A community-based approach for enriching microalgae that grow under high concentration of CO_2

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Abstract

Certain species of microalgae are capable of growing under high concentration of carbon dioxide (CO_2), and these microalgae have potential to be used for sequestering CO_2 released as industrial pollutants using phototrophic carbon fixation system. While some CO_2-tolerant algal species have been identified from existing algal culture collections, this study explored a community-based approach to enrich and isolate CO_2-tolerant microalgae. Meanwhile, we monitored the change of bacterial and microalgal communities during the CO_2 enrichment period based on the 16S and 18S rRNA gene sequences. Four different treatments were set up in the laboratory to test the effect of nutrient and CO_2 on the natural planktonic community. At the end of enrichment experiment (17 days), green algae (Chlorophyta), especially *Scenedesmus* species, dominated the microalgal community when the water samples were enriched with high CO_2 (10%) and nutrient. The dominance of species in the CO_2-enriched samples was also evident in the clonal isolation of microalgae at the end of experiment. This study clearly demonstrates that the amendment of high level of CO_2 to a natural phytoplankton community is an efficient way to enrich and isolate CO_2-tolerant microalgae. The community-based approach described here poses several advantages over traditional culture-based screening method for isolating microalgae with specific characteristics.

Keywords: CO_2 tolerant microalgae, 18S rRNA gene, 16S rRNA gene, microbial community, *Scenedesmus*

1. Introduction

Carbon dioxide (CO_2) accounts for nearly 80% of the total greenhouse gas (GHG) emissions worldwide, and most members of the United Nations have committed themselves to significantly reduce their GHG emissions.[1] Exhaust gases from power

plants attribute to ca. 40% of the U.S. annual CO_2 emission in 2010, and the concentration of CO_2 in power plant exhausts varies from 10-15% depending on the source of fuels.[2] CO_2 is a food source of phototrophic algae. Phototrophic carbon fixation through microalgae cultivation has been proposed as a biological way to mitigate CO_2 pollution, especially for the sequestration of CO_2 from industrial exhaust gases such as flue gases.[3-7] Therefore, the ideal microalgae candidates for sequestering CO_2 in flue gases should be able to grow under high CO_2 concentration (e.g. $\geq$ 10%).

Photosynthesis performed by microorganism (including cyanobacteria and eukaryotic microalgae) is an ancient process.[8, 9] Photosynthetic eukaryotes inhabited coastal waters ca. 1.4–1.9 billion years (Gyr) ago.[10-12] Green algae such as Chlorophytes (for example, *Scenedesmus* and *Chlamydomonas*), are thought to be the dominant phytoplankton in the Mesoproterozoic ocean (0.9–1.6 Gyr ago) and have become much less abundant in the Paleozoic period (0.25-0.54 Gyr ago).[13] Kasting [14] suggested that the concentration of atmospheric CO_2 decreased from 10% (v/v) at 2.5 Gyr ago to 1% (v/v) at 1 Gyr ago, and continue to decrease. The geological records on algal species and atmospheric CO_2 level suggest that some Chlorophytes species thrived in the high CO_2 environment during the Mesoproterozoic period, and the concentration of atmospheric CO_2 has decreased gradually by CO_2 fixation of Chlorophytes over the geological time. While the atmospheric CO_2 decreased, other types of microalgae that can use CO_2 more efficiently emerged.[15] It is possible some nowadays Chlorophytes species still keep the ability or strategy to grow under high CO_2 concentration. The key mechanisms governing the microalgal tolerance to high CO_2 concentration could involve the photosynthetic apparatus state transitions, rapid shutdown of CO_2-concentrating mechanisms, or adjustment of fatty acid composition of membranes.[16-19]

Some microalgal isolates are able to grow under high CO_2 concentration.[20-24] One green algal strain, *Chlorella* sp, was able to grow under 100% CO_2 and flue gas, although the maximum growth rate occurred at 10% CO_2 concentration.[22] Another *Chlorella* strain was found grow faster in 10% CO_2.[25] Hanagata et al. reported that the

green alga *Scenedesmus* sp. could grow under 80% CO_2 condition, but the maximum cell mass was observed in 10-20% CO_2 concentrations.[19] A *Scenedesmus* strain was able to grow in a large photobioreactor (500 L) bubbled with flue gas which contains 10-12% CO_2.[23] *Desmodesmus* spp. could grow under 100% unfiltered flue gas from coal combustion.[24] Other non-green algae can also grow in the high CO_2 environment. For example, red algae like some *Cyanidium* species can grow in pure CO_2.[26, 27] Growth of mixotrophic algae like *Euglena gracilis* was enhanced under elevated concentrations of CO_2 (5-45%).[28] Therefore, it is evident that microalgae in different algal lineages are able to thrive in high CO_2 condition.

The earlier studies mainly relied on available algal cultures to test their capability to grow in high CO_2 level. A high-throughput screen method has been used to screen for algal cultures that can grow in different concentrations of CO_2.[23] While the culture-based method has been widely used to select desirable algal strains for different purposes, the limitation of this method is multifold: 1) Only limited number of algal cultures can be tested; 2) Maintaining, growing, and monitoring of many algal cultures are very time consuming; 3) Selected algal strains may not be ideal for the local applications (i.e. use of local water); 4) Microalgae grow differently on the microplates compared to the large flasks., A recent study shows that community-based method can be used to enrich and isolate CO_2-tolerant microalgae,[24] suggesting that exposing natural phytoplankton communities to the desirable test condition enables us to quickly select target algal strains from a whole community of microalgae in a particular aquatic ecosystem.

Microalgae are very diverse in the natural environment. It has been estimated that more than 1 million algal species exist in nature, names for 44,000 of which have probably been published, and 33,248 names have been processed by AlgaeBase (http://www.algaebase.org).[29, 30] Many studies have contributed to better understanding the respective impacts of abiotic or biotic factors to natural environment community shifting. With the use of molecular sequencing technology, the change of prokaryotic and eukaryotic communities can be monitored

simultaneously.[31-34] Co-monitoring the variation of different microbial communities in response to a specific change or event (i.e. algal bloom) has become a powerful tool to study the interaction between organisms in the natural environment.[35] Ocean acidification showed significant effects on phytoplankton composition during the post-blooming period with negligible dissolved nutrients.[36] It would be interesting to know how microbial communities in the natural aquatic bodies respond to high CO_2 exposure or nutrient enrichment. By exposing the natural microalgal communities to high CO_2 condition, we also want to know which kind of microalgal populations will dominate the community at the end of the experiment.

In this study, we exposed a water sample collected from the Back River, Baltimore to 10% CO_2. The 10% CO_2 concentration was chosen as we intended to isolate algal strains that are suitable for sequestering CO_2 in flue gas of power plant. The goal here is to understand how bacterial and microalgal communities change when the natural water is exposed to a high CO_2 concentration. The bacterial and algal community will be analyzed by sequencing the partial 16S and 18S rRNA genes, respectively. Also, we identified and counted the cell density of major microalgae taxa. Ultimately, we isolated CO_2-tolerant algal strains at the end of CO_2 enrichment experiment.

