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GROWTH OF *Phormidium bigranulatum*-DOMINATED MAT IN RELATION TO NATURE OF THE SUBSTRATUM, TIME, pH AND NUTRIENT AVAILABILITY

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Abstract

The growth of a non-axenic *Phormidium bigranulatum*-dominated mat was tested under a range of chemical parameters and types of substrate. Total biomass yield (in term of mg chlorophyll *a*) of mats grown on muslin cloth (0.64 mg) and cotton mesh (0.62 mg) were significantly higher than those on other substrates, such as, nylon net (0.50 mg), sands of river Sone (0.43 mg) and river Ganga (0.30 mg), rice husk (0.38 mg) and saw dust (0.21 mg). The *Phormidium* mat would be easy to handle in bioremdation process as it grew well enmeshed in nylon net. The mat biomass increased with increasing phosphate enrichment of the medium up to 0.25 mM, whereas higher concentrations were inhibitory. At low levels of phosphate (<0.10 mM) in the medium, green algae, especially *Oocystis lacustris* (13%), *Scenedesmus obliquus* (10%), and *Chlorella vulgaris* (9%), became abundant in the mat community. Whereas ammonium chloride, ammonium nitrate, urea and glutamic acid (1 to 10 mM) failed to support the growth of the test mat, nitrate best supported the growth of the mat at concentrations above 10 mM. Low concentrations of nitrate (1 and 5 mM) could not sustain the growth of the mat for 30 days experimental period. The optimum sulfate concentration for mat growth was 0.10 mM. Air bubbles were seen entrapped in the mat due probably to the dense matrix of exopolymers which seem to have hindered the escape of oxygen and other gases. Conditions favoring high rate of photosynthesis, such as, optimal nutrient supply, led to the entrapment of a large number of air bubbles in the mat. Since the test mat requires high phosphate and nitrate concentrations for growth, it is a good candidate for removing nutrients from wastewaters.

Key words: air bubble, cyanobacterial mat, growth optimization, nutrient availability, *Phormidium bigranulatum*

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1. Introduction

Cyanobacterial mat is a kind of naturally immobilized system of algae, bacteria, and cyanobacteria embedded in exopolymeric matrix of these organisms. Massive growth of mats can adversely affect water fluidity and leisure activities in aquatic systems (Lembi, 2000). Excessive growths of floating mats may inhibit the growth of periphyton and phytoplankton by hampering the penetration of light in the waterbody (Jones and Pinn, 2006). However, mats can be used in several ways for the wellbeing of humans. They can be utilized in fish culture for growing tilapias (Bender et al., 1989). In fish culture, around 50% expenditure is incurred on fish feed; the use of mats can considerably cut down this cost (El-Sayed and Teshima, 1991). Diazotrophic cyanobacterial mats could be used for enhancing soil fertility. Cyanobacterial mats can also play an important role in biological production of hydrogen (Hill, 2001). They may also be employed in bioremediation of petroleum, heavy metals and also for the treatment of domestic wastewater for removing nitrogen and phosphorus (Bender and Phillips, 2004; Phillips and Bender, 1995). Insofar as bioremediation of metal pollutants is concerned, mat seems to be a

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better option than the other biosorbents, such as, planktonic algae, because of its self-immobilized nature, abundance of metal-binding exopolymers in it and ease in use in sorptive removal of metal ions (Kumar et al., 2010; Kumar and Gaur, 2011a, 2011b).

The conditions facilitating maximum growth of commercially important planktonic algae and cyanobacteria have been studied by several previous researchers (De Pauw et al., 1984; Borowitzka, 1999; Ugwu et al., 2008). A majority of such studies have employed single species cultures. In this context, it deserves mention that optimization of conditions for the growth of multispecies cyanobacterial mats, which have tremendous environmental and economical potential, is also extremely important. However, studies in this direction have unfortunately remained scarce except for a few reports (Bender and Phillips, 2004; Zamoro-Costro et al., 2008). In addition to impact on growth and development, nutrient supply and pH may also exert considerable influence on species composition of cyanobacterial mats. Several earlier researchers noticed considerable changes in composition of phototrophic communities of benthic systems under variable nutrient supply (Chételat et al., 1999; Stelzer and Lamberti, 2001; Vymazal and Richardson, 1995), and therefore this aspect deserves proper attention during optimization of conditions for the growth of cyanobacterial mats (Rejmánková and Komárková, 2005). Bender and Phillips (2004) mention the requirement of adequate surface conditioning for proper growth and development of cyanobacterial mats. They have recommended the use of grass clippings for encouraging the growth of *Oscillatoria*-dominated mat. Likewise, Zamoro-Costro et al. (2008) have used low density polyester for constructing a *Chroococcus* sp. and *Lyngbya* sp.-dominated cyanobacterial mat. In this context, diverse substrates that encourage mat development need to be tested.

In the light of the above, it seems necessary to identify conditions optimum for the growth of useful cyanobacterial mats so as to exploit them commercially. The present study works out optimum conditions for the growth of a *Phormidium bigranulatum*-dominated mat, which has been earlier found to efficiently remove metal ions from aqueous solution (Kumar et al., 2010; Kumar and Gaur, 2011a, 2011b).

