Bioresource Technology 164 (2014) 323–330

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

CO_2 biofixation and carbonic anhydrase activity in *Scenedesmus obliquus* SA1 cultivated in large scale open system

Samarpita Basu [a], Abhijit Sarma Roy [b], Kaustubha Mohanty [c], Aloke K. Ghoshal [b,c,*]

[a] Centre for Environment, Indian Institute of Technology Guwahati, Guwahati 781039, Assam, India
[b] Centre for Energy, Indian Institute of Technology Guwahati, Guwahati 781039, Assam, India
[c] Department of Chemical Engineering, Indian Institute of Technology Guwahati, Guwahati 781039, Assam, India

HIGHLIGHTS

- The novel microalga, *S. obliquus* SA1 was cultivated in large scale (open system).
- SA1 showed tolerance to 35% CO_2 concentration.
- Biomass production at 35% CO_2 was superior to the relevant literature reports.
- Extracellular (periplasmic) carbonic anhydrase activity was more prominent in SA1.
- Enhanced lipid and chlorophyll production was observed with increase in inlet CO_2.

ARTICLE INFO

Article history:
Received 20 March 2014
Received in revised form 3 May 2014
Accepted 5 May 2014
Available online 13 May 2014

Keywords:
Microalgae
CO_2 biofixation
Carbonic anhydrase
Open system

ABSTRACT

The present study deals with the large scale open system cultivation of the novel microalga: *Scenedesmus obliquus* SA1 (KC733762) previously isolated in our laboratory. SA1 strain was cultivated in open system at varying CO_2 levels ranging from 0.03% to 35% (v/v) and subsequently the carbonic anhydrase activity (CA) and the biochemical properties were monitored. Maximum biomass concentration (1.39 ± 0.023 g L^{-1}), CO_2 fixation rate (97.65 ± 1.03 mg L^{-1} d^{-1}) and total CA activity (166.86 ± 3.30 E.U./mg chla) were obtained at 35% CO_2. CA inhibitors: acetazolamide and ethoxyzolamide inhibited the external and internal enzyme activity in SA1. High CO_2 levels were favorable for the accumulation of lipids and chlorophyll. The present results suggested that SA1 possessed high CO_2 tolerance and high carbohydrate, lipid and chlorophyll content when cultivated in open system thus being suitable for CO_2 mitigation in outdoor ponds and subsequent generation of value added products.

1. Introduction

Increased CO_2 concentration in the atmosphere resulting from the combustion of fossil fuels such as coal, for energy is responsible for global warming and climate change. The production of 1 kWh of electricity from coal combustion results in the emission of 0.95 kg CO_2 (Chi et al., 2011). With heavy reliance on fossil fuels, worldwide CO_2 emissions still continue an upward trend. There are available measures to reduce CO_2 emissions like switching from high-carbon to low-carbon fuels, switching to renewable or nuclear energy; or CO_2 capture and storage (Chiang et al., 2011). CO_2 capture methods like chemical absorption and physicochemical adsorption present significant challenges from an economic point of view such as high energy consumption for regeneration. For e.g., while employing chemical absorption with regenerable alkaline aqueous solvents such as monoethanolamine (MEA), CO_2 is removed from the flue gas stream in the absorber column and then desorbed in a heated stripper column to give relatively pure CO_2 for compression and storage. However, solvent such as MEA tend to bind CO_2 tightly such that the energy loss in desorbing the CO_2 would result in a near doubling of the cost of electricity (Savile and Lalonde, 2011). Biological fixation and storage of CO_2 via microalgae are essentially photosynthesis, which can convert water and CO_2 into organic compounds without additional or extra energy consumption and without secondary pollution. Compared with other CO_2 capture methods, microalgal CO_2 fixation has several advantages, such as high photosynthesis rate, good environmental adaptability and low cost of operation (Zhao and Su, 2014). Microalgae have high CO_2 fixation ability and produce biodiesel and bioproducts through

* Corresponding author at: Department of Chemical Engineering, Indian Institute of Technology Guwahati, Guwahati 781039, Assam, India. Tel.: +91 0361 2582252; fax: +91 0361 2582291/2690762.
E-mail address: aloke@iitg.ernet.in (A.K. Ghoshal).