2. Materials and methods

2.1 Experimental design

The environmental water sample was taken from Back River, Baltimore, Maryland (Latitude: 39.300 ^{o}N, Longitude: 76.489 ^{o}W) on December 12, 2016, which located close to the Baltimore Back River wastewater treatment plant. Four treatments were set up to expose the environmental sample to 1) 10% CO_2 with high nutrient (BG11 medium); 2) 10% CO_2 without high nutrient; 3) air with high nutrient and 4) air only. Measurement of the CO_2 concentration was carried out using a GasLab® Sensor Configuration and Data Logging Software (https://www.co2meter.com/pages/downloads). All treatments were duplicated. A total of 8 bottles (2 L)

were exposed to same constant light at 21-23 °C under 30-100 μmol photons m^{-2} s^{-1} illumination from 54 watt natural white fluorescent lights. The light intensity was adjusted based on the cell density. The light intensity was 30 μmol photons m^{-2} s^{-1} at the beginning of this experiment when the cell density was lower. The light intensity was 100 μmol photons m^{-2} s^{-1} at the end of this experiment when the cell density was high.

2.1. Sample collection

Cell density (optical density at 600 nm) and pH were measured on the daily basis. A small subsample (10 ml) was taken from all treatments on day 0, 3, 7, 11, 17. All these samples were fixed with 1% glutaraldehyde (final concentration) for microalgae and bacteria counting, and stored in the dark at 4 °C. Microalgae cells were identified based on morphology according to literature.[37] Microalgae cells were then counted using a Neubauer Hemacytometer which have a center square, subdivided into 25 medium squares, 0.2 × 0.2 mm each in dimension. Counts from the center square are equivalent to counts per 0.1 μL of the sample. The center square was checked at a 100x magnification for even distribution of algae cells after which algae groups (Chlorophyte, diatom, Euglenophyte and dinoflagellate) in all 25 medium squares were counted. Only the top and right edges of each medium square was counted to avoid double counting cells. For day 17 of the treatments, nutrients and high CO_2 or air, samples were diluted to 10% before counting. Identification and counts were done at 100 × and 40 × magnification respectively using a microscope (Axiplan microscope, Zeiss).

Bacterial samples were stained with SYBR Gold and quantified by Chen, et al. [38]. Briefly, samples were stained with 2×SYBR Gold solution (final concentration) for 15 min in the dark after filtered through a 0.2 μm pore-size Anodisc membrane filter (Whatman Inc., Clifton, N.J.). All samples were enumerated under a fluorescent microscope (Axioplan, Zeiss), and visualized with blue excitation light.

2.2 Isolation and identification of microalgal strains

Pure culture was isolated from the samples enriched with nutrient and CO_2 by

streak plating on petri dishes containing BG11 medium and 1% agar on day 17. After isolation and cultivation of pure cultures, DNA was extracted by DNeasy® PowerWater Kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's instruction. The purity and concentration of DNA in samples were assessed using NanoDrop ND-1000 (Thermo-Fisher Scientific, Wilmington, DE). Genomic DNA of selected strains was extracted and 18S rRNA gene was amplified using the universal primers for eukaryotes.[39] The PCR product amplified from DNA of each isolate was confirmed as a single band by agarose gel electrophoresis. The sequences of PCR product were aligned to 18S rDNA sequences from the National Centre of Biotechnology Information Database using BLAST searches.

2.3 16S and 18S rRNA gene sample preparation and MiSeq Illumina sequencing

DNA samples for 18S rRNA gene and 16S rRNA gene sequencing were harvested on day 0, day 3 (only for 18S rRNA gene), day 11 (only for 18S rRNA gene) and day 17 from each group. Samples were immediately vacuum-filtered (adjusted based on the cell density) through a 0.7 μm pore-size glass microfiber filters (Whatman, GE Healthcare Life Sciences, Pittsburgh, PA) and 0.2 μm pore-size nylon membrane filters (Whatman), and then put together for sequencing. Samples were stored at -20 °C until further processing (< 1 month). DNA was extracted using DNeasy® PowerWater Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instruction. The purity and concentration of DNA in samples were assessed using NanoDrop ND-1000 (Thermo-Fisher Scientific, Wilmington, DE). The DNA was stored at -20 °C until further analyses. Bacterial amplicons were produced by targeting the 16S V3-V4 fragment, the 16S ribosomal gene primers used were: Forward Primer = 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGC CTACGGGNGGCWGCAG and Reverse Primer = 5'GTCTCGTGGGCTCGGAGATGTGTATAAG AGACAGGACTACHVGGGTATCTAA TCC).[40] The V9 region of the 18S rRNA gene was amplified with P73F- P47R primers (primer pair P73F = 5′AATCAGTTATAGTTTATTTGRTG GTACC3′ and P47R =

5′TCTCAGGCTCCCTCTCCGGA3′).[39] A total reaction volume of 25 µL for each PCR reaction mix contained 5 µL GoTaq® Flexi Reaction Buffers (Promega, USA), 0.0375 µM $MgCl_2$, 12.5 µM dNTPs, 0.5 U Taq, 13.9 µL H_2O and 2 µL template DNA and a total of 2×10^{-5} µM of both forward and reverse primer. For 18S rRNA gene amplification, PCR conditions consisted of an initial denaturation step of 94 °C for 1 min; followed by 37 cycles of 92 °C for 50 s, 57 °C for 50 s, 72 °C for 50 s with a final elongation step at 72 °C for 10 min. Negative control was no template controls for PCR amplification. PCR amplicons were verified for size by electrophoresis in 1% agarose gels. All samples were sequenced on a MiSeq (Illumina, San Diego, CA) at the BioAnalytical Laboratory of the Institute of Marine and Environmental Technology (IMET) of University of Maryland using a 2 × 300 MiSeq Reagent Kit v3. For eukaryotes (18S rRNA gene) there were 22.07 million raw sequence reads with 3.45 million passing quality control filters. The bacterial (16S rRNA gene) dataset consisted of 1.53 million raw sequence reads with 0.63 million passed by filters. A total of 511,527 reads of 16S rRNA gene, and 3,441,993 reads of 18S rRNA gene representing 104,173 and 343,495 operational taxonomic units (OTUs) after filtering, respectively, were available for diversity analyses. These sequences data are available on the NCBI (SRP128606).

