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Photosynthesis response of microalgae (*Tetradesmus wisconsinensis*) to different inorganic carbon sources probed with chlorophyll fluorescence analysis

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Abstract

Bicarbonate is an alternative carbon source for production of photosynthetic microalgae, although sparging of CO_2-enriched gas is commonly practised, its efficiency seems to be scarcely considered. This study evaluated the photosynthetic activities of microalgae (*Tetradesmus wisconsinensis*) using chlorophyll fluorescence under different concentrations of bicarbonate (HCO_3^-) and CO_2. Chlorophyll fluorescence analysis showed noticeable difference in photosynthetic activity between the bicarbonate (HCO_3^-) and CO_2 supplied cultures. The JIP-test parameters obtained for the cultures supplemented by HCO_3^- or CO_2 were distinct, but of similar values in quantum yield of PSII. The typical polyphasic rise, called the OJIP curves, showed a typical difference between the treatments at the J and I inflection of the fluorescence transient curve. Both HCO_3^- (20 mM) and CO_2 (5%, v/v) gave higher biomass yield than the other carbon regimes, *i.e*., 673 ± 12 and 658 ± 11 mg L^{-1}, respectively. We concluded that bicarbonate resulted in similar photosynthetic yield as CO_2 supplementation.

Additional key words: growth; medium; optical density; principal component analysis.

Introduction

Algae are effective to convert CO_2 and solar energy into various biomass, since they possess 20% higher photosynthetic efficiency compared to terrestrial plants (Wang *et al*. 2016). This is due to the fact that algae have CO_2-concentrating mechanism (CCM) in the carboxysome (*i.e*., intracellular structures filled with the enzyme Rubisco) which makes carbon fixation by Rubisco especially efficient (Nguyen and Rittmann 2016). In microalgae, carbon makes up ~ half the biomass dry mass and embodies about 70% of the electrons generated by PSII (Kim *et al*. 2010, Ducat *et al*. 2011, Nguyen and Rittmann 2016).

Recently, there has been substantial interest in using microalgae as the most productive biological systems for generating biomass and capturing carbon (Sayre 2010). Microalgae can be used to remove CO_2 from biogas in wastewater treatment, while also removing nitrogen and phosphorus from waste water (Meier *et al*. 2015).

Algae are able to use the different species of dissolved inorganic carbon, such as CO_2, HCO_3^-, and CO_3^{2-}, while the growth and physiology can be influenced by the type and amount of dissolved inorganic carbon (Chen *et al*. 2016). Commercial scale growth of microalgae in open and/or closed systems requires supplement of inorganic carbon as pure CO_2 (Gardner *et al*. 2013). However, cost efficiency of CO_2 supplementation as compared to bicarbonate supplementation is open to question, since during sparging of CO_2 gas into growth media, outgassing CO_2 is unavoidable. On top of that, the advantage of using bicarbonate in terms of growth, biomass, and bio-products did not get significant attention.

Photosynthetic activity can be a useful parameter to investigate the growth of microalgae supplemented either with CO_2 or bicarbonate as inorganic carbon source. In addition, it is well documented that there is a significant relationship between photosynthesis and chlorophyll (Chl) *a* fluorescence. Hence, Chl fluorescence is widely used as a reliable tool for measuring photosynthetic effi-

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Abbreviations: BBM – Bold's basal medium; CCM – carbon concentrating mechanism; NPQ – nonphotochemical quenching; OD – optical density; PQ – plastoquinone; Q_A – primary quinone acceptor of PSII; Q_B –secondary quinone acceptor of PSII.
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ciency, particularly of PSII (Maxwell and Johnson 2000, Strasser *et al*. 2000, Baker and Rosenqvist 2004, Baker 2008, Stirbet and Govindjee 2011). High excitation light is used to induce Chl fluorescence rise in photosynthetic organisms, and fluorescence signals during the fluorescence rise are recorded to obtain information about photosynthetic capacity of sample organisms (Lazár 2006).

The fluorescence rise typically exhibits several phases (*i.e*., polyphasic), for which the fluorescence signals can be plotted as OJIP curve, where O is the origin, the first measured minimal level, J and I are intermediate levels, and P is the peak (Strasser *et al*. 2000, Stirbet and Govindjee 2011). Because the fluorescence signals are assumed to originate from PSII, the OJIP curve is used to characterize the quantum yield of PSII photochemistry and the electron transport activity (Stirbet and Govindjee 2011). Studies have shown that the fast rise and declining phase of the fluorescence transient is correlated with an increase in the CO_2 assimilation (Strasser *et al*. 2000). On top of that, the PSII-operating quantum efficiency is linearly related with CO_2 assimilation (Genty *et al*. 1989).

The objective of this study was to investigate effects of inorganic carbon uptake on photosynthesis of the freshwater green alga, *Tetradesmus wisconsinensis*. The photosynthetic response to different sources and concentrations of inorganic carbon was investigated by fast Chl fluorescence transient responses ('JIP-test'). It is an additional aim to evaluate to what extent such a JIP-test method can be a useful tool to investigate how different inorganic carbons supplies influence algae production. The results are also discussed regarding insight on the supplementation of bicarbonates as an alternative carbon source in commercial growth of microalgae and monitoring photosynthesis using chlorophyll fluorescence parameters.

