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Simultaneous biomass production and mixed-origin wastewater treatment by five environmental isolates of Cyanobacteria

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Wastewater treatment by cyanobacteria is advantageous in many senses. In the present study, biological treatment of wastewater by five different environmental isolates of cyanobacteria was investigated individually under general tropical climatic conditions. Each cyanobacterial strain reduced the BOD, COD, nutrient levels and bacterial count of wastewater considerably. The marine isolate of *Phormidium* was found to be the most effective amongst the strains studied. Biochemical observations like those of chlorophyll a and protein content of wastewater treating strains were also made. Biomass production in wastewater was comparable to that of cultural media. Cyanobacterial biomass production side by side with wastewater biotreatment could have a better economic prospect.

Key words: bioremediation, wastewater, cyanobacteria, *Phormidium*, biomass, nutrient, sewage

INTRODUCTION

With the exponentially increasing population pressure and the huge amount of wastewater thus generated, biological wastewater treatment systems have drawn the interest of scientists in recent years [1, 2]. Biological treatment is a cheap, efficient and sustainable alternative to conventional chemical wastewater treatment and recycling. Cyanobacteria, being photoautotrophic and some of them being capable of atmospheric nitrogen fixation, are ideal organisms for biological treatment as they are inexpensive and easy enough to maintain. The use of cyanobacteria in wastewater treatment could prove beneficial as they would also increase oxygenation and mineralization. Cyanobacterial biomass is also economically useful for different byproducts such as amino acids, proteins and vitamins. Wastewater can thus be treated in waste stabilization ponds by cyanobacteria, alone or in symbiosis with heterotrophic bacteria, for reuse of treated wastewater in aquaculture or agriculture and for production of microbial biomass for beneficial uses [3].

Different species of cyanobacteria are normally found in wastewater [4, 5].The effect and extent of introduced and natural cyanobacteria in the removal of nutrients as nitrogenous and phosphorus compounds, which may lead to cultural eutrophication, have been studied by several workers [6–11]. Recently, workers all over the world are searching for novel strains of bacteria with high biomass yields and a high utilization fluorescent illumination (70–80 µmole photon m^{-2} s^{-1}, 14 + 10 light / dark phases) at a normal tropical temperature of 30 ± 2 °C with regular bubbling aeration.

PARAMETERS STUDIED

Wastewater samples were collected in sterilised bottles and preserved at a temperature below 4 °C. Analyses of physicochemical parameters (temperature, pH, BOD_5, COD, nitrate, ammonia and total phosphorus) and total bacteria were done within 24 hours by standard methods [15, 16].

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Wastewater treatment

As our prime objective was to study the effects of individual cyanobacteria on nutrient reclamation from wastewater and their biomass production, 1 l wastewater samples in bioreactors were inoculated with 5–8 ml of fresh cultures of different species (to reach an initial inoculum concentration of about 0.5 μg chlorophyll a ml^{-1} sample) and incubated in the cultural conditions given above. Aliquots of each sample were analyzed for physicochemical parameters on the 7th, 14th and 21st days of incubation, after harvesting cultures by 2000 g (10 min) centrifugation and filtration through Whatman-42. The aliquots were tested directly also for total bacterial count.

Biochemical estimation of biomass yield

After the necessary incubation period, microbial cultures were harvested and the chlorophyll-a content [17] and protein content [18] were estimated to assess cyanobacterial biomass yield.

Experimental design

Each experiment was replicated thrice, and also sample analyses were done in triplicate. Respective negative control sets were run simultaneously with wastewater treatment and biomass yield studies. Statistical analysis was performed in ANOVA at $p \leq 0.05$.

RESULTS AND DISCUSSION

The nutrient levels of raw wastewater were found to be moderate in nature (Table 1). The biological oxygen demand (BOD) and chemical oxygen demand (COD) levels were also high for the further direct reuse in agriculture or aquaculture. A preliminary study showed that all the cyanobacteria in question were growing quite efficiently in both diluted and raw wastewater. However, the biomass yield was higher in undiluted wastewater. As the bioremediation and biomass yield characteristics were more significant in undiluted effluent, only those results were presented here.

