

## **Photochemical and non-photochemical quenching study of microalgae and their consortia for CO<sub>2</sub> sequestration through carbonic anhydrase activity**

Adi Nath<sup>1,2</sup> and Shanthy Sundaram<sup>1</sup> \*

**Corresponding author email:** shanthy.cbt@gmail.com

1 Centre of Biotechnology, Nehru Science Centre, University of Allahabad, Allahabad-211002

2 Dept. of Botany, Nehru Gram Bharati, To be deemed University, Jamunipur Campus-221505

### **Abstract**

Factors such as light intensities and quality, water and nutrient availability, presence of heat, cold, herbicides, pesticides, pollution, heavy metals, disease and genetic make-up directly have an impact on the CO<sub>2</sub> assimilation, plant health and condition. All above factors are also reflected in the fluorescence signal in PSII. Therefore, by using chlorophyll fluorometer impact of these factors on plants to improve biomass and production program, and to better understand plant functions can be quantified easily. The loss of light energy from the reaction center as fluorescence comes primarily from the PSII reaction. When the chloroplasts are dark-adapted, the pools of oxidation-reduction intermediates for the electron transport pathway return to an oxidized state. Upon illumination of a dark-adapted condition, there is a rapid rise in the fluorescent light emission from PSII followed by a decrease to steady state fluorescence as CO<sub>2</sub> fixation starts to occur. Changes in the intensity of the fluorescence emission are sensitive to reflection of changes in the photosynthetic apparatus. Following many years of study, chlorophyll fluorescence has been shown to represent changes in the health of plants and photosynthetic organisms through photosynthetic processes. This includes all the reactions from the oxidation of water, charge separation, electron transport, development of the electrochemical gradient, the photo-protective mechanisms of the xanthophyll cycle, and the change in pH of the thylakoid lumen. Even changes in the micro-organism that affect stoma opening and gas exchange with the atmosphere are reflected by changes in the fluorescence characteristics of an organism. In the present chapter, the photochemical quenching (PQ) and non-photochemical quenching (NPQ) of microalgae and their consortia have been studied in cultivation broth; the  $f_v/f_m$  ratio of different algal strains was used for determination of maximum quantum yields.

### **6.2 Introduction**

The concentration of CO<sub>2</sub> in atmosphere has been increasing through anthropogenic activities (He *et al.*, 2012). The common statement of researchers about high concentration of CO<sub>2</sub> is the main atmospheric factor for global warming (IPCC 2007). The predictions about future that the concentration of atmospheric CO<sub>2</sub> will increase progressively if the release and primary production of atmospheric CO<sub>2</sub> is not regulated (Ramanathan 2006). A number of devices have been utilized for CO<sub>2</sub> sequestration. They have oceanic sequestration in Deep Ocean, geologic sequestration in earth crust and biological phenomenon (Reichle *et al.*, 1999). Although, they are not very better approach for CO<sub>2</sub> sequestration and further inspection is needed. In the sequestration processes, the CO<sub>2</sub> has to be pure (Han *et al.*, 2012). The CO<sub>2</sub> sequestered and captured at the production sites, it could be utilized for various purposes (Ono and Cuello, 2007). The synthesis of useful products by the CO<sub>2</sub> could be possible (Sun *et al.*, 2016). The capture of CO<sub>2</sub> from the flue gases, possess many transformations i.e. Aqueous phase dissolution, water hydration, carbonates formation and ionization. CO<sub>2</sub> hydration could be increased by carbonic anhydrase, which is the slowest process (Bond *et al.* 2001).

The quenching associated with photosystem II, in which electrons are accepted in its oxidized state. Quenching (q<sub>Q</sub>) associated with the oxidation- reduction status of Q is referred to as photochemical quenching (q<sub>p</sub>) (Baker, 2008). This is seen as the explanation for the initial rise in the fluorescence signal when CO<sub>2</sub> is suddenly removed from a microalgae sp. Once reduced, Q normally passes electrons, via other electron carriers, to photosystem I and finally to carbon dioxide via NADP (Campbell *et al.*, 1998). In the absence of CO<sub>2</sub>, NADPH<sub>2</sub> cannot be re-oxidized by CO<sub>2</sub>. The second quenching mechanism for energy quenching is called non-photochemical quenching (q<sub>NP</sub>). Infact, it is associated with the establishment of proton gradient (ΔpH) across the thylakoid membrane. This proton gradient normally discharges through the ATPase, generating ATP from ADP and Pi. In the absence of carbon dioxide, ATP is not consumed in photosynthetic carbon assimilation and ADP is therefore no longer available to discharge the proton gradient through the ATPase in this process (Vaz and Sharma, 2011, Holt *et al.*, 2004,. In these circumstances the discharged proton gradient appears to switch dissipation of excitation energy from fluorescence into thermal channels with the result that the fluorescence is quenched (Horton *et al.*, 2007, Maxwell and Johnson, 2000, Gilmore *et al.*, 1996). This, in part, is the source of decline in fluorescence which pushes the fluorescence signal from the initial maximum removal of CO<sub>2</sub> to a value even lower than before. When CO<sub>2</sub> is returned to

atmosphere from microalgae the converse sequence of events occurs (Bailey and Grossman, 2008). The first antennae can become re-oxidized in the presence of CO<sub>2</sub>, so that its fluorescence quenching is re-imposed. ATP is consumed in carbon assimilation making ADP available to discharge the proton gradient. As the proton gradient falls, some dissipation of excitation switches back, from thermal channels, into fluorescence (Kromdijk *et al.*, 2016, Zurek *et al.*, 2014). The fluorescence, initiated by a very weak measuring beam (F<sub>0</sub> value), remains undistributed by the saturating flash, thereby allowing accurate measurement of the differences between F<sub>0</sub> and F<sub>max</sub>. The entire process of light is doubling and the subsequent evaluations of the major quenching components by measurement and arithmetic have sometimes been referred to as quenching analysis (MacKenzie *et al.*, 2004, Miskiewicz *et al.*, 2002).

In the present work, quenching analysis for three cyanobacteria, three microalgae and three consortia of them, has been used to measure photosynthetic carbon assimilation i.e. the rate of carbon fixation can be accurately determined by evaluating the light emitted from microalgae as chlorophyll fluorescence. The rates of CO<sub>2</sub> fixation in a consortia and monocultures could be determined solely by fluorescence for CO<sub>2</sub> sequestration and mitigation.

## 6.3 Materials and Methods

### 6.3.1 Sampling

The different individuals microalga i.e. *Synechocystis* PCC 6803, *Anabaena cylindrica*, *Spirulina platensis*, *Chlorella* sp., *Scenedesmus dimorphus*, *Chlamydomonas reinhardtii*. and their consortia was grown axenically in batch cultures in BG-11 medium (Rippka *et al.*, 1979). No further treatments such as: herbicides or watering were applied, despite collecting biomass at the end of mid log phase. All observations and measurements: growth, generative primary abundance and total yield as well as Chl fluorescence were conducted at first at both locations: all parameters were measured in mid-log phase of interaction (Narwani *et al.*, 2017, Godwin *et al.*, 2017, Cho *et al.*, 2017, Yahya *et al.*, 2016, Gross *et al.*, 2014, Renuka *et al.*, 2013, Power and Cardinale, 2009). The description of consortia was given in table 11.

