Bioresource Technology 221 (2016) 498–509

Contents lists available at ScienceDirect

Bioresource Technology

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

CO_2 capture using limestone for cultivation of the freshwater microalga *Chlorella sorokiniana* PAZ and the cyanobacterium *Arthrospira* sp. VSJ

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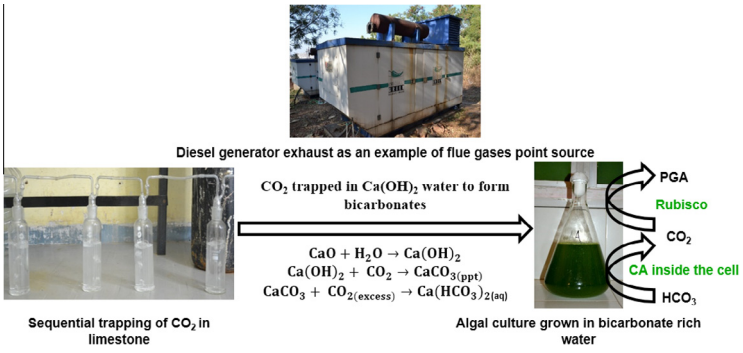
HIGHLIGHTS

- A practical approach for CO_2 mitigation without a huge capital investment.
- Strains isolated from saline soda lake of Lonar were explored.
- An insight into enhanced chemo-photosynthetic CO_2 sequestration.
- Exposure to UVA radiation enhanced carbon dioxide mitigation and growth yield of microalgae.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 23 July 2016
Received in revised form 14 September 2016
Accepted 17 September 2016
Available online 20 September 2016

Keywords:
CO_2 sequestration
Bicarbonate
UVA
Calcium oxide
Cyanobacteria
Microalgae

ABSTRACT

The present study reports a process wherein CO_2 is captured in the form of bicarbonates using calcium oxide and photosynthetically fixed into biomass. Microalgal cultures viz. *Chlorella sorokiniana* PAZ and *Arthrospira* sp. VSJ were grown in the medium containing bicarbonates. The rate of bicarbonate utilization by *C. sorokiniana* PAZ was higher when CO_2 trapped in the presence of 2.67 mM calcium oxide than in the presence of 10 mM sodium hydroxide and with direct addition of 10 mM sodium bicarbonate. For *Arthrospira* sp. VSJ the bicarbonate utilization was 92.37%, 88.34% and 59.23% for the medium containing CaO, NaOH and $NaHCO_3$, respectively. Illumination of photosynthetically active radiation (PAR) + ultraviolet A radiation (UVA) enhanced the yield of *C. sorokiniana* PAZ and *Arthrospira* sp. VSJ by 1.3 and 1.8 folds, respectively. FTIR analysis revealed elevation in the biosynthesis of specific metabolites in response to the UVA exposure.

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1. Introduction

The world is facing the problem of global warming due to the increased CO_2 emissions. The reason behind the increased CO_2 emissions is an excessive burning of fossil fuels (de Morais and Costa, 2007). Therefore, it is necessary to take action to minimize anthropogenic contributions to atmospheric levels of CO_2. According to the observation of Mauna Loa Observatory, Hawaii, the concentration of CO_2 increased dramatically from 370 ppm in 2002 to 400 ppm in 2014. One of the potential ways of CO_2 mitigation is capturing effluent gas under pressure from a point source and storing the material below 1 km of the Earth's surface. However, this process is technically challenging and is currently prohibitively

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http://dx.doi.org/10.1016/j.biortech.2016.09.079
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expensive at approximately US $ 67 per metric ton (MT) of CO_2 (Chi et al., 2011).

Mixed strategies for reducing net CO_2 emissions may prove the most promising approach to the daunting overall challenge given the diversity of socio-economic circumstances. One strategy to CO_2 mitigation is providing it to algal cultures for photosynthetic capture into biomass. In future economies, this may prove useful if the biomass contains high-value products whose production otherwise depends on energy intensive processes. Normally, 54,000 km^2 forest is required to assimilate 16.2 MT CO_2 per year while only 10,000 km^2 land is necessary for capturing 120 MT CO_2 per year via microalgae (Sudhakar et al., 2011). Typical algae farm is constructed on 100 ha land to consume around 31 kg of CO_2 m^{-2} $year^{-1}$ (Sudhakar et al., 2011). Availability of such big part of land near the source of CO_2 emission is almost impossible. Therefore, this option generally requires transport of captured carbon through pipelines up to the targeted site. The cost for compressing and drying CO_2 was US$8.48/MT CO_2 as well as US $3.30/MT CO_2 for pipeline transportation for 100 km distance (Kadam, 1997). The high cost of the transportation makes this approach unsuitable for extensive operation. Therefore, it is necessary to find an alternative solution. The use of CO_2 by converting it to a biologically available form such as bicarbonates and carbonates can be an effective solution. This form of biological CO_2 is stable and storable with a cost of transportation ranging from US $ 0.05–0.125 m^{-3} for 100 km distance (Zhou and Richard, 2005). This captured form of CO_2 may be subjected to long-term storage or used by photosynthetic organisms to minimize the level of CO_2 in the environment.

