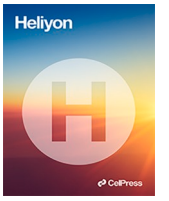
Heliyon 10 (2024) e32057

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Cultivation of algal biofilm and mat communities from the Garhwal Himalayas for possible use in diverse biotechnological applications

Pragati Kumari *, Dhananjay Kumar **

Laboratory of Algal Biotechnology, Department of Botany and Microbiology, School of Life Sciences, H.N.B. Garhwal University, Srinagar, Garhwal, 246 174, India

ARTICLE INFO

Keywords:
Microalgae
Biomass
Cyanobacterial mat
Nylon net
Harvesting

ABSTRACT

The current study aimed to screen biofilm-/mat-forming and fast-growing algal communities from the Garhwal Himalayas, India. A total of 15 biofilm/mat-forming algal samples were collected, 8 biofilms out of these could be cultured and analyzed for their growth and development with time. Light microscopy was used to identify different types of cyanobacteria and algae present in the different collected biofilms/mats. Four biofilm and mat communities, namely biofilms #E, #F, #G, and #H, were found to have fast growth and were quick to colonize the substratum. Nylon net was identified as the most cost-effective and best-supporting material for biofilm development and biomass production. The study also found that increasing the harvesting frequency from the nylon net-enmeshed biofilms at least once a week would enhance the final biomass yield compared to harvesting the community once after a longer growth duration. Nevertheless, the findings reported here will be useful for researchers in developing phototrophic biofilm-based technology using nylon net, as it will be mechanically strong, supportive, and easy to handle.

1. Introduction

Developing an integrated system that combines wastewater treatment, carbon dioxide sequestration, and biomass feedstock generation for diverse applications by employing microalgae is receiving a lot of attention these days [1,2] Several phytoplanktonic unialgal culture systems and the performance of different kinds of photobioreactors have been evaluated also in this regard [3–6]. However, maintaining a unialgal culture system and harvesting tiny planktonic microalgae are two major constraints that prohibit the large-scale application of any such approach [7]. In this context, the naturally immobilized phototrophic algal communities, such as algal biofilms, cyanobacterial mats, and periphytons seem to be a better option as being naturally prevailing consortia of cyanobacteria, microalgae and bacteria, they would not require any effort for upholding unialgal veracity, and their harvesting would also be easy due to inherent self-immobilized nature [8–10]. Unfortunately, these natural communities continue to remain sporadically investigated for beneficial uses [8,11–13] and have often been studied only for their negative consequences [2,14,15].

The fast growth rate is a prerequisite for employing any microalgal species for its large-scale industrial applications. Thus, the conditions facilitating maximum growth have been extensively examined by researchers for several planktonic microalgae and

* Corresponding author.
** Corresponding author.
E-mail addresses: pragati27@gmail.com (P. Kumari), drdkumar83@gmail.com (D. Kumar).

https://doi.org/10.1016/j.heliyon.2024.e32057
Received 19 December 2023; Received in revised form 27 May 2024; Accepted 28 May 2024
Available online 29 May 2024

cyanobacteria [16,17]. Conversely, very limited information is available regarding the growth, development, and cultivation of algal biofilms, mats, and periphytic communities. Some workers have reported the encouraging role of certain substrate materials in the primary development of mat and biofilm communities [8,18,19]. Thus, additional substrate materials need to be tested in this context. Diverse algal biofilms and mat communities comprise different species compositions, so they may show variable affinities and hence unpredictable growth responses for different substrate materials. Hence, it warrants checking out the suitability of a substrate for a variety of algal biofilms and mat communities before recommending it for generalized use. Moreover, it also needs to be taken into consideration that using any such substrate materials would not cause any inconvenience in utilizing algal biofilms or mats for the intended application(s).

The present study focuses on collecting algal biofilm and mat communities from different locations in the Garhwal Himalayas as this region has not been sufficiently explored, despite comprising a remarkable diversity of microalgae and cyanobacteria in its various habitats, such as thermal springs, cold-water streams, high-altitude water bodies, and snow-capped regions [20,21]. Here, we initially screened different algal biofilms for their fast-growing attribute. Thereafter, we tested different substrate materials to find the best one for promoting the growth and development of a chosen algal biofilm. Once the best substrate was identified, we examined its suitability for all other cultured algal biofilm communities. Finally, the impact of periodic biomass harvesting on the net productivity of the identified best algal biofilm community was also evaluated.

2. Materials and methods

2.1. Sample collection sites

The firm and mechanically strong algal biofilm communities were collected in clean sterile plastic vials and polybags using forceps and samplers from the diverse sites of Uttarakhand, Garhwal Himalayas [Fig. 1]. Samples were carefully transported to the laboratory and efforts were made to cultivate them using algal culture media. Though different media recipes, such as Allen and Arnon, Chu-10, BG 11, and Castenholz D (for hot spring mats) were tested, the BG 11 medium [22] was found suitable for almost all the selected mats and it was used in all the other experiments. Along with biofilms and mats, we also collected water samples from their growing sites and characterized them for their physicochemical parameters like pH, temperature, EC, and TDS. The species composition of the

