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Metabolic acclimation mechanism in microalgae developed for CO_2 capture from industrial flue gas

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ABSTRACT

Microalgae are the potential choice in diverting carbon emission from industrial plants. Owing to the high CO_2 concentrations, industrial flue gas can constrain the growth of most microalgae. A continuous transfer procedure was developed to select a tolerant microalgae species to feed on CO_2-rich industrial flue gas. The ability to capture CO_2 by the developed microalgae species is verified in a closed gas bag system and the bubble column reactors. A total of 432 metabolic molecules were collected from the microalgae culture subject to the ambient (0.04% CO_2) and the CO_2-elevated (15%) treatments, 37 of which showed significantly different concentrations. These 37 metabolites were found to enhance the cellular physiology mechanisms of the microalgae to thrive in the high concentrations of CO_2. The productivity of microalgae was shown to be improved for industrial applications.

1. Introduction

CO_2 capture with microalgae may be an attractive approach to reduce carbon emission from industrial plants. As a major greenhouse gas, CO_2 in atmosphere has risen greatly to 0.04% from 0.03% in just a century mainly due to industrial releases. The anthropogenic CO_2 emissions may contribute to climate change and ocean acidification [1]. In an industrial plant, flue gas is commonly denitrified and desulfurized. The main component of an industrial flue gas is CO_2 [2]. Recently, the use of microalgae to sequester CO_2 has been developed due to their higher growth and turnover rates as compared to those of the terrestrial plants [3–9]. Microalgae carbon capture methodology could save energy over a chemical absorption process [3] and is sustainable because microalgal biomass can serve as an alternative to fossil fuel [3–9].

Microalgal biomass is also rich in lipids, starches, proteins, beta-carotene and other nutrients, and thus is suitable as animal feed and food products [10]. Microalgae culture system integrated with CO_2 capture process can reduce the microalgal biomass production cost [11]. The flue gas of an industrial plant can provide a rich carbon source for microalgae. However, the CO_2 concentration of industrial flue gas is so high that it constrains the growth of most microalgae [3–9]. The flue gas can be captured and purified to provide the microalgae culture at desired concentrations. However, the capture and purification steps place an additional cost on flue gas delivery and distribution [12]. The screening for tolerant microalgae species to directly treat flue gas is therefore a better option to the microalgae CO_2 capture methodology [13].

Microalgae species can develop adaptive mechanisms to mitigate the stress caused by a high CO_2 environment [11,13–19]. The reported responses to the high CO_2 concentrations included regulations of CO_2 concentrating mechanisms (CCMs), inhibitions of photorespiration, rearrangements and transitions of membrane proteins, and triggering gametogenesis. In *Chlamydomonas reinhardtii*, the CCMs would be inhibited through the bicarbonate transporters and carbonic anhydrases (CAs) in the high concentration of CO_2. Giordano et al. suggested the high CO_2 concentration might affect nitrogen assimilation through the photo-respiratory nitrogen cycle, to satisfy the intracellular C:N ratio [17]. Similarly, the high CO_2 concentration reduced intracellular nitrogen in the chloroplast by suppressing the activities of nitrogen transporters [14]. Matsuda et al. described that the intracellular nitrogen levels, for instance, ammonia ions, could induce gametogenesis, a sexual reproduction state to form mature haploid gametes [20]. Based on those response mechanisms, transcription, translation and metabolite regulatory approaches have been developed for distinct proteins, expressed genes and the metabolic networks under varied CO_2 inputs [13–17,21–27].

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However, there is little understanding on the responses of the tolerant microalgae. Most reported molecular acclimation studies were based on *C. reinhardtii* whose photosynthesis activity was low in the high CO_2 concentration, and little information can be obtained about the mechanisms for a tolerant species [13–17,21–27]. To our knowledge, a comprehensive analysis of the molecular acclimation mechanisms to the high concentration of CO_2 for a tolerant microalgae species is not existent.

Improvements in microalgae-based CO_2 capture technology are making it possible to screen microalgae that can withstand high concentration of CO_2 [3–9]. For instance, Liu et al. described a high throughout gas bag system to rapidly identify high CO_2 affinity microalgae [28]. An adaptive procedure, like the continuous transfers, integrated with the gas bag system, has the potential to develop a tolerant species. Under the stress of the high concentration of CO_2, the microalgae transferred the genetic information for the acclimation to the offspring. The genetic information would be highlighted and maintained during the continuous transfers [29].

In this work, a closed gas bag system and the bubble column reactors were employed to develop microalgal candidate species. The molecular mechanisms that allowed microalgae to tolerate industrial flue gas were studied with metabolic analysis. Metabolic analysis provided a platform to profile all the detectable molecules and to analyze cellular physiology. Through this study, we examined how CO_2 enrichment could improve the productivity of microalgae for industrial applications.

