



Cotton Bed Promotes Faster Growth and Higher Biomass Production of Mat-Forming Cyanobacterium *Oscillatoria* sp.

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Abstract: The high biomass is the fundamental requisite for harnessing the commercial potentials of cyanobacteria. The present study demonstrates the faster production of high biomass of *Oscillatoria* sp. in the open-tray culture system. *Oscillatoria* sp. requires a suitable surface for forming its mat; therefore, initially, we have screened cotton, rice husk, gravels, and mud as supporting beds for the quicker growth of the *Oscillatoria* sp. Based on the expansion rate, the development of the *Oscillatoria* mat was the fastest on the cotton bed. Based on the result, the *Oscillatoria* mat grown on the cotton bed was superior in thickness, biomass quantity, and ability to produce phycocyanin to the naturally growing *Oscillatoria* mat. The *Oscillatoria* mat on the cotton bed was approximately 57.0% thicker than the naturally growing mat of *Oscillatoria* sp. Similarly, the *Oscillatoria* mat on the cotton bed generates almost double biomass and 15.0% higher phycocyanin content than that of the naturally growing mat of *Oscillatoria* sp. Finally, we compared four different harvesting methods, e. g. wiping, squeezing, centrifugation and vortexing with centrifugation for their efficiency to harvest the *Oscillatoria* biomass generated on the cotton bed. These four methods harvested more than 85.0% of the biomass of *Oscillatoria* sp., with more than 96.0% harvesting efficiency wiping was recorded as the most efficient harvesting method followed by vortexing and centrifugation with approximately 94.0% harvesting efficiency. The present findings suggest using the cotton bed as a supporting surface for the quick generation of a high volume of biomass of *Oscillatoria* sp. in the open culture system.

Keywords: Algal Culture, Biomass, Cyanobacterium, Green Energy, *Oscillatoria*

1. Introduction

Oscillatoria is a filamentous mat-forming cyanobacterium abundantly found in freshwater and marine environments [1]. In freshwater, the growth of *Oscillatoria* sp. is frequently observed in muddy ditches, sewer lines, and domestic water runoff in the form of a thick mucilaginous mat of blue-green color resulting in a musty odour [2]. *Oscillatoria* is named after the oscillating movement of its filament to orient the colony towards the light. The Genus *Oscillatoria* belonging to order oscillatoriales of cyanobacteria (also called Cyanophyta by phycologists) account for more than 50% of secondary metabolites of cyanobacterial origin [3].

Like other cyanobacterial members, *Oscillatoria* sp. produces several medicinally and commercially important

compounds. Harada et al. [4] reported the production of insecticidal compounds from *Oscillatoria agardhii* against mosquito larvae and thereby suggested *Oscillatoria* as the possible resource of environmentally safe and effective insecticidal compounds without any side effects for controlling mosquitoes, which are known to transmit several deadly diseases in the human society. A recent study [5] has demonstrated the efficiency of *Oscillatoria* sp. as an excellent antibacterial agent that efficiently inhibits the growth of several nuisance bacteria belonging to both gram-positive and negative groups. Previously, a group of researchers [6] has also reported the in vitro antioxidant and antibacterial activities of the freshwater *Oscillatoria agardhii*. Apart from producing antioxidant, antibacterial, and insecticidal compounds, several species of *Oscillatoria* are known to produce antinociceptive and anti-inflammatory compounds [7]. Further, *Oscillatoria*

also produces cyanotoxins known as Lyngbyatoxin-a and Aplysiatoxin with the capability of hepatotoxicity [8].

Several studies have proved *Oscillatoria* as an excellent feedstock for biofuel and bioenergy production using wastewater [9, 10]. Recently, Kallarakkal et al. [11] have optimized biobutanol production from a mixotrophic culture of *Oscillatoria* sp. In addition to the use of *Oscillatoria* for biofuel production, many studies have also advocated the importance of *Oscillatoria* sp. in environmental clean-up by removing toxic metals [12, 13] and organic compounds [14] from the contaminated environment.

