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Effects of surface material on growth pattern and bioactive exopolymers production of intertidal cyanobacteria *Phormidium* sp.

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A novel approach applying a conico-cylindrical flask (CCF) was used to study the growth of two morphologically identical *Phormidium* strains namely AP3 and AP9F. Further, the monosaccharide composition of the exopolysaccharide (EPS), its production, applicability in industrial processes and biological activity were evaluated. These evaluations were performed by employing contrasting cultivation modes (biofilm or planktonic) and different surface materials (hydrophobic polymethyl methacrylate (PMMA) and hydrophilic glass (G). Biofilm formation was seen to be elevated in PMMA-CCF cultivated *Phormidium* AP3 and AP9F than G-CCF mode. Among the two surface materials tested *Phormidium* AP3 and AP9F produced the highest amount of planktonic biomass in PMMA-CCF than in G-CCF mode. Average values of biofilm capsular/bound polysaccharide and polysaccharide released by cell suspension of *Phormidium* AP3 and AP9F were elevated in PMMA-CCF compared to the G-CCF setup. Protein concentrations in released polysaccharide (RPS) and biofilm of PMMA-CCF were higher in the *Phormidium* AP3 and AP9F when compared to the G-CCF cultivation. The antimicrobial, emulsifying and flocculating activities of EPS were assessed using standard assays.

[**Keywords**: Bioactive, Biofilm, Exopolysaccharide, Hydrophilic, Hydrophobic, Planktonic]

Introduction

Cyanobacteria are complex and widespread photosynthetic bacteria that can have significant phenotypic range permitting adaptation to extensively diverse and variable environments[1,2]. Benthic cyanobacteria grow beside the seashore on coarse sand, oolites, and shell fragments, generally in the intertidal zones. Their physiological adaptation studies were inadequate and more research is preferred. In such environments, they are exposed daily to a variety of stresses for example temperature, UV-radiation, light intensity, desiccation, and salinity[3,4]. The triumphant adaptation of microorganisms to the harsh habitats is principally because of their anaerobic energy generation, metabolic adaptation, and stress-protective mechanisms to safeguard against ecological stresses[5]. Periphyton mats of intertidal cyanobacteria are dominant primary producers and actively participate in the biotransformation of minerals in the sulfide-rich mangrove environments during phosphorus accumulation, carbon and nitrogen fixation, and photosynthesis[6].

Exopolymeric substances mainly formed of lipids, nucleic acids, humic substances, proteins and polysaccharides form high molecular mass polymers that help the biofilm to attach to the surfaces, survive in the extreme environmental conditions such as metallic trace elements, salinity, light intensity, extreme temperature, desiccation or other environmental stresses and protect it from the predators[7]. In nature, cyanobacterial biofilm EPS are continuously degraded and assimilated by all microbial community[8]. Cyanobacterial mats are dominant in hypersaline environment as major primary producers and carry outflow of energy. In the extreme environment, mats produce a thick matrix of EPS for protection from dehydration, exposure to UV and viral attack[9].

EPSAH is an exopolysaccharide (EPS) isolated from *Aphanothece halophytica* GR02 which acts as a polysaccharide adjuvant and found to increase both cellular and humoral immune responses in mice[10]. The exopolymers of *Anabaena* sp. showed shorter blood coagulation time, active thrombogenicity and antibacterial activity[11,12]. EPS also have many other roles such as protection from metal stress, antimicrobial activity, bioremediation and nutrient removal[13-17]. High Na^+ removal capability of the EPS from a consortium of cyanobacteria helps to enhance the vigor index, seed germination and mobilization

efficiency in rice, wheat and maize[18]. Presently attention is focused on the application of EPS in the industrial sector including food, cosmetic, textile, pharmaceutical, and laxatives[19]. This is due to the wide compositional diversity with distinctive properties including adhesion, structure, gliding motility, nutrient repositories, protection against abiotic stresses and bioweathering processes. Consequently, explorations into EPS works can have a cost-effective as well as a scientific relevance[20].

