Enhancement in growth and primary photochemistry of thylakoids of *Anabaena doliolum* treated with papermill wastewater.

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**Papers presented in National Seminars**

1. Effluent induced changes in the primary photochemistry of thylakoids and nitrogen fixation in *Anabaena doliolum* Bharadwaj

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2. Effluent induced changes in the vegetative cells of *Anabaena doliolum* Bharadwaj grown in different concentrations of papermill wastewater.

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Enhancement in growth and primary photochemistry of thylakoids of *Anabaena doliolum* treated with papermill waste water

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Abstract

Papermill waste water with important ions as its constituents has been shown to modulate growth and photosynthetic activity of *Anabaena doliolum*, a filamentous nitrogen fixing blue-green alga. The waste water stimulated growth in terms of chlorophyll content and cell density. The high growth rate of the organism was attributed to waste water induced enhancement of primary photochemical reactions associated both with photosystem I and photosystem II of the thylakoids. Photosynthetically essential ions such as Mg²⁺, Ca²⁺ and Cl⁻ which are major components of the waste water, in addition to its enrichment with nitrogen, may possibly modulate the efficiency of photochemical reactions of thylakoid membranes of the organism during treatment.

Introduction

Papermills are one of the major sources of waste water generation (Waghmare *et al.*, 1986). Since conventional physicochemical treatment methods are cost prohibitive, attempts are being made, particularly in developing countries, to search for low cost biological treatment methods. In order to produce suitable and efficient biological treatment systems, it is necessary to understand the mechanism of interaction between the waste water and the organisms so that improvement in the treatment systems can be achieved through manipulation.

Algae often serve as excellent indicators of pollution since they respond typically to many ions and toxicants (Elanbarawy and Welter, 1984). Indeed, cyanobacteria are ideally suited to play a dual role of cleansing waste water in the process of effective utilization of different constituents essential for growth leading ultimately to enhanced biomass production.

Although reports on the capacity of blue green algae in utilizing and assimilating toxic components are available (Walsh and Garnas, 1979; Walsh and Merrill, 1984; Subramanian and Shanmugasundaram, 1986), this work is the first attempt to examine the effect of these components on the photochemistry of thylakoids, the initial events of the photosynthetic process.
Materials and methods
Organism and culture condition
Strains of the filamentous, nitrogen-fixing blue-green alga, *Anabaena dolioiulm* Bharadwaj were obtained from the Department of Botany, Utkal University, Bhubaneswar, Orissa. The algae were cultured in BG110 medium without any combined nitrogen source (Stainer and Cohn-Bazire, 1977) at 30 ± 2°C in continuous cool white fluorescent light at an intensity of 12 Wm⁻². The culture medium was sterilized prior to inoculation of the organism and the algal culture was hand shaken twice daily.

Experimental work
The waste water collected from the combined waste water drain of Orient Papermills, Brajarajnagar, Orissa, was analysed to determine its physicochemical characteristics according to the APHA (1989). The waste water was filtered through cotton to separate the particulates and then sterilized before use.

The treatments employed were (1) sterilized BG110 nutrient medium used as a control; (2) sterilized 75% waste water supplemented with BG110 nutrient medium used in experiment one; and (3) sterilized 100% waste water without BG110 nutrient medium used in experiment two. The work was conducted under controlled conditions in Erlenmeyer flasks in triplicate for period of 20 days.

Biochemical analysis
For growth and pigment estimations the samples of algae were homogenized for 5 min in their respective nutrient media. The samples were then thoroughly shaken and scattering at 760 nm was measured with a UV-visible spectrophotometer (Shimadzu 150-02). Chlorophyll *a* was extracted in 80% chilled acetone at room temperature in dim light and estimated according to Talling and Driver (1961).