2.4 Operational taxonomic unit (OTU) assignments

Bacterial and eukaryotic reads were assembled on the CLC Bio Genomic Workbench software (version 8.5.1, CLC Bio, Cambridge, MA) using default settings, and annotated using QIIME version 1.9.0 (http://qiime.org/home_static/dataFiles.html). The Greengenes 13-8 reference database was used for 16S data, and the SILVA 128 was used as the reference database for 18S data. Both databases are available for download at (http://qiime.org/home_static/dataFile s.html). Quality filtered sequences were clustered into OTUs according to sequence similarity using a 97% similarity threshold against the respective reference databases. The remaining sequences were clustered into de novo OTUs with UCLUST [41] within QIIME. Taxonomy was assigned to these

sequences using the RDP classifier in QIIME.

2.5 Data analyses

Phylogenetic trees were constructed by placing representative sequences for each de novo OTU into the respective reference tree using the QIIME software. The weighted and unweighted UniFrac metric was used to assess shifts in community composition, which were visualized with principal coordinate plots. We calculated the Simpson diversity index, which takes into account richness and evenness, using QIIME software to assess changes in alpha diversity. Statistical analyses were performed and differences between treatments were compared using a one-way Analysis of Variance (ANOVA), considered statistically significant at $P \leq 0.05$). The Turkey's post hoc test was used to test for hypothesized differences which were met.

3. Results and Discussion

3.1 Physiological responses of high CO_2 and nutrients

When the water samples were enriched with nutrients, they turned into a dark green color gradually from day 6 to day 17, while the samples which did not receive nutrients remained relatively clear throughout the 17 days' incubation period. The growth of phytoplankton and bacteria in the nutrient-enriched samples was evident based on the OD600 reading, phytoplankton and bacterioplankton counts (Fig. 1). On day 17, phytoplankton and bacteria cell densities in the nutrient-enriched samples were significantly higher than those in the samples without nutrient addition (Fig. 1 B and C, $P < 0.05$). Phytoplankton cell density reached ca. 1.3E+08 cells/mL in the samples enriched with nutrients and CO_2, and 1.0E+08 cells/mL in the samples enriched with nutrients and air on day 17 (Fig. 1B). The total bacterial counts reached about 6.5E+08 cells/mL in nutrients and CO_2 group on day 17, and account for 9.0E+08 cells/mL in the nutrients and air group (Fig. 1C). Among the nutrient-enriched samples, the samples charged with CO_2 had higher OD600 reading and phytoplankton counts, but less bacterial counts compared to the samples without CO_2 charge (Fig. 1). Our result shows that water samples enriched with nutrients

triggered the fast growth of phytoplankton. In contrast, little growth of bacterioplankton and phytoplankton was seen if samples that received no nutrients. The supplement of CO_2 further enhanced the growth of microalgae in the samples enriched with nutrients. This is consistent with a recent study that showed that productivity of microalgal consortia was increased when exposed to supplementation with 10% pure CO_2.[24] Under eutrophic conditions, phytoplankton productivity may double as a result of doubling of the atmospheric CO_2 concentration.[42] Furthermore, in eutrophic freshwater systems doubling of atmospheric CO_2 may result in an increase of the productivity by more than 50%. Our results indicated that high concentration of nutrients (1.5 g/L nitrate, and 0.04 g/L phosphate) stimulates the rapid growth of phytoplankton, and addition of 10% CO_2 provided further benefit to the growth of phytoplankton.

The pH value was nearly 8 in the air treatment and about 6.5 in the CO_2 treatment after day 1 (Fig. S1). The pH value in the samples enriched with nutrients and CO_2 increased from 6.0 at day 2 to 7.4 at day 17, while the pH in the nutrients and air treatment increased from 7.9 to 9.5 during the same time frame (Fig. S1). When algal cells became dense in the later stage of our experiment, pH increased greatly in the samples enriched with nutrients. The increase in pH towards the end of experiment was mainly due to the decrease in free CO_2 concentration in the medium.[42] Significant basification during microalgae bloom development directly influences phytoplankton species growth rates, succession,[43-45] and the selection of species most capable of growth as pH increases.[44-47]

3.2 Changes of eukaryotic communities

One of the important aims of this study was to assess the impact of 10% CO_2 on eukaryotic and bacterial communities by comparing the 18S rRNA gene sequences and cell density obtained over a 17 days' experiment. Generally, the changes in eukaryotic communities based on the 18S rRNA gene were pronounced during the experiment (Fig. 2). At day 0 (control), the dominant taxa in the Back River water samples included diatoms (44.0 %), Chlorophyta (1.1 %), Dinoflagellata (8.2 %),

Chrysophyceae (1.3 %), Alveolata (23.6 %), Cercozoa Thecofilosea (10.1 %), Ciliophora Intramacronucleata (1.1 %), and unassigned eukaryotes (1.8 %). Together, these taxa comprised 91.2 % of the total 18S rRNA gene sequences in the original Back River water samples (Fig. 2-3).

Based on the relative contribution of 18S rRNA gene sequences, green algae (Chlorophyta) increased greatly in the samples enriched with nutrients (both air and CO_2), but remained relatively low in the samples with air or CO_2 only (Fig. 2). It appears that the supplement of nutrients and 10% CO_2 enriched for more green microalgae compared to the samples with only nutrients (Fig. 2). In the samples with nutrients and CO_2, green algae (Chlorophyta) made up about 35% of the eukaryotic community (Fig. 2). Throughout the incubation experiment (17 days), the relative abundance of Chlorophyceae increased to 30 % in the samples with nutrient and CO_2, nearly 40-fold increase compared to the control sample (Fig. 3). It is clear that addition of nutrients enriched for green algae Chlorophyceae, particularly in the samples with high CO_2 (Fig. 3). Meanwhile, much more green algae were identified in the samples with nutrient and CO_2 (Fig. 2-3).

The relative abundance of diatoms in the samples with nutrients increased on day 3, but declined rapidly after day 3 (Fig. 2). However, green algae (Chlorophyta) were able to thrive in the samples with nutrient, likely due to the nutrient enrichment and less grazing pressure (Fig. 2). It has been known that grazers prefer to prey diatoms over Chlorophyta because of the high content of polyunsaturated fatty acids (PUFAs) in diatoms. PUFAs derived from microalgae are essential for development of various larvae. Due to the deficiency in PUFA, Chlorophytes generally have low nutritional value and are the less preferred diet for animal larvae.[48] Diatoms seem to be less abundant in the samples with nutrient and CO_2, possibly due to the grazing pressure of zooplankton. Our results suggest that nutrient level and grazing pressure together seem to affect the shift of diatom and Chlorophyta communities during the enrichment experiment.

Corresponding to the relative abundance of microalgae, we counted the cell

density of main microalgae taxa in our experiment (Fig. 4). Chlorophyta and diatom were the dominant taxa in four groups (Fig. 4). Also, the cell density and percentage of Chlorophyta were higher in nutrient groups, especially the samples enriched with nutrient and CO_2 (Fig. 4 and Fig. S2). The cell density of Chlorophyta increased greatly in nutrient groups since day 3, especially in the nutrients and CO_2 group (Fig. 4). On day 17, the cell density of Chlorophyta in nutrients and CO_2 group increased to 1.3×10^8 cells/mL, accounting for more than 99% of total microalgae cell density (Fig. 4 and Fig. S2), and more of them are belong to Chlorophyceae. This abundance is consistent with the relative abundance result that high CO_2 condition could select for Chlorophyta, especially the Chlorophyceae.