Materials and methods

Microalgae and culture condition: The freshwater Chlorophyta, *Tetradesmus wisconsinensis*, was chosen as the model organism for this study. The *T. wisconsinensis* strain used for this study was isolated from a field water sample collected from Norsjø Lake in Telemark (59°12'N, 9°32'E), Norway, and has been maintained as a pure culture. The inoculum was cultured in a 250-ml conical flask without aeration under PAR of 30 µmol(photon) m^{-2} s^{-1} with a photoperiod of 18/6-h light/dark for two weeks prior to the start of the experiment.

A modified Bold's basal medium (BBM) (Oh-Hama and Miyachi 1988) was used as the culture medium. BBM was selected due to its extensive use for freshwater algal species. The medium was prepared with distilled water in a 10-L borosilicate flask, and was autoclaved at 120°C for 20 min. Then the medium was left overnight and 600 ml of the medium was transferred to 12 heat-sterilised culture flasks. Pre-weighed $NaHCO_3$ was added to the culture media used for the bicarbonate-treated cultures.

Experimental setup: The experiment was performed as batch cultivations using 1-L Erlenmeyer flasks with cotton plugs (Fig. 1). Two batch experiments were conducted using $NaHCO_3$ and CO_2 gas as inorganic carbon sources. In the first experiment, four treatments were made using different concentrations of $NaHCO_3$ (0, 5, 10, and 20 mM) added at the start of the experiment. For the second experiment, four inorganic carbon regimes were tested: (*1*) aeration only, (*2*) $NaHCO_3$ (20 mM) and aeration, (*3*) CO_2 gas (5%, v/v) and aeration, and (*4*) combination of $NaHCO_3$ (20 mM), CO_2 gas (5%, v/v), and aeration. In both batch experiments, there were three replicate cultures for each experimental treatment units. Each treatment medium was inoculated with 9 mL of the *T. wisconsinensis* inoculum culture.

The experiment was conducted in a fume hood at constant temperature of 19 ± 2°C. The hood was covered with black curtains to avoid interference from other possible light sources. The light source for the experimental cultures was provided by two *CIVILIGHT* (Eschborn, Germany) white LED tube batten (10 W, 584 × 25 ×

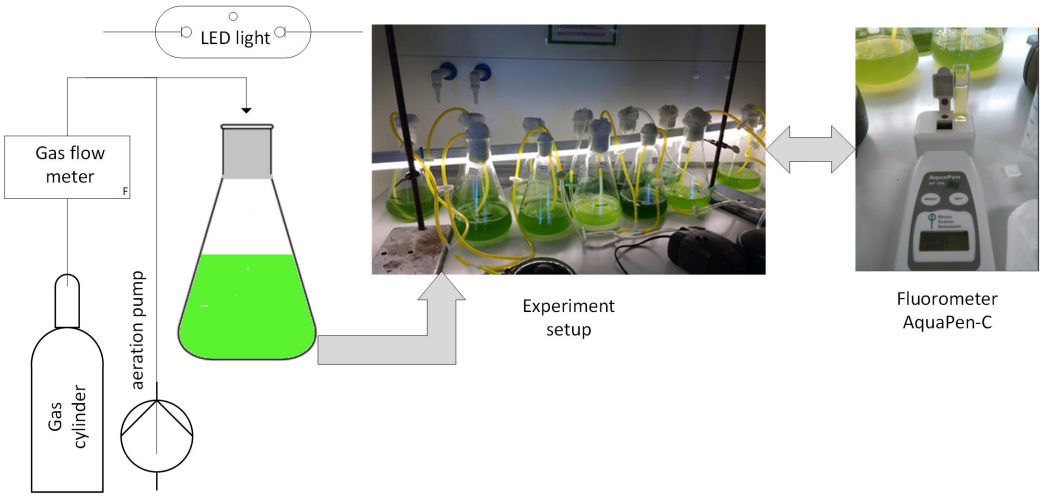

Fig. 1. Sketch and pictures of experimental setup and fluorimeter. The setup consisted of 12 Erlenmeyer flasks with cotton plugs, CO_2 gas supply, aeration pump, and LED light. Measurement of chlorophyll fluorescence was carried out with fluorimeter *AquaPen-C*.

35 mm) connected as one elongated light tube with a series cable connection. The light tube was placed in the middle of two parallel rows of *T. wisconsinensis* cultures, so that all cultures received the light from obliquely above the culture flasks. The measured average light intensities at each culture flask was 70–75 μmol(photon) m^{-2} s^{-1}. The light was measured using a *LI-COR* light meter (*LI-250*, Lincoln, Nebraska, USA) at several points on the surface of the culture flasks. The light period was set to 16/8 h of a light/dark cycle. The light/dark cycle was controlled by a digital control timer (*AX 300*). Moderate aeration sterilised through 0.25-μm pore size filter was provided by aeration pumps (*EHEIM*, Stuttgart, Germany) using tygon tubes (*Tygon*® tubing).

Sample collection and measurements: Optical density (OD) was used for the measurement of the growth of *T. wisconsinensis* cultures. OD was measured every other day using *Spectroquant Pharo 300* (*Merck KGaA*, Darmstadt, Germany) at the wavelength of 740 nm. Samples for the total dissolved inorganic carbon (TIC) were collected using syringes and filtered through 0.25-μm pore size disposable filters into 20-mL vials. The samples were filled to the top of the vials to have minimum air interference before the analyses. The pH of the nutrient solution was measured before and after addition of bicarbonates at the start of the experiment, and the pH of all cultures was measured at the end of the experiment.