2. Materials and methods

2.1. Test mat, culture medium and culture conditions

The non-axenic *Phormidium bigranulatum*-dominated cyanobacterial mat (referred hereafter as *Phormidium* mat), collected from the agriculture farmhouse of the Banaras Hindu University, Varanasi, India, was selected as the test mat. The mat was cultivated in BG 11 medium (Hughes et al., 1958), but different nitrogen sources and various concentrations of phosphate, nitrate and sulfate were tested to maximize the growth of the test mat. The medium was prepared using Milli-Q water, and pH of the medium was adjusted to 8 using 0.1N HCl or NaOH. Mat inoculation was done by adding 1 ml of homogenized mat suspension (A_{663}= 0.1) in Petri plates (10 cm diameter) containing 19 ml autoclaved BG 11 medium. The mats received 72 µmol m^{-2} s^{-1} PAR in a 12 h light and 12 h dark cycle at 27^0C. The mat was gently shaken 4-5 times every day. Each experiment was carried out in triplicate. The data were analyzed by one-way ANOVA followed by Tukey's HSD.

2.2. Development and growth of mat in relation to substratum, time and pH

The suitability of eight different substrates, namely, cotton band (75 mesh cm^{-2}), muslin cloth (300 mesh cm^{-2}), nylon net (100 mesh cm^{-2}), saw dust, two types of sand, and rice husk were tested for their potential in facilitating mat formation. The mat was allowed to grow in Petri plates containing mat-inoculated culture medium and the selected substrates for 30 days and thereafter harvested for the measurement of chlorophyll *a*. Growth response of the test mat was also studied in relation to time (i.e., up to 60 days). The number of air bubbles (diameter 0.5-10 mm) entrapped in the mat was recorded weekly. At 15 d time interval, mats were harvested from three Petri plates for the measurement of various parameters, such as, dry weight, pigment, protein and carbohydrate contents. Culture medium of each Petri plate of 30, 45 and 60 days set up was changed after every 15 days of mat growth so as to avoid nutrient limitation. A correlation was established between mat thickness and dry weight using individual datum of experiment conducted in triplicate.

To study the effect of pH on the growth and composition of the mat, a separate experiment was conducted. The mat inoculation was done in the medium having pH values 7, 8, 9, 10, and 11. The pH of the medium was maintained using 0.1 N HCl or NaOH, and stabilized during course of experiments by adding 1 mM final concentration of different biological buffers, such as, HEPES [(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)], AMPSO [N-(1,1-Dimethyl-2-hydroxyethyl)-3-amino-2-hydroxypropanesulfonic acid], and CAPS [N-cyclohexyl-3-aminopropanesulfonic acid] considering their pH maintaining range. Biological buffers were from Sigma Chemical Company, St. Louis, USA. After 30 days of growth, various measurements were performed.

2.3. Effect of nutrient supply on the growth and development of the mat

Separate experiments were conducted to understand the growth response of *Phormidium* mat in relation to availability of phosphate-phosphorus, various nitrogen sources and sulfate-sulfur. Desired concentrations (0.05 to 2.0 mM) of phosphate in the medium were obtained by adding $K_2HPO_4.3H_2O$ solution, while various forms of nitrogen (10 mM)

were added to the medium in the form of salts, such as, sodium nitrate, sodium nitrite, ammonium chloride, ammonium nitrate, urea and glutamic acid. Subsequently, sodium nitrate was identified as the most preferred nitrogen source, and therefore the growth of the mat was studied at different levels (1-40 mM) of sodium nitrate. Similarly, the growth of test mat was also studied at various concentrations of sulfate (0.01 to 2.0 mM) by adding $MgSO_4.7H_2O$ solution to the culture medium. The concentrations of K^+, Na^+ and Mg^{2+} in Petri plates, containing culture medium with various concentrations of phosphate-phosphorus, nitrate-nitrogen and sulfate-sulfur, were kept constant by adding non-inhibitory concentrations of NaCl, KCl and $MgCl_2$, respectively. In all the cases, each mat was allowed to grow for 30 days and the culture medium of each Petri plate was replaced by adding the same volume of fresh medium in the middle of the experiment so as to avoid nutrient limitation of mat growth. Various measurements were performed after 30 days of growth.

2.4. Species composition, thickness and dry weight determination

Phormidium mats subjected to various treatments were examined with a light microscope (Dewinter; model D select) for species composition and relative abundance of various taxa. Various genera and species of cyanobacteria and algae were identified following Desikachary (1959), Phillipose (1959) and others (John et al., 2002; Prescott, 1978; Rosen, 1990). Relative abundance of different algal species in the test mat was determined on the basis of their cell numbers. Cell counting was done with a Spencer's brightline hemacytometer. In the case of filamentous algae, total number of cells in a filament was counted and necessary corrections were incorporated as described by Lawton et al. (1999) and Olson (1950). Thickness of the mat was measured at the end of the experiment as described by Kumar et al. (2012). The final yield of the mat was measured in terms of dry weight after 30 days of growth. For dry weight determination, the mat was oven dried at 80°C till the constant weight was obtained.

2.5. Pigment, protein and carbohydrate estimation

A small known area of the mat was cut with the help of a cork borer and homogenized in 10 mL of deionized water. The homogenized suspension was centrifuged and the pellet was suspended in a known amount of acetone (80%). After overnight incubation at 4°C in the dark, it was centrifuged again and the resulting supernatant was used for measuring chlorophyll *a*, chlorophyll *b* and carotenoids. For the estimation of the pigments in the supernatant, absorbance was recorded at 663, 645 and 480 nm for chlorophyll *a*, chlorophyll *b* and carotenoids, respectively. The amount of chlorophyll *a* and *b* were calculated as per Mackinney (1941) and carotenoids quantified as per Myers and Kratz (1955).

Protein content was estimated following the method of Lowry et al. (1951) using lysozyme as the standard. Carbohydrate content was estimated by the phenol-sulfuric acid method (Dubois et al., 1956) using glucose as the standard.

