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Treating agricultural non-point source pollutants using periphyton biofilms and biomass volarization

Thomas Kiran Marella [a,1], Abhishek Saxena [b], Archana Tiwari [b,*], Aviraj Datta [a], Sreenath Dixit [a]

[a] International Crop Research Institute for Semi-arid Tropics (ICRISAT), Patancheru, 502 324, Telangana State, India
[b] Diatom Research Laboratory, Amity Institute of Biotechnology, Amity University, Noida, Uttar Pradesh, 201 313, India

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ABSTRACT

Untreated domestic wastewater and agricultural runoff are emerging as a potent cause of non-point source (NPS) pollutants which are a major threat to aquatic ecosystems. Periphyton biofilm-based technologies due to their high growth rate, energy efficiency and low input costs offer promising solutions for controlling nutrient pollution in agricultural systems. In this study we employed periphyton floway to treat NPS pollution from the agricultural watershed. The process performance of outdoor single pass algae floway (AFW) was evaluated. Steady state average biomass concentration of 11.73 g m^{-2} d^{-1} and removal rate of nitrogen: 0.60 g m^{-2} d^{-1}, phosphorus: 0.27 g $m^{-2}d^{-1}$, arsenic: 9.26 mg m^{-2} d^{-1}, chromium: 255.3 mg m^{-2} d^{-1} and lead: 238.6 mg m^{-2} d^{-1} was achieved. In addition, the microalgae and their associated bacterial diversity and dynamics were analyzed. The results revealed a high diversity and rapid variations in the microbiome structure with diatom and cyanobacteria dominance combined with high N fixing and P solubilizing bacteria during most of the operational period. Elemental analysis of periphyton biomass was done for its safe use as slow-release fertilizer. Biofuel feedstock potential and nanoparticle generation potential of the biomass were analyzed. This work highlights the potential use of periphyton biofilms in remediation and recycling of NPS pollutants with simultaneous resource recovery.

1. Introduction

Agriculture is one of the major economic growth engines to both developed and developing nations alike. It is of special importance to most populated countries like India, and china. Due to climate change and anthropogenic pressure, there are drastic changes in watershed soil fertility status which is leading to increased used of inorganic fertilizers especially nitrate and phosphate to support desired productivity (Emmerson et al., 2016). But due to uncontrolled fertilization practices and environmental factors majority of these nutrients are exiting the system through runoff leading to extensive eutrophication to aquatic ecosystems. Rapid urbanization, inadequate wastewater treatment infrastructure and indiscriminate use of fertilizers in agriculture have led to increased cultural eutrophication in both fresh and marine water habitats (Rajesh Banu et al., 2020). This has led to extensive plant growth, foul taste, and odor to drinking water, harmful algal blooms and fish kills. NPS pollution from agriculture is directly linked to atmospheric deposition, drainage, precipitation, seepage or hydrologic modification, and land runoff. Once spread, the NPS pollution is very difficult to control causing water bodies at their highest risk by negatively impacting the health of aquatic biota by elevating anthropogenic eutrophication (Rosov et al., 2020).

The greatest environmental challenge is to maintain safe water for various purpose since clean water is the primary requirement for nation-building as well as the survival of living beings (Walker et al., 2019). According to national water quality inventory reports agricultural runoff is the major source for water quality impairment to lakes, rivers and wetlands and contribute greatly to ground water contamination (US EPA,). Pollution generating from agricultural runoff has been believed to be affecting the level of eutrophication in surface waterbodies (Carpenter et al., 1998). Many studies emphasized that NPS pollutants impact more compared to point source pollutants in agricultural areas. In china's Taihu Lake Basin, agricultural NPS is accountable for 52% of the total load up of nitrogen (N) and 54% of total load up of phosphorous (P). Similarly, Italy has also experienced 24% and 71% of the load up of N and P whereas, in the USA, agricultural runoff is the leading cause of

* Corresponding author.
E-mail address: panarchana@gmail.com (A. Tiwari).

[1] Present Address: Algae Biomass and Energy System R&D Center (ABES), University of Tsukuba, Tennodai 1-1-1, Tsukuba, Ibaraki 305–8572, Japan.

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NPS pollution in lakes and streams, which is a serious concern (Xia et al., 2020). In most rural and peri urban watersheds, these pollutants are discharged directly into surface receiving waters. According to a recent estimate 75% of the domestic wastewater and almost 100% agricultural runoff in India is discharged without any treatment due to lack of decentralized wastewater treatment (Williams et al., 2019). Novel decentralized treatment technologies with high efficacy, low land and energy footprint and minimal maintenance cost are to be explored to minimize NPS pollutants detrimental effects on ecosystem diversity and health (Li, 2015).

Major activities which lead to NPS pollution from agriculture include irrigation, fertilization, planting, pesticide application, plowing, and harvesting. The main composition of agricultural runoff are nutrients, sediments, pesticides, and heavy metals, which can contaminate water bodies (Wang et al., 2018). To control eutrophication in aquatic bodies, it is necessary to systematically decrease agricultural NPS pollution to protect the environment and secure clean drinking and supply water quality (Qin et al., 2018). Primarily, the harmful impacts of agricultural NPS pollution can be controlled by adapting management practices in local conditions which include highly efficient irrigation equipment, limited use of fertilizers, and implementing nutrient management plans (Xia et al., 2020b). But it involves careful optimization and screening which is difficult to implement in developing countries. So, there is an urgent need to identify and implement novel strategies to reduce NPS pollution from agricultural watersheds.

Recently, microalgae have shown great promise in wastewater treatment field with their ability to bioremediate nutrients, heavy metals, and pharmaceuticals (Kiran Marella et al., 2020a). Algae turf scrubbers (ATS) are shallow depth ponds with solid substrate facilitating attached growth of periphyton and high-rate algal ponds (HRAP) which are paddle wheeled open raceways are increasingly studied for excess nutrient removal from various wastewaters including agricultural runoff (Leong et al., 2021). But cultivation of microalgae in agricultural watersheds using raceway ponds or photobioreactors is difficult due to large land and energy footprint. In this context, the use of periphyton biofilms which are a robust dynamic microcosm of microalgae and bacteria under symbiotic coexistence has been employed in treatment of domestic and agricultural wastewater (Mantzorou and Ververidis, 2019). But in developing countries due to lack of centralized wastewater treatment facilities segregation of agricultural wastewater at one location for its treatment is not feasible. So, in this study we want to explore a decentralized treatment method by using single pass algae floway system where the runoff is allowed to pass through the floway with no recirculation back on to substrate as in ATS systems. These systems can be advantageous as they can be directly installed in the farm ditches and other similar waterways with agricultural runoff, but the main drawback was limited hydraulic retention time (HRT). Periphytic biofilms are significant in transforming the nutrient utilization between overlying water and soil or sediment. However, their involvement in controlling and improving the NPS pollutant is still underrated (Wu et al., 2018) Periphyton biofilms consists of combination of bacteria and microalgae from different genera which can grow under varied trophic modes by symbiotic relationship with optimized mass balance (Kiran Marella et al., 2020a). Research lacuna still exists in factors influencing microalgae and bacterial dynamics especially in tropical climates for treating these pollutants with a path towards resource recovery. So, we have studied the microalgae-bacterial interaction at the phycosphere level to understand their synergetic relationship in facilitating wastewater bioremediation.

Algae biomass generated from treating different wastewaters contain various compounds like lipids, carbohydrates, proteins, and nutrients which can make ideal feedstock for biodiesel and bioplastics (Marella et al., 2020). But the use of wastewater grown microalgae biomass is limited due to legal restrictions by agencies like food and drug administration (FDA), which restricts its use for human consumption due to risk of heavy metal contamination and presence of pathogenic microbes. So, finding novel ways to volarize this biomass needs further research (Chandra et al., 2019). Use of periphyton biomass as slow release biofertilizer is a novel approach but bioaccumulation of heavy metals in biomass and their further release into soil can be detrimental to soil health (Mulbry et al., 2005). So, in the present study we performed elemental analysis of the biomass to explore its safe use as biofertilizer.

Recently, bio-based nanoparticles are considered a promising, cost-effective, and eco-friendly approach because they are coated with biosurfactants, natural capping agents with enhanced stability, reduced toxicity, and excellent biocompatible behavior (Mishra et al., 2020). Microalgae extracts provide controlled environment, low precursor concentrations, large surface area and inert conditions for nanoparticle synthesis as they strongly resist diffusion of strongly charged ions or highly reactive groups into the deepest layer of the biofilm matrix (Tanzil et al., 2016). So, in this work we studied the potential of periphyton biomass extracts to biosynthesize silver nano particles and efficacy of silver nano particles as antibacterial agents.

