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Performance evaluation of a green process for microalgal CO_2 sequestration in closed photobioreactor using flue gas generated *in-situ*

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HIGHLIGHTS

- CO_2 fixation from industrial flue gas using *Chlorella* sp.
- *Chlorella* sp. tolerates gas stream with 5% of CO_2 v/v.
- Different strategies to minimize toxic effect of flue gas compounds were evaluated.
- Biofixation efficiency was improved by 54%.

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ABSTRACT

In the present study, carbon-dioxide capture from in situ generated flue gas was carried out using *Chlorella* sp. in bubble column photobioreactors to develop a cost effective process for concomitant carbon sequestration and biomass production. Firstly, a comparative analysis of CO_2 sequestration with varying concentrations of CO_2 in air-CO_2 and air-flue gas mixtures was performed. *Chlorella* sp. was found to be tolerant to 5% CO_2 concentration. Subsequently, inhibitory effect of pure flue gas was minimized using various strategies like use of high initial cell density and photobioreactors in series. The final biofixation efficiency was improved by 54% using the adopted strategies. Further, sequestered microalgal biomass was analyzed for various biochemical constituents for their use in food, feed or biofuel applications.

1. Introduction

The anthropogenic activities such as excessive use of fossil fuel reserves, deforestation and intensive industrialization has led to unceasing rise in greenhouse gas emissions. In recent times, the CO_2 concentration in the atmosphere has reached an alarming level of 400 ppm (Tans, 2015). It is envisaged that CO_2 levels greater than 450 ppm could be destructive to global climate (Hansen et al., 2007). Therefore, demand for effective carbon-dioxide (CO_2) mitigation technologies is the need of hour. Of all the post combustion CO_2 capture technologies viz. chemical and physical absorption, geological and oceanic storage, and biological fixation; microalgal mediated CO_2 fixation offers several advantages such as faster growth rates and higher CO_2 fixation rates (10–50 times more) than terrestrial plants. Hence, microalgae are regarded as prime candidates for biological fixation of CO_2 (Yoo et al., 2010; Cheng et al., 2013). As a result of CO_2 fixation, microalgal biomass accumulates significant amounts of lipids, carbohydrates, proteins and other valuable compounds, such as pigments and vitamins, which can be used as active ingredients in nutraceutical, food and feed supplements or in the production of biofuels (Francisco et al., 2010; Cheah et al., 2015).

An effective microalgal cultivation system is designed in order to achieve high surface area per volume ratio and good hydrodynamics to give more surface area for the light penetration and gaseous CO_2 transfer (Cheah et al., 2015). An efficient CO_2-fixation system must ensure good mixing, high gas–liquid transfer rates, and even distribution of light. Broadly, microalgal cultivation is done in two types of systems, open and closed. Usually open microalgal cultivation is carried out in raceway ponds which are low cost, ease in operation and maintenance but suffer from poor light penetration, contamination and poor biomass productivity. On the other hand, closed system includes different configuration of photobioreactors (PBRs) (air-lift, flat plate, tubular) and generally have better mass transfer rates, higher productivity and better control over process parameters than open ponds. Due to their simplicity, robustness and relatively high process efficiency, closed

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system especially bubble column reactors are widely used (Kumar and Das, 2012). Various strains of microalgae have been utilized for CO_2 fixation. *Chlorella* sp. is widely studied strain which has been used in several industrial applications and found to be a fast growing microalgal strain capable of fixing CO_2 from flue gases (Van Den Hende et al., 2012). It contains high amount of protein (51–58%), carbohydrate (12–17%) and lipid (14–22%) that could be used for various applications like health food, nutritional supplements, animal feed, biofuels, etc. (Becker, 1994; Cheah et al., 2015). Flue gas is inexpensive and rich source of CO_2, approximately 400 times more concentrated than atmospheric CO_2 and thus can be exploited for microalgal mediated CO_2 fixation (Cheah et al., 2015). However, the major constraints are due to the presence of toxic compounds such as NO_x, SO_x and CO which are inhibitory for microalgal growth and biomass productivity (Kumar et al., 2014), primarily due to acidification of the growth medium (Van Den Hende et al., 2012). Flue gas from various sources has been used to cultivate microalgae for CO_2 fixation (Van Den Hende et al., 2012; Kumar et al., 2014). The direct use of flue gas is detrimental for microalgal growth and generally requires expensive pre-treatments processes. The efficiency of CO_2 fixation from flue gas by microalgae generally found to be less than 50% (Cheng et al., 2013). In order to treat flue gas by microalgae in a cost-effective manner, there is need to develop effective strategies which will not only reduce the cost of its pretreatment but also increase CO_2 fixation.

Therefore, in this study, strategies were developed to directly use coal-fired flue gas for the cultivation of microalgae and simultaneous flue gas remediation. Firstly, a comparative study was conducted to evaluate the performance of *Chlorella* sp. for CO_2 fixation using pure CO_2 and waste flue gas. Subsequently, inhibitory effect of pure flue gas was minimized using various strategies like use of high initial cell density and photobioreactors in series. Further, commercial potential of the sequestered microalgal biomass was assessed for their use in food, feed or biofuel applications.

