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Physiological studies of native cyanobacterial species *Lyngbya contorta* and *Phormidium foveolarum* in sewage waste water

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Abstract

A variety of Cyanobacterial species predominantly ensheathed forms occurs in sewage water receiving areas. A study was conducted to analyse the potential of using native *Lyngbya contorta* and *Phormidium foveolarum* isolated from sewage water irrigated soils, for biomass production under sewage waste water. The native Cyanobacterial strains were characterised and changes in their biochemical composition in response to different concentrations of sewage waste water were investigated. Results showed that biomass(3.5-6.6mg $10ml^{-1}$, 2.6-5.6mg $10ml^{-1}$) and photosynthetic pigment contents increased with incubation time (chlorophyll 1.21-3.09 μg ml^{-1}, 1.92-9.51 μg ml^{-1}; carotenoid 20.8-34.8 μg ml^{-1}, 16.4-32.8 μg ml^{-1}) and decreased thereafter as nutrients became limiting. On the other hand, soluble proteins, after showing a decline, recovered faster with maximum concentration(42.6-63.3 μg ml^{-1} and 59-79.8 μg ml^{-1}) recorded on day 8. Total carbohydrate content also increased (19.27-31.45 μg ml^{-1}, 14.1-28.21 μg ml^{-1}) in response to various concentrations of sewage waste water. The overall response was better for 50% sewage waste water concentration which showed that these native strains were suitable candidates for cultivation after proper dilution.

Key words

Biomass, Carbohydrates, Cyanobacteria, Photosynthetic pigments, Soluble proteins

Introduction

Cyanobacteria are prokaryotic group of algae, which have attracted commercial interest for their potential to produce valuable products in bulk with their high biomass production (Masojídek *et al*., 2008). Several aspects of cyanobacteria such as oxygenic photosynthesis, high productivity, non-food based feedstock, growth on non-productive and non-arable land, utilization of wide variety of water sources (fresh, brackish, seawater and wastewater) and production of valuable co-products along with biofuels have combined to capture the interest of researchers and entrepreneurs(Parmar *et al.,* 2011). The most basic approach to cyanobacterial mass production involves use of large area, open ponds, if sewage waste water can be utilized for their mass cultivation, it will be able to save fresh water resources. Sewage waste water may contain many different classes of contaminants including pathogens, viruses, heavy metals and nutrients, as well as organic compounds (Gallegos *et al.,* 1999). Organics in raw wastewater include humic substances, faecal matter, kitchen wastes, detergents, oils, drugs and industrial wastes. Concentration of these compounds in sewage depend on the waste input and treatment technology used in wastewater treatment plants(Khatri and Dhankhar, 2003).

Effect of inorganic and organic chemicals influence growth rate and development of cyanobacterial cells(Alexander, 2005). However, moderate stress is essential for normal growth and differentiation of metabolic and physiological systems of an organism. Cyanobacteria are affected by sewage waste water containing major nutrients such as phosphorus and nitrogen. They are also known to take up and assimilate organic compounds, which can play secondary, albeit important roles in their metabolism(Venugopal *et al.,* 2006) and produce plant growth promoting substances(Sergeeva *et al.,* 2002). However, bio-mixture of *Nostoc Muscorum* and *Anabaena subcylindrica* grown in sterilized sewage wastewater, *Anabaena subcylindrica* grown in El-Soda Company and Verta Company sterilized wastewater showed low content of pigments and protein compared to synthetic medium (El-Enany and Issa, 2000). Sedimentary humic acids in presence of iron reduced growth and production of chlorophyll"a" by 50% as compared to the cultures exposed only to iron at same concentration (Kosakowska, 2007).

The present investigation aimed towards quantifying the effects of sewage water concentration on Cyanobacterial growth and biomass production and to test sewage waste water as a suitable medium for cultivation of Cyanobacteria.