In this framework, the main aim of this study is to reduce the NPS pollutants from agricultural runoff through sustainable practices and to explore novel way to volarize the biomass. The specific objectives of this work are as follows: (i) to treat NPS pollutants generated from agricultural run-off using AFW, (ii) to evaluate the performance of AFW in recycling and removal of excess nutrients and heavy metals, (iii) to investigate microalgal-bacteria biodiversity and its dynamics, and (iv) to explore new ways to volarize the periphyton biomass. So, this study was envisioned to explore the capability of periphyton biofilms in nutrient conversion processes such as absorbing and accumulation of N and P with concomitant recycling as well as recovery of NPS pollutants and resource recovery from agricultural runoff. This work paves the way for the agriculturist to figure out and execute cost-effective ecofriendly technologies in improving nutrient utilization from farm to the watershed by employing microalgae bacteria consortia to reduce NPS pollution in artificial and natural wetland ecosystems and recycle them back to farmland to increase the agricultural productivity.

2. Materials and methods

2.1. Study area

Study was conducted in RL22 watershed (17°29′31.8″N 78°16′31.5″E) located inside International Crop Research Institute for semiarid tropics (ICRISAT) campus at Hyderabad, India. This watershed is exclusively used for paddy cultivation. Primary source of irrigation was from bird lake through gravity flow. Detailed land use and land cover map of the watershed was shown in Figure. S1 in supplementary information (SI). Environmental data on temperature, rainfall, photosynthetically active radiation (PAR) during the study period were collected from ICRISAT meteorology lab.

2.2. Algae floway operation and sampling

A single pass AFW with three dimensional (3D) solid substrate was constructed as mentioned elsewhere (Marella et al., 2019a). The AFW was fed with wastewater collected from drainage ditch of the rice field and allowed to pass on to the AFW by gravity flow through 10 cm polyvinyl chloride (PVC) pipe at 30 L min^{-1}. A water flow meter was connected before the inlet to measure water flow. We have operated the AFW during three periods which were defined as before (month 1–5; Jan–May), during (month 6–10; Jun–Oct) and after (month 11-2; Nov–Dec) the cropping season. Naturally occurring algae could grow on the floway as periphyton biofilm. Biomass sampling was done every month from the AFW as described elsewhere (Marella et al., 2019a).

2.3. Wastewater sampling

Sampling for runoff nutrient analysis was done at the outlet before entering the drainage ditch of the rice field in triplicate every month before, during and after the cropping season and the same sample was used to conduct algae floway experiments. Water samples for nutrient analysis were collected manually at 10 cm below the water surface and stored in clean narrow-mouth plastic polyethylene bottles with screw caps put on ice immediately after collection and during transport to the laboratory.

2.4. Nutrient and heavy metal analysis

Dried periphyton biomass samples (~20 mg) were directly analyzed for carbon and nitrogen content using PerklinElmer 2400 CHN elemental analyzer (Perklin-Elmer, New Delhi, India) (Culmo et al., 1989) in triplicate. Elemental analysis of dried algae biomass was done using inductively coupled plasma spectrometer (ICP-MS) (Kropat et al., 2011). Water samples were analyzed for total nitrogen (TN) and total phosphate (TP) using standard spectrometric methods (APHA, 1998).

Determination of the heavy metals, arsenic (As), chromium (Cr), lead (Pb), copper (Cu) and zinc (Zn) from runoff and periphyton biomass samples were carried out by ICP-MS (PE USA). The heavy metal concentration was determined by comparing with calibration curves using standard solutions.

2.5. Algae community analysis

Dominant algal community analysis during different culture seasons was done by collecting algae samples from algae scrubber and examining them manually between a slide and coverslip using Olympus BX50 binocular light microscope in duplicate for each sample (Kangas et al., 2017). Identification of algae class was done using specific literature related to each major algal class Bacillariophyta (Krammer and Lange-Bertalot, 1986, 1988, 1991), Cyanobacteria (Komarek and Anagnostidis, 1998; 2005), Chlorophyta (Prescott, 1982; John and Williamson, 2009) in addition to using some regional floras (Pandey et al., 2016).

2.6. Microbiome profiling

Illumina sequencing was used to track microbial population dynamics in the biofilm. All samples were quantified via the Qubit Quant-iT dsDNA High Sensitivity Kit (Invitrogen, Life Technologies, Grand Island, NY) to ensure that they met minimum concentration and DNA mass expectations. 16 S metagenome analysis of V3–V4 region is performed using QIIME workflow. The Illumina paired end V3–V4 reads (300*2) were demultiplexed using bcl2fastq1 tool. The paired end reads were quality checked using FastQC2. The raw reads having primer sequence and high-quality bases were selected. The reads were further stitched using Fastq-join3. These stitched reads were considered for further analysis using QIIME4 pipeline. The query sequences were clustered using UCLUST5 method against a curated chimera free 16s rRNA database (Greengenes6 v 13.8). The taxonomies were assigned using RDP7 classifier to these clusters at ≥ 97% sequence similarity against the reference database which resulted in the generation of a biome file. The biome was taken ahead for further advance analysis and visualization.

2.7. Lipid analysis

Lipid were extracted from dried biomass (1 g) using modified Bligh and Dyer method for algal lipids (Bligh and Dyer, 1959). Total lipid content of the biomass per dry cell weight (DCW) was estimated by gravimetric method. Fatty acid methyl ester (FAME) analysis from crude lipid extract was done according to Marella et al. (2018). Biodiesel properties like degree of unsaturation (DU), cetane number (CN), saponification value (SV), iodine value (IV), long chain saturated factor (LCSF), cold filter plugging point (CFPP) and cloud point (CP) were calculated from FAME profile (Talebi et al., 2013).

2.8. Biosynthesis of silver nanoparticles (AgNP) from periphyton biofilm extracts and their antibacterial assays

AgNP's were generated using aqueous biomass extract according to Mishra et al. (2020). Briefly, 1 g of dried biomass was extracted with 100 ml of MilliQwater and 5 ml of extract was mixed with 45 ml freshly prepared 2 mM silver nitrate ($AgNO_3$). After briskly stirring the mixture for 3 h it was left overnight at room temperature under light. A red-brown color change in the reaction mixture indicated the formation of AgNP. The biosynthesis of AgNP was further confirmed by taking absorption spectra using UV–vis spectrophotometer (Shimadzu, UV-1800) and shape, size and surface morphology were studied by scanning electron microscopy (SEM) (ZEISS EVO 1800; Germany), operating at 20.00 kV arranged with an energy dispersive X-ray spectrometry (EDX) model.

AgNP synthesized from biomass extracts was tested against both Gram-positive (*Bacillus subtilis, Streptococcus pneumoniae,* and *Staphyloccocus aureus*) and Gram-negative bacteria (*Aeromonas and E. coli)* using the microdilution assay method followed by agar well diffusion technique (Mishra et al., 2020).

2.9. Statistical analysis

The nutrient and heavy metal content, removal, algae growth, nutrient, and lipid productivity rates used for statistical analyses were the means ± standard deviation (SD). All statistical analyses were carried out using SPSS version 21.0 software (SPSS Inc., Chicago, IL, USA). A one-way analysis of variance was used to compare the significance levels of variations in the mean between groups. Statistical values of $P < 0.05$ were considered significant rejecting the null hypothesis between groups.

3. Results and discussion

3.1. Nutrient and heavy metal concentration in agricultural runoff

During the study we monitored major nutrients nitrate and phosphate as TN and TP from the runoff. Cropping season started during June month and lasted till August during this phase there are three nutrient fertilizations were done as basal, tillering and panicle fertilization. During the no cropping season before basal fertilization average TN and TP concentration is 6.24 and 2.13 mg L^{-1} respectively. After basal fertilization, these values reached a maximum of 13.2 mg L^{-1} TN and 5.6 mg L^{-1} TP which indicates excess nutrient runoff event (Fig. 1A). The same trend of nutrient runoff was observed after panicle fertilization (10th month). The trend of TN and TP increase and concentration (10–15 mg L^{-1}) in runoff during cropping season and its concentration was similar to previous studies in paddy cultivation (Cho, 2003). As the rice plants grow, they will undergo two important stages i.e. tillering and panicle initiation, which will ultimately have an affect yield. So, farmers apply fertilizers during these growth stages which directly influences overall productivity. But incidental nutrient loss and overuse of fertilizers are known to contribute to increased surface runoff from agriculture especially paddy fields (Liu et al., 2019).