The percentage of BOD reduction was highest on the seventh day, except for *Spirulina platensis* strain-I, which had reduced BOD by 60.9% over control by the third week of incubation (Fig. 1a). *Spirulina platensis* strain-II showed

Table 1. **Physicochemical and microbial parameters of raw wastewater**

Temperature (°C)	30.6 ± 2.3
pH	7.3 ± 0.2
BOD_5 (mg l^{-1} O_2)	93.4 ± 4.1
COD (mg l^{-1} O_2)	135.0 ± 11.5
Nitrate-content (mg l^{-1})	0.81 ± 0.06
Ammonia (mg l^{-1})	22.8 ± 1.7
Total phosphorus (mg l^{-1})	25.55 ± 0.24
Total bacterial count (cfu)	4.6×10^8

a maximum reduction of 69.6% on the 7th day. While in all other cases the BOD reduction levels decreased over the second and third weeks, *Phormidium valderianum* BDU 40271 maintained the BOD reduction level at about 60% and even elevated it to 68.6% in three weeks. During the initial period, BOD was reduced very quickly by the photoautotrophic activity of cyanobacteria. The oxygen generated by the cyanobacteria increased the amount of dissolved oxygen in samples, thus reducing the BOD values.

Data of COD reduction showed an interesting variation from the BOD reduction studies (Fig. 1b). COD reduction was highest on 14th day. *Spirulina platensis* strain-I was found to remove COD too, uniformly with time, as in the case of BOD. *Phormidium valderianum* BDU 40271 was most suitable to reduce both BOD and COD. The result was different from the observation made by Govindan [4] in case of dairy wastewater where COD removal was not as good as in the case of domestic water.

Unicellular green algae were found to be effective in the removal of nutrients such as nitrate and phosphorus [19]. In the present study, there was a significant removal of nitrate in most cases. The decline in nitrate content was proportionate with incubation time and the growth of organisms (Fig. 1c). *Phormidium valderianum* BDU 40271 showed