Some detailed studies to enhance the production and study the composition of EPS have been carried out on cyanobacteria. Singh *et al*.[21] depicted some differentiation in the EPS quantity and composition of *Anabaena* sp. PCC 7120 which had been cultivated under various levels of calcium chloride concentrations. *Nostoc minutum* was grown in different culture conditions (with respect to media and illuminations) and different EPS yields were recorded for each condition[22]. *Nostoc* spp. were cultured in diverse salt combinations, trophic conditions, nitrogen-replete and nitrogen-deplete conditions for comparing EPS production and cell growth. No connection between EPS production and cell growth was proven in strains cultivated in various forms of nitrogen[23]. The EPS production of *N. flagelliforme* cells were extensively increased while cultivated in BG-11 medium with red and blue wavelengths compared to white and yellow. The ratio of monosaccharides and uronic acid constituents displayed major differences when treated with different light wavelengths[24].

Cultivation of cyanobacteria in glass (G) and polymethyl methacrylate (PMMA) conico-cylindrical flask (CCF) can give the planktonic and biofilm growth[25-27]. Hence, cultivation in the presence of enhanced surface area has the potential advantage of biofilm production. The study was aimed to find out whether the two cyanobacterial strains could be cultivated in glass and PMMA-CCF vessels for the production of the EPS and their industrial application and biological activity. Furthermore, it was considered to be of interest to grow these organisms using different surface materials and mode of growth because this could possibly open up a way to enhance yield and activity of EPS. The EPS were isolated, purified and analyzed for their sugar compositions.

Material and Methods

Cultivation of cyanobacteria

The cyanobacterial strains were isolated from the Lothian (21°39′1″ N 88°19′37″ E) and Sagar (21°44′7″ N 88°7′2″ E) Islands, *Sundarbans* (India) following Pramanik *et al*.[28] and were taxonomically identified using existing literature. Two morphologically similar strains belonging to the same order were chosen for the current research. The isolated strains are deposited in the National Centre for Microbial Resource, Pune, Maharastra, India with accession numbers MCC 3171 and MCC 3172 for *Phormidium* sp. (AP9F) and *Phormidium* sp. (AP3), respectively. The cyanobacterial strains were maintained in ASN-III medium at 27 ± 2 °C with a light intensity of 36.45 µmol photons m^{-2} s^{-1} in a light/dark (14/10 h) cycle and were routinely sub-cultured at an interval of 15 days. The 30 days old culture were harvested by centrifugation at 10,000 *g* (Eppendorf model 5810R, rotor F-34-6-38) for 10 min, the excess water was removed by sterile blotting paper and was used as mother inoculum for further experiments.

Flask configurations, operating and culture conditions

Hydrophobic polymethyl methacrylate (PMMA) and hydrophilic glass (G) were used to make the conico-cylindrical flask (CCF) (500 ml). 50 % (v/v) benzalkonium chloride (immersed in for 5 h) were used to sterilize the disassembled CCF components, washed carefully in sterile water and dried at 60 °C. Afterwards, UV light (TUV15W/G15T8, Philips, The Netherlands) in a laminar airflow bench were used for 30 min for surface sterilization of all the components of the CCF. Equal inoculums and 150 ml of ASN-III medium were added to each PMMA and G-CCF flask.

Measurement of planktonic and biofilm biomass

30 days old planktonic biomass culture was harvested by centrifugation at 10,000 *g* for 10 min. At the end of the experiment, the biofilm was scraped off from the surface of the slides using the sharp scalpel. Salts present in biomass was removed by washing thrice with sterile distilled water and further dried at 50 °C. Lastly, using the analytical balance (Sartorius AG, Germany model GD103) the constant dry weight of biomass was determined.

Extraction of EPS

The dried cyanobacterial pellet 10 mg (biofilm and planktonic) was incubated with 0.1 mol l^{-1} 5 ml sulfuric acid at 95 °C for one hour to get capsular/bound polysaccharides (CPS). Following incubation, samples were centrifuged (4,000 *g*, 5 min; Eppendorf model 5810R, rotor F-34-6-38) and the

supernatant was separated from the pellet. Likewise, RPS was also extracted from the cell-free culture liquids following Gacheva *et al*[15]. The supernatants were then precipitated with ethanol in a ratio of 1:3 (v/v) (supernatant: 99 % ethanol) and the polysaccharides were pelletized at 10,000 *g* for 10 min. Finally, the pellets were washed with 65 % ethanol, thrice to remove any contaminants left and dried at 37 °C[25].

