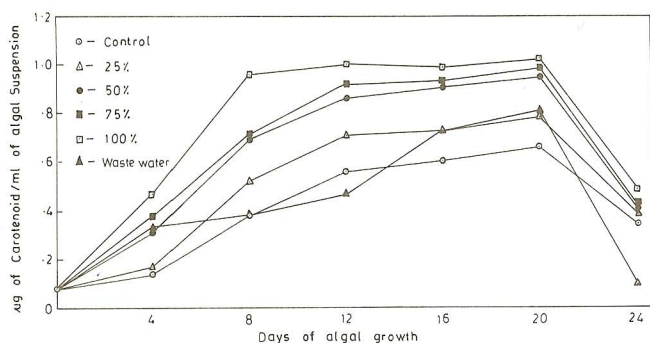


Fig. 2. Carotenoid content during the growth of *W. prolifica* in sewage enriched paper mill waste water

There was also a 4 day postponement in the declining phase in the culture medium containing sewage in comparison with the medium without sewage, indicating the contribution of the nutrients from sewage to algal growth. The biomass was recorded on harvesting day (24 th day) when the decline phase began. Therefore, the percentage increase in terms of biomass was relatively less than the increase recorded for protein and pigment content at their optimum growth phases.

Earlier studies undertaken by the same authors on the growth of *Westiellopsis prolifica* in paper mill waste water indicate an optimum growth at 100 % waste water with basal nutrient medium and the nutrients such as phosphate, boron, zinc, iron are lacking in waste water without BNM limiting the growth of the alga (Dash and Mishra 1996a). *W. prolifica* also showed higher biomass, chlorophyll, carotenoid and protein content when grown in paper mill waste water manipulated with nutrients than in waste water alone (Dash and Mishra 1996b). Thus addition of sewage to paper mill waste water during algal treatment to reduce pollution load in waste water may be a suitable and economic method of pollution control.

#### ACKNOWLEDGEMENTS

AKD is thankful to Sambalpur University and the Government of Orissa for financial assistance and to the Head of the Dept. for laboratory facilities.

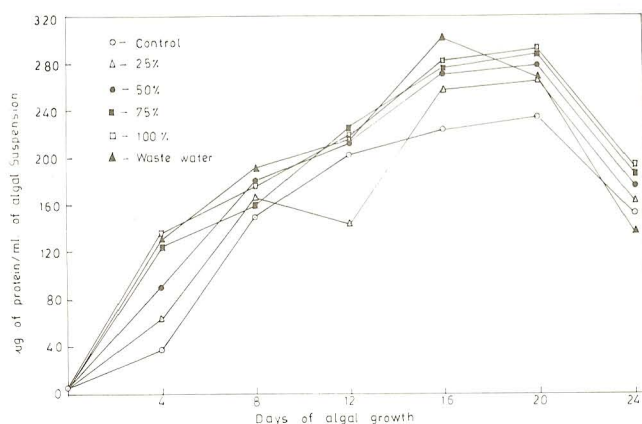


Fig. 3. Protein content during the growth of *W. prolifica* in sewage

#### REFERENCES

- Adhikary S.P. (1982). Utilization of organic substrates by a blue-green alga *Westiellopsis prolifica* under various growth conditions. *J. Indian Bot. Soc.* 61, 268-277.
- Adhikary S.P., Bastia A.K. and Tripathy P.K. (1992). Growth response of the nitrogenfixing cyanobacteria *Westiellopsis prolifica* Janet to fertilizer factory effluents. *Bull. Environ. Contam. Toxicol.* 49, 137-144.
- Allen M.B. and Arnon D.I. (1955). Studies on the nitrogen fixing blue-green algae. I. Growth and nitrogenfixation by *Anabaena cylindrica* Lemm. *Plant Physiol.* 30, 366-72.
- Amin P.M. (1969). Studies on certain aspects of sewage purification. Ph.D thesis, M.S. University of Baroda, Baroda.
- APHA, AWWA and WPCF (1976). *Standard methods for the examination of water, sewage and industrial wastes*. 14 edn, New York.
- Dash A.K. and Mishra P.C. (1996a). Changes in pigment and protein content of *Westiellopsis prolifica*, a blue-green alga grown in paper mill waste water. *Microbios* 84, 257-266.
- Dash A.K. and Mishra P.C. (1996b). Changes in biomass, pigment and protein content of *Westiellopsis prolifica*, a blue-green alga grown in nutrient manipulated paper mills waste water. *Cytobios* 88, 11-16.
- El-Baroudi H.M. and Moaward S.K. (1967). Rate of BOD reduction by oxidation ponds. Part I. *J. Water Pollut. Control. Fed.* 39, 1626-1646.
- Elnabarawy M.T. and Welter A.N. (1984). Utilization of algal cultures and assays by industry. In: *Algae as Ecological Indicators*. (L.E. Shubert, Ed.) Academic Press, London, pp. 317-328.
- Fogg G.E. (1949). Growth and heterocyst production in *Anabaena cylindrica* Lemm. in relation to carbon and nitrogen metabolism. *Ann. Bot.* 13, 241-59.
- Ganapathi S.V. and Amin P.M. (1972). Studies on algal bacterial symbiosis in low cost waste treatment systems. In: *Taxonomy and Biology of BGA* (T.V. Desikachary, Ed.), pp. 483-493.



- Gotaas H.B. and Oswald W.J. (1955). *Transaction of the use of solar energy*. The Scientific Basis, Tucson Vol. 4, pp. 95-114.
- Lichtenthaler K.H. (1987). Chlorophyll and carotenoid pigments of photosynthetic biomembranes. *Meth. Enzymol.* 148, 350-382.
- Lowry O.H., Rosebrough N.J., Farr A.L. and Randall R.J. (1951). Protein measurement with folin-phenol reagent. *J. Biol. Chem.* 193, 265-275.
- Oswald W.J. and Gotaas H.B. (1957). Photosynthesis in sewage treatment. *Trans. Am. Soc. Civic Engis.* 122, 73-105.
- Oswald W.J. and Gotaas H.B., Ludwig H.F. and Lynch A.V. (1953). Algae Symbiosis in oxidation ponds. II. Growth and characterization of *Chlorella pyrenoidosa* in sewage. *Sewage Indian Wastes* 25, 27-36.
- Routray B.K., Baliarsingh P.K., Mohanty B. and Padhi S. (1991). Effect of industrial effluent on *Westiellopsis prolifica*. *J. Curr. Biosci.* 8, 35-38.
- Saxena P.N., Tewari A. and Khan M.A. (1974). Effect of *Anacystis nidulans* on the physico-chemical and biological characteristics of raw sewage. *Proc. Indian Acad. Sci.* 79, 139-146.
- Shaw B.P., Sahu A. and Panigrahi A.K. (1989). Effect of effluent from a chlor-alkali factory on nitrogen fixation ability of *Westiellopsis prolifica*. *Microbios Lett.* 42, 91-96.
- Subramanian G. and Shanmugasundaram S. (1986). Sewage utilization and waste recycling by cyanobacteria. *Indian J. Environ. Health* 28, 250-253.