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Comparative studies on biomass of *Anabaena ambigua* grown in an external loop airlift photobioreactor using cross-shaped and circular sparger

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An external loop airlift photobioreactor of 5 litre capacity was fabricated with glass as per the design. Blue green algae *Anabaena ambigua* was cultured in this external loop airlift photobioreactor using cross-shaped and circular sparger. For each type of sparger different sets of air velocities viz., 1 m/s, 1.5 m/s, 2 m/s, 2.5 m/s and 3 m/s were maintained and, once the culture reached stationary phase, the biomass was estimated by gravimetric method. For cross-shaped sparger maximum biomass was obtained at air velocity of 1.5 m/s and for circular sparger maximum biomass was obtained at air velocity of 2 m/s. Statistical analysis two-way analysis of variance (ANOVA) was performed using Microsoft Excel 2007. It was observed that there is a significant change in the biomass formed by changing the air velocity as well as changing the type of sparger. A maximum biomass of 0.809 g/L was obtained using cross-shaped sparger at air velocity of 1.5 m/s.

Keywords: External loop airlift photobioreactor, blue green algae, *Anabaena,* cross-shaped sparger, circular sparger, biomass, two-way ANOVA

Introduction

The biomass of blue green algae *Anabaena ambigua* has potential applications in wastewater treatment, used as biofertilizers, and in food supplements. Phycobiliproteins are brilliantly coloured and fluorescent pigments. The colour is due to presence of chromophores bilins which are covalently attached to cysteine residues of apoproteins[1]. Because of their unique fluorescent properties, they find their applications in flow cytometry, fluorescent immunoassays, fluorescence microscopy, and biomedical research[2] and also used as protein markers for electrophoretic techniques[3]. The phycobiliproteins present in *A. ambigua* have similar applications as mentioned above. Biomass consists of light harvesting pigments like phycocyanin (PC), allophycocyanin (APC), and phycoerythrin (PE) which is used as natural colorants in food industry[4-6]. Due to industrialization, water bodies receiving effluents have high biological oxygen demand (BOD), chemical oxygen demand (COD) and chloride levels that cause health hazards. Many methods are available in remediating these pollutants and phytoremediation is one among those. Phytoremediation is a process which uses plants or microorganisms like microalgae, cyanobacteria for removal of contaminants like organic compounds, nutrients and heavy metals from wastewaters[7-8].

The biomass of *A. ambigua* has several advantages as phytoremediating agent over other microorganisms as they grow fast and it is less expensive when compared to physical and chemical means for degrading organic pollutants. Hence, mass culturing of *A. ambigua* species, extracting, and purifying the pigments is gaining great prominence[9]. Due to the above applications the biomass of *A. ambigua* is cultivated in closed systems like photobioreactors[10]. They generate more biomass and have better control on growth parameters such as light intensity, pH, and [illegible] systems[11]. Generally in biological process airlift photobioreactors with external loops are used, as external circulation allows less gas hold-up, proper liquid circulation which thereby leads to efficient mixing[12]. An external loop airlift photobioreactor has two separate columns connected at the top and bottom. This type of design has an advantage of separate gas disengagement section which helps in maximum deaeration. Gas holdup is one of the important parameters that determines the residence time of gas in the liquid. As the gas holdup increases, the rate of mass transfer also increases. Effect of mass transfer rates were studied by many investigators[13-15].

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External loop airlift photobioreactor offers efficient temperature control and effective heat transfer. It can reach total gas disengagement, which gives rise to higher difference in densities and better liquid circulation rates when compared to internal loop airlift photobioreactors. Detailed studies have been made by many investigators on this type of reactors[16-17]. The purpose of mixing is to permit all cells to access the lightened zones equally and periodically[18]. Proper mixing reduces cell shading, settling of the cells to bottom of the reactor and photoinhibition on the reactor surfaces[19-20]. Hence, in present study an external loop airlift photobioreactor was fabricated and *A. ambigua* was cultured by using two spargers viz., cross-shaped and circular at different velocities and biomass was compared.

