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Research article

Change in physico-chemical character of nutrient media and carpet effluent in presence of six algae monocultures and their consortia

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Abstract: Synthetic microalgae ecology is a promising approach to regulate the concentrations of nutrients in wastewater reservoirs. The aim of experiments was to evaluate the role of six microalgae monocultures and their consortia in restoring the water quality of water reservoirs represented here in the form of media BG11$^+$ (nitrate rich), BG11$^-$ (nitrate poor) and wastewater from carpet effluent (CE). The algal species utilized in the present experiment were *Anabaena* sp., *Chlorella* sp., *Oscillatoria* sp., *Nannochloropsis* sp., *Scenedesmus* sp., *Phormidium* sp., and their synthetic consortia. The water parameters analyzed were dissolved oxygen (DO), water conductivity, pH and concentrations of nutrients (nitrates, phosphates, nitrites). We observed that *Chlorella* sp. produced maximum biomass of 2.217±0.126 g.L^{-1} in BG11$^+$ media followed by *Anabaena* sp. 1.897±0.045 for BG11$^-$ media and 1.233±0.29 g.L^{-1} for synthetic consortia of BG11$^-$ media. On other hand, biomass yields of 1.233±0.017 and 0.460±0.010 g.L^{-1} were obtained respectively for consortia in the nitrate deficient BG11$^-$ media and CE water. Consequently, with inoculation of consortia in BG11$^+$ and CE media nitrate level was increased by 295.27% (748.08 mg.L^{-1}) and 123.14% (1.33 mg.L^{-1}) respectively, while decreased in BG11$^-$ by 68.91% (5.1 mg.L^{-1}). Nitrite concentration was enhanced up to 35.52, 0.038 and 0.028 mg.L^{-1} in BG11$^+$, BG11$^-$ and CE respectively. In contrast, with inoculation of the consortia, the phosphate levels were found to be depleted by 35.35% (1.31 mg.L^{-1}) in BG11$^+$, 95.72% (3.64 mg.L^{-1}) in BG11$^-$ and 94.97% (1.7 mg.L^{-1}) in CE respectively. The range of pH value and DO for different media was found between 8.25–8.87 and 6.14–10.54 mg.L^{-1} respectively, and conductivity was increased with the growth of algae. The study suggests that beneficial algae and their consortia can be logically selected for biomass production and for improving the water quality.

Keywords: Wastewater treatments - Microalgae consortia - Water parameters - Biomass - Carpet effluent.

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INTRODUCTION

The idea of employing the microalgae for wastewater treatments has been originated about six decades ago (Oswald *et al.* 1957). Today microalgae are widely utilized in industrial and household wastewater treatments. These microalgae can perform photosynthesis to generate their own energy and releases excess amount of O_2 to the atmosphere. Microalgae wastewater treatment plants require minimal supply of energy resources that reduces the use of sophisticated instruments such as energy-intensive electromechanical blowers (Green *et al.* 1995), catalytic hydrothermal gasification (Elliott 2008), anaerobic digestion (McCarty *et al.* 2011) and hydrothermal liquefaction (Chakraborty *et al.* 2012). In conventional wastewater treatments nitrate is converted into the N_2 gas through the oscillation within nitrification or denitrification system while phosphates are

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precipitated by metal salts. Microalgae remove nitrates and phosphates in more eco-friendly manner. Through mixotrophic metabolism, microalgae consortia could perform better and can be used as a sustainable method for treatment of wastewater. Mixotrophic metabolism utilizes nitrate and phosphate from wastewater to enhance microalgae biomass (Henkanatte-Gedera *et al.* 2015). Microalgae consortia based wastewater treatments has numerous advantages over the conventional methods. The consortia could be efficient in detoxification of organic and inorganic pollutants from wastewater as compared to the individual microalgae (Subashchandrabose *et al.* 2011). Natural consortia improve water quality (bioremediation of hazardous waste *viz.* nitrates and phosphates), soil quality (nutrient recycling), plant health etc., and help in maintaining the major ecosystem (Lugtenberg & Kamilova 2009, Gilbert *et al.* 2012, Craggs *et al.* 2013, Samorı` *et al.* 2013). Engaging microalgae consortia for the nutrient recycling in different wastewater reservoirs to produce biomass for a broad range of value added bio-chemicals (Martins *et al.* 2010) has revitalized the concept of synthetic microalgae ecology as emerging technology (Pittman *et al.* 2011, Craggs *et al.* 2013).

