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Performance evaluation of hydroponic system for co-cultivation of microalgae and tomato plant

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ABSTRACT

Conventional hydroponic units producing disease free plants with 30–40% faster growth rate are regarded as a promising agricultural alternative in case of unfertile/metal contaminated soil. However, most nutrients in hydroponics medium remain unused and are drained off creating environmental pollution. To reduce the nutrient load and eutrophication effects, cocultivation of plant and microalgae in the hydroponic units has attracted the attention of researchers over the past few years. The present study aims to explore the influence of initial inoculum concentration of a native algal consortium over the performance of hydroponic system. Cocultivation of tomato plant with varying initial inoculum algal concentration of 0.2–0.8 mg/ml, showed that 0.8 mg/ml concentration resulted in positive interactions between microalgae and plant, with algal and plant productivity of 0.149 ± 0.024 and 0.328 ± 0.087 g/m^2/d respectively after 42 days. Higher chlorophyll accumulation, along with nutritionally rich algal and plant biomass revealed lack of unwanted competition during cocultivation. Increase in dissolved oxygen during cocultivation was corroborated with efficient root respiration. Highly developed roots also provided adequate metabolic energy thus significantly increasing the nutrient uptake and accumulation, thereby reducing the nutrient load during drainage. The supernatant after algal harvesting (recycled media) supported plant growth for 24 days due to limited nutrients. Such system with microalgae symbiotically favouring plant productivity and simultaneous nutrient load reduction would ultimately support in attaining sustainable agriculture.

1. Introduction

Land availability and water supply for agricultural crops is becoming limited from the past centuries due to urbanization, population increase and depletion of soil quality (Dobermann et al., 2013). Hence, modern practices for achieving sustainable agriculture came into picture in lieu of the above-mentioned issues (AlShrouf, 2017). Hydroponics systems are currently regarded as one of the most efficient crop production strategy, with 30–50% faster growth rate, and comparatively less space occupancy compared to the soil-based farming (Huo et al., 2020). Although these soilless plantation systems provide high-quality fruits and vegetables free of toxins with relatively lesser use of insecticides and pesticides, the high capital investment and operational costs involved in control and maintenance often hinders their field-scale applications (Ronga et al., 2019). Also, the waste discharge from the conventional hydroponics unit contains about 200–300 mg/L nitrate and 30–100 mg/L phosphorous, thereby causing eutrophication when discharged into water bodies (Hultberg et al., 2013). There is a need to integrate these systems to supplement the growth of microalgae, where the later could provide benefit to plant growth and metabolism.

Microalgae has the capacity to capture nutrients from wastewater, atmospheric carbon dioxide (CO_2) or industrial flue gas, sunlight and undergo photosynthesis for their biomass generation (Behera et al., 2019a). Algal biomass is a rich source of lipids mainly used for biodiesel production, proteins used as food supplements and carbohydrates as feedstock for bioethanol and biohydrogen (Rangabhashiyam et al., 2017; Behera et al., 2018). In addition to these applications, microalgae is also incorporated into modern agriculture as it has the potential to produce plant growth-promoting biostimulants for better performance of plant against abiotic stress and efficient uptake of nutrients (Chiaiese et al., 2018). However, several studies have projected that large scale algal

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cultivation system currently employed often requires huge capital expenditure associated specifically in the upstream growth phase and the downstream harvesting stage (Behera et al., 2019b; Ronga et al., 2019). Therefore, there is an immense need to find out alternative cheaper and environmental sustainable strategies for culturing microalgae for reaping multiple advantages in a biorefinery concept to make the overall process economically feasible.

Over decades, microalgae has been reported to be naturally growing in hydroponics systems, which under uncontrolled conditions caused organic loading and clogging of pipes. Symbiotic association of microalgae and plant often exists in the hydroponic system providing oxygen from microalgal photosynthesis for plant root respiration (Zhang et al., 2017). Though a general consensus exists with the beneficial effects of microalgae over plants as detailed by several researchers (Chiaiese et al., 2018; Ronga et al., 2019; Supraja et al., 2020), there is only limited scientific evidence underpinning their effects over plants in a hydroponic system, compared to other microbial plant biostimulants. Cortés-Jiménez et al. (2014) and Escalante et al. (2015) reported better growth of tomato plants in Bold Basal medium when inoculated with *Chlorella vulgaris*. Camargo et al. (2015) reported that *C. vulgaris* could be grown successfully without interfering with the growth of *L. sativa*, in LC oligo medium, showing the suitability of hydroponics unit to be utilized as the low-cost reactor for culturing algal biomass. Zhang et al. (2017) projected higher algal biomass productivity of 32 g/m^3/d and plant productivity of 54.24 g/m^3/d in a hydroponic unit employing *Chlorella infusionum* and tomato plant compared to the monoculture. *C. vulgaris* and *S. quadricauda* were simultaneously grown in hydroponics medium with tomato plants showing synergistic and positive effects on growth of both autotrophs (Barone et al., 2019). Huo et al. (2020) reported an increased total nitrogen and phosphorous removal of 97.6% and 98.8% with better algal and leafy vegetable yield under cocultivation mode with *Chlorella vulgaris*. Most of the studies done so far on hydroponics under cocultivation mode with microalgae have been restricted to generally *Chlorella* sp., and *Scenedesmus* sp. Vast diversity of native algal species still remain unexplored and little work has been done to evaluate their biofertilizing potential. Studies done with microalgae in a cocultivation unit have mostly utilized algal inoculum having an initial cell density of 1×10^5 cells/ml (Camargo et al., 2015; Huo et al., 2020). However, initial concentration influences the algal growth rate and indirectly the dissolved oxygen level in a hydroponic unit, analysing the influence of different algal cell density over the performance of these units is necessary. Also as microalgae and plants coexist in a single system, the overlap of specific operational conditions for both the autotrophs often result in unwanted competition, thus declining the efficiency of these systems in terms of yield as well as nutrient removal (Addy et al., 2017). This often leaves a thin margin for the cocultivation system to succeed. Though several studies by Zhang et al. (2017), Huo et al. (2020), Barone et al. (2019) have proposed the integrated system as an efficient approach, yet the process has not been scaled up due to the above-mentioned issues.

The present study focused on 'zero residual nutrient discharge' with simultaneous removal of nutrients by microalgae and plants in a hydroponic system. The coexisting potential of a natively isolated algal consortium and tomato plant cultivated in a hydroponic nutrient solution was analyzed. Different concentration of algal consortium was utilized as inoculum to study its effect over the plant growth rate and algal biomass productivity. The study also evaluated the efficiency of the cocultivation unit in terms of nutrient removal and accumulation efficiency for each of the phototroph. Parallel experiments were also conducted with the used urine based media (after algal growth) to study its influence as a hydroponic nutrient reservoir, emphasizing the nutrient recycling strategy. To the best of author's knowledge, this is the first study on cocultivation with variable initial inoculum of mixed consortium and use of recycled urine media in hydroponics. Such studies are essential for realizing the symbiotic potential of microalgae and plants to develop sustainable agricultural economy simultaneously making the large scale algal cultivation process economically feasible at field scale.

2. Materials and methods

2.1. Microalgal and plant cultures

2.1.1. Microalgal culture

Mixed consortium of microalgae consisting mainly of *Chlorella* sp., along with *Scenedesmus* sp., *Synechocystis* sp., *Spirulina* sp., were obtained from the wastewater ponds of National Institute of Technology (NIT) Rourkela. It was enriched with 6.5% (v/v) of diluted human urine and was grown at ambient temperature of 30 ± 5 °C with a light intensity of 205 μmol photons/m^2/s for 8:16 light:dark cycle. The conditions for algal growth and productivity in diluted human urine has already been preoptimized by authors (Behera et al., 2020).