Materials and Methods

Culturing of *Anabaena*

The strain *A. ambigua* (Accession Number: 2785) was obtained from National Collection of Industrial Microorganisms (NCIM), Pune, India. It was grown in BG-11 medium[21]. It was prepared by dissolving the given constituents in 1 L of distilled water: $NaNO_3$ (1.5 g), K_2HPO_4 (0.04 g), $MgSO_4.7H_2O$ (0.075 g), $CaCl_2.2H_2O$ (0.036 g), citric acid (0.006 g), ferric ammonium citrate (0.006 g), EDTA (disodium salt) (0.001 g), Na_2CO_3 (0.02 g), trace metal mix (1 mL). The trace metal mix was prepared by dissolving the following constituents in 1 L of distilled water: H_3BO_3 (2.86 g), $MnCl_2.4H_2O$ (1.8 g), $ZnSO_4.7H_2O$ (0.222 g), $NaMoO_4.2H_2O$ (0.39 g), $CuSO_4.5H_2O$ (0.079 g), $Co(NO_3)_2.6H_2O$ (49.4 mg). A 10% (v/v) culture was used as inoculum and subsequently transferred aseptically into a conical flask of 250 mL capacity consisting of 100 mL of sterile BG-11 media. The light intensity is an important parameter for the growth of microalgae[22]. Cool white fluorescent tubes were used for providing the light and the light intensity was measured using a Lux meter (LX-1010B).

Flask containing BG-11 media was kept in an orbital shaker at 100 agitation speed/min, maintained at temperature of 28°C and at a light intensity of 80 µmol/m^2s. After each day the optical density of culture was measured using a UV-visible spectrophotometer[23] at 680 nm and the dry weight of *A. ambigua* was measured by separating the biomass from the supernatant liquid and drying it in hot air oven. This procedure was repeated for different optical density values. A standard graph was plotted between biomass concentration (g/L) vs optical density (OD) at 680 nm. Now, instead of measuring the dry weight of *A. ambigua* at each time, the OD of the samples was measured using a UV-visible spectrophotometer at 680 nm. The samples were diluted suitably so that the OD_{680} lies in the range of 0.1 to 1.0. Based on the OD values obtained during the growth of *A. ambigua* the biomass concentration (g/L) was estimated from standard graph shown in Figure 1. Once the biomass reached stationary phase it was collected separately and allowed to settle for 6 hours. Using Whatmann filter paper the biomass was separated from the supernatant liquid and dry weight of biomass was measured after drying in hot air oven. (triplicates were measured at stationary phase).

Fabrication of External Loop Airlift Photobioreactor and Spargers

The capacity of external loop airlift photobioreactor is 5 L and is fabricated with glass as per given specifications. The height of the reactor is 550 mm and the diameter of the riser is 110 mm and the downcomer is 15 mm. The aspect ratio (height/diameter) is 5. An external loop of 350 mm length is connected to photobioreactor at an angle of 45°, at a distance of 35 mm from the top and 85 mm from the bottom as shown in Figure 2. This external loop acts as the downcomer. The sparger was located in such a way that when air is passed through it, it should displace the liquid in upward direction in the riser. The displaced liquid reaches the top of the photobioreactor, enters into the external loop and

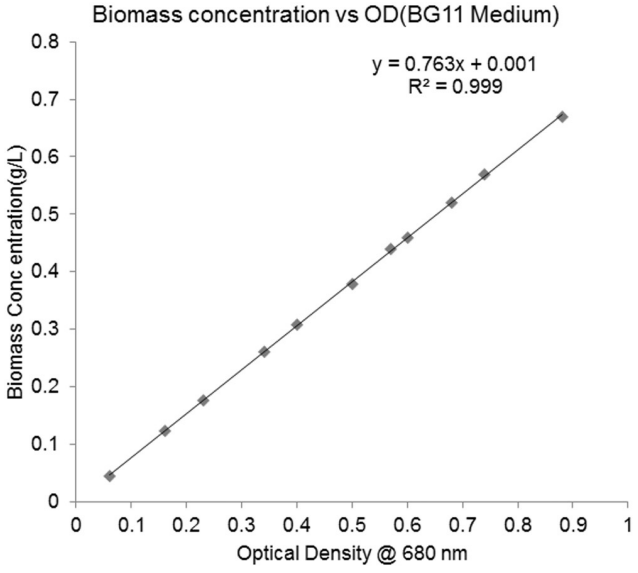

Fig. 1— Standard graph for estimation of biomass of *Anabaena ambigua* using BG-11 medium.

