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Modified conventional bioreactor for microalgae cultivation

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Microalgae, a renewable source for third generation biofuel production, have a great potential if cultivated in high concentration economically. Bottleneck lies with designing economical and efficient photobioreactor. In addition, proportional C and N inputs in the known media does not support high specific growth rate and high biomass build-up. Nitrates in fermentation media, f/2 for *Nannochloropsis* sp. and Zarrouk's for *Arthrospira platensis*, were modified. Aeration and agitation were altered in conventional bioreactor (BIOFLO 110) to reduce power consumption, increase mixing time and prevents settling. This was achieved by introducing four way flow regime supporting uniform nutrient and cell distribution in media. Volumetric cell productivity for *Nannochloropsis* sp. and *A. platensis* were achieved as 0.618 g/l/d and 0.774 g/l/d, respectively. This photobioreactor also supported the maximum specific CO_2 sequestration rates to the level of 0.42 g/g/h and 0.39 g/g/h for *Nannochloropsis* sp. and *A. platensis*, respectively, confirming efficient and effective operation.

[**Key words:** Photobioreactor; Sparger; Impeller type; CO_2 sequestration; Four way flow regime]

World oil demand is rising exponentially to the value of 96.95 mb/d in the fourth quarter of the year 2016 with deficit 0.96% (International Energy Agency, 2017). Deficits are increasing due to the uneven distribution of natural fuels. To overcome this, alternative sources and economical technology are required. Third generation biofuel as biodiesel from renewable microalgae emerged as a potential resource to meet the deficit. In addition, microalgae perform CO_2 sequestration (1–3).

Microalgae cultivation have been achieved in open systems (Raceway ponds) as well as closed systems (Photobioreactors). Microalgae cultivated in raceaway ponds faces difficulties as large space requirement, high evaporation, low CO_2 sparging efficiency, high contamination risk, variable biomass quality, low biomass concentration (0.5–0.7 g/l) and high harvesting cost in comparison with photobioreactors (4,5). Photobioreactor configurations also face challenge of high energy input but capable of achieving high cell density accompanying high CO_2 sequestration (6,7).

Major configurations of conventional bioreactors include a flat plate, tubular (horizontal/vertical), bubble column/airlift and stirred tank bioreactors (8,9). All mentioned configurations have been used in several studies for microalgae batch cultivation but provided insignificant biomass build up of 0.726–3.5 g/l (10–15). Improvements in engineering design of parameters such as illumination, aeration and agitation certainly result in further biomass yield and productivity. Literature depicts that gas sparging velocity and large bubbles in bubble column; mechanical pumping in tubular reactors, dead zones in flat plate reactors and flow profiles due to impeller actions in stirred tank bioreactor encountered hydrodynamic shear stress (16). High hydrodynamic stress to the cells leads to subsequent cell damage during photobioreactor operation (17–21). Despite, stirred tank bioreactors are preferred over mentioned configurations due to simple structure, ease of operation, better provision of aeration and agitation providing suitable hydrodynamic conditions, controlled operating conditions and its scalability (22–25). If designed systematically, shear sensitive microalgae cultivation can be performed with high cell density production in conventional stirred tank bioreactor. Additional illumination of intensity up to 450 μEinst m^{-2} s^{-1} constructs it to a photobioreactor for microalgae cultivation (26). Higher light intensity for shorter photoperiod favours cell growth within mentioned intensity range otherwise observes photoinhibition (27). A suitable combination of parameters in a photobioreactor would not only alleviate diffusion limitation through enhanced cell circulation but also promote high cell density and reduced production cost. Biomass being affected by growth promoting substrates, nitrate and CO_2 supply is necessary at an optimum rate during growth in photobioreactor (28). The focal point of the present research is to overcome the challenges related to aeration and agitation. Paper aims at modifying the nutrient inputs (C/N ratio), illumination, and aeration/agitation assembly in a conventional stirred tank bioreactor to convert it into a photobioreactor suitable for shear sensitive microalgae cultivation. The performance of this photobioreactor was also tested by conducting batch cultivations using *Nannochloropsis* sp. and *Arthrospira platensis* in their respective modified medium. In brief, the present research work endow with a photobioreactor suitable for microalgae cultivation using CO_2 as carbon source.

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MATERIALS AND METHODS

Microalgae culture, maintenance and fermentation medium *Nannochloropsis* sp. and *A. platensis* were received from Algal Research and Supply (Carlsbad, CA, USA). These were initially maintained in the Basal salt medium. Inoculum size was 2% (v/v). The culture was incubated at 30 °C for 5 days keeping photoperiod of 12:12 (night:day) in incubator equipped with 100 W halogen bulb combined with red light which was manually controlled for maintaining 12:12 photoperiod. This livestock was used as inoculum for seed culture. The seed culture was prepared in 1 L Erlenmeyer flasks containing 200 ml sterile medium by inoculating 10% (v/v) of livestock. This prepared fermentation broth was used as an inoculum for further studies in a photobioreactor. f/2 and Zarrouk's medium were used for the cultivation of *Nannochloropsis* sp. and *A. platensis*, respectively (29–32). Nitrate concentration of both media was further modified based on its optimum value for microalgae growth.

