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Designing efficient floating bed options for the treatment of eutrophic water

Sampurna Nandy[a,b], Divya Kalra[a] and Atya Kapley[a,b,*]
[a] Environmental Biotechnology and Genomics Division, CSIR-National Environmental Engineering Research Institute, Nagpur 440020, India
[b] Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201002, India
*Corresponding author. E-mail: a_kapley@neeri.res.in

ABSTRACT

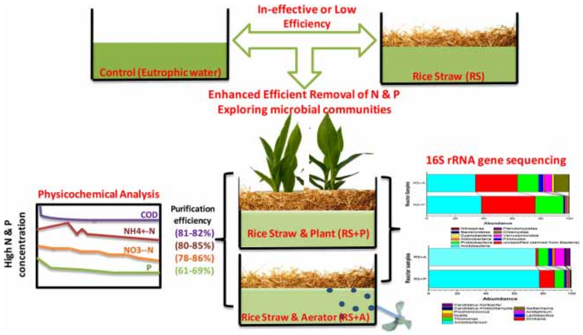
Developing solutions for lake eutrophication has emerged as a priority area to address the loss of ecosystem balance, reduction in aquatic biodiversity, and the potential production of toxins. Floating bed solutions offer an effective methodology to address this issue. This study uses rice straw as a base for floating bed treatment. Treatment of simulated eutrophic water was analyzed with and without plants in combination with rice straw beds (RS and RS + P). Treatment efficiency was also tested under increased aeration conditions (RS + A). Results demonstrated that average removal efficiencies of the ecological beds assembled with plant and aerator ranged from 81 to 82%, 80 to 85%, 78 to 86%, 61 to 69% for Chemical Oxygen Demand (COD), NH_4^+-N, NO_3^--N, and phosphates, respectively. The microbial community structure was also analyzed from the water samples taken from ecological beds assembled with plant and aerator by 16S rRNA gene sequencing. Based on the above results, systems assembled with plants and aerators proved to be efficient for the treatment of eutrophic water.

Key words: aeration, ecological floating beds, eutrophication, phytoremediation, rice straw

HIGHLIGHTS

- Combinations of ecological floating beds were tested for excess nutrient removal.
- Bio-carrier rice straw used facilitated anchorage to microbes and help their growth.
- Rice straw with plants (RS + P) as well as aeration (RS + A) showed enhanced N and P removal.
- Microbial communities were investigated using 16S rRNA gene sequencing.
- Study provides environmentally benign techniques eliminating toxic treatment protocols.

GRAPHICAL ABSTRACT



1. INTRODUCTION

One of the impacts of the growing human population on the environment is an increase in the rate at which nitrogen (N) and phosphorus (P) enter the biosphere, which is expected to increase far more in the next few decades (Yao *et al.* 2021). These large amounts of nutrients being discharged into water bodies via domestic, agricultural, and industrial effluents lead to environmental pollution, thereby causing eutrophication (Boeykens *et al.* 2017). Consequently, a growing number of researchers around the globe are focusing their efforts on eutrophication mitigation and minimizing the level of nitrogen and phosphorus in water for safeguarding aquatic environments as well as for human well-being (Chowdhury & Behera 2021).

Bioremediation of nutrients from eutrophic water bodies is one of the widely accepted techniques that has been extensively applied in the field of ecological engineering for the treatment of surface water as well as wastewater which generally includes the use of plants (phytoremediation), owing to their ability to absorb nutrients and create ideal environments for microbial decomposition of organic materials (Muisa *et al.* 2020). However, phytoremediation strategies such as constructed wetlands end up occupying a massive area of land despite their significant nutrient removal capabilities as well as low costs for the remediation of eutrophic water bodies. At the same time, ecological floating beds have been widely used for river and lake water quality restoration as an *in situ* water treatment solution. They have been shown to be more efficient and convenient for the treatment of eutrophic waters than traditional phyto-based constructed wetlands in terms of their low cost, ease of management, lower ecological footprint, and eliminating any requirement for an on-site operator (Lu *et al.* 2015). However, the treatment efficacy of floating beds is limited because it relies entirely on their plant roots, making them hard to use in the clean-up of hyper-eutrophic water bodies. Plant roots give surface area for microbial adhesion in traditional floating beds comprising plants and floating rafts (Yuan *et al.* 2021). One of the primary reasons for the low treatment efficiency of floating bed systems is the lack of substrate to retain substantial microbial biomass, which is critical in the remediation of hyper-eutrophic waters (Cao *et al.* 2016).

To circumvent these issues, several ecological floating beds are coupled with hydrophytic plants as well as biofilm carriers (Sun *et al.* 2009). These biofilm carriers prove to be an additional substrate for microbial anchorage. Biofilm carriers used in these systems can be of two types: inert or biodegradable. Inert biofilm carriers have been observed to be ineffective for nutrient removal for two reasons: lacking bio-available and biodegradable organic matter that can be easily utilized by denitrifying bacteria and relatively long biofilm-forming culture time, thus providing less biomass for bioremediation of eutrophic waters.