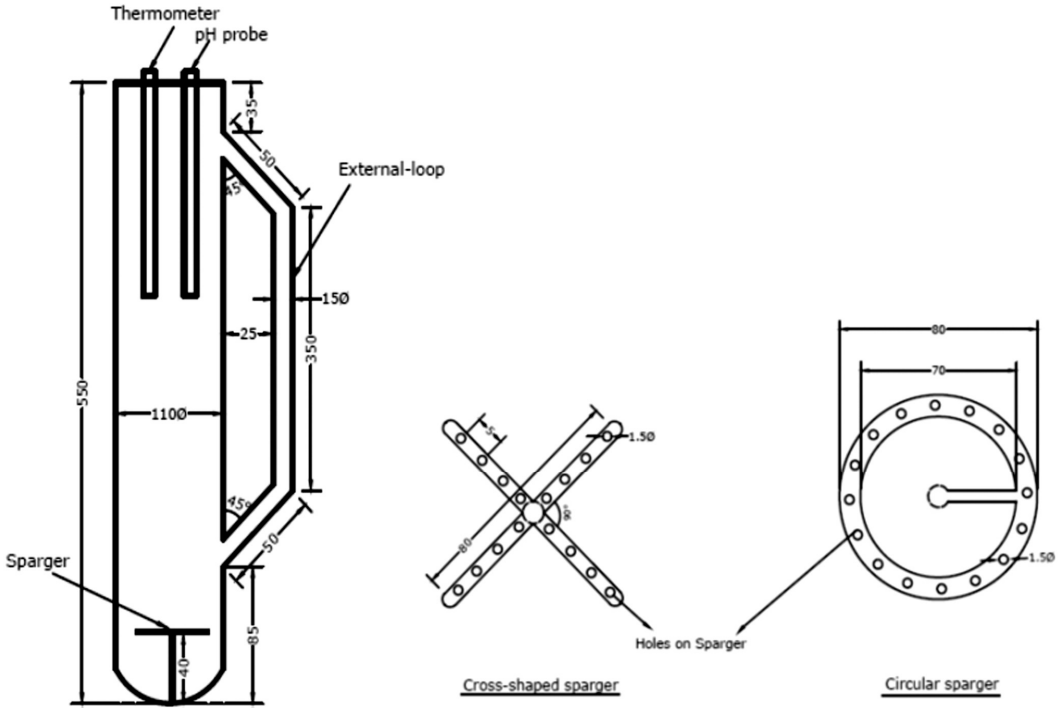

Fig. 2 — Schematic drawing of an external loop airlift photobioreactor with cross-shaped and circular sparger. (all dimensions are in millimeters).

comes down through it under the influence of gravity. The external loop (downcomer) is connected to photobioreactor at an angle of 45˚ as the back flow of the fluid and bubble formation in the external loop is minimum at this angle. Both the spargers viz., cross-shaped and circular spargers are made of glass having 16 holes on each of them with diameter of each hole as 1.5 mm as shown in the Figure 2.

At the top of the photobioreactor a provision was made for two electrodes so that the pH and the temperature can be measured at any time. A mesh of very fine pore size was covered at the top so that the air passed through the sparger escapes out of the photobioreactor. An electrical magnetic air pump (Model No: ACO-001) of RESUN make with voltage 220 - 240 v/ 50Hz was used for pumping air into the photobioreactor. A calibrated rotameter of 5 L/min capacity of INDUS make was used for measuring the air flow rate. The photobioreactor was externally illuminated by white fluorescent tubes placed equidistant in a closed chamber. Light intensity was measured using a Lux meter (Model no: LX-1010B) of MexTech make.

Results and Discussion

Experiments were conducted using cross-shaped and circular sparger at different air velocities. Based on air flow rates air velocities were evaluated. The

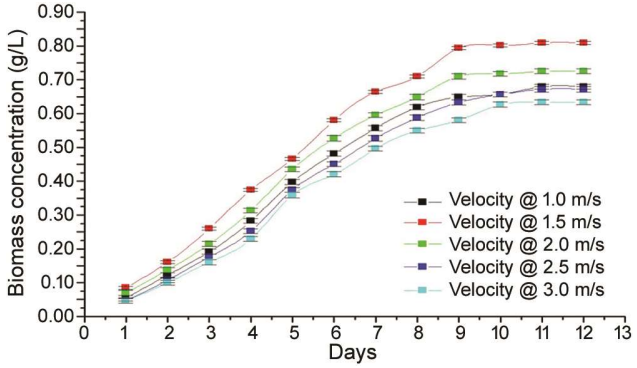

Fig. 3 — Effect of different velocities on biomass concentration of *Anabaena ambigua* grown in an external loop airlift photobioreactor using cross-shaped sparger.