For the extraction of commercially valuable compounds, generation of biofuel, and remediation of contaminated sites, quicker and higher production of biomass of the targeted microalgae and cyanobacteria is the primary and essential requirement. However, *Oscillatoria* sp., despite having enormous commercial and environmental significance, are extremely slow-growing cyanobacterial species, especially under controlled conditions in the laboratory. Moreover, the outdoor cultivation of cyanobacteria is usually not economically feasible. It is also cumbersome to get pure cyanobacterial culture due to the possibility of contamination with other microbes [15]. Hence, the present study has demonstrated a simple and economically feasible method for the rapid generation of higher biomass of *Oscillatoria* sp. in the laboratory using cellulosic cotton fibers as supporting surface floating on the culture medium.

2. Experimental Section

2.1. Isolation, Purification, and Culture of the Test Organism

The test organism, *Oscillatoria* sp. (cyanobacterium) used in the present study, was isolated from the drainage line containing domestic wastewater of IGNTU campus, Amarkantak. *Oscillatoria* sp. was identified microscopically (EVOS XL Core digital microscope, Thermo Fisher Scientific, USA) based on morphology using identification keys given by Desikachary [16]. Further, it was purified in the laboratory using serial dilution and plating onto the agar plates having BG 11 culture medium [17] following the standard procedures described for the purification of phototrophic microbes. Subsequently, the purified colonies of *Oscillatoria* sp. were inoculated in the 250 ml Erlenmeyer flasks containing 75 ml of BG 11 liquid growth medium. Further, the flask inoculated with *Oscillatoria* sp. were kept in the algal culture room at standard conditions of light and temperature as elaborated by Tripathi et al. [18] for the suspension culture of cyanobacterium.

2.2. Preparation of Inoculum and Setting up the Open Tray Culture Containing Four Different Supporting Surfaces/Beds

The commercial applications require huge biomass of *Oscillatoria* sp. However, the biomass of *Oscillatoria* sp. is frequently available in natural habitats, e. g. sewer lines, domestic wastewaters, etc. But the presence of unwanted

chemicals/materials present on these habitats may compromise the quality of the biomass of *Oscillatoria* sp. for industrial application. *Oscillatoria* sp., which usually grows in the form of a mat due to the intertwining of its filaments, showed extremely slow growth in laboratory conditions. Therefore, we have optimized the cultivation of *Oscillatoria* sp. for its faster growth and higher and qualitatively superior biomass. For this purpose, sterilized mud obtained from the natural habitat of *Oscillatoria*, small-sized gravels, rice husk, and thin cotton bed were screened in open tray culture in controlled laboratory conditions. For this purpose, initially, a part equal to the size of 1.0 cm² with an approximately 1.0 mm thick mat of 30 days old *Oscillatoria* sp. growing in liquid BG 11 medium was harvested as stock culture and vortexed vigorously using sterilized glass beads in 15.0 ml Falcon tube containing 5.0 ml autoclaved distilled water. The resulting homogenous suspension of *Oscillatoria* sp. obtained after vortexing was used as inoculum for its cultivation on four different supporting beds in an open tray culture established in the algal culture room.

A setup for the open tray culture was established in the algal culture room before preparing the inoculum of the test cyanobacterium. For this purpose, a polypropylene tray with the dimension of 45x30x7.5 cm was used and divided into four different chambers using thermocol sheets with the help of a permanent adhesive to prevent the mixing of culture medium between the chambers as elaborated in the diagram (Figure 1A). These chambers separated by thermocol sheets were filled with sterilized mud obtained from the natural habitat of *Oscillatoria*, small-sized gravels, crop husk, and thin cotton bed, separately. A 100 g mud was obtained from the natural habitat (domestic drainage in the IGNTU campus) of *Oscillatoria* sp. and dried overnight in the hot air oven and subsequently sterilized using an autoclave. Similarly, small-sized gravels brownish in color were collected from the Son river originating from Amarkantak and processed following the same method as described for the mud. The growth of *Oscillatoria* was noticed on naturally occurring brownish gravels on the bank of the Son river near Anuppur town. Therefore, these gravels were selected as one of the materials for optimizing the growth of *Oscillatoria* sp. in the laboratory. Likewise, the luxuriant growth of *Oscillatoria* sp. was also noticed in the drainage lines of the local villages, which were filled with various crop residues. Therefore rice husk was obtained from a local farmer of an adjacent village and used for the supporting surface as crop husk after autoclaving. For the preparation of a thin cotton supporting bed or surface, a medical cellulosic bandage procured from the market was used due to its chemical inertness, easy availability, and the possibility of easy harvesting of the biomass. After setting up these surfaces using the above-mentioned materials in four different chambers in the plastic tray, each chamber was filled with 125 ml of autoclaved BG 11 culture medium. Thereafter, the 100 µl homogeneous inoculum of *Oscillatoria* sp. as described earlier was inoculated in one corner of each chamber in form of a single drop using a micropipette. After the inoculation of *Oscillatoria* sp. the tray was placed on the culture rack in the algal culture room, maintaining the standard conditions of light and temperature as mentioned earlier.