An additional obstacle is rooted in the physico-chemical nature of inorganic carbon transport in aqueous medium conversion of CO_2 to photosynthetic biomass may be inefficient due to the low dissolution rate of CO_2 into the water. Consequently, gassing of large-scale cultures is likely to result in the release of more CO_2 into the atmosphere than is fixed photosynthetically. The forward reaction rate constant for hydration of CO_2 in water is 6.2×10^{-3} s at 25 °C in the absence of a catalyst (Sullivan et al., 1993). Moreover, this conversion is critical for biological fixation because microalgae and cyanobacteria largely depend upon relatively efficient systems for the uptake of bicarbonate. Consequently, the low rate of hydration of CO_2 dissolved in water often limits the growth of cyanobacteria and microalgae. At the same time, the equilibrium proportions of the different and interconvertible molecular forms of dissolved inorganic carbon changes as the pH of the system changes. When the pH of the water is acidic, the major form of inorganic carbon that is present in the water is dissolved CO_2, while HCO_3^- (bicarbonate) is the dominant species at pH 8.0, and CO_3^{2-} (carbonate) is major species at pH 10. Accordingly, the limitations on capture by aquatic photosynthetic organisms could be mitigated under alkaline culture conditions. Use of NaOH and KOH for trapping CO_2 is an effective approach, but it results in unwanted effects on algal growth due to its high ionic strength and these substances are costly to use at a higher scale. Among all materials used for trapping CO_2, limestone (CaO) has an enormous potential due to its availability at low cost. The waste water of alkaline pulp and paper industry can be the readily available source of $Ca(OH)_2$ for trapping emissions (Pérez-López et al., 2008). This process of capturing CO_2 is similar to the natural process that occurs through marine carbonate weathering (Murray and Wilson, 1997).

The main advantage of using photosynthetic microbes for CO_2 sequestration is that the consumption and conversion of CO_2 is powered by solar energy. After the complete utilization of bicarbonates, formed biomass is converted into useful products like biodiesel (Lam and Lee, 2013), polysaccharides (Chen et al., 2012) and phycocyanin (Zeng et al., 2012). When an organism utilizes dissolved CO_2 in the form of bicarbonate, it produces OH^-, which makes the water alkaline and again regenerates the substrate for CO_2 capture without spending any extra energy. In this study, a new chemo-photosynthetic approach is used for CO_2 sequestration. The given process addresses the challenge to overcome the high cost of CO_2 capture and transportation by capturing CO_2 at the point source and converting it into utilizable form i.e. bicarbonates. Moreover this form of CO_2 can be stored during night when algae grow poorly. Cyanobacteria and microalgae utilize this form of CO_2 efficiently and help to mitigate the CO_2 from the environment. Further, the effect of UVA radiation on the utilization of bicarbonates by cyanobacteria and microalgae was assessed.

2. Material and methods

2.1. Isolation and culture maintenance

Strains were isolated from a unique aquatic ecosystem of the Lonar Lake, which is a soda lake situated in the Buldhana district of Maharashtra, India. Phytoplankton's-containing water sample were cultivated in BG-11 (Stanier et al., 1971) and Zarrouk's medium (Zarrouk, 1966) incubated at 28 ± 2 °C and 50 μmol m^{-2} s^{-1} light intensity with a 16 h light: 8 h dark photoperiod until visible growth was observed. Then, they were removed and serial dilutions were being made in respective medium. Samples were also streaked on Petri plate containing 1.5% agar. All flask and plates were incubated under same condition up to 10 days. After incubation isolated colonies were picked up and inoculated in the medium. The purity of cultures was checked by repeated sub culturing and daily observation under the microscope (Nikon Eclipse 200).

Chlorella sorokiniana PAZ was maintained in BG-11 medium while *Arthrospira* sp. VSJ was maintained in Zarrouk's medium. All the culture flasks were incubated at 28 ± 2 °C and 50 μmol m^{-2} s^{-1} light intensity with a 16 h light: 8 h dark photoperiod up to 10 days. Respective cells were harvested in the log phase of their growth and used as an inoculum for the further experiments.

2.2. Molecular identification

For molecular identification of isolates, genomic DNA was extracted and used for PCR amplification of the 16S rRNA gene from *Arthrospira* sp. VSJ using universal primers 8F 5′ AGAGTTTGATCCTGGCTCAG 3′ and 1492R 5′ CGGTTACCTTGTTACGACTT 3′ (Youssef et al., 2015). PCR amplification reaction was done in 50 μL of reaction mixture containing PCR buffer, 1X (Herculase II); dNTP mix, 0.25 mM; Herculase II fusion DNA polymerase, 1 μL; primer, 0.25 μM, DMSO, 0–8% final concentration and template DNA, 100 ng. Amplification was carried out according to following condition: 5 min at 95 °C, then 35 cycles including 30 s at 95 °C, 45 s at 50 °C and 1 min 30 s at 72 °C, and final step of 15 min at 72 °C. The PCR product was purified from agarose electrophoresis gel using QIAquick® gel extraction kit (QIAGEN®, USA) and the mixtures containing the PCR product and primers were subjected to sequencing (Oklahoma State University Molecular Biology Core Facility). Sequencing and molecular identification of *Chlorella sorokiniana* PAZ was done at a commercial lab Triyat Scientific, Nagpur, India using primers 18S FP 5′GCCTGTCTCAAAGATTAAGCC 3′ and 18S RP 5′ CACCTACGGAGACTTTGTTAC 3′. PCR reaction was done in 20 μL of reaction mixture containing PCR buffer, 1× (Kappa, SA); $MgCl_2$, 3 mM; dNTP mix, 0.25 mM; *Taq* DNA polymerase, 0.05 U, primer, 1 picomol and template DNA, 50 ng. Amplification was carried out according to following condition: 2 min at 94 °C, then 30 cycles including 50 s

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