http://dx.doi.org/10.1016/j.biortech.2014.05.017
0960-8524/

their biomass (Yoo et al., 2010). Microalgae and cyanobacteria fix CO_2 via C_3 biochemistry (Calvin cycle) (Sinetova et al., 2012). These organisms face several challenges in acquiring CO_2 from the environment. The first challenge is presented by the properties of the enzyme ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco) which fixes CO_2 in the Calvin cycle, converting it into organic carbon. From Michaelis–Menten enzyme kinetics it is known that there is a hyperbolic relationship between the rate of an enzyme-catalyzed reaction and the concentration of the substrate. The rate of reaction when the enzyme is saturated with substrate is the maximum rate (V_{max}) and K_m (Michaelis–Menten constant) is the concentration of substrate which permits the enzyme to achieve half of the maximum reaction rate (V_{max}). Thus in photosynthesis, K_m, implies the concentration of CO_2 at which the rate of photosynthesis attains one-half of its maximum value and V_{max} implies the maximum rate of photosynthesis (Shelp and Canvin, 1980). When the rate of photosynthesis is highest, Rubisco is fully saturated with CO_2 and has maximum catalytic capacity. However at ambient CO_2 levels, Rubisco can function at only about 25% of its catalytic capacity because the concentration of the substrate (CO_2) is lower than the concentration of CO_2 at which the rate of photosynthesis attains K_m. Also, ribulose-1,5-bisphosphate and CO_2 are the substrates for Rubisco in the Calvin cycle but the oxygenation of ribulose-1,5-bisphosphate is competitive with the carboxylation reaction. Hence the relatively high concentration of O_2 competes with CO_2 as a substrate for Rubisco (Moroney and Ynalvez, 2007; Fang et al., 2012).

Also, algae often experience significant fluctuations in dissolved inorganic carbon ($C_i = CO_2 + HCO_3^-$) levels and pH, which change the availability of dissolved CO_2 and HCO_3^- for photosynthesis. At an acidic pH, the vast majority of C_i is in the form of dissolved CO_2, while at alkaline pH, C_i is mostly in the form of HCO_3^-, with dissolved CO_2 making up only a small fraction of the available C_i. Another challenge these organisms face is that the diffusion of CO_2 in an aqueous solution is 10,000 times slower than the diffusion of CO_2 in air (Moroney and Ynalvez, 2007). Algae have adapted to these challenges through the development of a CO_2 concentrating mechanism (CCM). It permits the maintenance of the photosynthetic activity at low concentration of CO_2 in the environment. Carbonic anhydrase (CA) (EC 4.2.1.1), an important component of CCM, is a zinc-containing metalloenzyme which catalyzes the inter-conversion of HCO_3^- and CO_2, i.e. $CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$, a reaction which occurs spontaneously, but too slowly to meet the physiological needs of a cell (Jaiswal et al., 2005). CA is characterized by a diversity of structures and physiological functions, and by different locations in cells (Dudoladova et al., 2007).

The microalgae culture system plays an important role in the CO_2 mitigation process. Two distinctive culture systems being used for CO_2 sequestration with microalgae include open pond system and closed photobioreactor system. There is ongoing research regarding the use of these two systems for CO_2 sequestration. The successful mitigation of CO_2 using microalgae also requires that microalgae are sorted according to their growth rate, CO_2 fixation rate and tolerance to high levels of CO_2. In our previous study we isolated and identified a potential microalga *Scenedesmus obliquus* SA1 (KC733762) capable of growth and CO_2 sequestration at 13.8 ± 1.5% CO_2 and at elevated temperature of 40 °C (Basu et al., 2013). SA1 proved to be a potential candidate for CO_2 sequestration from flue gas in closed system. In the present study, we used the isolate SA1 for large scale cultivation studies in open vessel mimicking an outdoor pond under varying CO_2 concentrations ranging from ambient to 35%. Open culture system was our preferred choice since it is the most suitable system for handling the large quantity of flue gas generated from thermoelectric power plants. Also, open culture system proves to be simple (in terms of construction and operation), suitable for mass cultivation of algae and relatively economical to use for the CO_2 sequestration process. Much literature data is not available on microalgae cultivation in open system and mostly correspond to cultivation in closed photobioreactors. The carbonic anhydrase activity in the SA1 strain was monitored periodically for culture receiving ambient, 15% and 35% CO_2 to study the effects of the varying CO_2 concentrations on the enzyme activity. Much report is not available on the carbonic anhydrase activities in microalga *S. obliquus* under high levels of CO_2. Also, the effect of the CA inhibitors, ethoxyzolamide (EZ) and acetazolamide (AZ) on CA activity were investigated to more closely understand the cellular distribution of this enzyme in the SA1 strain. Since SA1 can tolerate CO_2 concentration up to 35%, it is interesting to study the role of enzyme carbonic anhydrase in this microalga with changes in the available CO_2. The corresponding changes in the growth kinetic parameters and the biochemical composition have also been monitored at varying CO_2 concentrations.