Most of the cocultivation studies with microalgae have been done with a single initial cell density of 1×10^5 or 10^6 cells/ml (Huo et al., 2020). However, the level of DO and thereby the plant and microalgal productivity in the cocultivation medium is influenced by the initial inoculum of microalgae added. Thus, the present study aims to explore the effect of variation in concentration of inoculum over the synergistic interaction with the plant species during cocultivation. Therefore, three initial inoculum concentrations were utilized: 0.2 mg/ml, 0.5 mg/ml, 0.8 mg/ml with approximate cell density of 2×10^6, 6×10^6 and 12×10^6 cells/ml respectively. These inoculum levels were selected based on the fact that the maximal reported algal density utilized in cocultivation studies was 1×10^6 cells/ml as reported by Cortés-Jiménez et al. (2014).

2.1.2. Plant seedlings

Tomato seeds were obtained from Hi-Tech Genetic Crop Science Pvt. Ltd, (India) and germinated in coco-peat by watering twice a day in morning and evening. The seed germination was observed after 4 days. The seedlings reached two and four-leaf stage after 7 and 12 days from germination. The seedlings in the 4 leaf stage were used for the further cocultivation experiments.

2.2. Experimental conditions for the cocultivation of plant and microalgae

The cocultivation experiments were conducted in a 500 ml glass beaker (11 cm height and 9 cm diameter) covered with 1 cm thick polystyrene sheet. Plant holders filled with coco peat were used as supporting material for crop fixation on the polystyrene sheet. Modified Hoagland media with the composition as provided by Zhang et al. (2017) had initial ammonium and nitrate-nitrogen concentration of 82.89 and 325.55 ppm respectively were utilized in the study. This modified media had supplementation of other micronutrients as 129.67 ppm phosphate, 235 ppm potassium and 200 ppm calcium. Since the concentration of nutrients were found to be well above the minimal amount required for the plants to sustain (Jones, 2016), the same nutrient solution could be used to support the growth of microalgae.

The used nutrient medium after microalgal harvesting (recovered media after harvesting microalgae from 6.5% (v/v) diluted urine media) contained relatively low initial nutrients such as 56.62 and 24.65 ppm of nitrate and ammonium nitrogen, 77.38 ppm of

phosphate, 18.88 ppm of calcium and 14.58 ppm of sodium. The entire experiment was conducted in a closed room providing artificial light source at 205 μmol photons/m^2/s (fluorescent white lights) with a photoperiod of 12 h/day for a period of 42 days. The ambient air and water temperature was maintained at $30 \pm 2^{\circ}C$ and $25 \pm 2^{\circ}C$ respectively. The pH of the nutrient solution was maintained between 5.5 and 6.5 for the plant to effectively utilize all the required nutrients. Aeration was supplied to all the experimental beakers for 12 h/day by bubbling air at a flow rate of 800 ml/min using aeration pumps, to allow efficient blend of nutrients, prevent anaerobiois and provide sufficient distribution of algal cells to colonise the plant roots. All operational conditions have been selected were well within limits for both phototrophs based on Zhang et al. (2017) and Nguyen et al., (2019).

Different treatments (numbered from 1 to 8) of microalgae and plant conducted were as follows:

(1) Control: Fresh media + Plant
(2) MC1: 0.2 mg/ml microalgae + Media
(3) CC1: 0.2 mg/ml microalgae + Media + Plant
(4) MC2: 0.5 mg/ml microalgae + Media
(5) CC2: 0.5 mg/ml microalgae + Media + Plant
(6) MC3: 0.8 mg/ml microalgae + Media
(7) CC3: 0.8 mg/ml microalgae + Media + Plant
(8) R1: Recycled media + Plant

All treatments (MC and CC representing monoculture and coculture respectively) were randomized and each treatment was carried out in four replicates. Original media volume in all the beakers was maintained by adding distilled water. The notations of the treatments mentioned above has been utilized for the rest of this manuscript.

2.3. Plant and microalgal growth rate analysis

Growth of plants was measured and compared in terms of increase in shoot length and number of leaves for all the treatments for every 6 days until the 42nd day.

The microalgal growth rate both in the monoculture and cocultivation system was determined by taking the optical density (OD) at 680 nm using a double beam UV–Vis Spectrophotometer. Specific algal growth rate was determined by the equation provided in Behera et al. (2020). To compare the growth and plant respiration rate, the DO was measured between (5–5:30 p.m., every 6 days, until 42nd day) using portable DO meter (Model HI98193).

2.4. Estimation of nutrient depletion in the growth medium

The growth medium from all the experimental beakers was collected for every 6 days, centrifuged at 5000 rpm for 5 min and the supernatant was used to estimate the amount of nitrate, phosphate, ammonium using standard methods (APHA, 1955). Potassium concentration was detected by using a microprocessor-based Flame Photometer (Model 1385, ESICO, India). The nutrient (N, P, K) removal efficiency for all treatments was estimated using the equations as given in Behera et al. (2020).

2.5. Post-harvest analysis

2.5.1. Morphological and physical characterization of harvested plant and microalgae

Tomato plants grown in all the experimental beakers were harvested at the end of 42nd day. The final shoot and root length were measured using a ruler, and the number of leaves were counted. Shoots and root regions of plants were segregated and the fresh weight of both the parts were measured. Comparison was done between the fresh weight of both shoots and roots. Segregated root and shoot biomass was then dried at 70 °C for 3 days in a hot air oven (Labotech, India), until a constant weight was obtained. The dried biomass of root and shoots were determined, and the root to shoot (dried biomass) ratio was estimated. The plant productivity was calculated using Eq. (1).

$$\textit{Plant productivity}\left(g \,/\, \mathrm{m}^2 \,/\, d\right) = \frac{\textit{dry weight of plant biomass}(g)}{\textit{Area of production } (m^2) \times \textit{time}(day)} \quad \text{Eq. (1)}$$

Microalgae was recovered from the experimental beakers at the end of 42nd day by using Centrifuge 5430 R (Eppendorf, Germany) at 5000 rpm for 20 min. The fresh weight of the microalgae harvested (pellet) was noted and the biomass was also dried at 70 °C for 3 days in a hot air oven (Labotech, India), until a constant weight was obtained, and the dry weight was estimated. The algal productivity was calculated using Eq. (2)

$$\textit{Algal productivity}\left(g \,/\, m^2 \,/\, \mathrm{d}\right) = \frac{\textit{dry weight of microalgae}(g)}{\textit{Area of production}(m^2) \times \textit{time } (day)} \quad \text{Eq. (2)}$$

2.5.2. Chemical composition analysis of harvested plant and algal biomass

Total phosphorous (P) content present in the plant and microalgal biomass was estimated by using method outlined in Koenig and Johnson (1942). Potassium (K), sodium (Na) and calcium (Ca) concentration was measured using flame photometer (Model 1385, ESICO India). Elemental characterization was performed to calculate the percentage of Carbon (*C*), Hydrogen (*H*), Nitrogen (*N*), Sulphur (*S*) and Oxygen (*O*) contents using *CHNS* analyzer. Chlorophyll *a* (*Chl a*), chlorophyll *b* (*Chl b*) and total chlorophyll content (total *Chl*) were measured according to the method of Arnon (1949). Total carotenoid content was estimated according to Sumanta et al. (2014).

