

Micro-algal consortia complexity enhances ecological biomass stability through CO₂ sequestration

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Abstract

The micro-algae are autotrophic, photosynthetic plants which are the greatest gift of nature to humans. These micro-algal species have the property of CO_2 fixation. In the present study, an experiment was performed in two sets A and B, each of which had two consortia, namely A1, A2 and B1, B2 respectively. In set A, Bioenergy rich cyanobacterium, namely *Synechococcus* PCC 7942, an edible Chlorelin antibiotic producing micro-algae, *Chlorella* sp. and two nitrogen fixing cyanobacteria, *Nostoc muscorum, Oscillatoria* sp. and an edible protein rich cyanobacteria *Spirulina platensis* were tested in BG-11(+) media, i.e. Consortia-A1 and in Zarrouk media, i.e. consortia-A2. While, in set B, Biofuel rich cyanobacteria *Quantum black signal and edible* protein rich cyanobacteria *Spirulina platensis* were tested in BG-11(+) media, i.e. Consortia-A1 and in Zarrouk media, i.e. consortia-A2. While, in set B, Biofuel rich cyanobacteria *Anabaena cylindrica, Lyngbya* sp. and an edible protein rich cyanobacteria *Spirulina platensis* were grown in BG-11 (+) media, that is consortia-B1 and in Zarrouk media, which is consortia–B2. The aim of the study was to evaluate how these organisms behave in community. Therefore, enzymatic, activity of carbonic anhydrase was evaluated. Instead estimation of photosynthetic pigments, carbohydrate, proline, total protein, and photochemical quenching were evaluated and the total productivity of community was measured in terms of the dried biomass produced at the end of cultivation. The total carbohydrate and biomass yields of different consortia show their enhanced quantum efficiency. Increased activity of carbonic anhydrase enzyme in consortia reflects the better CO_2 fixation by these organisms.

Keywords: Microalgae, Consortia, CO₂ mitigations, Non-photochemical quenching, Sequestration.

Introduction:

In micro-algal consortia species have a high opportunity to increase effective size as compared to the best producing individual genera. The reason for the development of consortia was co-variance in the complementarity effect in consortia and the activity of species individually (Cardinale et. al 2006.) Elton's hypothesis that more diverse ecosystems are more stable has received much attention, but Darwin's proposal that more diverse plant communities are more productive, and the related conjectures (Tilman et al 1996). Elucidating structural and functional properties in polyculture is a matter of great curiosity which helps us to know the interaction of consortia of microalgae with the environment. Previous studies have proved that more diverse communities of various species drastically utilize and capture available limited resources from the environment and the vast amount of standing stock/ biomass production than less diverse of communities (Fox et al 2004). The reason for huge standing stock / aggregate abundance or total biomass production was photosynthetic mechanism of micro-algal participation. In this context Korner (2004) suggests that consortia could enhance not only photosynthetic rate but also capture and store Carbon dioxide from their surroundings, although this would not co-relate a stability of biomass by carbon storage due to increased aggregate abundance or total biomass (Gross et al 2014). Plants through photosynthesis process excrete out oxygen and intake/uptake CO₂ and yield critically quantified biomass. In this direction various studies have been generally made to show how preliminary action takes place in photosynthetic activity in photosynthetic micro-organism. In the present study, we have shown that, if the richness of species is enhanced in ratio, there is a depletion of available resources by resource pool method, thereby leading to the enhancement of micro-algal consortia biomass stability (Zimmerman et al 2014, Weis et al 2008). The stability of micro-algal biomass analyses either in diverse habitat or best yielding biomass of single strain of best consuming species by transgressed excess yielding (Cardinale et al 2007). The change in strain of algae and cyanobacteria in the media with continuous stepwise mixture of DDT (1, 1, 1 trichloro 2, 2-bis p-chlorophenyl ethane) may provide a profitable bio indicator of wastewater treatment (Meghora *et al* 2000). Members of cyanophyta *Anabaena* and *Nostoc* sp. have capability of dinitrogen-fixation to convert DDT to DDD (1, 1 dichloro-2, 2 bis p- chlorophenyl di ethane). On the other hand members of chlorophyceae (*Chlorella* sp.) inter-convert DDT to DDE (1, 1dichloro -2, 2 bis p-chlorophenyl ethylene (Subashchandrabose *et al* 2011). The strains of Chlorophyceae (*Chlorella* sp., *Scenedesmus* sp.) and Cyanophyceae (*Nostoc* sp. and *Lyngbya* sp.) were able to grow without any dangerous effect in concerning media containing 1% black oil. The cyanobacteria protect algae from toxic effects of the oil, and also provide other extracellular Carbon-rich exudates. Abed (2010) suggested that the capability of *Oscillatoria* sp. and other cyanobacteria mats to degrade hydrocarbons in oil was not due to themselves but due to the combination of aerobic heterotrophic bacteria. (Shukla *et al* 2012)