Measurement of Chl *a* fluorescence parameters: The measurement of the Chl fluorescence was done using a hand-held fluorometer *AquaPen-C AP-C 100* (*Photon Systems Instuments*, Drasov, Czech Republic). The *AquaPen* is equipped with blue and red LED emitter, to deliver PAR values of up to 3,000 μmol(photon) m^{-2} s^{-1} measuring light to generate maximal fluorescence (F_m) and other fluorescence parameters (Strasser *et al*. 2000). The fluorometer uses blue excitation light (455 nm) for Chl excitation especially for measuring Chl fluorescence in algal cultures. Samples of 4 to 5 mL were dark-adapted for 10 min in the *AquaPen* cuvettes before the measurement. The dark-adaptation time was chosen prior to commencement of the experiment by measuring the time giving the highest Q_Y values (*i.e.*, the maximum quantum yield of PSII in dark-adapted state) in three samples of *T. wisconsinensis* cultures grown with aeration. The samples were diluted accordingly with the BBM medium when the OD measurement exceeded 0.5. The *FluorPen* software (*Photon Systems Instruments*) was used to conduct JIP-test and extract the data. The OJIP parameters listed in Appendix were measured for each sample at different growing days.

Measurement of nutrients and pH: Concentrations of major nutrients, NO_3^- and PO_4^{3-}, were monitored during the experiment. Samples were collected using syringes and filtered through 0.25-μm pore size disposable filters into 20-mL vials and stored at 4°C before the analysis. The measurements were conducted for two replicate cultures for each treatment using ion chromatography (*Dinex ICS-500*, Slunnyvale, CA, USA). The pH was measured with an *inoLab pH7110* pH meter (*WTW*, Weilheim, Germany).

Data analysis: The huge amount of data generated by fluorometer *AquaPen-C* in the *FluorPen* software was transferred to *Microsoft Excel* for data processing. The means of each parameter measurements between the three parallels were used for statistical comparisons. Data analyses were done for outlier effect, normality test, equality, and analysis of variance (*ANOVA*). For the OJIP analysis, the recorded curves (n = 3) were averaged and normalized to F_0 and F_m in order to distinguish changes in the intermediate steps. Principal component analysis was applied to identify the patterns of the fluorescence parameter and variations in the experimental values. The *R* programming statistical tool (*R version 3.4.2*, www.r-project.org) was used for the data analysis, statistical test, and PCA.

Results

Growth of *T. wisconsinensis*: Optical density of the cultures was measured as an indication of changes in biomass concentrations during the experiments. In the first batch experiment, the culture, which grew in the culture media containing 20 mM bicarbonate (HCO_3^-), showed the highest biomass concentration followed by the cultures that were grown in the media containing 10 and 5 mM bicarbonate, respectively (Fig. 2*A*). The culture that was grown in the media without bicarbonate addition (*i.e.*, no HCO_3^- culture) showed the lowest biomass concentration during the experiment. Rapid increases of biomass concentration were observed from day 9 to 14 in all cultures with the lowest increase in the culture without HCO_3^- supplementation. Meanwhile, cultures that were grown in the media containing 10 and 5 mM bicarbonate showed the decline in growth after 14 d.

In the second batch experiment, the OD results showed that cultures grown in 20 mM HCO_3^-, CO_2, and 20 mM $HCO_3^- + CO_2$ gave higher biomass increases in the cultures compared with the cultures grown only with aeration (Fig. 2*B*). There was no significant difference in the OD measured between cultures grown in 20 mM HCO_3^- and CO_2 cultures, while both cultures exhibited higher biomass concentrations compared to the mixture of 20 mM $HCO_3^- + CO_2$ cultures.

In both batch experiments, the differences between the OD measurements among the cultures were noticeable after 9 d. The results indicated that single source of inorganic carbon, either 20 mM HCO_3^- or CO_2, enhanced biomass growth compared to supplementation of mixture of these two carbon sources. At the end of the experiment, 20 mM HCO_3^- and CO_2 cultures showed nearly similar biomass concentrations of 673 ± 124 and 658 ± 117 mg L^{-1}, respectively, and their biomass concentrations were significantly higher than that of treatments supplied only by aeration.

Moreover, the nutrient analysis showed that none of the cultures was limited by the supply of nitrogen or

phosphorus (data not shown). The original pH of BBM before adding HCO_3^- was 6. The pH increased when bicarbonate was added (Fig. 3), while the pH of CO_2 culture media dropped at the start from 6 to 5 and stayed in the range of 5 to 5.6 during the whole experimental period.