Microzooplankton in all 4 treatments decreased from about 40% of total abundance on day 0 to lower than 9 % on day 3 (Fig. 2). It is likely that microzooplankton did not adapt to the laboratory culture system (i.e. light, temperature, etc) at the beginning of experiment. For example, the temperature of original water sample was ca. 7 ^oC, but our laboratory culture system was about 22 ^oC. The abundance of microzooplankton has decreased greatly to lower than 6 % in the samples charged with high CO_2 after day 0 (Fig. 2), in which pH remained between 5.8 and 6.8 (Fig. S1). Low pH can have negative impacts on calcification by planktonic foraminifera,[49-52] and could also lead to more variable proton concentrations in the cell surface boundary layers of marine microzooplankton,[53] which could affect numerous cellular processes that rely on proton pumps.[54] The relative abundance of microzooplankton, major grazers of phytoplankton were maintained in low abundance in the samples charged with high CO_2 since day 3 (Fig. 2 and 3), likely due to the inability of zooplankton to grow in low pH. Microzooplankton can be sensitive to high concentration of CO_2.[55] In the samples with nutrient and CO_2, pH increased when microalgae began to grow (Fig. S1). The pH in the samples with nutrient and CO_2 increased from 5.9 to 7.6 by day 17 (Fig. S1). Furthermore, numbers of diatoms and Chrysophyceae sequences increased in nutrients and CO_2 group on day 3, and then “others” taxa increased at day 11, both of

them are preferred food sources for many grazers (Fig. 3). The abundance of microzooplankton increased quickly in the samples with nutrient and CO_2 on day 11 and 17, likely due to the increased pH in the samples.

High pH may decrease net growth of grazer populations and hence decrease feeding on phytoplankton.[56] The pH in the samples with nutrient and air increased from 7.9 to 9.5 in our experiment (Fig. S1). The relative abundance of diatoms and Chrysophyceae decreased greatly in the samples with nutrient and air since day 3. Also, the relative abundance of microzooplankton sequences in the samples with nutrient and air decreased slightly at day 17 (Fig. 2), probably due to the large contribution of algal sequences.

For the samples without nutrients (air or CO_2 group), the "other" phytoplankton (e.g. Charophyta, Heterokontophyta and unassigned) increased dramatically towards to the end of experiment, accounting for greater than 80% of total 18S rRNA gene sequences (Fig. 2). In contrast, the abundance of other phytoplankton remained relatively low in the samples with nutrients (air or CO_2) (Fig. 2). Relative abundance of golden algae (Chrysophyceae) increased slightly at day 3, and remained in relatively low abundance in the four treatments (Fig. 2). Dinoflagellates remained in low abundance (lower than 10%) throughout the experiment (Fig. 2).

Irrespective of the treatments, alpha diversity of eukaryotic sequences was relatively low at day 3 compared to the control sample with Simpson diversity index values (Fig. S3A). This was probably because the culture condition in the laboratory (i.e. temperature and light) was different from the original environment in Back River, e.g. the temperature in the laboratory was ca. 22 °C and the original environment was ca 7 °C when the samples were collected. Some eukaryotic species do not grow well in new environment, and many species need some time to adapt and grow well in the new environment. The samples with nutrients (air and CO_2, respectively) had much higher alpha diversity than other treatments (Fig. S3A). This is probably because the samples had enough nutrients to support growth when charged with high nutrients. Both weighted (quantitative) and unweighted (qualitative) variants of UniFrac are

widely used in microbial ecology, where the former accounts for abundance of observed organisms, while the latter only considers their presence or absence. Enriched nutrient samples had higher beta diversity compared to day 11 and 17 of samples not enriched with nutrients (Fig. S3B-C). The results suggest that nutrient enrichment can greatly influence the beta-diversity of eukaryotic community. Previous studies also reported that elevated CO_2 and nutrients addition could cause the shift of plant and microbial community compared to ambient CO_2 treatment.[57-59] Therefore, nutrient enrichment resulted in more changes of eukaryotic community compared to those without nutrient enrichment.

3.3 Changes of prokaryotic community

Patterns in the relative abundance of dominant taxa provide insight into potential linkages between community composition and the physiological function of those communities in the four treatments. Dominant taxa present at the Back River water sample (control sample) included Cyanobacteria (1.4 %), Proteobacteria (51.2 %), Actinobacteria (16.3 %), Bacteroidetes (15.4 %), Verrucomicrobiae (2.6 %), Parcubacteria (OD1) (0.5 %), and unassigned (10.3 %), and these taxa comprised 97.7 % of the total 16S rRNA gene sequences (Fig. 5-6). Bacterial communities associated with the Back River water were largely represented by Alpha-, Beta-, Gamma- and Delta- Proteobacteria, Bacteroidetes, Actinobacteria and Verrucomicrobia (Fig. 5), a finding that is consistent with the research on bacterioplankton community in Chesapeake Bay.[60]

The relative abundance of Cyanobacteria (*Synechococcus*) increased about 31 fold and made up nearly half of bacterial community in the samples with nutrient and air (Fig. 5-6). Also, several cyanobacteria blooming species are capable of sustaining growth under the alkaline conditions resulting from active photosynthesis during blooms.[61] So, the increased pH in the samples with nutrient and air likely triggered the rapid growth of cyanobacteria (Fig. S1). However, in the samples with nutrient and CO_2, cyanobacteria only contribute to a small portion (ca. 1%) of bacterial community (Fig. 5-6), suggesting that cyanobacteria (*Synechococcus*) are not a good candidate for

CO_2 mitigation. The samples with nutrient and CO_2 contain more unassigned bacterial sequences (37 %) (Fig. 5-6), suggesting that high CO_2 environment selects for different types of bacteria, and most of which are not well studied.

Within the domain bacteria, Proteobacteria constitutes the largest and phenotypically most diverse phylogenetic lineage, and play key roles in the carbon, sulfur and nitrogen cycles on our planet.[62] The relative abundance of Proteobacteria in nutrients and CO_2 group, air group and CO_2 group was nearly the same to Back River water sample at day 17, and made up higher than 42 % of total community (Fig. 5-6). Alphaproteobacteria accounted for more than 30 % of total abundance in these three groups (Fig. 5). Alphaproteobacteria can thrive under elevated CO_2 (550 ppm).[63] The abundance of alphaproteobacteria increased from 28.9 % in the control sample to 37.1 % in the samples with nutrient and CO_2 and 30.5 % in the samples with CO_2 (Fig. 5). The relative abundance of gammaproteobacteria decreased from 5 % in the control sample to ca. 0.5 % in the samples containing CO_2 (Fig. 5). Interestingly, in the samples with nutrient and air, the abundance of Proteobacteria group decreased from 51 % to 19% at day 17 (Fig. 5-6), and alphaproteobacteria decreased greatly from 29 % to 9 % of total abundance at day 17 (Fig. 5). Alphaproteobacteria and betaproteobacteria become less abundant in the samples with nutrient and air. It is not clear whether this is due to the "bloom" of cyanobacteria from 1 % to 44% at day 17 in the samples with nutrient and air.