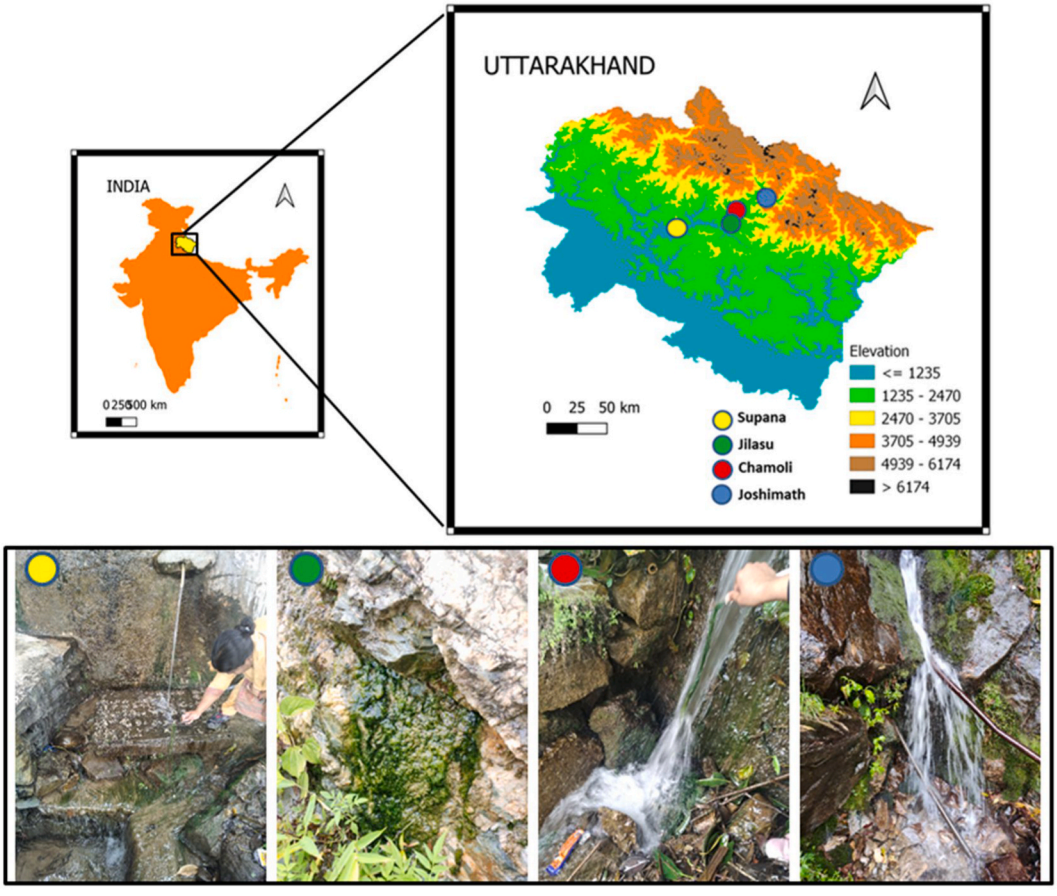


Fig. 1. Sample collection sites in the Garhwal Himalayas, Uttarakhand, India.

collected algal biofilms was determined using a compound light microscope (CX21iLED; Olympus) attached to a digital camera (Magnüs, MIPS-5 MP, MagCam DC Series, Noida, India). The different cyanobacterial and algal taxa inhabiting the biofilms/mats were identified by consulting relevant literature [23–28]. We also consulted Algaebase, CBdata base, and ANSP algal image database for this particular purpose.

2.2. Cultivation of algal biofilms in the laboratory

Initially, efforts were made to cultivate the collected algal biofilms and mats into Petri dishes. For this, the 1 mL homogenized inoculum of each biofilm or mat community was added into Petri dishes containing 19 mL full-strength liquid BG11 medium prepared using double-distilled water. The different constituents were added as per the recipe provided by Hughes et al. [22]. The biofilms/mats

Table 1
Details of algal biofilm/mat communities collected from the different sites of Garhwal Himalaya, India.

S. No.	Site	Assigned name	Attributes					Species composition	
			Biomass	Colour	Habitat	Water Temperature (°C)	pH	Dominant	Others
1	Khanda freshwater stream, Pauri Garhwal	Biofilm # A	Soft floating community	Green	Freshwater	18.6 ± 1.0	8.5 ± 0.2	*Phormidium* sp.	*Chlorella* sp., *Lyngbya* sp.
2	Ringigarh Hot Spring, Chamoli, Garhwal	Biofilm #B	Firm biofilm	Dark green	Hot spring	37.8 ± 1.9	6.7 ± 0.3	*Mastigocladus* sp.	*Scytonema* sp., *Oscillatoia* sp.
3	Tapovan hot spring	Biofilm #C	Fragile thin biofilm	Green	Hot spring	40.9 ± 1	6.7 ± 0.3	*Mastigocladus* sp.	*Nostoc* sp., *Oedogonium limnosa*
4	Roadside wall area, Joshimath	Biofilm #D	Rocky biofilm	Light green	Freshwater	18.3 ± 0.5	8.2 ± 0.1	*Oscillatoia* sp.	*Chlorella* sp., *Navicula* sp.
5	Supana, Alaknanda river, Dhari devi road, Garhwal	Biofilm #E	Thick sheet	Brownish	Freshwater	18.4 ± 1.0	8.6 ± 0.2	*Pseudophormidium* sp	*Pinnularia* sp., *Scenedesmus* sp., *Chlamydomonas* sp.
5	Supana, Alaknanda river from the road side	Biofilm #F	Fragile biofilm	Light green	Freshwater	19.6 ± 0.2	8.3 ± 0.5	*Phormidium* sp.	*Synecococcus elongatus*
7	Supana, roadside wall area, Garhwal	Biofilm #G	Soft Biofilm	Dark green	Freshwater	18.6 ± 1.2	8.3 ± 0.3	*Oscillatoia* sp.	*Chlorella* sp., *Synecococcus* sp.
8	Supana, Garhwal	Biofilm #H	Fragile biofilm	Green	Freshwater	20.1 ± 0.6	8.2 ± 0.2	*Synecococcus* sp.	*Pinnularia* sp., *Chlamydomonas* sp.
9	Saldhar hot spring, Chamoli, Garhwal	Biofilm #I	Fragile Biofilm	Black	Hot spring	45.5 ± 0.8	7.4 ± 0.6	*Leptolyngbya* sp.	*Mastigocladus* sp., *Tribonema* sp.
10	Badrinath Hot Spring Chamoli, Garhwal	Biofilm #J	Thick sheet	Green	Hot spring	41.2 ± 0.9	6.4 ± 0.3	*Chroococcus* sp.	*Lyngbya* sp., *Nostoc* sp., *Scenedesmus* sp.
11	Roadside area, Joshimath	Biofilm #K	Soft Biofilm	Dark Green	Freshwater	19.3 ± 1.4	8.6 ± 0.2	*Pinnularia* sp.	*Oocystis* sp., *Pseudokirchneriella* sp.
12	Chamdhar freshwater stream, Pauri Garhwal	Biofilm #L	Rocky biofilm	Dark Green	Freshwater	18.5 ± 1	8.6 ± 0.3	*Phormidium* sp.	*Cosmarium* sp., *Chroococcus* sp.,
13	Alaknanda river Dharidevi temple, Pauri Garhwal	Biofilm #M	Soft Gel-like Biofilm	Dark Green	Freshwater	22.2 ± 0.7	8.2 ± 0.6	*Pinnularia* sp.	*Oocysti* sp., *Cosmarium* sp., *Pithophora* sp.
14	Near Nand Prayag bathing area, Jilasu	Biofilm #N	Fragile thin Biofilm	Brownish	Freshwater	22.1 ± 1.2	7.9 ± 0.3	*Scenedesmus* sp.	*Chlorella* sp., *Chlamydomonas* sp.
15	Nand Prayag, from the wall near bathing area, Jilasu	Biofilm #O	Fragile thin Biofilm	Light Green	Freshwater	17.9 ± 1.6	8.1 ± 0.2	*Synecococcus* sp.	*Chroococcus* sp., *Pseudokirchneriella* sp.