In this situation, organic bio-carriers can provide both support to microbial growth and a substrate for plant growth (Wang *et al.* 2019). As a result, there has been an upsurge in the use of bio-carriers to absorb pollutants (nitrogen and phosphorus) as well as to improve the efficacy of an ecological floating bed in eutrophic water purification (Guo *et al.* 2019). Additionally, biodegradable organic bio-carriers aid in the treatment of eutrophic water bodies and have a direct impact on treatment efficiency as well as energy usage (Wu *et al.* 2016). Rice straw is one such biodegradable organic substrate for a microbial community used for bioremediation of such waters, mainly due to the following advantages: it provides an anchorage to microbial systems, it provides an organic substrate for enhanced microbial metabolism, it reduces the cost for treatment by 70–90%; it is a waste reduction and water, air, as well as soil protection strategy that is environmentally sustainable; and nearly all of the rice straw consumed produces no secondary pollution. The major idea for its application is the well-known effectiveness of 'solid phase denitrification' in biological denitrification for the removal of nitrogen and accumulation of phosphate-accumulating organisms (PAOs) for the removal of phosphate from eutrophic water bodies (Cao *et al.* 2016).

In the present study, rice straw was used as a bio-carrier as well as a substrate in an ecological floating bed to enhance its performance for nutrient (N and P) removal. It has also been demonstrated to be an advantageous medium for the growth of biofilms as well as the growth of wetland plants. Furthermore, to enhance the efficiency of the floating bed, the rice straw system is used in various combinations; only rice straw; in combination with wetland plant (*Canna indica*); coupled with aeration, and tested for improved nutrient removal efficacy in batch experimentation. 16S rRNA gene sequencing was also used to investigate bacterial communities during the nitrogen and phosphorus (nutrients) removal by the best-performing ecological floating beds. We also aimed to determine the various microbial communities involved in the removal of nutrients which helped in improving the quality of eutrophic water.

2. MATERIALS AND METHODS

2.1. Bio-carrier, hydrophyte, and aeration

Bio-carrier: Rice straw used as a bio-carrier, which is made up of lignin and cellulose, was trimmed to pieces and autoclaved for sterilization and color removal. The physical characteristics were as follows: porosity 82%, bulk density 45 g/L, and surface area 145 m^2/m^3.

Hydrophyte: C. indica was chosen as an ecological floating bed plant because it grows well in eutrophic waters indicating their effectiveness and tolerance against highly concentrated waters, ease of availability, and cost-effectiveness. Two sets of plants were used in a plant-based system.

Aeration: Enhancing aeration increases aerobic biodegradation capacity. Herein, a single-way air pump with a 1-m air tube spherical fine bubbles aerator equipment was used (SB998, SEBO Equipment Co. Ltd). For a water depth of 10 cm, an airflow rate for a single aerator was kept at 1–3 m^3/h.

2.2. Batch experimental study

Four laboratory-scale biological grid reactors were set up for the bioremediation experiment. The framework of the ecological floating bed was made up of a polyacrylamide sheet. Four different tanks with inner dimensions of 25 cm × 25 cm × 15 cm (length × width × depth) were used. The reactor system consisted of (1) control (containing only synthetic eutrophic water; SEW), (2) RS (SEW + rice straw), (3) RS + P (SEW + rice straw + *C. indica*), and (4) RS + A (SEW + rice straw + aerator). The schematics of the experimental reactor setup are given in Figure 1.

Mesh nets attached to the reactor's frameworks supplied buoyancy to ecological floating beds. The aerator was fitted at the side of one of the biological grid systems (Figure 1, reactor IV). Each tank was filled with 9 L of eutrophic water (SEW prepared in the laboratory) containing the average concentrations of the following parameters: chemical oxygen demand (COD) = 189 mg/L, ammonical nitrogen (NH_4^+-N) = 29.38 mg/L; nitrate nitrogen (NO_3^--N) 12.37 mg/L, and phosphates (P) = 7.01 mg/L. The SEW was maintained at a given concentration of various parameters using laboratory chemicals/reagents. The ecological floating beds were maintained for 15–20 days until steady-state biomass loading on the rice straw was established. The residual concentrations of COD, NH_4^+-N, NO_3^--N, and P levels were determined in water samples collected every alternate day for a period of 60 days.

2.3. Analytical methods

The experiment was carried out at ambient environmental temperature conditions. A multi-parameter water quality probe (YSI 6600V2, USA) was used to measure water temperature (T) and pH at regular intervals. Every alternate day water samples were collected and then filtered through a 0.45-m cellulose acetate membrane filter before proceeding for analysis; all samples were evaluated within 2 h of collection. NH_4^+-N, NO_3^--N, P, and COD were analyzed chemically using a UV-visible spectrophotometer (UV-3802, USA) in accordance with standard APHA guidelines (Nineteenth & Editions 2000). The data were estimated in triplicates of the samples.