*Table 1 Combination of microalgae strains for consortia development*

Consortia 1	All the members of cyanophyta i.e. <i>Synechocystis</i> PCC 6803, <i>Anabaena</i>
-------------	---

	<i>cylindrica, Spirulina platensis.</i>
Consortia 2	All the members of chlorophyta i.e. <i>Chlorella</i> sp., <i>Scenedesmus dimorphus</i> , <i>Chlamydomonas reinhardtii</i> .
Consortia 3	Complete i.e. <i>Synechocystis</i> PCC 6803, <i>Anabaena cylindrica</i> , <i>Spirulina platensis</i> , <i>Chlorella</i> sp., <i>Scenedesmus dimorphus</i> and <i>Chlamydomonas reinhardtii</i> .

### 6.3.2 Determination of the activity of Carbonic Anhydrase (CA) (Wilbur and Anderson, 1948)

#### 6.3.3 Extracellular CA

A volume of culture containing the adequate number of cells ( $10^6$  cells) was centrifuged at  $2000\times g$  and washed twice with 0.010M Tris buffer (pH 8.3) containing 1 mM dithiothreitol (DTT) and 1mM EDTA. The pellet consisting of intact cells whose integrity was checked through the microscope was re-suspended with the same buffer, in which the measurements of enzymatic activity were made immediately after the re-suspension of the cells.

#### 6.3.4. Total CA

A culture volume containing  $10^6$  cells was filtered using cellulose acetate membranes (0.45 $\mu$ M pore size diameter, from Micron Separation Inc.). After removal of the membrane, the microalgae material was ground in a mortar in presence of liquid nitrogen to get its fine powder and resuspended in 0.01M Tris buffer containing 1 mM DTT and 1mM EDTA (pH 8.3). The total extract was used to measure the enzymatic activity. Some extracts were checked at the microscope and no intact algal cells were found after this treatment.

#### 6.3.5 Measurement of enzymatic activity

Measurement of enzymatic activity were made using the potentiometric method of Wilbur and Anderson, with modifications; 1.5 mL of deionized water saturated with CO<sub>2</sub> at 0°C, were added to a volume of 3 mL of microalgae material resuspended in the buffer(Ta), or buffer

without microalgae (Tb). This reaction was performed in a closed flask kept at a temperature between 0 and 2 °C. The time necessary for the CO<sub>2</sub> saturated deionized water to lower one unit of pH of both solutions was measured and the enzymatic activity was calculated using the equation:

$$Tb/Ta-1=UA \times 10^6 \quad (1)$$

Equation 1 expresses the enzymatic activity as units of activity (UA) per cells since 10<sup>6</sup> cells were used for measurement of activity.

### 6.3.6 Measurements of Chl a fluorescence

Chl a fluorescence was measured simultaneously on both light and dark adapted time durations at the first fully developed mid-log phase, Dark adaptation (0.5 hour) of the middle preceded the measurement. Fluorescence was induced by saturating, red actinic light with energy of 3.500 mmol m<sup>-2</sup> s<sup>-1</sup>, and first 3 seconds of transient fluorescence, covering more than its exponential growing part, was registered with time intervals increasing from 10 ms within first 300 ms of the measurement up to 100 ms intervals for times longer than 0.3 sec ( Zurek *et al.*, 2014).

### 6.3.7 Quenching measurements

For the assessment of photosynthetic performance, chlorophyll fluorescence measurements were taken in dark adapted leaves of control and treated seedlings using hand held leaf fluorometer (FluorPen FP 100, Photos System Instrument, Czech Republic). The following fluorescence parameters: variable/maximum fluorescence ratio (Fv/Fm), photochemical quenching (qP) and non-photochemical quenching (NPQ) were measured. Measurements were taken in of six different microalga monocultures and their consortia (Holt *et al.*, 2004). The value could be calculated by the given formula.

$$qP=(Fm'-F)/(Fm'-Fo') \dots \dots \dots (1)$$

$$NPQ= (Fm-Fm')/Fm' \dots \dots \dots (2)$$

$$NPQ=qE+qT+qI \dots \dots \dots (3)$$

$$qN=(Fm-Fm')/Fm-Fo). \dots \dots \dots (4)$$

$$qL=qP(Fo'/F') \dots\dots\dots (5)$$

$$Y(NO)=1/NPQ+1+qL((Fm/Fo)-1) \dots\dots\dots(6)$$

$$Y(NPQ)=1-Y-Y(NO) \dots\dots\dots (7)$$

**6.4 Results and discussion**

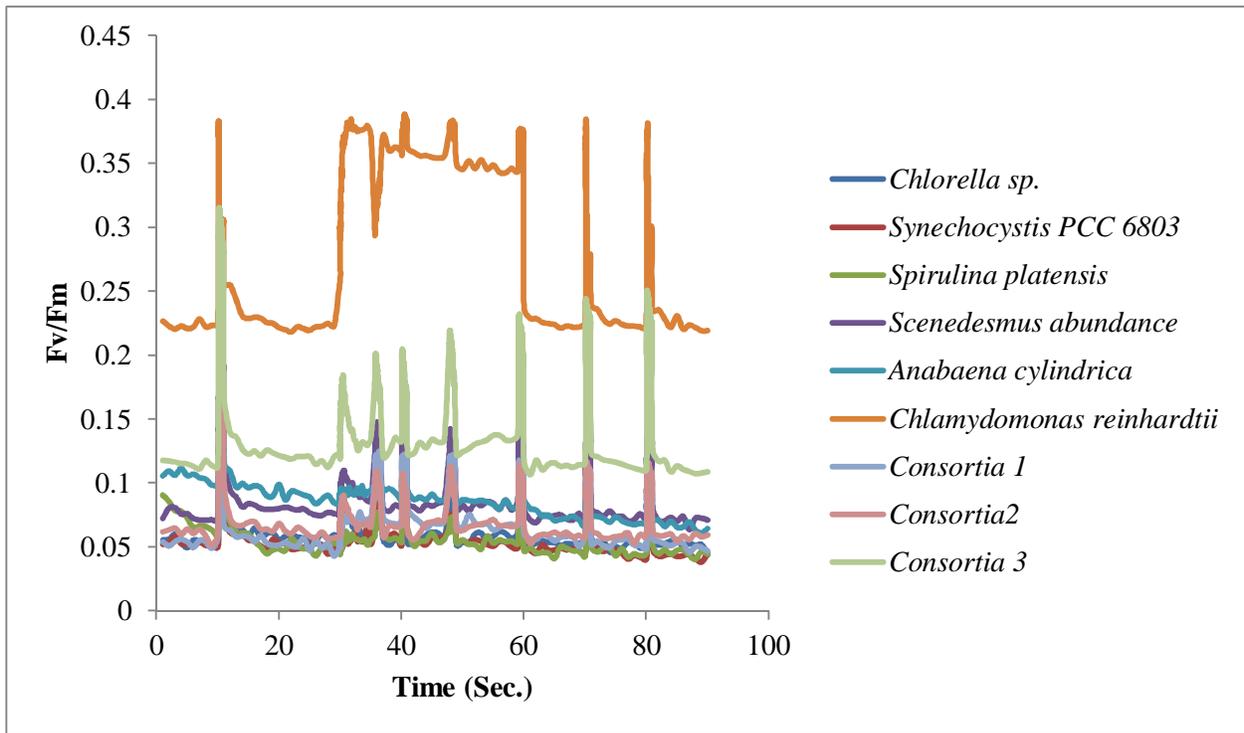
**6.4.1 Carbonic anhydrase activity of microalgae and consortia**

The regulatory services of microalgae and their consortia of CA showed different patterns. The total inorganic carbon and CO<sub>2</sub> concentration played effective role in CA activity. The shift of equilibrium inorganic carbon was lowering the CO<sub>2</sub> concentration. The higher activity of the external and total CA of microalgae consortia was obtained when compared to most producing individual alga. The activity of the internal enzyme was increased in consortia as a mechanism to increase CO<sub>2</sub> concentration through RUBISCO. On the other hand extracellular activity was increased by uptake of bicarbonate ion as a resource, i.e. major source of inorganic carbon as a result of carbon concentration mechanism (Kumar *et al.*, 2011, reported that in alleviating the impact of increasing CO<sub>2</sub> in the atmosphere sequestered CO<sub>2</sub>, Hu *et al.*, 2017 elaborated highly settleable microalgal consortia could be enriched by external pressure for stable biomass.

