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Continuous mode of carbon dioxide sequestration by *C. sorokiniana* and subsequent use of its biomass for hydrogen production by *E. cloacae* IIT-BT 08

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HIGHLIGHTS

- Operating continuous mode of operation for CO_2 sequestration using *C. sorokiniana*.
- Modeling and simulation of continuous culture of algae.
- Utilizing algal biomass as substrate for H_2 production using *E. cloacae* IIT-BT 08.
- Better H_2 energy using algal biomass as substrate over its use in biophotolysis.

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ABSTRACT

The present study investigated to find out the suitability of the CO_2 sequestered algal biomass of *Chlorella sorokiniana* as substrate for the hydrogen production by *Enterobacter cloacae* IIT-BT 08. The maximum biomass productivity in continuous mode of operation in autotrophic condition was enhanced from 0.05 g L^{-1} h^{-1} in air to 0.11 g L^{-1} h^{-1} in 5% air–CO_2 (v/v) gas mixture at an optimum dilution rate of 0.05 h^{-1}. Decrease in steady state biomass and productivity was less sensitive at higher dilution and found fitting with the model proposed by Eppley and Dyer (1965). Pretreated algal biomass of 10 g L^{-1} with 2% (v/v) HCl–heat was found most suitable for hydrogen production yielding 9 ± 2 mol H_2 (kg COD reduced)$^{-1}$ and was found fitting with modified Gompertz equation. Further, hydrogen energy recovery in dark fermentation was significantly enhanced compared to earlier report of hydrogen production by biophotolysis of algae.

1. Introduction

The renewable energy sources have important role for decreasing not only the greenhouse effect but also to provide alternative option in the current exponential increase in worldwide energy demand resulting into depletion of energy reserves at greater pace. Moreover, it is the responsibility of civilized society to explore the possibility of using clean, efficient, sustainable and renewable sources of energy. Hydrogen is widely recognized as suitable and potential candidate as it has highest energy density among any known fuels (143 GJ tonne^{-1}) and is the only common fuel that is not chemically bound to carbon. In addition, it produces only water on combustion (Levin et al., 2004). On the other hand, green algae have the ability to sequester CO_2 from flue gas and alleviate the impact of global warming due to increasing concentration of CO_2 in the atmosphere (Kumar et al., 2011). Though some of the green algae such as *Chlamydomonas reinhardtii*, *Chlorella sorokiniana* can be used for hydrogen production because of the presence of hydrogenase enzymes. But its H_2 producing potential is always under scanner as the rate of hydrogen production is not encouraging. However, their biomass as such can be utilized as substrate for hydrogen producing bacteria.

Enterobacter cloacae IIT-BT 08, a meshophilic facultative anaerobic, gram-negative, rod shaped bacteria is widely known for its potential in H_2 production (Kumar and Das, 2001; Khanna et al., 2011a,b). Wild type *E. cloacae* has been reported to produce a practical yield of 2.2 mol H_2 mol^{-1} glucose as against the theoretically limit of 4 mol H_2 mol^{-1} glucose (Kumar and Das, 1999). Previously, various studies on this potential strain have been conducted by many researchers based on process design, media optimization, effect of pH, temperature, different substrates, reduced partial pressure, use of different configuration of reactors and different modes of operation such as batch, continuous, immobilization to improve the yield (Kumar and Das, 2001; Das, 2009; Khanna et al., 2011a). In addition, various carbonaceous substrates such as sewage

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sludge (Kotay and Das, 2007), distillery waste (Vatsala et al., 2008), foodwaste (Ming et al., 2008), whey (Antonopoulou et al., 1985; Khanna et al., 2011b), pome (Ismail et al., 2010), and feedstock (Blackburn et al., 2009) were also investigated and optimized in order to decrease the substrate cost of the process.

The depleting fossil fuels based energy reserves and emission of greenhouse gas on its combustion has serious negative effects on environment. Both issues can be addressed simultaneously by green algae. Being photosynthetic microorganisms, algae have ability to fix CO_2 from flue gas and can thrive on the waste containing different volatile fatty acids. In addition, it has very high photosynthetic efficiency and requires very less water requirement. Unlike other crops like corn or soybeans, algae can use various water sources ranging from wastewater to brackish water and can be grown in small, intensive plots on denuded land. Microalgae can be cultivated in either batch, fed batch or continuous. However, continuous mode of operation has advantages over batch mode such as no downtime and regulating the productivity by holding a particular phase of cell. Further, it has advantage of testing the effect of different physico-chemical parameters for productivity. Use of algal biomass for energy purpose is the very fundamental matter for the sustainable development of alternative renewable energy in future. Their biomass as substrate can be fermented by bacteria similar to other organic wastes as it is rich in carbohydrates, protein and lipids. Algal biomass is considered as third generation biofuel and has advantages over first two generation biofuels. For example, it does not create obstacle in food supply and hence causes no negative impact on the global food market. Similarly the technology for the conversion of biomass to usable substrate is simple and economically viable which was major disadvantage with second generation biofuel (Brennan and Owende, 2010).