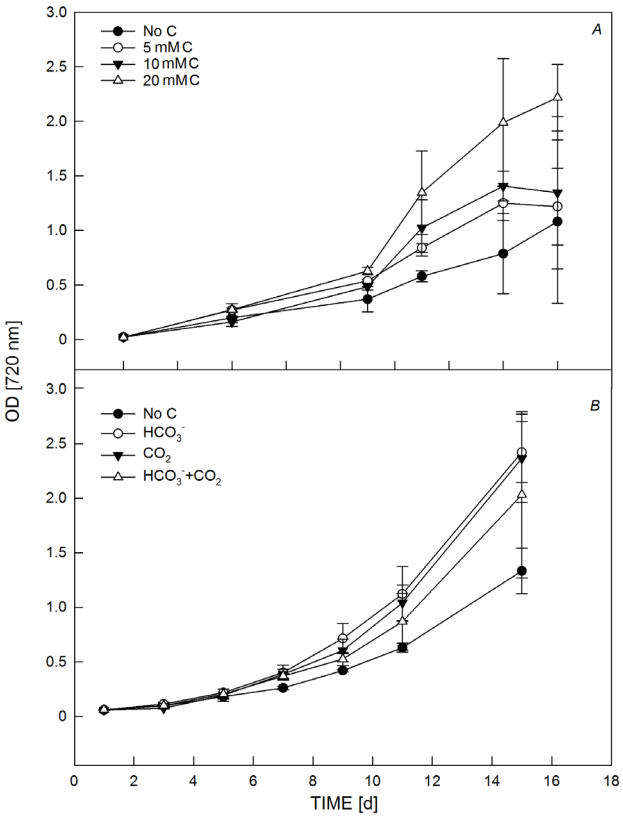

Fig. 2. Changes in optical densities of the microalgae *Tetradesmus wisconsinensis* batch cultures grown in the media prepared with different concentrations of HCO_3^- (*A*) and cultures grown in 20 mM HCO_3^-, CO_2 gas (5%, v/v), and combination of 20 mM HCO_3^- and CO_2 gas (5%, v/v) in the culture media (*B*). The error bar represents the standard deviation, $n = 3$.

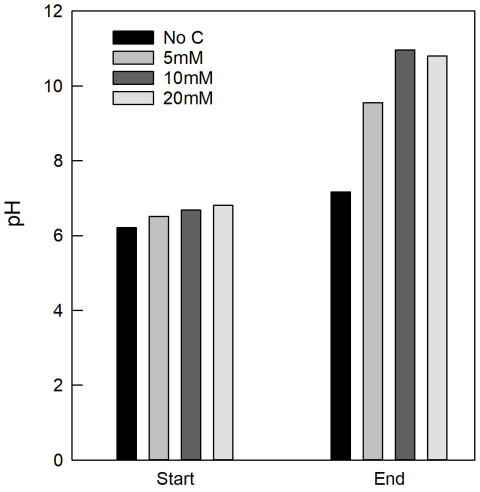

Fig. 3. The pH of original Bold's basal medium before adding HCO_3^- and after adding different concentration of HCO_3^-.

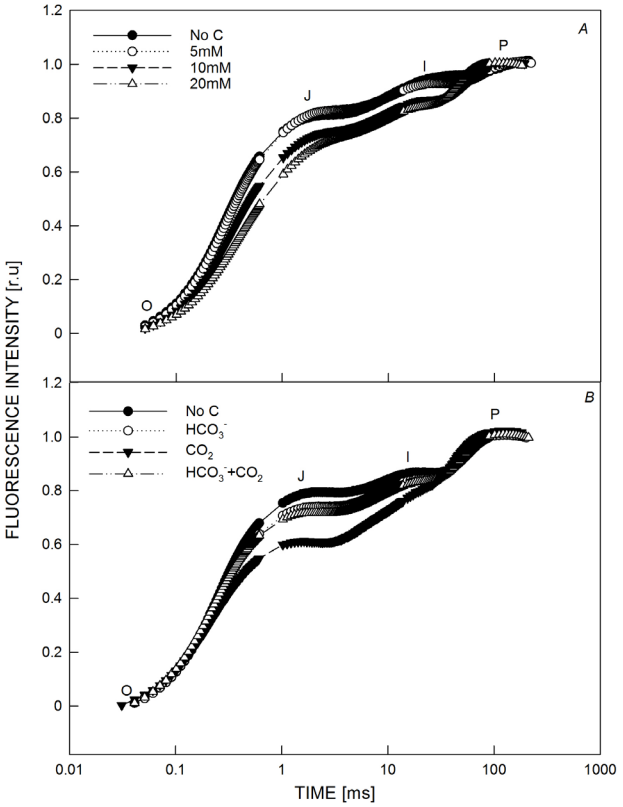

Fig. 4. Rapid chlorophyll fluorescence induction curves of *Tetradesmus wisconsinensis* during first experiment with different concentrations of $NaHCO_3$ (0, 5, 10, and 20 mM) (*A*) and second experiment with four inorganic carbon regimes [aeration only, $NaHCO_3$ (20 mM), CO_2 gas (5%, v/v), and combination of $NaHCO_3$ (20 mM) and CO_2 gas (5%, v/v)] (*B*). The induction curves were measured after dark adaptation of the culture for 10 min. The recorded curves ($n = 3$) were averaged and normalized to F_0 and F_m in order to distinguish changes in the intermediate steps (J and I) that represent various reduction states of the PSII electron carriers. r.u. = relative units.

OJIP curve: In both experimental tests, the typical polyphasic rise, called the OJIP curve was observed (Fig. 4). There was a typical difference between the treatments in the J and I inflection curve of the fluorescence transient curve. The high J peak was also observed for the treatment with low inorganic carbon and a dip after the J inflection for the application of carbonates as pure CO_2.