3. Results

3.1. Development of the cyanobacterial mat on various substrates

Growth of the test mat on different substrates was determined after 30 days of mat inoculation by measuring total biomass yield in terms of chlorophyll *a* (Fig. 1). Mats grown on muslin cloth (0.64 mg) and cotton mesh (0.62 mg) had significantly higher biomass yield than the other substrate types, such as, nylon net (0.50 mg), sands of river Sone (0.43 mg) and river Ganga (0.30 mg), rice husk (0.38 mg) and saw dust (0.21 mg). Moreover, growth of the mat without any substrate (referred to as the control) was equally good since the final biomass yield was 0.59 mg chlorophyll *a*.

Effect of different substrates on growth of the test mat was also reflected in air bubble formation. Entrapment of air bubbles in matrix of mat growing on cotton mesh, muslin cloth and the control begun after 7-9 days of inoculation. However, it was delayed for both types of sands and no air bubble was formed till the end of the experiment in the case of rice husk and saw dust. Microscopic observations revealed changes in species composition of the test mat growing on different substrates.

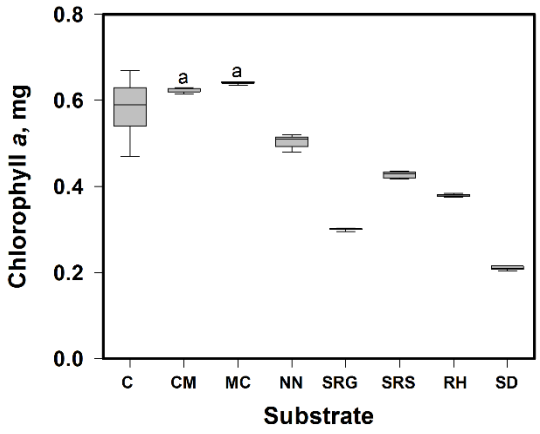

Fig. 1. Biomass, measured in term of chlorophyll *a*, of *Phormidium* mat grown on different substrates [C = control (no substrate), CM = cotton mesh, MC = muslin cloth, NN = nylon net, SRG = sand river Ganga, SRS = sand river Sone, RH = rice husk, and SD = saw dust]. Chlorophyll *a* was measured after 30 days of mat growth; boxes marked with the same letter are not significantly different from control ($P<0.05$)

Although *Phormidium bigranulatum* dominated the mat in all the tested conditions, green algae, such as, species of *Chlorella*, *Oocystis*, *Scenedesmus*, etc. were also observed in plates containing cotton mesh, muslin cloth, sand, and rice

husk. Green algae were not seen on the mat growing on nylon net.

3.2. Mat growth in relation to time and pH

Growth and development of *Phormidium* mat were studied in relation to time. A very thin light blue-green film appeared after 6-7 days of inoculation (Fig. 2). Entrapment of air bubbles in mat matrix was observed for the first time on the 7th or 8th day after inoculation, but the number of air bubbles reached the maxima in 21 days old mat and subsequently decreased with aging (Fig. 3a). Several parameters, such as, thickness, dry weight, pigments, protein and carbohydrate contents were recorded after 15, 30, 45, and 60 days of mat growth (Fig. 3b and Fig. 3d). Mat thickness, dry weight and carbohydrate content showed a regular increase with time. Conversely, chlorophyll *a* content did not show any marked change with time (Fig. 3d). Protein content of the mat increased up to 30 days, and then it remained almost unchanged subsequently. Interestingly, mat thickness showed a strong positive correlation with mat dry weight (r^2 = 0.938, Fig. 3c). Species composition of the test mat remained unchanged with time as *Phormidium bigranulatum* remained the dominant organism (relative abundance >96%) in the mat community. A few cells of green algae, such as, *Chlorella vulgaris*, *Oocystis lacustris*, and *Scenedesmus obliquus*, were also seen in the mat after 45 days of growth.

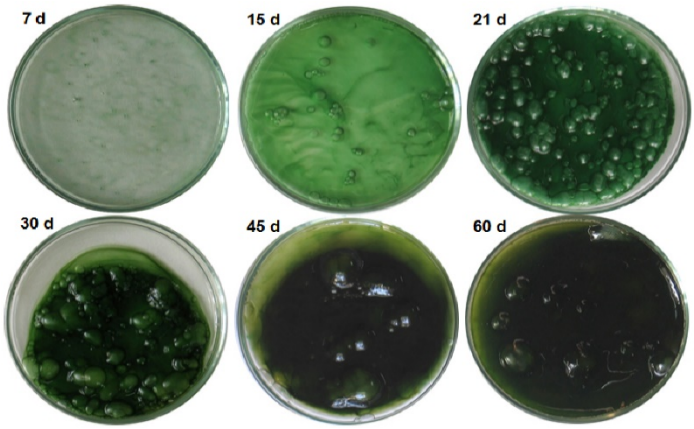

Fig. 2. Development and growth of *Phormidium* mat in relation to time (days). Mat was grown in Petri plates in BG 11 medium. Diameter of each Petri plate is 10 cm