2.3. Measurement of Colonization of *Oscillatoria* sp. on Different Supporting Beds

The growth of *Oscillatoria* sp. was measured in terms of the rate of expansion of its mats on different supporting surfaces. For this purpose, the area of the mat of *Oscillatoria* sp. was determined manually with the help of scale and basic geometrical tools at every 5th day from the date of inoculation (day 0) till 30th day. The mat area was divided by the number of days, and the resulting value was termed as the rate of expansion. The rate of the expansion of the mat of *Oscillatoria* sp. was expressed in the units of mm² day⁻¹.

2.4. Assessment of the Toxicity of the Leachates of Different Materials Used as Supporting Bed

For this purpose, initially, 10.0 g of each supporting bed material (cotton, rice husk, gravels, and mud) were added to the 10.0 ml of sterilized distilled water in the falcon tubes. These tubes were vortexed vigorously for 3.0 min, and after that, suspended materials were kept in the shaking incubator at 200 rpm overnight. Subsequently, the leachates (released in the supernatant) of different supporting materials were obtained after centrifugation of these tubes at 5000 g for 10 min. The resulting supernatants containing the leachates of different materials were assessed for the toxicity on *Oscillatoria* sp. A small portion of *Oscillatoria* mat (1.0 cm²) was incubated in each leachate (10.0 ml) for 6 h. Then photosynthetic pigments of *Oscillatoria* were extracted with 80% acetone and the amount of the chlorophyll *a* was quantified using the methods given by Mackinney [19].

2.5. Quantification of Thickness, Biomass, and Phycocyanin Generated by the Mat of *Oscillatoria* sp. Grown on the Cotton Surface

After noticing the faster growth of *Oscillatoria* sp. on the cotton surface, we have quantified the thickness, biomass, and phycocyanin content of *Oscillatoria* sp. mat to ensure that faster growth on the cotton surface has not compromised the commercial significance of the test organism in the open culture system. Production of higher biomass and a sufficient quantity of commercially valuable compounds, such as phycocyanin, are important criteria for selecting microalgae for various industrial applications. Therefore, the biomass produced by *Oscillatoria* sp. growing on the cotton surface after the 30th day was quantified in terms of final yield following the method as given by Tripathi et al. [18]. The quantification of phycocyanin content produced by *Oscillatoria* sp. was done after the 30th day of its growth on the cotton surface in open tray culture following the method as described by Zavrel et al. [20]. For this purpose, 1.0 mg 30 days old mat of *Oscillatoria* sp. was homogenized in 1.0 ml of pre-cooled PBS (phosphate-buffered saline) buffer (pH 7.4). After homogenization, the samples were kept on ice for 60 min followed by centrifugation at 15000 g at 4°C for 5.0 min. The phycocyanin content present in the resulting supernatant was measured spectrophotrically and the amount

of phycocyanin was calculated using the formula [20]. The thickness of a 30 days old mat of *Oscillatoria* sp. grown on the cotton bed was measured manually with the help of geometrical tools. We have measured the thickness of the *Oscillatoria* mat using a ruler and divider. The thickness, biomass, and phycocyanin content of a naturally growing *Oscillatoria* mat were also determined for comparing the results with the cotton bed grown mat of *Oscillatoria* sp.