communities received 72 μmol m^{-2} s^{-1} PAR in a 12 h light and 12 h dark cycle at 27 ° C. The Petri plates were gently shaken 4–5 times every day to facilitate the colonization and growth of the mat-/biofilm-forming organisms. The growth and development of each biofilm community were recorded in terms of the colour appearance of the initially formed biofilm, the behaviour of the entrapment of air bubbles in the matrix, and the change in pH of the culture medium as a result of the photosynthetic activity of the biofilm or mat community. The culture medium of each plate was replaced by the fresh sterilized BG 11 medium fortnightly. Each experiment was carried out in triplicate.

2.3. Screening of suitable substratum for the growth and development of algal biofilms

Some earlier workers have tested the suitability of different substrate items for facilitating the growth and development of biofilms and mats [8,10,19]. Thus, we also tried to investigate the significance of providing good substrate conditioning on the growth and development of algal biofilms. Cotton bandage (75 mesh cm^{-2}), muslin cloth (300 mesh cm^{-2}), nylon net (mesh size 2 mm), rice husk, and sawdust were tested for this purpose considering the algal biofilm G as the test community, and the growth response was estimated in terms of total chlorophyll.

2.4. Assessing the ability of different algal biofilms to grow enmeshed with nylon net

After conducting substrate screening with algal biofilm G, we studied different biofilms (E, F, G, and H) to determine their ability to grow and develop enmeshing nylon net discs. This was done considering the advantages of nylon net-enmeshed algal biofilm in bioremediation-related applications, as highlighted by others [10,19]. An experiment was also conducted to evaluate the effect of periodical harvesting on the productivity of a nylon net-enmeshed growing algal biofilm. For this, two sets of Petri plates containing growth medium and nylon net disc were inoculated with mat inoculum considering triplicate. The productivity of the algal biofilm was estimated in terms of total chlorophyll. The biomass of one set was scraped from a nylon net every week to determine total chlorophyll. On the other hand, the plates in the other set were allowed to grow for 21 days and their biomass was harvested only once, at the end of the experiment. For each set, the medium was replaced weekly with fresh sterilized BG 11 liquid medium.

2.5. Determination of pigment content, dry weight and change in medium pH

Different pigments, like chlorophyll *a, b* and carotenoids were estimated following standard protocols as described elsewhere [12]. For this, a small known area of each mat was cut with the help of a cork borer and homogenized in 10 mL of deionized water. In the case of the nylon enmeshed mat, the cork borer was used to mark the specific area, and mat biomass was scrapped with the help of a brush and forceps. The homogenized suspension of mat biomass was centrifuged and the pellet was suspended in the known amount of 80 % acetone and incubated at 4 °C in the dark. It was centrifuged again and the resulting supernatant was used for the estimation of the pigments in the supernatant. The absorbance was recorded using a Systronics UV–vis spectrophotometer (Systronics India, Ahmedabad, India; model no. 117) 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 [29], while carotenoids were quantified as per Kratz and Myers [30].

To determine the final yield of the algal biofilm productivity, the dry weight was considered. A small, known area of the algal biofilm was cut using a cork borer and placed onto Whatman filter paper. The biomass was then dried in an oven at 80 °C until a constant weight was achieved. The pH of the culture medium was recorded weekly when the used culture medium was replaced with a fresh one. All experiments were conducted in triplicate.

3. Results

3.1. Characteristics of algal biofilms collected from different sampling sites

Fig. 1 represents the different sites of the Garhwal Himalayas from where a total of 15 algal biofilm and mat communities were collected for this study. The characteristics of all these algal communities related to the nature of the biomass, colour, habitat, and the dominant microalgal form have been provided in Table 1. The biomass of some of the biofilms was soft and fragile, while some communities were firm and thick. Some algal biofilms and mats were free-floating but some were growing firmly adhering to pebbles and rocks. The colour of communities ranged from light green to intense blue-green. However, a few samples, particularly floating mats, were deep black. Nevertheless, most of the collected biofilms and mats were mainly dominated by cyanobacterial species but also comprised species of green algae and diatoms. However, two collected benthic mat communities were dominated by diatoms, though the main entangling forms were again cyanobacteria. Four collected biofilms belonged to hot spring habitats from zones having temperatures as high as 40 °C.

3.2. Response of algal biofilm and mat communities upon cultivation in the lab

Efforts were made to grow all the collected algal biofilms and mats in Petri dishes providing BG 11 as a growing medium. However, of fifteen tested biofilms and mats, only eight responded properly. The other communities were identified as extremely slow-growing communities and, hence were not used in further study. Further, we noticed the initial growth response of the eight selected communities in the Petri plates [Fig. 2(A–H)]. In most of the cases, a thin film appeared after 6–7 days of inoculation which became thick

with time. It was observed that a few communities started proliferating attaching to the bottom of the plates but very soon detached from it and started floating in the medium. However, some remain attached to the bottom during the entire experimental duration. One community grew floating from inoculation to the end of the time course study of 45 days. Mostly the growing mat communities were green in colour except for biofilm #E, which was brownish and formed as a thick mat [Table 1]. Among all the studied biofilms, #E, #F, #G, and #H appeared better in terms of their fast growth [Fig. 2(E–H)].