2.5.3. Nutrient accumulation efficiency of cocultivation units

Nutrient accumulation efficiency in terms of the essential

nutrients like nitrogen (*N*), phosphorous (*P*) and potassium (*K*) was calculated for both the tomato plant and algal biomass harvested after 42 days. The nutrient accumulated in the plant biomass was calculated by taking the concentration of the nutrient (N, P, K) multiplied by the amount of dried biomass (g). The nutrient accumulation efficiency was obtained using Eq. (3).

$$\textit{Nutrient accumulation efficiency}\ (\%) = \frac{\textit{Nutrient accumulated}}{\textit{Total nutrient supplied}} \times 100 \qquad \text{Eq. (3)}$$

The total nutrient accumulation was obtained by adding the nutrient accumulated by plant and microalgae in each of the treatment together.

2.6. Statistical analysis

The mean and standard error of the data were calculated using XL STAT incorporated in Microsoft Excel (2016). OriginPro 2016 64-bit along with Tukey test was used to calculate one-way ANOVA to compare the mean and differences were considered as significant with probability value $p < 0.05$. Details of ANOVA are provided in Supplementary Tables 1 and 2 Data are represented in

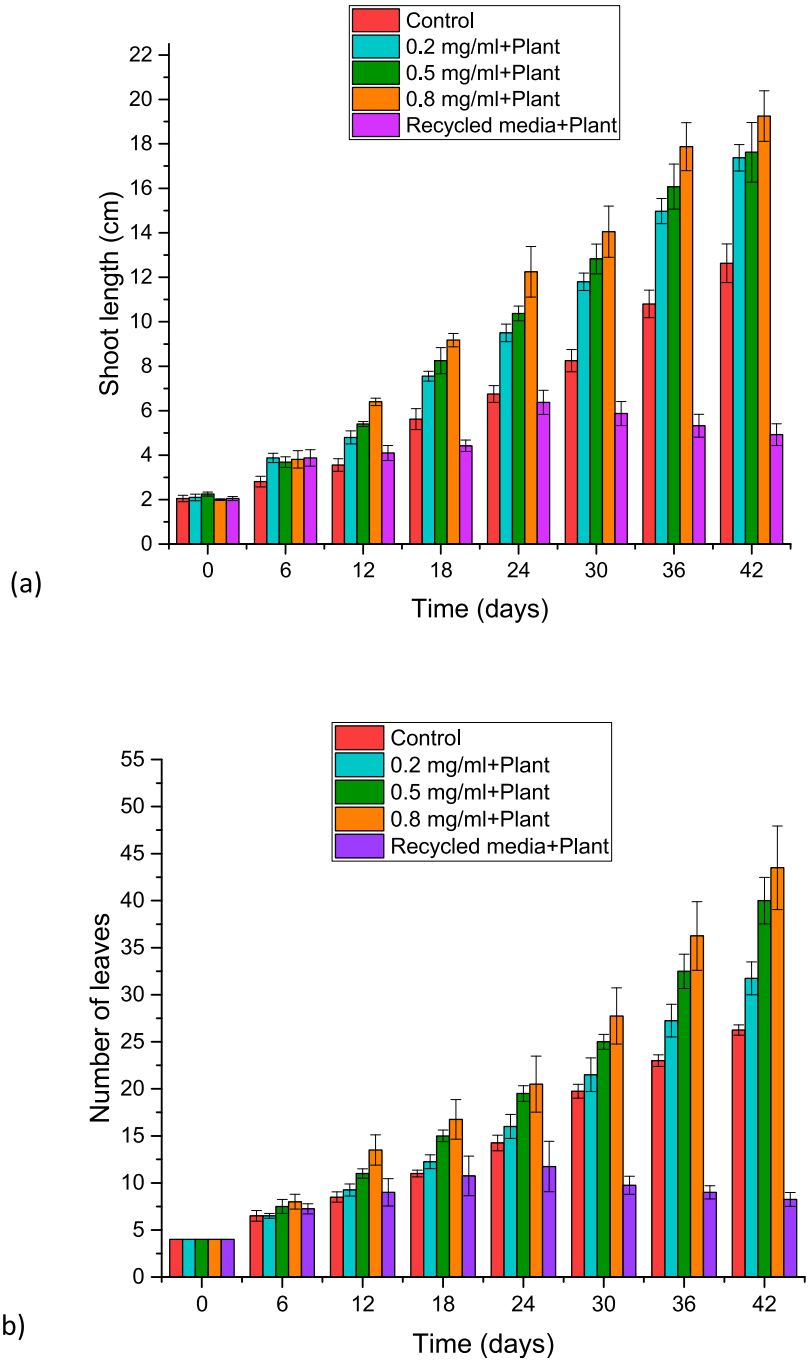

Fig. 1. Comparison between (a) shoot length and (b) number of leaves of plants grown with 0.2, 0.5 and 0.8 mg/ml microalgal inoculum and recycled media with the control Data represented are Mean ± Standard Error at n = 4.

Mean ± Standard Error (SE) at n = 4.

3. Results and discussion

3.1. Measurement of physiological parameters of plant during the growth phase

3.1.1. Shoot length measurement

Growth of plant was analyzed in term of the changes in shoot length (Cortés-Jiménez et al., 2014). Fig. 1(a) shows the shoot length measurement of the plants taken every 6 days during the period of growth until the harvest (42nd day). From the figure, it was observed that the shoot length of all the plants cocultivated with the three microalgal concentrations was more compared to the control, where microalgae was completely absent. Also, plants grown in CC3 showed a shoot length of 19.25 ± 1.14 cm (n = 4), an increase of 52.4 ± 1.01% (n = 4) statistically at $p < 0.05$ compared with the control, which is also highest among other treatments. Huo et al. (2020) also reported higher shoot growth rate for purple kohlrabi (0.80 cm/d) with microalgae compared to 0.70 cm/d in the hydroponic unit free of algal biomass. Similar to the above study, Barone et al. (2019) also reported an increase in shoot growth of tomato plant cocultivated with *S. quadricauda*. Cortés-Jiménez et al. (2014) reported an increase of stem length of tomato seedlings inoculated with *Chlorella vulgaris* in hydroponic nutrient solution. The association of microalgal consortium during the cocultivation increases the root respiration and nutrient uptake, thereby resulting in increased nutrient assimilation and better shoot growth in plants (Barone et al., 2019). The putative mechanism showing better growth can also be linked to colonization by microalgal consortium which secretes secondary metabolites and other allelochemicals like phytohormones that enhanced the plant growth (Bharti et al., 2019).

Recycled media used as nutrient source for plants showed an increase in plant shoot length initially, until 24 days with maximum shoot length corresponding to 6.375 ± 0.54 cm (n = 4). Later, it was observed that due to drying up of the leaves, the shoot length of plants declined with the progress of time. Thus, recycled media as nutrient source with limited nutrient availability helped for plant growth only to a certain extent.

3.1.2. Number of leaves measurement

Increase in number of leaves is often used to assess the growth of plants (Cortés-Jiménez et al. (2014). Fig. 1(b) shows the comparison between the number of leaves calculated for every 6 days in case of all treatments. It was observed that there was a significant increase in the plant growth rate in terms of number of leaves per plant with an increase in the initial microalgal inoculum concentration. Also, the number of leaves per plant cocultivated with 0.8 mg/ml initial microalgal inoculum was found to be 43.5 ± 4.43 (n = 4), (i.e.,) 65.7 ± 2.49% greater and statistically significant at $p < 0.05$ compared to the control. Recycled media as a nutrient source for plants supported the plant growth until the end of 24th day with 11.75 ± 2.67 (n = 4) leaves (n = 4). Escalante et al. (2015) reported an increase in number of leaves as well as the leaf length in tomato plant in hydroponic nutrient medium inoculated with *Chlorella vulgaris*. Positive interaction between microalgal consortium and the plant during cocultivation resulted in healthier leaves with higher chlorophyll content (Bharti et al., 2019). The biostimulatory effect via the availability of carbon and nitrogen based metabolites in the periphery of plant roots colonized by microalgae increases the leaf chlorophyll content (Mattner et al., 2018; Ronga et al., 2019).