The micro-algae are non-vascular, autotrophic, photosynthetic plants and nature's greatest gift to mankind. The scope and application of micro-algae is broad in food, fodder and medicine. Increased industrial demand and utilization of fossil fuels result in increased CO_2 and other greenhouse gases into the environment. A number of efforts are going on globally to mitigate CO_2 from air. The photosynthetic organisms use solar energy to convert the atmospheric CO_2 into energy rich compounds- starch, cellulose, hemicellulose and oils. These renewable energy sources can be efficiently approached to solve the energy crisis and fulfil its demands without increasing the atmospheric CO_2 levels (Tans *et al.*, 2010). Nowadays the most important scope and application of algae is the optimization of carbon and oxygen quantity in environment due to process of photosynthesis. The recent hazardous problem, i.e. Green- house effect (GHG), means increase in temperature or global warming due to increase in carbon dioxide concentration in the atmosphere. During the past 150 years, the carbon-dioxide content in our atmosphere has increased approximately between 270-340 ppm. And 81% of it is caused by developed countries. (IPCC Report-2007, Vienna) .CO₂ was important resource, because of green plants, through the process of photosynthesis which produces its own food material, but when the concentration of CO_2 increases in atmosphere, it becomes problematic. There are two important factors by which the concentration of CO_2 in atmosphere increases rapidly.

Due to combustion of fossil flue gases, the content of CO₂ released due to increasing rate results in lower surface of atmosphere, i.e. the surface of earth produces high temperature which lowers the humidity in soil and rain due to the increase of acidity in oceanic water resulting in the melting of continental glaciers and peak of mountains (Carvalho et al, 2006).

It is CO₂which alone is 60% responsible for global warming. Nowadays, the human activities increase the heat absorbing gases or global effective gases. The exact amount of solar radiation, i.e.44% of it is directly absorbed by earth's atmosphere (Ono & Cuello, 2006). The micro-algal species has the property of CO2-fixation. The photoautotrophic algal culture has the potential to diminish the release of CO₂ in atmosphere, helping to alleviate the trend toward global warming (ONO et al 2006). To realize workable biological CO2 -fixation, the system selection of optional micro-algal species is vital. There were various CO₂ devices by which we know how to optimize the CO₂ concentration in the atmosphere, viz. Carbon credit, Carbon footprint, Carbon catcher, Carbon-index, Carbon emission, Absorbed carbon content and Clean development mechanism. (Brenan and Owende, 2010). The present study was done with the scope, application and utilization of fresh and dried biomass and the analysis of different bioorganic molecules, such as Optical density, estimation of chlorophyll-a, Carotenoid, Phycocyanin, protein, proline ,carbohydrate, photosynthesis analysis by flurowin fluorescence and Carbonic anhydrase enzymatic activity. In the present study there was a bioenergy rich cyanobacteria Synechococcus PCC7942, a micro-algae Chlorella sp., and nitrogen-fixing cyanobacterium Nostoc muscorum, Oscillatoria sp., and protein rich cyanobacteria Spirulina platensis. All the above species were grown in BG-11(+) media whilst Spirulina platensis was grown in Zarrouk media and all these were grown in two sets of community, one in BG-11(+) media, i.e. Consortia-A1, and the other set grown in Zarrouk media i.e. Consortia-A2. Like these, a biofuel rich cyanobacteria Synechocystis PCC 6803, a micro-algae Scenedesmus dimorphus and nitrogen fixing cyanobacterium Anabaena cylindrica, lyngbya sp. and Spirulina platensis were individually grown in BG-11(+) media and Spirulina platensis in Zarrouk media. All these species were grown in two sets of community, one set in BG-11 (+) media, i.e. consortia-B1, whilst other in Zarrouk media, namely consortia -B2.