sub-cultured species *A. ambigua* was inoculated into a 5 liter capacity external loop airlift photobioreactor consisting of sterile BG11 medium with all the process parameters maintained at optimal conditions obtained in the shake flasks (i.e light intensity of 80 µmol/m^2s at photoperiod of 18 : 6 and pH of 7.4). The experiments were performed with cross-shaped sparger by passing air at different velocities viz., 1 m/s, 1.5 m/s, 2 m/s, 2.5 m/s and 3 m/s. At each velocity the biomass formed was estimated after stationary phase was attained by gravimetric method. At the end of each day biomass was estimated from standard graph by using optical density values at 680

nm. Figure 3 shows the biomass concentration of *A. ambigua* versus days for cross-shaped sparger. From Figure 3 it was observed that as air velocity increased from 1 m/s to 1.5 m/s the biomass formation increased, later on increase in the velocity from 1.5 m/s to 3 m/s the biomass formation decreased.

Similar set of experiments were performed with circular sparger by passing air at different velocities of 1 m/s, 1.5 m/s, 2 m/s, 2.5 m/s and 3 m/s. The biomass formed was estimated at stationary phase. Figure 4 shows the biomass concentration of *A. ambigua* versus days for circular sparger. From Figure 4 as the air velocity increased from 1 m/s to 2 m/s the biomass formation increased and further when velocity increased from 2 m/s to 3 m/s the biomass formed was decreased.

At velocity of 1 m/s using cross-shaped sparger and at velocities of 1 m/s and 1.5 m/s using circular sparger, it was observed that the fluid particles tend to settle down to the bottom of the photobioreactor as they do not posses enough energy required for their movement inside the photobioreactor. As the fluid particles settle down, the photosynthesis process becomes slow and biomass formation decreased.

At velocity of 1.5 m/s using cross-shaped sparger and at velocity of 2 m/s using circular sparger, it was observed that the fluid particles move in an orderly fashion inside the photobioreactor so that they spend enough time in light and dark zones. The residence time spent by individual fluid particles is almost the same in such types of flow. Hence the process of photosynthesis was effective which increased the biomass formation.

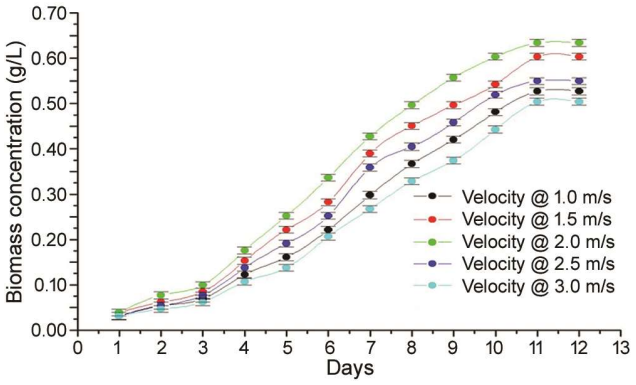

Fig. 4 — Effect of different velocities on biomass concentration of *Anabaena ambigua* grown in an external loop airlift photobioreactor using circular sparger.

At velocities above 1.5 m/s using cross-shaped sparger and at velocities above 2 m/s using circular sparger, the movement of the fluid was in an erratic fashion. The particles collided with each other, particles bypassed the route and cell shading was observed. Hence, less time was spent by the cells in the light zone inside the photobioreactor and also the residence time spent by individual particles in external loop airlift photobioreactor was different. Improper utilization of light energy by the cells due to above reasons had affected the photosynthesis process and the biomass formed was decreased.

It was also observed that at the end of stationary phase the biomass formed by using cross-shaped sparger was more than the biomass formed by using circular sparger for the same velocities (Table 1) (average of triplicates).

Statistical analysis two-way ANOVA was performed to check whether change in the type of the sparger and change in air velocities had significant effect on the biomass of *A. ambigua* formed.

From the Table 1 two null-hypotheses (H_0) were framed. They are: 1) There was no significant change in the biomass formed based on the type of sparger and 2) There was no significant change in the biomass formed when air velocity was changed. The alternate hypotheses (H_1) for the above two statements are - 1) There was a significant change in the biomass formed based on the type of sparger and 2) There was a significant change in the biomass formed when air velocity was changed. Two-way ANOVA was performed using Microsoft Excel 2007 and results were shown in Table 2.