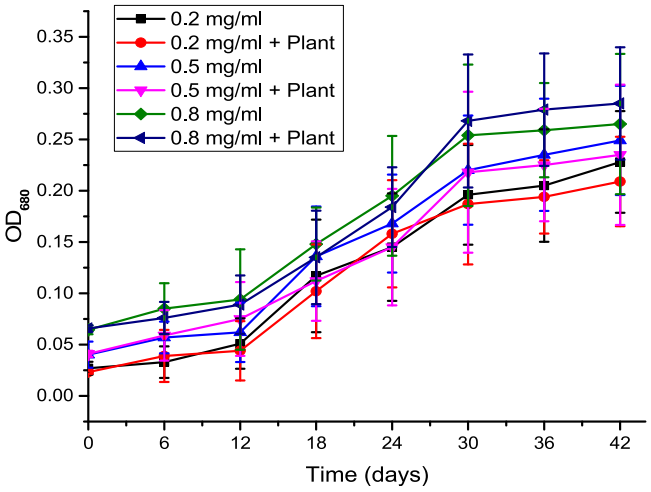

Fig. 2. Comparison between 0.2, 0.5 and 0.8 mg/ml microalgal growth rate (OD_{680}) in the microalgal monoculture and cocultivation systems
Data represented are Mean ± Standard Error at n = 4.

3.2. Microalgal growth rate analysis

Growth rate of microalgae can be measured in terms of increase in algal concentration with time (Zhang et al., 2017). Fig. 2 shows the increase of microalgal concentration in terms of optical density in all the monoculture and cocultivation system. It was observed that for all the treatments, there exists a lag phase for almost 12 days because of acclimatization of the microalgal biomass harvested from 6.5% (v/v) of diluted human urine media to Hoagland media. It was found initially that the algal growth rate in monocultures of all the inoculum concentration was higher compared to the cocultivation system. For 0.2 and 0.5 mg/ml algal inoculum, the specific growth rate in monoculture was 11% and 9% higher compared to cocultivation. In case of 0.8 mg/ml inoculum, the specific growth rate during cocultivation was found to be 0.057 day^{-1}, about 8% higher than the monoculture with 0.053 day^{-1}. Higher growth rate of *Chlorella infusionum*, *Chlorella* sp. and *Scenedesmus* sp., has also been reported during the cocultivation with tomato plants in the studies by Zhang et al. (2017) and Barone et al. (2019) respectively. Camargo et al. (2015) also reported higher growth of microalgae cocultivated with lettuce plants in hydroponic solution comparable to the conventional nutrient media. The availability of stable soluble carbon (C) pool in the cocultivation nutrient solution due to the process of root respiration and exudation by plants, increases the microalgal growth rate (Zhang et al., 2017). The lower growth rate of microalgae in case of 0.2 and 0.5 mg/ml inoculum inside the cocultivation system might be attributed to the fact that the algal concentration might not be sufficient enough to create a positive interaction between the phototrophs, thereby the dominance of plant would have further inhibited microalgal growth. Thus, it is essential to maintain an appropriate microalgal inoculum inorder to achieve the requisite synergistic effects for ensuring the success of hydroponic unit.

3.3. Nutrient removal efficiency in the cocultivation system

Hoagland nutrient media provided to the plants and microalgae consists of all the essential nutrients for their growth (Zhang et al., 2017). Nutrient removal efficiency in terms of nitrate, ammonium, phosphate and potassium were estimated for all the experimental systems, for every 6 days over 42 days and the final efficiency achieved has been given in Table 1. It was observed that the

Table 1
Nutrient removal efficiency of different treatments.

Treatment	Nitrate removal efficiency (%)	Ammonium removal efficiency (%)	Phosphate removal efficiency (%)	Potassium removal efficiency (%)
Control	49.59 ± 2.36	90.87 ± 2.58	54.04 ± 3.26	81.11 ± 2.05
MC1	41.65 ± 1.52	88.53 ± 3.62	60.14 ± 3.09	82.52 ± 1.65
CC1	52.86 ± 2.00	92.89 ± 2.44	64.94 ± 2.57	83.60 ± 1.07
MC2	45.88 ± 2.62	91.81 ± 2.39	72.11 ± 2.66	83.98 ± 2.03
CC2	53.85 ± 1.56	92.44 ± 3.05	80.26 ± 1.89	86.24 ± 1.86
MC3	52.75 ± 2.19	94.68 ± 2.34	80.64 ± 3.38	88.95 ± 1.66
CC3	69.36 ± 2.24	99.01 ± 1.21	91.53 ± 1.59	93.89 ± 1.69
R1	84.81 ± 4.47	100 ± 0	94.56 ± 1.66	95.84 ± 1.77

Data represented are Mean ± Standard Error at n = 4.

utilization of ammonium as the nitrogen source was rapid compared to the nitrate in all the experimental conditions over the entire period of growth. Plant cocultivated with 0.8 mg/ml microalgal inoculum concentration (CC3) has the highest ammonium removal efficiency of 99.01 ± 1.21%,(n = 4) at $p < 0.05$ compared to the control with 90.87 ± 2.58%, (n = 4) at $p < 0.05$ removal, and corresponding monoculture having 94.68 ± 2.34%, (n = 4) at $p < 0.05$ removal efficiency. Similarly, cocultivation units with 0.2 and 0.5 mg/ml also showed the maximum ammonium removal efficiency than their corresponding monoculture. The ammonium removal efficiency during the cocultivation with 0.8 mg/ml algal inoculum was 10% higher compared to the control and about 8% more than other two cocultivation units. Higher removal efficiency achieved during cocultivation was probably due to the higher root respiration leading to efficient uptake of ammonium by both phototrophs (Barone et al., 2019). Similar to the present study, Huo et al. (2020) also reported higher ammonium removal in cocultivation system using *Chlorella* sp. and purple kohlrabi. Plants grown on recycled media (R1) showed complete ammonium removal of 100% as the nutrients supplied were having lesser ammonium concentrations compared to the control.

Nitrate, a form of nitrogen is considered as one of the most essential nutrients required for plant growth and increase in biomass (Huo et al., 2020). From Table 1, it is evident that experimental nitrate removal efficiency was lesser than ammonium, as phototrophs prefer ammonium better than nitrate. The highest removal efficiency of 69.36 ± 2.21%, (n = 4) at $p < 0.05$ was recorded in the case of plant cocultivated with 0.8 mg/ml microalgal inoculum concentration, which was 41% more than the control, 32% and 30% greater than the cocultivation units having 0.2 and 0.5 mg/ml algal inoculum respectively. 70% higher total nitrogen removal efficiency during the cocultivation of purple kohlrabi with *Chlorella* vulgaris compared to the control was also reported by Huo et al. (2020). Synergistic association of phototrophs promote better uptake of nitrate and thereby increase the growth of both species (Ronga et al., 2019). Recycled media as nutrient source gave an efficiency of 84.81 ± 4.47% removal of nitrate, advocates the methodology worth-while for reducing the eutrophication of water bodies.