The algal cells were homogenized by the solid shear method (Graham and Smillie, 1971). A thick paste of 1 g wet weight of algal cells was ground with glass beads in a glass homogenizer for 20 min. Electron transport activity was assayed polarographically using a Clark type of oxygen electrode (Hansatech, England) according to Murthy *et al.* (1989). The 2 ml of reaction mixture for the assay of whole chain electron transport contained a suspension buffer at pH 6.5 (20 mM phosphate buffer, 10 mM NaCl, 10 mM KCl, 2.5 mM MgCl₂, 10 μM EDTA, 0.34 M sucrose and 0.5 mM methyl viologen). The reaction mixture for reduced (dichlorophenol indophenol) DCPIP/ascorbate supported photosystem I (PS I) catalysed electron transport consisted of suspension buffer at pH 6.5, 5 mM ascorbate, 0.1 mM DCPIP, 2 μM 3(3,4-dichlorophenyl)-1, 1-dimethyl urea.
(DCMU), and 0.5 mM methyl viologen. The reaction mixture used for the assay of photosystem II (PS II) catalysed electron transport contained suspension buffer (pH 7.5) and 1 mM ferricyanide (FeCN). In all the assays, chloroplasts equivalent to 30 μg of chlorophyll were used. The reaction rates were expressed as μmole O₂ evolved or consumed per mg chlorophyll per h.

**Results**

Pilot experiments were carried out for a concentration profile analysis. In this experiment the growth in terms of increase in biomass of *A. doliolum* in response to different concentrations of papermill waste water (data not shown) revealed an increase of up to 75% concentration in combination with nutrient medium compared with BG₁₁₀ nutrient medium alone (control).

At 100% waste water without nutrient medium the growth was retarded. The enhancement of growth of *Anabaena* at 75% waste water with BG₁₁₀ nutrients might be due to the phosphorus supplement from nutrient medium along with magnesium and other nutrient components. The retarded growth of *Anabaena* in the 100% waste water...

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tbody>
<tr>
<td>Colour</td>
<td>Brown</td>
</tr>
<tr>
<td>pH</td>
<td>8.3</td>
</tr>
<tr>
<td>Biological oxygen demand (BOD)</td>
<td>200</td>
</tr>
<tr>
<td>Chemical oxygen demand (COD)</td>
<td>800</td>
</tr>
<tr>
<td>Dissolved oxygen (DO)</td>
<td>2.5</td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>0–3</td>
</tr>
<tr>
<td>Calcium</td>
<td>65–120</td>
</tr>
<tr>
<td>Sodium</td>
<td>148–190</td>
</tr>
<tr>
<td>Potassium</td>
<td>6–14</td>
</tr>
<tr>
<td>Magnesium</td>
<td>20–25</td>
</tr>
<tr>
<td>Chloride</td>
<td>55–80</td>
</tr>
<tr>
<td>Sulphate</td>
<td>83–120</td>
</tr>
<tr>
<td>Total alkalinity (as CaCO₃)</td>
<td>88–130</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0–3</td>
</tr>
</tbody>
</table>

Except for pH, the data are given in parts per million (ppm).

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water may be attributed to a negligible amount of phosphorus in the papermill waste water, high sodium content and the brownish colour of the effluent contributing to a screening effect for light absorption. Based on a pilot study 75% waste water with nutrients of BG_{11(0)} medium, and papermill waste water without nutrients of BG_{11(0)} were chosen for further experimental research.

The physicochemical characteristics of papermill waste water (Table 1) revealed that the waste water was alkaline with a brownish colour and had a high biochemical oxygen demand. It did not conform to the ISI standard (Indian Standards Institute) prescribed for discharge of industrial waste water into open waters.

*Anabaena doliolum* was found to grow both in 75% waste water supplemented with BG_{11(0)} nutrients as well as without nutrients, but the growth was relatively slow in 100% compared with 75% waste water with nutrient medium (Figure 1). Similar findings were obtained with the chlorophyll a content (Figure 2). Thus 75% waste water supplemented with BG_{11(0)} nutrient medium was the concentration which had a stimulatory effect on the growth of the organism.

![Figure 1](image_url)

*Figure 1* Changes in the growth characteristics of *A. doliolum* treated with different concentrations of waste water. O, Control; •, 75% effluent; A, 100% effluent.
Figure 2  Changes in the content of chlorophyll a of *A. dolio lum* treated with different concentrations of waste water. ○, Control; ●, 75% effluent; △, 100% effluent.

To determine whether the primary reactions of photosynthesis were affected by different concentrations of papermill waste water, the whole chain electron transport activity of the alga was measured. Methyl viologen dye which is known to accept electrons from the reducing side of PS I, was used to measure whole chain electron transport (from H₂O to MV). For PS I mediated partial photochemical reactions, reduced dichlorophenol indophenol (DCPIP/ascorbate) was used as the electron donor and PS II photochemical efficiency was probed using ferricyanide as an artificial electron acceptor. The rate of O₂ evolution was measured independently under saturating illumination.