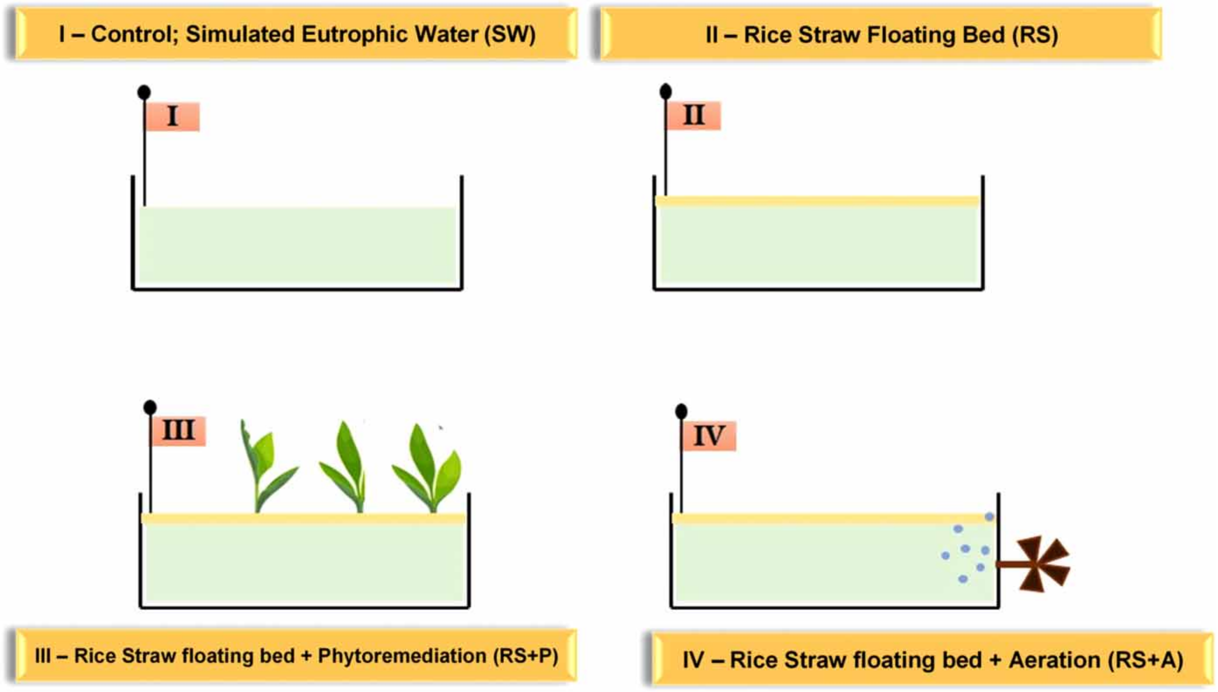


Figure 1 | Schematic of different ecological floating bed reactors: (I) Control (containing SEW only, (II) SEW + Rice straw (RS), (III) SEW + Rice straw + *Canna indica* (RS + P), and (IV) SEW + Rice straw + Aerator (RS + A).

2.4. DNA extraction and polymerase chain reaction (PCR) amplification

The best-performing reactor water samples were subsequently subjected to high-throughput 16S rRNA sequencing. Total DNA was isolated from water samples using a DNA extraction kit (MP Biomedicals' Fast DNA Spin Kit) and analyzed via gel electrophoresis. The V3–V4 regions of the bacterial 16S rRNA gene were amplified using universal primers 16S rRNA F (5′-GCCTACGGGNGGCWGCAG-3′) and 16S rRNA R (5′-ACTACHVGGGTATCTAATCC-3′). These products were stored at −20 °C before 16S rRNA gene sequencing.

2.5. Sequencing and statistical analysis

The sequencing data were analyzed using the MG-RAST database with the default parameters. QIIME was used for the filtration of low-quality sequences and to optimize the connection of paired-end sequences to ensure the accuracy and reliability of the analysis results (Edgar 2010). The Classifier in MG-RAST was used to collect the taxonomy categorization information relating to each operational taxonomic unit (OTU) (confidence threshold was set to default), and at 97%, OTU representative sequences were classified at the similarity level (Chu *et al.* 2015).

The species richness and evenness of the samples were calculated using Alpha diversity indices. PAST software was used to construct Shannon–Weiner, Simpson inverse Simpson, and Fisher alpha to show and compare the species richness and evenness of both water samples. The alpha diversity index is generally calculated using the Shannon index, but the Simpson index provides a sufficient number of genera to reflect environmental heterogeneity (Chen *et al.* 2021). The PCA plot was developed to analyze the taxonomic diversity as well as the difference in abundance associated with both samples for a better understanding of the comparison between the best-performing reactor studies (Breda *et al.* 2017).