Chl *a* fluorescence parameters (JIP-test parameters): The bicarbonate and CO_2-supplemented cultures showed similar pattern in F_m during the growth period (Fig. 5). F_m increased to an optimum and became stable afterwards, but it was different between the treatments. The maximum photochemical efficiency of PSII (F_v/F_m) was 0.7 in the first measurement (*i.e.*, 3 d of growth), but started to decrease with the growth of algae and decreased to 0.4 for cultures grown in CO_2 (Fig. 6*A*). Similarly, F_v/F_0 declined markedly in bicarbonate- and CO_2-supplemented cultures over the growth period until it reached steady state after 9 d (Fig. 6*B*). F_v/F_m and F_m/F_0 values were high and no significant difference between the treatments was found

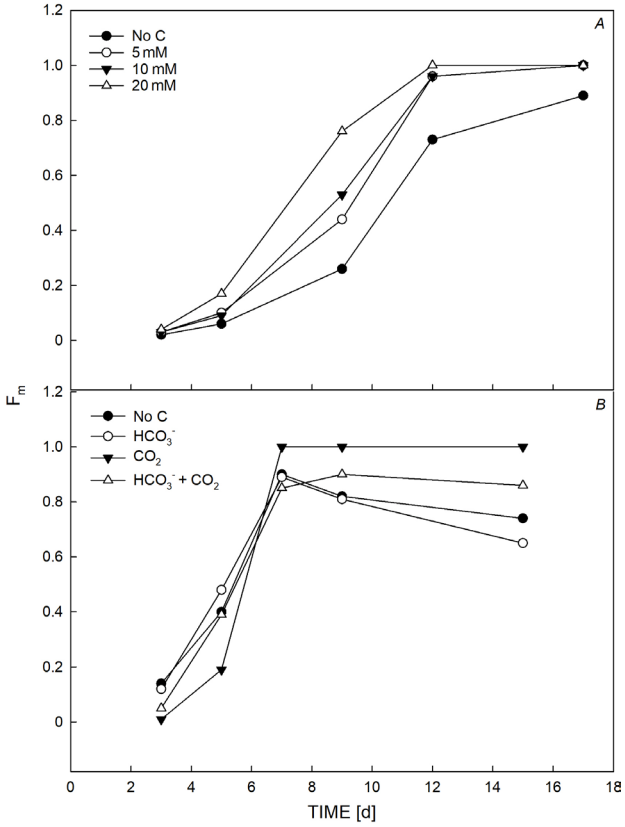

Fig. 5. Changes in maximum fluorescence intensity (F_m) in the first experiment with different concentrations of $NaHCO_3$ (0, 5, 10, and 20 mM) (*A*) and second experiment with four inorganic carbon regimes [aeration only, $NaHCO_3$ (20 mM), CO_2 gas (5%, v/v), and combination of $NaHCO_3$ (20 mM) and CO_2 gas (5%, v/v)] (*B*). The recorded measurements for each treatment culture were averaged. The error bar represents the standard deviation, $n = 3$.

during the early growth period for both bicarbonate and CO_2-supplemented culture. Meanwhile, the number of Q_A reducing RC_S per PSII antenna Chl (ABS/RC) increased with the decrease of F_v/F_m and F_v/F_0 (Fig. 6*C*).

Principal component analysis (PCA): Fluorescence parameters were selected for the PCA analysis to identify the variables that reflect the maximal changes. The dimension 1 (principal component 1) explained most of the variability which accounted for 61.3% and dimension 2 (principal component 2) accounted for 22.7%, respectively (Fig. 7). The positive and negative correlation between the parameters also shows the variation of the parameters in the respective principal components (dimensions) (Table 1). For instance, the Chl fluorescence parameters F_0, φ_{Do}, and DI_0/RC were highly correlated with each other and negatively correlated with F_v/F_m and F_v/F_0 in the dimension 1.

Discussion

Growth of *T. wisconsinensis*: OD measures microalgae cell growth based on the effect on the optical property,