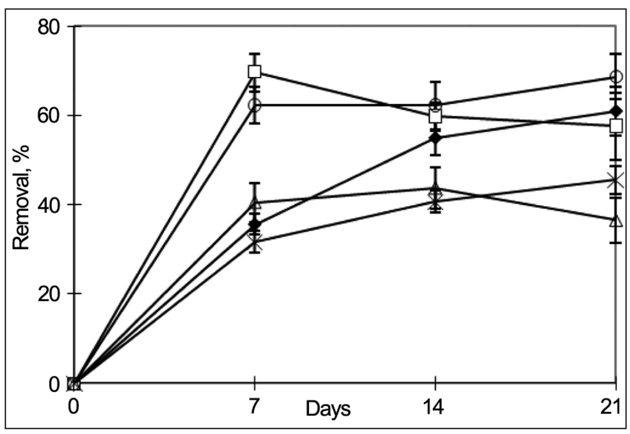

Fig. 1a. Change of BOD, %

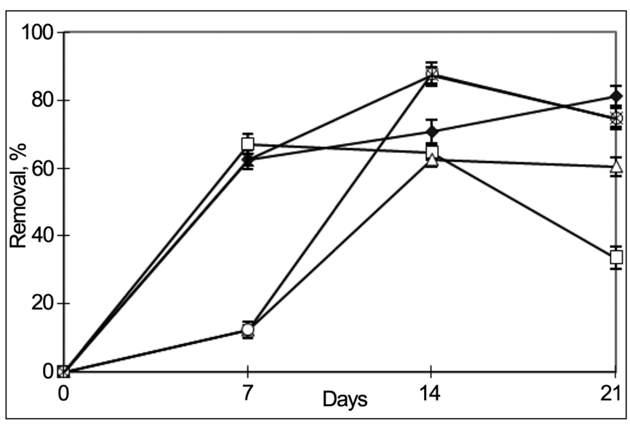

Fig. 1b. Change of COD, %

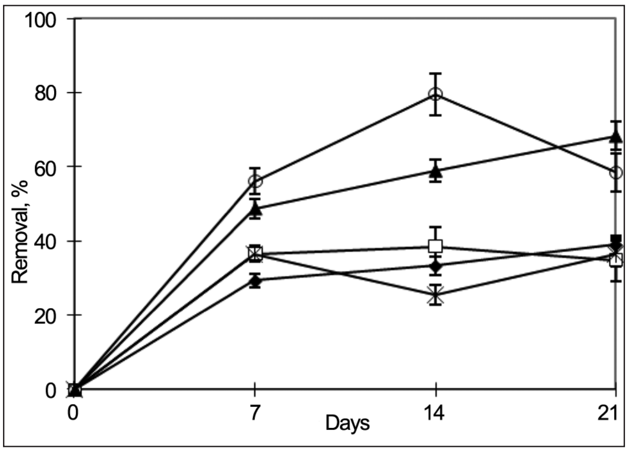

Fig. 1c. Change of nitrate, %

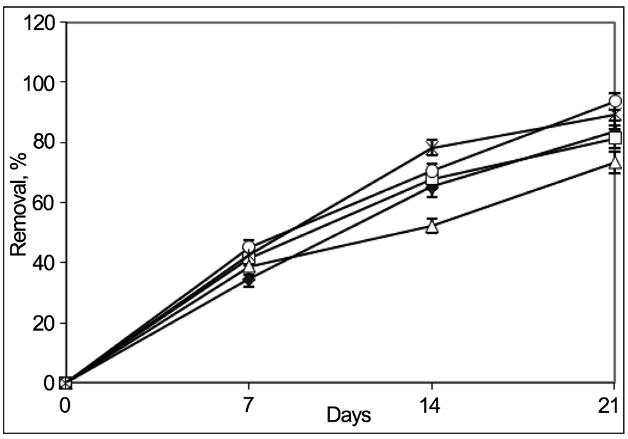

Fig. 1d. Change of ammonia, %

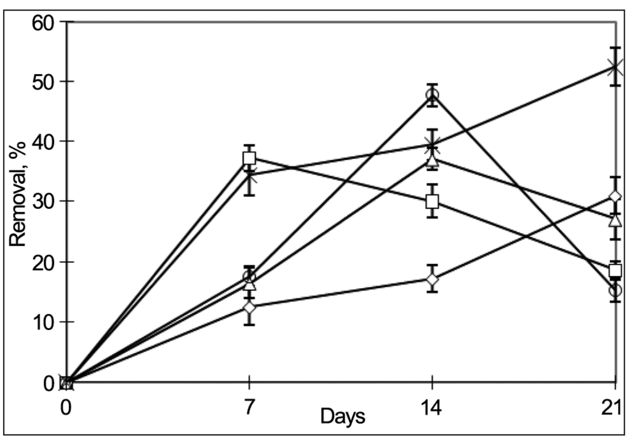

Fig. 1e. Change of total phosphorus, %

Fig. 1(a–e). Weekly change of physicochemical parameters of wastewater under incubation with respective cyanobacterial strains. Results shown over respective control sets and in mean values

good results in removing these nutrients. The positive relationship of nitrate removal with growth indicates that these cyanobacteria might be utilizing nitrate from wastewater. These autotrophic cyanobacteria, which could be easily filtered out, represent a possible nitrate-removal treatment system instead of heterotrophic bacteria for groundwater and drinking water [20].