2.6. Data availability

Nucleotide sequences of the sequenced samples were submitted to NCBI Sequence Read Archive (SRA) having the following biosample accession numbers: SAMN28649270 (RS + P) and SAMN28649181 (RS + A).

3. RESULTS AND DISCUSSION

3.1. Physical parameters of ecological floating beds

Temperature and pH were regularly monitored in all four reactors. The temperature of water in the floating bed system differs significantly between working stages, with a maximum temperature variation of ∼8 °C. However, the variation in the temperature can be attributed to the ambient environmental condition as the floating bed systems were placed in an open environment. Microbial metabolism reactions can also play a significant role in varying the temperature of systems (Scofield *et al.* 2015). The pH of floating bed reactors did not vary much during the operation and remained around near neutral (6–8.5) as shown in Figure 2.

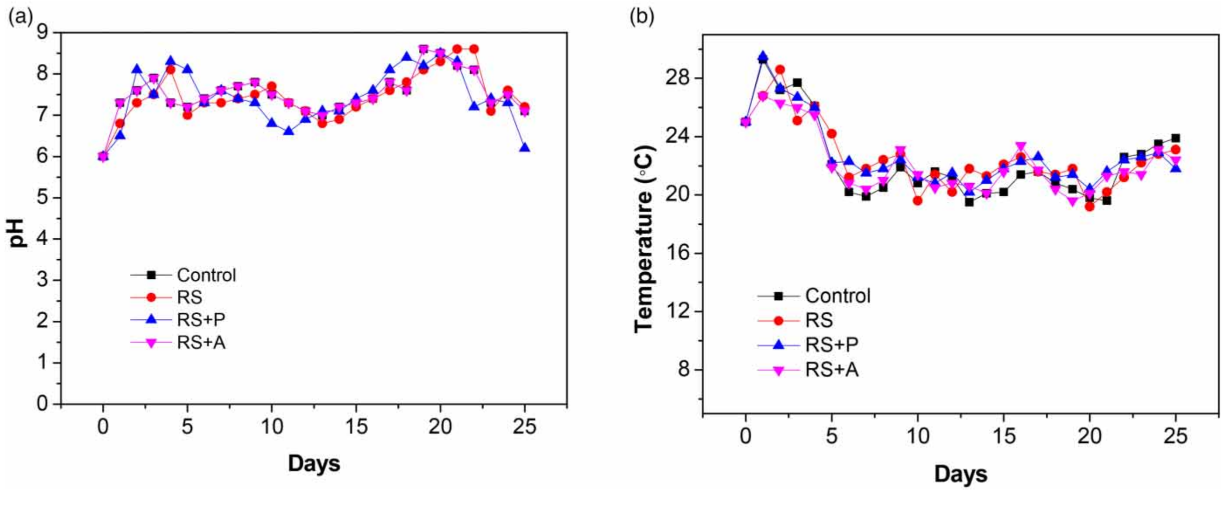


Figure 2 | Variation in the (a) pH and (b) temperature during the treatment process in different systems such as control, SEW + Rice straw (RS), SEW + Rice straw + *Canna indica* (RS + P), and SEW + Rice straw + Aerator (RS + A).

3.2. COD removal in reactor systems

Figure 3 demonstrated the COD removal efficiency in different reactor systems. It can be observed that both RS + P and RS + A reactors were the most efficient, wherein 66.1 and 67.8% of COD removal was obtained in 3 days. Afterward, both reactor systems maintained their efficacy for COD removal. However, a slight reduction of 12.7% in COD removal was also observed in the control reactor. The reactor RS showed its maximum removal efficiency of 44.5% by the 10th day which became consistent afterward indicating its significant role in COD reduction.

Notably, RS + P and RS + A showed significantly higher COD removal efficiency than that of the control, demonstrating that the combination of approaches in a floating bed system had a synergistic effect on COD removal. Rice straw in the floating bed reactors played a significant role by facilitating growth medium for both, wetland plants and bacterial adhesion for biofilm formation (Ahmad *et al.* 2016; Yuan *et al.* 2016). Therefore, additional growth of bacteria leading to the formation of biofilm along with phytoremediation and aeration were the major reasons for the enhanced COD removal. Aeration raised the water's dissolved oxygen content, which resulted in an increase in COD removal in the RS + A system, which was consistent with previous findings (Shi *et al.* 2018). The efficiency of all the reactors in terms of COD removal was obtained in the following order: control > RS > RS + P > RS + A. The COD removal rate for the two ecological floating beds (RS + P and RS + A) remained high and the harvesting operation had no effect on the floating bed system. The addition of a framework of rice straw acted as a plant growth substrate that helped in increasing biomass activity.

