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Growth characteristics of *Chlorella sorokiniana* in airlift and bubble column photobioreactors

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ABSTRACT

The present study investigated the feasibility of bioCO_2 sequestration using *Chlorella sorokiniana*. It was found that 5% CO_2 (v/v) in air was the most suitable concentration for the growth of this organism. At this concentration, the maximum rate of CO_2 sequestered and the biomass obtained were found to be 1.21 gL^{-1} d^{-1} and 4.4 gL^{-1} respectively. Modeling and simulation of the growth profile was obtained using the logistic equation. Further, at higher CO_2 concentrations, pH drop in the growth media, TAP [-acetate], was prevented by replacing NH_4Cl by $NaNO_3$. Additionally, the study evaluated the performance of two reactors namely: bubble column and airlift reactor based on their growth profile and transport properties like K_La and mixing time. The growth profile was better in airlift reactor and it provides cyclic axial mixing of media. K_La of downcomer was significantly lower than the riser in airlift reactor.

1. Introduction

Global warming has reached an alarming level due to increase in CO_2 concentration in the atmosphere. Recent studies conducted at Mauna Loa Observatory (Hawaii, US) in December, 2011 found that the concentration of CO_2 in air was nearly 391 ppmv. Thus, it is imperative to identify and improve the process of CO_2 sequestration. In view of this, microalgae have been identified as a potential candidate for sequestering CO_2. Besides mitigating CO_2, they are known to have several other uses. They can be used for the production of biofuels (e.g. biodiesel, bioethanol, biohydrogen) and other important products like industrial biofilters, food products and secondary metabolites (Loubiere et al., 2009; Kumar et al., 2011).

Microalgae, *Chlorella sorokinina*, is playing an important role as food and feed because of the multiuse of its biomass previously known to be a rich source of carbohydrate, vitamins, and proteins. The high protein content makes it a suitable raw material for the production of single cell protein (Mahasneh, 1997) while the high vitamin contents makes it a suitable feed for aquaculture systems (Gapasin et al., 1998). Besides, under sulfur deprived condition (Chader et al., 2009) *C. sorokiniana* is also known to produce clean energy, biohydrogen. Additionally, it has been used for the production of commercially important antioxidants like lutein, α/β carotene, α/β tocopherol, zeaxanthin (Matsukawa et al., 2000).

Several factors are known to affect the CO_2 sequestration process; like choice of photobioreactor, culture/strain, temperature, pH, light intensity, culture density, concentration of CO_2, SO_x and NO_x, CO_2 mass transfer, O_2 accumulation etc. (Kumar et al., 2011). Among these, most notably, a suitable photobioreactor is essential for improved CO_2 sequestration and better utilization of light. Ease of operation, scalability, lower land requirement, higher biomass productivity and cost effectiveness are some of the significant features of an ideal photobioreactor (Kumar et al., 2011). Airlift and bubble column were considered as ideal photobioreactors for the present study because they are known to possess all the above-mentioned properties. In the past, both airlift and bubble column photobioreactors have been studied extensively for the cultivation of shear sensitive microorganisms (Barbosa et al., 2003; Chisti, 1989; Suh and Lee, 2001). Similarly, Ranjbar et al. (2008) and Harker et al. (1996) performed studies on airlift photobioreactor for astaxanthin and carotenoids production respectively. However, till date, these photobioreactors have not been completely exploited for the cultivation of photosynthetic microorganisms. A comparative analysis of both the photobioreactors based on the growth profile of the organism, mixing time and volumetric mass transfer coefficient may provide a thorough and comprehensive knowledge of both of the reactors. However, only few studies are available on the interaction of CO_2 mass transfer, light availability, hydrodynamic stress in the airlift photobioreactor (Chisti and Young, 1993; Contreras et al., 1998; Sánchez Miron et al., 2004; Hulatt and Thomas, 2011).

It is well known that alga grows efficiently only at optimal CO_2 concentrations while, any further increase or decrease of CO_2

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concentration limits its growth (Chiu et al., 2008). Similarly, pH is another factor limiting the growth of the microalgae. Mostly the microalgae maintain neutral or slightly alkaline cytosolic pH, as many enzymes are highly pH dependent and become inactive in acidic pH (Gimmler, 2001). Moreover, choice of nitrogen source in the medium is critical as it serves as a nitrogen source as well as, controls the pH of the medium. TAP medium containing NH_4Cl has been extensively used by the researchers for the growth of the biomass that can be used for the hydrogen production (Skjanes et al., 2008). However, use of $NaNO_3$ as nitrogen source is considered as better than the NH_4Cl especially in presence of high CO_2 concentration in the reactor. Chen et al. (2011) reported that inhibition of cell growth at higher CO_2 concentration in the presence of NH_4Cl may be due inability of cells to regulate passive diffusion of the NH_3, which is in equilibrium with NH_4^+, across the plasma membrane. Moreover, consumption of $NaNO_3$ by algae may increase the pH of the medium as also, it may balance the pH drop due to high dissolved CO_2 and carbonic acids (Hulatt and Thomas, 2011).

Thus, the present study aimed to determine the growth characteristics of *C. sorokiniana* in airlift and bubble column photobioreactors based on different aspects like, mixing time, K_La, and growth profile. Besides, the study investigated the effects of factors like pH and CO_2 stress on algal growth profile. Further, an attempt was made to modify the existing TAP [-acetate] medium by substituting NH_4Cl by $NaNO_3$ to improvise algal cultivation at higher CO_2 concentrations.