2. Methods

2.1. Organism and culture medium

S. obliquus SA1 (KC733762), a freshwater isolate of Guwahati, Assam (26°11′15″N 91°45′4″E) was maintained in our laboratory in BG-11 medium (ATCC Medium 616) containing 1.5 g L^{-1} sodium nitrate ($NaNO_3$), 0.04 g L^{-1} dipotassium hydrogen phosphate (K_2HPO_4), 0.075 g L^{-1} magnesium sulfate ($MgSO_4{\cdot}7H_2O$), 0.036 g L^{-1} calcium chloride ($CaCl_2{\cdot}2H_2O$), 0.006 g L^{-1} citric acid ($C_6H_8O_7$), 0.006 g L^{-1} ferric ammonium citrate ($C_6H_5 + 4yFexNyO_7$), 0.006 g L^{-1} EDTA ($C_{10}H_{14}N_2Na_2O_8{\cdot}2H_2O$), 0.02 g L^{-1} sodium carbonate (Na_2CO_3), 1 ml L^{-1} trace metal mix. The trace metal mix consisted of 2.86 g L^{-1} boric acid (H_3BO_3), 1.81 g L^{-1} manganese chloride ($MnCl_2{\cdot}4H_2O$), 0.222 g L^{-1} zinc sulfate ($ZnSO_4{\cdot}7H_2O$), 0.39 g L^{-1} sodium molybdate ($Na_2MoO_4{\cdot}2H_2O$), 0.079 g L^{-1} copper sulfate ($CuSO_4{\cdot}5H_2O$) and 0.0494 g L^{-1} cobalt nitrate ($Co(NO_3)_2{\cdot}6H_2O$).

2.2. Growth conditions

The SA1 strain was cultivated in 5 l culture vessel (0.17 m diameter, 0.27 m length) with a culture depth of 0.17 m. A constant inoculum size of 40 mg L^{-1} was used in each experiment. The culture was subjected to varying concentrations of CO_2 supply, ranging from 15% to 35% and the growth of the organism was monitored periodically. A control culture was maintained which received only the ambient CO_2 (0.03%) for growth. At 15% inlet CO_2 concentration, the culture depth was varied from 0.0425 m to 0.17 m (which implied a change in culture volume from 0.964 L to 3.8 L) to assess the height of the culture at which maximum CO_2 was sequestered. All the culture vessels were of open type mimicking the natural pond type algal cultivation used in practice. The cultures were incubated at 25 ± 1 °C and illuminated with daylight-type 20 W fluorescent tubes with a light intensity of 6000 lux maintained on the surface of the culture vessels using a Digital Light Meter (model LX-101A, Lutron, Taiwan). Fourteen hours of light and 10 h of dark period was maintained during each experiment. These growth conditions employed in our current study were optimized in our earlier study (Basu et al., 2013). The flow rates of CO_2 employed were 1.2, 2, and 2.8 l per hour (LPH) respectively for CO_2 supply of 15%, 25%, and 35%, respectively. CO_2 and N_2 from gas cylinders were mixed and the mixture gas was supplied to the culture at a flow rate of 8 LPH. The flow rates were maintained using pre-calibrated rotameters.