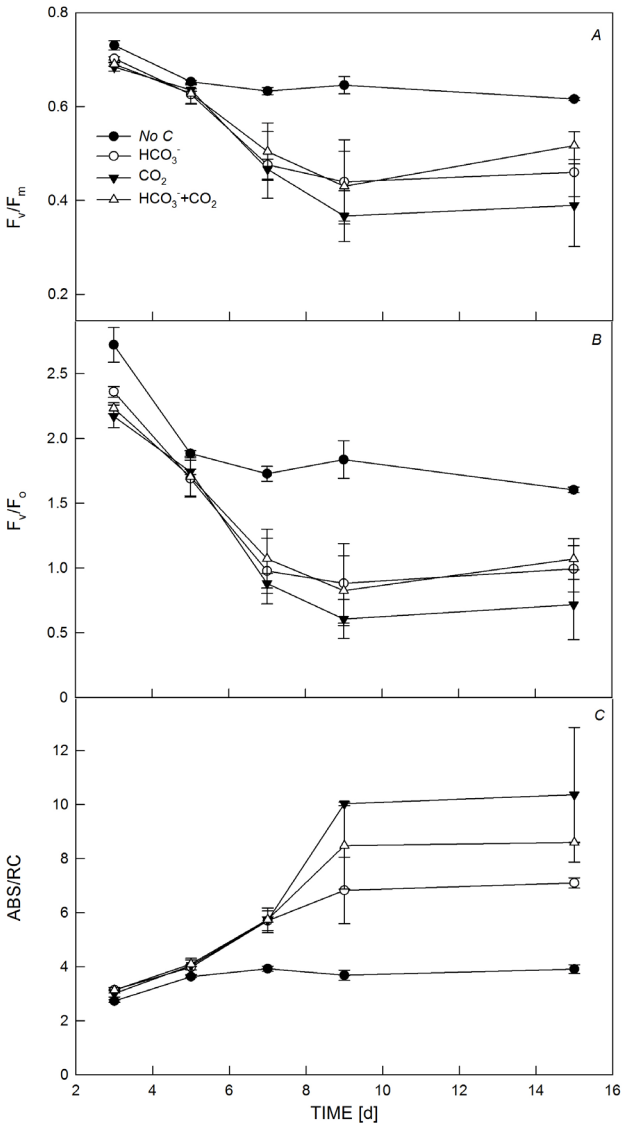

Fig. 6. Changes in fluorescence parameters, F_v/F_m (the maximum quantum yield of PSII) (*A*), F_v/F_0 (conformation term for primary photochemistry) (*B*), and ABS/RC (the number of Q_A reducing RCs per PSII antenna Chl) (*C*). The recorded measurements for each treatment culture ($n = 3$) were averaged. The error bar represents the standard error.

i.e., absorbed, transmitted, and scattered light. Microalgae growth typically shows three different phases in batch culture: lag, exponential, and stationary phase (Lee *et al.* 2015). The optical density exhibited typical difference between the treatments after 9 and 7 d for the first and second batch experiments, respectively (Fig. 2). This could be due to the growth phase in both cultures had few days of the lag phase followed by several days of the exponential growth phase, which prolonged until the end of the experiment. Due to the low pH in the CO_2-gas sparging medium, the early growth period had a noticeable few days lag phase in biomass production. Apparently, the first batch culture started to reach the stationary phase of the growth at the end of the experiment. Since OD values

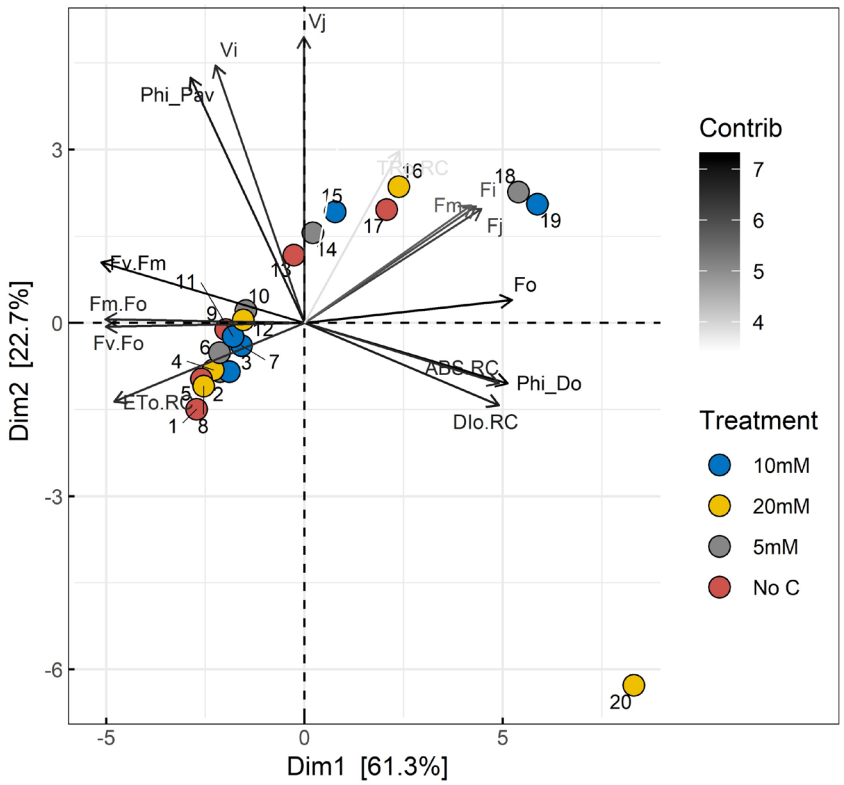

Fig. 7. The principal analysis with four treatment conditions. The PCA is based on the chlorophyll fluorescence data and the loading vector, *i.e.*, arrows represent the fluorescence parameters on the corresponding dimensions (Dim1 and Dim2), where Dim1 explain most of the variability in the data.

Table 1. The correlation and *P*-value of the different chlorophyll parameters in the two dimensions, *i.e.*, Dim1 and Dim2. Explanation of the OJIP parameters is listed in Appendix.

Description of dimension 1	Correlation	*P*-value	Description of dimension 2	Correlation	*P*-value
F_0	0.98	8.8×10^{-15}	V_J	0.93	2.8×10^{-9}
φ_{Do}	0.96	8.2×10^{-12}	V_I	0.84	3.9×10^{-6}
DI_0RC	0.92	7.9×10^{-9}	Phi Pav	0.79	2.4×10^{-5}
ABS/RC	0.92	8.0×10^{-9}	F_v	0.65	1.7×10^{-3}
F_J	0.84	3.8×10^{-6}			
F_I	0.81	1.4×10^{-5}			
F_m	0.79	3.4×10^{-5}			
TR_0/RC	0.45	4.6×10^{-2}			
ET_0/RC	–0.90	5.6×10^{-8}			
F_v/F_0	–0.94	5.1×10^{-10}			
F_m/F_0	–0.94	3.6×10^{-10}			
F_v/F_m	–0.96	8.7×10^{-12}			

of microalgae suspension depends on time due to the growth of microalgae cells, OD could vary with the growth time, accumulation of pigment and lipid, cell division, and metabolism (Heng and Pilon 2014, Zhao *et al*. 2018). Provided that the cultures were affected by source and concentration of organic carbon, they showed different growth characteristics. However, the bicarbonate and CO_2 carbon sources were closely of similar growth intensity in our study. Therefore, our result suggests that bicarbonate and CO_2 can be used alternatively with similar effect on biomass growth of algae. In some studies, bicarbonates as an inorganic carbon source gave higher biomass and lipid production (Abinandan and Shanthakumar 2016, Mokashi *et al*. 2016).