Material and Methods

Test strains

Seven cyanobacteria test strains- *Synechococcus* PCC 7942, *Nostoc muscorum*, *Oscillatoria* sp., *Spirulina platensis, Synechocystis* PCC 6803, *Anabaena cylindrica* and *Lyngbya* sp. were raised. Besides these, two Chlorophycean microalgae *Chlorella* sp., and *Scenedesmus dimorphus* were previously preserved at the Centre of Biotechnology University of Allahabad. The test strains were raised in BG -11± media (P^H 7.8) in control as well as in community (Table 1)

Name of species	Name of Interaction	Culture condition
Synechococcus PCC7942, Nostoc muscorum, Oscillatoria sp. Spirulina platensis, Chlorella sp.	Consortium A1	All were grown in BG-11 (+) media.
Synechococcus PCC7942, Nostoc muscorum, Oscillatoria sp., Spirulina platensis, Chlorella sp.	Consortium A2	All were grown in Zarrouk media.
Synechocystis PCC6803,Anabaena cylindrica, Lyngbya sp.,Spirulina platensis, Scenedesmus dimorphus	Consortium B1	All were grown in BG-11 (+) media.
Synechocystis PCC6803,Anabaena cylindrica, Lyngbya sp.,Spirulina platensis, Scenedesmus dimorphus	Consortium B2	All were grown in Zarrouk media.

Table1.	Micro alga	I strains	employed
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The test strains were raised in BG-11(+) medium p^H^(7.8) with 1.5 gm. sodium nitrate (Stainer *et al* 1971). The flask and media were sterilized in an autoclave (Yarco, India), maintaining 15 lb./in² or kg /cm² pressure for 30 min. ±25 ml inoculums were suspended in 225 ml sterile medium taken in 500 ml .EM flask (three sets), maintaining the same O.D. after dilution at 750 nm., While as 5ml of each culture inoculums in each and every consortium. Cultures were allowed to grow for 25 days at 30© under light intensity of 200 lux provided by 20 W fluorescent tubes following a 16:8 h. light/ dark regime. Repeated shaking was done at regular intervals. The biomass was harvested by filtration through 50 ml plastic tubes.

Growth measurements

The optical density of test Cyanobacteria, green algae and consortia were determined over a period of 25 days by recording O.D. of 2ml. Algal suspensions at 750 nm .through cuvette of UV-visible spectrophotometer-SPECORD-200 at regular alternate intervals were observed. The growth absorption data was supported by biomass data (dry weight).

Microscopy

Microalgae cultures were collected from late logarithmic phase, washed twice with phosphate buffer (pH, 7.8), rinsed with sterile water and enriched in fresh sterile BG11⁺ media. 20 μ L of each culture was placed on clean glass slide, covered with slips and seen below 63X objective. The micrographs were recorded at 20 μ m bar scale. The compound

light microscopy was performed using Olympus microscope. Different micrographs were compared, and used to see the changes in community structure and niche partitioning of each monoculture in artificial habitat (Cardinale, 2011).

Biochemical analysis

Estimation of Chlorophyll

Chlorophyll content was estimated according to Rippka *et al*.1979. Absorption was measured at the 660 nm. Methanol was used as the standard.

Estimation of Carotenoid

Carotenoid content was estimated according to Hellebust and Craige, 1978. Absorption was measured at the 480 nm 100% methanol was used as the standard.

Estimation of Phycobiliprotein

Phycobiliprotein content was estimated according to Siegelman and Kygia 1978. Absorption was measured at the 620 nm ±0.05M Phosphate buffer was used as a standard.