The mean concentration (mg L^{-1}) of heavy metals Pb, Zn, Cu, As and Cr in runoff were 0.71, 1.2, 0.16, 0.16 and 0.66. The mean highest concentration of heavy metals was found during the summer season (3rd to 6th month) (Fig. 1B). Cropping season did not significantly influence heavy metal concentration, the reason for this increase might be due to increased concentration in inlet water as the field in the present study is primarily irrigated with lake water which might contain higher heavy metal concentration due to summer evapotranspiration. Higher Pb and Zn in runoff can also be attributed to frequency of tillering combined with wastewater irrigation which is the case with the present study site

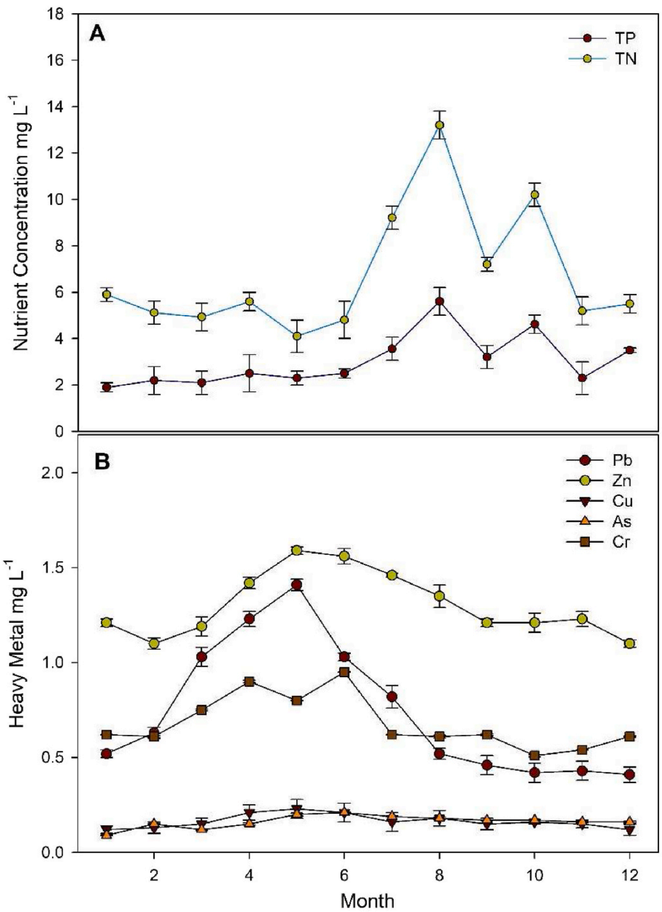

Fig. 1. Nutrient (A) and heavy metal concentration in agricultural runoff (Total Nitrate: TN and Total Phosphate: TP). Error bars represent ±SD.

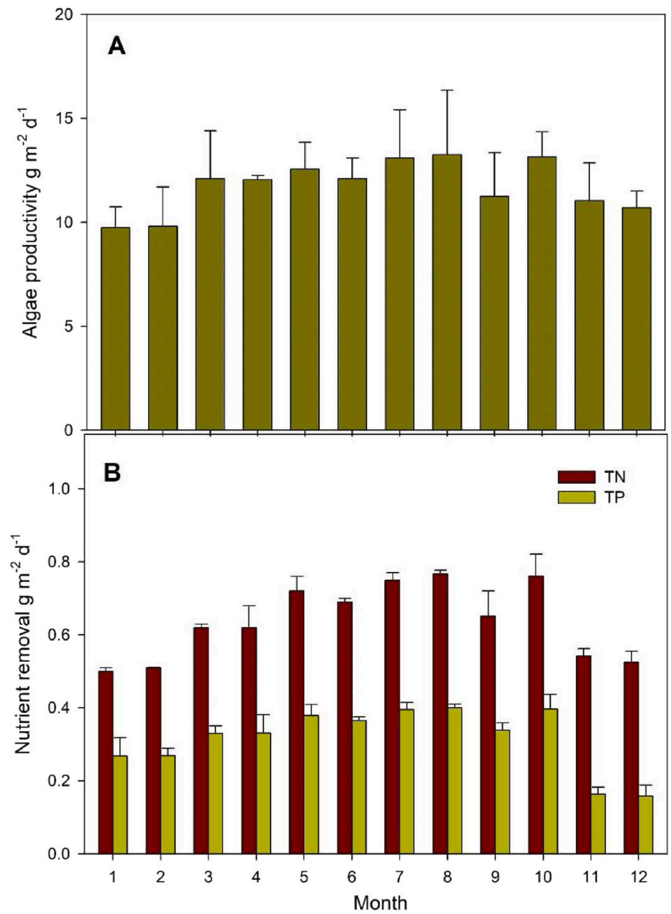

Fig. 2. Periphyton biomass productivity (A) and TN and TP removal rate (B). Error bars indicate ±SD.

(Liu et al., 2005). But the concentration of heavy metals observed was within their respective permissible limits set by FAO (1985).

3.2. Biomass productivity and nutrient removal

Periphyton biomass productivity was estimated from dried biomass collected from AFW every month. Mean biomass production during entire study period reached 11.45 g m^{-2} d^{-1} with the highest productivity of 12.56 g m^{-2} d^{-1} during cropping season followed by 10.92 g m^{-2} d^{-1} during before cropping season (Fig. 2A). We observed no significant difference ($p > 0.05$) between mean productivity before (10.92 g m^{-2} d^{-1}) and after (10.87 g m^{-2} d^{-1}) cropping season. The highest monthly productivity (13.1–13.25 g m^{-2} d^{-1}) was achieved during the months of 7 and 8 which coincides with high N and P loading (Fig. 1). Previous studies with the use of ATS for treating agricultural runoff from Lake Okeechobee watershed achieved 11.67–14.18 g m^{-2} d^{-1} biomass productivity, which is almost similar to the productivity achieved in the present study (Hydromentia Inc., 2005). Nutrient loading combined with higher solar irradiation positively influenced biomass productivity. Middle of the cropping season (7th and 8th month) resulted in higher N (11.20 mg L^{-1}) and P (4.55 mg L^{-1}) runoff due to basal and tillering fertilization and higher light availability (1230 PAR μmol s^{-1} m^{-2} (Table S1 in SI). As the periphyton community is dominated by photosynthetic autotrophs light availability can be a major limiting factor influencing growth (Marella et al., 2019a). Compared to previous studies using dairy, domestic wastewater biomass productivity obtained in the present study is low, this could be due to low nutrient and organic matter concentrations in the agricultural runoff which limited the increase of biomass production (García-Galán et al., 2018). Based on temperature and light availability for algae production India is among one of the eight countries where biomass yields of 13–15 g m^{-2} d^{-1} can be achieved (Moody et al., 2014). The productive achieved in the present study is close to this predicted range. Despite having the advantage of favorable conditions for algae biomass productivity the amount of research and pilot projects on microalgae-based wastewater treatment is very few compared to other temperate countries (Mohsenpour et al., 2021).

The nutrient removal rate of the periphyton biofilm was calculated from the elemental composition analysis of dried biomass. Total N and P % of the biomass ranged between 4.9%-5.7% and 1.48%–3.02% (by weight) respectively during the entire growth period. The mean seasonal removal rate of N was 0.56, 0.72 and 0.53 g m^{-2} d^{-1} and P were 0.29, 0.37 and 0.16 g m^{-2} d^{-1} for before, during and after the cropping season (Fig. 2B). Both nutrient loading and biomass productivity positively influenced nutrient removal rate with higher N and P availability can result in higher biomass productivity and nutrient removal (Mulbry et al., 2005). Nutrient removal also depends on periphyton community dynamics, many species of diatoms along with some green algae like *Chlorella* sp. and blue green algae like *Anabena* sp. are known to be pollution tolerant species which can tolerate high nutrient and heavy metal concentration. In the present study, periphyton community is dominated by diatoms which are known for their efficient nutrient acquisition and due to the presence of bigger storage vacuole can store higher concertation of nutrients inside their cells which influences nutrient removal (Marella et al., 2020). The nutrient removal capacity of AFW is essential in controlling non-point source pollution. Since the use of fertilizer in the rice field is a major concern because it would have adverse environmental impacts through the leaching of nutrients via runoff into receiving water bodies.

Microalgae can bioaccumulate heavy metals and some of the heavy metals like Zn, Fe and Cu are used by microalgae for their metabolic activities. So, we have analyzed the heavy metal content in periphyton biomass and calculated the heavy metal removal rate of AFW. The mean seasonal heavy metal removal rate was shown in Fig. 3. Zn removal rate (499.3 mg m^{-2} d^{-1}) was highest among all the heavy metals analyzed, along with Cu (111 mg m^{-2} d^{-1}). Zn and Cu are essential micronutrients for microalgae growth due to this they bioaccumulate higher concentration of these metals. Microalgae employs different mechanisms to encounter heavy metal toxicity. They can both adsorb and absorb heavy metals depending on the type of heavy metal and the requirement of heavy metal for metabolic activities. Essential metals like Zn, Cu and Fe are directly absorbed through their cell wall whereas more toxic metals Cd, Cr, Pb and As are first detoxified through metal speciation and then absorbed (Marella et al., 2020). In the present study toxic heavy metals like Cr (255.33 mg m^{-2} d^{-1}), Pb (238.66 mg m^{-2} d^{-1}) and As (9.9 mg m^{-2} d^{-1}) were also removed from runoff. Although there are no previous reports on microalgae biofilm heavy metal removal rate many researchers have studied heavy metal absorption in varied microalgae species (Chan et al., 2014). Heavy metal contamination in the agricultural field is a major concern and considered a serious environmental problem due to their non-biodegradable nature and long half-life. Leaching of heavy metal through agricultural runoff to the aquatic bodies is toxic to human health and it can also bioaccumulate in the crops and enter the food chain. In the current study, the heavy metal accumulation by AFW is quite promising showing substantial uptake of different heavy metals. As high concentration of Cu and Zn is rather toxic whereas Pb shows carcinogenic effects on humans and other biota alike (Hu et al., 2017).