We observed the entrapment of air bubbles in the matrix of biofilms and mats, which generally appeared one or two days after the visible appearance of a thin phototrophic biofilm. The test biofilms #G usually showed early entrapment of a good number of air bubbles, though smaller in size, in its matrix. The number of entrapped air bubbles was greatly influenced by the age of the mat. It increased up to 19–20 days, while thereafter decreased regularly with increasing the age and thickness of the biofilm. Other biofilms like #B, #C, and #D either did not entrap any air bubbles or their numbers vary from one to four only. Moreover, in the case of these latter biofilms, the sizes of the air bubbles were far larger than was noticed for biofilm #G.

Microscopic observations of laboratory-grown biofilms and mats were performed to compare the change in species composition from the naturally collected ones. Results reveal that in the case of nearly all the communities, the dominant photosynthetic forms remained unaffected. The relative abundance of the dominant species belonging to the test mats, namely#E, #F, #G, and #H was considerably higher (>70 %) when they grew enmeshing nylon net discs. Some other microalgal forms that remained present in different biofilm and mat communities were *Chlorella* sp., *Oocystis* sp., *Cosmarium* sp. *Chroococcus* sp., *Scenedesmus* sp.; and *Pseudokirchneriella* sp. Some species of diatoms like *Pinnularia* sp. and *Navicula* sp. were also reported in cyanobacteria-dominated mats, however, they disappeared during subculturing. Other microalgal forms had very low relative abundance (<1 %), hence they were not specified.

3.3. Screening of different substratum for enhanced biomass production

To maximize the productivity of the test biofilms and communities, the selection of a suitable substratum was necessary. Hence different substrata were tested here to examine their ability to support growth and development considering biofilm #G as the test community. After 30 days of growth on different substrata, biomass productivity was measured in terms of chlorophyll *a* (mg g^{-1}). Slow growth and poor development of the test community were noticed on sawdust (0.527 ± 0.02), rice husk (0.416 ± 0.02), and cotton bandages (0.516 ± 0.02). The entrapment of air bubbles was also delayed. A very thin film was formed after a fortnight which did not become thick even after a month. Growth without any substratum was also comparable with different substrata (0.472 ± 0.01). Growth on muslin cloth (0.867 ± 0.01) was better and appeared in the form of a thin biofilm after 7–8 days of inoculation and the first bubble appeared after 12 days. However, the nylon net (1.158 ± 0.03) was identified as a good substratum for the development and growth of the biofilm [Fig. 3(A and B)]. In this case, a thin biofilm appeared after 4–5 days which showed good thickness after 30 days and successfully enmeshed the nylon net discs.

3.4. Screening of algal biofilm communities for enmeshing nylon net discs

Besides biofilm #G, three other biofilms, #E, #F, and #H, also showed a better affinity for colonizing nylon net discs [Table 2]. In the case of these communities, a thin film appeared after 4th day of inoculation that became thicker with time. Trapping of air bubbles was as usual as noticed in the control of the respective biofilm where they were grown in a Petri dish without any substratum.

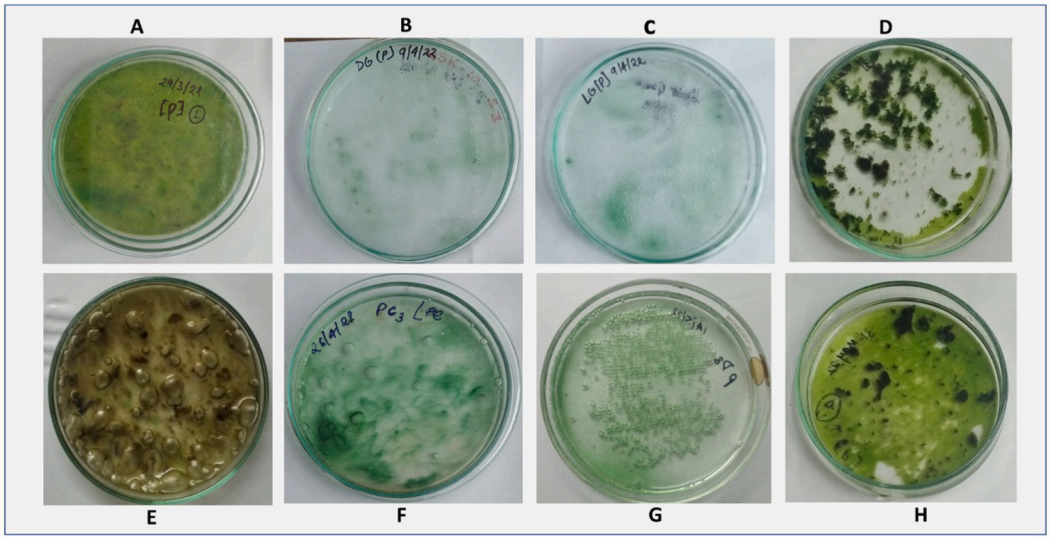


Fig. 2. Growth response of different algal biofilms/mats (A–H) after 10 days of inoculation in culture medium.

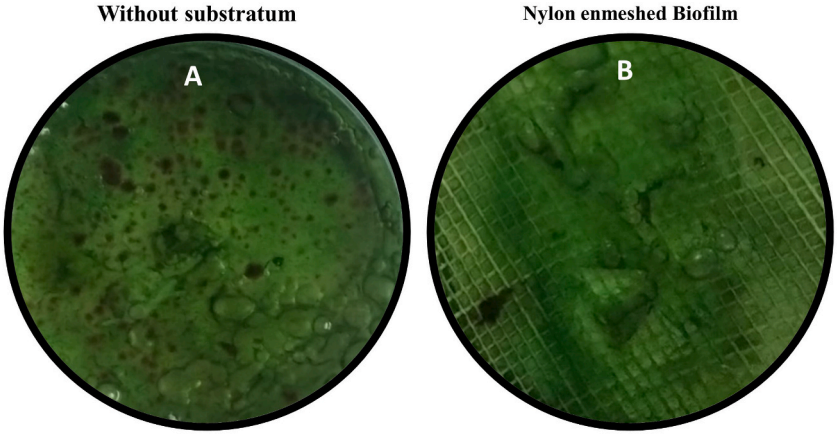


Fig. 3. Nylon net enmeshed growth of one of the test biofilms, (A) without nylon net and (B) with nylon net. The photograph was taken after 21 days of biofilm growth.

