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Carbon sequestration in batch cultures of three *Phormidium* species isolated from Sunderbans brackish-water habitats

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Abstract

Three species of filamentous cyanobacteria,viz. *Phormidium laminosum*, *P. valderianum* and *P. tenue* were isolated from different habitats of Indian Sunderbans and tested as potential strain for large-scale bio-sequestration of atmospheric CO_2. They were assessed for biomass growth and their C -fixation capacity in batch culture mode under laboratory conditions over a period of 30 days. The CO_2 sequestration rates were determined in terms of specific growth rate (μ) and organic carbon content of the studied taxa. Maximum biomass productivity was recorded in *P. laminosum* (1.389 g / L), while maximum μ was observed in *P. valderianum* (0.215 d^{-1}). Maximum CO_2 sequestration rate (168.1 mg L^{-1} d^{-1}) was attained by *P. Tenue* in terms of maximum OC content (44.3-47.9%) and biomass productivity.

Keywords: *Phormidium*; CO_2 bio-mitigation; CO_2 sequestration rate; cyanobacterial growth

Introduction

Cyanobacteria form a very large and diverse phylum of prokaryotes with the ability for oxygenic photosynthesis, and nitrogen fixation in many species (Stanier et al. 1987). Their dominance in aerated microbial communities has led to the several assessment studies of different species of cyanobacteria for bio-sequestration of atmospheric CO_2, bio-remediation of excess nutrients in wastewater and biomass utilization (Ono and Cuello 2007; Pandit et al. 2012; Sánchez Fernández et al. 2012; Clares et al. 2014). The capacity for CO_2 sequestration in different species, however, have been shown to depend on the culture conditions, including factors such as level and duration of irradiance, and concentration of CO_2 supplied to the medium (Berberoğlu et al. 2008; Jacob-Lopes et al. 2008; Lv et al. 2014). Attempts have been made for enhanced CO_2 bio-mitigation by genetically modified cyanobacteria (Chen et al. 2012).

The vast mangrove swamps and tidal mudflats of the Indian Sunderbans (21° 31´N - 22° 53´N and 88° 37´- 89° 09´E) form a sensitive eco-system with high biodiversity. The river Hooghly alone provides a huge influx of nutrients, including nitrogen (0.49 mol / m^2 mangrove) and phosphorus (0.043 mol / m^2 mangrove), annually into this region, which sustains a rich and varied microbial population in the sediments of this region (Mukhopadhyay et al. 2006; Ramanathan et al. 2008). Blooms of phytoplankton species seasonally enhance the bio-sequestration of atmospheric CO_2. Shallow brackish-water wetland habitats within this region are sustained by algal photoautotrophy (Satpati et al. 2012, 2013). Post-monsoon (August-November) periods see vigorous blooms of cyanobacterial species, which are mostly filamentous forms such as *Lyngbya*, *Anabaena* and *Phormidium*. This study was aimed at assessing the capacity of C sequestration by three Phormidium species isolated from brackish-water communities of the Indian Sunderbans.

Materials and Methods

Microorganism of study

Trichome-forming cyanobacteria *Phormidium laminosum*, *P. valderianum* and *P. tenue* were collected from different brackish-water ponds in Indian Sunderbans (21°31´ to 22°53´N and 88°37´ to 89°09´E) and identified in laboratory from proper monographs. Axenic cultures were established in laboratory conditions. Exponential phase cultures were used as inocula for following experiments.

Batch culture conditions

Batch cultures were grown in 500 mL Erlenmeyer flasks containing 300 mL ASN-III medium modified to simulate brackish-water conditions: NaCl 1.0 g/L, $MgSO_4.7H_2O$ 3.5 g/L, $MgCl_2.6H_2O$ 2.0 g/L, $NaNO_3$ 0.75 g/L, $K_2HPO_4.3H_2O$ 0.75 g/L, $CaCl_2.2H_2O$ 0.5 g/L, KCl 0.5 g/L, $NaCO_3$ 0.02 g/L, Citric acid 3.0 mg/L, Ferric ammonium citrate 3.0 mg/L,

Mg EDTA 0.5 mg/L, Vitamin B_{12} 10.0 µg/L, H_3BO_3 2.86 mg/L, $MnCl_2{\cdot}4H_2O$ 1.81 mg/L, $ZnSO_4{\cdot}7H_2O$ 0.222 mg/L, $NaMoO_4{\cdot}2H_2O$ 0.39 mg/L, $CuSO_4{\cdot}5H_2O$ 0.079 mg/L, and, $Co(NO_3)_2{\cdot}6H_2O$ 49.4 µg/L. Media pH was 7.4 ± 0.2 at 22 ± 2˚C temperature with 14 h:10 h light dark cycle and light intensity 75 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR).