2. Materials and methods

2.1. Species and culture conditions

2.1.1. Species development

Eight microalgae species were selected as candidates for developing a flue gas-tolerant species. The microalgae species were collected from the South China Sea and the lakes around Guangzhou, China. All the species were preserved in the biochemistry greenroom of Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences. The eight microalgae species (*GIEC-28 Scenedesmus sp.*, *GIEC-171 Scenedesmus sp.*, *GIEC-173 Scenedesmus sp.*, *WZKMT Scenedesmus sp.*, *GIEC-38 Chlorococcum sp.*, *GIEC-44 Chlorella sp.*, *and GIEC-22 Desmodesmus sp.*, *and GIEC-101 Desmodesmus sp.*) were selected from a large pool of candidates due to their high growth. All the selected microalgae species were incubated in 96-well plates in flexible Tedlar® polyvinyl fluoride (PVF) gas bags (Dalian Delin Gas Packing Co., Dalian, China) filled with simulated flue gas (15% CO_2, 85% N_2, NO_x 100 ppm and SO_2 200 ppm) [3–9]. Each species was cultured in one column (8 wells) of the 96-well plates. The transfers of the selected 8 species were conducted continuously until at least one of the species adapted to the flue gas environment. For the identification and phylogeny, the 18 s rDNA sequence of the tolerant species was purified according to the DNA purification kit procedure (Sangon industry, Shanghai, China). The genetic sequencing was conducted with the help of Sangon industry.

Microalgae cell development was monitored by checking the optical density at 680 nm (OD_{680}). When the highest OD_{680} of the 96 wells-plate reached 2, the wells with the highest OD_{680} of each species were transferred to a new plate (1% inoculum). Transfers continued until one of the wells reached 2.0 OD_{680} within 3 days, which was considered our benchmark for the microalgal acclimation to the environment.

The process for developing flue gas tolerant species is illustrated in the Supporting material Fig. 1. Briefly, gas bags were connected with pressure meter, gas cylinder, gas pump and GC. The growth of the developed species was firstly tested in the 2.0 L PVF gas bags, 100 rpm (rounds per minute) (Supporting material Fig. 3) and secondly in the bubble column reactors (Supporting material Fig. 5), with 150 μmol m^{-2} s^{-1} photosynthetically active radiation and maintained at 25 ± 1 °C. The medium used to grow cultures was either BG11, or one with a modified composition with recycling water as described to examine the effects of inorganic ions [30]. For the water recycling study, the cells were harvested by centrifuging at 2000 rpm for 1 min. Samples for analysis were collected and analyzed every 24 h.

Each set of experiments was performed at least in triplicate. The average values ($n = 7$, $n = 8$) and corresponding standard errors (SDs) were shown for the metabolic analysis.

2.1.2. The performance of developed species in 2 L gas bags

The carbon capture abilities of the developed microalgae in materials and methods 2.1.1 were firstly studied in 2 L PVF gas bags with 150 μmol m^{-2} s^{-1} photosynthetically active radiation maintained at 25 ± 1 °C. Gas-liquid ratios were set at 20, 10, 9, 5.5 and 4, accordingly. The tolerant species in logarithmic growth phase was inoculated into gas bags with an OD_{680} of 0.1. The gas bag treatments included 100% recycled water, 75% recycled water, 50% recycled water, 100% recycled water supplemented with salts, and 100% recycled water supplemented with NO_3^-. The growth of the tolerant species was studied in six batches, and the gas bags were refilled with simulated flue gas having a CO_2 concentration < 1%.

2.1.3. The performance of developed species in the bubble column reactors

The growth of the developed microalgae was studied in the 250 mL bubble column reactors (lab made, Supporting material Fig. 5). The gas was injected to the bottom of the bubble column reactors at 50 mL/min. The biomass accumulation rates of the microalgae were studied in the ambient CO_2 (0.04% CO_2) and in the elevated CO_2 (15% CO_2) environments, with 50%, 75%, 100% recycled water, and fresh water, with or without phosphate buffers for pH control experiments. The pH control experiments were to examine pH acclimation mechanism of the tolerant species.

2.1.4. Metabolic analysis for the cellular physiology adjustment to high CO_2

Metabolic analysis based on gas chromatography time-of-flight mass spectrometry was used to identify the distinct metabolites, and those metabolites were involved in the cellular adjustment at 15% CO_2. The tolerant microalgae were cultured with the bubble column reactors as described in materials and methods 2.1.3, which were divided into two groups. Group A was bubbled with ambient CO_2 (0.04% CO_2, $n = 8$) and group F was bubbled with elevated CO_2 (15% CO_2, $n = 7$), and the total metabolites were assessed to determine the metabolite profile of the microalgae treated with different concentrations of CO_2. 100 mL samples of the developed microalgae in logarithmic growth phase were collected with 1.2 μm Whatman GF/C filter paper, and immersed in the liquid N_2 immediately to stop their metabolic activities. Samples were stored at − 80 °C until GC-Mass analysis [31].

For the metabolic analysis, the principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA) and orthogonal partial least-squares discriminant analysis (OPLS-DA) were performed with SIMCA software package (Umetrics, Umea, Sweden). Detailed information for metabolic analysis is included in supporting materials.

2.2. Monitoring and analytical methods

2.2.1. Growth measurement

The OD_{680} of culture in 96-well plates were measured by spectrophotometer (Enviro-USA American Manufacturer, LLC, USA). Biomass of each culture sample was measured after filtering through a pre-weighed 1.2 μm pore size Whatman GF/C filter paper (Whatman plc company, UK), dried at 80 °C in an oven for 12 h, cooled, and then weighed. The differences between the final weight and weight before filtration were the dry weight of the samples.

Cell numbers were counted using a haemocytometer (Qiujing, Shanghai, China), after appropriate dilutions. Salt analysis was conducted by ion chromatography (761 Compact IC, Metrohm, Switzerland), according to the manufacturer's instructions. In the