Determination of EPS hydrophobicity

Hydrocarbons assay as described by Bhatnagar *et al.*[11] were used to determine the EPS hydrophobicity. For this, 50 mg (1 %) of EPS was suspended in the 5 ml of phosphate-buffered saline (PBS) after obtaining optical density (OD) at 550 nm (A0 = initial absorbance). Following this, 300 µl hexadecane was added and the mixture was vortexed for 1 min. After 15 min of resting, the optical density of the aqueous phase was calculated (A1 = absorbance after phase separation). The degree of hydrophobicity (DH) was determined as {(A0-A1)/A0)} X 100.

Analysis of EPS

Total carbohydrate and protein in the RPS and CPS were measured by Anthrone and Bradford method[29]. Estimations were made by taking 10 mg of EPS in 1 ml of double distilled water. Glucose (0–100 µg mL^{-1}) and bovine serum albumin (0–100 µg mL^{-1}) were used as standards for carbohydrate and protein, respectively.

Uronic acid (UA) content in the EPS was determined subsequently following method described by Taylor & Buchanan-Smith[30]. For this, 3 ml of sulfuric acid-borate reagent (0.80 g sodium borate (tetrahydrate) was suspended in 16.67 ml water; 83.33 ml concentrated sulfuric acid) was added to 0.4 ml of EPS solution (4 mg of dry EPS) in a test tube. To this, 100 µl of carbazole reagent (Carbazole, 0.1 % (w/v), was made up in absolute ethanol) solution prepared in absolute ethanol was added and mixed in a test tube. The test tube was then placed in a water bath (40 °C) for 4 hours followed by cooling to room temperature. The pink- to red-color developed at room temperature was measured against a blank (distilled water treated similarly) at 530 nm. For the calibration, Glucuronic acid (0–100 µg mL^{-1}) was used as a standard.

Flocculating activity

The flocculating activities were measured using the method prescribed by Chen *et al.*[31]. For this, 99 mL of 0.5 mL of 10 % $CaCl_2$ solution, 0.5 mL of EPS solution, and 0.2 % kaolin suspension were mixed together. The absolute concentration of $CaCl_2$ solution was 0.5 g/L. The prepared mixture was then vortexed at 150 rpm for 30 min and was rested for 30 min. 2 ml of supernatant was removed from the upper layer of mixture and was measured for its absorbance at 550 nm using a spectrophotometer. The measurement of absorbance of a blank sample was done by replacing EPS fraction with deionized water. The flocculating activity was calculated as per the equation given below:

$$Flocculating\ activity = {}^{1}\!/_{OD550a} - {}^{1}\!/_{OD550b}$$

Emulsifying activity

Emulsifying activity was measured following method described by Han *et al.*[32]. For this analysis, briefly, 3 ml of n-hexane was added to 2 mL of 1 % EPS solution in a glass tube and vortexed for 5 min. After 24 h, the emulsion index (E24) was calculated as given below (depending on the assay):

$$E24 = he/hT \times 100$$

Where, he (mm) is the height of the emulsion layer and hT is the overall height of the mixture.

All the tests were performed in triplicate and the samples were stored at room temperature.

Antimicrobial activity

Antimicrobial properties of the cyanobacterial EPS were checked against the bacterial strains of *Staphylococcus aureus* MTCC 2940, *Bacillus subtilis* MTCC 441 and fungus *Candida albicans* MTCC 227. The bacterial and fungal strains were cultured (Mueller–Hinton Broth (MHB)) and incubated overnight at 37 °C. After 12–18 h of incubation, the microbial strains were harvested and the turbidity was adjusted to 10^6 CFU ml^{-1}. EPS were primarily dissolved in sterile double distilled water at concentration 5 mg ml^{-1}. 30 µl of 3.3 X strength MH broths and Aliquots of 50 µl of EPS or solvent (negative control) were added to the appropriate microtitre wells. Lastly, 10 µl of the microbial suspension was added to each well to attain a concentration of 5×10^5 CFU ml^{-1}. Following incubation (37 °C for 18–24 h), 10 µl of resazurin was added and kept in incubator shaker for 4 h. The color change was then examined by using 96 well plate reader at excitation 560 and emission 590 nm[25].