2.6. Comparison of Different Harvesting Methods

The economy of the algal industries largely depends on harvesting methods used for collecting the biomass of algae and cyanobacteria from the cultivation sites. We have compared four simple and economically viable methods e. g. wiping, squeezing, centrifugation, vortexing, and centrifugation for the harvesting of the *Oscillatoria* mats grown on the cotton surface. The wiping method was performed by collecting the cyanobacterial mat growing on the cotton bed in open tray culture with the help of a handheld wiper. For this purpose, the cotton bed containing *Oscillatoria* mat was initially taken out from the tray. Subsequently, mat was wiped off using a hand-held wiper and biomass was collected in a glass beaker. For squeezing, the cotton bed was taken out from the tray and it was squeezed manually to harvest the biomass in a beaker.

Similarly, the cyanobacterial mat along with the cotton bed was collected and kept in the large volume centrifuge tube (250 ml), and an appropriate volume of sterilized distilled water was added in each such tubes followed by centrifugation at 5000 rpm for 2 min. The cotton bed was settled as pellet and cyanobacterial biomass got suspended in the supernatant. Subsequently, the supernatant was again centrifuged at 5000 rpm for 15 min to harvest the biomass as pellet settled at the bottom of the tube. The sticky nature of the *Oscillatoria* mat makes the separation of cyanobacterial biomass from the cotton bed a little cumbersome during the simple centrifugation-based harvesting method. Therefore, a step of vortexing was performed before centrifugation for the easy separation of *Oscillatoria* mat from the surface of the cotton bed. For this purpose, the *Oscillatoria* mat and cotton bed were collected and placed into a large volume centrifuge tube (250 ml). An appropriate volume of sterilized distilled water was added to the tube. Further, tube was vigorously vortexed for 2-3 min and thereafter centrifuged at 5000 rpm for 3 min leading to the separation of cotton bed (pellet) and cyanobacterial biomass suspended in the supernatant. Subsequently, the supernatant was again centrifuged at 5000 rpm for 15 min to harvest the biomass in the pellet form. For each method, initially, the weight of the *Oscillatoria* mat and cotton bed were recorded. The biomass recovered after each harvesting method was also weighed to calculate the harvesting efficiency of the methods used in this study.

2.7. Statistical Analyses

The student's t-test was used for the comparison of means in the present study at $P < 0.05$.

3. Results and Discussion

Cyanobacteria, known for their tremendous industrial significance, are capable of colonizing a variety of habitats. The present study deals with the quick production of higher biomass of *Oscillatoria* sp., a vital cyanobacterium. Figure 1 (A-D) shows the colonization of *Oscillatoria* sp. on four different supporting beds e. g. cotton bed, rice husk, gravels, and mud. As evident from the photographs, the growth of *Oscillatoria* sp. was faster on the cotton bed than other materials. *Oscillatoria* sp. formed a thicker, continuous, and healthy mat on the cotton bed (Figure 1E). Further, microscopic examination of the mat growing on the cotton beds confirmed the formation of mono-algal mat free from the contamination of other algal species as only *Oscillatoria* sp. was found in the mat (Figure 1F).

Further, the data on the rate of the expansion of *Oscillatoria* mat on different supporting beds is also according to the morphological observations (Figure 2A). The growth of *Oscillatoria* mat measured in terms of expansion rate was significantly higher ($P < 0.05$) in mat grown on the cotton bed than in mat on rice husk (Figure 2A). The expansion rate of the *Oscillatoria* mat on the cotton bed was approximately two times higher than mats on rice husk. Whereas, *Oscillatoria* sp. almost failed to colonize on gravels and mud. Slower mat formation on rice husk compared to the cotton bed is possibly associated with rice husk-mediated inhibition of the growth of *Oscillatoria* sp. Zhang et al. [21] have also noticed the inhibition of the growth of *Chlorella* on the rice husk. Additionally, the hydrophilic nature of the supporting materials also seems to be important in controlling the formation of the *Oscillatoria* mat. The cotton bed consists of thin cotton fibers intertwined with each other in the form of a net that absorbs and retains more water content than rice husk and gravels. Therefore the growth of *Oscillatoria* was faster in cotton bed than that of rice husk and gravels, which absorb almost no water content. Whereas, mud being the natural soil retains a high amount of water. But as evident from the results, *Oscillatoria* sp. could not colonize on mud in the present study. However, available data do not suggest the precise reason behind the unsuccessful colonization of *Oscillatoria* sp. on mud obtained from the natural habitat of *Oscillatoria* sp. Perhaps adsorption of nutrient ions to the soil particles present in the mud leading to the unavailability of ionic forms of essential nutrients [22] could be a possible reason for almost no growth *Oscillatoria* sp. on mud. However, more experimentation is required to elucidate the facts.

