An algal-bacterial symbiotic system of carbon fixation using formate as a carbon source

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Abstract

Algae are an attractive option for CO_2 sequestration due to their natural ability to simultaneously fix CO_2 and accumulate algal biomass for value-added products. However, the commercial implementation of such technology for efficient capture of CO_2 from fossil-derived flue gases is not a reality yet due to several major challenges, such as low gas-liquid mass transport efficiency and relatively high light irradiance demand of algal growth. This study explored an algal-bacterial symbiotic system to utilize formate, a potential intermediate liquid compound of CO_2, as carbon source to support microbial growth. The algal-bacterial assemblage, after an adaptive evolution using the formate medium, demonstrated a new route to assimilate CO_2 without using high pH cultivations and promote biomass production under low light irradiance condition. The formate based culture system not only resolves CO_2 mass transfer limitation, but also expels algae grazers in non-sterilized cultivation conditions. Continuous

cultivation of the assemblage on formate led to a carbon capture efficiency of 90% with biomass concentration of 0.92 g/L and biomass productivity of 0.31 g/L/day, which is significantly better than the control cultivation on saturated CO_2. In addition, isotope tracing and microbial community analysis offer new insights into formate metabolism and algal-bacterial symbiosis under light and carbon conditions. This study demonstrates a promising route of using electrochemical-derived formate to support algal biorefining.

Key words: Adaptive evolution, *Bacteroidetes*, Carbon capture, *Chlorella sorokiniana* MSU, Isotopic tracing, and *Proteobacteria*

1. Introduction

Greenhouse gas emission, particularly CO_2, must be controlled and reduced in a fast and efficient manner. The deterioration of natural environment caused by greenhouse gas emission is approaching a critical point and presents a very serious challenge to humankind. The global average surface temperature has increased by 0.3-0.7°C since 1986 and is expected to increase another 0.3-4.8°C by 2100 (I. Price, 2008). Reduction of CO_2 emission and capture of emitted CO_2 are the ways to address this challenge. Many capture and utilization methods have been developed to counteract increasing level of CO_2, which are generally classified into three categories: physical, chemical, and biological approaches (Lackner, 2003). Physical methods such as geologic injection or membrane separation have advantages of utilizing abandoned space, relatively easy operation, and suitability for large-scale CO_2 sequestration (Lackner, 2003). However, physical methods have drawbacks such as expensive separation equipment and facility, specific requirement of geological structures (Aaron & Tsouris, 2005). Chemical

sequestration methods are a high-efficiency technology for CO_2 emission control, but large amounts of reagents (e.g., amine) lead to other environmental concerns of corrosive and harmful chemical release and high energy consumption (Dutcher, Fan, & Russell, 2015) . Among the three capture and sequestration approaches, biological sequestration represents a very promising route, which combines both capture and utilization steps into one-single process to directly convert CO_2 into useful cell components (e.g., protein, carbohydrate, and lipid) (Klinthong, Yang, Huang, & Tan, 2015). However, the development and implementation of commercial-scale biological sequestration applications are behind the other two approaches (Huang & Tan, 2014).

Photosynthetic organisms such as microalgae can capture CO_2 (Wang, Li, Wu, & Lan, 2008) and convert it into macromolecules of protein, carbohydrate, and lipid. These macromolecules can be used directly or indirectly to produce value-added commodities, such as animal feedstock, pharmaceutical compounds, cosmetic areas, and health care products (de Morais & Costa, 2007). However, microalgae growth is largely restricted by gaseous mass transfer(Fu et al., 2019; Ho, Chen, Lee, & Chang, 2011). Bicarbonate has been used as a vehicle of enhancing CO_2 transfer in culture media to address this issue, though, requirements of high alkalinity and fine pH control limit its applications(G.-Y. Kim, Roh, & Han, 2019). Therefore, this study aimed to develop an algal-bacterial symbiotic system to utilize formate, a liquid product from CO_2 reduction that is stable under a wide range of pH, as a carbon source to support growth of the algal-bacterial assemblage. In addition, use of formate could also address another challenge of algal predators for large-scale cultivations (Flynn, Kenny, & Mitra, 2017). Formic acid is a well-known toxicant against many insects (Chen, Rashid, & Feng, 2012). It has been reported that freshwater organisms and marine crustaceans were adversely affected by

formic acid at concentrations ranging between 111 – 400 mg/L (EPA, 1990). The presence of formate at a relatively high level (e.g., greater than 1,000 mg/L) during the algal-bacterial cultivation may serve as a contamination control strategy to repel insects and other species in non-sterilized environments (e.g., open-pond cultivation) and realize long-term culture stability.

There are many investigations that have explored algal-bacterial communities for stable and effective phototrophic applications, including wastewater treatment (Mujtaba & Lee, 2016), biomass production (Y. Wang et al., 2015), and lipid accumulation (Magdouli, Brar, & Blais, 2016). In these algal-bacterial systems, algae provide photosynthetic products like dissolved oxygen and waste sugars for bacteria growth. Meanwhile, the bacteria typically promote algae growing through provision of some metabolites (Riquelme & Avendaño-Herrera, 2003), and also keep the whole environment stable by preventing invading organisms from becoming established (Kazamia et al., 2012). The algal-bacterial symbiotic strategy is quite useful especially for large-scale operations where the culture media cannot be easily sterilized and the culture conditions are largely influenced by other environmental factors (such as open pond cultivation)(Narala et al., 2016).However, algal-bacterial communities using formate as a carbon source has not been reported to date.

Compared to other reduction products of CO_2 (e.g., acetic acid, carbon monoxide, methane, and methanol), formate requires relatively less energy (Albo, Alvarez-Guerra, Castano, & Irabien, 2015; S. Kim, Kim, Lee, Yoon, & Jung, 2014) and is a dense, stable, and soluble compound that can bypass the issues of gas-liquid mass transfer limitation and pH requirement. In addition, formate also plays an important role as a metabolic intermediate in many diverse reactions among diverse microbial species (Hwang et al., 2020; Liu et al., 2017). It has been used as a compound to promote ATP and NADH generation in microorganisms(Ferry, 1990; Hou,

Patel, Laskin, & Barnabe, 1982). One common pathway is formate degradation through formate dehydrogenase, in which formate was dehydrogenated into carbon dioxide and releases energy ($HCOO^- \rightarrow CO_2 + H^+ + 2e^-$) (Ferry, 1990). Therefore, considering both prokaryotic mechanism of formate dehydrogenation and the symbiosis of algae and bacteria, adaptive evolution of an algal-bacterial symbiotic system was adopted by this study to utilize formate as a carbon source to accumulate microbial biomass. Formate dehydrogenation pathway of bacteria in the assemblage first dehydrogenates formate into localized CO_2 to improve carbon assimilation and algal growth, and the algal growth then releases oxygen and other metabolites to support bacterial growth.

The objectives of this study are to elucidate formate utilization between bacteria and alga, to understand effects of formate on microbial community of the algal-bacterial assemblage, and to demonstrate performance of the symbiotic system on carbon capture. Correspondingly, batch and continuous cultivations along with isotopic tracing and amplicon sequencing were carried out to fulfill the objectives and conclude a novel route of CO_2 fixation.

2. Materials and methods

2.1 Algal assemblage and cultivation system

The algal assemblage containing a selected microalga *Chlorella sorokiniana* MSU from the Great Lakes region and several bacteria (mainly *Bacteroidetes* and *Proteobacteria* etc.) (Cutshaw et al., 2020) was continuously cultured in flasks on Tris-Acetate-Phosphate (TAP) medium (X. Wang et al., 2015) at room temperature under constant fluorescent light to use for seeding the algae photobioreactors (APBs). Modified liquid TAP medium (without acetic acid and tris base) was used for microalgal cultures, which contains 7.5 mmol L^{-1} of NH_4Cl, 0.34

mmol L^{-1} of $CaCl_2 \cdot 2H_2O$, 0.4 mmol L^{-1} of $MgSO_4 \cdot 7H_2O$, 0.68 mmol L^{-1} of K_2HPO_4 (anhydrous), 0.45 mmol L^{-1} of KH_2PO_4 (anhydrous), 0.09 mmol L^{-1} $FeCl_3 \cdot 6H_2O$, and 1ml TAP trace elements solution. The modified TAP medium was unsterilized. The microbial community was analyzed before seeding the photobioreactors.

2.2 Photobioreactors

Both lab-scale and pilot-scale APBs were used. Lab-scale APBs were modified based on 10 L Eppendorf BioFlo®/CelliGen® 115 Benchtop fermenters with a working volume of 7.5 L (Fig. S1a). Metal shells with adjustable LED light strips installed inside were placed around the fermenters. The lab-scale APBs were used for kinetic study and continuous cultivation of formate utilization.

The pilot-scale APB was located at the T.B. Simon Power Plant at Michigan State University. The effective volume of the pilot-scale APB is 100 L (Fig. S1b). The pilot-scale APB configuration and operating mechanism were described in a previous study (Cutshaw et al., 2020). The control of continuous culture on saturated CO_2 was carried out using the pilot-scale APB.