3.3. Phytoplankton and bacterial diversity

A rich diversity of microalgae was noticed in the periphyton biofilm during the present study with identification of different classes of phytoplankton such as Bacillariophyta, Chlorophyta, Cyanophyta, Euglenophyta, Dinophyta, and Cryptophyta (Fig. 4A). Bacillariophyta (49.8–69.7%) which constitutes diatoms were the major group followed by Chlorophyta (14.7–35.6%) and Cyanophyta (6.0–10.6%) during all three cropping seasons. Bacillariophyceae was the most dominant periphyton community of algae turf scrubbers for treatment of NPS pollutants (Adey et al., 2013), algae diversity analysis from the present work supported this observation. Chlorophyta and Cyanophyta abundance increased during cropping season compared to another dominant group. The reason for this increase can be attributed to an increase in phosphate concentration with fertilizer input, as higher P levels can promote cyanobacteria growth. Majority of the Chlorophyta observed during the study are filamentous forms which form a canopy on the substrate on which diatoms grow. Due to decrease of diatoms during cropping season the availability of light might have increased to bottom dwelling species this might be the reason for an increase in Chlorophyta during the cropping season. Temperature and light are the key physicochemical factors along with pH influencing microalgae growth and diversity wastewater (Gonçalves et al., 2017). The diatoms require less light due to the presence of fucoxanthin as major photosynthetic pigment this might be the reason for their dominance during winter months before and after the cropping season. For microalgae-based wastewater treatment, the use of poly cultures instead of single celled cultures is more preferred as wastewater warrants a robust biological system that can function and adapt to varying environmental conditions and nutrient loads (Fouilland, 2012). In the present study we employed a natural consortium of microalgae which self-seeded onto the substrate and contains high diversity in terms of different algae groups, similar biofilm algae profiles were reported from biofilms treating river wastewater (Kangas et al., 2017). The use of mixed natural consortia for wastewater treatment has many advantages like increased biomass productivity and nutrient removal efficiency due to cooperative interactions between different species (Bacellar Mendes and Vermelho, 2013).

To estimate the microbial diversity of the periphyton biofilms we analyzed the DNA sequences of mature periphyton biofilm obtained using Illumina based sequencing for the presence of hypervariable V3 and V6 regions of rRNA gene fragments, which is an established protocol to study bacterial diversity in diverse ecosystems. To understand the

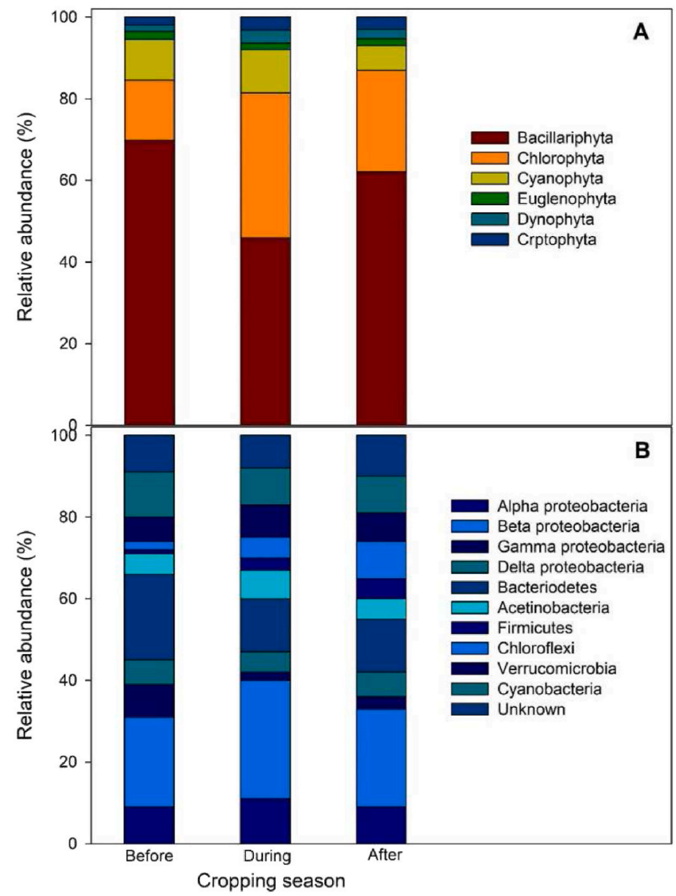

Fig. 4. Relative abundance of phytoplankton (A) and bacterial (phylum level) (B) diversity of periphyton biofilm.

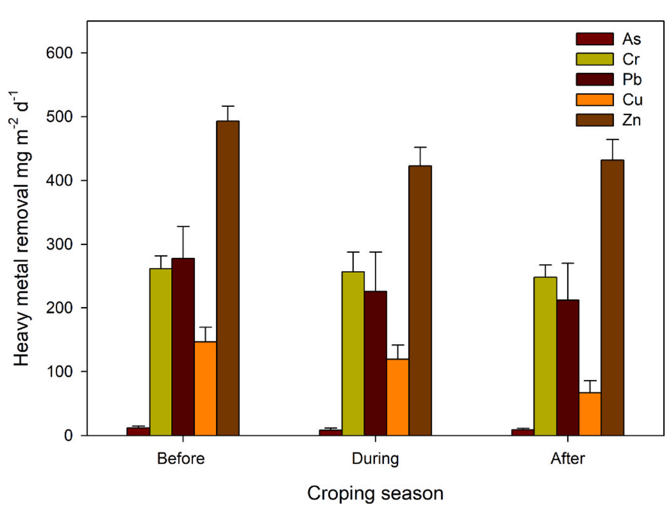

Fig. 3. Heavy metal removal rate of periphyton biofilm. Error bars indicate ±SD.

microbiome diversity and species abundances for each of the individual sample, QIIME analysis was performed on individual samples. Read count statistics resulted in a mean total of paired end reads between 254860-320010 of which total identified rRNA sequences raged between 149174-159985 with a total operational taxonomic unit (OTUs) 1449–2090. In addition, a mean Shannon-Weaver index of 5.7 was observed along with Chao1 richness estimates (1957–2908) among samples from different cropping seasons, indicating significant assessment of bacterial species diversity. Of the OTUs identified to taxa level, the bacteria identified in samples before, during and after cropping season were alpha-, beta-, elta-proteobacteria, bacteroidetes, firmicutes, chloriflexi, verrucomicrobia and acetinobacteria. Majority of the bacteria identified are opportunistic heterotrophs, phototrophs and filamentous bacteria with known mechanisms related to N fixation, P solubilization, iron oxidation and metal speciation (Newton et al., 2011) Relative abundance from the different OTUs is presented in Fig. 4B. By percentage reads betaproteobacteria was the most dominant group (29%) followed by bacteroidetes (21%) and alfa proteobacteria (11%). But there is no significant difference between bacterial diversity between cropping seasons. Proteobacteria and bacteriodetes are known to be associated as symbionts with many algae classes which includes diatoms, green algae, and cyanobacteria (Ramanan et al., 2016). Alfa proteobacteria are known to promote algae growth and aid in nitrogen fixation which helps in nutrient recycling at the phycosphere. *Rhizobium* an alfa proteobacteria not only promotes algae grown but also benefits from supply of vitamins, phytohormones and carbon exchange from algae. Mutual relationship between bacteriodetes and green alga *Chlorella vulgaris* enhanced growth and lipid production (Cho et al., 2015). Acetinobacteria is known to facilitate phosphate removal from wastewater. Our findings are based on limited sampling from the periphyton biofilms during the cropping season, so generalizations cannot be made regarding their functions and significance. A deeper understanding of algae microbe interactions and their potential environmental and ecological implications require robust studies with intensive sampling across spatio-temporal distributions.

3.4. Lipid productivity and biodiesel quality

To estimate the suitability of lipids extracted from periphyton biomass for biodiesel production we have extracted lipids from dried biomass and analyzed lipid percentage, productivity, and fatty acid profile (Table .1 and supplementary file figure. S2). The percentage of lipid as DCW was highest during just before (19.2%) and initial phase (18.2%) of the cropping season. But mean seasonal lipid% was higher before (15.58%) compared to during (11.94%) cropping season. Nutrient limitation is known to induce higher lipid production in a variety of microalgae, before and after the cropping season the nutrient load on the AFW is considerably lower compared to during cropping season, this might be one of the possible causes for higher lipid content (Adeniyi et al., 2018). Mean lipid productivity reached 1.60 g m^{-2} d^{-1}, 1.64 g m^{-2} d^{-1} and 1.75 g m^{-2} d^{-1} before, during and after cropping season, respectively. The combination of nutrient limitation and domination of diatoms during winter period (Nov–Dec) might have induced higher lipid productivity but not biomass productivity as there is no significant difference between biomass productivity during different cropping seasons ($p > 0.05$). Comparatively diatoms can accumulate lipids up to 50–60% of their DCW which is higher than any other algae class (Hildebrand et al., 2012). Lipid % as DCW and productivity achieved in this study is higher than previously reported studies using agricultural effluents, but lower than studies that used domestic wastewater, higher biomass productivity achieved due to higher nutrient loading might have resulted in higher lipid productivity (Marella et al., 2019b; Mulbry et al., 2010).