Previous reports suggest the toxic effect of leachates released from the bio-carrier used as a supporting bed for algal growth [21]. After witnessing the differential colonization patterns of *Oscillatoria* sp. on four different supporting beds, the toxicity assay of the leachates extracted from these supporting beds was also performed. Based on the toxicity assay, the leachate obtained from the supporting materials (cotton, rice husk, gravels, and mud) used in this study was not toxic to *Oscillatoria* sp., as evident from the

insignificant ($P > 0.05$) impact of these leachates on Chl *a* content of *Oscillatoria* sp. (Figure 2B). Hence, the present data ultimately declines the possibility of any toxic effect of leachates of rice husk, gravels, and mud on the growth and colonization of *Oscillatoria* sp. in the present case.

After observing the faster growth and colonization of the *Oscillatoria* mat on the cotton bed than in other materials (Figures 1 and 2), the mat of *Oscillatoria* sp. was raised on a cotton bed in open tray culture for the study of other parameters. The morphologically, mat grown on the cotton bed was thicker, continuous, and darker in colour compared to the *Oscillatoria* mat growing naturally (Figure 3D). The cotton-bed grown *Oscillatoria* mat was significantly thicker ($P < 0.05$) than the naturally growing *Oscillatoria* mat. The thickness of the *Oscillatoria* mat grown on the cotton bed (1.73 mm) was significantly higher compared to the naturally developing *Oscillatoria* mat (1.1 mm) (Figure 3A). Similarly, the *Oscillatoria* mat grown on the cotton bed generated almost two times higher biomass measured as fresh weight compared to the naturally growing *Oscillatoria* mat (Figure 3B). The development of a thicker and darker mat on the cotton bed compared to the naturally growing mat occurs due to the availability of sufficient nutrients [23] without any competition for the resources in the open-tray culture of *Oscillatoria* sp. on the cotton bed. Whereas in nature, the same resources are shared by other organisms (microalgae, bacteria, sometimes fungi), and therefore the availability of the nutrients becomes lesser for a particular organism [23]. Perhaps, the availability of sufficient nutrients also enhances the synthesis of photosynthetic pigments in the test organism leading to a darker mat of *Oscillatoria* sp. [22] on the cotton bed compared to the natural one. The mechanical forces caused by continuously moving water currents in the natural system do not permit the growth of continuous mat in nature [24], contrasting to the mats grown in the controlled laboratory conditions.

Oscillatoria mat grown on the cotton bed also performed better than naturally developing *Oscillatoria* mat in producing the valuable compounds. The phycocyanin contents of the *Oscillatoria* sp. mat raised on the cotton bed were more than 15.0% higher than that of the naturally growing *Oscillatoria* mat (Figure 3C). The availability of sufficient nutrients in laboratory conditions keeps *Oscillatoria* sp. physiologically active [22]. The physiologically active cyanobacteria may produce more amount of pigments, including phycocyanin [25]. However, the content of phycocyanin was determined using an equal-sized mat area (1.0 cm²). Therefore, a thicker cyanobacterial mat on a cotton-bed would possess more filaments of *Oscillatoria* sp. than a naturally growing mat that was relatively thinner in thickness. The higher number of filaments in the unit area of the mat may also be the possible reason for the higher content of phycocyanin in the *Oscillatoria* sp. mat grown on the cotton bed than its naturally growing mat.