In general, phosphorous supplied as phosphate acts as one of the major nutrients for plant and microalgae (Behera et al., 2020). All the cocultivation units showed higher phosphate removal compared to the monoculture (Table 1). Phosphate removal efficiency for plant cocultured with 0.8 mg/ml microalgal inoculum concentration (CC3) was highest corresponding to 91.53 ± 1.59% (n = 4) at $p < 0.05$, compared to the control with 54.04 ± 3.26%, (n = 4) at $p < 0.05$ removal efficiency (Table 1). Cocultivation system with 0.2 and 0.5 mg/ml microalgal inoculum also showed efficient removal in comparison with their monocultures, yet 44% and 15% lower than CC3. Higher root respiration increases the availability of soluble phosphates near the roots which are readily absorbed during cocultivation compared to the control (Barone et al., 2019). Similar to the present study, Huo et al. (2020) also reported significant increase in phosphorous removal (>98%) in cocultivation units of leafy vegetables inoculated with *Chlorella* sp.. Similar to nitrogen and phosphorous, the potassium removal efficiency of the cocultivation system with 0.8 mg/ml inoculum was also found to be highest corresponding to 93.89 ± 1.69%, (n = 4) at $p < 0.05$ compared to other treatments. Recycled media showed phosphorous and potassium removal efficiency of 94.56 ± 1.66% and 95.84 ± 1.77% respectively.

It could be concluded that the nutrient removal efficiency depends on the extent of positive interaction between the species during cocultivation. It was evident that the cocultivation unit having 0.8 mg/ml algal inoculum showed higher growth rate of microalgae as well as tomato plant in terms of increase in shoot length and leaf number with the maximal nutrient (N, P, K) removal. The probable mechanism might be correlated to the better exchange of gases (O_2 and CO_2) due to synergistic interaction of plants with appropriate concentration of algal inoculum, that makes the availability of nutrients in requisite soluble form in the hydroponic solution. These soluble nutrients are readily absorbed during cocultivation resulting in better removal efficiency compared to that by only plants or microalgae. Better nutrient removal often results in increased growth of both the species as also reported in the study by Zhang et al. (2017); Barone et al. (2019) and Huo et al. (2020). Ronga et al. (2019) also described that hydroponic cocultivation system employing microalgae would provide economic benefits as a substitute of conventional costly chemical fertilizers in terms of generation of healthy crops, and algal biomass that could be processed into multitude of bioproducts. Further it facilitates the efficient nutrient removal, thereby reducing the process costs associated with the treatment of waste discharge.

3.4. Post-harvest analysis

3.4.1. Physical and morphological analysis of plants after harvest

Cocultivation with algal consortium increases the plant growth that can be observed in terms of variation in morphological features. Table 2 shows the shoot and root length, number of leaves, fresh weight of shoots and roots of the plants harvested after 42 days. All the cocultivation treatments positively influenced the morphological traits of tomato plants. It was observed that the final shoot length of 19.25 ± 1.14 cm, number of leaves as 43.5 ± 4.43, and root length (25.75 ± 2.3 cm in case of plant cocultivated with 0.8 mg/ml microalgal inoculum concentration (CC3), which was highest compared with the control (statistically significant with n = 4 at $p < 0.05$) and other treatments. This was also visibly evident from the morphological features of plants shown in Fig. 3. Increased root and stem length of tomato seedlings inoculated with *Chlorella vulgaris* in Bold Basal medium compared to the control

Table 2
Table showing the number of leaves, final plant length, fresh and dry weight and productivity of plants obtained from the hydroponic systems.

Treatment	Number of leaves	Final plant length (cm)		Fresh weight (g)		Dry weight (g)		Plant productivity ($g/m^2/d$)
		Shoot	Root	Shoot	Root	Shoot	Root	
Control	26.25 ± 0.54	12.63 ± 0.87	8.12 ± 0.54	1.15 ± 0.06	0.43 ± 0.07	0.072 ± 0.006	0.041 ± 0.007	0.061 ± 0.003
CC1	31.75 ± 1.74	17.37 ± 0.59	10.37 ± 2.41	2.19 ± 0.29	1.78 ± 0.32	0.168 ± 0.029	0.053 ± 0.012	0.120 ± 0.016
CC2	40 ± 2.47	17.62 ± 1.34	22.75 ± 1.28	4.62 ± 0.09	2.62 ± 0.28	0.332 ± 0.031	0.077 ± 0.006	0.221 ± 0.016
CC3	43.5 ± 4.43	19.25 ± 1.14	25.75 ± 2.30	6.05 ± 0.42	3.82 ± 0.44	0.502 ± 0.145	0.105 ± 0.022	0.328 ± 0.087
R1	8.25 ± 0.74	4.92 ± 0.49	5.67 ± 0.63	0.52 ± 0.11	0.25 ± 0.07	0.022 ± 0.006	0.010 ± 0.003	0.017 ± 0.004

Data represented are Mean ± Standard Error at n = 4.

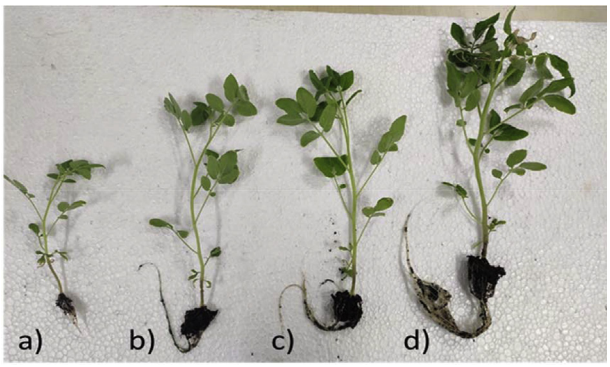

Fig. 3. Comparison between plant growth in (a) Control, (b) 0.2 mg/ml of algae + Plant, (c) 0.5 mg/ml of algae + Plant, (d) 0.8 mg/ml of algae + Plant cocultivation unit.

was reported by Cortés-Jiménez et al. (2014). Escalante et al. (2015) also reported an increase in leaf number as well as stem length of tomato plant during the cocultivation with *Chlorella* sp. Barone et al. (2019) projected 130% increase in root length of tomato plant with values corresponding to 18.5 and 15.5 cm during cocultivation with *C. vulgaris* and *S. quadricauda* respectively. It was also observed in the study that the increase in root length was more compared to the shoot length during cocultivation. Rapid increase in length of roots compared to shoots might be attributed to the process of cell distension due to rapid uptake of water and its accumulation inside vacuoles of root cells (Taiz et al., 2015).

Better morphological features of tomato plants during cocultivation can also be positively correlated with the fresh and dry weight of shoots and roots as well as the overall plant productivity compared to the control as represented in Table 2. Microalgal consortium colonized in the region of roots facilitates higher root respiration rate, thereby better availability of energy for complex metabolic functions and faster uptake and transmission of nutrients (N, P, K) which help in built up of plant biomass (Bharti et al., 2019). In the present study, the fresh weight of shoots (6.05 ± 0.42 g) and roots (3.82 ± 0.44 g) were found to be highest (n = 4, at $p < 0.05$) in case of plant cocultivated with 0.8 mg/ml microalgal inoculum concentration (CC3). Cocultivation system with 0.2 and 0.5 mg/ml inoculum also gave higher fresh weight of shoot and roots compared to the control although lesser than CC3. In CC3, the dry weight of plant shoot and root was also found to be highest with 0.502 ± 0.145 g and 0.105 ± 0.022 g respectively with the plant productivity of 0.328 ± 0.087 $g/m^2/d$ at n = 4, with $p < 0.05$, significantly higher compared to the control. Often the rapid uptake of water along with the nutrients during the cocultivation promotes accumulation inside vacuoles as reported by Taiz et al. (2015) resulting in higher fresh weight of roots than the shoots. Thus, the study clearly demonstrates the effect of cocultivation with the mixed algal consortium over the tomato plant, similar to that of the studies by Zhang et al. (2017) and Barone et al. (2019) using *Chlorella* sp., and *Scenedesmus* sp. respectively. Highly developed roots with more dry weight (8.04 g) along with greater crop productivity for tomato plant (54.24 $g/dm^3/d$) harvested after 76 days was also reported in an eco-friendly cocultivation system employing *Chlorella infusionum* by Zhang et al. (2017).

