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# Standing stock production by micro-algal consortia for $CO_2$ Sequestration and mitigation

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## Abstract

Micro-algae comprise the members of Cyanophyta and Chlorophyta that have the different morphological types. These are the only known prokaryotes with oxygenic photosynthesis. Due to anthropogenic activities a drastic loss in their biodiversity has been observed in recent years. The literature shows that how they could be actively engage in increasing the overall productivity of community by mitigating the atmospheric $CO_2$ and providing opportunities to explore them for a number of purposes. Present study was performed to evaluate the role of taxonomically different genera based synthetic microalgae consortia in improving the sequestration and mitigation of atmospheric $CO_2$. The growth of monocultures and different consortia was studied, and at the end of cultivation functional changes in terms of photosynthetic pigments, total protein, proline, total carbohydrate, reducing sugars, photosynthetic acclimation, and bicarbonate-anhydrase activity were evaluated. Besides, total productivity was measured in the terms of dried biomass while structural changes in the community were monitored under the microscope. Drastic observations were observed in the functional and structural characteristics of different consortia.
Based on experimental observations, we advise that the communities with more species take greater advantage in an environment, and it allow the diverse systems to capture a greater proportion of biologically available resources such as nitrogen and $CO_2$. Therefore, the present synthetic algal technology could be a helpful tool in developing the sustainable biomass production.

*Keywords:* Microalgae, Consortia, Mitigation, PAM, Biomass and Biofuels.

## 1. Introduction

Biologists have suggested that the detrimental environmental effects are mainly responsible for the extinction of micro-algal species and loss of biodiversity. Yet, it was not until after the 1990 that research efforts could formalize the hypothesis that species diversity might influence the fluxes of energy and matter that are fundamental to all the ecological processes, including those which control the abundance, biomass and distribution of organisms. A throughput study suggested that loss of photosynthetic micro-algal species is directly related to the decrease in the productivity of community and increasing in the atmospheric $CO_2$ levels (Cardinale*, et. al*. 2006). But the interpretation of these studies provoked the considerable debate (Cardinale, 2011) and subsequent work produced several counter examples that questioned about the generality of these biodiversity effects. Standing stocks were calculated as the aggregate abundance or biomass of all organisms per unit area or volume. Depletion of resources was calculated as an instantaneous rate of consumption (Gross *et. al* 2014). Over the past few decades, researchers have examined that how the biodiversity loss influences the functioning of ecosystems, as well as the cascading impacts on the goods and services of ecosystems (Zimmerman *et.al* 2014). The relationship between the biodiversity and ecosystem functions quantified in prior worldwide efforts suggests that initial losses of biodiversity have relatively small impacts on functioning like community biomass production; however, beyond some threshold, more species loss lead to accelerating declines in overall

function. Some peoples have questioned whether a saturating relationship between diversity and community biomass production is an artifact of overly simplified experiments that manipulate diversity in homogeneous conditions over short time-scales in which niche differences may not be realized. Others have questioned that even the modest effects of biodiversity observed in the experiments would be discernible in natural systems where they could be over ridden by the stronger influence of abiotic factors (Power and Cardinale 2009).

## 2. Materials and methods

### 2.1 Test strains

The monocultures included in the study were belonging to the Cyanophyceae; *Westiellopsis prolifica, Calothrix sp., Aphanothece nageli, Microcystis aeruginosa, Scytonema sp.,*and *Gloeocapsa sp,* and Chlorophyceae; *Chlamydomonas reinhardtii, Scenedesmus quadricauda, Chlorella sp.*, *Chlamydomonas reinhardtii, Scenedesmus abundance* and *Scenedesmus dimorphus* respectively.

Total six consortia were synthesized in two different sets and each set had three different consortia respectively. The organism in 1$^{st}$ consortia of set one, included *Westiellopsis prolifica, Calothrix sp., Aphanothece nageli, Chlamydomonas reinhardtii, Scenedesmus quadricauda* and *Chlorella sp*, while 2$^{nd}$ consortia consisted *Chlamydomonas reinhardtii, Scenedesmus quadricauda, Chlorella sp.* and 3$^{rd}$ consortia had *Westiellopsis prolifica, Calothrix sp., Aphanothece nageli* respectively. Fourth, fifth and sixth consortia were synthesized by group of second set of organisms. The 4$^{th}$ consortia consisted the cyanobacteria *Microcystis aeruginosa ,Scytonema sp. , Gloeocapsa sp.,* and green algae *Chlamydomonas reinhardtii, Scenedesmus abundance, Scenedesmus dimorphus*; whilst 5$^{th}$consortia included *Chlamydomonas reinhardtii, Scenedesmus abundance, Scenedesmus dimorphus*; and 6$^{th}$ consortia consisted *Microcystis aeruginosa, Scytonema sp.,Gloeocapsa sp.*