*Table 2 Total and extracellular carbonic anhydrase activity in microalgae and consortia (Wilbur and Anderson, 1948)*

<b>Name of microorganism</b>	<b>Extracellular CA activity</b>	<b>Total CA activity</b>
<i>Synechocystis</i> PCC 6803	0.279±0.001	0.68±0.062
<i>Spirulina platensis</i>	0.372±0.003	0.84±0.063
<i>Anabaena cylindrical</i>	0.291±0.002	0.69±0.061
<i>Chlorella</i> sp.	0.371±0.003	0.82±0.060

<i>Scenedesmus dimorphus</i>	0.310±0.002	0.64±0.059
<i>Chlamydomonas reinhardtii</i>	0.407±0.004	0.94±0.071
Consortia 1	0.403±0.004	0.92±0.069
Consortia 2	0.423±0.004	0.97±0.069
Consortia 3	0.498±0.005	1.13±0.079



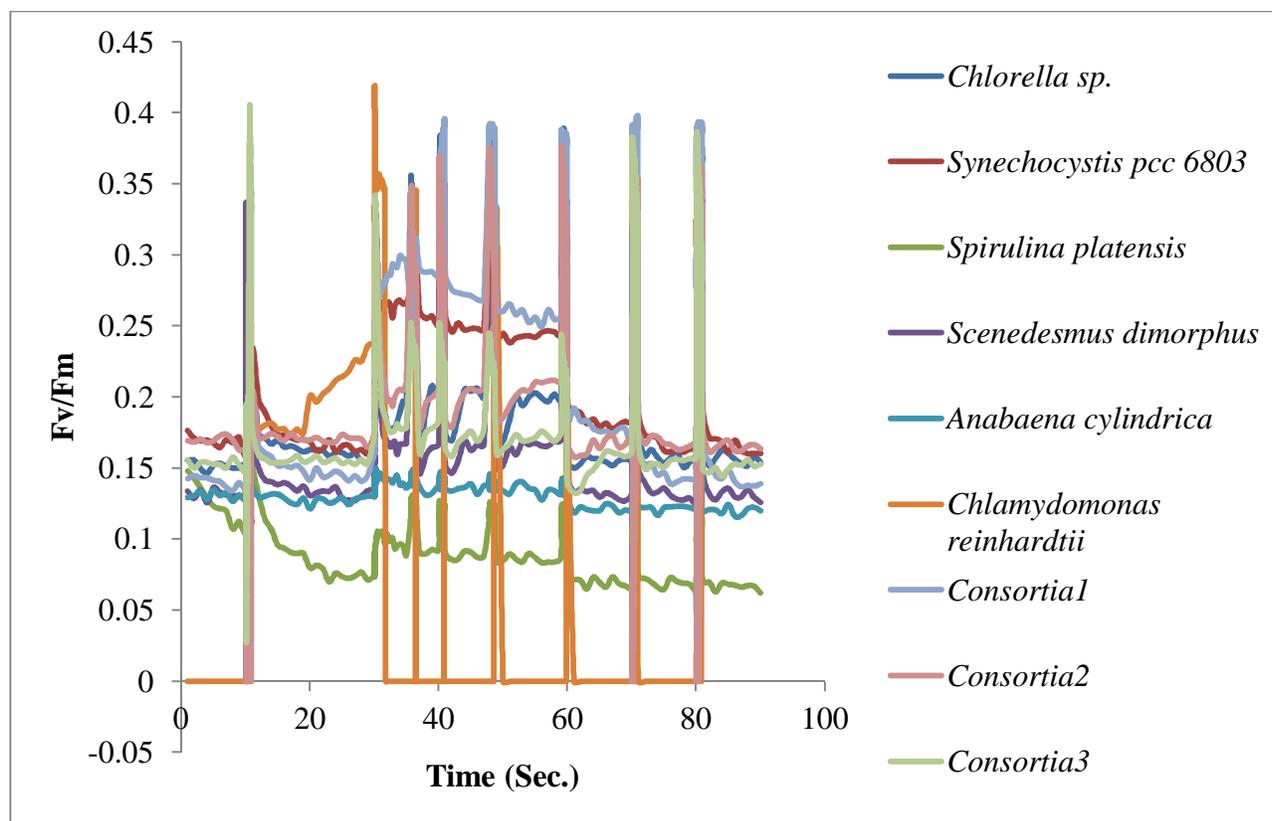


Fig 1 Fluorescence variable analysis both dark and light adapted conditions in microalgae and consortia

#### 6.4.2 Chlorophyll fluorescence response on CO<sub>2</sub> fixation

In each case the sample was first dark adapted. The test was started and  $F_0$ , or minimal fluorescence, was measured without actinic light. Then a saturation pulse was provided and completely closed all the receptors in PSII by completely reducing PSII. After the saturation pulse, an actinic light was turned on and the fluorescent signal declines slowly with the onset of CO<sub>2</sub> fixation until it reached steady state. In this communication the transfer of stable biomass in consortia resulted in an initial decline of Fv/Fm ratio rather than monocultures. Increased quantum efficiency observed in microalgae consortia with respect to the ratio of dark and light adapted culture condition compared to their respective monocultures (Fig 3a, b). In this regard Adamczyk et al., 2016, the maximal biofixation rate and cell concentration characterized by higher CO<sub>2</sub> fixation rate.