Percentage inhibition was calculated following formula given below:

$$\%\ of\ inhibition = \frac{(Control - test)}{control} \times 100$$

Results

Effect of surface property on the hydrophobicity of released polysaccharide

The G-CCF cultivation of *Phormidium* sp. (AP3 and AP9F) EPS showed increased hydrophobicity (31.1 ± 6.6 and 60.27 ± 4.3 %) than PMMA-CCF cultivation (16.3 ± 5.5 and 38.8 ± 4.8 %; Fig. 1).

Effect of surface property on planktonic growth and biofilm formation

The initial inoculums of both the strains settled and grew at the bottom of the glass and PMMA-CCF. After 15 days, the culture started to grow to form biofilm on the rectangular strips (glass and PMMA slides) of the inner arrangement of the CCF cultivation. The biofilm formation (as recorded by average dry weights) was found to be high in PMMA-CCF cultivated *Phormidium* sp. (AP3 and AP9F) (0.0054 ± 0.0013 and 0.0083 ± 0.0005 g) than G-CCF cultivation (0.0043 ± 0.0016 and 0.006 ± 0.0014 g). Amongst the surface materials tested for cyanobacterial cultivation, *Phormidium* sp. (AP3 and AP9F) produced highest amount of planktonic biomass in PMMA-CCF (0.1166 ± 0.006 and 0.1296 ± 0.002 g) than in G-CCF (0.1056 ± 0.006 and 0.0875 ± 0.004 g) growth (Fig. 2).

Effect of surface property and mode of growth on protein and carbohydrate composition in EPS

Average values of biofilm CPS were higher in PMMA-CCF than to that of G-CCF. Similarly, CPS obtained from the planktonic biomass of *Phormidium* sp. (AP3 and AP9F) in the PMMA-CCF contained much higher proportion of polysaccharides than G-CCF (Table 1a & 1b). Further, cell suspension RPS-polysaccharides (Table 1c), Protein concentration of biofilm and planktonic biomass (Table 2a & b) and Protein concentrations in RPS (Table 2c) were recorded to be higher in the PMMA-CCF cultivation of *Phormidium* sp. (AP3 and AP9F) than the G-CCF setup.

Effect of surface property on the uronic component

The EPS from *Phormidium* sp. (AP9F and AP3) of PMMA-CCF culture also contained higher amount of uronic acid than G-CCF cultivation (Table 3).