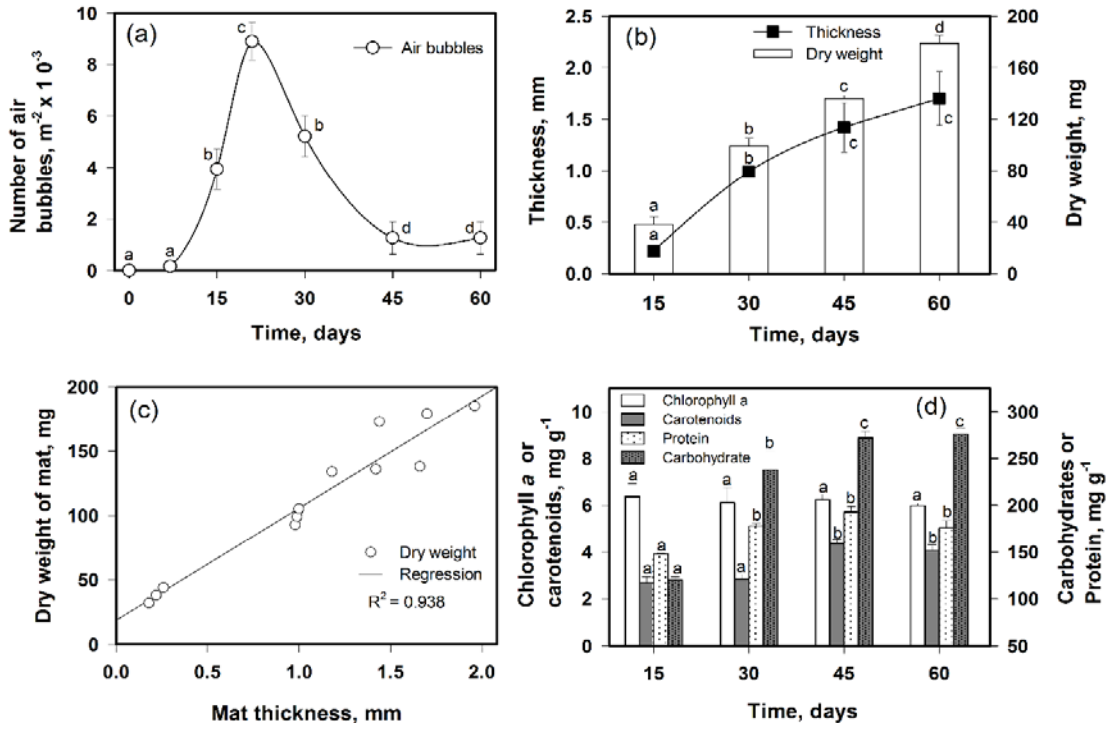

Fig. 3. Time-dependent growth of *Phormidium* mat measured in terms of different parameters, such as, number of air bubbles trapped in mat matrix (a), thickness and dry weight (b), and chlorophyll *a*, carotenoids, protein and carobohydrate contents (d), and linear relationship between thickness and dry weight of mat (c). Vertical bars show mean±SD of three replicates. Values marked with the same letter are not significantly different (P<0.05) from each other

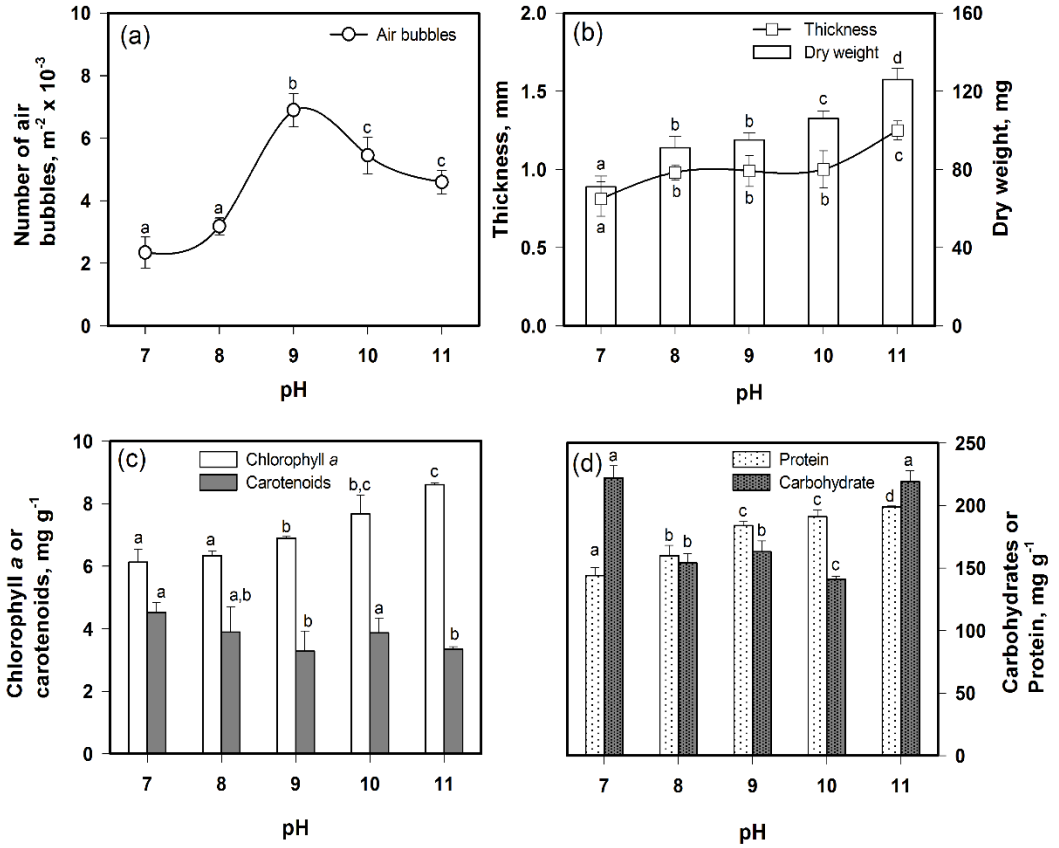

Fig. 4. Growth response of *Phormidium* mat cultivated on BG 11 medium at various pH values. Various parameters of the mat, such as, number of air bubbles entrapped in mat matrix (a), thickness and dry weight (b), chlorophyll *a* and carotenoid (c) and protein and carbohydrate contents (d) were estimated after 30 days of mat growth. Vertical bars show mean±SD of three replicates. Values marked with the same letter are not significantly different (P<0.05) from each other