Determination of growth and C sequestration parameters

Each liquid culture was sampled at 3-day intervals for 30 days of incubation. Biomass concentration on dry cell weight (dcw) basis was measured by centrifugation of 300-mL cultures at 5000 rpm for 10 min and subsequent oven-drying of pellets at 60°C to get a constant weight. Each sample was also analysed for organic carbon (OC) content (%dcw) following the method of Walkley and Black (1934).

Specific growth rate (µ) at each sampling was determined by the following equation:
μ_t (d^{-1}) = [ln(X_t) - ln(X_{t-3})] / 3, where X_{t-3} and X_t are two subsequent sampled biomass concentrations (g L^{-1}) at t-3 and t days respectively.

Rate of CO_2 sequestration (R) at each sampling was determined by the following equation:

R_t (mg L^{-1} d^{-1}) = (44/12) x [C_t x X_t – C_{t-3} x X_{t-3}] x 0.01 / 3, where X_{t-3} and X_t are two subsequent sampled biomass concentrations (mg L^{-1}) at t-3 and t days respectively; C_{t-3} and C_t are correspondingly sampled OC contents (%dcw) at t-3 and t days respectively; molecular weight of C is 12; and, molecular weight of CO_2 is 44.

Results

Growth kinetics of all three *Phormidium* species exhibited similar sigmoid patterns (Fig. 1A). Lag phase continued for 0-6 d for all three species. Exponential phase continued for 6-15 d of incubation for *P. laminosum*; while both *P. valderianum* and *P. tenue* exhibited longer exponential phases at 6-21 d of incubation. However, *P. laminosum* exhibited a distinct phase of deceleration at 15-24 d, after which the culture entered decay phase. In contrast, *P. valderianum* entered decay phase at 21 d, while *P. tenue* underwent stationary phase at 21-27 d before entering decay phase. Also, OC content was found to follow sigmoid patterns for each species (Fig. 1B). For each species, OC content increased slightly after 6 d of incubation, reached maxima at 21 d and subsequently fell to minimum level. However, these variations in OC content were almost insignificant without affecting CO_2 sequestration rates (Fig. 2A).

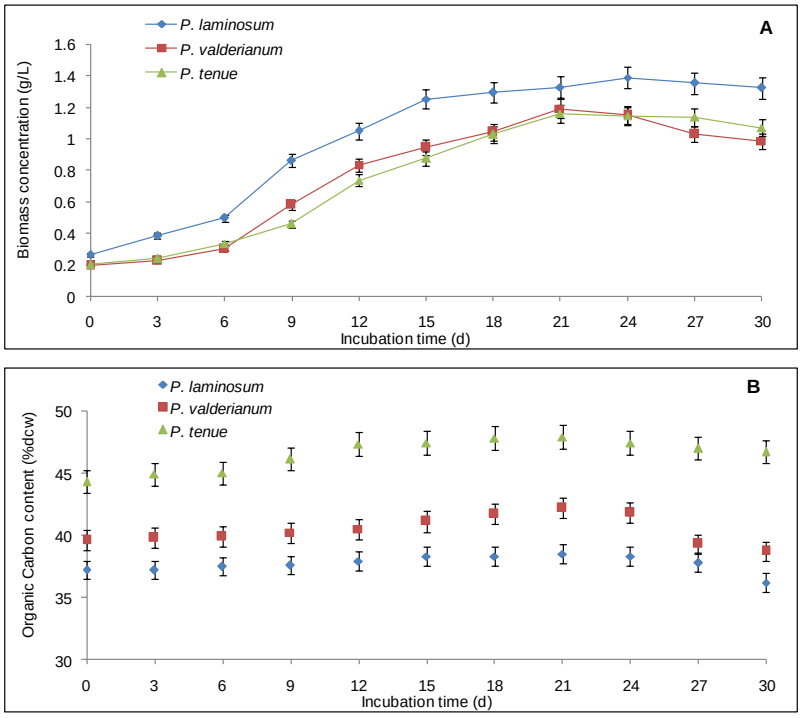

Fig. 1. Growth kinetics (A) and OC contents (B) of three *Phormidium* species at 0-30 days of incubation. Each datum is represented as mean ± standard error of duplicate samples.

Temporal changes in rates of CO_2 sequestration (R) and specific growth (μ) showed marked congruency for each species (Fig. 2). For *P. laminosum* and *P. valderianum*, both R and μ increased significantly within 6-9 d interval, and then gradually decreased till 30 d. For *P. tenue*, pattern of steep increase (9-12 d) and subsequent step-wise decrease (12-30 d) began at a further lag of 3 d.