Ammonia present in wastewater is comparatively high in concentration and should be removed before disposal. Strains showed a good removal percentage after two weeks of incubation (Fig. 1d). *Phormidium* strain removed more than 90% of ammonia in three weeks. Ammonia is one of the principal sources of nitrogen for bacteria. Oswald and Gotaas [21] showed almost all ammonia in sewage to become converted in the form of algal cell-protein.

The increasing use of detergents has increased the content of phosphates in wastewater. The conventional technology for microbial removal of phosphorus is activated sludge treatment, sometimes accompanied with the enhanced biological phosphate removal (EBPR) technology. However, until recently, very few bacteria able to remove phosphates have been singled out [22]. Several cyanobacteria were known to be active in phosphorus reclamation [23, 24]. The total phosphate content was reduced moderately proportionately with incubation time (Fig. 1e).

Cyanobacterial incubation reduced the total bacterial count even within seven days of incubation in all cases, except in control where the total count actually increased (Table 2). This study showed that these cyanobacterial strains could grow well in wastewater and could reduce the nutrient level as well as microbial count to a moderately significant level. Although the *Phormidium* strain concerned was a marine one, it was proved to be a good nutrient removal agent.

The biochemical changes in wastewater were estimated in terms of chlorophyll a. The rate of growth was very high during the first week, but later it decreased (Fig. 2). The growth was almost stagnant after three weeks, seemingly because of nutrient depletion. In most cases, the growth was optimum on the second week after which it somewhat decreased. The protein content (on the fourteenth day after the optimum growth) was found to be comparable with that of an organism grown in appropriate culture media without adding an extra carbon source or minerals (Fig. 3). Bhagwat and Apte also noticed a similar trend for *Anabaena* sp. [25].

From the above studies it may be concluded that the wastewater concerned is suitable for mass-culture of cyanobacteria which, in turn, can sufficiently reduce nutrient levels and microbial count in sewage. The strains showed varied reduction levels of nutrients. These cyanobacterial isolates thus represent a suitable alternative or rather a supplementary option to be incorporated in the conventional system. In waste recycling systems like Solar Aquatics [26, 27], the potentiality of cyanobacterial biomass production is immense. Large-scale studies are to be made to select an appropriate wastewater-cyanobacterial system for biological treatment as well as biomass production under definite environmental conditions.

Table 2. **Change of total bacterial counts in wastewater under incubation with Cyanobacteria**

Organisms	Weekly change of total bacterial count			
	Day 0	Day 7	Day 14	Day 21
Spirulina platensis-I	4.6×10^8	8.7×10^6	8.1×10^3	3.5×10^4
Spirulina platensis-II	4.6×10^8	2.2×10^7	6.0×10^5	2.4×10^5
Spirulina subsulsa	4.6×10^8	6.4×10^7	1.4×10^6	4.8×10^6
Phormidium valderianum	4.6×10^8	6.2×10^7	9.2×10^5	2.6×10^6
Oscillatoria-Lyngbya	4.6×10^8	3.8×10^7	4.5×10^4	5.8×10^3
Control	4.6×10^8	3.5×10^9	8.2×10^8	5.0×10^8