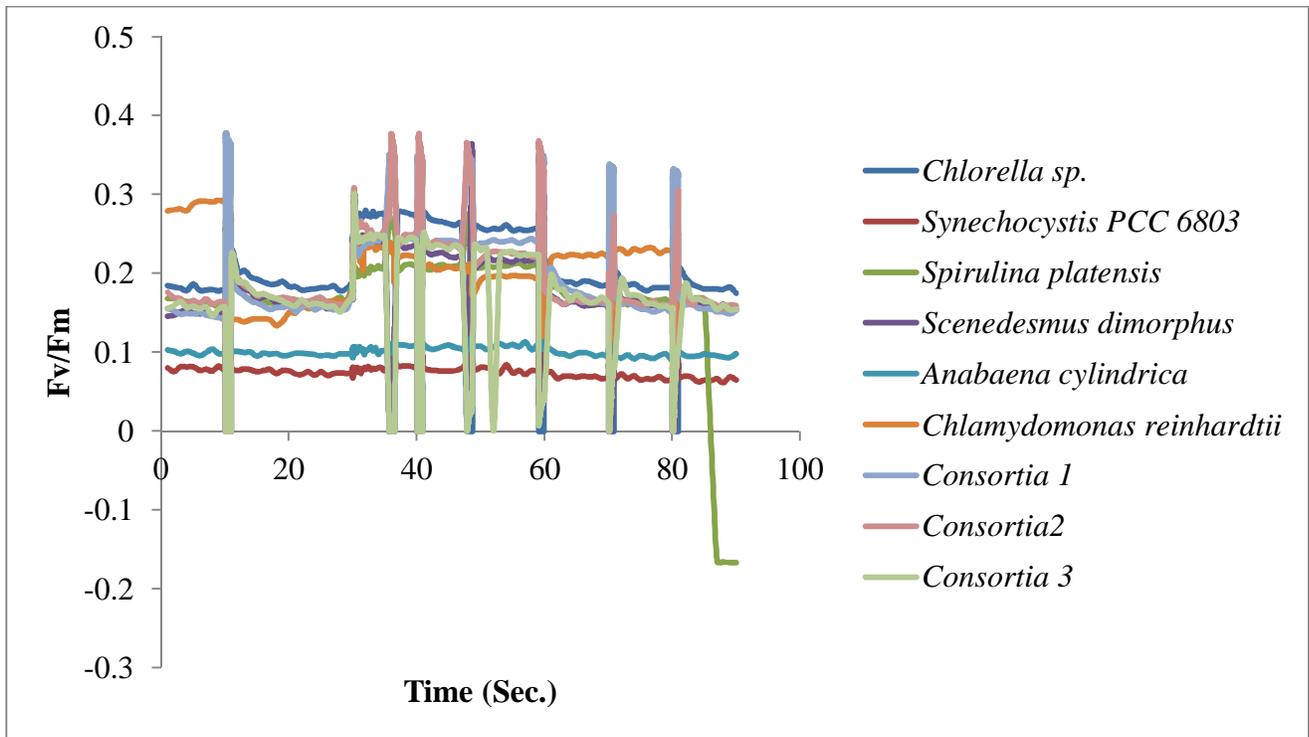
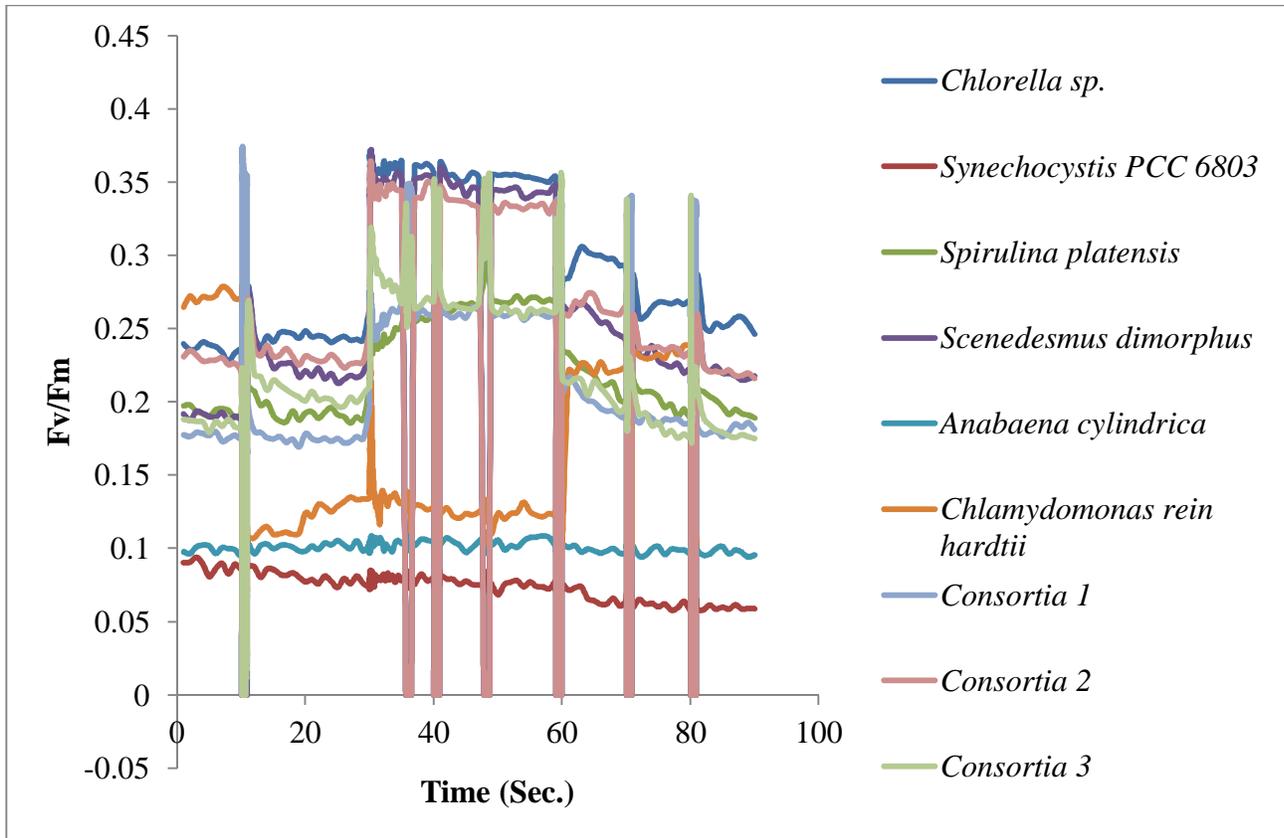


Fig 2 Fluorescence analysis of microalgae monocultures and consortia on both dark and light adapted conditions with mixing carbon saturated water in media

### 6.4.3 Quenching analysis of monocultures and consortia

Quenching parameters allow the quantification of both effective photochemical state of the PS II regarding the fraction of PSII centers that remain open or oxidized at any time, and the non-photochemical photosynthetic mechanisms involved in photo-protection, state 1 and state 2 transition quenching, photo-inhibitor and photo-damage. The NPQ was found to be higher in microalgae consortia as compared to individual alga, there must be an inverse relationship between chlorophyll a fluorescence emission from microalgae and photosynthetic carbon assimilation. It was argued must an indicator of “richness”. The red excited state of chlorophyll available heat or light loss as dissipation that produced more excitation energy in microalgae consortia through fluorescence induction. This was seen that first illumination after a period of darkness. When CO<sub>2</sub> was abruptly removed from microalgae, more excitation energy became available for dissipation that alternatively increased the fluorescence emission in community rather than individual microalga. In microalgae consortia increase NPQ in response to monocultures indicates high irradiance resistant to photo inhibition due to an increased photosynthetic capacity by stable biomass production.