3.5. Comparative growth response of nylon net enmeshed algal biofilm communities

Four different algal biofilm communities, namely #E, #F, #G, and #H were further studied to compare their biomass productivity when grown with and without a nylon net disc [Fig. 4a]. The biomass yield (measured in terms of milligram dry weight) of each community was substantially higher when grown with the nylon net. All the tested communities showed a severalfold increase in biomass production when allowed to grow from 30 to 45 days. Interestingly, the biofilm communities H, G, and F initially produced biomass at a slower rate for up to 30 days, but then accumulated more biomass in the next 15 days, reversing the order of biomass production as seen in the first 30 days of growth. Moreover, after 45 days of growth, the maximum biomass yield was noticed for biofilm #H, which was closely followed by #G, #F, and #E.

The different photosynthetic pigments, namely chlorophyll *a, b*, total chlorophyll, and carotenoids were also measured for the tested algal biofilm communities [Fig. 4b]. Results revealed a significant increase in the contents of all the estimated pigments as the growth duration increased from 30 to 45 days. However, this time the increase was not as prominent as it was noticed for biomass dry weight. Moreover, the order of pigment production by the different biofilm communities was also different from what was noticed in the case of biomass dry weight estimation.

The pH level of the medium was observed for communities that were growing on nylon nets and those without a substratum, at 15, 30, and 45 days after being inoculated. All of the studied communities showed a significant increase in the pH of the culture medium compared to the initially adjusted pH (8.0). However, the increase was relatively higher for biofilm #G, although its magnitude decreased as growth duration increased from 15 to 45 days, compared to the increase noticed for biofilms #E, #F, and #H. Moreover, no definite trend was observed in pH alteration between the plates of the different biofilm communities grown with and without nylon nets [Fig. 5].

3.6. Effect of periodical harvesting on biomass yield of biofilm and mat communities

A study was conducted to analyze the effect of periodic harvesting on the productivity of nylon net-enmeshed biofilm #G. The results showed that the productivity of the algal biofilm, as measured in terms of total chlorophyll, was almost double when it was harvested weekly for up to three weeks, as compared to the productivity of a biofilm that was harvested only once after an undisturbed growth of 21 days [Table 3].

4. Discussion

Self-immobilized phototrophic biofilms and mats are recently appreciated as 'Productive systems' by some researchers [31]. Unlike phytoplanktonic algae, these systems are immobilized in a matrix, and therefore, their biomass can be easily harvested. Given this, biofilm and mat communities were collected from different sites in the Garhwal Himalayas and initially characterized for their different attributes. The collected biofilm and mat communities were mostly dominated by cyanobacterial forms, except for benthic diatoms-dominated mats. This nature of the phototrophic biofilms coincides with the findings of several other researchers who also reported cyanobacterial forms as dominant entities in microbial mats and algal biofilms obtained from diverse habitats [15,19,32]. However, some studies have characterized thin algal biofilms dominated by unicellular green algae [33]. Nevertheless, some other researchers have reported loosely entangled net-like structures formed by *Spirogyra*, *Cladophora*, and *Pithophora*-like macroalgae as mat [34].

The selection of substratum is a very important component for getting quick initial development and ensuring enhanced biomass production of biofilm and mat communities. The nature of the substratum can affect the initial adhesion strength of algal cells and the

Table 2
Characteristics of the test cyanobacterial biofilm growing with and without substratum after 7 days of growth.

Substratum (Nylon net 2 mm)	Characteristics			
	Biofilm #E	Biofilm #F	Biofilm #G	Biofilm #H
With substratum	A brown colour film appeared after four days of inoculation and became a thick and strong mat with the passage of time. Bubble formation appeared after one week.	A thin blue-green biofilm was developed and it enmeshed the nylon net disc with the passage of time. Entrapment of a few bubbles was noticed after 7–8 days.	The Nylon net disc provided support, and a light green colour mat was developed. This mat started entrapping a large number of small-sized air bubbles from the very beginning of its growth.	Dark green in colour, grew firmly adhering to the substratum and entrapped a smaller number of air bubbles of variable sizes.
Without substratum	Slow growth as compared to mat growing on nylon net; Bubbles appeared after 10 days.	Slow growth without substratum No bubbles formed even after 4 weeks.	The mat grew adhering to the bottom of the Petri dishes, but thickening and bubble entrapment were delayed.	Growth behaviour was similar to Mat #G. However, Bubble entrapment was absent.

Note: Biofilms #A, #B, #C and #D did not grow well enmeshing nylon net disc.