Carbohydrate quantification

The total carbohydrate from Synechococcus PCC 7942, Nostoc muscorum ,Oscillatoria sp., Spirulina platensis , Synechocystis PCC 6803, Anabaena cylindrica, Lyngbya sp., Chlorella sp., Scenedesmus dimorphus, consortia A1, consortia A2, consortia B1 and consortia B2 was quantified using the Anthrone method of Loweus (1952) from equal biomass. One mL culture from initial stationary phase was taken from each experimental set up in separate eppendorf tubes and pelleted at 10000 rpm, 25© for 10 minutes. The pellet was washed with sterile distilled water twice and dried. Equal dried biomass from each strain was considered for study. The biomass was treated with 1mL of 1 N-NaOH by vigorous vortexing, and was kept in boiling water bath for 5 minute. The mixture was sonicated at±40kH frequency for 5 cycles of 30 seconds on and 30 seconds off. The crude homogenate was centrifuged at 2500g, for 5 minutes, and 100 mL supernatant from each was used for the determination of total carbohydrate. Glucose (1mg mL-1) stock was used for preparing the standard. The anthrone reagent was prepared by dissolving 0.2 g anthrone in 95%, 100mL ice chilled H₂SO₄ (stored at 4°C). 100mL processed supernatant of each cyanobacteria, green algae and their consortia was added to separate test tubes and the volume was crest upto1 mL by adding 900µL sterile distilled water. Added 4mL anthrone reagent (to five times diluted samples), incubated the content at R.T. for 10 minutes, boiled in boiling water bath for 10 minutes to stop the reaction, greenish blue colour of different intensity was seen, immediately chilled in ice bath for 10 minutes and measured the absorption at 625nm. The corresponding O.Ds. were subjected to linear regression analysis (Graph Pad prism 5.0) along with the O.D. values of glucose standard, and interpolated the unknown concentration from the glucose standards.

Estimation of Protein

Protein was estimated according to Folin-Lowry *et al*, 1951. Absorption was measured at the 665nm. BSA was used as the standard.

Estimation of Proline

Proline was estimated according to Bates *et al*, 1973. Absorption was measured at the 520 nm. BSA was used as the standard.

PAM- Analysis

In order to select the efficient high-CO₂ algal strains, control as well as consortia photosynthetic activity of algal strains were measured using Pulse Amplitude Modulation (PAM) Fluorometer (Photon System Instrument Pvt. Ltd., Czech Republic). All algal cells were grown at constant pH levels in7.8 with±0.4 v fed continuously into the culture

during cultivation. The algal pellets were collected by centrifugation (1500 \times g for 5 min) and suspended in a fresh culture medium at a density of 50µg.Chl mL-1. The algal suspension was placed in a cylindrical transparent vessel of a Chlorophyll-fluorometer for non-quenching PAM Analysis of Chlorophyll fluorescence.

Estimation of Dried Biomass (Greenberg et al., 1980)

Prior to the oven-drying process, the sample was either centrifuged or filtered. Each biomass sample was oven-dried at 105 °C in accordance with standard cyanobacteria sampling to separate the biomass from the culture medium. Samples of different micro-algae and consortia were filtered with whatman filter papers (11 mm pore size) to separate the biomass from dissolved nutrients. The carbon content of selected microalgae samples was also determined. Post –hoc test analysis and AIC Likelihood test analysis done to prove interaction in consortia community and decide the graph quality.

Carbonic anhydrase assay

Carbonic anhydrase activity was determined electrometrically according to Wilbur & Anderson, 1948. The measurements were carried out in five replicates by monitoring the rate of pH change during the carbon dioxide hydration using a fast-response.

Statistics

Various experimental data were analyzed by either one way or two way ANOVA as and where needed by using the Graph Pad Prism 5.0 or Sigma Plot 12.0 statistical tools. The significant differences in various treatments were considered on the basis of Fisher ratios (F value) and probability ($p\leq0.05$) at 95% confidence levels.

Results and discussion

Growth behavior

Cyanobacteria/Algae have diverse in monoculture and consortia

The different cyanobacteria available at the Centre of Biotechnology, University of Allahabad, and Allahabad, India were of diverse morphology, and they were light to dark greenish in color. The Consortia grew much rapidly than other cyanobacteria under the same environmental conditions in their respective growth media. The growth rate of Consortia significantly differed from that of other cyanobacteria and commenced from 3rd day with regular doubling to 25th day and finally stabilized in stationary phase. The growth curves and morphology of various organisms are provided in Fig.-1& Fig-2. The statistically significant differences in growth rates of Synechocystis PCC 6803, Synechococcus PCC 7942, Spirulina platensis, Nostoc muscorum, Oscillatoria sp., Anabaena cylindrica, Lyngbya sp. and Scenedesmus dimorphus, Chlorella sp. and different Consortia were observed (p20.05). Conversely, the more challenging task of producing 1-butanol from CO₂ in cyanobacteria remains more elusive, and only under dark conditions at the expense of internal carbohydrate storage (Power & Cardinale, 2009). This supports the diversityproductivity and diversity-sustainability hypothesis. Our results demonstrate that the loss of species threatens ecosystem functioning and sustainability (Tillman et al 1996). These results suggest that interspecific interactions tend to stabilize consortia biomass in diverse communities. Contrary to the prevailing theory, we found no evidence that species responses to environmental variation in monoculture predicted the strength of consortia stabilizing effect. Together, these results deepen our understanding of increasing species richness that stabilizes consortia biomass (Gross et al 2014).But Cardinale et al (14) also showed that resource capture and biomass production by the consortia used in experiments begin to saturate at low level of richness and that diverse communities seldom capture more resources or produce more biomass than their most productive species.