Microalgae require nitrate and phosphate in a specific ratio for biomass production. Therefore, removal of nitrate depends on the levels of phosphate in wastewater and vice-versa. In household wastewater nitrate occurs between 15 to 90 mg.L^{-1} while levels of phosphate vary between 4 to 20 mg.L^{-1} (Christenson & Sims 2011, Abdelaziz *et al.* 2013, Cai *et al.* 2013). Microalgae utilize N and P in various biochemical reactions to produce a number of primary and secondary metabolites that alternatively determine the overall biochemical composition of biomass (Klausmeier *et al.* 2004, Loladze & Elser 2011). Nitrogen participates in the synthesis of protein while phosphorus helps in the synthesis of ribosomal RNA and ribosome (French *et al.* 2003, Dennis *et al.* 2009). A number of studies have shown that lower supply of nitrate and phosphate resulted in more accumulation of carbohydrate or lipid or both in their biomass. However, their higher levels increase the algae biomass as well as their concentration in water or media (Xin *et al.* 2010, Arbib *et al.* 2013, Samorı` *et al.* 2013, Beuckels *et al.* 2015). Published literature showed that the concentration of any one nutrient (either nitrate or phosphate) fluctuate at a time while other remains constant (Xin *et al.* 2010, Arbib *et al.* 2013, Samorı` *et al.* 2013, Zhang & Hong 2014). Therefore, levels of both nutrients vary simultaneously without changing their ratios (Aslan & Kapdan 2006, Akerström *et al.* 2014). At high levels of nitrate reduced absorption of phosphate from water has been reported (Pittman *et al.* 2011, Craggs *et al.* 2013). High amount of nitrate content reduces the nitrate uptake. In the presence of high amount of nitrate, some algae synthesize nitrate (Philips *et al.* 1992). Nutrient availability influences the biochemical composition of microalgae biomass that alternatively provokes the biosynthesis of some primary and secondary metabolites. Keeping their facts in view the aim of present study is to know up to what extent the microalgae monocultures and their consortia can mitigate the levels of nitrate, nitrite and phosphate in carpet effluent (CE), and how they respond to BG11^{+} and BG11^{-} media supplemented with and without nitrate respectively. We have also evaluated that up to what extent the water physico-chemical parameter in media and natural CE water can affect the physiology and biomass production.

MATERIALS AND METHODS

Algae Cultures

Six different microalgae species belonging to three phyla such as Cyanophyta (*Anabaena* sp., *Oscillatoria* sp., *Phormidium* sp.), Orchrophyta (*Nannochloropsis* sp.) and Chlorophyta (*Chlorella* sp., *Scenedesmus* sp.) were isolated and purified from the carpet industries effluent of Bhadohi district, Uttar Pradesh, India. These microalgae monocultures were regularly maintained on BG11^{+} agar plates as well as in the liquid BG11^{+} media. Each algae taxon was identified by following the standard procedures and relevant literature (Desikachary 1959, Philipose 1967, Hibberd 1981). CE media was sterilized through 0.22 µm membrane filter while BG11^{+} and BG11^{-} media were steam sterilized. All the cultures were incubated at 27±1 ºC temperature with an illumination of 92 µmole.m^{-2}.S^{-1} photons in a regime of 14:10 light-dark period in 500 mL microcosms.

Experimental design

A set of 21 experiments were executed in 200 mL of sterilized BG11^{+}, BG11^{-} media and CE water for six monocultures and their synthetic consortia in three replications. The BG11^{+} media was supplemented with 1.5 g.L^{-1} $NaNO_3$ while BG11^{-} media was maintained without nitrate supplement and CE acted as natural media. The algae biomasses were harvested after 35 days of cultivation, and dried biomass was obtained by the gravimetric method. At the end of experiments, different physico-chemical parameters such as changes in the pH values, dissolved oxygen, water conductivity, nitrates, nitrites and phosphate of the cell-free supernatants of different media were analyzed by using DREL 2800 Complete Water Quality Lab with Meter (Hach Co. USA).

Statistics

All the data for different physico-chemical parameters yields were analysed statistically by two-way ANOVA followed by post hoc Bonferroni analysis, significant differences among the treatments were considered at 95% confidence level (P value < 0.05). Various graphs were prepared using the Graph Pad Prism^{TM} (version 5.0, GraphPad Software). All the experiments were done in three replications, and data presented here as mean and standard deviation in graphs and in tables.