3.3. Nitrogen removal in reactor systems

Nitrogen in wastewater is mostly composed of ammonical nitrogen (NH_4^+-N) and nitrate (NO_3^--N) (Wang *et al.* 2014). As reported, denitrification, bio-accumulation, and sedimentation are responsible for the removal of 80 and 20% of the nitrogen (Samal *et al.* 2019). Microbial nitrification, plant absorption and assimilation, and the escape of free ammonia are the primary processes for removing NH_4^+-N from an aqueous medium (Gao *et al.* 2017).

The control reactor system showed ~20% of NH_4^+-N removal over the treatment period possibly due to auto-reduction (Zhuang *et al.* 2019), whereas the RS reactor showed a removal efficiency of 66% (Figure 4(a)). As already discussed, rice straw increased the biomass while inducing low biotoxicity which enhanced the growth and activity of nitrifying bacteria leading to higher removal in the RS system. On the other hand, the removal efficiency of NH_4^+-N was found to be 82 and 73% in RS + P and RS + A systems, respectively, which is much higher than the control and is consistent with the findings of a previous study on sewage treatment using various types of ecological floating beds (Lv *et al.* 2019). The increment in the RS + P system was due to synergy among plant root system, microbial membrane, and rice straw as a substrate which provides a favorable environment for nitrifying bacteria to thrive (Chen *et al.* 2016).

The RS, RS + P, and RS + A had much lower residual NO_3^--N levels than the control system. The concentration increased slightly when a considerable proportion of NH_4^+-N was converted to NO_3^--N. All three reactors, RS, RS + P, and RS + A, had

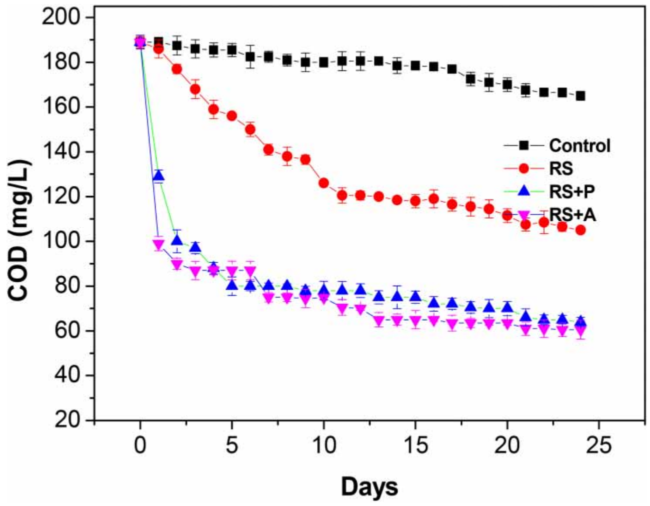


Figure 3 | COD removal performance of all four reactors (control, RS, RS + P, and RS + A).

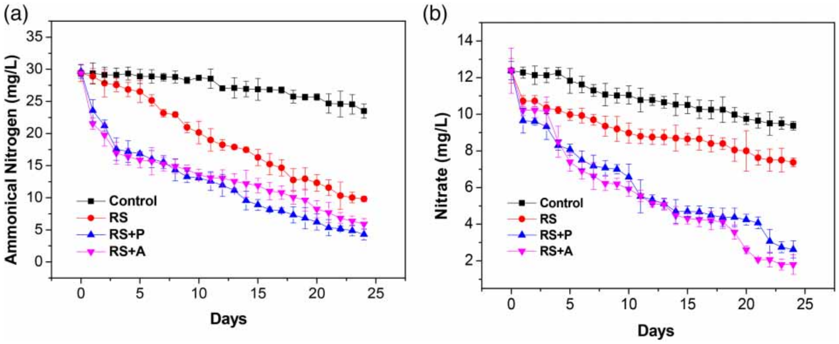


Figure 4 | (a) NH_4^+-N and (b) NO_3^--N removal efficiency in all four reactors: control, RS, RS + P, and RS + A.

removal efficiencies of 40, 78, and 85%, respectively (Figure 4(b)). The occurrence of bacteria and subsequent biofilm formation on the rice straw may have triggered the NO_3^--N removal phenomenon. Microbe enrichment in media has been shown to play a major influence in nitrogen reduction. This investigation demonstrated that RS + A was the most effective for removing NO_3^--N because rice straw served as a source of carbon for denitrification and the presence of dissolved oxygen provided ideal conditions for the process (Bartucca *et al.* 2016). The denitrification process utilized the rice straw, which was presumably broken down into soluble components by bacteria on its surface. This resulted in a significant decrease in nitrogen in the system. The deterioration of rice straw causes a denser biofilm to grow on its surface, in contrast to other inert bio-carriers. As a result, the thicker biofilm that was created made it easier to create the anaerobic conditions necessary for denitrification (Cao *et al.* 2016), whereas the control showed reduced nitrate removal performance with limited efficiency of 24% in the absence of anaerobic conditions as well as a denitrification carbon source.