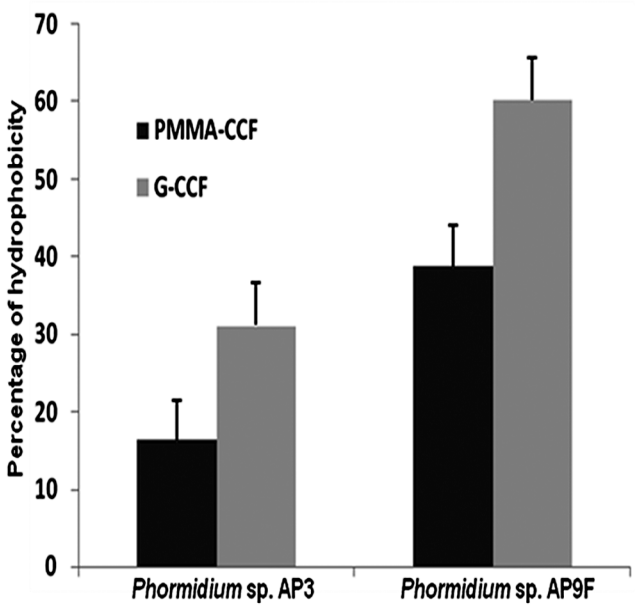

Fig. 1 — Hydrophobicity of released polysaccharide

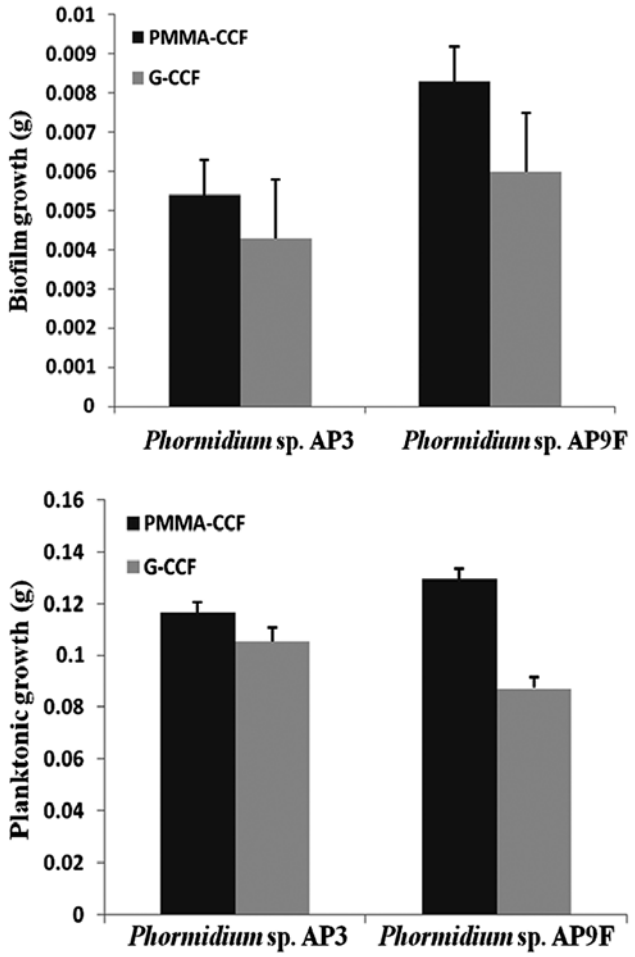

Fig. 2 — Effect of surface on growth of cyanobacteria

Table 1 — Carbohydrate from CPS of biomass (mg gram dry biomass^{-1} (gdb)) and RPS of cell suspension (mg g^{-1})

Strains	a) Biofilm		b) Planktonic		c) Cell suspension	
	PMMA-CCF	G-CCF	PMMA-CCF	G-CCF	PMMA-CCF	G-CCF
Phormidium sp. (AP3)	13.2 ± 1.6	7.4 ± 0.1	4.6 ± 0.8	3.9 ± 0.9	14.01 ± 3.3	12 ± 3.7
Phormidium sp. (AP9F)	8.9 ± 0.6	4.6 ± 2.2	10.7±1.4	8.6 ± 0.7	5.52 ± 0.92	4.29 ± 1.1

Values are means ± Standard deviation (n = 3)

Table 2 — Protein from CPS of biomass (mg gdb^{-1}) and RPS of cell suspension (mg g^{-1})

Strains	a) Biofilm		b) Planktonic		c) Cell suspension	
	PMMA-CCF	G-CCF	PMMA-CCF	G-CCF	PMMA-CCF	G-CCF
Phormidium sp. (AP3)	11.8 ± 0.1	10.8 ± 1.6	15.7 ± 3	10.2 ± 4	22.8 ± 5.8	15.8 ± 4.1
Phormidium sp. (AP9F)	12.9 ± 1.2	10.7 ± 0.1	16.0 ± 0.8	13±2.4	8.34 ± 0.49	4.51 ± 0.32

Values are means ± Standard deviation (n = 3)

Table 3 — Estimation of uronic acid in EPS (µg mg^{-1})

Strains	PMMA-CCF	G-CCF
Phormidium sp. (AP3)	16.8 ± 1.3	13.7 ± 1.2
Phormidium sp. (AP9F)	7.16 ± 0.35	2.77 ± 0.38

Values are means ± Standard deviation (n = 3)

Effect of surface property on a flocculating and emulsifying activity

It was revealed that the emulsifying activity of EPS of *Phormidium* spp. from PMMA-CCF culture with the hydrocarbon employed was found better than that of G-CCF cultivation at a concentration of 0.3 %, indicating that the EPS of PMMA-CCF possesses an improved emulsifying activity than G-CCF (Table 4a). As a result, the flocculating properties of EPS from *Phormidium* sp. with different surface materials were examined through kaolin clay as the suspended solid. The flocculation reactions were carried out with 0.5 mg of EPS l^{-1} concentration on flocculating activity. EPS of *Phormidium* sp. (AP3 and AP9F) from PMMA-CCF showed high flocculating activity than G-CCF cultivation (Table 4b).

