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

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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 proteinas, 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 proteinas, clorofila y carotenoides el dia 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 dias debido a la accesibilidad a nutrientes adicionales del agua de drenaje. El intervalo de crecimiento de la biomasa fue de 7 a 25 %. Asi 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 Shannugasundaram 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

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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 MgSO₄, NaCl, CaCl₂, K₂HPO₄, and Fe-EDTA and micronutrients such as MnCl₂, H₂BO₃, Na₃MoO₄, CuSO₄ and ZnSO₄. The cultures were maintained in Erlenmeyer flasks in triplicate at a temperature of 28±2°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 Brahajajnagar, 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 (µg/ml) = 12.25 A₆₄₆₈ - 2.79 A₆₆₃₂.

**Carotenoid content**

The carotenoid content was measured at 470 nm and calculated using the formula of Lichtenthaler (1987) i.e. total carotenoid content(µg/ml) = [1000 X A₄₇₀ -1.82 X total chlorophyll (µg/ml)]/198.

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

<table>
<thead>
<tr>
<th>Concentration of waste water (%)</th>
<th>Basal nutrient medium 10 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (DDH)</td>
<td>+</td>
</tr>
<tr>
<td>25</td>
<td>+</td>
</tr>
<tr>
<td>50</td>
<td>+</td>
</tr>
<tr>
<td>75</td>
<td>+</td>
</tr>
<tr>
<td>100</td>
<td>+</td>
</tr>
<tr>
<td>100</td>
<td>-</td>
</tr>
</tbody>
</table>

+ 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

<table>
<thead>
<tr>
<th></th>
<th>Chlorophyll <em>a</em> (mg/ml) WW</th>
<th>WW</th>
<th>SEW</th>
<th>% increase</th>
<th>Carotenoids (mg/ml) WW</th>
<th>WW</th>
<th>SEW</th>
<th>% increase</th>
<th>Protein (mg/ml) WW</th>
<th>WW</th>
<th>SEW</th>
<th>% increase</th>
<th>Biomass (mg/ml) WW</th>
<th>WW</th>
<th>SEW</th>
<th>% increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.384</td>
<td>2.460</td>
<td>3.187</td>
<td></td>
<td>0.584</td>
<td>0.662</td>
<td>13.356</td>
<td></td>
<td>223.582</td>
<td>234.045</td>
<td>4.679</td>
<td></td>
<td>1012.666</td>
<td>1101.333</td>
<td>8.755</td>
<td></td>
</tr>
<tr>
<td>25% WW with BNM</td>
<td>2.702</td>
<td>2.833</td>
<td>4.848</td>
<td></td>
<td>0.612</td>
<td>0.793</td>
<td>29.575</td>
<td></td>
<td>242.181</td>
<td>265.721</td>
<td>9.720</td>
<td></td>
<td>1053.333</td>
<td>1134.333</td>
<td>7.689</td>
<td></td>
</tr>
<tr>
<td>50% WW with BNM</td>
<td>1.952</td>
<td>2.945</td>
<td>50.870</td>
<td></td>
<td>0.762</td>
<td>0.953</td>
<td>25.065</td>
<td></td>
<td>223.582</td>
<td>278.508</td>
<td>24.566</td>
<td></td>
<td>1077.666</td>
<td>1206.666</td>
<td>11.970</td>
<td></td>
</tr>
<tr>
<td>75% WW with BNM</td>
<td>1.733</td>
<td>3.056</td>
<td>76.341</td>
<td></td>
<td>0.943</td>
<td>0.990</td>
<td>4.984</td>
<td></td>
<td>257.021</td>
<td>286.654</td>
<td>4.226</td>
<td></td>
<td>1114.333</td>
<td>1246.333</td>
<td>11.845</td>
<td></td>
</tr>
<tr>
<td>100% WW with BNM</td>
<td>3.120</td>
<td>3.485</td>
<td>11.698</td>
<td></td>
<td>1.001</td>
<td>1.154</td>
<td>15.284</td>
<td></td>
<td>281.124</td>
<td>293.991</td>
<td>4.576</td>
<td></td>
<td>1158.666</td>
<td>1293.666</td>
<td>16.651</td>
<td></td>
</tr>
<tr>
<td>100% WW without BNM</td>
<td>0.737</td>
<td>1.562</td>
<td>111.940</td>
<td></td>
<td>0.218</td>
<td>0.820</td>
<td>276.146</td>
<td></td>
<td>268.628</td>
<td>302.629</td>
<td>12.657</td>
<td></td>
<td>518.000</td>
<td>649.666</td>
<td>25.904</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>2.104</td>
<td>2.723</td>
<td>43.147</td>
<td></td>
<td>0.686</td>
<td>0.895</td>
<td>60.737</td>
<td></td>
<td>249.353</td>
<td>276.923</td>
<td>10.070</td>
<td></td>
<td>989.110</td>
<td>1105.332</td>
<td>12.969</td>
<td></td>
</tr>
</tbody>
</table>

WW - Waste water  
WWWS - Waste water without sewage  
SEWW - Sewage enriched waste water  
BNM - 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 µg 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](image)

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.

![Graph](image)

**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 (24th 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.

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**REFERENCES**