RESULTS AND DISCUSSION

Estimation of physico-chemical parameters

The pH of cultivation media was variably influenced by the growth of monocultures and their synthetic consortia, and an interesting pattern was observed. The pH values were found to be in the range between 8.25 to 8.87 for different monocultures and consortia. The initial pH value was found to be highest in the case of both nitrate enriched $BG11^+$ (9.02±0.00) and nitrate deprived $BG11^-$ (8.69±0.01) media, while it was lowest in natural CE (7.93±0.58) water. The pH invariably increased in CE with inoculation of monocultures and consortia, and highest change was found in the case of consortia. The pH value was considerably decreased in $BG11^+$ with growth of *Anabaena* sp. while in $BG11^+$ with *Nannochloropsis* sp. Significant changes in pH were recorded with growth of *Anabaena* sp. in $BG11^+$ ($P < 0.001$), CE ($P < 0.001$) and $BG11^-$ ($P > 0.05$, ns), and with *Chlorella* sp. in $BG11^+$ ($P > 0.05$, ns), $BG11^-$ ($P < 0.01$) and CE ($P < 0.001$). In contrast, *Oscillatoria* sp. in $BG11^+$ did not affect the pH ($P > 0.05$, ns) while it significantly affected in $BG11^-$ ($P < 0.001$) and CE ($P < 0.001$). On other hand, *Nannochloropsis* sp. affected the pH of CE ($P < 0.001$), *Scenedesmus* sp. significantly altered the pH in $BG11^+$ ($P < 0.05$), $BG11^-$ ($P < 0.01$) and CE ($P < 0.001$). Growth of *Phormidium* sp. also affected the pH significantly in $BG11^+$, $BG11^-$ ($P < 0.01$) and CE ($P < 0.001$). Consortia significantly altered the pH in $BG11^+$ ($P < 0.05$), $BG11^-$ ($P < 0.01$) and CE ($P < 0.001$) when compared with respective controls. The means of pH and their standard deviation are given in figure 1.

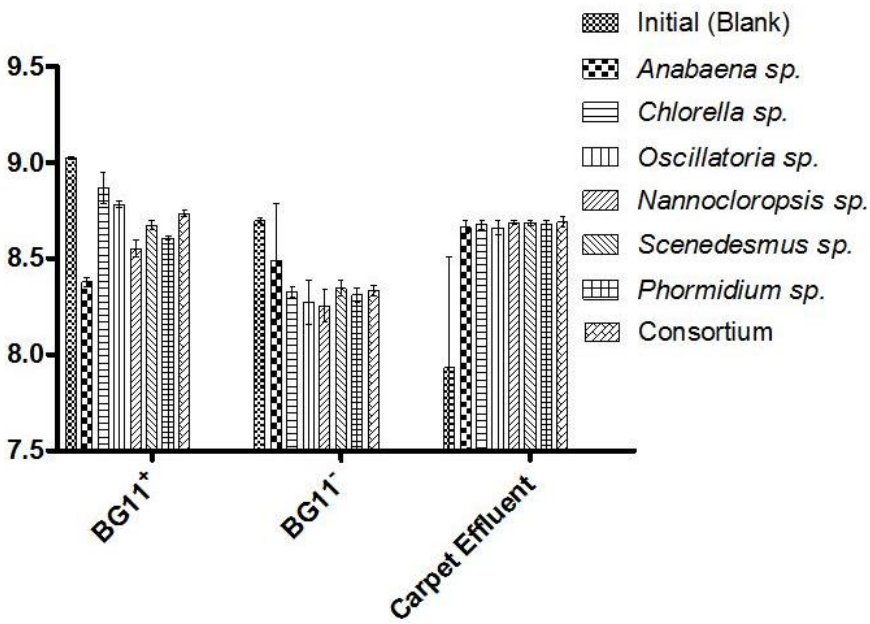

Figure 1. pH of media and carpet effluent of blank (Initial) after growth of monoculture and consortia.

The monocultures have some distinct inherent traits that determine their metabolic features and morphological appearance. However, variations in environmental conditions could highly influence their physiology and morphology (Smith & Schindler 2009). A little variation in the environment can affect their metabolite profile and biomass (Piersma & Drent 2003), and pH is one of the major factors that affect the physiology including growth rates, primary and secondary metabolite production. Most of the algae can make their own food through the process of photosynthesis which involves a number of enzymes that work optimally at a particular pH. When we cultured these monocultures and consortia, pH of different growth media fluctuated in a wide range. Any variation in the pH can affect the function or may denature those important enzymes which are involved in the photosynthesis. Alternatively, it affects the overall biomass yields. Since CO_2 is a major byproduct of respiration, microalgae utilizes CO_2 and water during photosynthesis to produce the photosyntax, carbonic acid that alternatively decreases the pH (Wurts & Durborow 1992). Therefore, pH values of the media and CE water oscillated between 8.25 and 8.87 for optimal CO_2 sequestration. As more CO_2 is absorbed into the solution, the acidity of the solution gradually increases. At the higher acidity point no more CO_2 can be absorbed. The change in pH is responsible for the increased synthesis of biomass by carbon fixation reactions. The pH of CE media was increased from 7.93 to 8.69 for all the organisms and consortia which indicate that it is the range of pH at which these organisms could grow well in natural water reservoirs (Yeh *et al.* 2005).