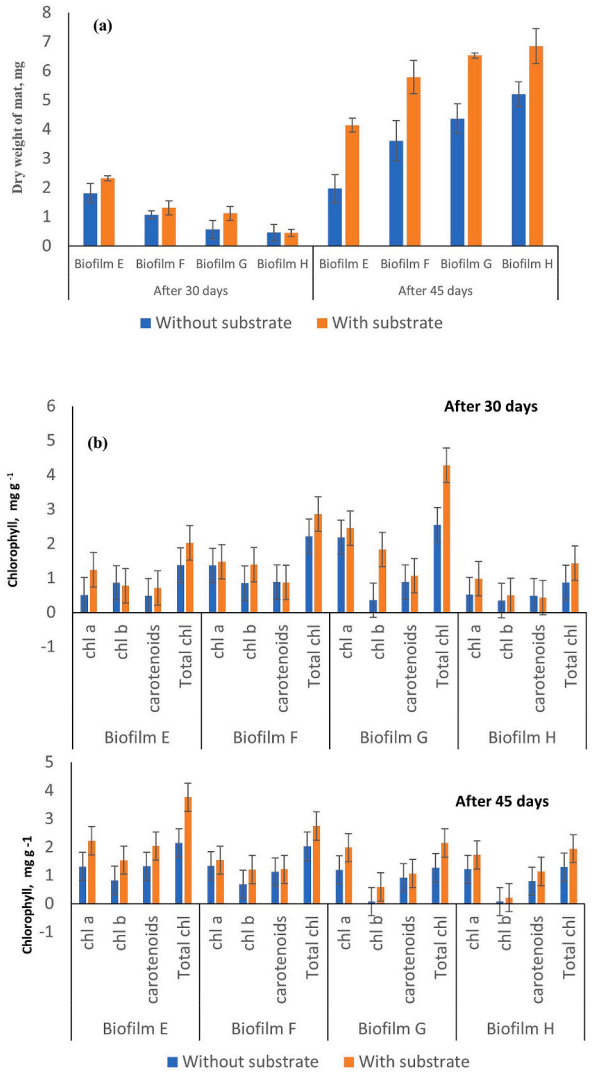


Fig. 4. Growth response of test biofilm communities on nylon net substratum measured in terms of (a) dry weight and (b) chlorophyll *a, b*, and carotenoids.

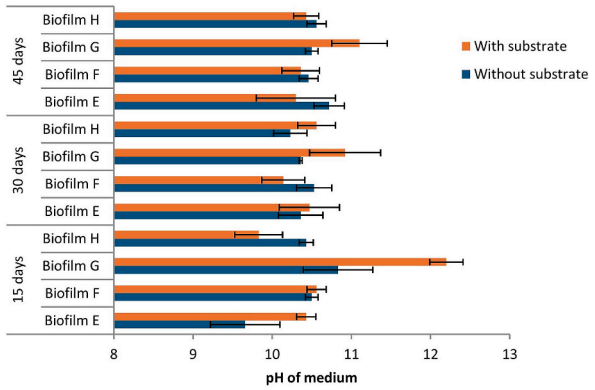


Fig. 5. Alteration in pH of culture medium supporting the growth of different algal biofilms in the absence and presence of nylon net substratum. The data was recorded after 15, 30, and 45 days of growth.

Table 3
Effect of periodical harvesting on biomass yield of biofilm #G.

Experimental design	Growth period (day)	Biomass yield (Total chlorophyll, mg)	Aggregate biomass yield (Total chlorophyll, mg)
Biomass harvested weekly from the nylon net enmeshed biofilm	7 days	0.763 ± 0.09	~2.0 mg
	Next 7 days	0.540 ± 0.20	
	Another 7 days	0.691 ± 0.09	
Biomass harvested from the nylon net enmeshed biofilm after 21 days of undisturbed growth	21 days	1.00 ± 0.17	~1.00 mg

composition of the algal community [35–41]. In the present study, different materials were tested for their suitability to act as a substratum for the development of test biofilm and mat communities. Of different tested materials, nylon net was found promising in facilitating the growth and development of at least four biofilm communities, namely #E, #F, #G, and #H. Perhaps the superiority of nylon net above other materials like cotton bandages, muslin cloth, rice husk, and sawdust, is attributed to its rough and hard surface. Moreover, being an inert material, it will also not leach something out in the medium which can inhibit the growth of photosynthetic forms inhabiting the mat communities. Likewise, some other workers also noticed the positive effect of different materials like grass clippings and polyester foams on the growth of phototrophic biofilms or mats [8,18]. The poor performance of rice husk, sawdust, and cotton bandages might be related to the leaching of certain inhibitory compounds from them. In this context, it deserves to be mentioned that wheat and barley straws are reported elsewhere for inhibiting algal growth [42,43]. Likewise, Kumar et al. [19] reported adverse effects of sawdust, rice husk, and sands of the river Ganges and Sone on the growth and development of a *Phormidium*-dominated mat.

The time-dependent accumulation of chlorophyll *a, b,* and carotenoids by the test biofilm communities was measured. Results reveal that the content of these pigments increased with time but it was not as prominent as it was noticed in the case of biomass dry weight. Perhaps these results indicate variable accumulation of exopolysaccharides and other similar compounds by different biofilms with time, and the increased content of these compounds contributed a significant share to the final biomass yield of the community. A high apportionment of exopolysaccharide content in the biomass of cyanobacterial mats and biofilms has also been emphasized by several other workers [9,44]. Moreover, it makes these mat communities a system endowing enormous abilities to bind metal ions and other pollutants from aqueous systems [10,12,45].

During the growth of various algal biofilms, either without any substratum or enmeshed on a nylon net disc, the entrapment of air bubbles was observed. The number and size of entrapped bubbles, however, varied with biofilm communities. The trapping of air bubbles primarily occurs due to the capture of oxygen produced during photosynthesis into the dense matrix of the exopolymers of the mat community [19,32]. Therefore, the varying numbers and sizes of air bubbles are apparently related to the differential rates of photosynthesis of the different cultivated biofilm communities. Nonetheless, these bubbles help the biofilm communities reach out to the upper layers of water and obtain excess light and other physicochemical parameters in their natural habitats. Although the primary gas in these bubbles during the light period is of course oxygen, other gases like carbon dioxide and nitrogen enter these bubbles to maintain pressure inside particularly during the darkness [46].

Algal biomass produced by nylon net-enmeshed biofilm communities can easily be harvested from the aqueous medium by scraping it off from the substrate surface. However, very little information is available regarding the effect of harvesting frequency on the productivity of algal biofilm and mat communities. In the present study, we found that scraping off the biomass of growing biofilm every week from a nylon net significantly enhanced the final biomass yield. This increase was in comparison to the yield obtained after 21 days of undisturbed growth of the test biofilm on the nylon net. Likewise, some other workers have also reported improved biomass production in response to regular harvesting by a biofilm community, dominated by unicellular green algae, cultivated in a bioreactor specially designed for this purpose [33]. It appears that regular harvesting of biomass improves the accessibility of nutrients to the growing cells that are possibly impeded due to attaining the thickness of growing biofilms with time. This phenomenon is similar to what was observed in a study where a thick cyanobacterial mat showed better tolerance to metal exposure compared to a younger, thinner mat. This was mainly due to the entrapment of metal ions in the thick matrix of the former communities [47]. Thus, the present study advocates periodic harvesting of biofilm in order to ensure high biomass yield.