Materials and Methods

The sewage waste water samples were collected from main sewage outlet of Rohtak city, Haryana, India, in sterilized screw capped bottles. The soil samples at depth of 0-10cm were collected from sewage irrigated fields located along sewage drain. Cyanobacterial strains, *Lyngbya contorta* and *Phormidium foveolarum,* were isolated from sewage waste water irrigated soil. Pure culture was obtained by agar plate spreading and serial dilution techniques (Andersen and Kawachi, 2005) using BG-11 medium(Stanier *et al.,* 1971). Identification of cyanobacterial strains was done with the help of keys given by Desikachary (1959). Purified Cyanobacterial strains were maintained at 28 ± 3°C and illuminated under 16:8 hr light–dark cycle, using cool fluorescent tubes. Cultures were also shaken by hand two to four times daily to prevent cell clumping.

Ten ml of different concentrations of sewage waste water namely 25%, 50%, 75% and 100% along with control (BG-11 media) was taken in test tubes in triplicates. Prior to analysis, cultures were homogenized using a homogenizer. Then, one ml of inoculum of exponentially growing *Lyngbya contorta*(O.D. 668nm) and *Phormidium foveolarum* (O.D. 641nm) from broth was transferred into each of the test tube aseptically and incubated at 16:8 hr light–dark cycle at 28±3°C. The growth of cultures was followed through dry weight estimation. Chlorophyll content was estimated by the standard methods of Mckinney (1941) and carotenoid pigments by (Jensen, 1978). Physico-biliproteins were estimated by cold extraction method (Siegelman and Kycia, 1978). Total proteins were estimated by the method of Herbert *et al.,* (1971). Total carbohydrates were estimated by anthrone reagent (Spiro,1966). All the experiments were conducted in triplicates.

Two-way analysis of variance (ANOVA) was applied for determining significant differences due to application of sewage waste water and different time intervals. The data were analyzed on Microsoft Excel and SPSS 7.5 software programmes.

Results and Discussion

Exposure to sewage water treatments promoted the growth performance in *Lyngbya contorta* and *Phormidium foveolarum* with high biomass content being achieved in all concentrations of sewage waste water except for 100% sewage concentration(Fig.1a,b). Several studies have shown the inhibitory effects of humic substances, organic matter, phenolic compounds on the growth of Cyanobacterial species (Prokhotskaya and Steinberg, 2007; Kosakowska, 2007; Sun *et al.,* 2005; Park *et al.,* 2006). Humic acid exerts toxic effect by reducing bioavailability of iron, which delays and/or decreases the growth of the Cyanobacterium (Sun *et al.,* 2005). But exposure to low concentration of humic substances stimulated better growth of the phototrophs (Bährs and Steinberg, 2012). In the present study, low concentrations of sewage water were growth promoting for both the cyanobacterial strains while at higher concentrations, only minor negative effects were noted. The maximum biomass was accumulated from 4th to 8th day in both the cyanobacterial strains. Both strains showed an increased biomass accumulation at fifty percent sewage waste water concentration, which attained peak value on 8th day, thereafter it decreased. Biomass content varied significantly with increase in incubation time as well as concentrations of sewage water.

Initially, both the strains showed reduced amount of chlorophyll content at all sewage waste water concentrations, which picked up with incubation time (Fig. 2a, b). The maximum value was recorded in 50% sewage waste water concentration on 8th day. The development of internal oxidative stress due to