The present study was performed to evaluate the synthetic consortia that consisted of genera based on different taxonomic family type. The Cyanophyceae monocultures were *Westeillopsis prolifica, Calothrix sp., Aphanothece nageli, Microcystis aeruginosa, Scytonema sp.* and *Glycothecium sp.,* Chlorophyceae monocultures included in the study were*, Scenedesmus quadricauda, Chlorella sp.*, *Chlamydomonas reinhardtii, Scenedesmus abundance* and *Scenedesmus dimorphus*, which were chosen on basis order feature. The test strains grown in BG11$^{+}$ media (pH 7.8) are presented in Table 1. 25 ml inocula were suspended in 225 ml sterile medium in 500 mL Erlenmeyer flasks. Equal volumes of cultures were inoculated from the mid-log phase grown cultures. Cultures were allowed to grow for 26 days at 30ºC under light intensity of 100 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 (Fox 2004*)*.

### 2.2 Growth measurements

The optical density of test cyanobacteria, micro-algae and consortia strains were determined for a period of 20 days on each interval of 24 h at 750 nm by using the U.V.-visible spectrophotometer-SPECORD-200. Dried biomass was quantified by gravimetric method (Patel *et al*., 2014).

### 2.3 Estimation of total carbohydrates

Total carbohydrate was estimated according to the Anthrone, 1966 with slight modifications. Absorbance was measured at the 625 nm. Glucose was used as the standard.

### 2.4 Estimation of reducing sugars

Reducing sugar content was measured by following the method of Nelson-Somogyi (1944).

### 2.5 PAM analysis

In order to select the efficient high-$CO_2$ sequestering algal strains, photosynthetic activity of the control as well as consortia algal strains were measured using the Pulse Amplitude Modulation (PAM) Flourometer (Photon System Instrument Pvt. Ltd., Czech Republic). All the algal cells were grown at constant pH 7.8 with 0.4 vvm continuously fed batch condition throughout the cultivation. The algal pellets were collected by the centrifugation (1500 × *g* for 5 min), and suspended in a fresh culture medium at a density of 50 µg *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 (Patel *et al* 2016).

**2.6 Carbonic anhydrase assay**

Carbonic anhydrase activity was determined electrometrically according to the Wilbur & Anderson, 1948. The measurements were carried out in five replicate.

**2.7 Statistics**

Various experimental data were analyzed by either one way or two way ANOVA as where needed by using the Graph Pad Prism 5.0 statistical tools. The significant differences in various treatments were considered on the basis of probability ($p \leq 0.05$) at 95% confidence levels.

**3.0 Results and discussion**

**3.1 Growth behaviour**

The different cyanobacteria available at Centre of Biotechnology, University of Allahabad, and Allahabad, India were of diverse morphology, they were light to dark greenish in colour. The consortia grew much rapidly than other cyanobacteria under the same environmental conditions in their respective growth media. The growth rate of consortia was significantly differed than other cyanobacteria and commenced from 3$^{rd}$ day with regular doubling to 20$^{th}$ day and finally stabilized in stationary phase. The growth curves and growth kinetics of various organisms are provided in Fig. 1a&b.