Initial dissolve oxygen recorded in $BG11^+$ was 6.79±0.02 $mg.L^{-1}$, in $BG11^-$ media 6.83±0.02 and in CE water 2.64±0.02 $mg.L^{-1}$. In all the experiments *Anabaena* sp. and *Chlorella* sp. significantly increased DO levels in media and effluent water at a range of 9.70±0.05 to 10.54±0.16 $mg.L^{-1}$. Monoculture namely *Anabaena* sp., *Chlorella* sp., *Oscillatoria* sp., *Scenedesmus* sp., *Phormidium* sp. and consortia significantly optimized ($P < 0.001$) the DO levels of media and CE water. *Nannochloropsis* sp. could not improve the DO content of $BG11^+$ and $BG11^-$ ($P > 0.05$, ns) while improved significantly in CE water ($P < 0.001$). Mean values and standard deviation of DO of cell-free supernatant for different culture media are shown in figure 2. In general, if consumption of DO is more than the production its level declines.

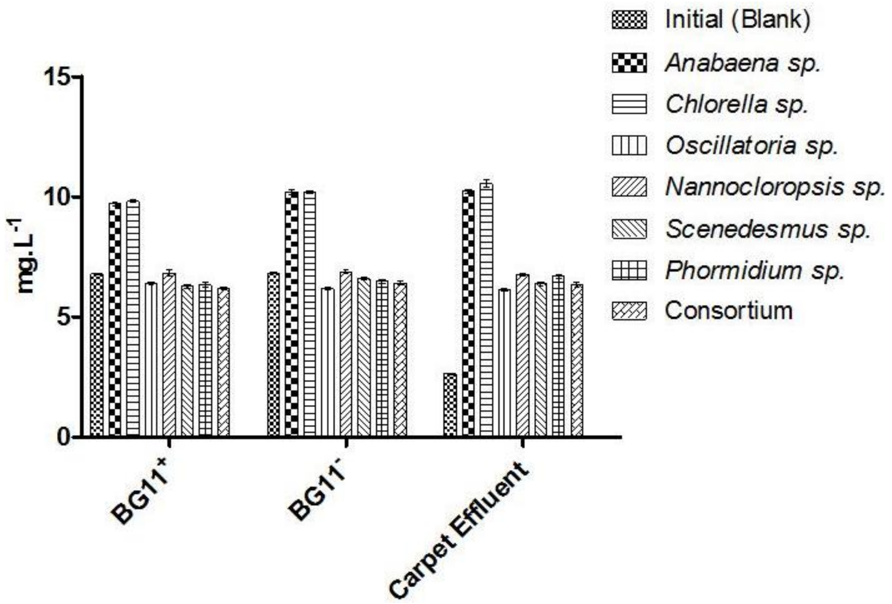

Figure 2. Dissolve oxygen of media and carpet effluent of blank (Initial) after growth of monoculture and consortia.

The microbial growth is directly related to the available amounts of nutrient and DO in water. Here, we found that the DO level of un-inoculated CE media was lowest when compared to $BG11^-$ and $BG11^+$ media. However, after inoculation, DO level significantly increase up to 5 folds. Microalgae growth significantly increased the DO levels by releasing the oxygen that added to the media of their growth. In the case of *Anabaena* sp. and *Chlorella* sp. highest DO levels were observed than other microalgae in all the three media with its maximum values in CE. Therefore, inoculation of *Anabaena* sp. and *Chlorella* sp. in CE water could significantly enhance DO levels. In contrast, the lowest increase in DO levels was observed in all the media with inoculation of algae consortia.

DO levels varies with temperature and altitude, cold water holds more oxygen than warm water but at the higher altitude water holds less oxygen. DO concentrations highly depend on the photosynthetic rates of microalgae and levels of nutrients in the water. In general, high temperature and salinity reduce oxygen levels. The maximum amount of DO (7.95 $mg.L^{-1}$) occurs at 27 ºC (Manasrah *et al.* 2006). In our study at the end of experiment DO was found to be in the range between 6.14–10.54 $mg.L^{-1}$. However, published literature of microalgae consortia showed the range of DO between 6.2–6.4 $mg.L^{-1}$ (Badran 2001, Renuka *et al.* 2013).

Initial conductivity recorded in $BG11^+$, $BG11^-$ media and CE water were 2440.0±10.0, 351.6±7.6 and 934.0±8.5 respectively. Conductivity increased in all set of experiments expect for *Anabaen*a sp. and *Chlorella* sp.in CE water. In $BG11^+$ and $BG11^-$ media, inoculation of all the monocultures and consortia significantly increased the water conductivity. *Oscillatoria* sp. significantly enhanced the water conductivity in all the media. *Anabaena* sp. significantly increased in $BG11^+$ ($P < 0.001$) and CE water ($P < 0.01$) whilst in $BG11^-$ ($P > 0.05$, ns) it did not affected significantly. On other hand, significant changes in conductivity were recorded with inoculation of *Chlorella* sp. (BG11$^+$, $P < 0.01$), *Oscillatoria* sp. (BG11$^+$ and CE, $P < 0.001$), *Nannochloropsis* sp. (BG11$^+$, $P < 0.001$), *Scenedesmus* sp. (BG11$^-$, $P < 0.05$), *Phormidium* sp. (BG11$^+$, $P < 0.001$) respectively. In contrary, the consortia could change the conductivity only in BG11$^+$ ($P < 0.001$). The mean values of conductivity along with their standard deviation are presented in figure 3.