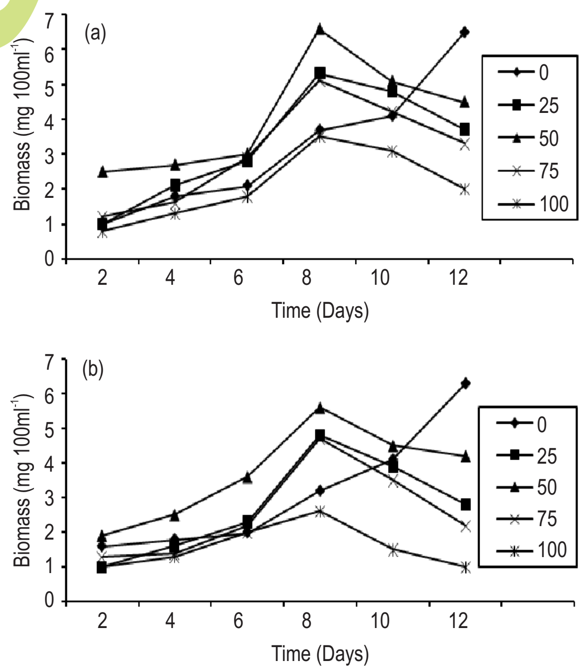

Fig. 1 : (a) Temporal variations of biomass content in *Lyngbya contorta* in response to different concentrations of sewage waste water **(b)** Temporal variations of biomass content in *Phormidium foveolarum* in response to different concentrations of sewage waste water

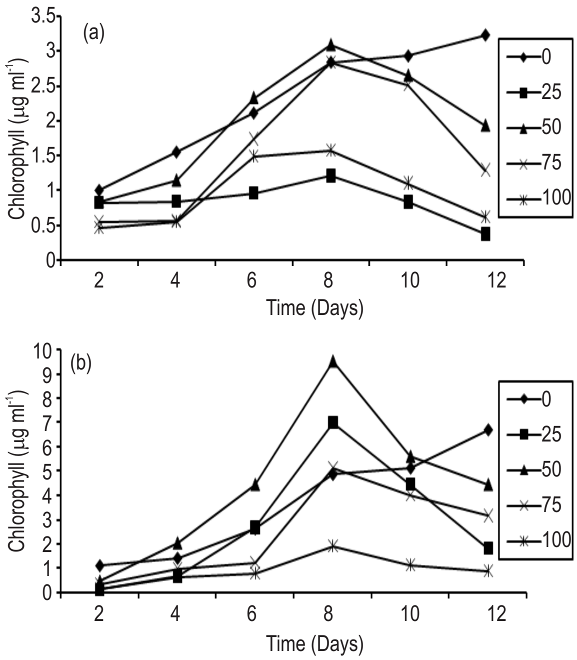

Fig. 2 : (a) Temporal variations of chlorophyll content in *Lyngbya contorta* in response to different concentrations of sewage waste water (**b**) Temporal variations of chlorophyll content in *Phormidium foveolarum* in response to different concentrations of sewage waste water

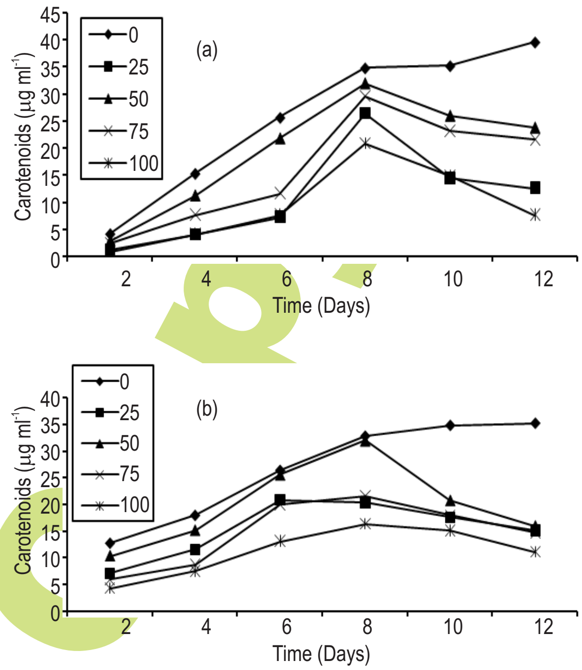

Fig. 3 : (a) Temporal variations of carotenoid content in Lyngbya contorta in response to different concentrations of sewage waste water (b) Temporal variations of carotenoid content in Phormidium foveolarum in response to different concentrations of sewage waste water