Figure 4 shows the harvesting efficiency of four different methods used to harvest *Oscillaria* mat grown on the cotton

bed. All four methods performed very well in harvesting the *Oscillatoria* biomass and recovered more than 85.0% of the total biomass developed on the cotton bed (Figure 4A). The data show the harvesting efficiency of these methods in the order of wiping > vortexing + centrifugation > squeezing > centrifugation (Figure 4B). However, we have not noticed any significant ($P > 0.05$) difference in the harvesting efficiencies of these four methods. But thicker size and presence of mucilage in mat of *Oscillatoria* sp. on the cotton

bed made wiping the easiest and most efficient method of harvesting the biomass. Whereas simple centrifugation and manual squeezing generate forces on the mat towards the inner side (cotton-bed side), causing filaments of *Oscillatoria* sp. to get strictly attached with the surface of the bed and thereby decline the amount of the harvested biomass. On the other hand, vigorous vortexing before centrifugation breaks the association of cyanobacterial mat with the surface of the cotton-bed providing more biomass for harvesting.

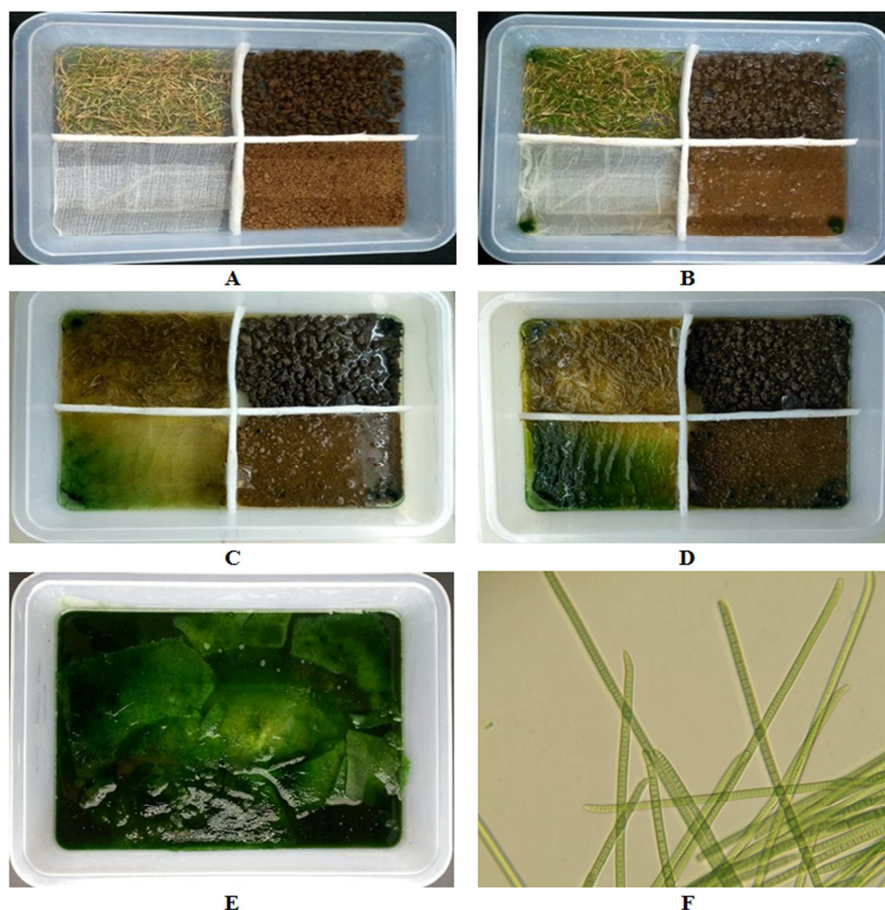


Figure 1. Screening of supporting beds for the cultivation of *Oscillatoria* sp. in the Open-tray culture (A) experimental set up (B) Inoculation of *Oscillatoria* sp. (C) commencement of the colonization of *Oscillatoria* sp. on different supporting beds (D) formation of *Oscillatoria* mat on different growth beds (E) growth of *Oscillatoria* mat on cotton bed (F) microscopic view of *Oscillatoria* sp. growing on cotton bed.