*Table 3 Quantum yield efficiency, photochemical quenching and non-photochemical quenching quantification in microalgae monocultures and consortia*

Name of organism	Chlorophyll florescence		
	Fv/Fm	qP	NPQ
<i>Synechocystis PCC 6803</i>	0.73±0.10	0.758±0.009	1.37±0.06
<i>Spirulina platensis</i>	0.75±0.07	0.791±0.011	1.36±0.06
<i>Anabaena cylindrica</i>	0.78±0.11	0.812±0.015	1.32±0.09
<i>Chlorella sp.</i>	0.80±0.14	0.876±0.019	1.29±0.08
<i>Chlamydomonas reinhardtii</i>	0.82±0.19	0.987±0.020	1.26±0.10
<i>Scenedesmus dimorphus</i>	0.69±0.10	0.782±0.017	1.46±0.09
Consortia 1	0.65±0.06	0.679±0.016	1.52±0.14
Consortia 2	0.61±0.04	0.621±0.016	1.59±0.19
Consortia 3	0.56±0.02	0.572±0.016	1.63±0.19

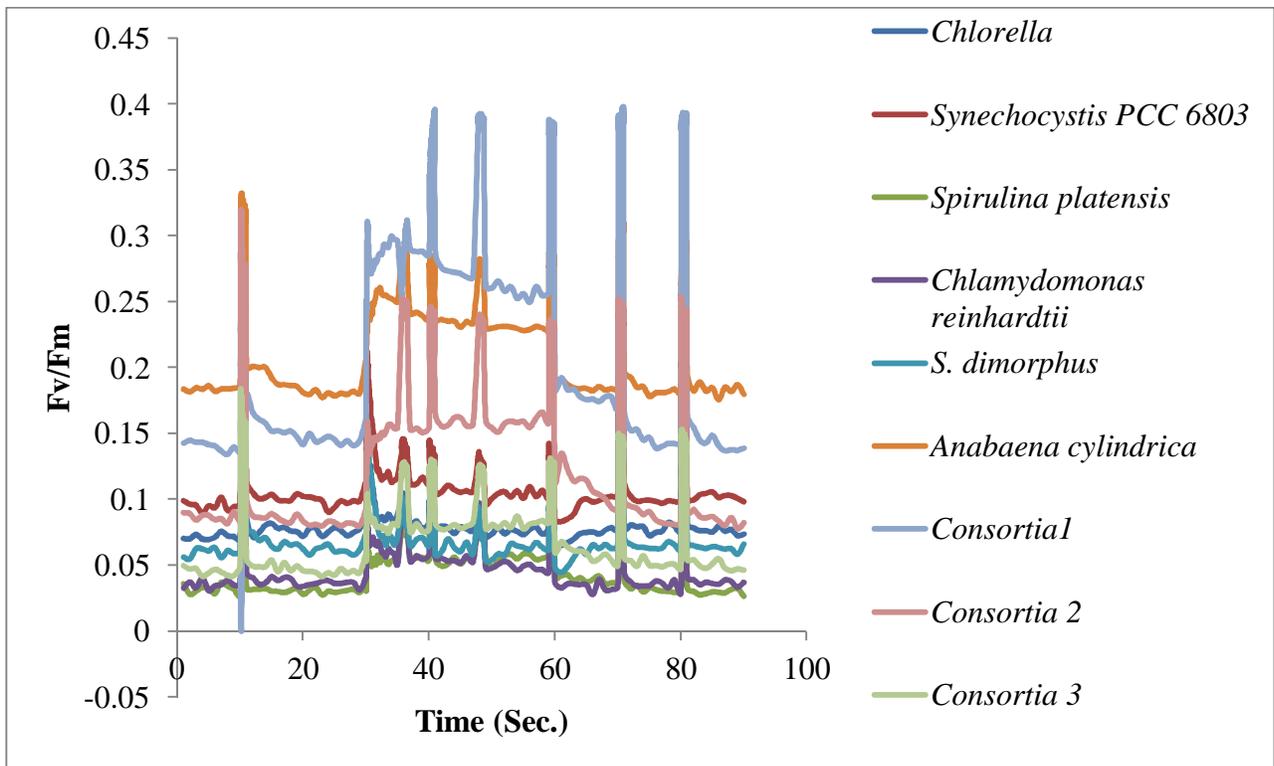
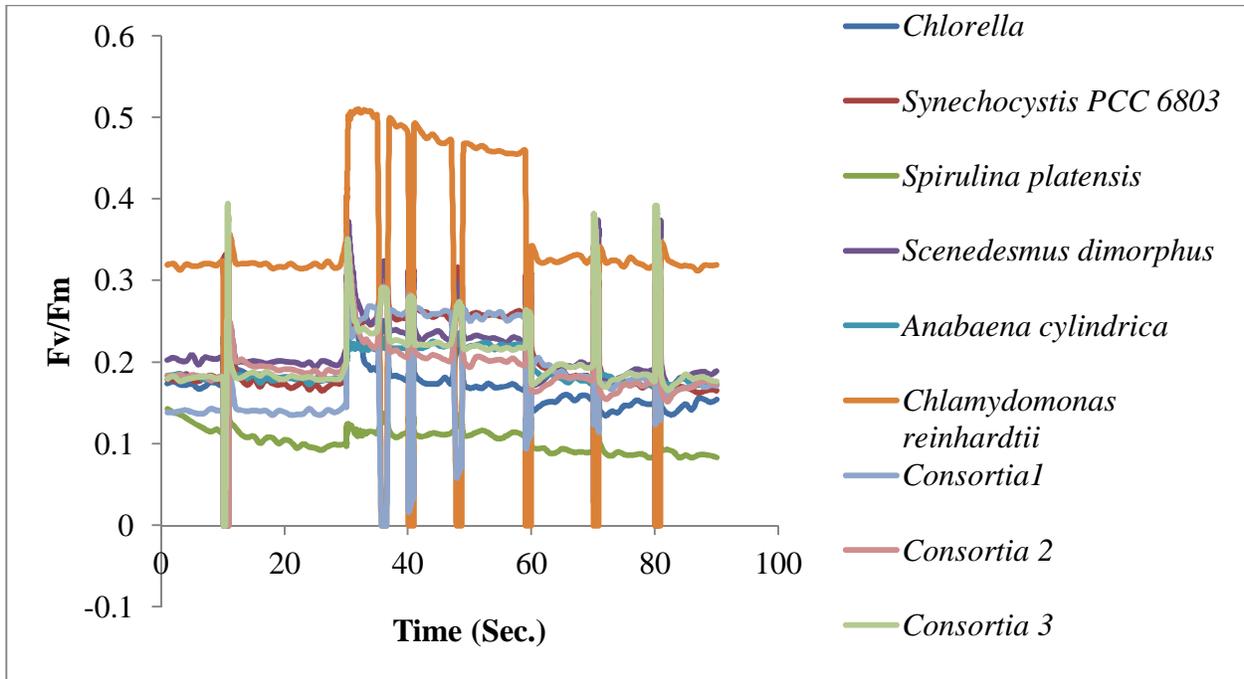


Fig 4 Fluorescence induction analysis of microalgae monocultures and consortia on both dark and light adapted conditions

#### 6.4.4 Photo protection response of monocultures and consortia

In a wide range of microalgae and their consortia with different pigment contents and morphologies, non-photochemical quenching varies with different species composition. Therefore, the species richness to which the population is photo synthetically acclimatized or not could be predicted from a readily measured light response curve of NPQ. The fluorescence-based estimate of electron transport and carbon dioxide fixation is very valuable. Fluorescence measurements are possible with monocultures samples; unlike gas exchange, they are specific to photobionts and so do not detect interference from homo and heterogeneity respiratory activity in mixed samples. The fluorescence transients arise largely from PS II, and so calculations based on fluorescence reflect PS II activity and electron transport through PS II. In extrapolating from fluorescence signals to photosynthesis, we therefore, rely on congruence between PS II activity, net electron transport, and overall photosynthesis. The light-harvesting pigment-protein complex of PSII- dependent fluorescence component does affect, microalgae consortia less rather than monocultures. Maximal fluorescence conditions in microalgae consortia reduce quenching indicates stabilize biomass production.