Zhang et al. (2017) have projected that the plant root respiration is directly proportional and can be linearly correlated with the dissolved oxygen (DO) levels in the nutrient medium. Due to aeration, DO levels remained between the threshold range of 4–6 mg/ml for all treatments for most period of plant growth. The highly developed root system observed during cocultivation is probably due to enhanced root respiration rate as evident from the high DO level. DO of all the coculture systems remained fairly above 7 mg/L after the 18th day as given in Supplementary Fig. 3. Maximal value of DO reached 9.5, 10.5 and 12 mg/L by 30th day for 0.2, 0.5 and 0.8 mg/ml inoculum concentrations respectively. However, in case of control, maximum DO of 7.5 mg/L was observed on 36th day after transplantation. Often algal addition, their photosynthetic activity, and the exchange of gases causes high DO during cocultivation, that increases the plant respiration rate, thereby enhancing the amount of energy available for the uptake of nutrients resulting in better plant productivity. Zhang et al. (2017) also reported consistently high DO levels resulting in higher root respiration rate and better growth of tomato plant during co-cultivation with *C. infusionum*.

The dry root/shoot ratio (on the basis of weight) for cocultivation with the algal consortium was found to be in the range of 0.21–0.32, shows lack of hypoxic stress. Similarly, dry root/shoot ratio of 0.21 for tomato plant under cocultivation with *Chlorella* sp. and *Scenedesmus* sp., was also reported by Barone et al. (2019). Thus, sufficient availability of essential allelochemicals from algal biomass around the root regions ensures the healthy growth of root and epigenous parts of the plant (Bharti et al., 2019).

Recycled media as nutrient source for hydroponics supported the plant growth only to a certain extent and hence the final shoot length of 4.92 ± 0.49 cm, root length of 5.67 ± 0.63 cm with 8.25 ± 0.74 leaves were obtained after the harvest, which was lesser than the control. Lowest plant fresh and dry shoot and root biomass, with productivity of 0.017 ± 0.004 $g/m^2/d$ was obtained after harvesting. It is noteworthy to mention that the recycled media can only support the growth of tomato plant for a period of 24 days, after which the growth ceases due to the lack of nutrients. Nevertheless, the study unleashes the potential of this media to partially support the growth of plants atleast till the nursery level (after which they might be transferred to soil), thereby reducing cost of nutrients. Further, recycling the used media after growing microalgae also decreases the pollution/eutrophication effects associated with the waste drainage.

3.4.2. Microalgal biomass content and productivity analysis in cocultivation system

Microalgal productivity in the cocultivation system is largely dependent on the adequate synergistic interaction with the plants

Table 3
Fresh and dry weight of microalgal biomass in monoculture and cocultivation systems.

Treatment	Microalgal fresh weight (g)	Microalgal dry weight (g)	Microalgal productivity (g/m^2/d)
MC1	1.24 ± 0.095	0.142 ± 0.033	0.077 ± 0.017
CC1	1.12 ± 0.12	0.122 ± 0.041	0.066 ± 0.023
MC2	2.56 ± 0.17	0.176 ± 0.059	0.095 ± 0.032
CC2	2.48 ± 0.14	0.162 ± 0.050	0.088 ± 0.027
MC3	3.05 ± 0.26	0.254 ± 0.037	0.137 ± 0.020
CC3	3.18 ± 0.17	0.276 ± 0.045	0.149 ± 0.025

Data represented are Mean ± Standard Error at n = 4.

as well as the environmental factors. As evident from Table 3, the algal growth (in terms of fresh and dry biomass, after 42 days of harvest) as well the productivity was higher during cocultivation with 0.8 mg/ml algal inoculum (CC3) compared to its corresponding monoculture. The fresh weight of algal biomass in CC3 was found to be highest with a value of 3.18 ± 0.17 g, (n = 4) compared to its monoculture with 3.05 ± 0.26 g, (n = 4) at $p < 0.05$. The system also showed maximal dry biomass of 0.276 ± 0.045 g harvested after 42 days, with 0.149 ± 0.025 g/m^2/d, (n = 4), and significant at $p < 0.05$ with the monoculture. Huo et al. (2020) reported algal biomass productivity of 1.41–1.80 g/m^2/d for *Chlorella* sp., cocultivated with leafy vegetables. Zhang et al. (2017) also reported a higher algal growth and biomass productivity of 32 g/m^3/d for *Chlorella* sp. cocultivated with tomato plant than the monoculture. Barone et al. (2019) projected that *Chlorella* sp., and *Scenedesmus* sp., cocultivated with tomato plant showed a biomass content of 0.77 g/L and 1.02 g/L respectively after 45 days of harvest. Higher biomass productivity of 0.019 g/L/d and 0.022 g/L/d for *Chlorella* sp. and *Scenedesmus* sp.was also reported by the same authors, which was comparatively higher than the monoculture. The efficient gaseous transfer due to the ongoing photosynthesis and respiration process during cocultivation often maintains an adequate soluble carbon pool that is being assimilated by microalgae resulting in increased algal growth and productivity (Zhang et al., 2017). Algal fresh and dry biomass weight as well as productivity was found to be lower in coculture with 0.2 and 0.5 mg/ml inoculum compared to the corresponding monoculture. This could be attributed to the fact that the absence of sufficient amount of initial algal inoculum might not be enough for promoting positive interaction, resulting in unbalanced growth due to competition with plant species.

3.4.3. Biochemical composition of microalgae and plants

Cocultivation of microalgal consortium with tomato plant in hydroponic solution is often limited by competition between the phototrophs. The unwanted competition often influences the growth as well as the biochemical composition of the plant and microalgal species (Addy et al., 2017). Thus, it is essential to quantify the elemental composition of both algae and plants in the cocultivation system. The elemental composition of microalgal consortium and plant biomass subjected to different treatments and harvested after 42 days has been shown in Table 4. Elemental composition (C, H, N, S, O, P, Na, K and Ca) in plant biomass harvested from control as well as the cocultivation units were nearly same. The cocultivation unit with 0.8 mg/ml of algal inoculum (CC3) showed higher C, H, N, S, P, Na, Ca content compared to the control. Thus, it could be concluded that the positive interaction between microalgae and plants resulted in better and healthier plant. Algae during cocultivation with plants increases the availability of oxygen, stimulating the rate of root respiration, thereby the overall nutrient uptake (Zhang et al., 2017). Also, algal consortium have been reported to secrete allelochemicals extracellularly that stimulate the multi-elemental composition of plants (Chiaiese et al., 2018). The similarity in elemental composition of the plants grown in hydroponic solution having microalgae with that of the control, showed the lack of unwanted competition between the species during cocultivation. Plants grown in recycled media showed significantly less elemental content compared to the control, owing to the limited presence of nutrients which were mostly exhausted while culturing microalgae. Elemental composition of algal biomass from monoculture and coculture was nearly same due to the luxury uptake of nutrients by microalgae. However, algal C content in case of CC3 (33.37 ± 2.4%) was more than monoculture (MC3) due to the better synergistic interaction, resulting in higher uptake of the available soluble carbon pool during the process of root exudation and respiration. Zhang et al. (2017) reported higher C content in *Chlorella* sp., during cocultivation with tomato plant.