2.3 Kinetic study using ^{13}C labeled formate

Kinetic labeling of culture metabolites was performed to trace carbon fates. Cultivation with inoculum of 0.1g/L of the seed was applied in two lab-scale APBs containing 4 L modified liquid TAP medium. The temperature was controlled at 22°C under a continuous light of either 50 or 500 μmol/m^2/s. The APB was mixed by a mechanical agitation at 250 rpm. pH was maintained between 6.5-7.2 by automatic addition of sulfuric acid (5% vol/vol). Cultures were

pulsed with concentrated ^{13}C-formate or ^{13}C -bicarbonate to obtain a final labeled carbon concentration of approximately 0.23 g/L. At specified timepoints after the pulse (1 min, 20 min, 4 h, 8 h, and 24 h), 20 mL of culture medium was sampled and quenched with an equal volume of medium salts (containing no carbon or nitrogen sources) in a liquid nitrogen bath. Samples were then pelleted at 4°C, the supernatant discarded, and stored at -80°C until further analysis. Additional 50 mL algal samples at each timepoint were collected for analysis of nutrient concentration, biomass concentration and carbon utilization.

2.4 Continuous algal cultivation on formate

One of the lab-scale APBs was used to run continuous algal cultivation on formate. The APB contained 4 L modified liquid TAP medium with an initial algal biomass concentration of 0.35 g/L. The light intensity was maintained at 180 μmol/m^2/s for the entire cultivation. The pH was maintained between 6.5-7.2 by automatic addition of sulfuric acid (5% vol/vol). The temperature was kept at 22±2°C. The APB was mixed by a mechanical agitation at 250 rpm. The culture was initiated as a batch culture for 48 hours. 1 g/L of formate was fed to the APB every 24 hours in the first 48 hours. After the initial concentration reached 0.7 g/L at the end of the 48-hour batch culture, the continuous cultivation started with a daily formate feeding rate of 1 g/L/day and a daily harvesting ratio of 30% (v/v). The experiment was continuously run for 14 days.

The control cultivation on the saturated CO_2 medium was run in the pilot-scale APB. The light intensity for the cultivation was 407 μmol/m^2/s. The natural gas fired flue gas, containing 7.2 v/v of CO_2 was directly pumped from the stack into the APB at a flow rate of 120 L/m^3/min to make CO_2 saturated in the culture medium. The modified liquid TAP medium was used as the

nutrients. The pilot-scale APB has been continuously running for 33 months on the saturated CO_2 medium using flue gas as the CO_2 source. The data that were used for the comparison were from a 20-day continuous cultivation with the same nutrient condition and same harvesting ratio of 30% (v/v).

2.5 Chemical analysis

Samples were analyzed for dry biomass weight, pH and nutrient (total nitrogen (TN), total phosphorus (TP), nitrate (NO_3-N) and ammonia (NH_3-N)) concentrations. Algal biomass was pelleted for dry weight measurement using a Thermo Electron Corporation IEC Centra CL2 Centrifuge at 3800 rpm for 5 minutes. Biomass was washed once and resuspended using deionized water, and then dried at 105°C for 24 hours. Sample pH was measured using a pH meter (Fisherbrand™ accumet™ AB15 + Basic, Fisher Scientific Co., Pittsburgh, PA). Nutrient concentrations were tested in the liquid supernatant using nutrient test kits (HACH Company, Loveland, Colorado) equivalent to EPA methods (hach.com/epa). Algal biomass composition was analyzed using the standard forage analysis method (Undersander, Mertens, & Thiex, 1993).

The C1 carbon incorporation into biomass proteins was quantified via GC-MS analysis. Amino acids were extracted and analyzed as previously described (Hollinshead, He, & Tang, 2019). Briefly, the pelleted biomass was hydrolyzed with 6 N HCL at 100°C for approximately 20 h. The supernatant was transferred to a new vial and dried with air for 12 h. The amino acids were then derivatized with N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide) TBDMS at 70 °C for 1 hour and analyzed on an Agilent GC (7820A)-MS (MS5970E) equipped with HP-5ms column and a temperature gradient previous described (Hollinshead et al., 2019).

Free metabolites were extracted from the biomass via methanol at 4°C for 8 hours. Samples were diluted with LC-MS grade water, frozen, lypholyzed, and reconstituted in 200 μL of 60:30:10 acetonitrile:methanol:water. Labeling was analyzed using a hydrophobic interaction liquid chromatography (HILIC) method on a Shimadzu Prominence-xR UFLC system and a SCIEX hybrid triple quadrupole-linear ion trap MS equipped with Turbo VTM electrospray ionization (ESI) source as previously described (Czajka, Kambhampati, Tang, Wang, & Allen, 2020).

Formate concentration of algal samples in kinetic study was determined by high performance liquid chromatography (HPLC) (Shimadzu Corp., Kyoto, Japan) equipped with an analytical column (Aminex HPX-87H, Bio-Rad Laboratories, Inc., Hercules, CA) and a refractive index detector (Shimadzu Corp., Kyoto, Japan). The mobile phase was 0.005 mol/L sulfuric acid at a flow rate of 0.6 mL/min. The oven temperature was set at 65 °C. Bicarbonate concentration of algal samples in kinetic study was determined by the alkalinity test kit (HACH Company, Loveland, CO).

2.6 Microbial community analysis

Samples (1 mL) collected for DNA analysis were kept frozen at -20°C until analysis. To remove nutrient media, algae sample was centrifuged using an Eppendorf 5416R centrifuge at 10,000 rpm for 5 min and the supernatant was discarded. The remaining pellet was used for DNA extraction using the DNeasy® PowerSoil® Kit (Qiagen, Germany). DNA was eluted with 100 μL of 10 mM Tris-HCl (pH 8.5) and the concentration and purity determined using a NanoDrop Lite spectrophotometer (Thermo Fisher Scientific, USA). The DNA extracts were stored at -80°C for several weeks and then used for PCR and Illumina DNA sequencing.

Illumina sequencing was performed for the 16S rRNA gene to assess the bacterial community. Prior to PCR, extracted DNA samples were diluted 10x due to high DNA concentrations. The PCR conditions were as follows: 1.0 μL DNA template (10x diluted), 0.5 μL of 100 μM forward primer (IDT, Pro341F), 0.5 μL of 100 μM reverse primer (IDT, Pro805R), 12.5 μL 2x Supermix (Invitrogen, USA), and 10.5 μL PCR grade water. The PCR program used for all assays is as follows: 96°C for 2 min, followed by 30 cycles of 95°C for 20 s, 52°C for 30 s, and 72°C for 1 min, and a final elongation period of 72°C for 10 min. After PCR, samples were diluted to normalize DNA concentrations within a range of 5-10 ng/μL. DNA concentration was determined using the PicoGreen® dsDNA quantitation assay (Invitrogen, USA) and Fluostar Optima microplate reader (BMG Labtech, Germany). The PicoGreen® conditions were as follows: 95 μL 1x TE buffer solution, 100 μL 1:200 diluted PicoGreen® reagent, 5 μL DNA template. Samples with known DNA concentrations were also prepared for standard curve generation. Illumina library preparation and sequencing were performed at the Michigan State University Genomics Laboratory, East Lansing, USA. QIIME 2™ was used for all sequence analyses (Bolyen et al., 2019).

The 16S rRNA gene sequencing was also used to determine *C. sorokiniana* in the assemblage (Cutshaw et al., 2020). It has been reported that *Cyanobacteria* have 85-93% of 16 rRNA gene sequences similar with *C. sorokiniana* (Burja, Tamagnini, Bustard, & Wright, 2001a, 2001b), while, color, shape, and size of both species are very different (Sukenik, Zohary, & Padisák, 2009). Therefore, after microscopic imaging verification of each sample, *Cyanobacteria* sequence was interpreted as microalga *C. sorokiniana* for all samples.

2.7 Statistical analysis

All data collected was analyzed using the statistical tools of R (version 3.6.3). In order to determine whether a parametric or non-parametric test was necessary, the data were first tested for normality and equal variance using a Shapiro-Wilk's test and an F-test, respectively. Data that were normal with equal variance were tested using an analysis of variance (ANOVA) and a Tukey test was used when applicable to compare individual factors. Data with non-normal distribution and unequal variance were tested using the Kruskal-Wallis test. All tests were performed with a significance value of $\alpha = 0.05$. Microbial community analysis was completed using Vegan, ggplot2, phyloseq, and MASS R libraries. Taxonomic/phylogenetic data was analyzed in order to graph relative abundances of samples.