Sundberg et al. have simultaneously measured fluorescence quenching parameters and CO<sub>2</sub> exchange in cyano-lichen to develop a model to predict gross photosynthesis from fluorescence parameters. They found the empirical relation

$$P = \phi_{PS II} \times I_i \times 1CO_2 \text{ fixed}/10 \text{ photons}$$

Where p= micromoles of CO<sub>2</sub> fixed per milliliter per second,  $\phi_{PS II} = (F_M' - F_S) / F_M'$ , I<sub>i</sub>=number of incident photons per milliliter per second and 1CO<sub>2</sub> fixed/10 photons is an empirical conversion factor.

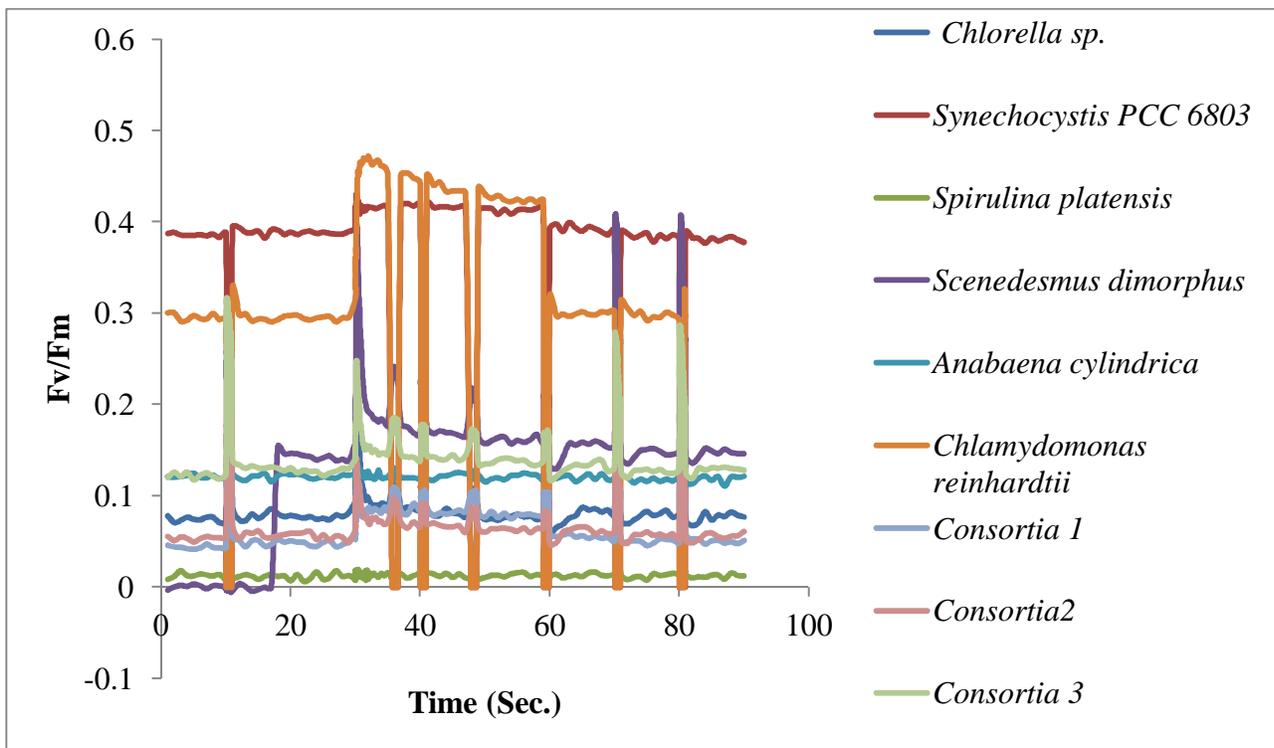
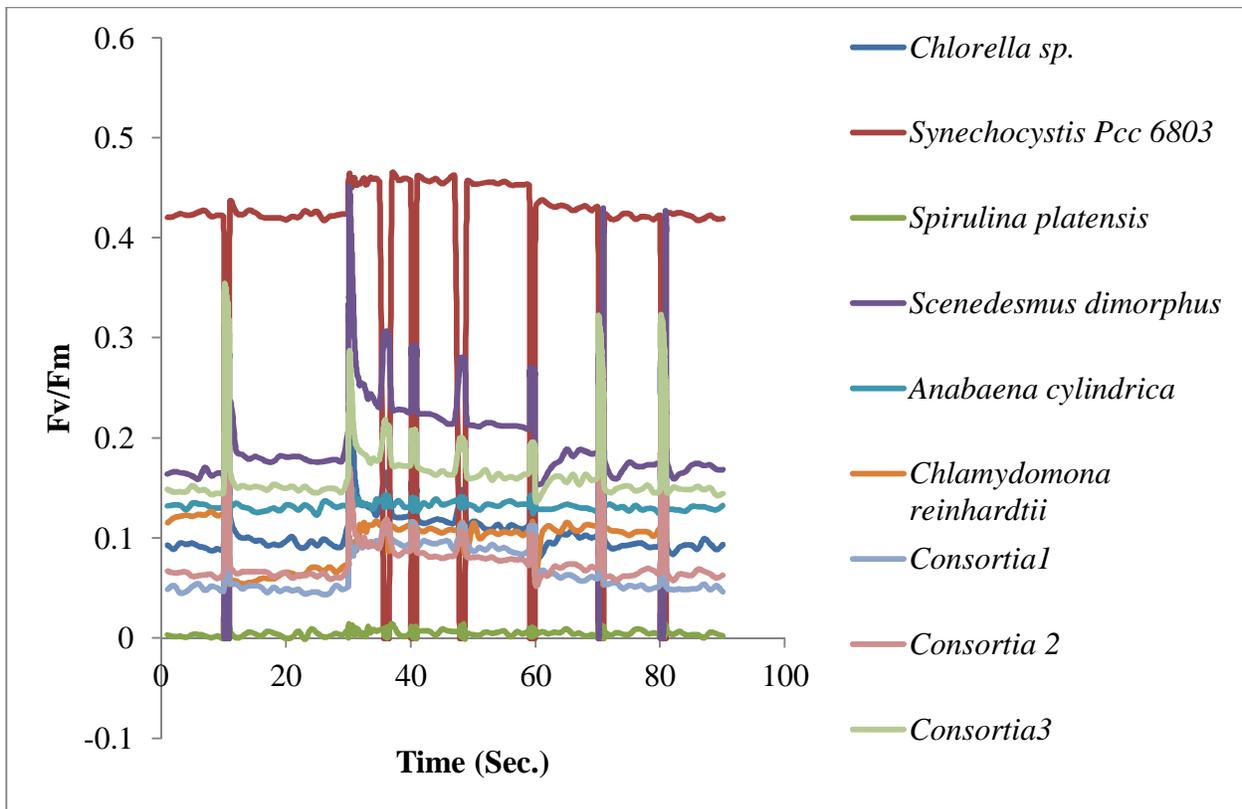


Fig 5 Protonation and de-protonation fluorescence response in dark and light adapted condition in monocultures microalgae and consortia