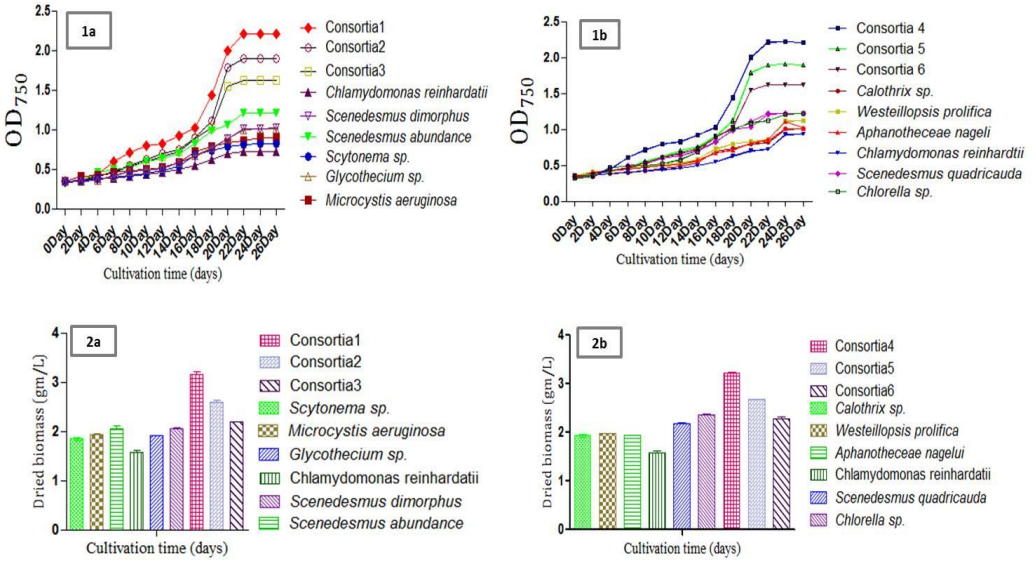


**Fig 1a, 1b: Growth curves of various cyanobacteria, microalgae and consortia.**
**Fig 2a, 2b: Dried biomass yields of various cyanobacteria, microalgae and consortia**

**3.2 Total Carbohydrate and reducing sugar yields**

The varying carbohydrate productivity was observed by different monocultures and consortia. Maximum total carbohydrate yield of 0.7130 mg $mL^{-1}$ was observed in the fourth consortia that consisted of *Calothrix sp., Weisteillopsis prolifica* and *Aphanothecae nageli* as constituting species. In contrast, reducing sugar content in that consortium was only 0.012 mg $mL^{-1}$. The consortia one, two, three, five and six produced 0.7130, 0.6028, 0.5987, 0.6823, and 0.6078 mg $mL^{-1}$ total carbohydrates. Amongst the monocultures; *Chlorella* sp. produced maximum total carbohydrate followed by *S. quadricauda, S. dimorphus, S. abundance, A. nageli, Glycothecium, W. prolifica, C. reinhardtii, Chlamydomonas sp, Calothrix sp., Scytonema sp.,* and *Microcystis aeruginosa* respectively. Two way ANOVA and Bonferroni post tests 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 providing environmental sustainability.

### 3.3 Biomass production

Overall the biomass production of consortia was found to be greater than monocultures and maximum biomass production was recorded in the case of consortia one followed by fourth, two, five, three, and six respectively. 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 Fig 2a&b.

### 3.4 Fluoroscence measurement

Fv/Fm ($F_{max}$-$F_{min}/F_{max}$) 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 in all the cases including all the monocultures and consortia. 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 poor in maximum quantum yield, than all the consortia in Fig 3a and b.

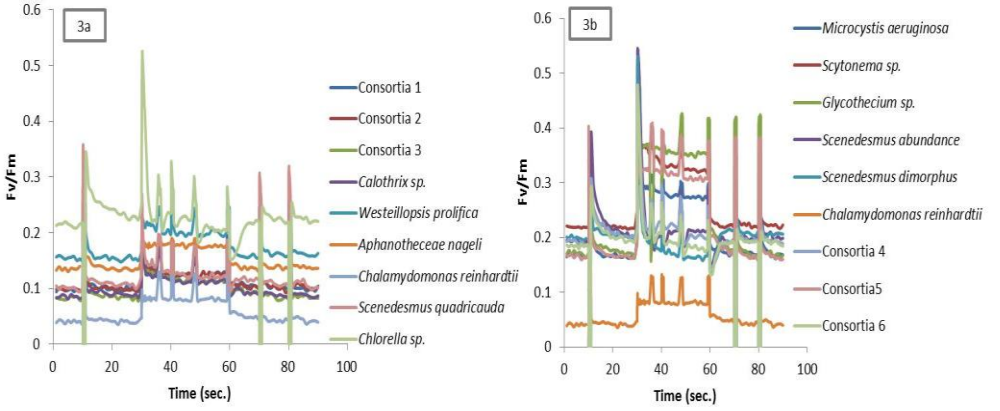


**Fig. 3a, 3b: Photosynthetic quantum yields by various cyanobacteria, microalgae and Consortia**

### 3.5 Carbonic anhydrase activity

The increased (decreased) intracellular carbonic anhydrase (CA) activity in consortia and monocultures confirm their progress towards $CO_2$ 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 $CO_2$ Capture and storage. In contrast, low $CO_2$ cells could utilize large amount of DIC within cells and excrete $CO_2$ in the cells. If CA activity or $CO_2$ ability is reduced this indicates that $K_m$ ($CO_2$) values for photosynthesis and photorespiration rate both increased respectively, thus showing high power of DIC utilization in high $CO_2$ concentration due to least(maximum) carbonic anhydrase activity in consortia. Conclusively the results indicate that in these organisms consortia functions better than individual alga in Table 1.