The test mat successfully grew at five selected pH values (i.e., 7, 8, 9, 10 and 11). Air bubble formation in the mat was optimum at pH 9 (Fig. 4a), while other parameters, such as, thickness, dry weight and chlorophyll *a* attained maxima at pH 11 (Fig. 4b). The carotenoid content of the mat remained unchanged with pH increment (Fig. 4c). A little increase in protein content was noticed above pH 8. The carbohydrate content of the mat was maximum at pH 7 and 11 (Fig. 4d). Species composition of the test mat was not affected in the entire range of the selected pH as *P. bigranulatum* dominated (relative abundance >97%) the mat irrespective of pH.

3.3. Mat growth in relation to nutrient supply

3.3.1. Phosphate

Air bubble formation in the mat was very sensitive to the concentration of phosphate in the medium. Number of air bubbles showed a steep increase as phosphate concentration increased from 0.05 to 0.25 mM. However, it showed a sharp decline when phosphate in medium exceeded 0.25 mM (Fig. 5a). Air bubble formation in the mat was delayed at low (0.05 mM) and high (0.5-2.0 mM) concentrations of phosphate. In both these cases, air bubbles appeared after 20 days of inoculation, which were normally observed after 7-8 days of inoculation at 0.1 and 0.25 mM of phosphate.

Dry weight and thickness of the mat considerably increased with phosphate enrichment up to 0.25 mM (Fig. 5a). However, further enrichment (>0.25 mM) of phosphate had inhibitory effect on growth of the test mat as thickness and dry weight declined significantly (Fig. 5a). The maximum value for chlorophyll *a* was recorded at the lowest tested concentration of phosphate (i.e., 0.05 mM). Except for this single instance, chlorophyll *a* content followed the same trend as those for thickness or dry weight. The lowest carotenoid content was recorded at 0.25 mM of phosphate, but it showed a considerable increase when phosphate concentration decreased or increased from 0.25 mM. Protein and carbohydrate contents attained maxima at 0.50 mM of phosphate. Moreover, the carbohydrate content of the test mat was always greater than its protein content.

Concentration of phosphate in the medium had significant impact on species composition of the mat (Fig. 6). At 0.05 mM of phosphate, the relative abundance of *P. bigranulatum* in the mat was 62%, while the rest 38% was contributed by green algae, such as, *Chlorella vulgaris*, *Oocystis lacustris* and *Scenedesmus obliquus*. At 0.25 mM of phosphorus, relative abundance of *P. bigranulatum* in the mat increased to 97%. Further phosphate enrichment again enhanced the apportionment of *P. bigranulatum* and green algae were almost eliminated from the mat when phosphate concentration was ≥ 1.0 mM.