presence of toxic compounds may lead to reduced photo synthetic activity by blockage of electron transport in Hill reaction in Photosystem-II. Later on, enhancement observed in photosynthetic pigments content may be due to nitrogen contribution by sewage waste water(Shrivastava and Banerjee, 2009). It also indicate that the requirements for a large investment of energy for biosynthesis and reorganization under stress are met by enhanced photosynthetic activity. Restoration of chlorophyll would account for faster growth rates measured in the higher sewage water concentrations. However, as growth depleted nitrogen content in the medium, chlorophyll was degraded to reutilize nitrogen for growth with chlorophyll level decreasing from day 8. As chlorophyll is a nitrogen-rich compound, it is utilized as an intracellular nitrogen pool to support further cell growth and biomass production as nitrogen becomes depleted (Li *et al.,* 2008). The photosynthetic pigments in *Lyngbya contorta* did not decrease at high concentration of sewage water containing higher levels of nitrogen. Mostafa *et al.* (2005) also reported that chlorophyll content of *N. muscorum* and *A. subcylindrica* grown in sterilized sewage waste water were higher than those grown in the standard medium. Concentration of sewage water and incubation time both significantly effected Cyanobacterial photosynthetic pigments as indicated by ANOVA.

A reduction in carotenoid content was observed for both strains treated with sewage water concentrations compared to control(Fig.3a,b). The peak concentration of carotenoid pigment was observed on 8^{th} day, at 50% sewage waste water concentration. The concentration of sewage waste water as well as time had significant effect on carotenoid pigments in *Lyngbya contorta* and *Phormidium foveolarum*. The retarding effect could be attributed due to presence of total residual chlorine (as chlorine is added in domestic water supplies as disinfectant) in the sewage waste water or due to formation of chloro-organics that could inhibit Cyanobacterial growth.

In both Cyanobacterial strains, there were tendencies towards increased carbohydrate accumulation after exposure to sewage water treatments. Both strains recorded appreciable amount of carbohydrate content under different sewage waste water concentrations(except at hundred percent) compared to control (Fig. 4a,b). Carbohydrate content increased with incubation time upto 8 days and decreased thereafter. The carbohydrate content ranged from 4.92 to 31.45 $\mu g\ ml^{-1}$ and 4.56 to 28.21 $\mu g\ ml^{-1}$ in *Lyngbya contorta* and *Phormidium foveolarum,* respectively. In contrast to *Lyngbya contorta*, *Phormidium foveolarum* showed maximum carbohydrate content on 8^{th} day at 75 % of sewage waste water concentration. The statistical test indicated significant increase in carbohydrate content with increase in concentration of sewage waste water and time. Increased development of pigments are indicative of high photosynthetic activity and consequently, probable high accumulation of

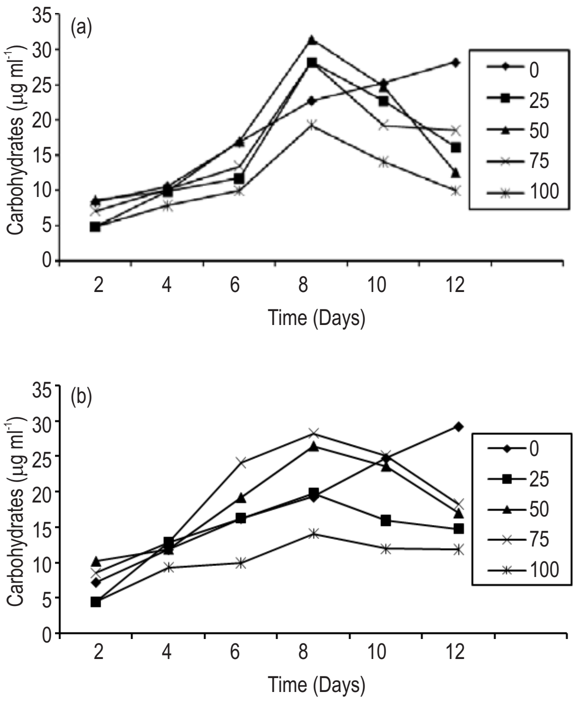

Fig. 4 : (a) Temporal variations of carbohydrate content in *Lyngbya contorta* in response to different concentrations of sewage waste water (b) Temporal variations of carbohydrate content in *Phormidium foveolarum* in response to different concentrations of sewage waste water