Fig. 1 Growth curves of various microalgae and their consortia.



Fig.2 Micrographs of various microalgae showing morphology (a) Chlorella sp. (b) Lyngbya sp (c) Oscillatoria sp. (d) Scenedesmus dimorphus (e) Consortia A1 (f) Consortia A2 (g) Consortia B1 (h) Consortia B2.

Total Carbohydrates were increased in community

The total carbohydrate was increased in the order Consortia B1>Consortia B2>Consortia A1>Consortia A2>*Nostoc m* uscorum>*Chlorella* sp.>*Synechococcus* PCC7942>*Lyngba* sp.>*Spirulina platensis>Anabaena cylindrica>Scenedes mus dimorphus>Oscillatoria* sp.>*Synechocystis* PCC 6803 producing 800, 788, 742, 720, 676, 625, 601.5, 594, 554.5, 524.5, 508, 403.5, 289 µg/ml. respectively(fig-3) Therefore, carbon storage was highest in all consortia, especially in *Nostoc muscorum* and *Chlorella* sp., whilst least in the *Synechocystis* PCC 6803 and *Oscillatoria* sp. An interesting thing was the enhanced carbohydrate content in community more than in all of the monocultures, indicating the positive role of these microbes in consortia. Two way ANOVA and Bonferroni posttests showed the statistically different carbohydrate contents in different monocultures (p-value ≤0.01, F=35.39). Therefore, these microalgae species and their consortia can be efficiently engaged to optimize Global temperature and to provide environmental sustainability. A large proportion of micro-algae are auxotrophs or facultative auxotrophs for vitamins, especially B12, and at least some of them can acquire B12 from more or less specific interactions with heterotrophic cyanobacteria. In some cases, heterotrophic cyanobacteria appear to provide Phyto-harmone like substances (indole-3-acetic acid) to enhance several micro-algal activities. This has been shown for the effect of culturing *Chlorella* sp. with the plant growth promoting cyanobacteria for an increased pigment, Carbohydrates, and population size of the micro-algae (Federico *et al.*2013)



Fig.3 Carbohydrate yields at the day of harvesting by different microalgae monocultures, their consortia.

Total dried biomass of consortia Impact Population-level stability

The total dried biomass increased in the order Consortia B2>Consortia B1>Chlorella was sp.>Scenedesmus dimorphus>Consortia A1>Consortia A2>Spirulina platensis>Nostoc muscorum>Lyngbya sp.>Synechococus PCC 7942>Synechocystis PCC 6803>Oscillatoria sp.>Anabaena cylindrica producing 3.547, 3.162, 2.2085, 2.197, 2.1672, 2.1425, 2.0625, 1.928, 1.8645, 1.86, 1.76625, 1.55425, 1.37365 gm./L(Fig-4) thus it, utilizes around 6.49101, 5.78646, 4.041555, 4.02051, 3.965976, 3.92075, 3.774375, 3.52824, 3.412035, 3.4038, 3.2322375, 2.8442775, 2.5137795 gm. CO₂ respectively(Brenann & Oened, 2006), which indicate micro-algal consortia used as a high light adaptability ,high temperature tolerance power, maximum carbon rate achieved property. The net effect is the species richness when values are averaged across all species and species combinations for a given level of richness. Our data suggest that algal species richness influenced the ratio of

production to respiration in vitro. Statistically, significant variation in biomass production was observed (p<0.001). Corresponding figures for biomass production by different consortia and monocultures are given in the consortia (Oikos,2009). Higher biomass and productivity in the consortia appeared to stem from co-dominance by two algal species that also achieved high biomass in monoculture (*Scenedesmus dimorphus* and *Chlorella* sp.), one of which also had the highest rate of biomass specific production. Our results suggest that consortia have both the rate at which CO₂ was removed from water, and the total amount of carbon accumulated and stored in biomass (Power *et al.* 2009).