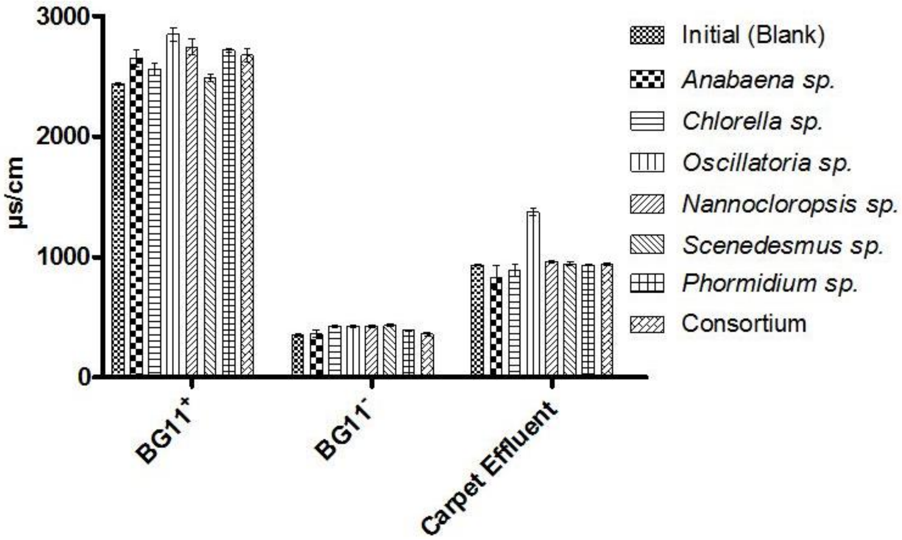

Figure 3. Conductivity of media and carpet effluent of blank (Initial) after growth of monoculture and consortia.

Since water conductivity is used to measure the ability of water to carry the electricity, pure water is considered as a poor conductor. It is the main reason that water shows significant conductivity in the presence of dissolved salts. In general, conductivity is directly proportional to the amount of dissolved salts in the water (Iyasele & Idiata 2015), and microalgae growth increases the water conductivity (Potapova & Charles 2003). Microalgae growth adds a number of minerals such as iron, nitrogen and phosphorus, which significantly involve in enhancing the water conductivity (Townsend *et al.* 2012). In addition, the increased water conductivity and the algae density have a direct relationship (Potapova & Charles 2003, Anonymous 2012).

Biomass Estimation

Biomass production was found to be highest in the case of *Chlorella* sp. (2.217±0.126 g.L^{-1}) in BG11$^+$ media, followed by *Anabaena* sp. (1.897±0.045 g.L^{-1}) and consortia (1.233±0.029 g.L^{-1}) in BG11$^-$ media; *Phormidium* sp. (1.103±0.057 g.L^{-1}), *Scenedesmus* sp. (1.020±0.026 g.L^{-1}) and consortia (0.923±0.025 g.L^{-1}) in BG11$^+$ media. Biomasses produced by different cultures are shown in figure 4.

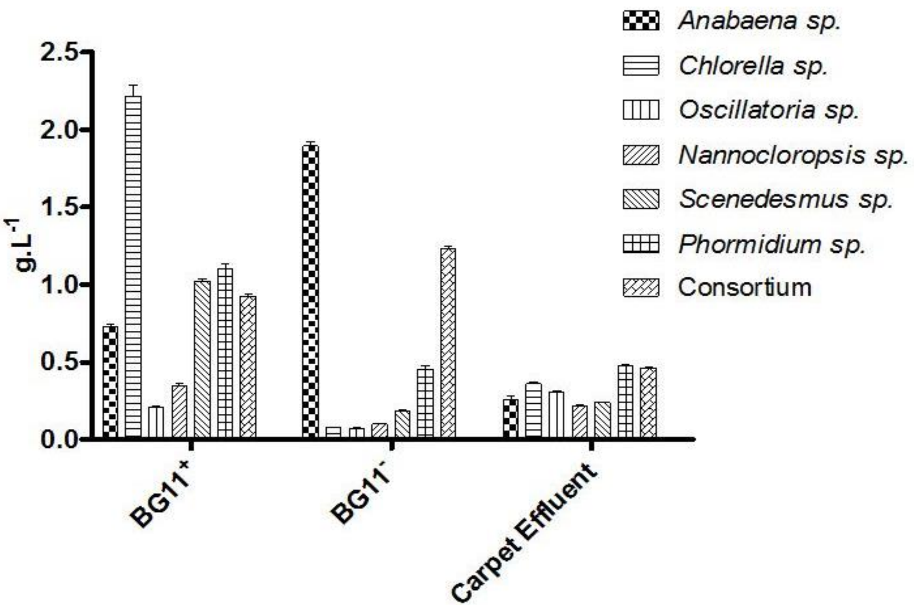

Figure 4. Biomass of media and carpet effluent of monoculture and consortia.