Table 1
Seasonal variation in FAME profile, lipid % and productivity of periphyton biomass. Values represent mean for each season.

FAME relative %	Cropping Season		
	Before	During	After
C14:0	9.2	5.7	10.3
C14:1n-5	2.4	2.1	2.9
C16:0	26.2	20.2	20.2
C16:1n-7	16.3	25.2	20.3
C17:0	2.4	1.2	n.d.
C18:0	19.1	16.2	9.5
C18:1	12.3	13	15.4
C18:2n-6	0.2	1.4	2.4
C18:3n-6	1.2	2.1	2.1
C20:0	2.9	1.2	1.2
C20:4n-6	0.5	1.9	2.1
C20:5n-3	7.1	8.7	12.7
C20:6n-3	0.2	1.1	0.9
SFA	59.8	44.5	41.2
MUFA	31	40.5	38.6
PUFA	9.2	15	20.2
Lipid % DCW	14.67	13.15	16.75
Lipid productivity g m^{-2} d^{-1}	1.6	1.6	1.75

Along with lipid quantity (total lipid content per DCW) lipid quality (fatty acid profile) is also a crucial factor which defines the suitability of lipid for biodiesel production. To estimate biodiesel quality, we have done FAME profiling of lipid extract from different seasons (Table .1.). Saturated fatty acids (SFA) consisting of C14:0, C16:0 and C18:0 were the major fatty acids followed by monounsaturated fatty acids (MUFA) across all seasons. Polyunsaturated fatty acid (PUFA) content was higher during the winter period after the cropping season. Fatty acid profile in the present study reflected a typical pattern related to microalgae with C16:0 and C18:0 being dominant fatty acids (Hu et al., 2008). Saturated fatty acid (SFA) content was higher before (59.8%) and during (44.5%) cropping season whereas PUFA content was high after cropping season (20.2%). This change can be due to availability of nutrients and light as degree of unsaturation in microalgal fatty acids is influenced by these two parameters, with higher concentration leading to SFA and lower leading to PUFA increase (Bromke et al., 2015).

Biodiesel quality indicators like centane number (CN), degree of unsaturation (DU), long chain saturated factor (LCSF), cold filter plugging point (CFPP) and cloud point (CP) were calculated from FAME profiles (Table. S2 in SI). DU of periphyton biomass derived biodiesel ranged between 49.4 and 79.0 during the growth period. The lowest DU value (49.4) was obtained before cropping season due to the presence of a higher MUFA:PUFA ratio whereas values were higher during other two cropping seasons. Lower DU is preferred for good quality biodiesel due to its influence on storage factors with higher DU value resulting in higher oxidation (Schenk et al., 2008). CN value of 37.8 (before), 36.9 (during) and 30.6 (After cropping season) was achieved in the preset study, so biodiesel from before cropping season biomass with higher CN was ideal with shorter ignition delay. LCSF relates to saturation of H atoms on fatty acid carbon chain with close relation to other parameters like CN and CFPP. CFPP is the crystallization temperature of biodiesel and it defines the use of biodiesel at the particular climatic region, with low CFPP ideal for temperate and high CFPP for tropical climates. In the present study high CFPP value of 30.95 was obtained with biomass from before cropping season and the lowest CFPP of 8.60 from after cropping season although both the values are ideal for specific climatic zones a mixture of both high and low CFPP biodiesel can also yield good biodiesel quality. CP is biodiesel solidifying temperature. CP value of periphyton biomass derived biofuel raged between 5.63 and 8.79 which can be ideal for climatic regions with high to medium temperatures. Although biodiesel quality achieved in the present study is not ideal but mixing of biodiesel from different cropping season can result in better quality biodiesel. Biodiesel from microalgae is known to safe due to its non-flammable nature and its wide applicability in areas such as power generation, energy and heat generation, transportation, lubricant, etc. (Chisti, 2007). The biodiesel quality parameters from periphyton biomass grown using agricultural wastewater was not reported earlier but are similar to previous reports regarding single celled microalgae species and mixed species biofilms grown using domestic wastewater (Marella et al., 2019a; Talebi et al., 2013).

3.5. Nano particle biosynthesis using periphyton biomass extract and antibacterial activity

Synthesis of nanoparticles using biological extracts reduces the risks associated with the use of harmful organic solvents and crates a path for green synthesis. So, we have used an aqueous extract of periphyton biomass to generate AgNP. During synthesis, a change in color of $AgNO_3$ the precursor solution was observed from colorless to reddishbrown which confirms the synthesis of AgNP after overnight incubation. The synthesis was further ascertained by taking UV spectra as presented in Fig. 5. A sharp peak obtained at ʎmax 420 nm in the visible region is due to the excitation of freely available electrons from lower energy to higher energy thus set up a surface plasmon resonance (SPR) band since both the valence as well as conduction band falls close to each other during metallic nanoparticle synthesis. The SPR band obtained at a particular wavelength can determine the size of the nanoparticle (Gupta et al., 2018). From the SPR band and SEM analysis it is concluded that the size of the AgNP synthesized using periphyton biomass extract falls in the nanometer range of approximately 100 nm. The biosynthesized AgNP can be utilized for many applications like active pharmaceutical ingredients, paint industry, electronic industry, textiles, etc. Active biomolecules like phenols, fatty acids, alkaloids and pigments in algae extracts are reported to act as metal reducing, stabilizing and capping agents (Hamouda et al., 2019). Reducing agents facilitate the initiation of nucleation process leading to formation of colloidal silver from $AgNO_3$. The hydroxyl (OH) group of fucoxanthin which is the main photosynthetic pigment present in the most dominant phytoplankton bacillariophyceae in the present study is a known strong reducing agent this along with other essential biomolecules in the presence of light might have led to the development of stable AgNP synthesis (Lechner and Becker, 2015). In the present study detailed chemical analysis of periphyton extracts was not performed but further work in this area is initiated in our lab to optimize the nanoparticle biosynthesis.

The AgNP synthesized from biofilm aqueous extracts exhibit very strong activity showing a broad-spectrum antibacterial effect against both gram -ve and gram + ve bacteria (Table 2). AgNP showed MIC of <1.95 μg ml^{-1} against *E. coli*, *S. aureus*, and *Aeromonas* and 1.95 μg ml^{-1} *against S. pneumonia* and *B. subtilis* respectively. However, the pure aqueous extracts without nano particles taken as control did not exhibit antibacterial activity. The control experiment showed a MIC value of >250 μg ml^{-1} against both gram-negative as well as gram + bacteria. The MIC observed in the present study is much lower compared to similar studies using single cells microalgae this could be due to the generation of more stable AgNP with mixed microalgae consortium with varied pigment and reducing agent concentration (Mishra et al., 2020). The mechanism involved in AgNP antibacterial activity is still unclear, but it may be hypothesized that positively charged Ag ions are attached to the negatively charged bacterial cell due to electrostatic interaction causing drastic change in membrane structure and free radical generation which can lead to cell death (Qais et al., 2019).

Recent advances in nano science have led to use of metal nanoparticles for different biotechnological applications, in this context silver nanoparticles are increasingly explored for its anti-inflammatory, anti-cancer and anti-bacterial effects (Stensberg et al., 2011). But using physical and chemical synthesis methods has disadvantages like high energy consumption and toxic byproduct generation. In this study to our knowledge, for the first time we successfully used wastewater grown microalgae biomass extract as a green synthesis option for silver nanoparticle generation. Periphyton biomass aqueous extracts provided enough reducing and stabilizing power to produce nano silver with no need for additional physical and chemical energy. Due to this green synthesis methods downstream processing of metal nanoparticles for biomedical applications becomes easier as no toxic chemicals are used which adds further processing steps for their removal. 3.6. Elemental composition of Periphyton biomass and potential as slow-release fertilizer.

Monthly samples during three cropping seasons were pooled and dried biomass was used for elemental analysis. Mean elemental concentration of the biomass from cropping season along with Algae turf scrubber (ATS) biomass grown using dairy wastewater and conventional manures from farm waste and vermicompost was given in Table 3. N, P, K concentration of periphyton biomass was 46.9, 32.4, 18.7 g kg^{-1} respectively. While N concentration is slightly lower when compared with ATS biomass is grown using dairy wastewater, but P and K concentration is higher ((Mulbry et al., 2005). The reason could be the nutrient loading rate due to higher loading of nutrients through fertilizer runoff might have resulted in higher bioaccumulation. In comparison with traditional manures although N content is similar P and K content is very low in algae biomass. Additionally, we have estimated heavy metal concentration of periphyton biomass, Cu (293 mg kg^{-1}) and Zn (750 mg kg^{-1}) content are slightly higher compared to ATS biomass. Heavy metal concentration in biomass from this study was well below US EPA ceiling concentration for manure application (As-75; Cr-3000; Cu-4300; Pb-840; Zn-7500 mg kg^{-1}) (EPA, 1994). Algae biomass grown using wastewater contains valuable nutrients which can be recycled back to the farm one such application was to use the biomass as a slow-release fertilizer. As algae entrap the nutrients inside their cell wall and they can be released when the cell wall is broken this gives algae derived biomass unique character which is specific to slow-release fertilizers.