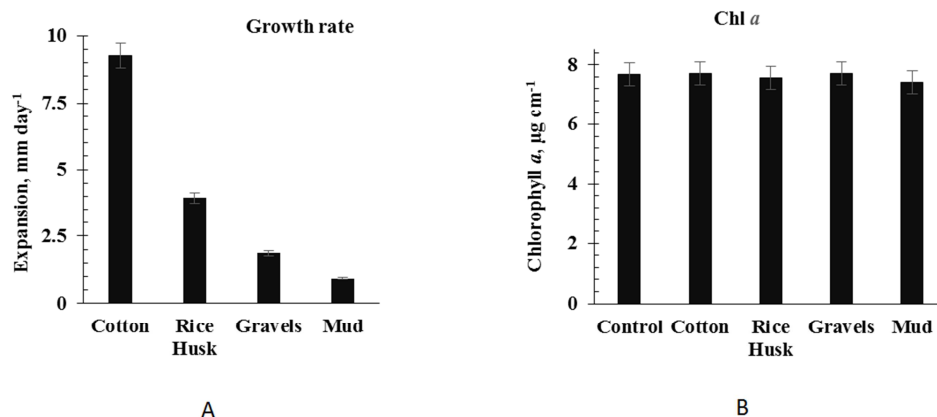


Figure 2. (A) Rate of expansion of *Oscillatoria* sp. mat on different supporting beds. (B) Toxicity assay of the leachates of different supporting beds on *Oscillatoria* sp. measured in the terms of Chlorophyll a content.

