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# Carbon dioxide sequestration in wastewater by a consortium of elevated carbon dioxide-tolerant microalgae

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ABSTRACT

The emission of the green house gas (GHG) carbon dioxide ($CO_2$) in the atmosphere at an increasingly high rate is the primary cause of global warming. A study was performed to isolate an elevated $CO_2$-tolerant microalgal consortium (CMAC) and then characterize growth-influencing environmental factors, $CO_2$ sequestration capacity and the potential applications of CMAC for elevated $CO_2$ sequestration. The CMAC was isolated from a wastewater treatment plant under a selection condition consisting of 50% $CO_2$ in air (v/v). The CMAC species were identified as *Chlorella* sp., *Scenedesmus* sp., *Sphaerocystis* sp. and *Spirulina* sp. Multiple variables including 20% $CO_2$, culture medium pH of 8–9, and an illumination intensity of 50–80 $\mu$mol $m^{-2}$ $s^{-1}$ were found to be optimal for high density growth of CMAC for uptake of elevated $CO_2$, although the CMAC were demonstrated to grow well in up to 50% $CO_2$. The CMAC showed high $CO_2$ sequestration (53–100%; 150–291 mg $g^{-1}$) with strong growth performance in wastewater. The lipid content of CMAC was high ($350 \pm 0.31$ mg $g^{-1}$), which gave a high biodiesel yielding capacity ($420 \pm 0.43$ mg $g^{-1}$). CMAC was also found to have high nutrient removal abilities ($PO_4$-P, up to 59% and $NH_4$-N, up to 39%). These characteristics all indicate that the isolated CMAC could be used as an efficient tool for biofuel generation from wastewater as well as bioremediation of pollutants. Thus by coupling the identified $CO_2$ sequestration potential of the CMAC with the wastewater tolerance characteristics, there is novel potential to integrate wastewater treatment with $CO_2$ sequestration and biomass utilization in order to mitigate the problems of increased GHG in response to global warming.


## 1. Introduction

Climate change and global warming are two serious challenges to the global environment. Global warming is induced due to the increasing emissions of the green house gases (GHGs) as a result of anthropogenic activities, causing severe changes to the global climate [1,2]. In addition to methane, nitrous oxide and other fluorinated gases, carbon dioxide ($CO_2$) is one of the major constituents of GHG emissions; for example, in 2012, 82% of the USA GHG emissions were comprised of $CO_2$ [3]. Increased combustion of fossil fuels such as coal and petroleum, and flue gases from power and steel plants (>7% of global $CO_2$ emissions) [4] are the major sources of energy-related $CO_2$ emissions in the world [5]. Therefore, high $CO_2$ emissions into the atmosphere are of great concern and have received increasing attention, yet global demand for energy and therefore fossil fuels are increasing [6].

Various actions are being taken to mitigate the GHG emissions from anthropogenic activities, especially for large point source emissions, through various mechanisms and protocols. $CO_2$ capture and storage (CCS) technologies are considered as an integral part of these measures for GHG emissions abatement [2,7]. One of the promising solutions is to convert $CO_2$ to organic matter through biological processes [8,9]. Photosynthesis has long been recognized as a means to sequester anthropogenic $CO_2$ and algae have been identified as fast growing photosynthetic agents whose carbon fixing rates can be much higher than those of terrestrial plants [10] and have the ability to efficiently uptake and utilize dissolved inorganic forms ($CO_2$ and $HCO_3^-$) of carbon in an aqueous environment [11]. However, rather than being used strictly for CCS, algae have potential for $CO_2$ capture and subsequent utilization. Carbon fixed by microalgae is incorporated into biomass, particularly carbohydrates and lipids, which in turn

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could be utilized for a variety of applications including for bioenergy, fine chemicals, or foods [12–14]. For example, high lipid yield from the microalgal biomass can be extracted and transesterified for biodiesel production [15,16]. Microalgal biomass could also be used for biofuel production by pyrolysis, direct combustion or thermal chemical liquefaction [17,18].

To realize the potential of microalgal-based $CO_2$ capture and utilization, selection of $CO_2$-tolerant microalgal species from different habitats and characterization of growth influencing environmental factors are required. Few studies have been carried out concerning the selection of $CO_2$-tolerant microalgae, determination of environmental factors such as light intensity, temperature, $CO_2$ concentration, and pH for the optimization of $CO_2$ sequestration, and subsequent utilization of the generated algal biomass for food or energy. Although most $CO_2$-sensitive microalgae are inhibited by $CO_2$ concentrations above 2–5%, some highly $CO_2$-tolerant microalgae strains have previously been isolated [19–23], such as a freshwater green alga, named *Chlorella* HA-l, which was isolated from a paddy field by Watanabe et al. [19]. In another study, *Chlorella* KR-1 showed maximum growth at 5 and 10% $CO_2$ but the growth rate decreased remarkably with increasing concentration of $CO_2$ higher than 10% [23]. It has also been reported that concentrations of $CO_2$ above 5% can enhance the growth of certain microalgae strains [24–26]. For example, Hanagata et al. [21] reported that *Scenedesmus* and *Chlorella* species had high growth rates when grown in up to 50% $CO_2$ and stable growth rates against high temperature, but provided no information about the stability of the strains toward pH changes. Furthermore, Kodama et al. [20] reported that a high $CO_2$-tolerant marine microalga, *Chlorococcum littorale* was suitable for high density culture.