The present study also recorded a considerable increase in medium pH during the growth of algal biofilms on nylon nets especially for biofilm #G. This is perhaps related to the efficient photosynthetic utilization of bicarbonate present in the BG 11 recipe. Moreover, an increase in the pH of the matrix of algal biofilm communities during the active phase of photosynthesis has also been reported elsewhere as a promising attribute to employ these communities for the precipitation-dependent removal of heavy metals from the aqueous systems [7,48].

In a nutshell, the findings of the present study suggest that cultivating mat community enmeshing nylon net is a promising proposition. This method facilitates the growth and development of biofilms and makes biomass harvesting easier. Nylon net reels could be inoculated with the mat community and used for cultivation in suitable systems involving gleaning nutrients from wastewater [10,13]. The biofilm and mat biomass obtained thereby can be used as a feedstock for various purposes such as the production of biodiesel, biochar, and fine chemicals, or as fish feed, soil conditioner, and biofertilizer [2,8,15]. However, dedicated efforts are needed in this direction.

5. Conclusions

The present study recommends the use of nylon net-embedded algal biofilm and mat communities for various biotechnological applications as it simplifies the cultivation and biomass harvesting process. Among several biofilm communities studied, biofilm #G, dominated by *Oscillatoria* sp., was found to be promising due to its rapid growth and easy enmeshment in the nylon net substratum. Additionally, other biofilms #E, #F, and #H, respectively dominated by *Pseudophormidium* sp., *Phormidium* sp., *Synechococcus* sp., were also found comparably good. Furthermore, the study emphasizes the need for periodic biomass harvesting from the growing mat community to ensure improved biomass production.

Data availability statement

All data to support the conclusions have been provided in the manuscript.

CRediT authorship contribution statement

Pragati Kumari: Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Dhananjay Kumar:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements and Funding

We thank the Head of Department of Botany & Microbiology for the necessary facilities. PK acknowledges the Department of Science and Technology, New Delhi for awarding the Women Scientist Scheme-B fellowship, grant no. DST/WOS-B/AFE-23/2021(G).

References

[1] M.I. Khan, J.H. Shin, J.D. Kim, The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products, Microb. Cell Factories 17 (1) (2018) 36.
[2] J.H. Moreno Osorio, A. Pollio, L. Frunzo, P.N.L. Lens, G. Esposito, A review of microalgal biofilm technologies: definition, applications, settings and analysis, Front. Chem. Eng. 3 (2021) 737710.
[3] G. Randrianarison, M.A. Ashraf, Microalgae: a potential plant for energy production, Geol Ecol Landscapes 1 (2) (2017) 104–120.
[4] A. Pawlowski, J.L. Guzmán, M. Berenguel, F.G. Acién, S. Dormido, Event-based control systems for microalgae culture in industrial reactors, in: Prospects and Challenges in Algal Biotechnology, Springer Singapore, Singapore, 2017, pp. 1–48.
[5] E. Posadas, C. Alcántara, P.A. García-Encina, et al., Microalgae-based biofuels and bioproducts, in: C. Gonzalez-Fernandez, R. Muñoz (Eds.), Microalgae Cultivation in Wastewater, Woodhead Publishing, 2017, pp. 67–91.
[6] S.Y. Lee, K.W. Chew, M.K. Lam, J.W. Lim, S.H. Ho, P.L. Show, A review on microalgae cultivation and harvesting, and their biomass extraction processing using ionic liquids, Bioengineered (2020) 116–129. Dec;11(1).
[7] S.K. Mehta, J.P. Gaur, Use of algae for removing heavy metal ions from wastewater: progress and prospects, Crit. Rev. Biotechnol. 25 (3) (2005) 113–152.
[8] J. Bender, P. Phillips, Microbial mats for multiple applications in aquaculture and bioremediation, Bioresour. Technol. 94 (3) (2004) 229–238.
[9] D. Kumar, L.K. Pandey, J.P. Gaur, Evaluation of various isotherm models, and metal sorption potential of cyanobacterial mats in single and multi-metal systems, Colloids Surf. B Biointerfaces 81 (2) (2010) 476–485.
[10] J. Rai, D. Kumar, L.K. Pandey, A. Yadav, J.P. Gaur, Potential of cyanobacterial biofilms in phosphate removal and biomass production, J. Environ. Manag. 177 (2016) 138–144.
[11] W.H. Adey, K. Loveland, Dynamic Aquaria: Building Living Ecosystems, Academic Press, New York, 1998, p. 498.
[12] D. Kumar, J.P. Gaur, Growth and metal removal potential of a *Phormidium bigranulatum*-dominated mat following long-term exposure to elevated levels of copper, Environ. Sci. Pollut. Res. 21 (2014) 10279–10285.
[13] M. Pećić, S. Grašić, D. Gajić, S. Popović, G.S. Simić, D. Predojević, Periphyton efficiency in phosphorus accumulation affected by phytoplankton dynamics in the reservoir for water supply, Ecol. Eng. 191 (2023) 106963.
[14] M. Kesaano, R.C. Sims, Algal biofilm-based technology for wastewater treatment, Algal Res. 5 (2014) 231–240.
[15] S. Thapa, A. Bharti, R. Prasanna, Algal biofilms and their biotechnological significance, in: Algal Green Chemistry, Elsevier, 2017, pp. 285–303.
[16] M.A. Borowitzka, Commercial production of microalgae: ponds, tanks, tubes and fermenters, J. Biotechnol. 70 (1999) 313–321.
[17] C.U. Ugwu, H. Aoyagi, H. Uchiyama, Photobioreactors for mass cultivation of algae, Bioresour. Technol. 99 (2008) 4021–4028.
[18] J. Zamoro-Costro, J. Panigua-Michel, C. Lezama-Cervantes, A novel approach for bioremediation of a coastal marine wastewater effluent based on artificial microbial mats, Mar. Biotechnol. 10 (2008) 181–189.
[19] D. Kumar, L.K. Pandey, J.P. Gaur, Growth of *Phormidium bigranulatum*-dominated mat in relation to nature of the substratum, time, pH and nutrient availability, Environ. Eng. Manag. J. 17 (2) (2018).
[20] P. Singh, N. Kumar, M. Jethva, et al., Riboswitch regulation in cyanobacteria is independent of their habitat adaptations, Physiol. Mol. Biol. Plants 24 (2018) 315–324.
[21] S.F. Ikram, D. Kumar, V. Singh, B.N. Tripathi, B.H. Kim, Microalgal and cyanobacterial diversity of two selected hot springs of Garhwal Himalaya, Uttarakhand, India, Fundam. Appl. Limnol. 195 (2021) 111–127.
[22] E.O. Hughes, P.R. Gorham, A. Zehnder, Toxicity of unialgal culture of Microcystis aeruginosa, Can. J. Microbiol. 4 (1958) 225–236.
[23] T.V. Desikachary, Cyanophyta, Indian Council of Agricultural Research, New Delhi, 1959.
[24] G.W. Prescott, How to Know the Freshwater Algae, third ed., Wm C Brown Company, Dubuque, IA, 1978.
[25] R. Patrick, C.W. Reimer, Diatoms of the United States, exclusive of Alaska and Hawaii, in: Monographs of the Academy of Natural Sciences of Philadelphia No. 13, vol. 2, Academy of Natural Sciences of Philadelphia, Philadelphia, PA, 1975.