OJIP curve: The rapid O–J rise (it ranges from 2 to 5 ms) is associated with primary photochemical reactions of PSII and reflects the accumulation of the reduced primary acceptor of PSII, Q_A^- (*i.e.*, closing of the open PSII) (Strasser *et al*. 2000, Malapascua *et al*. 2014). It indicates quick reduction of Q_A^- and closing of the PSII at low supply of the inorganic carbon. Hence, the high J peak for treatment with low inorganic carbon was due to the over reduction of Q_A and Q_B electron acceptors of PSII. Cultures with limited supply of inorganic carbon show the distinct J peak and shape of OJIP curve (Malapascua *et al*. 2014).

On the other hand, the dip after the J inflection reflects the movement of electrons from Q_A to Q_B which leads to high electron transport after the application of carbonates as HCO_3^- and/or pure CO_2 reflected by a low I inflection (Fig. 4). It is reported that a high I inflection is due to high level of Q_A and Q_B reduction and slowdown of electron transport beyond the P maximum (Govindjee 2004, Malapascua *et al*. 2014). The electron transport in the photochemistry leads to CO_2 fixation, otherwise the reduced electron transport results in low photosynthetic process (*i.e.*, the Calvin-Benson cycle is slowed down) and slow growth of the microalgae. Moreover, the photosynthetic activity and growth of microalgae species can be shown by different J and I inflection points that are indications of efficiency in the photosynthetic electron transport (Malapascua *et al*. 2014).

Chl *a* fluorescence parameters: In this study, Chl *a* fluorescence rise was measured to study effects of HCO_3^- and CO_2 on photosynthetic activities of *T. wisconsinensis*. The fluorescence parameters enabled to examine the photosynthesis efficiency, in particular, the PSII activities of *T. wisconsinensis* cultures grown with different HCO_3^- and CO_2 concentrations. For instance, the F_0 and F_m fluorescence parameters measures the absorption flux (*i.e*., number of photons) per cross section (Strasser and Srivastava 1995, Strasser *et al*. 2000), which is a function of Chl content and antenna size of the PSII. The fast increase in F_m with the bicarbonate, CO_2, and mixed culture of bicarbonate and CO_2 grown *T. wisconsinensis* showed a relative increase in the Chl content and better absorption of photons (Fig. 5). In our study, the bicarbonate supplementation gave maximum fluorescence comparative to the pure CO_2 supplementation.

On top of that the JIP-test has several parameters extracted from the fast Chl *a* fluorescence transient based on the PSII energy flux model (Strasser *et al*. 2000). F_v/F_m is one of the frequently used JIP-test parameters because it is a robust indicator of the maximum quantum yield of PSII chemistry. Principally, none of stressed microalgae show the value of F_v/F_m ranging between 0.7 and 0.8, and it correlates with the maximum quantum yield of photosynthesis (Masojídek *et al*. 2013, Murchie and Lawson 2013, Malapascua *et al*. 2014). In this study, F_v/F_m was 0.6 to 0.7 in the first measurement (*i.e*., 3-d growth) and started to decrease with the days (Fig. 6*A*). The lowest F_v/F_m was 0.4 for microalgae grown in a continuous flow of pure CO_2 (5%, v/v), and the decrease in F_v/F_m during the experiment was 42%. Whereas in the microalgae grown in a medium with no additional inorganic carbon the decrease in F_v/F_m was not significant. Similar studies has shown that F_v/F_m declined by over 62% with the growth of algal cells (Oukarroum 2016). It is reported that change in F_v/F_m could be due to nonphotochemical quenching (Maxwell and Johnson 2000).

We also observed that the F_v/F_0, which measures the efficiency of the water-splitting complex on the donor side of PSII (Kalaji *et al*. 2011) decreased after the first measurement on day 3 (Fig. 6*B*). When compared with F_v/F_m, the F_v/F_0 decreased significantly over the experimental period, *i.e*., with the growth of the algal culture. For instance, for microalgae grown in a continuous flow of pure CO_2 (5%, v/v), F_v/F_0 decreased over 60% after the first measurement, while it was 36% for the microalgae grown in a medium with no additional inorganic carbon source. This parameter is more sensitive even in a nonstressed microalgae culture but with high cell density and pigment content it decreases significantly. The decrease of F_v/F_m indicates inhibition of electron transport from the PSII reaction centers to the plastoquinone pool (Strasser *et al*. 2000, Kalaji *et al*. 2011, Oukarroum 2016).