Among Proteobacteria, alpha- and beta-proteobacteria are the two abundant groups (Fig. 5). Betaproteobacteria decreased in abundance from 16.3 % in control sample to lower than 4 % in adding nutrients group (Fig. 5). Betaproteobacteria probably did not have a competitive advantage compared to other species when enriched with nutrients. Verrucomicrobia are ubiquitous in the world's oceans, averaging 1.8 % of sequences in one global survey, and are more abundant in coastal waters.[64] Verrucomicrobia in the samples charged with air only are relatively more abundant (3.5%) than the samples charged with CO_2 only (0.8%) (Fig. 6), suggesting that Verrucomicrobia could be more sensitive to high concentration of CO_2.

3.4 Selection high CO_2 tolerant microalgae

Chlorophyta (green algae) appear to thrive in the samples with high nutrient and 10% CO_2 (Fig. 2 and Fig 4). The cell density of Chlorophyta can reach ca. 1.3×10^8 cells/mL, and account for higher than 90% of total eukaryotic community (Fig. 4 and Fig. S2). We tried to isolate algae from the samples with nutrient and CO_2 at the end of experiment, 14 clonal isolates of microalgae were recovered and characterized by their morphology and 18S rRNA gene sequences. 12 of them turned out to be closely related to *Scenedesmus* (Fig. 7). Growth of these algae were measured, and these algae grew well with 10% CO_2 (Fig. S4). This result suggests that water samples enriched with high CO_2 and nutrient select for green algae, especially *Scenedesmus* species. According to the earlier study based on the strain selection, the most of algal strains that can grow in high level of CO_2 are *Scenedesmus*.[23, 65, 66] Our study is also consistent with a recent study where high CO_2 condition also selected for green algae like *Desmodesmus* spp..[24] Our study further supports that enrichment of natural phytoplankton community with high CO_2 can be an efficient way to select for CO_2-tolerant microalgae.

Selection of optimal microalgal species is vital to sequestering CO_2 using phototrophic carbon fixation system. Here we present a comprehensive study showing how eukaryotic and prokaryotic communities respond to the nutrient enrichment and high concentration of CO_2. We also demonstrate that desired algal strains can be obtained by exposing a natural community to the selected conditions. By exposing aquatic communities to high concentration of CO_2, green algae (Chlorophyta) became the dominant algae. Upon algal isolation, we found that microalga *Scenedesmus* species are the winner under the high CO_2 growth condition. Compared to the traditional culture-based method, the community-based approach allows us to search for target microalgae from a diverse community of phytoplankton. We believe that the community-based method can be an efficient way to select desirable microalgae for other selection purposes such as temperature or salinity tolerant strains.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

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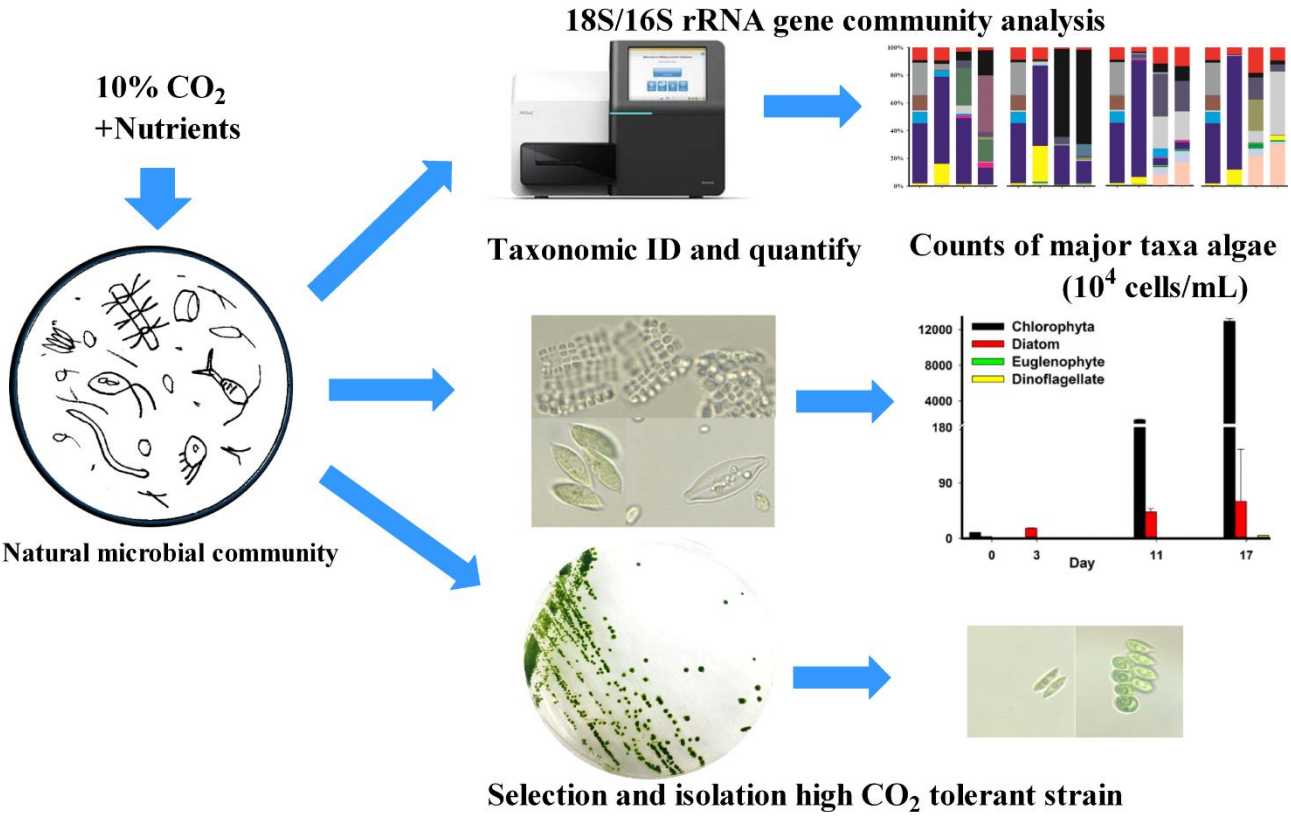