A number of published articles are available dealing with how the different species of *Chlorella* sp. behaves in the nitrogen enriched environment, and utilised for the large-scale biomass production. In general, various species of *Chlorella* sp. needs nitrogen and nutrient enriched water for their healthy growth. Therefore, it grew well in nitrogen enriched media and able to produce higher biomass in $BG11^{+}$ media (Min *et al.* 2011). The algae consortia produced second highest biomass in nitrate deficient $BG11^{-}$ media after *Anabaena* sp. It clarifies that under nitrogen depleted conditions and in the presence of phosphate *Anabaena* sp. can efficiently fix the nitrogen to produce biomass (Buikema & Haselkor 1991). However, when *Anabaena* sp. was grown in consortia the nitrogen fixed by it got used up by other non-nitrogen fixing organisms for their healthy growth. Possibly, some of these organisms inhibited the growth of *Anabaena* sp. by producing the biochemicals that have allelopathic interactions (Hu & Hong 2008). *Anabaena* sp., it may be responsible for inhibiting the growth of other organisms in the consortia. Therefore, biomass yield of consortia was found to be less than *Anabaena* sp. In the CE maximum biomass yield was recorded with *Phormidium* sp. (0.48±0.010 $g.L^{-1}$) followed by algae consortia (0.460±0.010 $g.L^{-1}$). As *Phormidium* sp. has a strong capability to grow in nutrient enriched or polluted water (De-Bashan & Bashan 2010), and carpet effluent is a natural source of a list of hazardous heavy metals, therefore, due to its inherent traits to utilise all these things as supplements for healthy growth could ultimately help it to produce the highest biomass in CE water.

Nitrate, Nitrite and Phosphate estimation

The initial value of nitrate recorded in $BG11^{+}$ was 253.35±1.75 in $BG11^{-}$ 7.40±0.05 media and in CE 1.08±0.10 $mg.L^{-1}$. From the growth of synthetic consortia in $BG11^{+}$ media nitrate content was enhanced by 295.27% (748.08 $mg.L^{-1}$) while its cultivation in nitrate deficient $BG11^{-}$ media resulted in a decrease of 68.91% (5.1 $mg.L^{-1}$) due to biosorption of nitrate from media (Table 1). However, in CE media 123.14% (1.33 $mg.L^{-1}$) increase in the nitrate content was obtained. Overall in nitrogen rich $BG11^{+}$ media, algae consortia synthesized the nitrate and efficiently participated in the bio-addition process. In $BG11^{+}$ media *Nannochloropsis* sp., *Phormidium* sp. and consortia synthesized nitrate while other algae used the nitrate from the media and significantly contributed in biosorption process. Cultivation of nitrogen fixing microalgae *Anabaena* sp. in $BG11^{-}$ media significantly enhanced the nitrate content by 1258.10% (93.1 $mg.L^{-1}$). In contrast, almost all the microalgae species and consortia depleted the nitrate to the surrounding habitat in the CE water that resulted in the valuable quantity of biomass production.

Table 1. Nitrate concentration of initial, monoculture and consortia.

Algae	**$BG11^{+}$ (Nitrate $mg.L^{-1}$)**	**$BG11^{-}$ (Nitrate $mg.L^{-1}$)**	**Carpet effluent (Nitrate $mg.L^{-1}$)**
Initial (Blank)	253.35±1.75	7.40±0.05	1.08±0.10
Anabaena sp.	113.23±0.87	100.50±1.25	2.13±0.15
Chlorella sp.	85.45±0.56	3.58±0.35	3.18±0.07
Oscillatoria sp.	152.53±2.10	1.3±0.05	44.26±1.04
Nannochloropsis sp.	271.80±1.32	1.94±0.04	2.31±0.07
Scenedesmus sp.	191.96±6.04	3.75±0.15	2.48±0.07
Phormidium sp.	341.43±3.46	1.90±0.05	2.31±0.07
Consortia	1001.19±6.53	2.30±0.15	2.41±0.07

Table 2. Nitrites concentration of initial, monoculture and consortia.