**Table1: Carbonic anhydrase activity of cyanobacteria, microalgae and their consortia.**

| Name of species | Enzyme with extract (T) | Enzyme without extract($T_0$) | Unit of activity | (WAU=2($T_0$-T)/T) |
|---|---|---|---|---|
| *Scytonema sp.* | 26.50 | 84.21 | | ±1.371 |
| *Microcystis aeruginosa* | 26.50 | 86.46 | | ±1.386 |
| *Gloeocapsa sp.* | 26.50 | 85.50 | | ±1.380 |
| *Chlamydomonas reinhardtii* | 26.50 | 88.38 | | ±1.400 |
| *Scenedesmus abundance* | 26.50 | 86.20 | | ±1.385 |
| *Scenedesmus dimorphus* | 26.50 | 87.27 | | ±1.393 |
| Consortia 1 | 26.50 | 108.20 | | ±1.506 |
| Consortia 2 | 26.50 | 103.13 | | ±1.486 |
| Consortia 3 | 26.50 | 102.34 | | ±1.482 |
| *Calothrix sp.* | 26.50 | 85.21 | | ±1.378 |
| *Westiellopsis prolifica* | 26.50 | 86.34 | | ±1.386 |
| *Aphanothece nageli* | 26.50 | 85.51 | | ±1.380 |
| *Chlamydomonas reinhardtii* | 26.50 | 88.18 | | ±1.399 |
| *Scenedesmus quadricauda* | 26.50 | 86.39 | | ±1.387 |
| *Chlorella sp.* | 26.50 | 89.42 | | ±1.407 |
| Consortia 4 | 26.50 | 114.27 | | ±1.536 |
| Consortia 5 | 26.50 | 107.37 | | ±1.506 |
| Consortia 6 | 26.50 | 103.19 | | ±1.486 |

**Table 2 Total and reducing carbohydrate quantification**

| Name of species | Total Carbohydrate (mg/ml.) | Reducing sugar (mg/ml.) |
|---|---|---|
| *Scytonema sp.* | 0.3139 | 0.084 |
| *Microcystis aeruginosa* | 0.3157 | 0.023 |
| *Gloeocapsa sp.* | 0.4993 | 0.092 |
| *Chlamydomonas reinhardtii* | 0.4627 | 0.043 |
| *Scenedesmus abundance* | 0.5139 | 0.097 |
| *Scenedesmus dimorphus* | 0.5350 | 0.099 |
| Consortia 1 | 0.7130 | 0.011 |
| Consortia 2 | 0.6028 | 0.013 |
| Consortia 3 | 0.5987 | 0.012 |
| *Calothrix sp.* | 0.4247 | 0.059 |
| *Westiellopsis prolifica* | 0.4948 | 0.083 |
| *Aphanothece nageli* | 0.5127 | 0.049 |
| *Chlamydomonas reinhardtii* | 0.4623 | 0.082 |
| *Scenedesmus quadricauda* | 0.5753 | 0.099 |
| *Chlorella sp.* | 0.5898 | 0.098 |
| Consortia 4 | 0.7231 | 0.012 |
| Consortia 5 | 0.6823 | 0.014 |
| Consortia 6 | 0.6078 | 0.017 |

**Conclusion**

Concept of consortia is a modern tool to develop the affordable biomass production and $CO_2$ sequestration. However, a little effort has been made to evaluate their potential till date. In the present study, we evaluated these cyanobacteria and green algae species in $CO_2$ mitigation and biomass production. The results could be implemented for *in situ* studies in natural reservoirs. The green algae species showed better activity towards the biomass production whilst cyanobacterial species were more efficient towards $CO_2$ sequestration. Therefore, to get the carbohydrate enriched biomass with higher $CO_2$ sequestration potential, it is very important to establish the consortia of the selected algae and cyanobacterial species. Improving the quality of consortia by proper selection of species could be a good approach towards sustainable improvement and environmental restoration. Better understanding of the natural assemblages of microbial communities and engineering microbial consortium with enhanced abilities, can guide us toward the dual mission of pollutant degradation and commercial production of metabolites of biotechnological importance and simultaneous mitigation of $CO_2$ by its photosynthetic fixation (Subashchandrabose *et. al.*2011).

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