3 Results and discussion

3.1. The algae assemblage utilizing formate as a carbon source

^{13}C labeled formate and bicarbonate under different light intensities were used to study growth kinetics of algae assemblage and elucidate the effects of carbon source and light intensity on the algae assemblage (Fig. 1). Under high light intensity of 500 μmol/m^2/s, biomass concentrations reached 0.12±0.00 and 0.16±0.03 g/L at 24 hours of the culture for the formate and bicarbonate carbon sources, respectively (Fig. 1a). Under low light intensity of 50 μMol/ m^2/s, the corresponding biomass concentrations were 0.15±0.02 and 0.13±0.02 g/L for formate and bicarbonate (Fig. 1b). The growth patterns under individual light intensities and carbon sources show that the algae assemblage grew slightly faster on bicarbonate than formate under the high light intensity of 500 μMol/m^2/s. There were no significant ($P>0.05$) differences on assemblage growth between two carbon sources after 4 hours of the culture under the low light intensity of 50 μmol/m^2/s. The statistical analysis further concluded that biomass concentrations

of all four cultures were not significantly ($P>0.05$) different at the end of the culture (24 hours). However, consumptions of formate and bicarbonate by the assemblage show different patterns between different carbon sources and light intensities (Fig. 1c and d). Under the high light intensity, 79% of bicarbonate was consumed in 24 hours of the culture. While, 49.5% of bicarbonate was consumed by the culture under the low light intensity. Corresponding sodium bicarbonate concentrations were 0.32±0.10 and 0.46±0.02 g/L for 500 and 50 μMol/m^2/s at the end of the culture. As for formate consumption, the data show that the assemblage consumed formate much slower than bicarbonate under corresponding light intensities, though, two studied light intensities had no significant ($P>0.05$) influence on formate consumption of the assemblage. 25 and 26% of the provided formate were consumed under 500 and 50 μMol/m^2/s, respectively after 24 hours of cultivation. The corresponding final sodium formate concentrations were 0.84±0.05 and 0.81±0.11 g/L, which were also not significantly ($P>0.05$) different as well. Despite similar final biomass concentrations, different observed rates of formate and bicarbonate consumption between different conditions led to significant differences of biomass yield. After 24 hours of the cultivation, biomass yields were 0.44±0.01, 0.23±0.06, 0.49±0.04, and 0.28±0.05 g/g for formate under high light intensity, bicarbonate under high light intensity, formate under low light intensity, and bicarbonate under low light intensity, respectively (Table 1). The biomass yields of the cultures grown on formate were significantly ($P<0.05$) higher than the cultures grown on bicarbonate, even under the high light intensity. The data indicates that even though formate was consumed at a slower rate than bicarbonate, the carbon source leads to higher biomass yields irrelevant of the light intensity.

TN and TP concentrations were also monitored during the culture (Fig.1e, f, g, and h). The cultures from the different conditions show similar trends. The TN reductions were 25.2,

26.6, 22.2, and 26.2 mg/L, for bicarbonate under 500 μMol/m^2/s, formate under 500 μMol/m^2/s, bicarbonate under 50 μMol/m^2/s, and formate under 50 μMol/m^2/s, respectively, and the corresponding TP reductions were 4.7, 8, 8.4, and 8.9 mg/L. There were no significant ($P>0.05$) differences on TN reduction between four cultures under different carbon sources and light intensities. As for TP reduction, the culture on bicarbonate under high light intensity had significantly ($P<0.05$) less TP reduction than other three cultures. There were no significant ($P>0.05$) differences on TP reduction on the other three cultures. Since a large quantity of TP and TN remained at the end of 24 hours, they were not the limiting nutrients during growth. Therefore, carbon and light intensity are the main factors that influence growth of the algal assemblage under the studied conditions.

The metabolic activity of the community was assessed using ^{13}C pulse-trace experiments. Labeled formate or bicarbonate was pulsed to the cultures at the beginning of the culture, and the resulting label incorporation into the metabolites or proteins was determined (Fig. 2). The turnover rates of free metabolites (sugar phosphates and Calvin cycle intermediates) show that at the beginning of the culture (within the first 4 hours), formate incorporation into the central metabolism of the assemblage was slower than bicarbonate under both light intensities (Fig. 2a and b; Fig. S2). This is most likely caused by the additional steps needed for formate metabolization, bacterial conversion of formate to CO_2, transportation of CO_2 to the algae, and incorporation of CO_2 into the Calvin cycle. Free metabolites related with glycolysis and the Calvin cycle had similar turnover rates between high and low light intensities, with the exception of glycerol-3-phosphate (Glycerol-3P). Cultures on bicarbonate and formate under 50 μMol/m^2/s had glycerol-3P turnovers of 30.8 and 12.2% at 4 hours, respectively, which were much higher than corresponding turnovers of 18.2 and 5.6% under 500 μMol/m^2/s. Glycerol-3P is a key

intermediate related with glycerol production and lipid accumulation (Kennedy pathway) and its accumulation indicates that low light (50 μMol/m^2/s), causing insufficient NADPH and ATP from photosynthesis, prevented precursor Glycerol-3P from consumption for lipid synthesis since lipid synthesis requires large amount of ATP and NADPH. However, as mentioned above, turnover rates of free metabolites on glycolysis and Calvin cycle were not largely influenced by light conditions in the presence of both formate and bicarbonate. Thereby, cultures under low light intensity generated the same amount of algal biomass compared to the cultures under high light intensity (Fig. 1a, b, c and d), which indicates the bicarbonate and formate improved the growth robustness of algal-bacteria community under low light conditions.

The labeled amino acid data confirms that formate was used as a carbon source to synthesize biomass of the algal assemblage (Fig. 2c and d; Fig. S3). Under high light intensity, the culture on formate shows moderately faster rates of labeling in proteinogenic amino acids than the cultures grown with bicarbonate (Fig. 2c). Labeled serine and methionine from the culture on formate were 41.5±0.9 and 41.7±3.1%, respectively, at 24 hours, which were significantly ($P<0.05$) higher than the culture on bicarbonate (35.7±3.05 and 31.1±0.0% respectively). Under low light intensity, amino acid labeling in formate and bicarbonate cultures were higher (Fig. 2c and d) than the culture under high light intensity condition. This observation could be explained by the increased contribution of atmospheric unlabeled CO_2 fixation to biomass synthesis under high light conditions. There were no significant ($P>0.05$) differences between the labeling contents for the measured amino acids (Alanine, glycine, serine, phenylalanine, aspartic acid, glutamic acid, and methionine) between the two carbon sources at 24 hours of the culture (Fig. 2d). The results were consistent with the biomass yield of different cultures, with the cultures on formate resulting in higher biomass yields than the cultures grown

on bicarbonate, and the beneficial impact of low light intensity on biomass yield (Table 1). In addition, it is important to note that protein labeling experiments were conducted over a longer duration (24 hours) than free metabolites labeling experiments (4 hours). The results indicate that formate has extended benefits for biomass growth over extended period of time (24 hours), which could guide the design of continuous cultures (presented in the next section).

The kinetic data and ^{13}C pulse-trace data indicate that syntrophic interactions between alga and bacteria were established by the algal assemblage using formate as the carbon source (Figure 3). Bacteria in the assemblage first utilizes formate as an energy source for their metabolism and release CO_2 that satisfies the need of algal photosynthesis; photosynthesis then generates oxygen and other micro-nutrients to support bacterial growth. Such syntropy was further enhanced under low light condition.

Amplicon sequencing was applied to the samples taken at the beginning and end of the cultivation. The results show that the dominating phylum was Chlorphyta for all four cultures at 24 hours (Fig. 4a), which corresponds to *Chlorella sorokiniana* in the assemblage. The relative abundances of *C. sorokiniana* were 92±1.35, 80.8±1.9, 86.9±3.6, and 83.6±0.4% for the cultures with formate under 500 μMol/m^2/s, formate under 50 μMol/m^2/s, bicarbonate under 500 μMol/m^2/s, and bicarbonate under 50 μMol/m^2/s, respectively. Compared to its abundance at the beginning of the culture (86.2%), the cultures under high light intensity maintained similar abundances of *C. sorokiniana*, while its abundances in the cultures under low light intensity were significantly ($P<0.05$) reduced. Correspondingly, the bacterial community percentage in the cultures under low light intensity at 24 hours were significantly ($P<0.05$) increased compared to the cultures grown under high light intensity (Fig. 4b). *Rhizobiales* were the dominant bacterial order in the cultures. An unclassified *Rhizobiales* family and *Methylobacteriaceae* are two

dominant *Rhizobiales* families (Fig. 4c and d). Carbon sources and light intensity had significant ($P<0.05$) influences on their abundances in the culture. The data clearly show that *Methylobacteriaceae* were more abundant from the cultures on formate (3.35 and 10.4% for 500 and 50 μMol/m^2/s, respectively) than them on bicarbonate (0.7 and 0.6% for 500 and 50 μMol/ m^2/s, respectively). Unclassified *Rhizobiales* family was more abundant on the cultures with bicarbonate than formate. As it is well known, *Methylobacteriaceae* are one of methylotrophs that are capable of growth on single carbon compounds (Lidstrom, 2006). It has also been reported that *Methylobacteriaceae* are accumulated under abiotic stress, which is consistent with the observation of this study (Kelly, McDonald, & Wood, 2014; Kumar et al., 2019). The abundance of *Methylobacteriaceae* in the culture with formate under 50 μMol/m^2/s (the most abiotic condition among four studied cultures) was much higher than other three cultures. This result, along with data of free metabolites, amino acids, and biomass yields, further confirmed the high metabolic resilience of algal-bacterial symbiotic system under a wide range of light and carbon conditions. Particularly, such resilience enables the algal-bacterial system to efficiently utilize formate as a carbon source.