Chlorophyll and carotenoids are the pigments present in chloroplast, which plays a vital role in photosynthesis and are mainly involved in harvesting light energy to perform photochemical redox reactions (Lefever et al., 2017). Fig. 4 (a) and (b) shows the

Table 4
Macro and micro elemental composition of plant and microalgal biomass obtained after harvest.

Treatment	C (%)	H (%)	N (%)	S (%)	O (%)	P (mg/g)	K (mg/g)	Na (mg/g)	Ca (mg/g)
Plant Biomass									
Control	34.71 ± 0.77	1.53 ± 0.07	4.34 ± 0.23	1.43 ± 0.11	57.98 ± 1.04	0.71 ± 0.08	1.15 ± 0.05	0.16 ± 0.02	0.06 ± 0.01
CC1	34.01 ± 1.47	2.48 ± 1.05	4.27 ± 0.31	2.06 ± 0.47	57.17 ± 3.32	0.79 ± 0.06	1.09 ± 0.03	0.18 ± 0.04	0.05 ± 0.03
CC2	34.59 ± 0.95	2.42 ± 1.43	5.03 ± 0.39	1.06 ± 0.61	56.88 ± 2.16	0.64 ± 0.07	1.08 ± 0.04	0.21 ± 0.05	0.07 ± 0.02
CC3	35.26 ± 1.28	2.40 ± 1.35	4.87 ± 0.33	1.87 ± 0.36	55.58 ± 3.33	0.81 ± 0.09	1.13 ± 0.06	0.24 ± 0.06	0.09 ± 0.02
R1	26.99 ± 1.64	0.66 ± 0.12	1.80 ± 0.35	0.54 ± 0.31	69.98 ± 1.55	0.26 ± 0.04	0.76 ± 0.04	0.08 ± 0.02	0.03 ± 0.01
Microalgal Biomass									
MC1	31.54 ± 1.00	2.26 ± 0.72	3.86 ± 0.38	1.89 ± 0.145	60.44 ± 0.52	1.9 ± 0.20	0.8 ± 0.32	1.1 ± 0.23	0.48 ± 0.02
CC1	31.81 ± 1.23	2.49 ± 0.65	2.91 ± 0.76	1.70 ± 0.54	61.08 ± 0.73	1.6 ± 0.42	1.2 ± 0.56	0.9 ± 0.35	0.54 ± 0.03
MC2	31.57 ± 1.31	2.29 ± 0.75	3.35 ± 0.30	1.31 ± 0.23	61.57 ± 0.06	2.1 ± 0.51	1.1 ± 0.24	1.2 ± 0.48	0.52 ± 0.05
CC2	32.29 ± 1.30	2.19 ± 0.95	3.19 ± 0.65	1.94 ± 0.43	60.38 ± 0.73	1.9 ± 0.35	1.5 ± 0.48	1.0 ± 0.32	0.45 ± 0.04
MC3	31.48 ± 1.30	2.51 ± 1.38	3.99 ± 0.48	1.43 ± 0.39	58.68 ± 2.93	2.4 ± 0.45	1.2 ± 0.59	1.11 ± 0.57	0.58 ± 0.03
CC3	33.37 ± 2.40	2.46 ± 0.75	4.83 ± 0.40	1.89 ± 0.44	59.32 ± 2.00	2.3 ± 0.52	1.0 ± 0.52	1.23 ± 0.45	0.48 ± 0.04

Data represented are Mean ± Standard Error at n = 4.

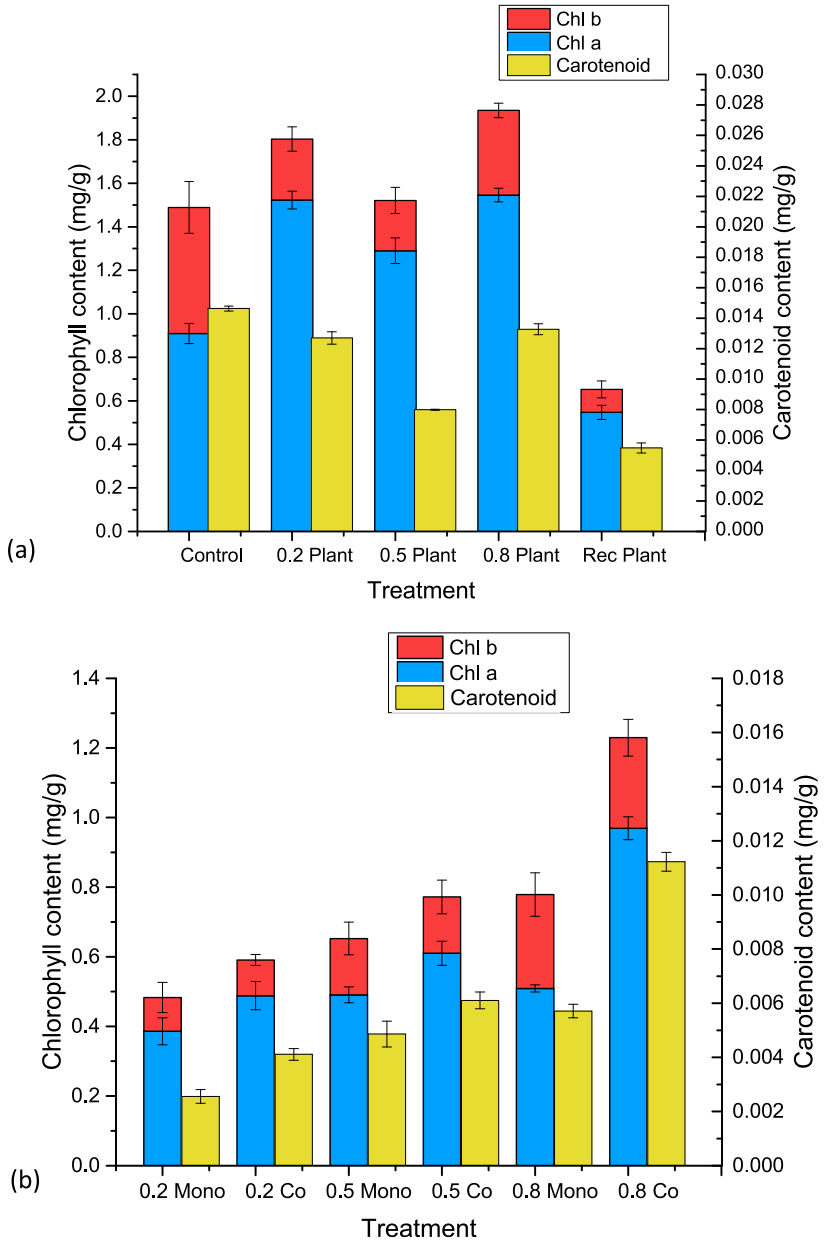

Fig. 4. Comparison between chlorophyll and carotenoid content present in (a) plant and (b) microalgal biomass obtained with 0.2, 0.5 and 0.8 mg/ml inoculum concentrations in the monoculture and cocultivation systems
Data represented are Mean ± Standard Error at n = 4.