Bioreactor setup BIOFLO 110 bioreactor (M/S Eppendorf, USA) was used as a base reactor. It comprises of a vessel (diameter 13.32 cm (ID), height 23.5 cm) equipped with four baffles, a point sparger, and motor driven agitator shaft containing two Rushton impellers.

Modified bioreactor (Photobioreactor) consisted of a combination of Rushton and marine impellers, sintered stainless steel microporous sparger and 100 W halogen bulb with a red filter as an illumination source as shown in Fig. 1a–c. 2N KH_2PO_4 and 2N K_2HPO_4 solutions were used as acid and base, respectively, for controlling pH because NaOH caused precipitation in medium while used as base.

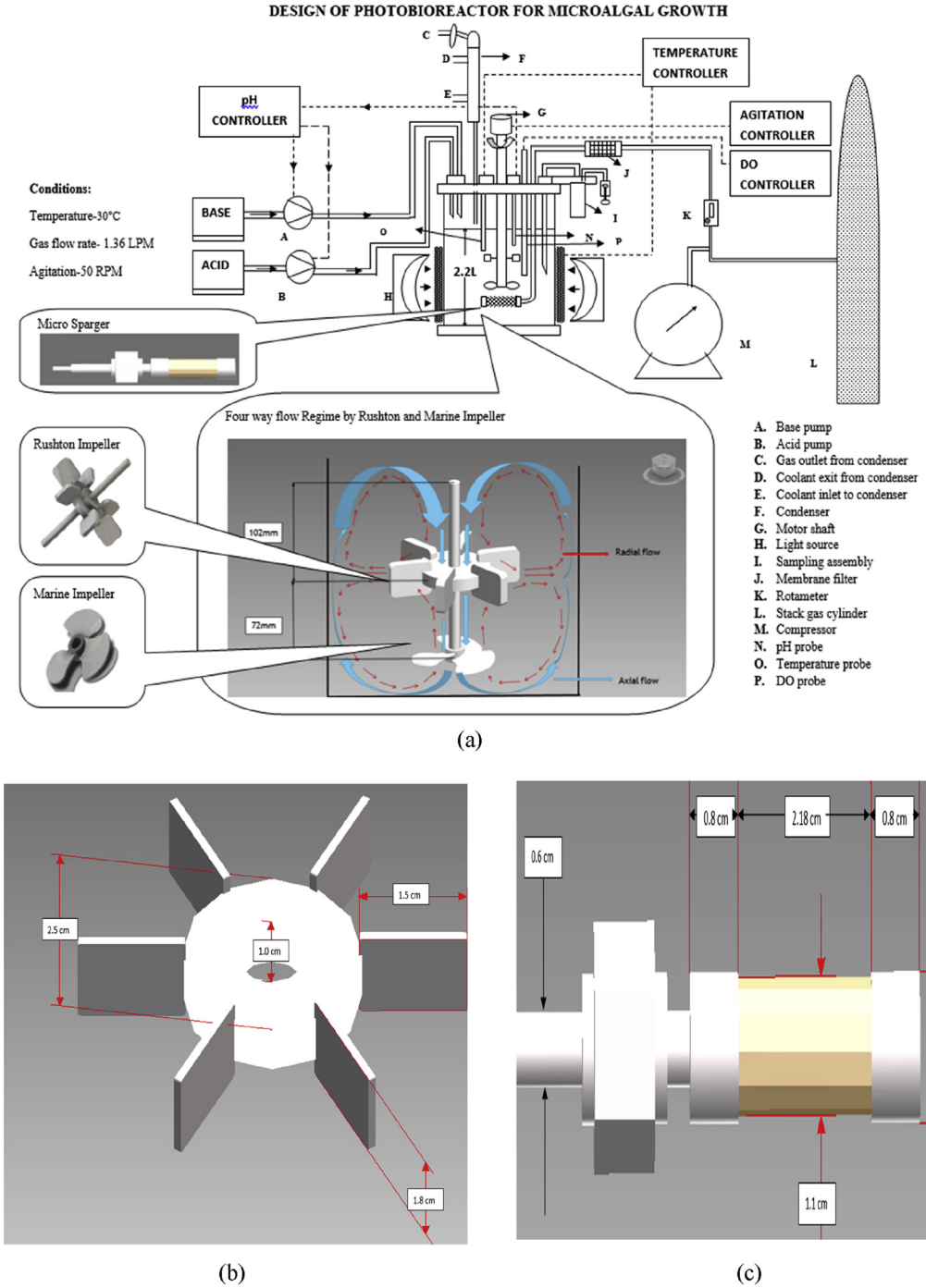

FIG. 1. (a) Schematic diagram of bioreactor. A, base pump; B, acid pump; C, gas outlet from condenser; D, coolant exit from condenser; E, coolant inlet to condenser; F, condenser; G, motor shaft; H, light source; I, sampling assembly; J, membrane filter; K, rotameter; L, stack gas cylinder; M, compressor; N, pH probe; O, temperature probe; P, DO probe. (b) Rushton impeller design in AutoCAD. (c) Sintered micro-porous sparger design in AutoCAD.

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