3.2. Continuous culture of the algae assemblage using formate as a carbon source

The algal assemblage was fed at 1 g sodium formate/L/day

to study the performance of carbon capture and biomass accumulation during steady state continuous cultivation. 30% of the culture volume was harvested daily. A control was run on saturated CO_2 using the same harvesting amount and light intensity. An intermediate light intensity of 180 μMol/m^2/s was used for the continuous culture. Figure 5 shows effects of carbon sources on the continuous culture of the assemblage. Under steady state cultivation, there

were no significant ($P>0.05$) differences in phosphorous and nitrogen consumption between two cultures (Fig. 5c and d). As for biomass concentration and productivity, biomass concentrations of the cultures on formate and CO_2 were 0.92±0.12 and 0.97±0.19 g/L, respectively, with no significant difference ($P>0.05$) from each other (Fig. 5a). Biomass productivity of the culture on formate (0.31±0.04 g/L/day) was significantly ($P<0.05$) higher than that of the culture on CO_2 (0.24±0.08 g/L/day) (Fig. 5b). 1 g/L formate was completely consumed by the algal assemblage every day. Elemental composition of the biomass from the formate culture measured as C (47.2±2.7%), H (6.9±0.3%), and N (8.0±0.9%), which were not significantly ($P>0.05$) different from the biomass composition from the CO_2 cultures (48.9±0.5, 7.4±0.0, and 8.8±0.6% for C, H, and N, respectively) (Table 2). Based on carbon content in the biomass, the carbon balance calculation shows that carbon capture efficiency of the culture on formate was 89.8±10.2%, which is much higher than the control culture on CO_2 (48.2±11.4%) (Fig. 5e). The results indicate that the algal assemblage utilizing formate can be a new route to fix carbon and accumulate algal biomass, which much more efficient than the algal cultivation grown with saturated CO_2.

Microbial community analysis was further conducted on both continuous cultures to elucidate effects of carbon sources on alga and bacteria in the assemblage. 16S rRNA gene sequences were rarified at 10,000 read, which indicated sufficient sample coverage. A rank abundance curve with a gentle slope between 10 and 60 species shows an even distribution of gene sequences. Statistical analysis on alpha-diversity (Shannon's index, *H*) and evenness (Pielou's evenness, *J*) shows that carbon sources had significant ($P<0.05$) influence on microbial diversity (Table 4). The community of the culture on formate had much higher *H* and *J* (1.5±0.2

and 0.5±0.1, respectively) that the community on CO_2 (0.4±0.1 and 0.1±0.0, respectively), which means that more microbial species were unevenly distributed in the culture on formate.

Under steady state culture condition, the relative abundances of alga at the domain level for the cultures on formate and CO_2 were 57.9 and 92.3%, respectively, and corresponding abundances of bacteria at the domain level were 42.3 and 7.6% (Fig. 6a). The microbial community of the continuous culture on CO_2 was similar to the community of the culture on CO_2 in the kinetic study and a previous study (Cutshaw et al., 2020), while, community data of the culture on formate clearly demonstrate that formate significantly ($P<0.05$) shifted the community and increased bacterial distribution to facilitate formate utilization. The dominant bacterial phyla of the culture on formate were *Bacteroidetes* (19.3%) and *Proteobacteria* (22%) (Fig. 6b). *Flavobacteriaceae* (3.3%) and unclassified *Bacteroidetes* family (12.8%) are two major families in the phylum *Bacteroidetes* (Fig. 6c). Unclassified *Alphaproteobacteria* family (3.2%), Unclassified *Rhizobiales* family (3.4%), *Methylobacteriaceae* (7.3%), Unclassified *Betaproteobacteria* family (5.3%) are four key families in the phylum *Proteobacteria* (Fig. 6d). Compared to the previous kinetic study, continuous cultivation of the algal assemblage on formate shifted the bacterial community further. Besides the two dominant *Proteobacteira, Methylobacteriaceae* and the unclassified *Rhizobiales*, an unclassified *Bacteroidetes* became another dominant bacterial family. It is well known that *Bacteoridetes* are mainly responsible for degrading carbohydrates in the medium. Enrichment of them in the assemblage of the culture on formate could be interpreted as some carbohydrates from alga needing to be degraded to provide nutrients to other bacteria to grow and convert formate into CO_2 which enhances the symbiosis of alga and bacteria to utilize formate. The results from microbial community analysis of the

continuous cultivation further proved the symbiosis of alga and bacteria in the assemblage of formate utilization (Fig. 3).

4 Conclusions

A new and robust algal-bacterial assemblage containing *C. sorokiniana* and bacteria (*Proteobacteria* and *Bacteroidetes*) has been adaptively evolved and selected to utilize formate as a carbon source. Formate significantly enhances the methylotrophic population in the assemblage. Isotope tracing results conclude significant contribution of formate as a carbon source for biomass growth. Formate can be used as an alternative carbon form to replace bicarbonate or CO_2, which promotes algal growth and resolves CO_2 mass transfer limitation that plague current photosynthetic algae cultivation. Particularly, formate as a carbon source allows algal growth under a wide range of pH, from weakly acidic to alkaline conditions. In addition, the assemblage of formate utilization has strong resilience under different light intensities. The result shows that formate facilitated algal carbon fixation under low light intensity. A high carbon capture of 90% was achieved from continuous cultivation of the assemblage on formate. Therefore, with advancement of current research on electrochemical conversion of CO_2 to formate, this study provides a novel, flexible, and efficient route to fix CO_2 into algal biomass for value-added uses. Finally, the capability of the assemblage to handle relatively high formate concentration is highly advantageous to repel insects and other contaminated species during long-term, continuous cultivation.

5 Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

6 Acknowledgements

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Figure Captions:

Figure 1. Kinetics of algae cultivation on formate and bicarbonate
(a). Biomass concentration under 500 μMol/m^2/s
(b). Biomass concentration under 50 μMol/m^2/s
(c). Formate/bicarbonate concentration under 500 μMol/m^2/s
(d). Formate/bicarbonate concentration under 50 μMol/m^2/s
(e). TP concentration under 500 μMol/m^2/s
(f). TP concentration under 50 μMol/m^2/s
(g). TN concentration under 500 μMol/m^2/s
(h). TN concentration under 50 μMol/m^2/s

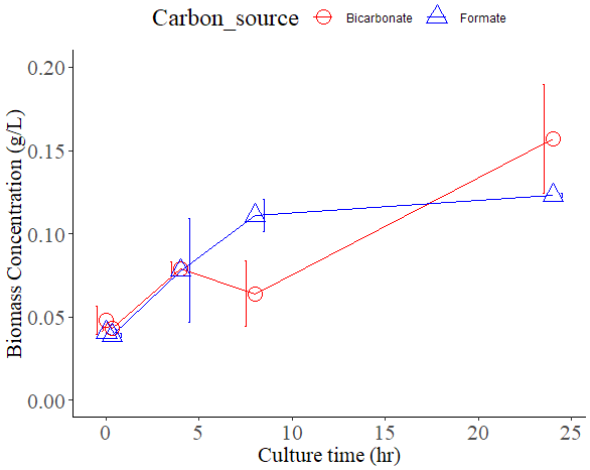
Figure 2. Labeled free metabolites and proteinogenic amino acids from the cultures on formate and bicarbonate. a. Free metabolites under 500 μmol/m^2/s; b. Free metabolites under 50 μMol/ m^2/s; c. Amino acids under 500 μmol/m^2/s; d. Amino acids under 50 μMol/m^2/s
*Free metabolites: 3PGA is 3-phosphoglyceric acid; F6P is D-fructose-6-phosphate; Glycerol 3P is glycerol 3-phosphate; G6P is glucose 6-phosphate; MAL is malate; PEP is phosphoenolpyruvic acid; SUC is succinate; and GAP is D-glyceraldehyde-3-phosphate.
**Amino acids: ALA is alanine; GLY is glycine; SER is serine; PHE is phenylalanine; ASP is aspartic acid; GLU is glutamic acid; and MET is methionine.

Figure 3. Symbiosis of alga and bacteria on formate

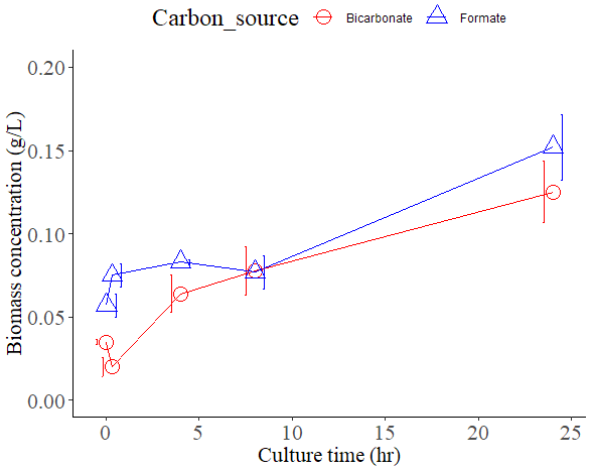
Figure 4. Changes of microbial communities from the batch cultures on formate and bicarbonate*. a. *Eukarya*; b. *Bacteria*; c. Unclassified *Rhizobiales* family; d. *Methylobacteriaceae*
*: Relative abundances of *Eukarya*, *Bacteria*, Unclassified *Rhizobiales* family, and *Methylobacteriaceae* were 86.2, 13.7, 10.0 and 0.3% at the beginning of the cultures. They were presented as the red lines in the figures. The detailed relative abundances of all communities were presented in Figure S4.