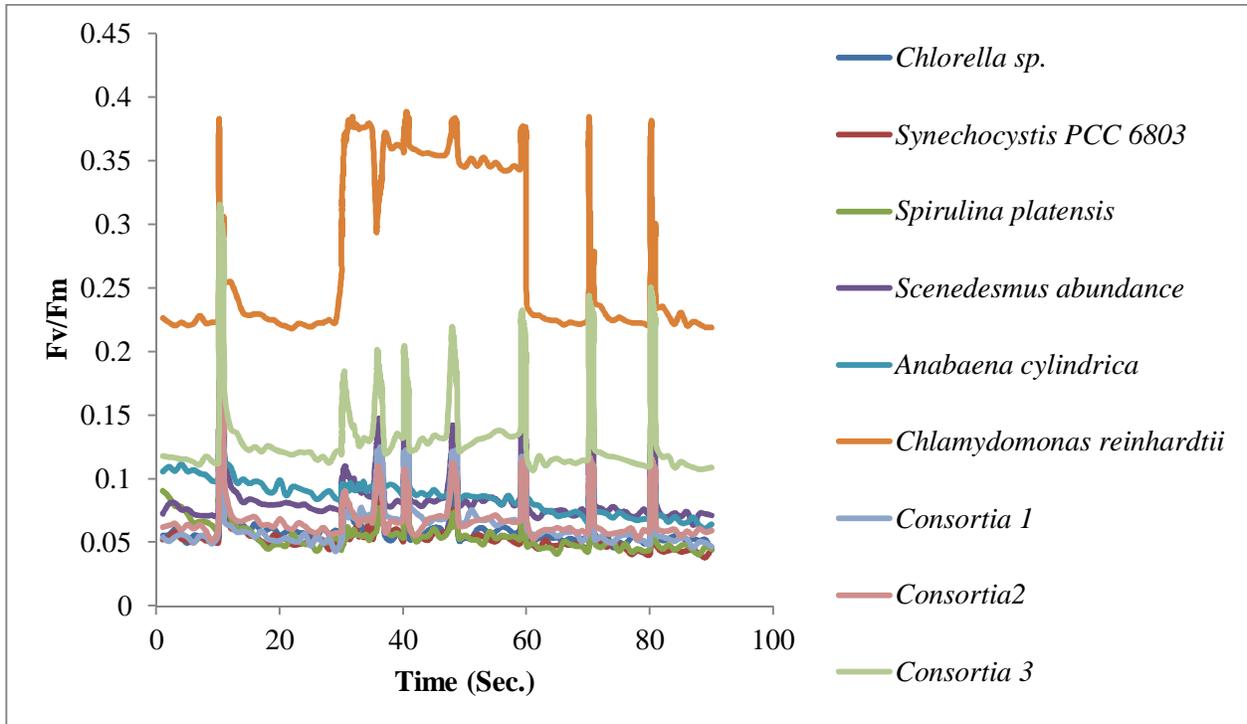
#### 6.4.5 Applying fluorescence analysis in microalgae monoculture and in consortia

We have tested the methods described in three cyanobacteria, three green algae and consortia of them representing a wide range of pigment contents, carbonic anhydrase induced activity, CO<sub>2</sub> concentrated activity, and protonation and de-protonation activity. *Anabaena cylindrica*, a heterocystous filamentous strain which strongly regulate its content of phycoerythrin and phycocyanin according to relative supply of green and red light. Quenching analysis showed that energy captured by phycoerythrin was transferred from the phycobilisome to PSI to maintain balanced electron transport. This transfer was reflected in a high non-photochemical quenching and serve as a good demonstration that does not usually reflect excitation dissipation but, rather, reflects the transfer of excitation to PS I at the expense of PS II. Upon transfer to red light or prolonged growth under red light, this excitation transfer stops, the PS II fluorescence yield increases and NPQ drops. *Synechocystis* PCC 6803 is a unicellular strain which contain phycoerythrin. In microalgae consortia, the dark-light state II – state I transition tends to be small, probably reflecting microalgae-cyanobacteria interaction in respiration and carbohydrate consumption.

Production of carbohydrate by microalgae and their consortia is an important criterion in terms of their use in bioenergy as bioethanol. The aforementioned microalgae strain showed a good growth under different environments such as variable concentration of bicarbonate, carbon dioxide gas and light. The carbon fixation behaviors of the microalgae and their consortia under fluorescence of light and dark adapted bicarbonate level were shown in fig 2a, b. Nutritional supplement implies the biochemical constituents of a microalgae and their consortia, mainly comprises of protein, lipid and carbohydrate. Gaseous carbon dioxide was also supplied to the culture flask in variable amounts. When enormous of gaseous carbon dioxide was supplied, it was observed that the cells tend to settle down and clump but they continue to grow slowly. It was noticed that a higher flow rate inhibited microalgae growth in monoculture much rather than consortia. It resulted in decreasing pH as it leads to carbonic acid production.

Ribulose 1,5-bisphosphate (RuBP) carboxylase/oxygenase (RubisCO) catalyzes the key reaction by which inorganic carbon may be assimilated into organic carbon. Phylogenetic analyses indicate that there are three classes of RubisCO proteins; I, II, and III, that all catalyze the same reactions. In addition, there exists another form of RubisCO, form IV, which does not

catalyze typical RubisCO reactions. Form IV is actually a homolog of RubisCO and is called the RubisCO-like protein (RLP). Both RubisCO and RLP appear to have evolved from a methanogenic archaeal ancestor protein and comprehensive analyses indicate that the various forms (I, II, III, and IV) contain various subgroups, with individual sequences derived from representatives of all three kingdoms of life. The diversity of RubisCO molecules, many of which function in distinct conditions, have provided convenient model systems to study the ways in which the active site of this protein has evolved to accommodate necessary molecular adaptations. Such studies have proven useful to help provide a framework for understanding the molecular basis for many important aspects of RubisCO catalysis, including the elucidation of factors or functional groups that impinge on RubisCO carbon dioxide/oxygen substrate discrimination. The enzyme mediated activity response in consortia increased in respect to monoculture indicates an increase photoprotection capability. The xanthophyll cycle-dependent non-photochemical quenching in microalgae consortia and discrepancies between their monocultures may be attributed to the energy transfer processes of PSII.