Electron transport activities through the entire electron transport chain, and partial photochemical reactions of PS I and PS II activities were maximal in the organism grown in 75% waste water in combination with BG₁₁₀ nutrient medium, and minimal in 100% waste water without nutrient medium in comparison with controls (Table 2). Furthermore, the activities were maximal on day 12 or in
Table 2 Electron transport activities in *A. doliolum* grown in papermill waste water.

<table>
<thead>
<tr>
<th>Days</th>
<th>Treatment</th>
<th>Assay (μmole O₂ consumed or evolved mg chlorophyll⁻¹ h⁻¹):</th>
<th>Whole chain</th>
<th>PS I</th>
<th>PS II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>Control (BG11(0) nutrient medium only)</td>
<td>58.44 ±14.45</td>
<td>233.51 ± 14.0</td>
<td>83.411 ± 13.95</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Control</td>
<td>95.0 ± 10.34</td>
<td>310.64 ± 9.45</td>
<td>119.48 ± 8.46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>75% waste water with nutrient medium</td>
<td>113.05 ± 9.78</td>
<td>322.21 ± 19.96</td>
<td>141.31 ± 9.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100% waste water without nutrient medium</td>
<td>83.00 ± 20.74</td>
<td>282.06 ± 11.98</td>
<td>110.67 ± 12.0</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Control</td>
<td>75.59 ± 11.89</td>
<td>261.13 ± 23.8</td>
<td>96.2 ± 11.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>75% waste water with nutrient medium</td>
<td>85.96 ± 11.45</td>
<td>277.73 ± 19.84</td>
<td>99.19 ± 19.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100% waste water without nutrient medium</td>
<td>68.38 ± 14.8</td>
<td>239.34 ± 13.9</td>
<td>94.02 ± 29.6</td>
<td></td>
</tr>
</tbody>
</table>

Whole chain: H₂O→PS II→PS I-MV. PS I with Cyt b₉ ascorbate/DCPIP→MV. PS II: H₂O→FeCy.

the logarithmic phase of growth. On day 20 the electron transport activities declined in the controls as well as in the treated samples (Table 2).

**Discussion**

It was recorded that 75% waste water supplemented with BG₁₁(0) nutrient medium had a stimulatory effect on the growth of *Anabaena*. Inoculated cyanobacteria are known to grow fairly well in different types of waste water and the growth is measured in terms of chlorophyll *a* content (Sallal, 1986; Subramanian and Shanmugasundaram, 1986). The growth of the alga was relatively slow in 100% waste water without nutrients. The relative slow growth in 100% effluent may be attributed to a brownish colour with high COD and sodium ion concentration (Manoharan and Subramanian, 1992). The brownish colour of the effluent obstructed photosynthetically active light thus imparting a screening effect. Furthermore, the papermill waste water contained a negligible amount of phosphorus necessary for algal
growth (Table 1). This was evident from the observation that the chlorophyll a content was maximal when the organism was grown in 75% waste water (Figure 2) supplemented with BG110 nutrient medium containing phosphorus as one of the major components.

In view of the stimulating action of the effluent in combination with BG110 on growth of the alga, the precise mechanism of growth stimulation was examined. The effluent contained a significant amount of Mg²⁺, Ca²⁺, and Cl⁻, which are the ions necessary to carry out the light reaction of photosynthesis. Whether or not these ions might enhance the growth rate of the organism by modulating the photochemical reactions associated with PS I and PS II of the thylakoids is unknown. These ions specifically modulate the structural stability of the oxygen-evolving complex PS II in the thylakoids (Murata and Miyao, 1987). The maximum electron transport activities of the alga in 75% waste water supplemented with BG110 may be due to the presence of large amounts of essential nutrients like Mg²⁺, Ca²⁺, and Cl⁻ in addition to nitrogen in a moderate quantity from the waste water, and a significant amount of phosphorus from the growth medium. The low electron transport activity of the organism in 100% effluent in the absence of phosphorus, known to be a major limiting factor for cyanobacterial growth under natural conditions (Whitton, 1992), may contribute to its reduced growth rate and chlorophyll a content. The declining electron transport activities on day 20 in controls as well as treatments were due to the nutrition depletion phase of the organism. In addition, any shading effect could be the cause of a decline in photochemical activities.

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