2. Methods

2.1. Microalgae and culture medium

The culture of *C. sorokiniana* was obtained from Dr. Kari Skjanes (Norwegian Institute for Agricultural and Environmental Research, Bioforsk, Oslo, Norway). The microalgae were cultured in TAP medium having composition as described below (Skjanes et al., 2008). TAP media contained 2.42 g L^{-1} Tris base, 25 ml L^{-1} TAP salt stock solution (15.0 g L^{-1} NH_4Cl, 4.0 g L^{-1} $MgSO_4$ $7H_2O$, 2.0 g L^{-1} $CaCl_2$ $2H_2O$, 0.375 ml L^{-1} PO_4 stock solution (28.8 g per 100 ml K_2HPO_4, 14.4 g per 100 ml KH_2PO_4), 1 ml L^{-1} Hutner trace metals (21.6 g per 100 ml H_2O EDTA: Titriplex II, 11 g per 50 ml H_2O $ZnSO_4 \times 7H_2O$, 5.7 g per 100 ml H_2O H_3BO_3, 2.53 g per 25 ml H_2O $MnCl_2 \times 4H_2O$, 0.805 g per 25 ml H_2O $CoCl_2 \times 6H_2O$,0.785 g per 25 ml H_2O $CuSO_4 \times 5H_2O$, 0.55 g per 25 ml H_2O $(NH_4)_6Mo_7O_{24} \times 4H_2O$, 2.495 g per 25 ml H_2O $FeSO_4 \times 7H_2O$), 1 ml L^{-1} Vitamins stock solution (0.5 mg L^{-1} Cyanocobalamin (B_{12}), 100 mg L^{-1} Thiamine HCl, 0.5 mg L^{-1} Biotin), 1 ml L^{-1} glacial acetic acid. Glacial acetic acid was absent in TAP [-acetate] medium. TAP [-acetate] medium was modified by substituting NH_4Cl by 1.5 g L^{-1} of $NaNO_3$ (mTAP [-acetate] to be used elsewhere in the manuscript). This amount was based on $NaNO_3$ concentration in standard BG_{11} medium largely used for the growth of cyanobacteria (Rippka, 1988). However, its concentration was not optimized for this strain. Initial pH of the medium was maintained at 8 and 9 in TAP [-acetate] and mTAP [-acetate] medium respectively.

2.2. The photobioreactor

The bubble column photobioreactor was constructed using plexiglass (polymethyl methacrylate). The bubble column bioreactors were of dimensions dia. 7 cm × 42.3 cm with two sides opening near the top and bottom of the reactor. The opening at the top was used as an exhaust for the disengaged bubbles. The wall thickness was 5 mm. CO_2 rich stream was introduced into the base by a sparger attached at the bottom (Fig. 1a and b). Bubble column was converted into airlift bioreactor by inserting a draft tube into them. Height of the draft tube and the clearance zone for airlift reactor were 28 and 3 cm respectively.

With the addition of the draft tube of diameter 3 cm, bubble column photobioreactor was converted into airlift reactor both having constant surface by volume (S/V) ratio of 0.57 cm^{-1} while the A_d/A_r was 4.4 where, A_d and A_r are the area of downcomer and riser, respectively (Table 1). S/V ratio of 0.57 cm^{-1} was in good accordance with the reported literature (Contreras et al., 1998). However, design criteria related to A_d/A_r was found to be higher than 1.041 reported for an ideal airlift photobioreactor (Barbosa et al., 2003). During all the experiments temperature was maintained at 30 ± 2 °C. Photobioreactors were constantly illuminated by light intensity of 100 μmol $m^{-2}s^{-1}$ at the surface of the reactor from cool white fluorescent tubes placed at one side of the reactors.

2.3. Optical density, dry weight and the amount of CO_2 sequestration determination

Optical density (OD) of cells was determined in spectrophotometer at 682 nm (Chemito). Dry cell weight (Dwt) was calculated using following formula generated using OD data and a calibration plot.

Dwt (mg ml^{-1}) = 0.28 $OD_{682\ nm}$.

Amount of CO_2 sequestered (g L^{-1}) = 1.83 Dwt (g L^{-1}).

Assuming 50% of carbon in dry weight of microalgae which corresponds to 1.83 g of CO_2 required for the cultivation of 1 g of microalgae dry cell weight (Chisti, 2007).

2.4. Net specific growth rate

Net specific growth rate was calculated from Eq. (1) (Issarapayup et al., 2009):

$$\mu_{net} = \frac{(\ln N_2 - \ln N_1)}{t_2 - t_1} \quad (1)$$

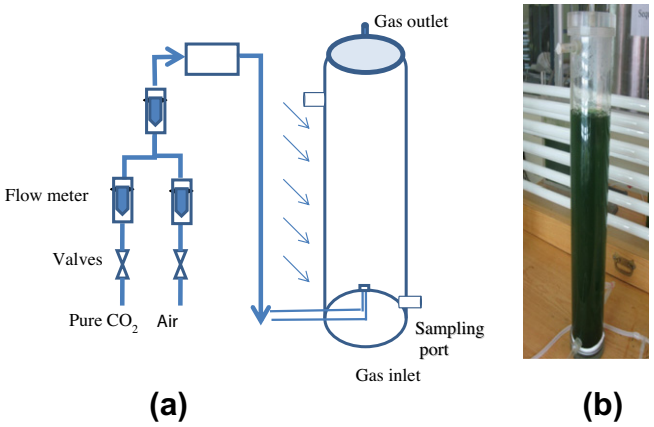

Fig. 1. (a) Schematic diagram of CO_2 sequestration process and (b) Experimental set up of an airlift bioreactor.

Table 1
Design parameters of airlift photobioreactor.

Criteria	Present study	Barbosa et al. (2003)	Contreras et al. (1998)
A_d/A_r	4.4	>1.041	–
S/V (cm^{-1})	0.57	–	0.44