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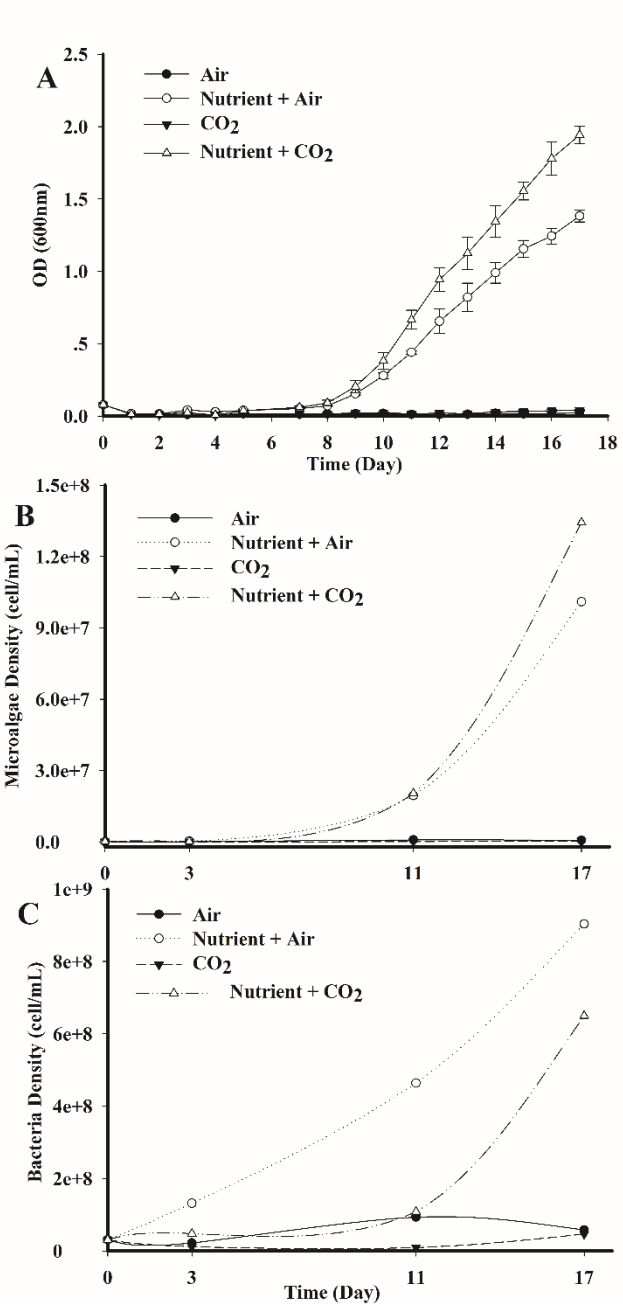

Fig. 1 The growth of phytoplankton and bacteria under different treatments. Optical density (OD) (A), Microalgae cell density (B), Bacteria cell density (C). Vertical bars indicate the standard deviations (n = 2).

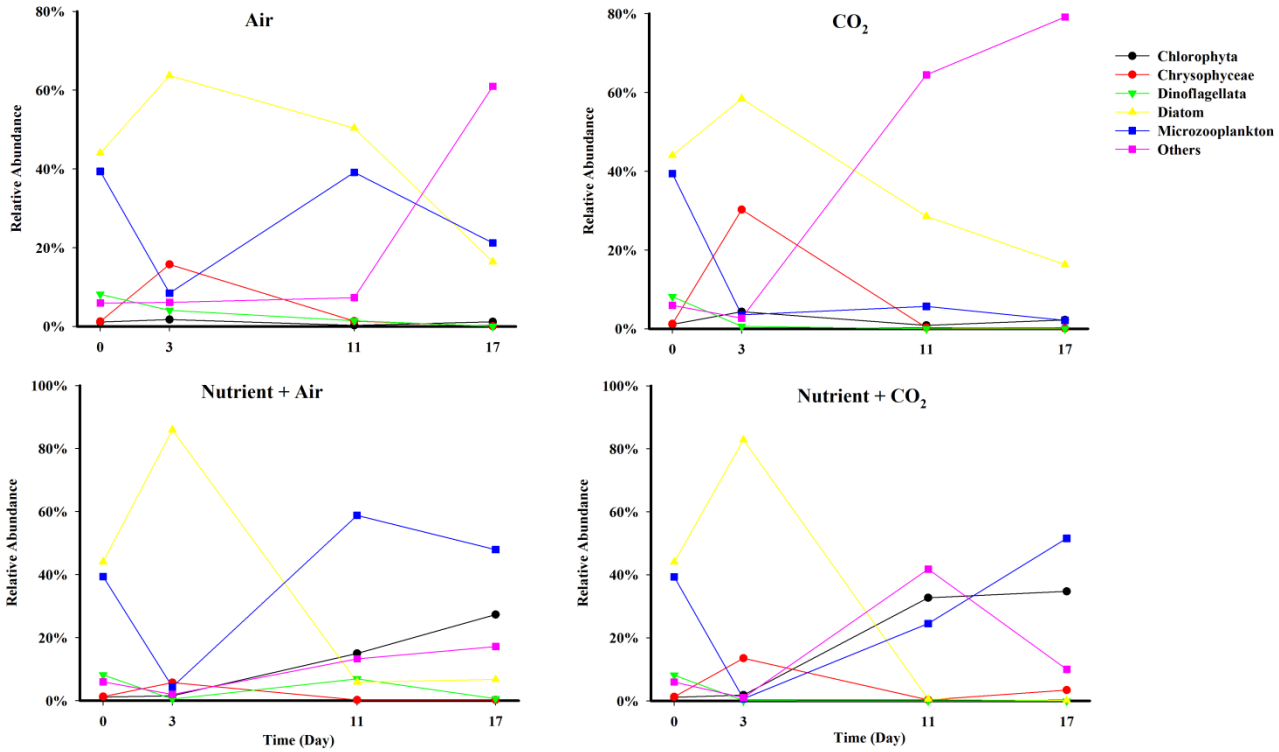

Fig. 2. Relative abundance of major microalgae groups and microzooplankton based on 18S rRNA gene sequences. Others refer to unassigned and rest of eukaryotic sequences.