Effect of surface property on an antimicrobial activity

Cyanobacterial EPS displayed diverse antimicrobial activity when cultivated under different surface materials. The EPS of PMMA-CCF-cultivated *Phormidium* (AP3) and (AP9F) showed higher antibacterial activity (90.1 ± 13.9 and 24.5 ± 0.1 %, respectively) against *S. aureus* than G-CCF (86.5 ± 5.4 and 0 %, respectively). Whereas, The EPS of G-CCF-cultivated *Phormidium* (AP3) and (AP9F) exhibited mild activity (11.5 ± 5.4 and 13.45 ± 0.9 %, respectively) against *B. subtilis* compared to MMA-CCF (0 and 10.7 ± 5.2 %, respectively). No significant activity was recorded when all the EPS were tested against *C. albicans* (0 – 6.9 ± 1.9 %; Table 5).

Discussion

In the present study, EPS from different strains (AP3 and AP9F) of *Phormidium* spp. were cultivated in hydrophobic polymethyl methacrylate (PMMA) and hydrophilic glass (G) surfaces. Based on the surface property it is revealed that strains cultivated on hydrophilic surfaces showed increased hydrophobicity than those cultivated on the hydrophobic surface. In the current study, the hydrophobicity of the cyanobacterial EPS was corroborated with uronic acid content. When, uronic acid content was higher; it caused retention of the water content and hence increase in the hydrophobic activity in PMMA-CCF cultivation. Uronic acid contributes to anionic property of EPS and the presence of very high fucose and rhamnose monosaccharide contents may also be responsible for increasing the hydrophobic activity[33]. In this investigation, increased production of uronic acid by the cyanobacterial strains was supportive of EPS hydrophobicity.

All the strains grew at a faster rate as suspension at the bottom of the glass and PMMA-CCF, yielding higher planktonic biomass. Considering both the vessels showed productivity in terms of total biomass (planktonic + biofilm), growth depends on the species

Table 4 — Emulsifying and flocculating activity of EPS

Strains	a) Emulsifying activity (0.3 %)		b) Flocculating activity (500 µg)	
	PMMA-CCF	GS-CCF	PMMA-CCF	GS-CCF
Phormidium sp. (AP3)	73.31 ± 0.89	68.47 ± 1.6	55.7 ± 5.5	35.6 ± 3.7
Phormidium sp. (AP9F)	14.2 ± 1.3	9.2 ± 0.2	18.6 ± 1.1	14.9 ± 0.9

Values are means ± Standard deviation (n = 3)

Table 5 — Antimicrobial activity of cyanobacterial EPS cultured in different surface material

Cyanobacteria	% of inhibition					
	B. subtilis		*S. aureus*		*C. albicans*	
	G-CCF	PMMA-CCF	G-CCF	PMMA-CCF	G-CCF	PMMA-CCF
Phormidium sp. (AP3)	11.5 ± 5.4	0	86.5 ± 5.4	90.1 ± 13.9	4.7 ± 0	0
Phormidium (AP9F)	13.45 ± 0.9	10.7 ± 5.2	0	24.5 ± 0.1	0	6.9 ± 1.9

and surface material. The biofilm growth was highest for *Phormidium* sp. AP3 and AP9F when cultivated in PMMA-CCF. The density of the biofilm (attached biomass) differed with the surfaces. The co-culture of hydrophobic algal suspension of *Synechocystis salina* with *Chlorella vulgaris*, *Pseudokirchneriella subcapitata* and *Microcystis aeruginosa* influenced the microalgal recovery efficiencies[34]. *Synechococcus* cell surface produced protein-related amine groups, lipids and LPS-related carboxyl groups based on different growth conditions. These data showed the surface physico-chemical properties of microalgal settling and could play a significant role in the microalgal removal percentage and free energy of hydrophobic interaction. The select low net zeta potential of surface material is an important assumption in using cyanobacterial cultures for high recovery efficiency[35].