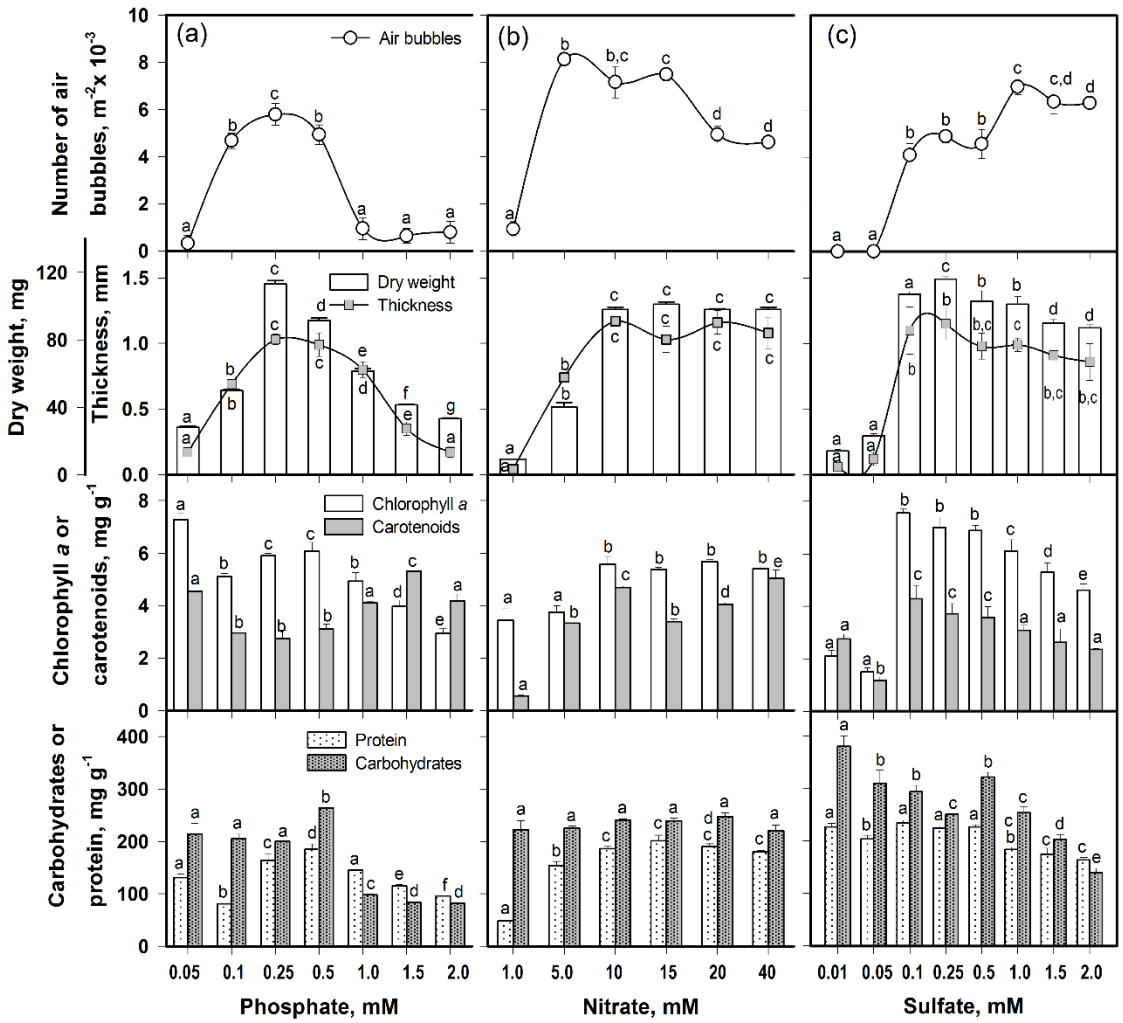

Fig. 5. Growth response of *Phormidium* mat cultivated on BG 11 medium containing different concentrations of phosphate (a), nitrate (b) and sulfate (c). All the parameters were estimated after 30 days of mat growth. Vertical bars show mean±SD of three replicates. Values marked with the same letter are not significantly different (P<0.05) from each other

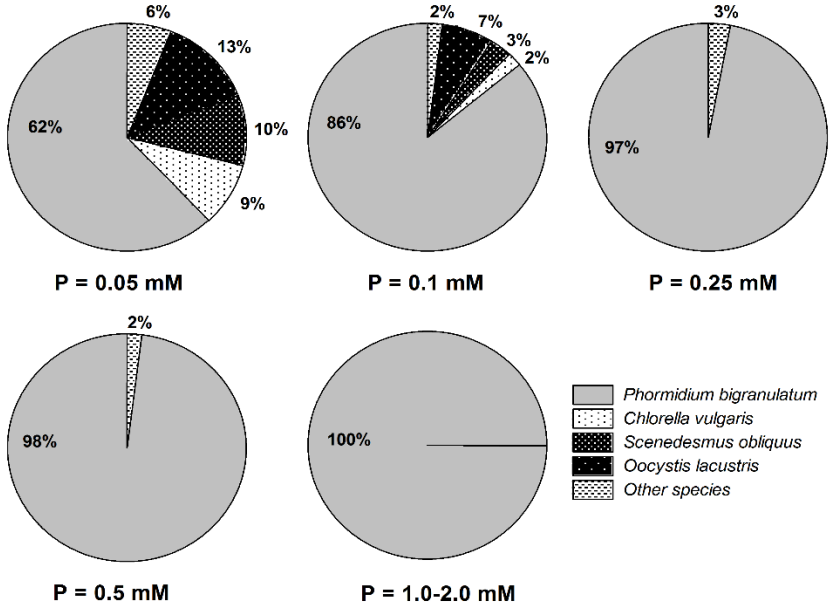

Fig. 6. Relative abundance of different species in the *Phormidium* mat grown on BG 11 medium at different concentrations of phosphate. Other species included both cyanobacteria and green algae, but they are not specified because of their very low relative abundance value (<1%)

3.3.2. Nitrogen

Six nitrogenous compounds were tested for their ability to support the growth of the test mat. Mat development was observed only in sodium nitrate- and sodium nitrite-containing plates, but it did not take place in plates containing ammonium chloride, ammonium nitrate, urea or glutamic acid. The medium devoid of any combined source of nitrogen also failed to support the growth of the mat. The mat growing on nitrate-nitrogen had slightly greater thickness and dry weight in comparison to that developing on medium containing nitrite-nitrogen (Table 1). A similar tendency was observed for chlorophyll *a* and carotenoid content. However, the mats growing on sodium nitrate and nitrite containing culture media did not differ in their protein and carbohydrate contents. In both sodium nitrate and nitrite, *P. bigranulatum* dominated the mat community.

Since sodium nitrate best supported the growth of the test mat, effects of its different concentrations were also studied (Fig. 5b). At low concentrations of nitrate (1 and 5 mM), thin blue-green biofilm appeared after 6-7 days of inoculation, but it became yellowish on the 12th day. Conversely, growth of the test mat at high concentration of nitrate (i.e., 10-40 mM) was almost normal during 30 days tenure of the experiment. Air bubble formation in mat increased as nitrate concentration increased from 1 to 15 mM; however, it showed a small decline at 20 and 40 mM of nitrate (Fig. 5b). Other parameters, such as, thickness, dry weight and chlorophyll *a*, protein and carbohydrate contents increased as nitrate concentration increased 1 to 10 mM; higher concentrations were saturating for the growth of the test mat (Fig. 5b). Species composition of the test mat remained unchanged at various concentrations of nitrate; *P. bigranulatum* always dominated the mat community (relative abundance >97%).