3.4. Phosphate removal in reactor systems

Organic phosphorus (Org-P) and inorganic orthophosphate (PO_4^{3-}-P) are the two types of phosphorus found in eutrophic water. Microbial action converts organic phosphorous to dissolved or inorganic form, which is then assimilated by microbes (Sheng *et al.* 2013). The rate of biomass development, nutrient storage capacity, root structure, and the types of tissues present in the vegetation all influence phosphorus uptake (Sun *et al.* 2009). Plant absorption, substrate adsorption, and microbial fixation are the keys to the removal of phosphate from water using ecological floating beds (Samal *et al.* 2018). In comparison to nitrogen and COD reduction, phosphate removal was rather slower in the reactor systems. The RS + A system was observed to be the best-performing reactor with the highest removal efficiency of 68.7%. This effective performance of RS + A can be attributed to the additional aeration leading to the gas disturbance causing the water to come into full contact with the rice straw. Such changes could possibly promote the phosphate adsorption by rice straw leading to enhanced reduction of phosphate concentration (Figure 5). Shen and co-workers found that employing biosorbent as substrate in an ecological floating bed resulted in a higher P removal rate (Shen *et al.* 2019). The removal efficiency in the plant system (RS + P) was estimated to be around 61%. The possible reasons for reduced phosphate removal in such systems can be due to various reasons such as (1) the time required for physical adsorption and biological absorption of phosphate by plant roots is comparatively long; (2) a large number of experiments indicated that plants have a significant effect on the removal of P in the traditional floating bed, but is insignificant as wetland substrate (Muisa *et al.* 2020). Previous reports have also indicated reduced P removal efficacy in plant systems. For example, Yao *et al.* used *C. indica* that removed 50.9–74.0% of phosphates from SEW (Yao *et al.* 2021). In a similar study, an ecological floating bed composed of *Typha domingensis* could only remove 37% phosphate from household wastewater (Benvenuti *et al.* 2018). Saeed *et al.* found that an ecological floating bed of *C. indica* and *Phragmites australis* removed 23.4% phosphate from polluted surface water bodies (Saeed *et al.* 2014). The phosphate removal efficiency was found to be a little higher (87.5%) when the river water samples were treated with *Equisetum* and *Ipomoea* sp. (Sheng *et al.* 2013).

The obtained results indicated that RS + P and RS + A reactor systems performed the best for the removal of nutrients in the SEW.

It is reported in the existing literature that there can be various methods for remediation of polluted water bodies which can be categorized into chemical, physical, and biological with engineering modifications (Gao *et al.* 2018; Bai *et al.* 2020). It has

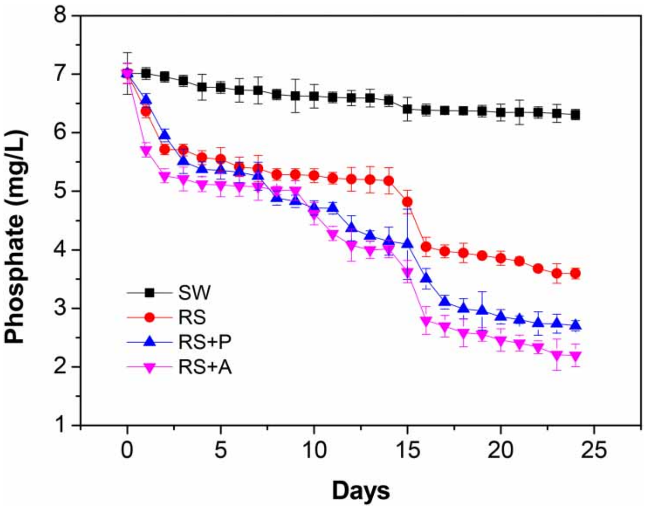


Figure 5 | Phosphate removal efficiency in all four reactors: control, RS, RS + P, and RS + A.

been seen that engineering and physical methods that include mechanical aeration and dilution can effectively improve the quality of water but impose a huge economic burden (Peilin *et al.* 2019). Similarly, chemical treatment methods facilitate rapid remediation through the dosing of chemical reagents such as coagulants (Fe/Al-based), oxidizing agents (chloramines, peroxides), and disinfectants which can reduce the suspended solids (SS) (Anawar & Chowdhury 2020). However, the production of secondary pollutants such as high chroma index, residual metal ion concentration (Al^{3+}), and disinfection byproducts (DBPs) could severely impact the ecological environment of water bodies. In comparison to these methods, the biological methods involving microorganisms, biofilm, and membrane bioreactor are economical and ecologically feasible processes (Wang *et al.* 2012; Peilin *et al.* 2019). However, their actions require an extended time framework (several months). It is also seen that no single method is adequate for the complete purification of heavily contaminated water bodies. Therefore, a hybrid method combining different strategies can be beneficial for enhanced remediation. In such cases, the developed treatment process of enhanced ecological floating beds combining biofilm formation, phytoremediation, and aeration requiring minimum natural precursors (straw) with reduced footprint is an economically viable and environmental friendly option for remediation of eutrophic water bodies.