EPS from cyanobacteria act as a buffer region among the cell and the environment, and its composition and structure vary depending on the environment and cyanobacterial taxa[36]. Intertidal environments are effectively influenced by changes in physical and chemical parameters[37]. Cyanobacteria from shallow marine intertidal zone are subjected to high levels of solar irradiation and to adapt such periodical fluctuations and wave activities, they form biofilms by overexpression of genes responsible for the biosynthesis of EPS[38]. Our previous studies[25] have confirmed that benthic cyanobacteria produce a high concentration of exopolysaccharide associated with biofilm mode of cultivation. The outcomes of the current study highlighted the relative account of the EPS production between PMMA-CCF and the G-CCF vessels. Test cyanobacteria produced extensively higher amounts of EPS in PMMA-CCF than in the G-CCF. The EPS formation was dependent on biofilm formation and biomass production. Compared to the previous work done by Veerabadhran *et al*.[25], the tested *Phormidium* spp. (AP3 and AP9F) produced a low quantity of EPS than *Oscillatoria* sp. AP17, *Leptolyngbya* sp. AP3b and *Chroococcus* sp. AP3U. These are all bottom dwellers and adapting to the planktonic mode of growth in natural conditions might be the reason for obtaining higher planktonic growth in both the vessels, although higher than the biofilm growth. Increased protein content in PMMA-CCF biofilm, planktonic and RPS indicated the higher photosynthetic activity. However, it was recently reported that in *Synechocystis* sp. and *Chroococcus minutes* of aquatic and desert environment, the exposure of UV-B radiation significantly affected the protein stability and photosynthetic activity[39]. Pereira *et al*.[16] reported that cyanobacterial EPS production could not track the access patterns of bacteria and most of the protein related to EPS production.

Various heterocystous and non-heterocystous species grown under photoautotrophic condition have been shown to excrete bioflocculant EPS. The EPS of *Anabaena* sp. BTA992 produced high flocculation EPS consisting of neutral sugars and uronic acid[40]. The present study also strongly supported the presence of high uronic acid concentration in PMMA-CCF with increased flocculating activity in EPS from PMMA-CCF. Bioflocculant exopolysaccharide isolated from *Anabaena* sp. BTA990 and *Nostoc* sp. BTA97 exhibiting high flocculation activity were positively correlated with higher uronic acid production[41]. Protein content was always high in the biomass (biofilm and planktonic) and RPS of PMMA-CCF was responsible for increasing the emulsifying activity. Proteins in EPS take part as a vital role in the emulsifying activity. The high content of protein is accountable for emulsifying activity against the hydrophobic substrate[5].

Furthermore, deproteinized EPS (Apo-EPS) affects the emulsification activity of the EPS, and thereby resulting in reduced emulsification than the native EPS[42]. The presence of hydrophobic character plays a significant role in the emulsifying activity[43]. The potential of intertidal cyanobacterial strains to produce EPS inhibiting the growth of gram-positive and negative bacterial strains was also recorded in this study. Antimicrobial screening of EPS, extracted from two cyanobacterial strains cultivated in different surface material, was carried out using the resazurin assay. The relatively high percentage of antibacterial activity is noticed in the PMMA-CCF extracted EPS, might be due to the suitability of the PMMA surface for enhancing antibacterial EPS production. When checked in bacteria, however, PMMA-CCF and G-CCF EPS were found highly active against *S. aureus* and *B. subtislis*. The aqueous fraction of EPS from *Arthrospira platensis* was active against *Staphylococcus epidermis*[44]. Exopolysaccharides of *Gloeocapsa* sp. and *Synechocystis* sp. exhibited activity against *S. aureus*, *C. albicans* and *Pseudomonas aeruginosa*[45].

In conclusion, the aim of this study was to make use of a recently designed conico-cylindrical flask, with the enhanced glass and PMMA surface area, a substitute to the generally used laboratory Erlenmeyer flasks. The PMMA-CCF vessel with higher internal surface could increase the production of RPS, CPS, uronic acid, and monosaccharide composition. The mode of growth and surface material mainly influences the production. Cyanobacteria grown in PMMA-CCF cultivation showed maximum production of RPS, CPS, protein, and uronic acid among the tested cyanobacteria. This feature could be effectively used in purposes containing direct pharmaceutical and industrial applications. Unevenness in the activity, EPS and biomass production between cyanobacteria grown in different vessels were to a large extent attributed to the growth rate which was reliant on vessel surface characteristics.

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Conflict of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Author Contributions

JM: Conceptualization, Project administration, Writing - review & editing; VM: Formal analysis, Investigation, Methodology, and Writing - original draft; SB: Formal analysis, Investigation, and Methodology; DB: Formal analysis, Investigation, and Methodology.

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