Moreover, the number of Q_A reducing RCs per PSII antenna Chl (ABS/RC) increased with the decrease of F_v/F_m and F_v/F_0 (Fig. 6*C*). ABS/RC was nearly similar for all treated groups. However, it increased and showed small difference between the bicarbonate and CO_2 supplemented cultures after 7 d. This could be due to the decrease of the fraction of active reaction centers of microalgae in highly growing cultures (*i.e*., high cell density environment) and/or ageing microalgae cells. Study shows that the accumulation of inactive reaction centers is associated with the increased efficiency for dissipation of absorbed light energy as heat (Kalaji *et al*. 2011, Oukarroum 2016). Overall, the three parameters, *i.e*., F_v/F_m, F_v/F_0, and ABS/RC, enable to compare the photosynthetic characteristics of bicarbonate- and CO_2-supplemented cultures and the change of these parameters depends on algal cell growth, cell density, and pigment content.

Principal component analysis (PCA): The PCA is an effective statistical approach special for Chl fluorescence parameter data to investigate the change in the data set (Kalaji *et al*. 2014). The Dim1 (*i.e.*, principal component 1, PC1) (Fig. 7) explained most of the variability in the data set. The Dim1 was positively correlated with Chl parameters of F_0, φ_{Do}, DI_0/RC, and ABS/RC and negatively correlated with parameters of ET_0/RC, F_v/F_0, F_m/F_0, and F_v/F_m, respectively (Table 1). It shows the strong correlation that existed between these parameters. Parameters, which had equal weight, for instance DI_0/RC and ABS/RC, indicate that the parameters are strongly correlated with each other. The Chl fluorescence parameters could be different with treatment conditions and give different contribution in the principal components (Kalaji *et al*. 2014). Here, the treatments were represented as points in the plane with their color marks (Fig. 7). In the biplot, the positon of samples values (*i.e*., loadings) in the plane shifted mainly with the growth stage of the algae, which was indicated in heterogeneity of the population with respect to the fluorescence parameters. Therefore, it was hardly possible to group the treatments into separated clusters. However, the formation of the Dim1 and Dim2 as well as the negative and positive correlation between the fluorescence parameters were attributed significantly to the change in photosynthetic activity. Therefore, it is related with growth of the microalgae (*i.e.*, cell age).

Conclusions: The photosynthesis characteristics of the microalgae *T. wisconsinensis* were studied in cultures supplemented with either bicarbonate or CO_2 gas using

chlorophyll fluorescence as a photosynthesis monitoring tool. Our results showed that both CO_2 and bicarbonate were comparably effective inorganic source for microalgae photosynthesis and biomass production. Low pH of the medium in CO_2 gas sparging resulted in few days of lag phase in biomass production and adjustment of pH was required by a close monitoring. Furthermore, the JIP-test also supported the result of the growth measurements of *T. wisconsinensis*, where the cultures supplemented of either bicarbonate or CO_2 were distinct from others, and these cultures showed similar values in quantum yield of PSII. The typical polyphasic rise, called the OJIP curve, was observed and it showed a typical difference between the treatments, especially in the J and I inflection curve of the fluorescence transient curve. Besides, the analysis of chlorophyll *a* fluorescence transient was a useful tool for monitoring performance of organisms under different treatments and growth stage. Hence, we propose that the measurement of fluorescence transient is useful as a rapid, on-site monitoring of performance in photosynthetic microalgae production.

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Appendix. Measured chlorophyll fluorescence and OJIP parameters obtained using the *AquaPen* fluorometer. Descriptions and formulas of the OJIP parameters are according to Strasser *et al*. (2000).

Parameter	Explanation
F_0	Fluorescence intensity at 50 μs (dark-adapted)
F_J	Fluorescence intensity at J-step (at 2 ms) (dark-adapted)
F_I	Fluorescence intensity at I-step (at 60 ms) (dark-adapted)
F_m	Maximum fluorescence intensity (dark-adapted)
F_v	$F_v = F_m – F_0$ (maximal variable fluorescence)
V_J	$V_J = (F_J – F_0)/(F_m – F_0)$, relative variable fluorescence at J-step
V_I	$V_I = (F_I – F_0)/(F_m – F_0)$, relative variable fluorescence at I-step
F_m/F_0	Ratio of maximum fluorescence to fluorescence intensity at 50 μs
F_v/F_0	Conformation term for primary photochemistry
F_v/F_m	The maximum quantum yield of primary PSII photochemistry
M_0	$M_0 = TR_0/RC – ET_0/RC = 4(F_{300} – F_0)/(F_m –F_0)$
Area	Area between fluorescence curve and F_m (background subtracted)
S_M	$S_M = Area/(F_m – F_0)$ (multiple turnover)
φ_{Po}	$\varphi_{Po} = 1 – (F_0/F_m)$ (or F_v/F_m)
ψ_0	$\psi_0 = 1– V_J$
φ_{Eo}	$\varphi_{Eo} = [1– (F_0/F_m)] \times \psi_0$
φ_{Do}	$\varphi_{Do} = 1 – \varphi_{Po} – (F_0/F_m)$
Phi Pav	Phi Pav $= \varphi_{Po} \times (S_m/t_{Fm})$, t_{Fm} = time to reach F_m (in ms)
ABS/RC	$ABS/RC = M_0 \times (1/V_J) \times (1/\varphi_{Po})$
TR_0/RC	$TR_0/RC = M_0 \times (1/V_J)$
ET_0/RC	$ET_0/RC = M_0 \times (1/V_J) \times \psi_0$
DI_0/RC	$DI_0/RC = (ABS/RC) – (TR_0/RC)$