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Illumination wavelengths effect on *Arthrospira platensis* production and its process applications in River Yamuna water treatment

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ABSTRACT

Illumination triggers the metabolic activities in microalgae. Wavelength governs the energy fluxes and activates the photosystem I and II. Treating the river water is the prime project in India due to disposal of effluent discharge. Lack of well-defined illumination wavelength for microalgae cultivation aimed this study to find optimum wavelength for promoting growth of *Arthrospira platensis* and hence, treatment of Yamuna water enriched with effluent discharge. Seven experimental set-up each comprising of an Erlynmeyer flask containing Zarrouk's medium supplied with sterile air and kept under different wavelengths, were used. Effects of different energy fluxes were investigated on the production of *A. platensis*. Optimum illumination wavelength selection was based on the comparison of maximum specific growth rate (μ_{max}). Sunlight exhibited μ_{max} of 0.45 day^{-1} and maximum biomass productivity of 16.77 mg/L/h, i.e. highest among others. Energy flux per unit power for the corresponding source of illumination was 6.05×10^{-21} s/m^2. Optimum wavelength was further used for microalgae cultivation in Yamuna water. Culture was acclimatized to Yamuna water using sequential increased levels to maintain the μ_{max}. Biomass productivity of 10 mg/L/h and removal efficiencies of 99.76% for nitrate, 99.22% for lead, 100% for nickel, 90.80% for cadmium and 68.57% for copper, were achieved. Biomass productivity was 40.3% lesser than that of Zarrouk's medium but lipid content of cells increased by 16.45%. Maximum productivity of value added by-products chlorophyll and phycocyanin was 2.93 and 7.34 mg/g cells/day respectively. *A. platensis* has great potential for wastewater treatment and production of valuable by-products.

1. Introduction

Illumination energy and intensity affects photosynthesis rate and growth rate in photoautotrophic microalgae [1–3]. Photosystems I and II triggered by photons having energy ($h\nu$) utilizes CO_2 for metabolic activation [4]. CO_2 sequestration rate, biomass production, and lipid content varies with the availability of light [5,6]. Photosystem comprises of chlorophyll A, phycocyanins and associated proteins; in addition, it is held within a protein matrix on the surface of photosynthesis membrane [3,7]. Both chlorophyll and phycocyanins capture light in the different region of the light spectrum [8,9].

Recent studies on pigmentation development in microalgae shows their dependency on the combination of illumination characteristics and nutrients composition. Illumination characteristics such as wavelength dependence has been investigated only using artificial light sources [10]. Illumination wavelength of ~680 nm of red light has been reported optimum, resulting in faster doubling rate without changing cell volume [11–13]. However, some microalgae exhibits highest growth rate under white and lowest under blue illumination [14]. If considering nutrients composition along with optimum illumination, literature provided high cell growth rate of *A. platensis* when supplied with sufficient nutrients in medium [15]. Domestic or industrial wastewater is also favourable as an economic alternative medium for cultivation due to the presence of adequate amount of growth supporting supplements [16,17]. Microalgae has the potential to selectively accumulate heavy metals Cu^{2+}, Pb^{2+}, Cr^{3+}, Ni^{2+}, Cd^{2+}, Co^{2+}, Fe^{2+}, Se^{2+} and Mn^{2+} by active biological transport [18,19]. However, different algae has found to have different capacity of accumulation. *Scenedesmus dimorphus* utilizes domestic secondary effluent nutrients for growth and production of lipid [20,21]. However, illumination have greater influence on the growth rate than that of nutrients [22]. But extensive studies were performed on various microalgae species in order to find optimum illumination wavelength to support uptake of nitrogen and phosphorous. Optimum illumination intensity for cultivating *Microcystis aeruginosa* for targeting production of microcystin was reported as 19–38 $\mu mol\, m^{-2}s^{-1}$ and illumination intensity $\geq 47\, \mu mol\, m^{-2}s^{-1}$ drastically decreases biomass [23]. Whereas, *A. platensis* exhibited maximum growth and C-phycocyanin

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production at optimum intensity of 300 μmolm^{-2}s^{-1} [24]. These studies have raised a dilemma for what kind of wavelength suits particular microalgae for targeting different production objectives. Literatures above highlight shortcomings in providing an optimum illumination wavelength for maximizing the consumption of nutrients from waste water coupled with production of value added products.

This study aims to provide an efficient illumination wavelength by comparing different wavelengths from artificial and natural sources; and observe effects of their energy flux on photosystem efficiency, specific growth rate, biomass yield, substrate consumption and specific photosystem efficiency during *A. platensis* cultivation in zarrouk's medium. Where, specific photosystem efficiency was obtained by estimating specific chlorophyll and phycocyanin content in cells. This paper further elaborates the application of optimum illumination wavelength for the cultivation of *A. platensis* in River Yamuna water (New Delhi, India), utilising its macro and micro elements (nitrates, phosphates, ammonia and heavy metals) to make an economical process of river water treatment and microalgae production.

2. Materials and methods

2.1. Strain and its maintenance

A. platensis, supplied by Algae Research and Supply (Carlsbad, CA 92008, USA) was used for present study. It was initially transferred in basal salt medium and kept for incubation at 30 °C for 5 days under sunlight. Sterile air was supplied to provide CO_2/O_2 for photosynthesis/respiration in culture broth. Culture was further maintained in basal salt agar medium plates and slants. After every 15 days, retransfer to fresh plates was carried out to preserve it in active state.