3.3.3. Sulfate

The mat grew poorly at 0.01 and 0.05 mM of sulfate, but showed good growth at higher concentrations. Air bubble formation was almost negligible at 0.01 mM of sulfate, but it increased as the concentration of sulfate increased from 0.01 to 1.0 mM (Fig. 5c). Moreover, a slight decrease in air bubble formation was noticed at sulfate concentration in the medium >1.0 mM. Dry weight, thickness and chlorophyll *a* and carotenoid contents of the test mat were recorded particularly low at 0.01 or 0.05 mM of sulfate. However, a marked sudden increase occurred in these parameters when sulfate concentration in medium increased from 0.05 to 0.10 mM. Sulfate levels exceeding 1 mM had a slight inhibitory effect on these parameters. Protein content of the mat community remained virtually unchanged within the sulfate concentration range 0.05 to 0.5 mM; however, significant decrease was noticed as sulfate concentration reached 1.0 - 2.0 mM (Fig. 5c). Contrary to protein, carbohydrate content of the mat was the maximum at 0.05 and the minimum at 2.0 mM of sulfate, respectively. At all the tested concentrations of sulfate, *P. bigranulatum* was the dominant cyanobacterial entity of the mat community. A few cells of *Chlorella* and other green algae were also observed at the lowest tested concentrations of sulfate.

4. Discussions

The growth of the mat in the laboratory followed a definite pattern; thickness and dry weight of the mat consistently increased with time. There was a highly significant (r^2 = 0.938), positive and linear relationship between thickness and dry weight of the mat. Therefore, thickness can very well be recommended as a non-destructive and easy-to-measure parameter for monitoring the growth of the mat. As the mat grew, chlorophyll *a* content did not change much with time, whereas carotenoid and protein contents slightly increased. The most dramatic change nevertheless has been great enhancement, almost three-fold, of carbohydrate content of the mat. This was obviously necessary for the proper development of the mat (Stal, 2000).

In natural waters, the mat develops on the surface of the sediment and then it gradually gains buoyancy due to gases accumulated in the matrix and moves up to the water surface. Since grass clippings (Bender and Phillips, 2004) and low density polyester (Zamoro-Costro et al., 2008) encourage the formation and development of cyanobacterial mats, several other substrates were tested for their capability in stimulating mat formation. Cotton mesh and muslin cloth were found good for mat development due probably to their rough surface, which seems to have favoured the attachment of filamentous cyanobacteria, the dominant microbial entities of the tested mat community.

Table 1. Thickness, dry weight, chlorophyll *a*, carotenoid, protein, and carbohydrate contents of the mat grown in BG 11 medium containing 10 mM concentrations of various nitrogen sources. All estimations have been done after 30 days of mat growth

Nitrogen source*	***Thickness (mm)***	***Dry weight (mg)***	***Chlorophyll a (mg g^{-1})***	***Carotenoids (mg g^{-1})***	***Protein (mg g^{-1})***	***Carbohydrates (mg g^{-1})***
Sodium Nitrate	1.20 ± 0.1	100 ± 0.2	5.7 ± 0.1	3.67 ± 0.3	175± 8.6	237 ± 2.0
SodiumNitrite	1.05 ± 0.1	95 ± 0.1	5.48 ± 0.1	3.03 ± 0.1	177± 4.8	212 ± 4.2

**The mat failed to grow at 10 mM concentration of ammonium chloride, ammonium nitrate, urea, glutamic acid, as also in medium devoid of any combined source of nitrogen.*

However, ease in attachment is not the only factor which promoted the growth of the cyanobacterial mat. It seems that degradation of cotton fibers in the substratum may have provided certain organic nutrients to the developing mat community and thus enhanced its growth. Bender and Phillips (2004) have also envisaged the positive role of organic nutrients, released by microbial degradation of grass clippings, during the growth of an *Oscillatoria* sp.-dominated mat. The development of mat on nylon net was a little bit lesser in comparison to the control. This may be due to smooth surface of the substratum; however, nylon net-enmeshed mat can be easily used in bioremediation process. Both types of sand inhibited the development of the mat. It may be that some toxicants adsorbed onto them have been released in the medium during the growth of the mat. Rice husk and saw dust inhibited the development of the mat, which is in consonance with earlier reports showing inhibition of algal and cyanobacterial growth by barley straw (Ball et al., 2001; Waybright et al., 2009).

Nutrients are important in regulating structure and functions of all communities including the cyanobacterial mats. The present study demonstrates the importance of phosphorus and nitrogen in the growth of *Phormidium* mat. Growth of the mat increased with enhancement of phosphate concentration in the medium up to 0.25 mM. Rapala et al. (1993) noted increase in growth of *Anabaena flos-aquae*, *Anabaena mendotae* and *Aphanizomenon flos-aquae* as concentration of phosphate-phosphorus increased in medium from 0.05 to 5.5 mg l^{-1}. Perusal of literature suggests that 0.05% phosphate is a normal concentration added to the various algal culture media (Fogg et al., 1973). In this context, phosphate requirement of *Phormidium* mat is relatively high, but this particular capability may make the test mat a suitable candidate for bioremediation of phosphorus-enriched wastewaters. Moreover, these findings are in consonance with the fact that cyanobacteria are phosphorus-loving prokaryotes, requiring a lot of phosphorus for their growth. Cyanobacteria assimilate large amounts of phosphorus from the medium and accumulate it in the form of polyphosphate bodies (Fogg et al., 1973). Other than cellular accumulation, *Phormdium* mat may accumulate a lot of phosphorus in its matrix as Whitton (1967) found accumulation of large amounts of inorganic phosphorus in extracellular mucilage of *Nostoc verrucosum*. Nevertheless, in the present study, very high concentrations of phosphate (>0.25 mM) were inhibitory for the growth and development of the mat. Inhibitory effect of phosphate (0.1-0.5%) on algal cells, specifically growing in the presence of elemental nitrogen, was noticed elsewhere (Fogg et al., 1973). Ernst et al. (2005) noted inhibitory effect of high concentrations of both phosphate and nitrate on the growth of *Synechococcus sp*. In fact, inhibitory effect of high concentrations of phosphate has also been reported in a variety of angiosperms. The symptoms of phosphate toxicity in plants comprise chlorosis, necrosis, senescence, and inhibition of growth (Silber et al., 2002). Whereas the mechanism of inhibition by high phosphate concentrations is not very well understood, the latter authors and many others believe it to be related with P-Zn interactions (Silber et al., 2002). Precipitation of zinc as phosphate is one such interaction.