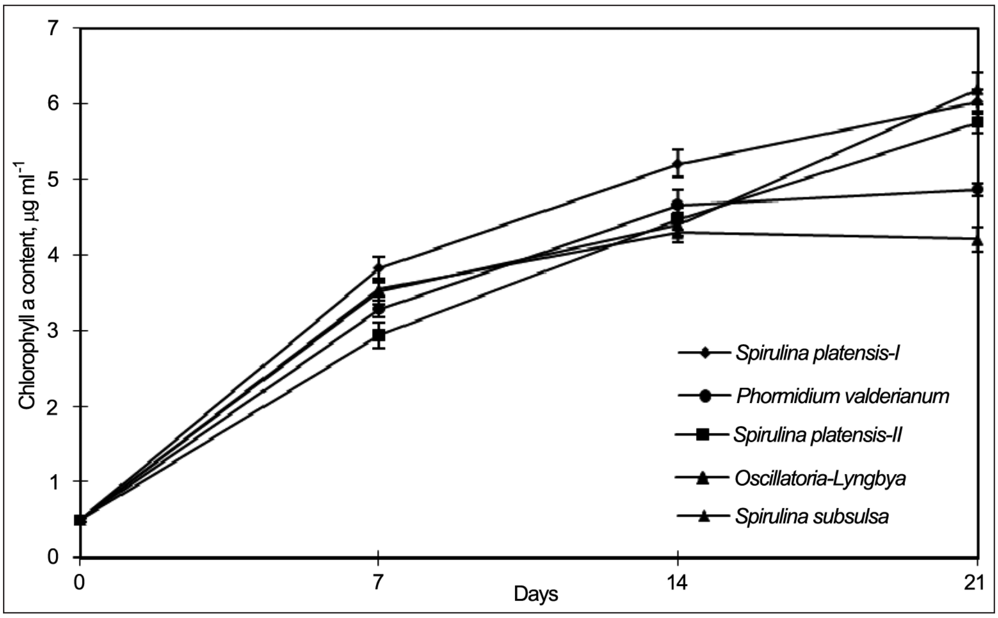

Fig. 2. Biomass yield (chlorophyll a content) of Cyanobacteria in wastewater effluent

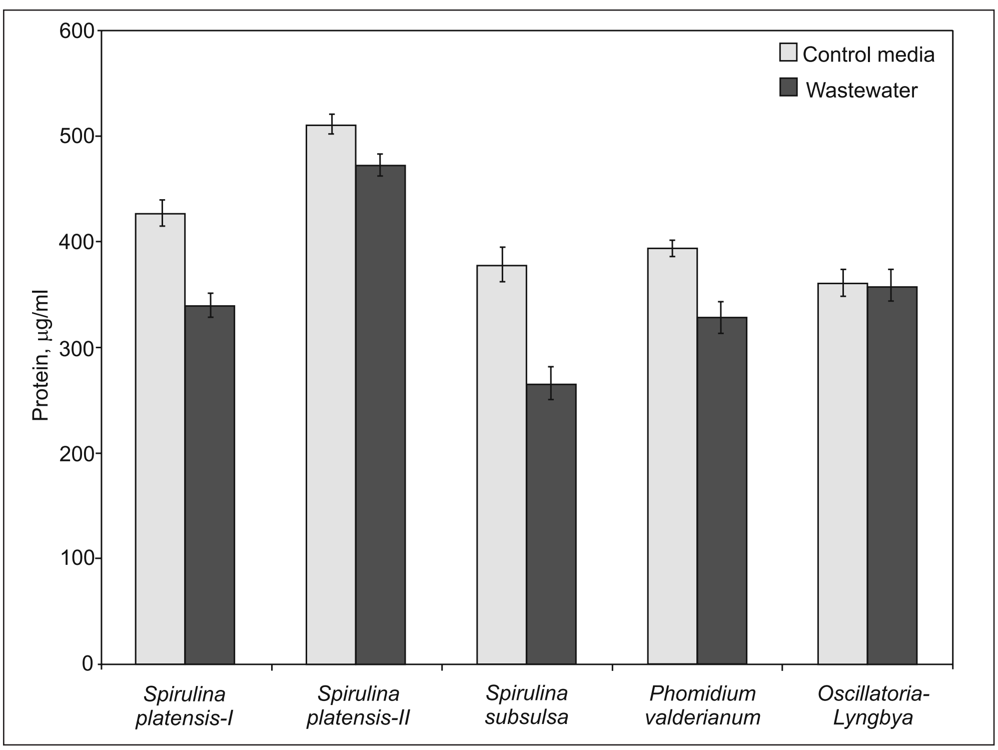

Fig. 3. Comparison of total protein content of Cyanobacteria after two weeks of incubation in control medium and wastewater

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