Fig.4 Biomass yields of various microalgae and consortia at stationary phase.

In consortia responses fluctuations predict the strength of sampling effect on Protein and Proline

The total protein was by Lowry *et.al,* obtained in the order *Spirulina platensis*>Consortia B1>*Chlorella* sp.>Consortia B2>*Scenedesmus dimorphus*>Consortia A2>*Anabaena cylindrica*>*Synechococcus* PCC7942>*Oscillatoria* sp.>Consortia A1>*Lyngbya* sp.>*Synechocystis* PCC 6803>*Nostoc muscorum* producing 4.1085, 2.9255, 2.8824, 2.631, 2.06225, 2.0875, 2.00585, 1.99165, 1.84305, 1.5545, .71065, .523, .385 mg/MI respectively(Fig-5a), whilst total proline was obtained in the order *Synechocystis* PCC6803>*Spirulina platensis*>*Nostoc muscorum*>Consortia A1>Con sortia B2>Consortia B1>*Oscillatoria* sp.>Consortia A2>*Scenedesmus dimorphus*>*Chlorella* sp.>*Lyngbya* sp.>*Anabae na cylindrica*>*Synechococcus* PCC 7942 producing 8.99035, 8.791, 8.7788, 8.35071, 8.3365, 8.27065, 7.9143, 7.80211, 7.3277,7.2927, 7.2782, 6.34245, 6.26964 mg/ml. respectively(Fig-5b). The reason for the richness of Protein and Proline content in *Spirulina platensis* is Protein rich alga. The biomolecules for nonconventional consortia naturally efficient to perform specific tasks need to be improved and shared among public and private sectors. More specific cases that would benefit from the use of synthetic micro-algal consortia should be identified to stimulate creative thinking on synthetic circuit design (Federico *et al* 2013).



Fig.5a&b Protein and Proline yields of various microalgae and their Consortia

Consortia impact on community stability into its effect on the mean and variance of Pigments Biomass

The chlorophyll-a estimation

The chlorophyll content was estimated for 25 days and the final value obtained was in the order *Spirulina platensis>Scenedesmus dimorphus>*Consortia B1>Consortia B2>*Chlorella sp.>Oscillatoria* sp., *Synechocys tis* PCC 6803>*Lyngbya* sp.>*Nostoc muscorum*>Consortia A2>Consortia A1>*Anabaena cylindrica*>*Synechococcus* PCC7942 producing 7.225, 5.6145, 4.5285, 4.4765, 3.927, 3.6165, 3.6165, 3.268, 2.8935, 2.7455, 2.7095, 1.9795,

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(a)

1.366mg/ml respectively(Fig-6a) more or less continued growing order, whilst carotenoid was

estimated on 0, 4, 8,12 days,the results were in the order *Spirulina platensis>Anabaena cylindrica>Lyngbya* sp.>Con sortia B2>Consortia B1>*Chlorella* sp.>*Synechococcus* PCC7942>Consortia A2>Consortia A1>*Scenedesmus dimorp hus*>*Oscillatoria* sp.>*Synechocystis* PCC 6803>*Nostoc muscorum* producing 7.14, 5.395, 4.905, 3.97, 3.94, 3.66, 3.455, 1.805, 1.395, 1.22, 1.195, 0.765, 0.12 mg/ml. respectively(Fig-6b) ,On the other hand, Phycocyanin was estimated also 0, 4, 8, 12 days and the results were in the order *Spirulina platensis*>*Chlorella* sp.>*Synechococcus* PC C7942>*Scenedesmus dimorphus*>*Oscillatoria* sp.>*Synechocystis* PCC6803>*Lyngbya* sp.>*Nostoc muscorum*>*Anaba ena cylindrica*>Consortia A1>Consortia A2>Consortia B1>Consortia B2 producing 3.879, 2.998, 2.898, 2.786, 2.211, 1.997, 1.989, 1.898, 1.883, 1.873, 1.452, 1.121, 1.009 µg/ml. respectively(Fig-6c). In the same sequence, chl-a and carotenoid content was maximum in *Spirulina platensis*. More chl-a in *Spirulina platensis* allows it for producing more standing biomass and utilizing the more photons from sunlight. Under excess light, PSI and PSII of *Spirulina platensis* thylakoid membranes will protect it more efficiently than *Spirulina platensis* and *Synechococcus* PCC7942 due to higher carotenoids, while the excess of photons absorbed by chl-a can be dissipated either as heat or can be utilized for more photosynthesis in *Spirulina platensis*. The reason for the dominance of these *Spirulina platensis* filaments in varying environment is the presence of rich carotenoids.