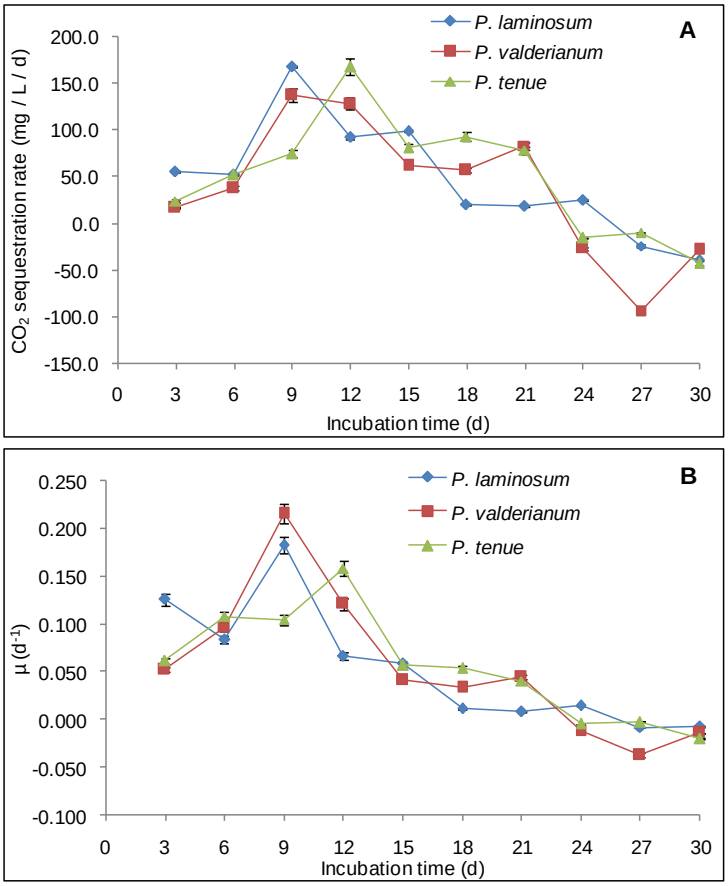

Fig. 2. CO_2 sequestration (A) and specific growth (B) rates of three *Phormidium* species at 3-30 days of incubation. Each datum is represented as mean ± standard error of duplicate samples.

Discussion

The kinetic patterns of R and μ discernibly followed the corresponding trends of biomass growth, but were not very faithful to the trends followed by OC contents (Fig. 1-2). Although *P. tenue* showed the highest levels of mean OC content (44.3-47.9% dcw), followed by *P. valderianum* (38.7-42.2% dcw) and then *P. laminosum* (36.2-38.5% dcw), this trend was not reflected in CO_2 sequestration rates. Maxima for CO_2 sequestration rates was highest (168.1 mg L^{-1} d^{-1}) for *P. tenue*, followed by *P. laminosum* (167.9 mg L^{-1} d^{-1}) and *P. valderianum* (137.2 mg L^{-1} d^{-1}). Maxima for specific growth rates followed the opposite trend, with *P. valderianum* showing the highest value (0.215 d^{-1}), followed by *P. laminosum* (0.183 d^{-1}) and *P. tenue* (0.158 d^{-1}). So, level of CO_2 sequestration depended on specific growth rate, and by extension the phase of the culture; but maximum capacity of CO_2 sequestration of a culture was a factor of both OC content of the species and biomass concentration in the culture.

Peak CO_2 sequestration rates obtained for *Phormidium* species are comparable to those obtained for another filamentous cyanobacterium, *Nostoc flagelliforme*, which sequestered CO_2 at the rates of 100 and 170 mg L^{-1} d^{-1} at CO_2 concentrations of 5 and 20% respectively (Lv et al., 2014). It is to be noted that the *N. flagelliforme* cultures were maintained under continuous illumination in non-saline BG11 media and for 15 d only. The differences in culture conditions make direct comparisons of CO_2 sequestration rates among the various species difficult, but the values nevertheless fall within a short range of 100-200 mg L^{-1} d^{-1}. However, a *Phormidium* strain cultured in BGN medium under continuous illumination and 15% CO_2 concentration for 7d was shown to attain maximal CO_2 sequestration rate of 18.8 mg L^{-1} min^{-1} or 27072 mg L^{-1} d^{-1} (Francisco et al., 2010). This value is more than that of *N. flagelliforme* (Lv et al., 2014) and also than the current study in the flow of ambient air. This implies that potential

capacity for CO_2 sequestration not only depends on the strain of cyanobacterium but also on different aspects of culture conditions.

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