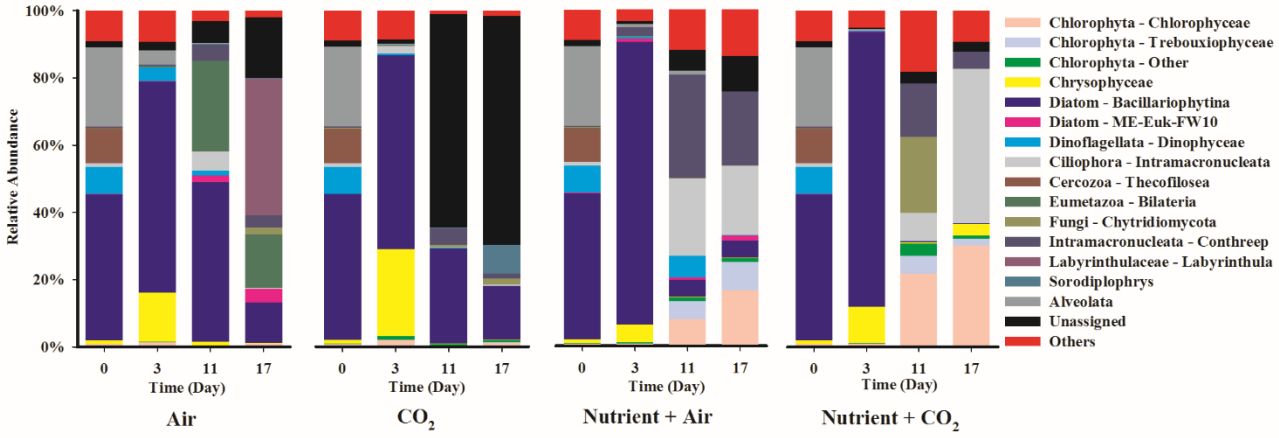

Fig. 3 Relative abundance histograms for 18S rRNA gene sequences across different treatments. Others include taxa which contributed less than 3.5% of total abundance.

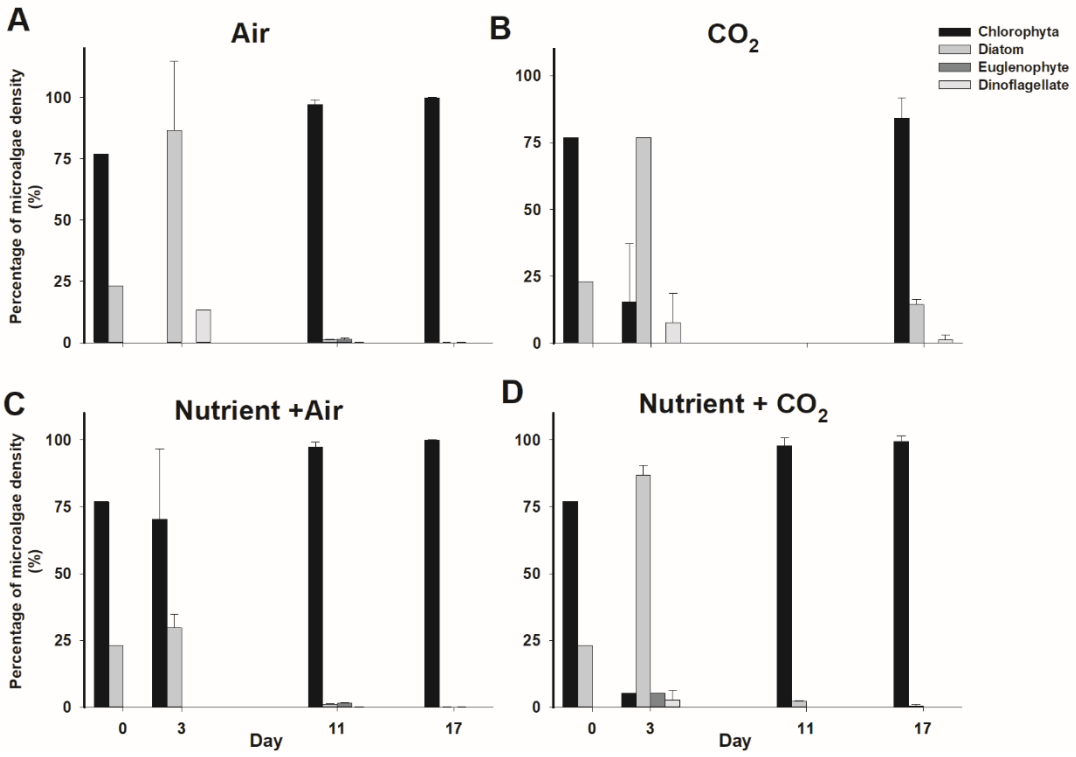

Fig.4 Cell density of main microalgae taxa in four treatments. Air group (A), CO_2 group (B), Nutrient and Air group (C), Nutrient and CO_2 group (D).

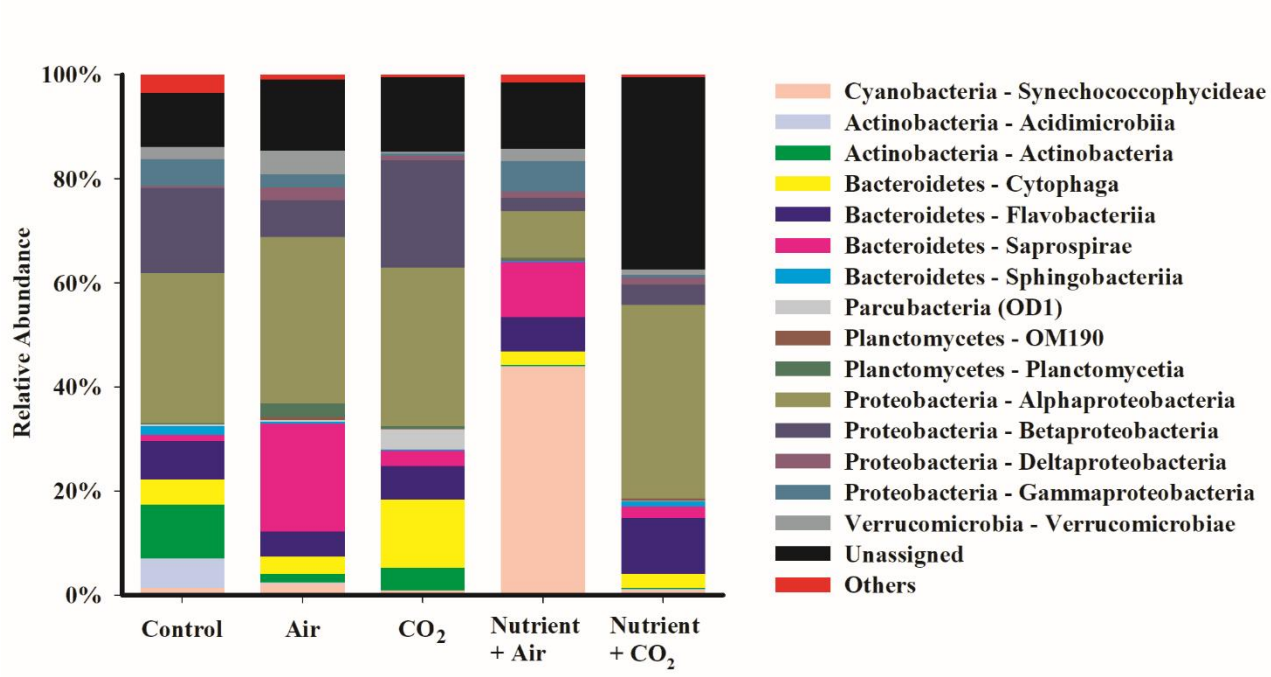

Fig. 5 Relative abundance of 16S rRNA gene sequences dominant taxa in the control (day 0) and all four samples on day 17. Only taxa with at least 0.5% abundance in the total dataset are identified in the legend. Others include taxa which contributed less than 0.5% of the number of reads in the total dataset.