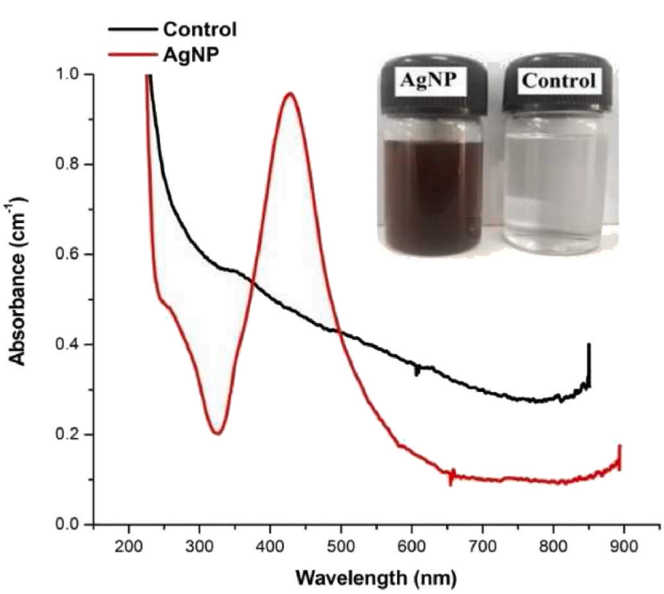

Fig. 5. UV–vis spectra of biosynthesized AgNPs synthesized from biofilm extract in comparison with control along with picture showing change in color during AgNP synthesis. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Minimum inhibition concentration (MIC) (μg ml^{-1}) of AgNP synthesized using periphyton biofilm extract in comparison with control with no nano particles.

Bacterial Strains	AgNP	Control
E. coli	<1.95	>250
S. aureus	<1.95	>250
Aeromonas	<1.95	>250
S. pneumoniae	1.95	>250
B. subtilis	1.95	>250

Table 3
Elemental composition of different manures used as biofertilizers in comparison with periphyton biomass.

Elemental concentration (mg kg^{-1})	Vermicompost	Farmyard manure	Fertilizer- Garden tone 4-6-6	Algae Turf Scrubber (ATS) Raw dairy	Periphyton biomass (Agricultural runoff)
N	17,900	17,100	48,000	49,100	46,900
P	39,800	20,300	25,200	8000	9400
K	13,200	18,100	28,100	7200	8724
C	190,000	183,700	325,000	n.d.[a]	n.d.
Ca	16,500	22,350	30,000	5470	6859
Mg	6521	3510	5000	1900	3214
Fe	1431	936	10,000	3000	10,102
Mn	181	126	500	450	320
Cu	7.9	5.54	500	155	293
Zn	49.2	35.8	500	560	750
As	n.d.	n.d.	n.d.	n.d.	0.58
Pb	n.d.	n.d.	n.d.	6.5	21.63
Cr	n.d.	n.d.	n.d.	n.d.	19.7

Elemental values of ATS raw dairy, garden tone 4-6-6 are from Mulbry et al. (2005), farmyard manure, vermicompost and periphyton biomass were determined in our laboratory. ATS biomass manure was anaerobically digested.
[a] n.d.: Not determined.

Previously algae biomass from ATS using dairy and swine effluents were tested for its use as fertilizers (Mulbry et al., 2008). In the present study we have done the elemental comparison of periphyton biomass grown using agricultural runoff and found that the composition is similar to previous studies and can be used as fertilizer with no detrimental effect on soil health. Microalgae biomass contain plant promoting substances like phytohormones, vitamins, amino acids and antibacterial and anti-fungal compounds along with macronutrients and trace metals. The use of microalgae based biofertilizers influences plant growth, fruit quality, carotenoid content and overall productivity (Coppens et al., 2016). Previous studies reported that use of 12% algal biomass as bio-fertilizer in millet resulted in efficient P uptake (Castro et al., 2020b). So, wastewater grown periphyton biomass can be used as slow-release fertilizer which can be a novel pathway to already existing volarization methods like biodiesel and bioplastic generation. Use of agricultural runoff grown biomass for soil application is an interesting valorization option as it reduces the use of chemical fertilizers there by reducing nutrient runoff (Castro et al., 2020a). Recycling the excess nutrients from agricultural runoff back to watershed using microalgae is a sustainable option in the context of circular bioeconomy.

4. Practical applications and future research prospects

In developing countries urban and peri urban watersheds are increasingly irrigated with treated and untreated domestic wastewater, this combined with excessive fertilization is leading to increased cultural eutrophication. Entrapping the excess nutrients using periphyton biofilms is advantageous in watersheds which lack operational and technical knowhow and facilities for centralized wastewater treatment. So, use of low cost, energy efficient options like AFW becomes effective and feasible due its ease of operation and maintenance even at the farm level. Installation of AFW in existing water channels and ditches by lining their surface with 3D substrates can be an option which reduces the need for dedicated land and pumping systems and makes the process decentralized. Finding novel options to volarize the algae biomass generated from wastewater is paramount to achieve environmental sustainability goals by means of circular bioeconomy. Present volarization routes are confined to bioenergy and biofertilizer sectors which are low-cost options compared to nanotechnology and nanomedicine sector option explored in the present study. Exploring the microalgae bacteria nexus in wastewater treatment is crucial to understand their synergy which can be channelized to further optimize the process parameters for microalgae-based wastewater treatment. From the algae bacterial diversity data obtained in the present study we deduced some metabolic pathways which are influencing their growth and subsequent impact on nutrient recycling. But future research should focus on the aspect of functional diversity in combination with species diversity. Despite numerous advantages related to use of periphyton biofilms for wastewater treatment their use is still confined to a few countries and research lacuna still exists in terms of process optimization and scale up from varied climatic zones. Conclusion.

Periphytic biofilms due to their faster growth and synergy between algae and bacteria play a critical role in nitrogen and phosphorus bio assimilation, heavy metal adsorption, nitrogen fixation and nutrient cycling. Accordingly, they facilitate in reducing NPS pollution, but their importance is usually undermined. So, ongoing research efforts should focus on understanding the underlaying mechanisms involved in nutrient and other pollutant removal using microorganisms. The present finding indicates that the periphyton biofilm-based treatment has the potential to treat agricultural wastewaters with low environmental footprints. Use of periphyton biomass for green synthesis of metal nanoparticles and their subsequent use for biotechnological applications can open novel high value pathways for wastewater biomass volarization. Further improvements in facilitating nitrogen fixing, phosphate solubilizing microorganisms and their synergy with microalgae in biofilms can increase the feasibility of the periphytic biofilms to improve nutrient utilization efficiency leading to enhanced NPS pollution abatement.

Author credit

1. Thomas Kiran Marella-substantial contribution to conception and design of the experiment, drafting the manuscript.
2. Abhishek Saxena-Nanoparticle synthesis, data interpretation.
3. Aviraj Datta- Analysis and interpretation of data.
4. Sreenath Dixit- Analysis and interpretation of data.
5. Archana Tiwari- Supervision, Experimental planning, manuscript editing, research funding

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

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References

APHA, 1998. Standard Methods for Examination of Water and Wastewater.

Adeniyi, O.M., Azimov, U., Burluka, A., 2018. Algae biofuel: current status and future applications. Renew. Sustain. Energy Rev. 90, 316–335. https://doi.org/10.1016/J.RSER.2018.03.067.

Adey, W.H., Laughinghouse, H.D., Miller, J.B., Hayek, L.-A.C., Thompson, J.G., Bertman, S., Hampel, K., Puvanendran, S., 2013. Algal turf scrubber (ATS) floways on the Great Wicomico River, Chesapeake Bay: productivity, algal community structure, substrate and chemistry [1]. J. Phycol. 49, 489–501. https://doi.org/10.1111/jpy.12056.

Bacellar Mendes, L.B., Vermelho, A.B., 2013. Allelopathy as a potential strategy to improve microalgae cultivation. Biotechnol. Biofuels. https://doi.org/10.1186/1754-6834-6-152.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.

Bromke, M.K, Sabir, J.S, Alfassi, F.A, Hajarah, N.H, Kabli, S.A, Al-Malki, A.L, et al., 2015. Metabolomic profiling of 13 diatom cultures and their adaptation to nitrate-limited growth conditions. PLoS ONE 10 (10), e0138965. https://doi.org/10.1371/journal.pone.0138965.