Algae	**$BG11^{+}$ (Nitrate $mg.L^{-1}$)**	**$BG11^{-}$ (Nitrate $mg.L^{-1}$)**	**Carpet effluent (Nitrate $mg.L^{-1}$)**
Initial (Blank)	0.0±0.000	0.00±0.000	0.011±0.002
Anabaena sp.	0.397±0.012	0.154±0.005	0.026±0.004
Chlorella sp.	1.427±0.008	0.022±0.004	0.022±0.003
Oscillatoria sp.	4.732±0.023	0.025±0.005	0.208±0.006
Nannochloropsis sp.	12.807±0.169	0.025±0.004	0.017±0.004
Scenedesmus sp.	18.420±0.270	0.119±0.003	0.039±0.004
Phormidium sp.	13.710±0.135	0.036±0.005	0.018±0.003
Consortia	35.520±0.665	0.038±0.004	0.039±0.004

In comparison to nitrate the concentration of nitrites was increased in all the experiment. With inoculation of algae consortia the maximum increase in the concentration of nitrite was recorded in the BG11$^+$ media, followed by BG11$^-$ and CE respectively (Table 2). Microalgae consortia and monocultures showed varying performance towards bio-addition activity in different cultivation media. Maximum activity was recorded by consortia in the case of BG11$^+$ (35.52 mg.L^{-1} from zero value) followed by BG11$^-$ (0.038 mg.L^{-1} from zero value) and CE 254% (0.028 mg.L^{-1}). Therefore, cultivation of these photosynthetic organisms significantly added the nitrate and nitrite in the nitrate enriched BG11$^+$ media.

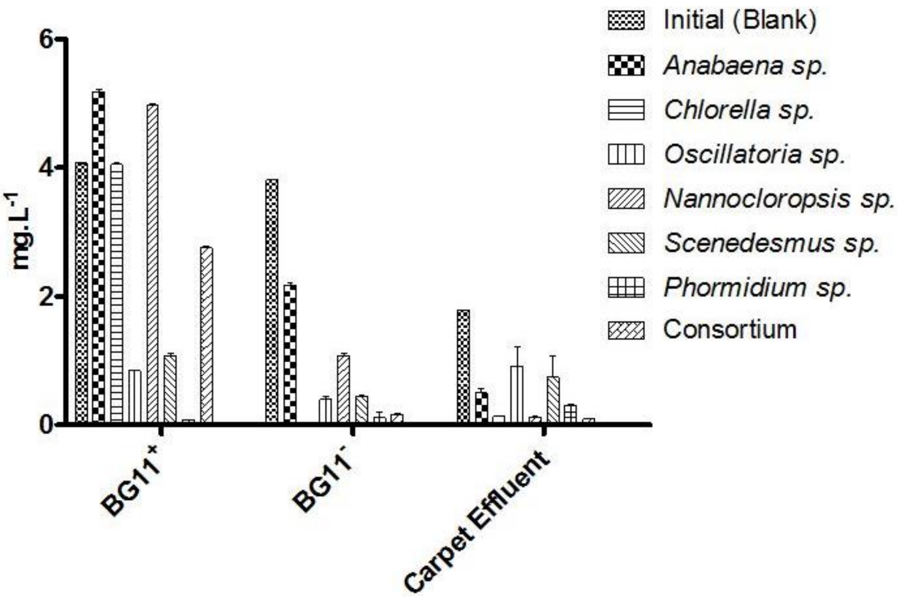

Figure 5. Phosphate of media and carpet effluent of blank (Initial) after growth of monoculture and consortia.

Initial phosphate recorded in BG11$^+$ was 4.07±0.02 mg.L^{-1}, in BG11$^-$ media 3.81±0.00 mg.L^{-1} and CE 1.79±0.00 mg.L^{-1}. In BG11$^+$ media *Anabaena* sp. and *Nannochloropsis* sp. added 27.10% (1.10 mg.L^{-1}) and 22.28% (0.90 mg.L^{-1}) phosphate respectively whilst other algae and consortia were actively involved in its biosorption in figure 5. Bio-sorption in BG11$^+$ and BG11$^-$ by algae consortia was 35.35% (1.31 mg.L^{-1}), 95.72% (3.64 mg.L^{-1}) and in CE water 94.97% (1.7 mg.L^{-1}) respectively. All monocultures and consortia significantly affected the phosphate absorptions ($P < 0.001$) except *Chlorella* sp. in BG11$^+$ ($P > 0.05$, ns) media.