3.4.1. Taxonomic profiling at the phylum level

Due to the improved efficacy of RS + P and RS + A for nutrient removal, water samples from these two reactors were collected and further subjected to 16S rRNA gene sequencing to know their bacterial diversity. This was done by estimating bacterial diversity at two levels, namely phylum level and genus level.

The phyla levels were compared with an average abundance of >1% after dividing the sequences in each sample at different classification levels. The dominance of bacterial phyla is observable in both the samples while their abundance was varying as shown in Figure 6. For the RS + P system, *Acidobacteria* (38.3%) was dominating followed by *Proteobacteria* (19.7%) and *Firmicutes* (1.09%). The order of dominance in RS + A was similar to the RS + P system wherein the abundances vary as *Acidobacteria* (34%), *Proteobacteria* (14.96%), and *Firmicutes* (3.16%). Similar results of dominance have also been reported previously in the rivers samples contaminated with domestic and agricultural wastewater (Zhang *et al.* 2019). Occurrences of *Acidobacteria* and *Proteobacteria* were frequently found in wastewater treatment plants indicating that both phyla played a role in pollutant removal (Cheng *et al.* 2020; Di Cesare *et al.* 2020; Zhou *et al.* 2021).

The presence of *Nitrospira* phyla was also observed in both samples which strongly validated the possibility of the removal of nitrogen from the simulated eutrophic water.

3.4.2. Taxonomic profiling at the genus level

Results demonstrated in Figure 7 showed that most genera exhibited substantial variations in abundance between the two reactor samples at the genus level. The *Acidobacteria* and *Acidiphillum* were the most dominating genera in RS + P,

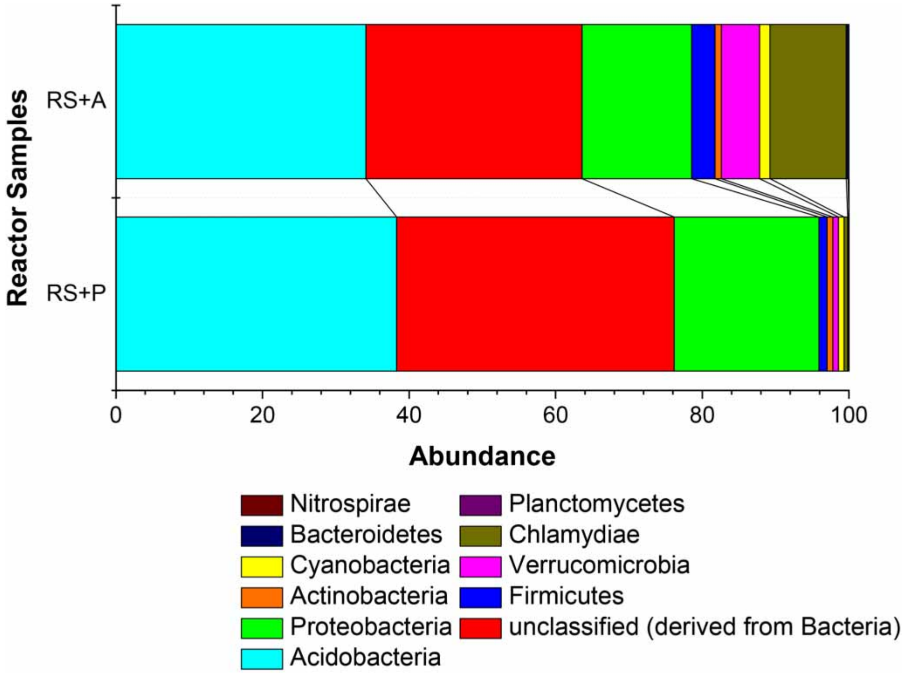


Figure 6 | Relative abundance of microbial community for RS + P and RS + A at the phylum level (top 10).