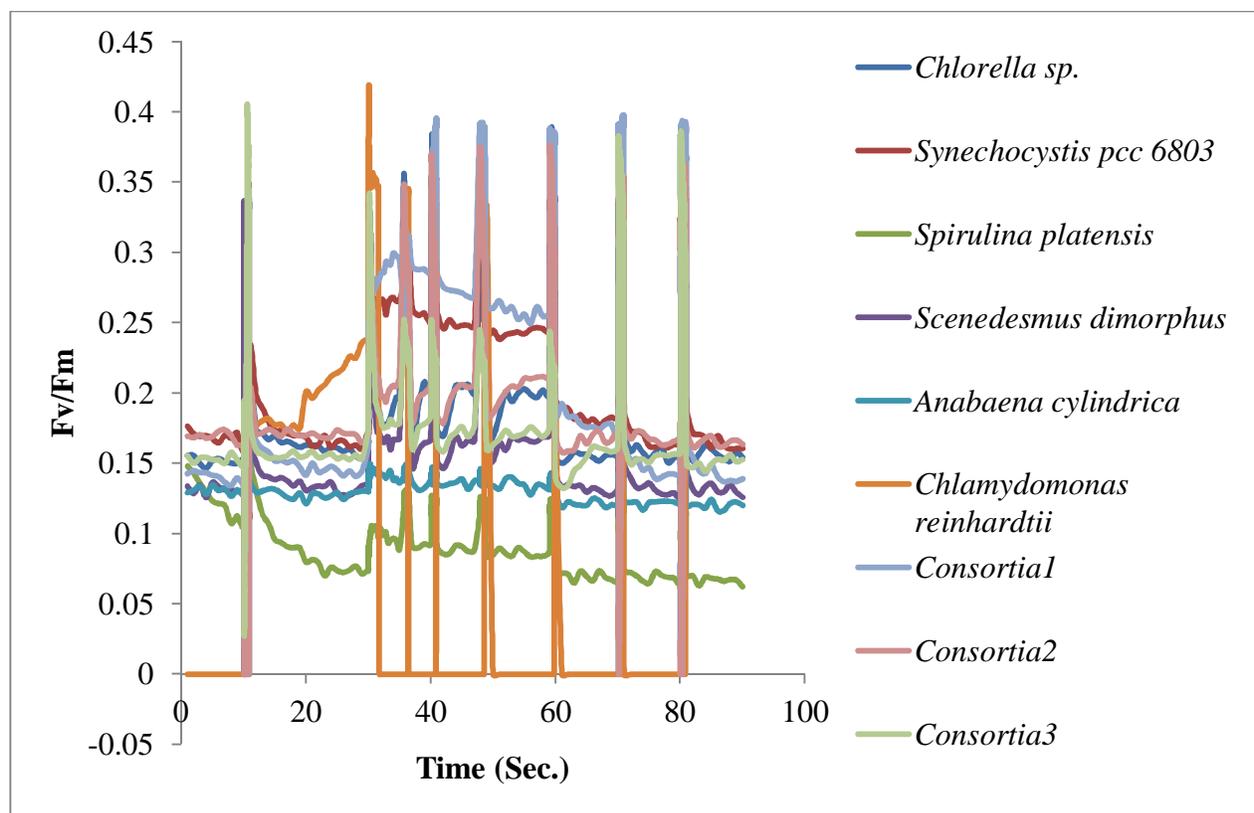


Fig 6 Fluorescence response of intracellular bicarbonate enzyme in monocultures microalgae and consortia

#### 6.4.6 Exploiting RubisCO Diversity to Learn More about Function

Phylogenetic, bioinformatic, and evolutionary considerations provide thought-provoking discussions, but how can these analyses help us in solving some of the “mysteries” of RubisCO catalysis, such as the molecular basis for CO<sub>2</sub>/O<sub>2</sub> discrimination or why the affinities for CO<sub>2</sub> and O<sub>2</sub> vary? Our approach has always been to use what nature provides. Thus, the different forms and structural adaptations of RubisCO are available, from organisms that assimilate and metabolize CO<sub>2</sub> under diverse and even extreme environments, that may provide useful insights as to how all RubisCO molecules function.

#### 6.5 Conclusion

The increased interest in microalgae consortia biotechnology, aimed to the production of biomass, high-value products, or even bio-fuels, has prompted the application of on-line measurements for monitoring growth and obtaining rapid evidence of unfavourable conditions affecting the performance of primary production by cultures. For these applications the use of

modulated fluorometers, which enable the actual photochemical quantum yield to be measured at a given light intensity during the day. Indeed, it is relatively easy to generate fluorescence data, thus care must always be taken to select and calculate sensible parameters. This is particularly true when dealing with microalgae consortia and monocultures response, where growth limitations, such as light, temperature and other unfavourable factors can occur side by side. As long as this is kept in mind, Chl fluorescence represents a powerful technique which allows rapid monitoring of physiological status, the use of which has been steadily increasing in the studies of microalgae efficiency.

### **Acknowledgements**

Adi nath is thankful to UGC New Delhi, India for providing him UGC-D.Phil. research fellowship.

### **Author's contributions**

Shanthy Sundaram and Adi Nath designed the experiments; Adi Nath performed all the experiments. Adi Nath drafted the manuscript, Shanthy Sundaram reviewed the manuscript.

### **Works Cited**

Holt N E, Fleming G R, and Niyogi K K. (2004). "Toward an understanding of the mechanism of Nonphotochemical Quenching in Green Plants". *Biochemistry*, the American Chemical Society.10.1021/bi0494020

Vaz J. and Sharma P K. (2011). "Relationship between xanthophyll cycle and non-photochemical quenching in rice (*Oryza sativa* L.) plants in response to light stress". *Indian Journal of Experimental Biology*. Vol. 49, January 2011, pp.60-67

Bailey S. and Grossman A. (2008). "Photoprotection in cyanobacteria: Regulating of Light Harvesting". *Photochemistry and Photobiology*, 2008, 84: 1410-1420

Maxwell K and Johnson G N. (2000). "Chlorophyll fluorescence-a practical guide". *Journal of Experimental Botany*, Vol.51, No.345,pp.659-668, April 2000

Baker N R. (2008). "Chlorophyll Fluorescence: A probe of photosynthesis In Vivo". *Annual Review of Plant Biology*. 2008.59:89-113.032607.092759

Campbell D, Hurry V, Clarke A K, Gustafsson P and Oquist G. (1998). "Chlorophyll Fluorescence Analysis of Cyanobacterial Photosynthesis and Acclimation". *Microbiology and Molecular Biology Reviews*, Sept. 1998, p. 667-683 1092-2172/98

Gilmore A M, Hazlett T L, Debrunner P G and Govindjee. (1996). "Comparative Time-Resolved Photosystem II Chlorophyll a Fluorescence Analyses Reveal Distinctive Differences between Photoinhibitory Reaction center Damage and Xanthophyll cycle- Dependent Energy Dissipation". *Photochemistry and Photobiology*, 1996, 64 (3): 552-563

Horton P, Johnson M P, Perez-Bueno M L, Kiss A Z and Ruban A V. (2008). "Photosynthetic acclimation: Does the dynamic structure and macro-organization of photosystem II in higher plant grana membranes regulate light harvesting states?". *The FEBS Journal*. 275 (2008) 1069-1079 doi: 10.1111/j.1742-4658.2008.06263.x

Kromdijk J, Glowacka K, Leonelli L, Gabilly S T, Iwai M, Niyogi K K, and Long S P. (2016). "Improving photosynthesis and crop productivity by accelerating recovery from photoprotection". *Sciencemag.org* 18 November 2016. VOL 354 ISSUE 6314 857

Zurek G, Rybka K, Pogrzeba M, Krzyzak J and Prokopiuk K. (2014). "Chlorophyll a Fluorescence in evaluation of the effect of heavy metal soil contamination on perennial grasses". *PLoS ONE* 9(3): e 91475. doi:10.1371/journal.pone.0091475

Miskiewicz E, Ivanov A G, and Huner N P A. (2002). "Stoichiometry of the Photosynthetic Apparatus and Phycobilisome Structure of the Cyanobacterium *Plectonema boryanum* UTEX 485 Are Regulated both Light and Temperature". *Plant Physiology*. org/cgi/doi/10.1104/pp.008631

MacKenzie T D B, Burns R A, and Campbell D A. (2004). "Carbon Status Constrains Light Acclimation in the Cyanobacterium *Synechococcus elongates*". *Plant Physiology*, October 2004, Vol. 136, pp. 3301–3312, www.plantphysiol.org/cgi/doi/10.1104/pp.104.047936