2.2. Microalgae cultivation

Red, green, blue, yellow, 60 W UV and sunlight were selected as illumination wavelengths. The experimental setup comprises of a flask containing sterile medium supplied with sterile air from the compressor and filter assembly (Fig. 1). The chamber walls were painted black to absorb all the excessive radiation and to maintain the temperature at 28 ± 2 °C [25]. Also, 60 W incandescent bulbs were used as light source with colour filters to generate illumination of required wavelengths. Intensity filters were used to control the intensity of the sunlight to 60 W. The light intensity was obtained using HTC Instrument LX-103 Light meter Lux meter.

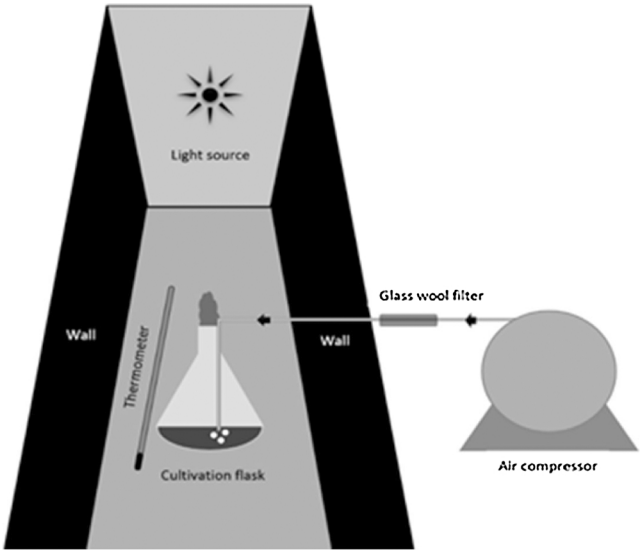

Fig. 1. Schematic diagram of setup.

A. platensis was cultivated in Zarrouk's medium with modified nitrate concentration of 5 g/L using 10% (v/v) inoculum. Samples were withdrawn at the beginning and end of the photoperiod (12:12) for analysis during cultivation cycle. Analytical assays were performed for estimation of biomass, nitrates, heavy metals, chlorophyll and phycocyanin concentration. Economic efficiency of energy to biomass was calculated using Eq. (1) [26].

$$E_{eff} = \frac{C_n - C_0}{kTP} \quad (1)$$

Where E_{eff} is the energy efficiency, C_n is the final biomass concentration (g/L), C_o is the initial biomass concentration (g/L), k is the coefficient of charge per unit power supply (INR/W), T is the time of culture (day), and P is the power of light source used for cultivation (W).

2.3. Acclimatization of A. platensis in Yamuna water

River Yamuna water was collected from its bank located in front of Pragati Thermal Power Plant, New Delhi at 1:45 PM on 3 September 2016. The river water flows from Wazirabad to Okhla Industrial Area. The collected water, kept for sedimentation, subsequently filtered and then autoclaved to kill all microflora. Dead biomass was allowed to settle, filtered aseptically to obtain the required medium for cultivation. Prior to cultivation, River Yamuna water composition was measured as 3.42 g/L of nitrate, 0.4 g/L of ammonia, 0.0025 g/L of nitrite and heavy metals having 0.00041 g/L of Ni, 0.00085 g/L of Cd, 0.00023 g/L of Cu and 0.0315 g/L of Pb. *A. platensis* was acclimatized to the inhibitory components with unknown concentration present in Yamuna water. Acclimatization was performed using a sequential increase in Yamuna water concentration in Zarrouk's medium. Batch cultivation of 5 days was performed with each sequential increase. The seed culture prepared in Zarrouk's medium was used as inoculum for acclimatization studies maintaining photoperiod of 12:12 under sunlight. The serial increases in Yamuna water concentration (as% (v/v)) in Zarrouk's medium and transfer of culture from the preceding batch, which was incubated for 5 days each, took a total of 30 days to acclimatize the strain to 100% Yamuna water. Ratios of Yamuna water to Zarrouk's medium (as% (v/v)) were used as 0:5, 1:4, 2:3, 3:2, 4:1, 5:0.

2.4. Analytical methods

Dry cell weight was measured using gravimetric method. Residual nitrate concentration in sample was measured using Ultraviolet Spectrophotometric method [27]. Filtered 5 mL sample was mixed with 1 mL of 1N HCl. Nitrate and organic molecules were measured as absorbance at 220 nm and 275 nm respectively. Standard curve was drawn using sodium nitrate solutions as standard. Residual nitrate was calculated using the correlation given below:

$$\text{Residual nitrate concentration} = \text{Absorbance}_{@220nm} - 2(\text{Absorbance}_{@275nm})$$

Residual ammonia in samples was measured as absorbance at 420 nm using Colorimetric method [28]. Nitrite in sample was measured as absorbance at 493 nm using spectrophotometric method [29]. Chlorophyll content was estimated using methanol extraction method and calculated using Eq. (2) [30], phycocyanin was assayed using Bennett and Bogorad method [8] and calculated using Eq. (3), and lipid was measured using Phospho-vanillin method [31].

$$Chlorophyll\ (g/L) = 16.5 \times OD_{665} - 8.3 \times OD_{650} \quad (2)$$

$$Phycocyanin\left(\frac{g}{L}\right) = \frac{OD_{615} - 0.474 \times OD_{652}}{5.34} \quad (3)$$

Samples of pre-cultivation and post-cultivation medium were analysed using Atomic Absorption Spectrophotometer (Model AAS4141, ECIL, India) for heavy metals. Instrument was operated maintaining