Figure 5. Performance of the algae assemblage on formate and (carbonic acid) dissolved CO_2
a. Biomass concentration; b. Biomass productivity; c. TN consumption; d. TP consumption; e. carbon capture*
*: The carbon capture is calculated by the carbon content in the harvested biomass divided by the carbon being dissolved in the media for both formate and CO_2. For the culture on CO_2, the amount of carbon dioxide was accounted for the carbon capture calculation is the dissolved CO_2, not the total CO_2 pumped into the reactor.

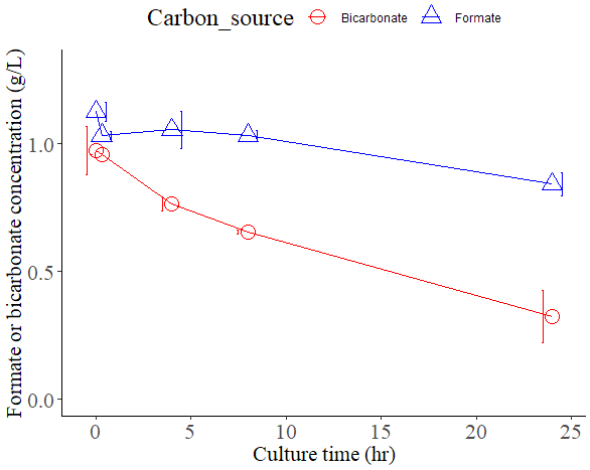
Figure 6. Microbial community of the algae assemblage on formate and CO_2
(a) Abundance at the domain level; (b) Bacteria abundance at the phylum level; (c) Bacteroidetes abundance at the family level; (d) Proteobacteria abundance at the family level



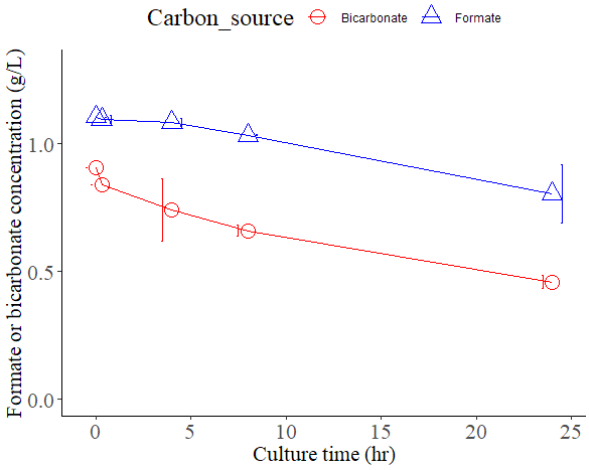
(a). Biomass concentration under 500 µMol/ m^2/s



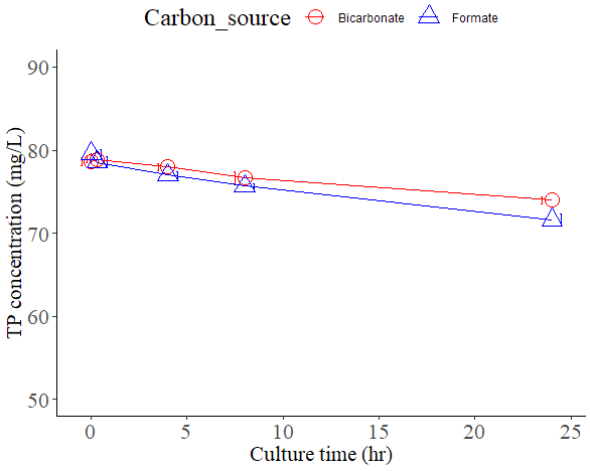
(b). Biomass concentration under 50 µMol/m^2/ s



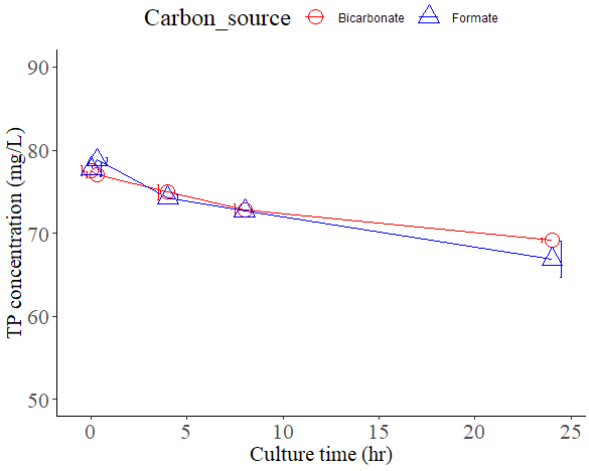
(c). Formate/bicarbonate concentration under 500 µMol/m^2/s



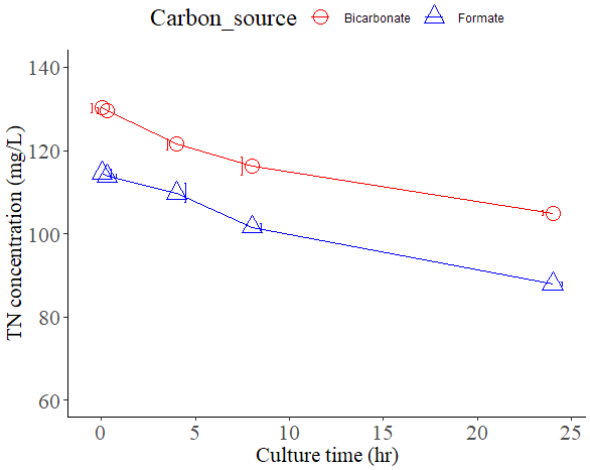
(d). Formate/bicarbonate concentration under 50 µMol/m^2/s



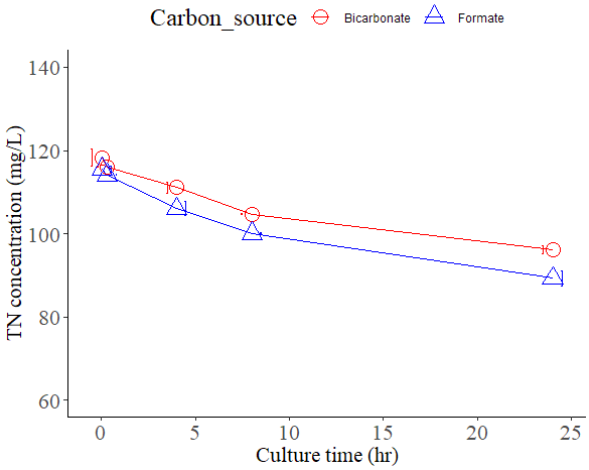
(e). TP concentration under 500 µMol/m^2/s