Thus the present study aimed to produce algal biomass of *C. sorokiniana* by CO_2 sequestration in continuous mode of operation and subsequent use of its biomass as substrate to produce H_2 in dark fermentation using *E. cloacae* IIT-BT 08.

2. Methods

2.1. Cultivation of Chlorella sorokiniana

The culture of *C. sorokiniana* was cultivated in airlift photobioreactor (Kumar and Das, 2012) at 30 °C under continuous light intensity of 120 μmol m^{-2} s^{-1} provided by white fluorescent lamps under continuous mode of operation in mTAP [-acetate] medium. The reactor was sparged with a gas mixture at the rate of 0.33 vvm in various concentration of CO_2. Different concentration of gas mixture of CO_2 was obtained by mixing air and pure CO_2 using rotameter. The dilution rate was varied from 0.01 to 0.11 h^{-1} with increment of 0.02 h^{-1}. Algal biomass was harvested by centrifugation at 5000 rpm for 5 min and washed thrice with distilled water. It was further lyophilized to get a powdered biomass.

2.2. Bacterial culture conditions and experimental set up for hydrogen production experiment

E. cloacae IIT-BT 08, an established H_2 producing bacterium was used for H_2 production in all the experiments. The strain was grown over night aerobically in Luria Bertani medium (Hi-Media, India) in an incubator shaker (New Brunswick Scientific, New Jersey, USA) at 200 rpm, 37 °C and routinely maintained aerobically on nutrient agar medium by monthly transfer.

Pretreatment study and algal biomass optimization was carried out in 100 ml septum bottle having working volume of 80 ml in an incubator shaker at 200 rpm. Batch study of hydrogen production experiment was conducted in double jacketed bioreactor having working volume of 500 ml kept on a magnetic stirrer. In batch study, glucose from standard MYG medium (malt extract – 1% w/v; yeast extract – 0.4% w/v; glucose – 1% w/v) was replaced with 1% w/v pretreated algal biomass. Magnetic bead was used to stir the medium at 200 rpm in the inner jacket. Water was circulated in outer jacket to maintain 37 °C. Inoculum volume in all the experiment was 10% (v/v). Nitrogen gas was used to purge the culture initially to establish the anaerobic condition for hydrogen production. Hydrogen gas was collected in gas collector under downward water displacement method.

2.3. Analytical procedure

The H_2 was quantitatively measured by gas chromatography (GC, Agilent Technologies, USA) with a thermal conductivity detector (TCD) with N_2 as the carrier gas while the volatile fatty acid composition of spent medium was determined by flame ionization detector (FID) method. The column used for gas determination was a stainless column (1/8 inch 15 ft) packed with 50/80 mesh Carboxen 1000 (Supelco). Optical density (OD) of cells was determined turbidometrically in spectrophotometer (Chemito) at 682 nm and 600 nm for *C. sorokiniana* and *E. cloacae* IIT-BT 08 respectively. Dry cell weight (Dwt) was calculated using a calibration plot between Dwt and OD. Carbon present in dry cell weight of microalgae was assumed as 50% which corresponds to requirement of 1.83 g of CO_2 for the production of 1 g dry cell weight of microalgae (Kumar and Das, 2012). Total sugar estimation was done using Phenol sulfuric method as described by (Loewus, 1952). The COD was measured according to APHA standard methods (APHA, 1998) using a COD measurement instrument set (DRB200 & DR2800 Portable Spectrophotometer, HACH, USA).

2.4. Algal biomass pretreatment

Prior to use of algal biomass as substrate for fermentation, lyophilized algal biomass was pretreated with HCl–heat to facilitate the release of intracellular complex polymeric form of carbohydrates bounded with rigid algal cell walls to simpler sugar (Kawaguchi et al., 2001). In HCl–heat pretreatment method, dried algal biomass powder was digested overnight with 2.0% HCl and autoclaved at 121 °C, 1 atm for 20 min. Hydrolysates were neutralized with 10% NaOH and used as substrate for H_2 production experiment.

2.5. Determination of hydrogen production kinetics using modified Gompertz model

Modified Gompertz equation was used to determine the hydrogen production kinetics of *E. cloacae* IIT-BT 08 using algal biomass as substrate and for the precise prediction of cumulative hydrogen production {H(t), ml} at any given interval of time (t, h) (Khanna et al., 2012).

Matlab (Curve fitting tool box ver 2.1) was used for the curve fitting the experimental values.

3. Results and discussion

3.1. Production of Chlorella sorokiniana biomass

C. sorokiniana was grown in batch followed by putting it in continuous mode in presence of air, 2.5% and 5% CO_2 (v/v). The results are shown in Table 1. The optimum dilution rate in each of the case was 0.05 h^{-1} with steady state biomass (X) of 0.92 g L^{-1}, 1.21 g L^{-1}