Fig.6 Pigment analysis of various microalgae and consortia (a) Chlorophyll-a (b) Carotenoid (c) Phycocyanin.

Interactions in non-photochemical quenching of Consortia affect correlations among species

Pulse amplitude modulation (PAM) is one of several forms of single modulation in which data are transmitted by varying the amplitude of the pulses in a regular timed sequence of electrical or electromagnetic pulses. A signal is sampled and regulated at regular intervals and is made proportionate and synchronous to the magnitude of the signal. According to the PAM graph obtained, we were able to find that all Consortia and Scenedesmus dimorphus was the best species (or the best strain) showing most efficient photosynthetic ability. Similarly SynechocystisPCC.6803, Synechococcus PCC 7942, S. platensis had a good photosynthetic efficiency as compared to the other strains studied. As we know, that non photosynthetic guenching gives a measure of the total quantum efficiency and is inversely related to it, and here the highest quenching is shown by A. cylindrica making it the least efficient strain regarding the photosynthetic efficiency. Intending to establish the functional position of photosynthesis rates of unicellular and filamentous cyanobacteria Fv/Fm ratio was measured. It imitated the maximum photosynthetic quantum yields of PSII reaction centers of various dark-adapted cyanobacteria cultures. In general, filaments were poor in maximum quantum yields. In all Consortia, Spirulina platensis and Chlorella sp. showed maximum Fv/Fm value followed by Anabaena cylindrica, Oscillatoria sp., Lyngbya sp., Nostoc muscorum and Synechcoccus PCC7942, and Scenedesmus dimorphus. The upper Fv/Fm ratios for Spirullina platensis point towards their higher photosynthetic rates than the other cyanobacteria. Thus, unicellular circles having more surfaces to volume ratio were found to have upper photosynthesis rates. Although Spirulina platensis yielded good biomass rather than trichome uniseriate, but it was the poorest strain in photosynthetic efficiency and the probable reason for it was the low content of chlorophyll and carotenoids in its elongated cells. Fv/Fm (Fmax-Fmin/Fmax) was monitored in all cultures and consortia cells grown at different levels of illumination. Fv/Fm is a useful parameter to evaluate photosynthetic efficiency in algae and mainly to highlight photo-inhibition due to excess illumination. PAM fluorometry results indicated that at optimal light and other growing parameters the quantum yield is most. In all the cases along with the increasing chlorophyll content, a lower Fv/Fm was also observed, indicating that the cells were growing and performing photosynthesis and were fighting photo-inhibition, although they were exposed to a low total amount of photons. The quenching analysis by PAM fluorometer gave the measure of photosynthetic efficiency in terms of quantum yield. The Fv/Fm series parallels the evolutionary sequence of these organisms and reflects several possible sources for improving the efficiency. Fv/Fm (Fmax-Fmin/Fmax) was monitored in all the cultures. Fv/Fm is a useful parameter to evaluate the photosynthetic efficiency in algae, and mainly used to highlight the photo-inhibition in excess illumination. PAM-fluorometry results indicated that at optimal light intensity, Fv/Fm ratios of different monocultures and consortia were found to be statistically different from each other (p<0.001). In all the cases, with increasing chlorophyll content, a lower Fv/Fm was observed, including all the monocultures and consortia (Fig-7). It indicates that the cells were efficiently growing and performing photosynthesis, and were actively involved in the photo-inhibition process. The quenching analysis by PAM-fluorometer provided the measure of photosynthetic efficiency in terms of quantum yield. In general, monocultures were poorer in maximum quantum yield, than all the consortia.



effects of non-photochemical quenching on photosynthetic rates of various Strains of cyanobacteria, green algae and their consortia.