[26] M.T. Phillipose, Chlorococcales, Indian Council of Agricultural Research, New Delhi, 1967.
[27] R. Rippka, R.Y. Stanier, J. Deruelles, M. Herdman, J.B. Waterbury, Generic Assignments, strain histories and properties of pure cultures of cyanobacteria, Microbiology (Reading, England) 111 (1) (1979) 1–61.
[28] E.J. Cox, Identification of Freshwater Diatoms from Live Material, Chapman & Hall, London, 1996.
[29] G. Mackinney, Absorption of light by Chlorophyll solutions, J. Biol. Chem. 140 (2) (1941) 315–322.
[30] W.A. Kratz, J. Myers, Photosynthesis and respiration of three blue-green algae, Plant Physiol. 30 (3) (1955) 275–280.
[31] L.A. Philipp, K. Bühler, R. Ulber, J. Gescher, Beneficial applications of biofilms, Nat. Rev. Microbiol. (2023) 1–15.
[32] L.J. Stal, H. Bolhuis, M.S. Cretoiu, Phototrophic microbial mats. Modern topics in the phototrophic prokaryotes, Environ. Appl. Aspects (2017) 295–318.
[33] L. Christenson, R. Sims, Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts, Biotechnol. Adv. 29 (2011) 686–702.
[34] C.A. Lembi, Relative tolerance of mat forming algae to copper, J. Aquat. Plant Manag. 38 (2000) 68–70.
[35] W.H. Adey, H.D.I.V. Laughinghouse, J.B. Miller, L.A.C. Hayek, J.G. Thompson, S. Bertman, K. Hampel, S. Puvanendran, Algal turf scrubber (ATS) floways on the Great Wicomico River, Chesapeake Bay: productivity, algal community structure, substrate and chemistry, J. Phycol. 49 (3) (2013) 489–501.
[36] D.M. Blersch, K. Kardel, A.L. Carrano, M. Kaur, Customized 3D-printed surface topography governs species attachment preferences in a freshwater periphyton community, Algal Res. 21 (2017) 52–57.
[37] D. Calahan, E. Osenbaugh, W. Adey, Expanded algal cultivation can reverse key planetary boundary transgressions, Heliyon 4 (2) (2018) e00538.
[38] M. Gross, D. Jarboe, Z. Wen, Biofilm-based algal cultivation systems, Appl Microbiol. Biotechnol. 99 (14) (2015) 5781–5789.
[39] A. Ozkan, Development of a Novel Algae Biofilm Photobioreactor for Biofuel Production, UT Austin, 2012. Dissertation.
[40] J.-H. Wang, L.-L. Zhuang, X.-Q. Xu, V.M. Deantes-Espinosa, X.-X. Wang, H.-Y. Hu, Microalgal attachment and attached systems for biomass production and wastewater treatment, Renew. Sustain. Energy Rev. 92 (2018) 331–342.
[41] O. Zeriouh, J.V. Reinoso-Moreno, L. López-Rosales, M.C. Cerón-García, A. Sánchez Mirón, F. García-Camacho, E. Molina-Grima, Assessment of a photobioreactor-coupled modified Robbins device to compare the adhesion of *Nannochloropsis gaditana* on different materials, Algal Res. 37 (2019) 277–287.
[42] Andrew S. Ball, et al., Algal growth control by a barley straw extract, Bioresour. Technol. 77 (2) (2001) 177–181.
[43] T.J. Waybright, D. Terlizzi, M.D. Ferrier, Chemical characterization of the aqueous algistatic fraction of barley straw (*Hordeum vulgare*) inhibiting *Microcystis aeruginosa*, J. Appl. Phycol. 21 (2009) 333–340.
[44] R. De Philippis, R. Paperi, C. Sili, Heavy metal sorption by released polysaccharides and whole cultures of two exopolysaccharide-producing cyanobacteria, Biodegradation 18 (2007) 181–187.
[45] D. Kumar, J.P. Gaur, Metal biosorption by two cyanobacterial mats in relation to pH, biomass concentration, pretreatment and reuse, Bioresour. Technol. 102 (2011) 2529–2535.
[46] T. Bosak, J.W.M. Bush, M.R. Flynn, B. Liang, S. Ono, A.P. Petroff, M.S. Sim, Formation and stability of oxygen-rich bubbles that shape photosynthetic mats, Geobiology 8 (2010) 45–55.
[47] D. Kumar, L.K. Pandey, J.P. Gaur, Metal sorption by algal biomass: from batch to continuous system, Algal Res. 18 (2016) 95–109.
[48] S.K. Liehr, H.J. Chen, S.H. Lin, Metal removal by algal biofilms, Water Sci. Technol. 30 (1994) 59–68.