Carpenter, S.R., Caraco, N.F., Correll, D.L., Howarth, R.W., Sharpley, A.N., Smith, V.H., 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecol. Appl. 8, 559–568. https://doi.org/10.1890/1051-0761(1998)008[0559:NPOSWW]2.0.CO;2.

Castro, J. de S., Calijuri, M.L., Ferreira, J., Assemany, P.P., Ribeiro, V.J., 2020a. Microalgae based biofertilizer: a life cycle approach. Sci. Total Environ. 724, 138138. https://doi.org/10.1016/J.SCITOTENV.2020.138138.

Castro, J. de S., Calijuri, M.L., Mattiello, E.M., Ribeiro, V.J., Assemany, P.P., 2020b. Algal biomass from wastewater: soil phosphorus bioavailability and plants productivity. Sci. Total Environ. 711, 135088. https://doi.org/10.1016/J.SCITOTENV.2019.135088.

Chan, A., Salsali, H., McBean, E., 2014. Heavy metal removal (copper and zinc) in secondary effluent from wastewater treatment plants by microalgae. ACS Sustain. Chem. Eng. 2, 130–137. https://doi.org/10.1021/sc400289z.

Chandra, R., Iqbal, H.M.N., Vishal, G., Lee, H.S., Nagra, S., 2019. Algal biorefinery: a sustainable approach to valorize algal-based biomass towards multiple product recovery. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2019.01.104.

Chisti, Y., 2007. Biodiesel from microalgae. Biotechnol. Adv. 25, 294–306.

Cho, D.H., Ramanan, R., Heo, J., Lee, J., Kim, B.H., Oh, H.M., Kim, H.S., 2015. Enhancing microalgal biomass productivity by engineering a microalgal-bacterial community. Bioresour. Technol. 175, 578–585. https://doi.org/10.1016/j.biortech.2014.10.159.

Cho, J.Y., 2003. Seasonal runoff estimation of N and P in a paddy field of central Korea. Nutrient Cycl. Agroecosyst. 65, 43–52. https://doi.org/10.1023/A:1021819014494.

Coppens, J., Grunert, O., Van Den Hende, S., Vanhoutte, I., Boon, N., Haesaert, G., De Gelder, L., 2016. The use of microalgae as a high-value organic slow-release fertilizer results in tomatoes with increased carotenoid and sugar levels. J. Appl. Phycol. 28, 2367–2377. https://doi.org/10.1007/s10811-015-0775-2.

Culmo, R.F., Swanson, K.J., Brennan, W.P., 1989. Application of the PE 2400 CHN and PE2410 N for Soils. Perkin-Elmer Publication EAN30. CT, Norwalk.

Emmerson, M., Morales, M.B., Oñate, J.J., Batáry, P., Berendse, F., Liira, J., Aavik, T., Guerrero, I., Bommarco, R., Eggers, S., Pärt, T., Tscharntke, T., Weisser, W., Clement, L., Bengtsson, J., 2016. How agricultural intensification affects biodiversity and ecosystem services. Advances in Ecological Research. Academic Press Inc., pp. 43–97. https://doi.org/10.1016/bs.aecr.2016.08.005

EPA, 1994. A Plain English Guide to the EPA Part 503 Biosolids Rule. U.S. Environmental Protection Agency, Washington, DC, p. 176. EPA/832/R-93/003.

FAO, 1985. Water Quality for Agriculture. Food and Agriculture Organization, Rome, Italy. http://www.fao.org/3/T0234E/T0234E00.htm.

Fouilland, E., 2012. Biodiversity as a tool for waste phycoremediation and biomass production. Rev. Environ. Sci. Biotechnol. https://doi.org/10.1007/s11157-012-9270-2.

García-Galán, M.J., Gutiérrez, R., Uggetti, E., Matamoros, V., García, J., Ferrer, I., 2018. Use of full-scale hybrid horizontal tubular photobioreactors to process agricultural runoff. Biosyst. Eng. 166, 138–149. https://doi.org/10.1016/j.biosystemseng.2017.11.016.

Gonçalves, A.L., Pires, J.C.M., Simões, M., 2017. A review on the use of microalgal consortia for wastewater treatment. Algal Res. https://doi.org/10.1016/j.algal.2016.11.008.

Gupta, S., Kashyap, M., Kumar, V., Jain, P., Vinayak, V., Joshi, K.B., 2018. Peptide mediated facile fabrication of silver nanoparticles over living diatom surface and its application. J. Mol. Liq. 249, 600–608. https://doi.org/10.1016/j.molliq.2017.11.086.

Hamouda, R.A., Hussein, M.H., Abo-elmagd, R.A., Bawazir, S.S., 2019. Synthesis and biological characterization of silver nanoparticles derived from the cyanobacterium Oscillatoria limnetica. Sci. Rep. 91 9 (2019), 1–17. https://doi.org/10.1038/s41598-019-49444-y.

Hildebrand, M., Davis, A.K., Smith, S.R., Traller, J.C., Abbriano, R., 2012. The place of diatoms in the biofuels industry. Biofuels 3, 221–240. https://doi.org/10.4155/bfs.11.157.

Hu, B., Jia, X., Hu, J., Xu, D., Xia, F., Li, Y., 2017. Assessment of heavy metal pollution and health risks in the soil-plant-human system in the Yangtze River Delta, China. Int. J. Environ. Res. Public Health 14 (9), 1042. https://doi.org/10.3390/ijerph14091042.

Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M., Darzins, A., 2008. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. Plant J. 54, 621–639. https://doi.org/10.1111/j.1365-313X.2008.03492.x.

Hydromentia Inc., 2005. S-154 Pilot Single Stage Algal Turf Scrubber Final Report. South Florida Water Management District Contract No. C-13933.

John, D.M., Williamson, D.B., 2009. A Practical Guide to the Desmids of the West of Ireland. Martin Ryan Institute, National University of Ireland, Galway.

Kangas, P., Mulbry, W., Klavon, P., Laughinghouse, H.D., Adey, W., 2017. High diversity within the periphyton community of an algal turf scrubber on the Susquehanna River. Ecol. Eng. 108, 564–572. https://doi.org/10.1016/j.ecoleng.2017.05.010.

Kiran Marella, T., Saxena, A., Tiwari, A., 2020. Diatom mediated heavy metal remediation: a review. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2020.123068.

Komarek, J., Anagnostidis, K., 2005. In: Budel, B., Krienitz, L., Gärtner, G., Schagerl, M. (Eds.), Cyanoprokaryota 2. Teil: Oscillatorialles, Süßwasserflora von Mitteleuropa 19/2. Elsevier, Munich, p. 758.

Komarek, J., Anagnostidis, K., 1998. In: Ettl, H., Gärtner, G., Heynig, H., Mollenhauer, D. (Eds.), Cyanoprokaryota 1. Teil: Chroococcales, Süßwasserflora von Mitteleuropa 19/1. Gustav Fisher, Jena, p. 548.

Krammer, K., Lange-Bertalot, H., 1991. Bacillariophyceae 3. Teil: centrales, fragilariaceae, eunotiaceae. In: Ettl, H., Gerloff, J., Heynig, H., Mollenhauer, D. (Eds.), Süßwasserflora von Mitteleuropa 2/3. Gustav Fisher Verlag, Stuttgart, p. 576.

Krammer, K., Lange-Bertalot, H., 1988. In: Ettl, H., Gerloff, J., Heynig, H., Mollenhauer, D. (Eds.), Bacillariophyceae 3. Teil: Naviculaceae, Süßwasserflora von Mitteleuropa 2/3. Gustav Fisher Verlag, Stuttgart, p. 876.

Krammer, K., Lange-Bertalot, H., 1986. In: Ettl, Surirelliaceae H., Gerloff, J., Heynig, H., Mollenhauer, D. (Eds.), Bacillariophyceae 3. Teil: Bacillariaceae, Epithemiaceae, Süßwasserflora von Mitteleuropa 2/3. Gustav Fisher Verlag, Stuttgart, p. 596.

Kropat, J., Hong-Hermesdorf, A., Casero, D., Ent, P., Castruita, M., Pellegrini, M., Merchant, S.S., Malasarn, D., 2011. A revised mineral nutrient supplement increases biomass and growth rate in Chlamydomonas reinhardtii. Plant J. 66, 770–780. https://doi.org/10.1111/j.1365-313X.2011.04537.x.

Lechner, C., Becker, C., 2015. Silaffins in silica biomineralization and biomimetic silica precipitation. Mar. Drugs 13, 5297–5333. https://doi.org/10.3390/md13085297.

Leong, Y.K., Chew, K.W., Chen, W.H., Chang, J.S., Show, P.L., 2021. Reuniting the biogeochemistry of algae for a low-carbon circular bioeconomy. Trends Plant Sci. 26, 729–740. https://doi.org/10.1016/J.TPLANTS.2020.12.010.

Li, A.M.L., 2015. Ecological determinants of health: food and environment on human health. Environ. Sci. Pollut. Res. 9002–9015. https://doi.org/10.1007/S11356-015-5707-9, 2015 2410 24.