Increased Carbonic anhydrase activities in consortia responses to Environmental fluctuations predict the strength of diversity, and affect the community stability

Carbonic Anhydrase catalyzes the reversible hydration of CO₂. This reaction involves a two-step mechanism. The first step is the nucleophilic attack of a zinc-bound hydroxide ion on CO₂. The second step is the regeneration of the active site by ionization of the zinc-bound water molecule and the removal of a proton from the active site. The increased (decreased) intracellular carbonic anhydrase (CA) activity in consortia and monocultures confirms their progress towards CO₂ assimilation. In consortia, the data showed that the activity of DIC (Dissolved Inorganic Carbon) utilization for photosynthesis is quite high. Thus, the activity of Carbonic anhydrase in all consortia was lower than that of CO₂ capture and storage. In contrast, low CO₂ cells could utilize large amount of DIC within cells and excrete CO₂ in the cells. If CA activity or CO₂ ability is reduced, this indicates that K_m (CO₂) values for photosynthesis and photorespiration rate both increased respectively, thus showing high power of DIC utilization in high CO₂ concentration due to least(maximum) carbonic anhydrase activity in consortia. The stability of micro-algal biomass analyses either in diverse habitat or best yielding biomass of single strain of most consuming species by transgressed exceed yielding. Conclusively, the results indicate that in these organisms consortia functions better than individual alga (see Table-1).

Name of species	Enzyme without extract (T₀)	Enzyme with extract (T)	Unit/mg activity WAU=2(T ₀ -T)/T
Synechococcus PCC7942	84	36	1.143
Nostoc muscorum	86	26	1.395
Oscillatoria sp.	88	25	1.432
Spirulina platensis	86	39	1.093
Chlorella sp.	90	60	1.022
SynechocystisPCC6803	90	38	1.155
Anabaena cylindrical	86	32	1.256
<i>Lyngbya</i> sp.	88	39	1.114
Scenedesmus dimorphus	89	46	0.966
Consortia A1	93	22	1.527
Consortia A2	92	26	1.435
Consortia B1	94	20	1.574
Consortia B2	93	22	1.526

Table-1 Carbonic anhydrase activity:

Conclusion

Photosynthetic fixation by micro-algal consortia for environment treatment, energy saving in Oxygen supply, CO₂ mitigation, efficient recycling of nutrients and revalorization of algal biomass, a pay-back to the plant operation cost, makes their utilization very valuable. Our work serves as a case study that suggests it is possible that loss of species diversity from environments could reduce both the accumulation and storage of Carbon in biomass. Species interactions also moderately positively show co-relation of algal species among species and promote community stability and enhanced the production of edible, medicinal and protein rich alga *Chlorella* sp. and *Spirulina platensis*. Our study contributes to an important ongoing debate on how changes in micro-algal consortia were likely to alter the

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function of ecosystem. Although there is no unequivocal evidence that micro-algal consortia in numerous behavior influences the biomass of those groups (Balvanera *et al*, 2006, Cardinale *et al* 2006, 2007, Stachowicz *et al*, 2007). It was much clear how consortia influences rates of important ecological processes, such as the rate at which consortia assimilate or release CO₂, O₂, or nutrients from/to the abiotic environment (Petchey 2003, Korner 2004, Shrivastava *et al*, 2009, Schmid *et al*, 2009). To understand how consortia affects rates of processes(as opposed to just biomass stocks) was essential if we were to predict whether modern changes in biodiversity would enhance ecosystem response to other forms of environmental change, such as increases in atmospheric level of CO₂ (Niklaus *et al*, 2001, Reich *et al*, 2001), widespread deposition of nutrients that cause eutrophication (Tilman *et al*, 1996, Reich *et al*, 2011, Fridley 2002), and the resulting change in CO₂ concentration that stems from eutrophication in natural habitats(Dodds, 2006). More diverse the species, more the production. The functioning, operation, and sustainability of ecosystem depend on the biological diversity. The consortia showed positive interactions amongst species present. The consortia showed the surge in effective biomass due to species richness. The carbohydrate and protein content was comparatively more in the consortia as against test cultures. The rate of photosynthesis was increased in consortia. Better carbon fixation rate in the consortia made with the variant richness of species was suggested by PAM analysis.

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