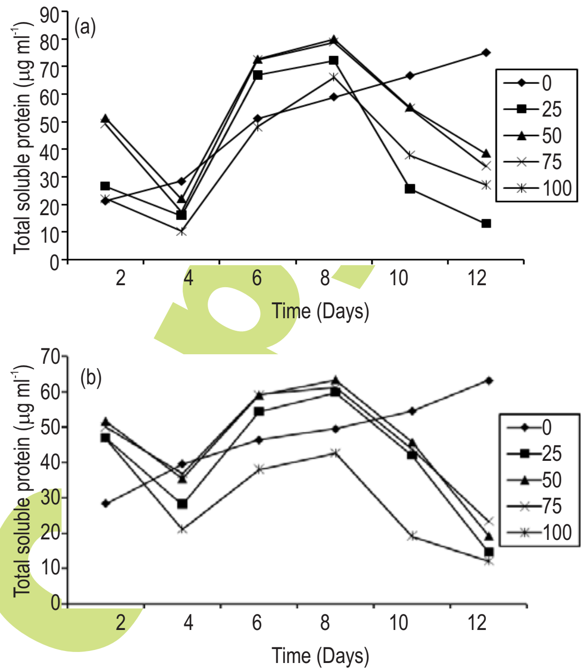

Fig. 5 : (a) Temporal variations of total soluble protein content in *Lyngbya contorta* in response to different concentrations of sewage waste water (b) Temporal variations of total soluble protein content in *Phormidium foveolarum* in response to different concentrations of sewage waste water

carbohydrate content in both cyanobacterial strains. During physical stress, microalgae tend to use available nutrients to preferentially produce carbohydrates instead of proteins(Rodolfi *et al.,* 2009).

Total soluble proteins showed an increase at the start of experiment (Fig.5a,b), then decreased on 4th day and recovered to levels above their initial concentrations from 6th –8th day and then decreased as nitrogen became limiting. The amount of soluble proteins in both strains on 8th day was maximum at 50% sewage waste water concentration. Two way analysis of variance for soluble protein content indicated significant increase in soluble protein content of both the Cyanobacterial strains with increase in sewage waste water concentration and incubation time. Protein concentrations were also related to nitrogen concentrations with cultures grown in lower nitrogen conditions *i.e.*, lower concentrations of sewage waste water, have lower protein concentrations. This may be due to the fact that nitrogen is required for protein biosynthesis, limited nitrogen affects protein concentrations that are required amongst other processes, for cell division (Ördög *et al.,* 2011). The increase in amount of total soluble protein may also be attributed to high amount of available phosphate content in sewage waste water. Phosphate assimilated by cells during growth is released upon decomposition in the form of soluble organic phosphate compounds and mineralized to orthophosphates resulting in an increase in available phosphate (Mandal *et al.,* 1999). After showing a decline on 4th day, the soluble protein content recovered and showed an increase on 6th day. This could be due to the fact that presence of various toxicants or deprivation of nutrient results in signaling the synthesis of new proteins which contribute to the survival of the organism (Pandey, 2006). Cyanobacterial strains in response to nutrient limitation are known to synthesize new enzymes (Grossman *et al.,*1994)that may mobilize the availability of a particular nutrient.

The studied strains showed tolerance towards contaminants of sewage waste water that seems to be inherent due to their long exposure to the presence of these contaminants in sewage water irrigated soil. Fifty percent sewage waste water could be efficiently utilized as a growth medium for Cyanobacteria, which may be further used for various commercial purposes, thus, proving beneficial from economic as well as environment conservation point of view.

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