(f). TP concentration under 50 µMol/m^2/s

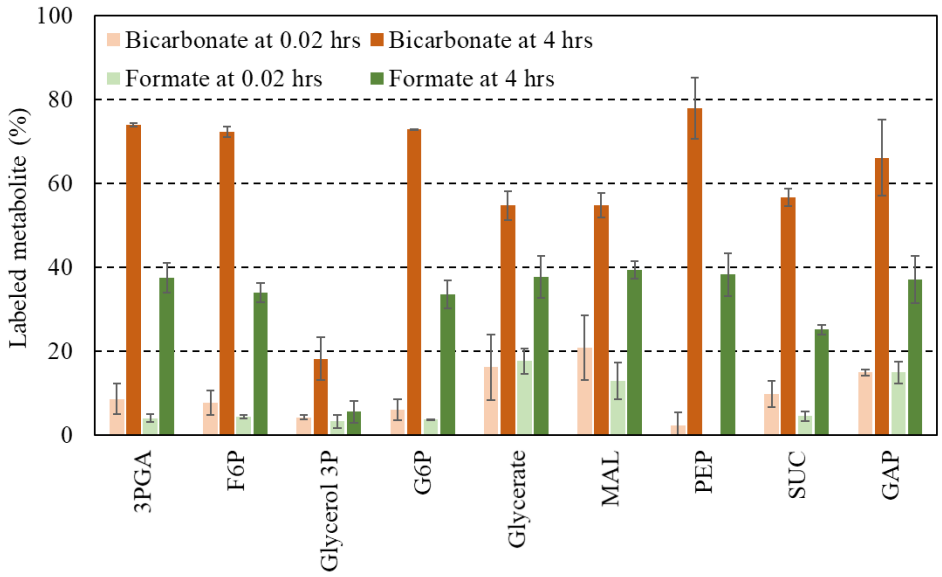


(g). TN concentration under 500 µMol/m^2/s

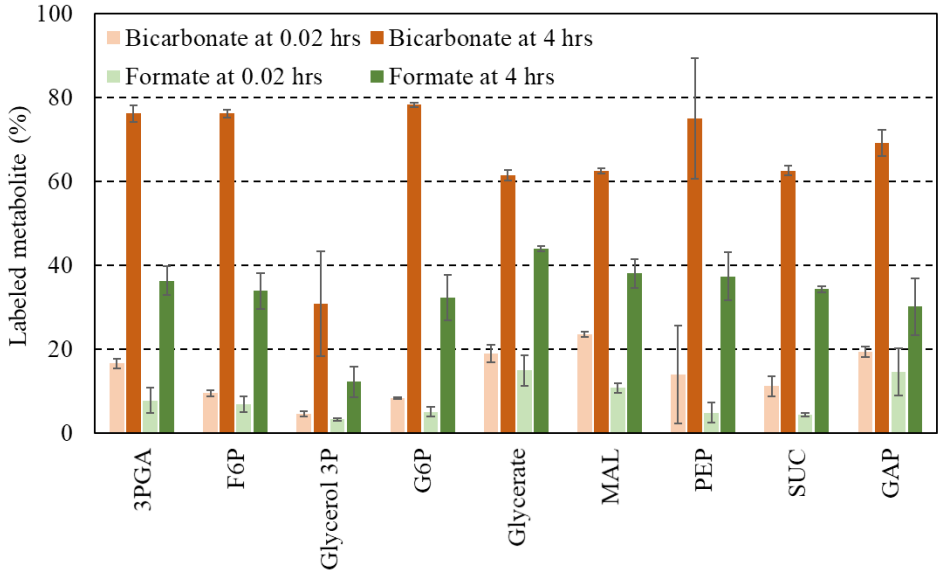


(h). TN concentration under 50 µMol/m^2/s

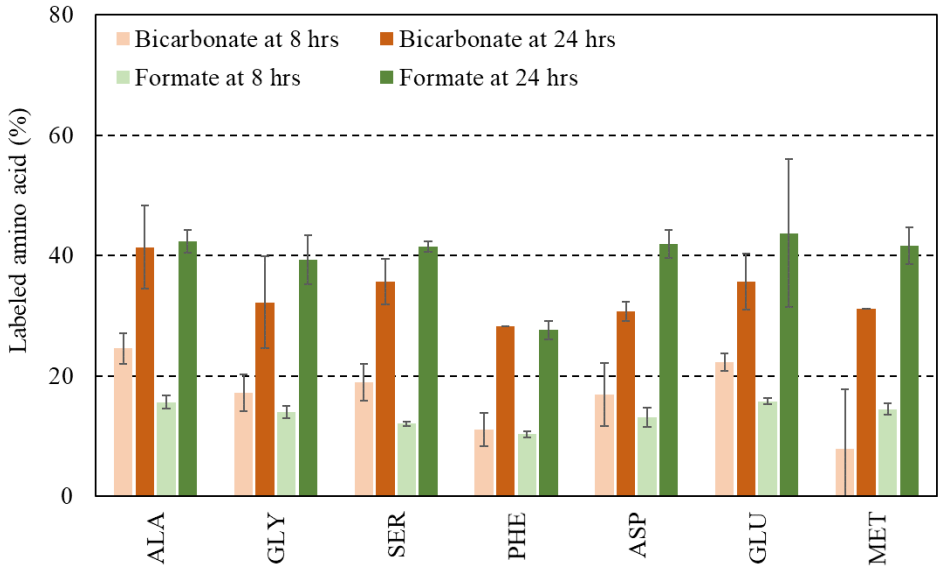
Figure 1


Bicarbonate at 0.02 hrs
Bicarbonate at 4 hrs
Formate at 0.02 hrs
Formate at 4 hrs
Labeled metabolite (%)
100
80
60
40
20
0
3PGA
F6P
Glycerol 3P
G6P
Glycerate
MAL
PEP
SUC
GAP

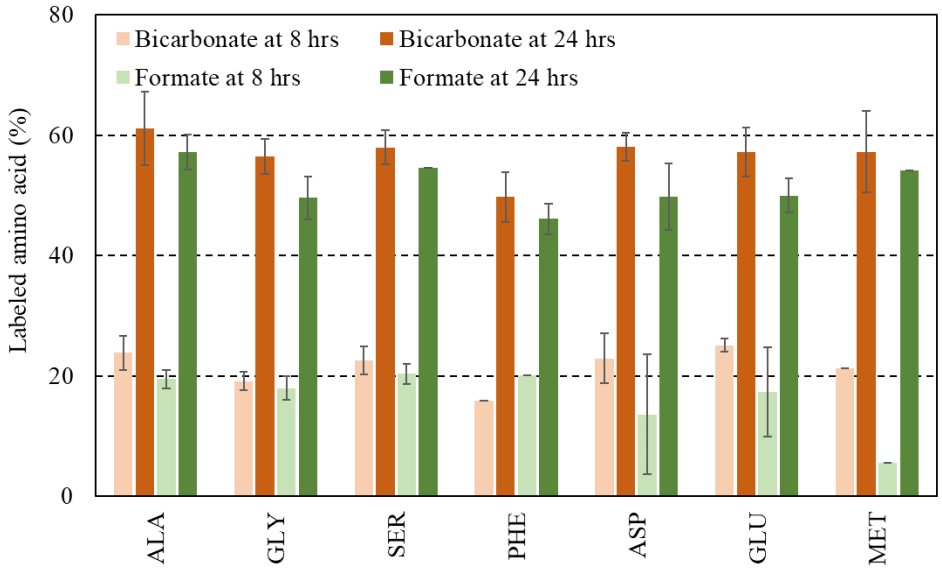
(a)


Bicarbonate at 0.02 hrs
Bicarbonate at 4 hrs
Formate at 0.02 hrs
Formate at 4 hrs
Labeled metabolite (%)
100
80
60
40
20
0
3PGA
F6P
Glycerol 3P
G6P
Glycerate
MAL
PEP
SUC
GAP

(b)


Bicarbonate at 8 hrs
Bicarbonate at 24 hrs
Formate at 8 hrs
Formate at 24 hrs
Labeled amino acid (%)
0
20
40
60
80
ALA
GLY
SER
PHE
ASP
GLU
MET

(c)


Bicarbonate at 8 hrs
Bicarbonate at 24 hrs
Formate at 8 hrs
Formate at 24 hrs
Labeled amino acid (%)
0
20
40
60
80
ALA
GLY
SER
PHE
ASP
GLU
MET

(d)

Figure 2.

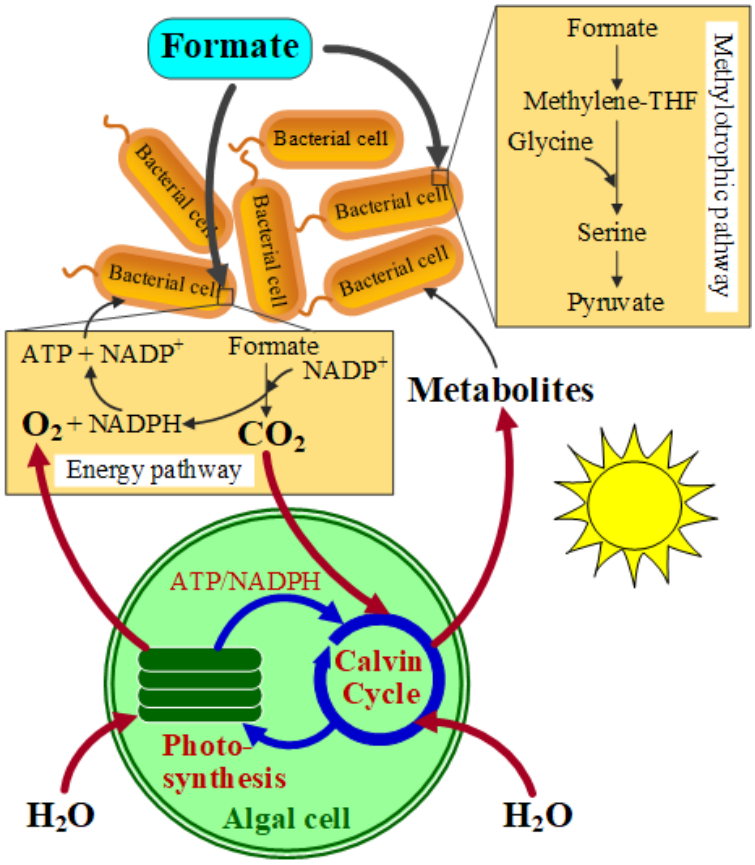

Formate
Bacterial cell
Bacterial cell
Bacterial cell
Bacterial cell
Bacterial cell
Bacterial cell
Formate
Methylene-THF
Glycine
Serine
Pyruvate
Methylotrophic pathway
ATP + NADP+
Formate
NADP+
O2 + NADPH
CO2
Energy pathway
Metabolites
ATP/NADPH
Calvin Cycle
Photo-synthesis
Algal cell
H2O
H2O