Beside growth, phosphate concentration also affected species composition of the test mat. Cyanobacteria are generally regarded as poor competitors of phosphate, and are often competitively replaced by green algae when concentration of phosphate is low in the medium (Thompson and Rhee, 1994). This is exactly what was evident in the present study. At very low levels of phosphate in the medium, green algae like *Chlorella vulgaris*, *Scenedesmus obliquus*, etc., became particularly abundant in the mat community. However, increase in concentration of phosphate in the medium led to elimination of green algae. Obviously, this resulted from enhanced growth of *P. bigranulatum* in the mat that outcompeted these green algae. These observations reinforce the view that green algae are better competitors of phosphate in comparison to blue-green algae.

The ability of various sources of nitrogen in supporting the growth of the cyanobacterial mat was evaluated. The mat failed to grow in medium devoid of any combined source of nitrogen, which means it lacks capability for diazotrophic growth. The present observations show that sodium nitrate followed by sodium nitrite served as good sources of nitrogen for mat development. Suitability of nitrite for the growth of cyanobacterial taxa, such as, *Anabaena doliolum* and *Anabaena cylindrica* is also noted by others (Fogg et al., 1973). NH_4^+ can be considered as a preferred source of nitrogen for growth of cyanobacteria, algae and plants because it is highly reduced and need not be reduced further in the cell (Raven et al., 1992). However, the present results disagree with the above view, as no mat development occurred in Petri plates containing ammonia-nitrogen in the culture medium. It may be that the concentration of ammonium taken for the study was so high that it inhibited the formation of the cyanobacterial mat. However, the mat failed to grow even when very low concentration (1 mM) of ammonium used in the medium (data not presented). Earlier researchers also discussed toxicity of ammonium-nitrogen to several cyanobacteria, such as, *Phormidium persicinum*, *Calothrix scopulorum* and *Anabaena doliolum* (Fogg et al., 1973). Several hypotheses have been proposed to explain the toxicity of NH_4^+, but none is considered satisfactory (Britto and Kronzucker, 2002). Other sources of nitrogen that were tested in the present study, namely, urea, ammonium nitrate, and glutamic acid, also failed to facilitate the growth of the mat. In this context, it deserves mention that the toxicity of urea is not uncommon for plants. This particular source of nitrogen increases lipid peroxidation in plants (Sakamoto, 1998). Inability of glutamic acid to support the growth of mat agrees well with the findings of Lynch and Gillmor (1966).

The mat grew optimally at nitrate concentrations 10 mM and above, and hence 10 mM concentration can be recommended for large scale cultivation of *Phormidium* mat. Addition of a large amount of nitrate-nitrogen (7.15 mM) in culture medium of cyanobacteria is well recommended by others (Fogg et al., 1973). However, some cyanobacteria also have the ability to grow at very low concentration of nitrate-nitrogen, such as, *Anabaena flos-aquae* and *Aphanizomenon flos-aquae* strains were reported to grow up to 21 days in the medium containing 0.0-0.99 mM nitrate-nitrogen (Rapala et al., 1993). An extremely low requirement of nitrogen by these species might be related to their dinitrogen fixing capability. However, some non-nitrogen fixing cyanobacteria could also have ability to grow at very low levels of nitrate-nitrogen. For example, a non-axenic strain of *Microcystis aeruginosa* grew successfully in medium containing 0.0118 to 1.18 mM of nitrate-nitrogen, and an axenic strain of the same species grew at 0.0086 to 1.107 mM of nitrate-nitrogen (Orr and Jones, 1998). Nevertheless, above discussion reveals that similar to phosphate, nitrogen requirement of *Phormidium* mat for optimum growth is also high. Thus, the test mat can be successfully used for remediation of phosphate and nitrate enriched wastewaters, and biomass produced during the process can be used as a feed stock for the production of algal biofuels, metal biosorbent and several other valuable products.

Trapped inside the mat were seen a large number of air bubbles, which were visible as protuberances. This entrapment is primarily due to dense matrix of exopolymers which does not allow oxygen and other gases to escape easily (Stal, 2000). Formation of air bubbles is a feature often reported in the case of mats with a high rate of photosynthesis (Stal, 2000). Air bubbles are formed on the surface also, wherefrom they are released in water. The number and size of entrapped air bubbles varied greatly in the present study. In general, the number of bubbles increased greatly under optimal availability of a particular nutrient. On the contrary, adverse conditions generally led to reduction in number of air bubbles. This is apparently related to the rate of photosynthesis. Conditions favoring a high rate of photosynthesis, such as, under optimal supply of a nutrient, led to formation of a large number of air bubbles. Although oxygen is the main constituent of air in these bubbles during the light period, other gases, notably carbon dioxide and nitrogen, may get into these bubbles in the period of darkness for maintaining pressure inside (Bosak et al., 2010).

5. Conclusions

The test mat could grow well over a wide range of pH (7 to 11) of the culture medium. It could also grow well on nylon net. Nylon net-enmeshed mat would be mechanically strong and easy to handle in bioremediation process. The mat grew optimally at 0.25 mM of phosphate-phsophorus, ≥10 mM of nitrate-nitrogen and ≥ 0.1 mM of sulfate-sulfur. Moreover, since the test mat had high phosphate and nitrate requirements for growth, it can be used in treatment of nutrient-enriched wastewaters.

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