2. Methods

2.1. Strain and culture medium

The strain *Chlorella* sp. (Chlorophyta, Chlorophyceae) obtained in this experiment was procured from National Environmental Engineering Research Institute, Nagpur, India (Fulke et al., 2010). The inoculum was grown on modified Bold Basal's medium (Nichols and Bold, 1965) and has the following composition (mg L^{-1}): $NaNO_3$ (750), $CaCl_2{\cdot}2H_2O$ (12.5), $MgSO_4{\cdot}7H_2O$ (150), $FeSO_4$ (6.27), K_2HPO_4 (62.4), KH_2PO_4 (225), NaCl (0.341), H_3BO_3 (5), MnSO4 (0.72), $ZnSO_4{\cdot}7H_2O$ (17.64), KOH (15.5), NaCl (12.5), $CuSO_4{\cdot}7H_2O$ (1.06), $NaMoO_3$ (0.6), $CoCl_2$ (0.2). The strain was maintained in liquid culture (100 mL) in an Erlenmeyer's flask (150 mL), at 28 ± 2 °C, under intermittent agitation and continuous illumination (24 μmol m^{-2} s^{-1}) and an atmosphere of air 0.04% CO_2 (v/v). Initial pH of the medium was adjusted to 6.8 ± 0.1. All chemicals used are of analytical grade and procured from Merck®, India. All experiments were performed in triplicates and expressed as mean with standard deviation (S.D).

2.2. Photobioreactor, experimental set-up and batch culture conditions

Batch experiments were performed in bubble column glass photobioreactors (BCR) (length, 33 cm; inner diameter, 4.5 cm; working volume, 500 mL). The average temperature during batch experiment was maintained at 28 ± 2 °C.

The initial pH of medium was noted to be 6.8 and the rate of flow of gas into the reactor was maintained at 0.5 vvm. Carbondioxide or flue gas was fed only during the light period and its supply was stopped during the dark period, however air was fed continuously. The light intensity falling from one side of reactor through cool white fluorescent tubes was measured using quantum meter (HTC LX 102 Lux Meter) to be approximately 106.6 μmol m^{-2} s^{-1} during the entire batch experiment in a 12:12 h (light/dark) photoperiod and. Ambient air or CO_2 enriched air was supplied through an aquarium sparger ($\Phi_{diameter}$ = 1.0 cm) located at the bottom of the BCR. The flue gas produced from burning of coal in the flue gas generator is mixed with air to create different v/v mixtures of air-flue gas before it is filtered using syringe filter and injected into the culture photobioreactors. The concentrations of air-pure CO_2 as well as air-flue gas mixture were adjusted and set to desired concentrations of 2.5%, 5%, 7.5%, 10% and 0.04% (ambient air) of the total mixture. A single batch culture was incubated for 7 days. Samples were removed once every 24 h to determine biomass concentration and CO_2 fixation rate and twice every 24 h to check the culture pH. Sample pH was determined using a pH meter (Deluxe pH Meter, Model LT-10). The effect of inoculum density was investigated by varying the cell density of initial inoculum viz. 0.06 g L^{-1}, 0.1 g L^{-1}, 0.15 g L^{-1} and 0.2 g L^{-1} respectively. Subsequently, the best inoculum density was selected to carry out the photobioreactor-in-series experiment to minimize the toxic effects of flue gas components in sequential reactors. *Chlorella* sp. was cultured in three bubble column PBRs connected in series and labeled sequentially as PBR 1st, PBR 2nd and PBR 3rd. Flue gas was fed into the sparger of PBR 1st and effluent from this PBR was fed to PBR 2nd and similarly the effluent from this PBR was fed to PBR 3rd (Supplementary Figs. A.1 and A.2).

2.3. Flue gas analysis

The flue gas used in the present study was obtained after burning of coal procured from Kolaghat Thermal Power Station (KTPS), West Bengal India. An indigenously designed and fabricated flue gas generator was used for burning the coal and collecting in situ generated flue gas. The flue gas generator was equipped with three-chambered double layered jacket in which water was circulated to cool down the temperature of flue gas from 120 °C to approximately 45 °C. The raw flue gas was collected from the upper position of stack and passed through a vacuum precipitator to remove the suspended particulate matter by a compressor pump (1HP). The flue gas was then transferred through pipelines to storage gas tank (volume: 41.3 L, pressure: 10 kg cm^{-2}) before feeding it to the microalgal PBR. The flue gas was analyzed by a portable on-line flue gas analyzer (Model: Indus FGA 53X). A schematic illustration of the flue-gas based cultivation system for *Chlorella* sp. is shown in Supplementary Fig. A.2. The flue gas was analyzed by a portable on-line flue gas analyzer (Model: Indus FGA 53X). The typical composition of the flue gas was 10 ± 2% (v/v) carbon dioxide, 0.554% (v/v) carbon-monoxide, 8.33% (v/v) oxygen, 61 ppm nitrogen oxides, 0.3% (v/v) sulfur oxides, 9 ppm other hydrocarbons.

2.4. Biomass concentration, biomass productivity and nitrate consumption profile

Biomass concentration (dry weight per liter) of microalgal cells was measured by taking optical density at 750 nm ($O.D_{750\ nm}$) in Agilent Cary 60 Scan UV/Visible spectrophotometer (Griffiths et al., 2011). Dry cell weight (dwt) was calculated using a calibration plot between Dwt and O.D. Dry weight biomass (g L^{-1}) = 0.44 × $O.D_{750\ nm}$. Sampling was done every 24 h to determine the cell concentration for further algal growth calculation. Each sample was diluted to give an absorbance in the range of 0.1–0.5 if the optical density was greater than 1.0. This is due to the fact that probability of obtaining erroneous result becomes