From the Table 2 it was seen that the probability value was less than 0.05 ($p < 0.05$) for both rows and

Table 1 — Biomass concentration with different spargers at different velocities

Type of sparger used	Biomass (g/L) formed at different velocities				
	1.0 m/s	1.5 m/s	2.0 m/s	2.5 m/s	3.0 m/s
Cross-shaped	0.68 ± 0.01803	0.809 ± 0.01212	0.725 ± 0.01323	0.672 ± 0.01929	0.634 ± 0.00954
Circular-shaped	0.527 ± 0.00458	0.603 ± 0.01127	0.634 ± 0.007	0.55 ± 0.04583	0.504 ± 0.02163

Note: The above values are average of triplicates + SD

Table 2 — ANOVA (Two-Factor) with different spargers and velocities

Summary	*Count*	*Sum*	*Average*	*Variance*		
Cross-sparger	5	3.52	0.704	0.004492		
Circular-sparger	5	2.818	0.5636	0.002896		
1.0 m/s	2	1.207	0.6035	0.011704		
1.5 m/s	2	1.412	0.706	0.021218		
2.0 m/s	2	1.359	0.6795	0.00414		
2.5 m/s	2	1.222	0.611	0.007442		
3.0 m/s	2	1.138	0.569	0.00845		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*Fcal*	*P-value*	*Fcrit*
Rows	0.0493	1	0.04928	53.64437	0.001849	7.708647
Columns	0.0259	4	0.00647	7.042018	0.042541	6.388233
Error	0.0037	4	0.00092			
Total	0.0788	9				

SS: Sum of squares, *df* : degrees of freedom, *MS*: Mean of squares, F_{cal} : F

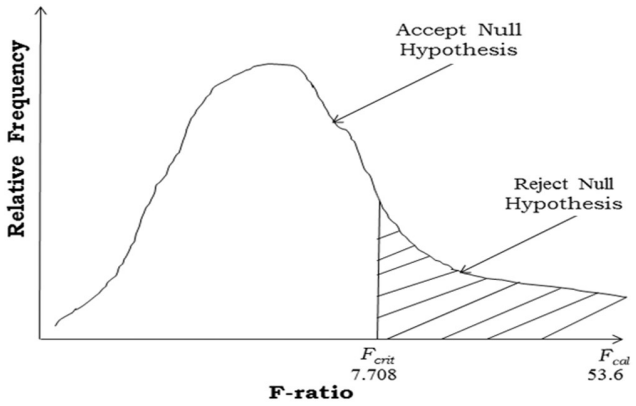

Fig. 5 — Effect of change in spargers on biomass formation.

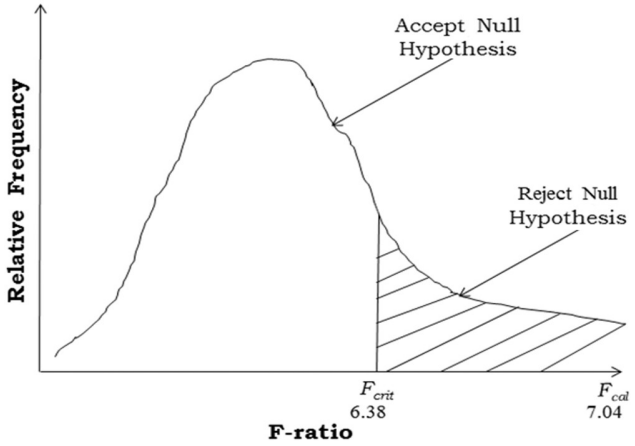

Fig. 6 — Effect of change of velocities on biomass formation.

columns (i.e., for types of spargers as well as for different air velocities). In Figures 5 & 6 the X-axis shows the F-values and Y-axis shows the relative frequency. Relative frequency indicates the ratio of the occurrence of a singular event and total number of outcomes in probability. It does not have any units. Fcal > Fcrit for both the rows and columns (Fig. 5 & 6). This indicates that there exists a significant change in the biomass formed by changing the type of sparger as well as changing air velocity. Hence null hypotheses (H_0) were rejected and alternate hypotheses (H_1) were accepted.

Conclusion

Blue green algae *A. ambigua* has wide biotechnological applications. It can be cultivated in open ponds as well as in photobioreactors. This study gives useful information regarding its cultivation in external loop airlift photobioreactor. Types of spargers that affect the biomass of *A. ambigua* and their role in mixing of the culture and proper light utilization. Among different combinations of types of spargers and air velocities the biomass formation was maximum (0.809 g/L) with cross-shaped sparger at air velocity of 1.5 m/s.

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