The ratio of nitrogen to phosphorus available for the growth of algae is an important parameter that determines the overall growth. The Redfield ratio of 106C:16N:1P is commonly used to indicate the required ratio of carbon, nitrogen and phosphorus (Redfield 1958). Nitrogen is the second limiting factor in plant growth taken up by cells in the form of ammonia, nitrate and nitrite (Evans 2001). Phosphorus is the third most important macronutrient that is absorbed by plant cells in the form of phosphate. It can bind to other ions and precipitate and enhance its limiting concentrations for optimal growth (Xin *et al.* 2010). With enhanced nitrogen levels bio-sorption of phosphorus decreases (Grobbelaar 2004). Hence, uptake of phosphate depends on the availability of nitrate. In BG11$^+$ media supplementation of sodium nitrate decreased the phosphate absorption. In BG11$^-$ media higher the phosphate absorption in the relation of BG11$^+$ was recorded. The higher concentrations of nitrate in media provoke the fixation of nitrogen by heterocystous filamentous and unicellular morphological types of algae (Philips *et al.* 1992). However, higher nitrate content reduces the biosorption of nitrate. In the presence of higher concentrations of nitrate, some algae synthesize nitrate (Manhart & Wong 1980, Philips *et al.* 1992). Nitrogen is mainly involved in the synthesis of proteins; therefore, low nitrogen supply limits the synthesis of required amounts of the proteins (Geider & Roche 2002, Loladze & Elser 2011) that alternatively results in a low number of ribosomes and the total amount of ribosomal RNA. Since most of the phosphate in the cell is stored in the ribosomal RNA, reduced numbers of the ribosomes are due to the lowering of cellular phosphate levels (Sterner & Elser 2002, Beuckels *et al.* 2015).

In microalgae consortia high nitrate content could not influence the higher biomass synthesis. However, low nitrate supply enhanced the higher biomass synthesis due to the association of nitrogen fixating as well as non-nitrogen fixating algae species. In general, at the low nitrogen supply higher biomass yields are reported (Arumugam *et al.* 2013). On other hand, higher content of nitrate in media reduces the biosorption of phosphate by microalgae. However, in CE algae growth released very little nitrate in wastewater. Therefore, with limited or lower supply of nitrates the rate of nitrate and phosphate bio-absorption was found to be higher (Ma *et al.* 2012, Zhang & Hong 2014). High nitrate concentration induced the 'nitrate reductase' activity to form nitrite which alternatively converted to the ammonium ion by the action of another enzyme 'nitrite reductase'. Nitrate reductase is inhibited by nitrite and ammonium ions, regulators of the *nir* operon (Jha *et al.* 2007). In non-nitrogen fixing cyanobacteria, nitrate, nitrites and ammonium ions chiefly regulate the *nir* operon by fine-tuning the activity of nitrate reductase (NR) enzyme. Nitrate is utilized as the chief source of inorganic nitrogen for the majority of plants (Li *et al.* 2010) and is converted to ammonium (Stitt *et al.* 2002). Nitrate reduces to ammonia in two consequent steps, first, the reduction of nitrate to nitrite which is catalyzed by $NADH_2$-nitrate reductase enzyme and secondly, the reduction of nitrite to ammonia that is catalyzed by ferredoxin-nitrite reductase. The presence of ammonia suppresses the uptake of nitrate by inactivating the nitrate reductase system by ammonia (Losada *et al.* 1970, Herrera *et al.* 1972, Serra *et al.* 1978). As a result, nitrate was synthesized with supplementation of high nitrate levels (Hansell *et al.* 2004). Some algae synthesize several types of phosphate enzymes such as alkaline phosphates, acid phosphates and protein phosphates. 'Phosphatase' is an enzyme that removes a single phosphate group from its substrate through hydrolysis of phosphoric monoesters into a phosphate (Babić *et al.* 2013). Therefore, uptake of phosphate is dependent upon the supply of nitrate and with low supply of nitrate biosorption of phosphate increases. Similarly, with high nitrate, biosorption of phosphate from waste water was decreased (Beuckels *et al.* 2015) with inoculation of these organisms.

CONCLUSION

Microalgae monocultures and synthetic consortia are capable biosorption of phosphate in the media and CE water, and played distinct roles in the ecosystem by altering the DO, pH and conductivity. It was observed in the present experiment where a higher amount of phosphate was removed from $BG11^-$ and CE water in comparison to nitrate enriched $BG11^+$ media. Microalgae consortia added nitrogen to the media which was already supplemented with considerable amounts of nitrogen *i.e*. $BG11^+$ media. Therefore, presence of nitrogen enhances the bio-addition of nitrogen to media. To optimize the services of these monocultures in their natural habitats, there is a need to screen the suitable candidate algae species that might be helpful in mitigating the nutrient levels, and that can produce affordable amounts of biomass. Consequently, by applying the modern principles of the synthetic ecology suitable consortia of selected algae species can be developed that could be a sustainable way of a better method for waste water treatment and biomass production at industrial scale. Synthetic consortia of $BG11^-$ could be helpful in getting the valuable amounts of biomass by adjusting the DO, pH, conductivity and biosorption of nitrate and phosphate. Carpet effluent is a rich source of nutrients, therefore, it can be targeted as media for such algae species which could improve the water quality and simultaneously can produce the biomass for various algae based value added bio-processes.

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