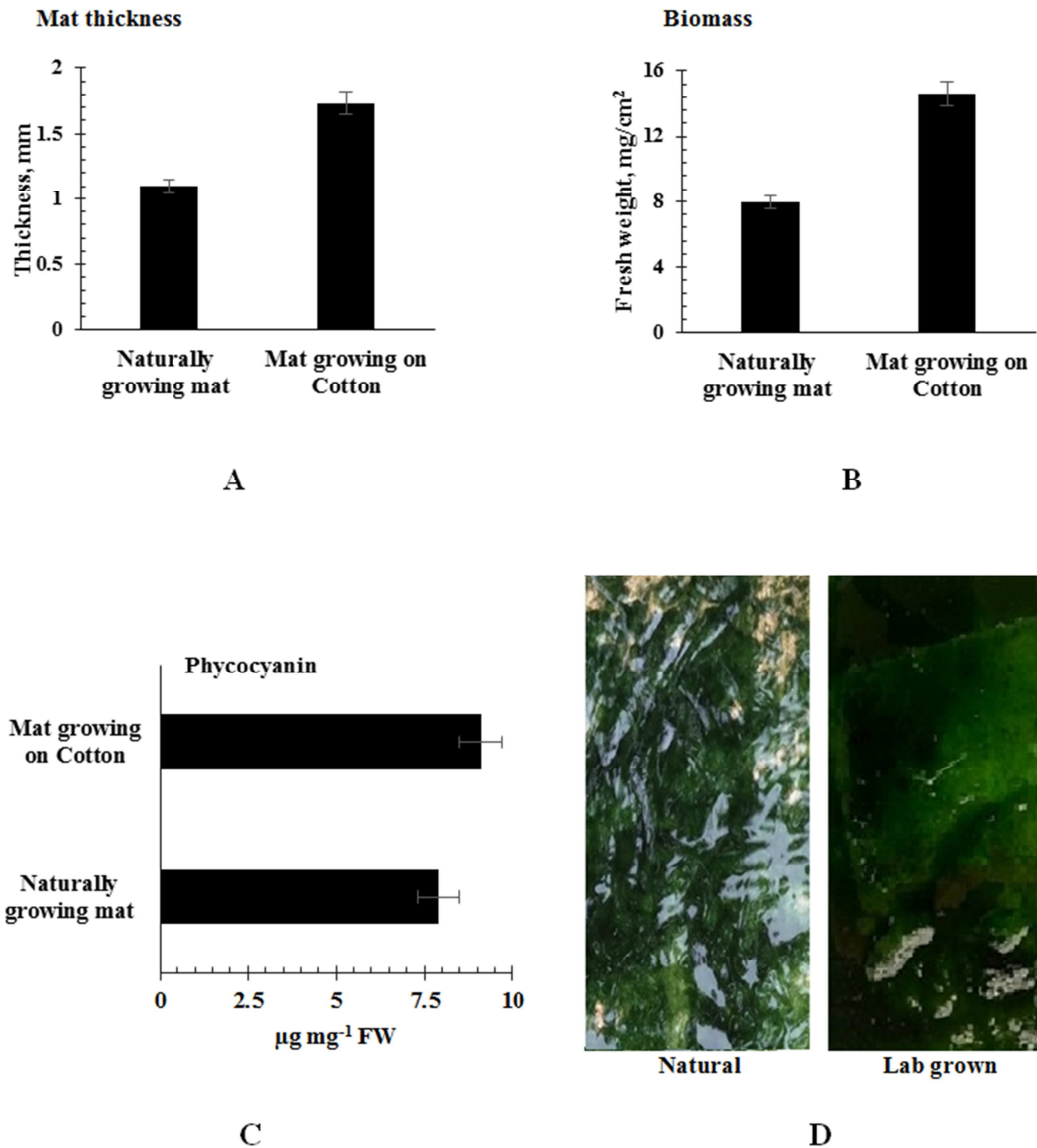


Figure 3. Comparative assessment of thickness (A), biomass (B), phycocyanin (C) and morphology (D) of *Oscillatoria* sp. mat grown on the cotton bed and naturally occurring *Oscillatoria* mat.

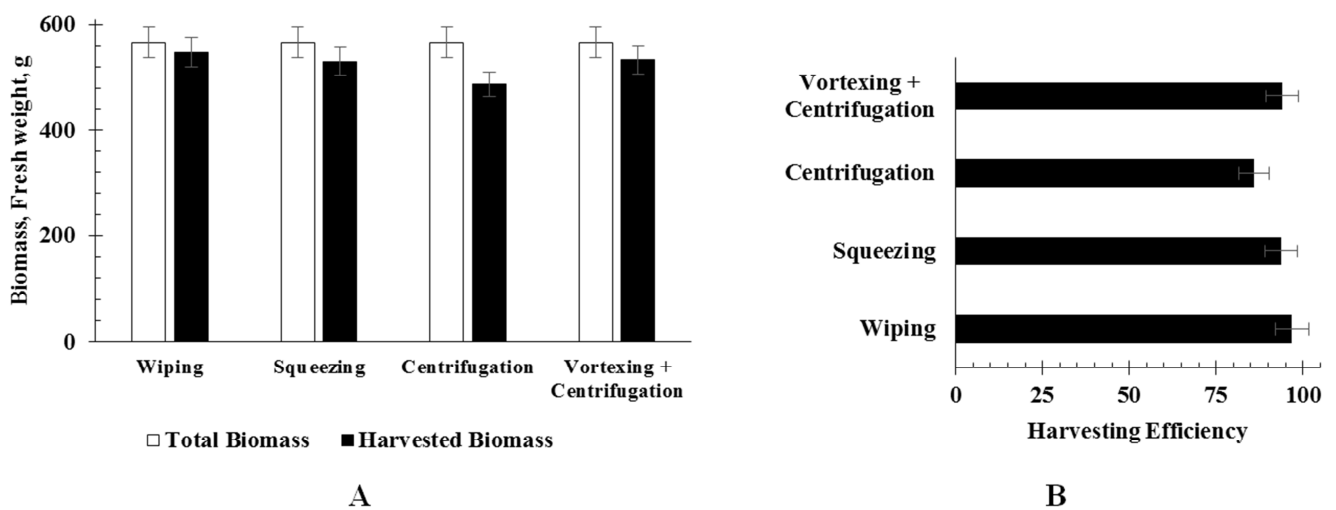


Figure 4. Amount of the harvested biomass of the *Oscillatoria* mat grown on the cotton bed by different harvesting methods (A) and the harvesting efficiency of different methods (B).

Based on the findings, the present study recommends using cotton-bed as a supporting surface to produce the high biomass of *Oscillatoria* sp. and other similar kinds of cyanobacteria for various commercial applications, especially in the controlled laboratory conditions. However, using the cotton bed to produce cyanobacterial biomass in the open pond system warrants more research to avoid the growth or contamination of non-targeted organisms and the test organism.

4. Conclusion

The present study concludes that cotton bed promotes the faster growth of *Oscillatoria* sp. and hence, generates higher biomass suitable for various applications e. g. biofuel production, extraction, and purification of industrially important molecules.

Statements and Declarations

The authors declare that they have no competing interests.

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