Figure 3

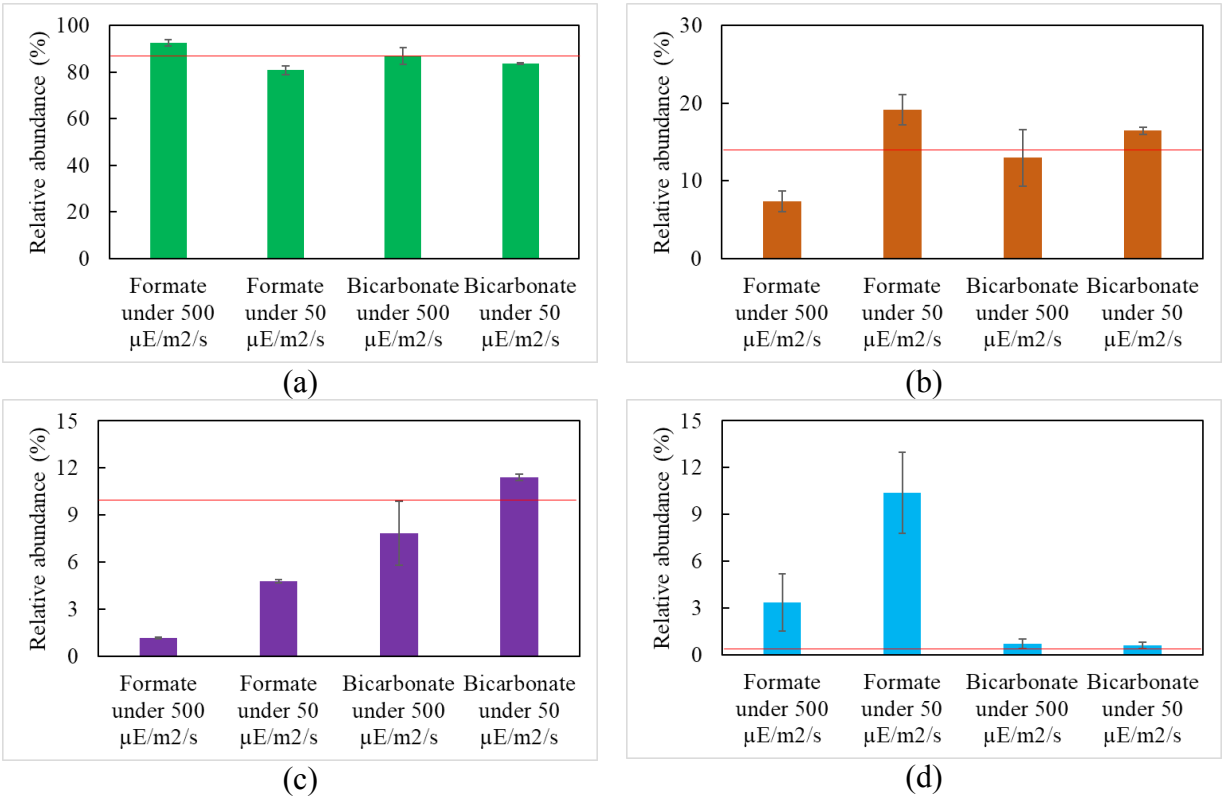
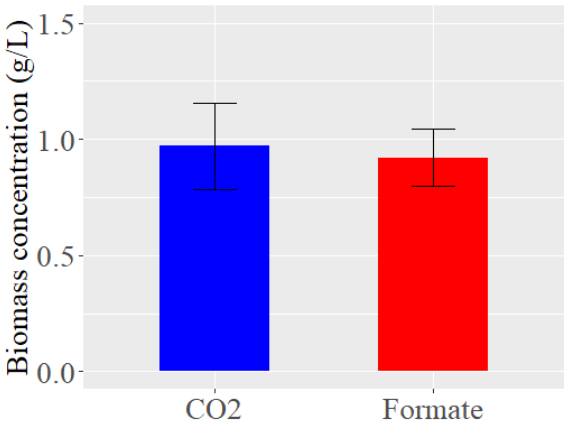
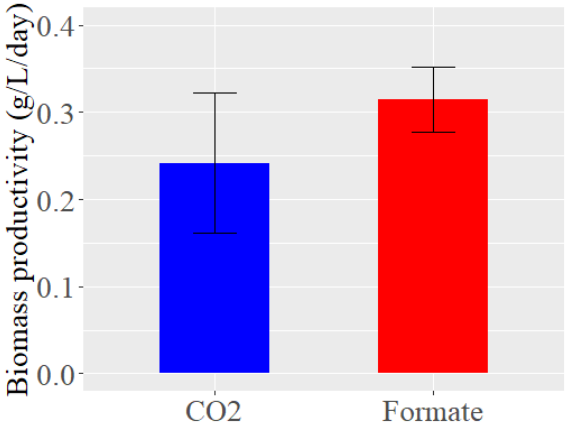

Relative abundance (%)
100
80
60
40
20
0
Formate under 500 µE/m2/s
Formate under 50 µE/m2/s
Bicarbonate under 500 µE/m2/s
Bicarbonate under 50 µE/m2/s
(a)
Relative abundance (%)
30
20
10
0
Formate under 500 µE/m2/s
Formate under 50 µE/m2/s
Bicarbonate under 500 µE/m2/s
Bicarbonate under 50 µE/m2/s
(b)
Relative abundance (%)
15
12
9
6
3
0
Formate under 500 µE/m2/s
Formate under 50 µE/m2/s
Bicarbonate under 500 µE/m2/s
Bicarbonate under 50 µE/m2/s
(c)
Relative abundance (%)
15
12
9
6
3
0
Formate under 500 µE/m2/s
Formate under 50 µE/m2/s
Bicarbonate under 500 µE/m2/s
Bicarbonate under 50 µE/m2/s
(d)

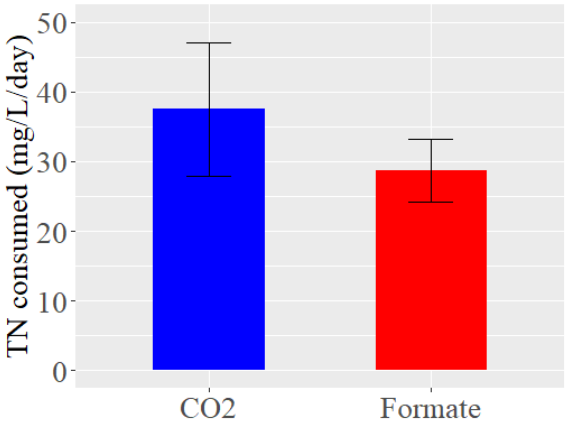
Figure 4


Biomass concentration (g/L)
1.5
1.0
0.5
0.0
CO2
Formate

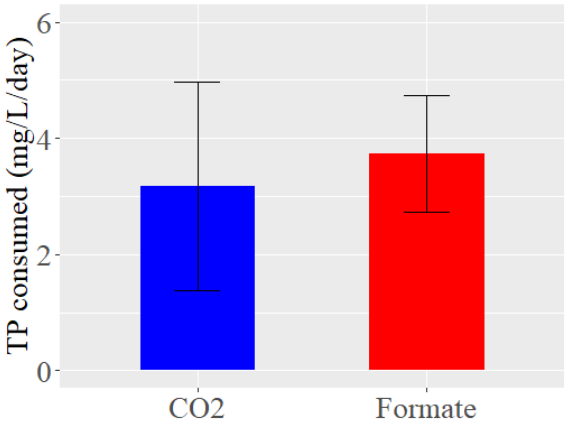
(a)


Biomass productivity (g/L/day)
0.4
0.3
0.2
0.1
0.0
CO2
Formate

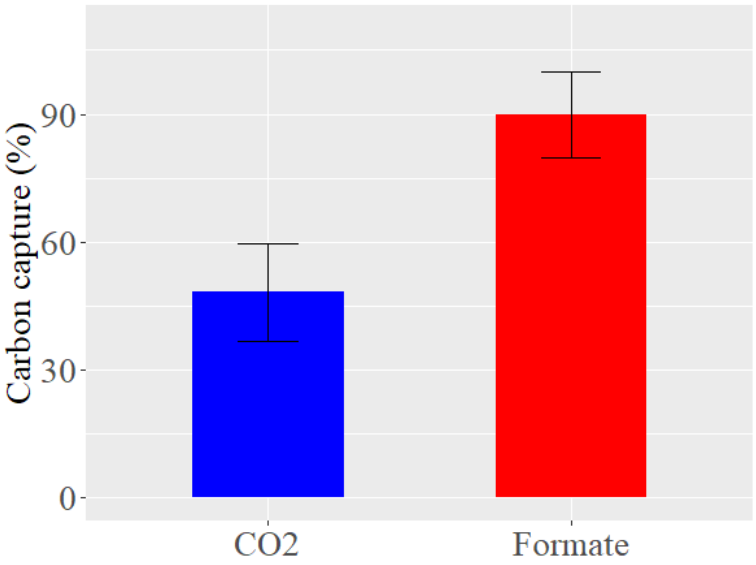
(b)


TN consumed (mg/L/day)
50
40
30
20
10
0
CO2
Formate

(c)

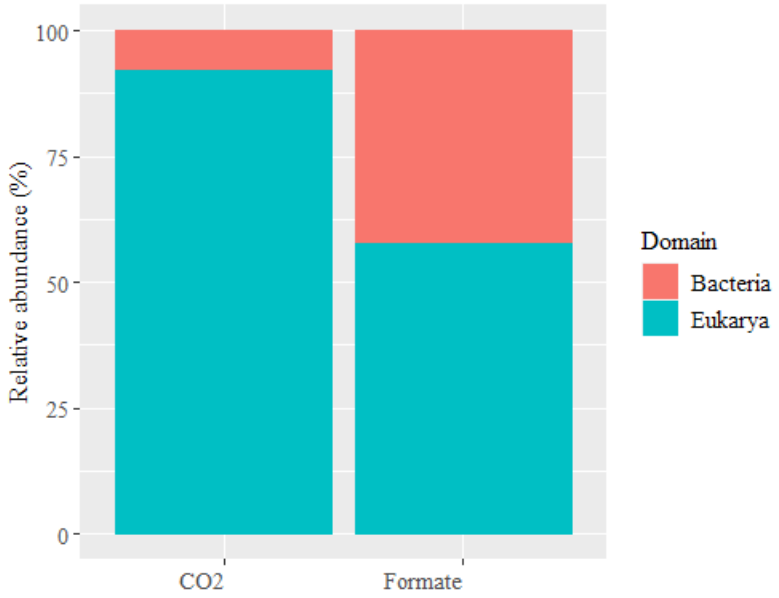

TP consumed (mg/L/day)
6
4
2
0
CO2
Formate

(d)