Liu, J., Liu, H., Liu, R., Mostofa Amin, M., Zhai, L., Lu, H., Wang, H., Zhang, X., Zhang, Y., Zhao, Xiaodong Ding, Y., 2019. Water quality in irrigated paddy systems. Irrigation in Agroecosystems. IntechOpen. https://doi.org/10.5772/intechopen.77339.

Liu, W.H., Zhao, J.Z., Ouyang, Z.Y., Söderlund, L., Liu, G.H., 2005. Impacts of sewage irrigation on heavy metal distribution and contamination in Beijing, China. Environ. Int. 31, 805–812. https://doi.org/10.1016/j.envint.2005.05.042.

Mantzorou, A., Ververidis, F., 2019. Microalgal biofilms: a further step over current microalgal cultivation techniques. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2018.09.355.

Marella, T.K., Datta, A., Patil, M.D., Dixit, S., Tiwari, A., 2019a. Biodiesel production through algal cultivation in urban wastewater using algal floway. Bioresour. Technol. 280, 222–228. https://doi.org/10.1016/j.biortech.2019.02.031.

Marella, T.K., Datta, A., Patil, M.D., Dixit, S., Tiwari, A., 2019b. Biodiesel production through algal cultivation in urban wastewater using algal floway. Bioresour. Technol. 222–228. https://doi.org/10.1016/j.biortech.2019.02.031.

Marella, T.K., López-Pacheco, I.Y., Parra-Saldívar, R., Dixit, S., Tiwari, A., 2020. Wealth from waste: diatoms as tools for phycoremediation of wastewater and for obtaining value from the biomass. Sci. Total Environ. 724, 137960. https://doi.org/10.1016/j.scitotenv.2020.137960.

Marella, T.K., Parine, N.R., Tiwari, A., 2018. Potential of diatom consortium developed by nutrient enrichment for biodiesel production and simultaneous nutrient removal from wastewater. Saudi J. Biol. Sci. 25 (4), 704–709.

Mishra, B., Saxena, A., Tiwari, A., 2020. Biosynthesis of silver nanoparticles from marine diatoms Chaetoceros sp., Skeletonema sp., Thalassiosira sp., and their antibacterial study. Biotechnol. Rep. 28, e00571 https://doi.org/10.1016/j.btre.2020.e00571.

Mohsenpour, S.F., Hennige, S., Willoughby, N., Adeloye, A., Gutierrez, T., 2021. Integrating micro-algae into wastewater treatment: a review. Sci. Total Environ. 752, 142168. https://doi.org/10.1016/J.SCITOTENV.2020.142168.

Moody, J.W., McGinty, C.M., Quinn, J.C., 2014. Global evaluation of biofuel potential from microalgae. Proc. Natl. Acad. Sci. U. S. A 111, 8691–8696. https://doi.org/10.1073/pnas.1321652111.

Mulbry, W., Kangas, P., Kondrad, S., 2010. Toward scrubbing the bay: nutrient removal using small algal turf scrubbers on Chesapeake Bay tributaries. Ecol. Eng. 36, 536–541. https://doi.org/10.1016/J.ECOLENG.2009.11.026.

Mulbry, W., Kondrad, S., Buyer, J., 2008. Treatment of dairy and swine manure effluents using freshwater algae: fatty acid content and composition of algal biomass at different manure loading rates. J. Appl. Phycol. 20, 1079–1085. https://doi.org/10.1007/s10811-008-9314-8.

Mulbry, W., Westhead, E.K., Pizarro, C., Sikora, L., 2005. Recycling of manure nutrients: use of algal biomass from dairy manure treatment as a slow release fertilizer. Bioresour. Technol. 96, 451–458. https://doi.org/10.1016/j.biortech.2004.05.026.
Newton, R.J., Jones, S.E., Eiler, A., McMahon, K.D., Bertilsson, S., 2011. A guide to the natural history of freshwater lake bacteria. Microbiol. Mol. Biol. Rev. 75, 14–49. https://doi.org/10.1128/mmbr.00028-10.
Pandey, L.K., Ojha, K.K., Singh, P.K., Singh, C.S., Dwivedi, S., Bergey, E.A., 2016. Diatoms image database of India (DIDI): a research tool. Environ. Technol. Innov. 5, 148–160. https://doi.org/10.1016/J.ETI.2016.02.001.
Prescott, G.W., 1982. Algae of the West Great Lakes Area. WMC Brown Company, Dubuque.
Qais, F.A., Shafiq, A., Khan, H.M., Husain, F.M., Khan, R.A., Alenazi, B., Alsalme, A., Ahmad, I., 2019. Antibacterial effect of silver nanoparticles synthesized using Murraya koenigii (L.) against multidrug-resistant pathogens. Bioinorgan. Chem. Appl. (2019) https://doi.org/10.1155/2019/4649506.
Qin, Y., Li, G., Gao, Y., Zhang, L., Ok, Y.S., An, T., 2018. Persistent free radicals in carbon-based materials on transformation of refractory organic contaminants (ROCs) in water: a critical review. Water Res. https://doi.org/10.1016/j.watres.2018.03.012.
Rajesh Banu, J., Merrylin, J., Kavitha, S., Yukesh Kannah, R., Selvakumar, P., Gopikumar, S., Sivashanmugam, P., Do, K.U., Kumar, G., 2020. Trends in biological nutrient removal for the treatment of low strength organic wastewaters. Curr. Pollut. Rep. https://doi.org/10.1007/s40726-020-00169-x.
Ramanan, R., Kim, B.-H., Cho, D.-H., Oh, H.-M., Kim, H.-S., 2016. Algae–bacteria interactions: evolution, ecology and emerging applications. Biotechnol. Adv. 34, 14–29. https://doi.org/10.1016/J.BIOTECHADV.2015.12.003.
Rosov, K.A., Mallin, M.A., Cahoon, L.B., 2020. Waste nutrients from U.S. animal feeding operations: regulations are inconsistent across states and inadequately assess nutrient export risk. J. Environ. Manag. 269, 110738.
Schenk, P.M., Thomas-Hall, S.R., Stephens, E., Marx, U.C., Mussgnug, J.H., Posten, C., Kruse, O., Hankamer, B., 2008. Second generation biofuels: high-efficiency microalgae for biodiesel production. Bioenergy Res. 1, 20–43.
Stensberg, M.C., Wei, Q., McLamore, E.S., Porterfield, D.M., Wei, A., Sepúlveda, M.S., 2011. Toxicological Studies on Silver Nanoparticles: Challenges and Opportunities in Assessment, Monitoring and Imaging, pp. 879–898. https://doi.org/10.2217/NNM.11.78 https://doi.org/10.2217/nnm.11.78 6.
Talebi, A.F., Mohtashami, S.K., Tabatabaei, M., Tohidfar, M., Bagheri, A., Zeinalabedini, M., Hadavand Mirzaei, H., Mirzajanzadeh, M., Malekzadeh Shafaroudi, S., Bakhtiari, S., 2013. Fatty acids profiling: a selective criterion for screening microalgae strains for biodiesel production. Algal Res 2, 258–267. https://doi.org/10.1016/J.ALGAL.2013.04.003.
Tanzil, A.H., Sultana, S.T., Saunders, S.R., Shi, L., Marsili, E., Beyenal, H., 2016. Biological synthesis of nanoparticles in biofilms. Enzym. Microb. Technol. 95, 4–12. https://doi.org/10.1016/J.ENZMICTEC.2016.07.015.
US EPA, O., n.d. Basic Information about Nonpoint Source (NPS) Pollution.
Walker, D.B., Baumgartner, D.J., Gerba, C.P., Fitzsimmons, K., 2019. Surface water pollution. In: Brusseau, M.L., Pepper, I.L., Gerba, C.P. (Eds.), Environ. And Pollut. Sci., third ed. Elsevier, pp. 261–292.
Wang, M., Zhang, D., Dong, J., Tan, S.K., 2018. Application of Constructed Wetlands for Treating Agricultural Runoff and Agro-Industrial Wastewater: a Review. Hydrobiologia. https://doi.org/10.1007/s10750-017-3315-z.
Williams, M., Kookana, R.S., Mehta, A., Yadav, S.K., Tailor, B.L., Maheshwari, B., 2019. Emerging contaminants in a river receiving untreated wastewater from an Indian urban centre. Sci. Total Environ. 647, 1256–1265. https://doi.org/10.1016/J.SCITOTENV.2018.08.084.
Wu, Y., Liu, J., Rene, E.R., 2018. Periphytic biofilms: a promising nutrient utilization regulator in wetlands. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2017.07.081.
Xia, Y., Zhang, M., Tsang, D.C.W., Geng, N., Lu, D., Zhu, L., Igalavithana, A.D., Dissanayake, P.D., Rinklebe, J., Yang, X., Ok, Y.S., 2020. Recent advances in control technologies for non-point source pollution with nitrogen and phosphorous from agricultural runoff: current practices and future prospects. Appl. Biol. Chem. https://doi.org/10.1186/s13765-020-0493-6.