chlorophyll and carotenoid contents for plant and microalgal biomass harvested after 42 days respectively. As evident from the figure, the cocultivation unit with CC3 showed maximal *Chl a* of 1.55 ± 0.031 mg/g and *Chl b* of 0.39 ± 0.033 mg/g with 0.013 ± 0.0003 mg/g carotenoids for plants. Maximal *Chl a* concentration of 0.51 ± 0.010 mg/g with 0.27 ± 0.062 mg/g Chl *b* and 0.006 ± 0.0002 mg/g carotenoids was also observed for the algal biomass in the same cocultivation unit. The pigment concentration in phototrophs varies with the quality of light as well as the uptake of N and P in the nutrient medium, and the nature of photosynthetic organisms (Lefever et al., 2017). Efficient uptake of the essential nutrients owing to the positive interaction between the algal consortium and plants in the hydroponic cocultivation units provides the metabolic active pool of N and P for incorporating into the backbone of the pigments formed (Sapkota et al., 2019). Mattner et al. (2018) also projected that the availability of C and N containing metabolites provided by the microalgae results in build up of plant biomass as well as increased chlorophyll content. Hydroponic cocultivation system employing *Chlorella* sp., and *Scenedesmus* sp., also showed better plant health in terms of the chlorophyll content of tomato plant (Barone et al., 2019). These results confirmed the beneficial effects of cocultivating both plants and microalgae.

3.4.4. Nutrient accumulation efficiency of plants and microalgae

Nutrient accumulation efficiency (NAE) of plants is often used as an index to assess the property of plants or agricultural systems with respect to the internal use of the supplied nutrients. NAE of the cocultivation system in terms of the assimilation of essential nutrients (N, P, K) have been provided in Table 5. The utilization

Table 5
Nutrient accumulation efficiency in monoculture and cocultivation systems.

Treatment	Nitrogen Accumulation Efficiency (%)		
	Plant	Algae	Total
Control	10.59 ± 0.45	0	10.59 ± 0.45
MC1	0	12.11 ± 0.69	12.11 ± 0.69
CC1	18.5 ± 1.27	14.26 ± 0.39	32.76 ± 0.83
MC2	0	20.95 ± 0.59	20.95 ± 0.59
CC2	35.78 ± 1.58	19.75 ± 0.43	55.53 ± 1.01
MC3	0	30.23 ± 1.28	30.23 ± 1.28
CC3	53.90 ± 2.35	34.40 ± 1.25	88.30 ± 1.80
R1	3.84 ± 0.21	0	3.84 ± 0.21
Treatment	**Phosphorous Accumulation Efficiency (%)**		
	Plant	**Algae**	**Total**
Control	7.50 ± 1.42	0	7.50 ± 1.42
MC1	0	19.07 ± 1.46	19.07 ± 1.46
CC1	14.32 ± 2.02	15.80 ± 1.40	30.12 ± 1.71
MC2	0	24.98 ± 1.60	24.98 ± 1.60
CC2	26.41 ± 2.80	23.25 ± 2.03	49.66 ± 2.41
MC3	0	37.69 ± 2.31	37.69 ± 2.31
CC3	39.16 ± 2.76	41.43 ± 2.83	80.59 ± 2.80
R1	4.16 ± 0.85	0	4.16 ± 0.85
Treatment	**Potassium Accumulation Efficiency (%)**		
	Plant	**Algae**	**Total**
Control	8.57 ± 0.25	0	8.57 ± 0.25
MC1	0	20.89 ± 0.23	20.89 ± 0.23
CC1	16.39 ± 0.76	18.11 ± 0.27	34.50 ± 0.52
MC2	0	26.13 ± 0.47	26.13 ± 0.47
CC2	30.14 ± 1.60	24.12 ± 0.53	54.26 ± 1.07
MC3	0	37.88 ± 0.72	37.88 ± 0.72
CC3	45.46 ± 2.19	41.20 ± 1.20	86.66 ± 1.69
R1	4.87 ± 0.20	0	4.87 ± 0.20

Data represented are Mean ± Standard Error at n = 4.

efficiency of all the nutrients for plants was found to increase on supplementation of algal consortium as the positive interaction between the species increased the rate of root respiration, which provided adequate energy to uptake and further internally assimilate the available nutrients. This could also be corroborated to the highly developed roots observed during the cocultivation compared to the control. Cocultivation unit with 0.8 mg/ml of algal inoculum (CC3) showed maximal N, P and K accumulation efficiency of 53.90 ± 2.35%, 39.16 ± 2.76%, 45.46 ± 2.19% respectively for plants which was significantly higher compared to the control. Zhang et al. (2017) reported 66.71% N and 21.51% P utilization for tomato plants cocultured with *C. infusionum*.

CC3 also showed N, P and K accumulation efficiency of 34.40 ± 1.25%, 41.43 ± 2.83% and 41.20 ± 1.20% respectively, which was 13%, 9% and 8% more than the monoculture (MC3). N and P accumulation efficiency of 18.16% and 22.04% respectively was reported by Zhang et al. (2017) for *Chlorella* sp., higher than the monoculture having 3.69% N and 15.87% P accumulation efficiency. The synergistic interaction between the plant and algae during cocultivation often maintains the adequate soluble carbon pool in the nutrient media, which provides sufficient energy for the assimilation of essential nutrients into microalgae.

It could be seen that with the addition of microalgal consortium, total NAE of the hydroponics system improved significantly. Maximum total N, P and K accumulation efficiency of 88.30 ± 1.80%, 80.59 ± 2.80% and 86.66 ± 0.85% was obtained respectively, which was significantly more than the control. The total NAE obtained in the study is more than the efficiency reported in most of the global crop production systems during soil or soiless cultivation systems (Zhang et al., 2017). Though the cocultivation studies of plants with microalgae by Barone et al. (2019); Bharti et al. (2019) and Huo et al. (2020) showed synergistic interaction between the microalgae and plants favoring the higher nutrient uptake rate and thereby increase in morphological features, very limited studies have provided NAE. Hydroponic cocultivation study by Zhang et al. (2017) employing *C. infusionum* and tomato plants showed a total N and P utilization efficiency of 84.87% and 43.55% respectively, higher than the control. Owing to the current advances in the field of bioeconomy, the higher NAE is essential for producing nutritionally rich crops and algal biomass in an ecological sustainable manner.

4. Conclusion

The present study evaluated the cocultivation of microalgae and plants in a hydroponic system with the input for crop production. Initial microalgal inoculum influences the dissolved oxygen concentration and thereby the growth rate of plant during cocultivation. Maximum algal and plant productivity of 0.149 and 0.328 g/m^2/d respectively were obtained with 0.8 mg/ml of mixed microalgal inoculum. Adequate inoculum concentration facilitates positive interaction between phototrophs thereby improving the overall system performance in terms of nutrient removal and accumulation efficiency. Recycled urine medium after harvesting microalgae could be utilized as a cheap alternative to conventional hydroponics nutrient solution to support the growth of tomato plant for 24 days. The cocultivation unit could provide sustainable economic benefits not only interms of multiple products (nutrient rich crops and microalgae) but by reducing the costs association with upstream process of cultivation and downstream steps of wastewater treatment due to reduction in nutrient load.

CRediT authorship contribution statement

Kolli Venkata Supraja: Methodology, Investigation, Formal analysis, Writing - original draft. **Bunushree Behera:** Methodology, Investigation, Writing - review & editing. **P. Balasubramanian:** Conceptualization, Supervision, Funding acquisition, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2020.122823.

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