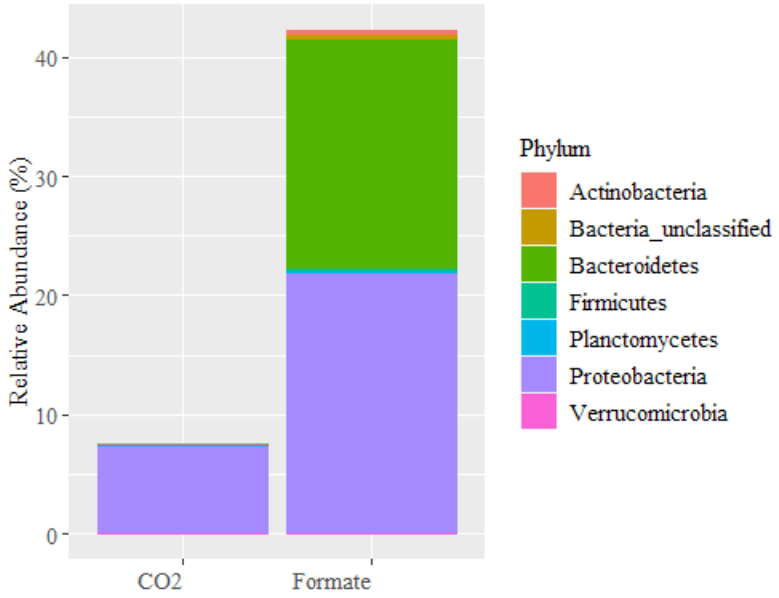


(e)

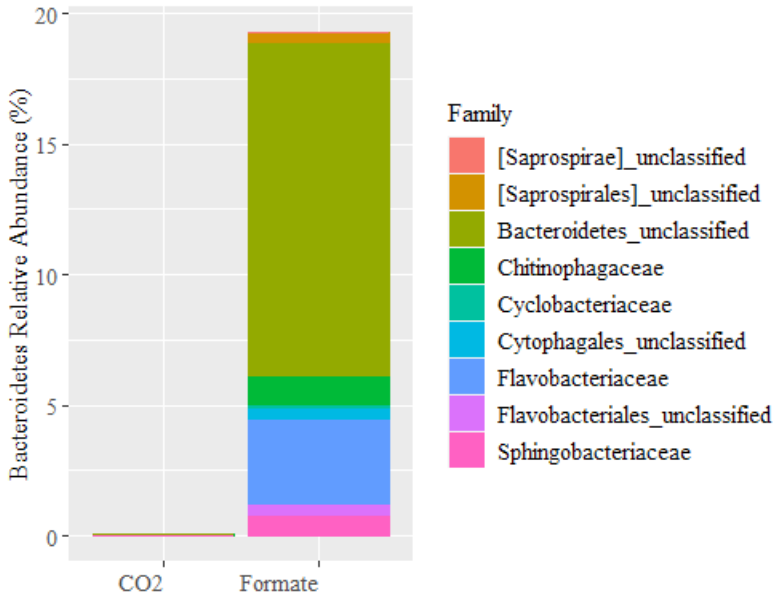
Figure 5


100
75
50
25
0
Relative abundance (%)
CO2
Formate
Domain
Bacteria
Eukarya

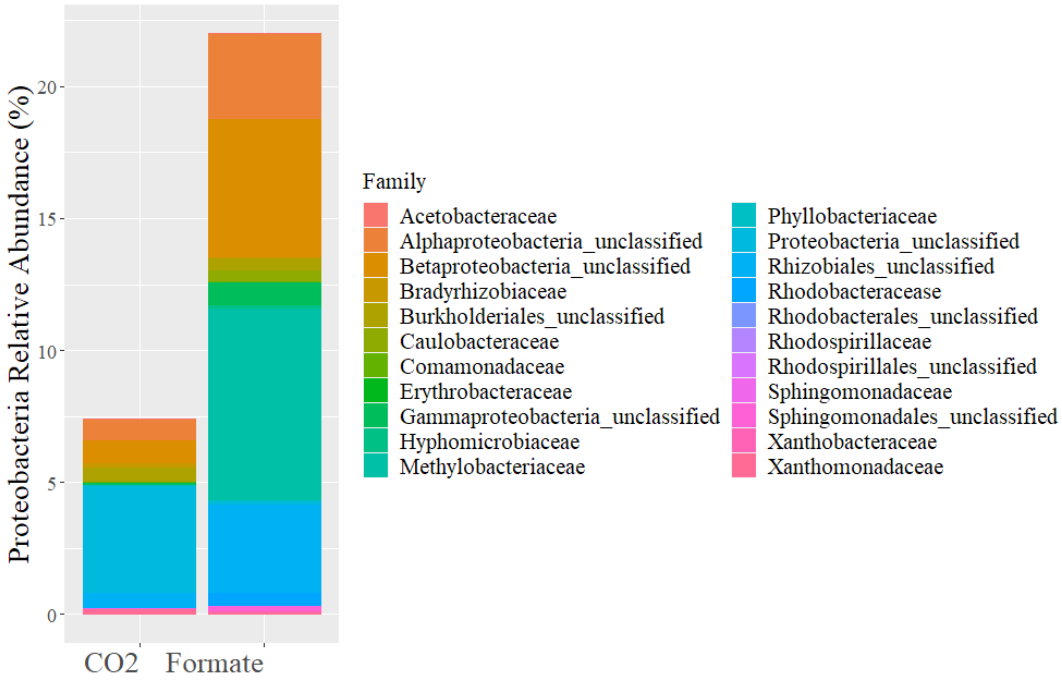
(a)


40
30
20
10
0
Relative Abundance (%)
CO2
Formate
Phylum
Actinobacteria
Bacteria_unclassified
Bacteroidetes
Firmicutes
Planctomycetes
Proteobacteria
Verrucomicrobia

(b)


Bacteroidetes Relative Abundance (%)
20
15
10
5
0
CO2
Formate
Family
[Saprospirae]_unclassified
[Saprospirales]_unclassified
Bacteroidetes_unclassified
Chitinophagaceae
Cyclobacteriaceae
Cytophagales_unclassified
Flavobacteriaceae
Flavobacteriales_unclassified
Sphingobacteriaceae

(c)


Proteobacteria Relative Abundance (%)
20
15
10
5
0
CO2
Formate
Family
Acetobacteraceae
Alphaproteobacteria_unclassified
Betaproteobacteria_unclassified
Bradyrhizobiaceae
Burkholderiales_unclassified
Caulobacteraceae
Comamonadaceae
Erythrobacteraceae
Gammaproteobacteria_unclassified
Hyphomicrobiaceae
Methylobacteriaceae
Phyllobacteriaceae
Proteobacteria_unclassified
Rhizobiales_unclassified
Rhodobacteracease
Rhodobacterales_unclassified
Rhodospirillaceae
Rhodospirillales_unclassified
Sphingomonadaceae
Sphingomonadales_unclassified
Xanthobacteraceae
Xanthomonadaceae

(d)

Figure 6

Table 1. Biomass concentration and yield on formate and bicarbonate

Culture conditions		Biomass yield with respect to carbon source (g biomass/g substrate)*	Final biomass concentration (g biomass/ L)
500 μMol/m^2/s	Formate	0.44 ± 0.01	0.12± 0.00
	Bicarbonate	0.23 ± 0.06	0.16± 0.03
50 μMol/m^2/s	Formate	0.49 ± 0.04	0.15± 0.02
	Bicarbonate	0.28 ± 0.05	0.13± 0.02

*: Biomass yield is calculated using biomass dry matter at the end of culture divided by total formate and bicarbonate ions (without counting sodium ion) consumed.

Table 2. Element contents of algal biomass from cultures on different carbon sources

Carbon source	Carbon (% dry matter)	Hydrogen (% dry matter)	Nitrogen (% dry matter)
Formate	47.24±2.67	6.92±0.27	7.99±0.85
CO_2	48.85±0.46	7.39±0.04	8.78±0.56

Table 3. Diversity and evenness of microbial communities of the algae assemblage on formate and CO_2

Carbon source	Frequency [a]	H[b]	J[c]
Formate	26	1.52±0.20	0.47±0.06
CO_2	18	0.39±0.08	0.13±0.03

[a] Frequency: numbers of observed frequency.
[b] H: Shannon's index which indicates the diversity of the microbial community.
[c] J: Pielou's index which indicates the evenness of the microbial community.