Wastewater from facultative treatment ponds is one of the richest $CO_2$-containing water bodies in the aquatic environment because of high metabolic activities of heterotrophic microorganisms. Consequently, the opportunistic adaptation of $CO_2$-tolerant microorganisms in wastewater should be common phenomena due to frequent exposure to high concentrations of $CO_2$. Therefore, wastewater is a potential source for isolating the high/elevated $CO_2$-tolerant green microorganisms, including microalgae. However, potential $CO_2$-tolerant microalgae have not been explored from wastewater medium so far.

Wastewater has been considered as a potentially sustainable medium for the cultivation of microalgae for biofuel production [27]. Previous studies have isolated and characterized microalgae strains from wastewater effluent with regard to biofuel and wastewater remediation potential [28,29], but such strains have not been examined for $CO_2$ tolerance. Furthermore, no study has been attempted to isolate $CO_2$-tolerant microalgal consortium from $CO_2$-enriched wastewater and examine an application for $CO_2$ sequestration. The potential to therefore integrate wastewater treatment by microalgae with $CO_2$ sequestration and biomass utilization, such as for biofuel production, would be a novel extension of microalgae biotechnology [30], but this type of study has not yet been demonstrated, particularly for a consortium of algae. Therefore, the objectives of this present study were to (1) identify an elevated $CO_2$-tolerant microalgal consortium (CMAC) from a wastewater sample, (2) optimize the growth-influencing environmental factors, (3) determine the $CO_2$ sequestration potential and (4) evaluate the applied significance of CMAC by assessing the growth efficiency under $CO_2$-enriched wastewater conditions, including a determination of lipid yield and thus biodiesel capacity, and bioremediation efficiency. Thus this study successfully describes the identification and initial proof-of-concept characterization of a microalgal consortium that achieves efficient $CO_2$ sequestration and wastewater tolerance and remediation characteristics.

## 2. Experimental

### 2.1. Wastewater sample

Wastewater samples (1 L each) were collected from four different locations of a facultative pond at a domestic wastewater treatment plant in Kalyani, WB, India at 11.00 a.m. Equal proportions (100 mL) of the four samples were blended properly in a sterilized 1 L conical flask to obtain a homogenous wastewater (mean values: pH 6.75, $CO_2$ 12.20 mg $L^{-1}$, chemical oxygen demand 287 mg $L^{-1}$, $PO_4$-P 0.546 mg $L^{-1}$, $NH_4$-N 2.90 mg $L^{-1}$) sample and stored in an algae culture chamber of the laboratory with a 16 h/8 h light/dark period at 31 ± 1 °C for use as a stock sample for algal strain isolation.

### 2.2. Isolation and identification of elevated $CO_2$-tolerant microalgae

A ten milliliter aliquot of the stock wastewater sample was inoculated into 90 mL of CHU 10 medium (CHU Basal Solution No. 10, 10×, Hi-media, India) and allowed to grow within a transparent plastic chamber provided with 50% (v/v) $CO_2$ in air (normal pressure) at 31 ± 1 °C under a 16 h/8 h light/dark period and an illumination intensity of 80 μmol $m^{-2}$ $s^{-1}$ for 7 days in an algae laboratory culture chamber. One milliliter of this sample was then used to determine the species composition of $CO_2$-tolerant microalgae present in the culture by morphological identification using a light microscope (Olympus, Olympus Pvt. Ltd., India). The identified microalgal sample was then designated as the elevated $CO_2$-tolerant microalgal consortium (CMAC), which was maintained in CHU 10 broth media in the algae culture chamber for subsequent studies.

### 2.3. Measurement of CMAC growth

The growth of CMAC was examined by measuring the optical density (OD) at 682 nm using a UV/Visible spectrophotometer (Simadzu Corporation, Tokyo, Japan) to evaluate the biomass concentration of cultured media.

### 2.4. Relationship of optical density (OD) with algal density and biomass

The relationship of optical density (OD) at 682 nm with algal density and biomass was established following the method of Chiu et al. [31] with slight modification. The CMAC was freshly cultured in CHU 10 media and diluted to different concentrations using the same media to give an absorbance in the linear range of 0.1–1.0, because the biomass will be underestimated when the optical density is out of the linear range. An aliquot of each diluted sample with a specific $OD_{682\ nm}$ value was used for direct cell number counting under the microscope using a Sedgewick Rafter cell counting slide. The CMAC dry biomass weight (DBW) was determined for each diluted sample by centrifuging at 8000 × *g* (REMI, Elektro Technik Ltd., India) for 10 min then washing twice with double distilled water (DDW). The DBW of the harvested CMAC pellet was measured after drying at 105 °C for 16 h [32].

The relationship of OD with counted algal density (cell number $mL^{-1}$) and DBW (g $L^{-1}$) was calculated by linear regression equation using EXCEL. There was a linear relationship between OD and cell density ($R^2$ = 0.9884; $p < 0.05$) and between OD and DBW ($R^2$ = 0.9809; $p < 0.05$) (Fig. 1). The OD values of CMAC were converted to DBW (g $L^{-1}$) using these regression equations in each experiment. Cell density and biomass were measured more easily by OD than by direct counting of cells or by cell dry weight [33].