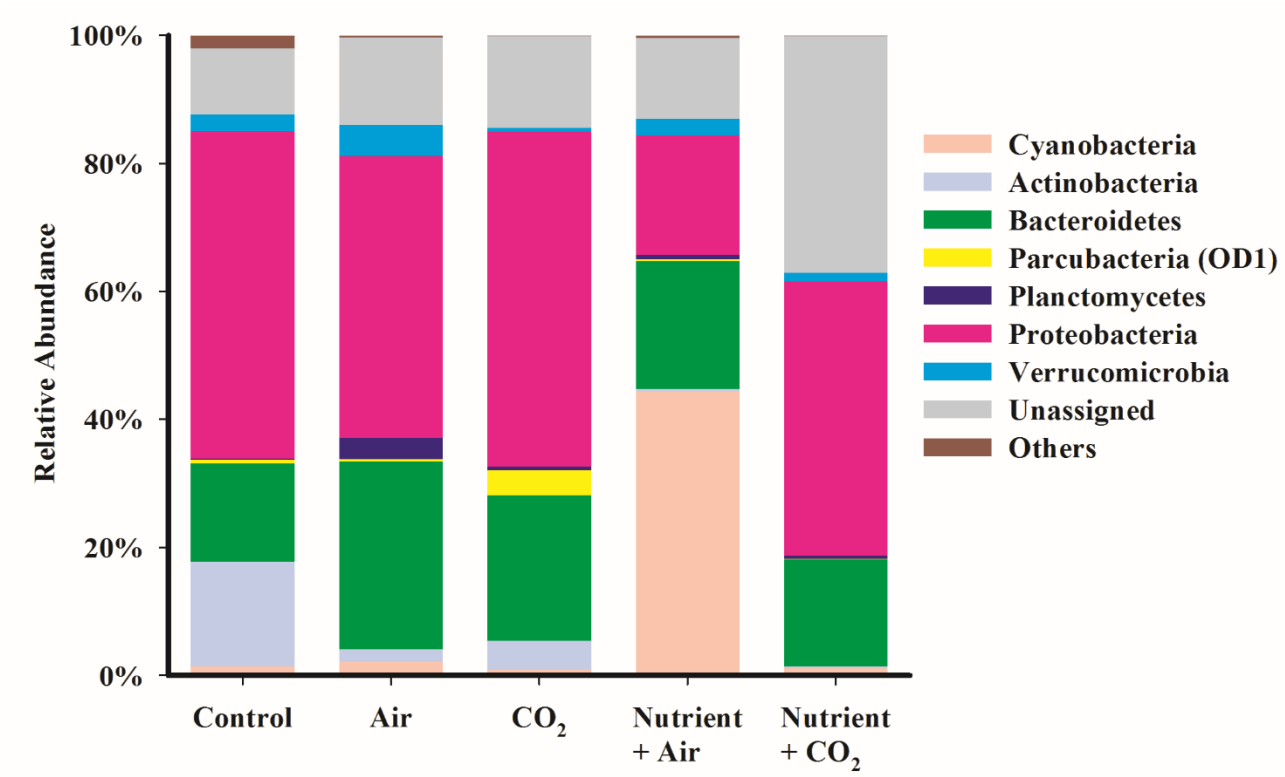

Fig. 6 Relative abundance of major prokaryotic groups including Cyanobacteria, Actinobacteria, Bacteroidetes, Parcubacteria (OD1), Planctomycetes, Proteobacteria, Verrucomicrobia and Unassigned based on 16S rRNA gene sequences. Others refer to the sequences that make up less than 0.5% of total data.

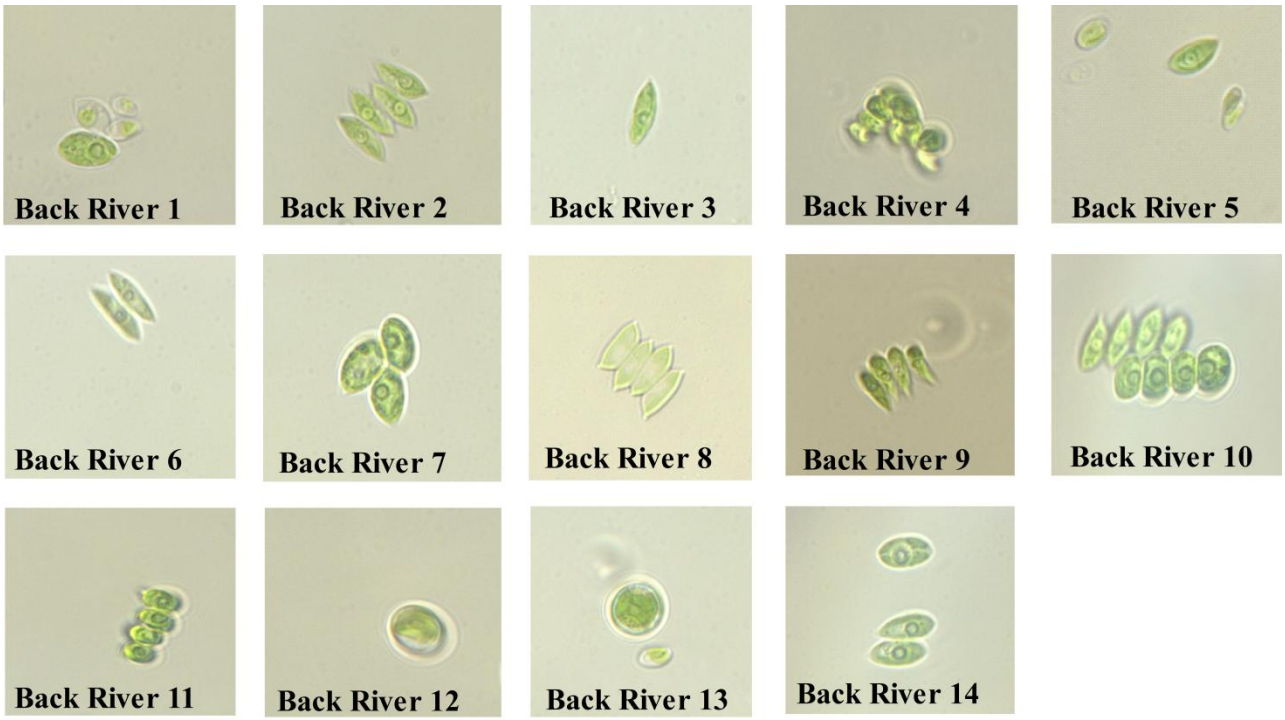

Fig. 7 Images of 14 clonal isolates of microalgae from Back River water samples.