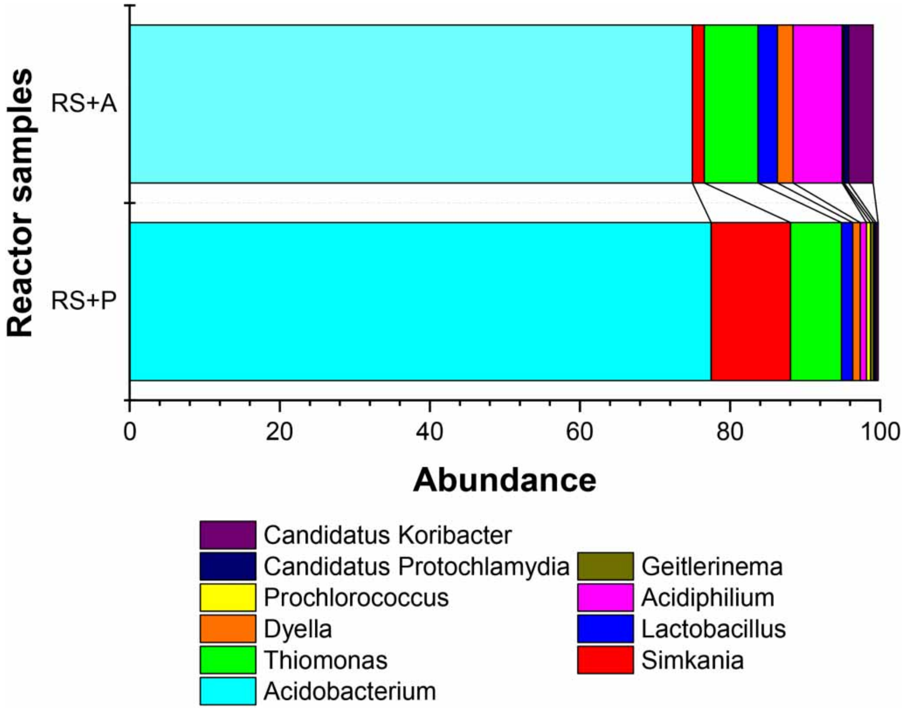


Figure 7 | Relative abundance of microbial community for RS + P and RS + A at the genus level (top 10).

which are vital for metabolizing the range of carbohydrates and play a key role in denitrification (Cao *et al.* 2017). The occurrence of *Simkania* demonstrates the nitrogen-fixing proteins' inherent property leading to nitrogen removal that is found in both reactors (Malit *et al.* 2021). The abundance of *Simkania* is a little higher in RS + P as compared to RS + A, indicating the higher efficacy of RS + P in terms of nitrogen removal (NH_4^+-N) which is consistent with obtained results (Figure 4(a)). However, the nitrate removal only varied between 5 and 7% in both reactors.

Thiomonas, being an autotrophic biotope, contributes significantly to nitrate removal. Its occurrence can be seen in both the systems, but was more dominant in the RS + A system, indicating higher nitrate removal which was consistent with the obtained results (Figure 4(b)). *Candidatus*, a prominent PAO genus that is predominantly active for excess phosphate breakdown, was also discovered in both samples. Comparatively, its abundance in the RS + A system was higher than RS + P which indicated a higher capacity of RS + A for P removal which also aligned with obtained results in Figure 5. Though this genus was not abundant, its presence indicates that it has a strong capacity for phosphate degradation in eutrophic water (Han *et al.* 2020).

High-throughput sequencing revealed the presence of microbial flora in the samples, as well as the diversity of microorganisms and emergence of microbes involved in the degradation of pollutants like nitrogen and phosphorus, indicating that rice straw provided a living environment for all different types of microbes and served as a good carrier for microbial growth.

3.5. Sequencing statistics and diversity indices assessment

3.5.1. Sequencing statistics

The quality filtration approach was used to process sequencing reads, yielding a total of 67,06,55,627 quality filtered paired-end reads with a read length of 100 bases. Megahit assembler was used to create contigs from filtered reads (Li *et al.* 2015). The average GC content of contigs was determined to be 54 ± 4% (RS + P) and 51 ± 4% (RS + A) with contigs N50 values ranging from 688 to 4,422. The RS + P system consisted of 50,929 sequences, whereas RS + A consisted of 90,051 sequences with dereplication sequences 84,622 and 47,985 respectively. Table 1 shows the sequencing data statistics and sequence assembly parameters.

3.5.2. Diversity and richness of microbial communities

Table 2 shows alpha indices of two samples (RS + P and RS + A), including richness estimator (Chao1 and Fisher alpha) as well as the diversity index (Shannon and Simpson). Shannon and Simpson's indices proved that microbial diversity increased at a starting phase and stabilized toward the end. The number of bacterial genera detected in water samples from RS + P and

Table 1 | Annotation of datasets obtained from sequencing statistics using MG-RAST

Feature	RS + P	RS + A
Total sequences average read length (bases)	22,237,391 bp 437 ± 55 bp	38,418,236 bp 427 ± 68 bp
Post QC sequence	2,944	5,429
Average GC content	53 ± 5%	51 ± 5%
Predicted protein feature	9	21
Predicted rRNA features	3,341	7,431
Identified protein features	0	6
Identified rRNA features	3,336	7,416

Table 2 | Bacterial abundance and alpha diversity indices

Alpha diversity indices	RS + P	RS + A
Taxonomy	207	295
Shannon_H	1.778	2.313
Chao-1	313.2	401
Fisher_alpha	27.77	38.97
Simpson_1-D	0.7118	0.7946

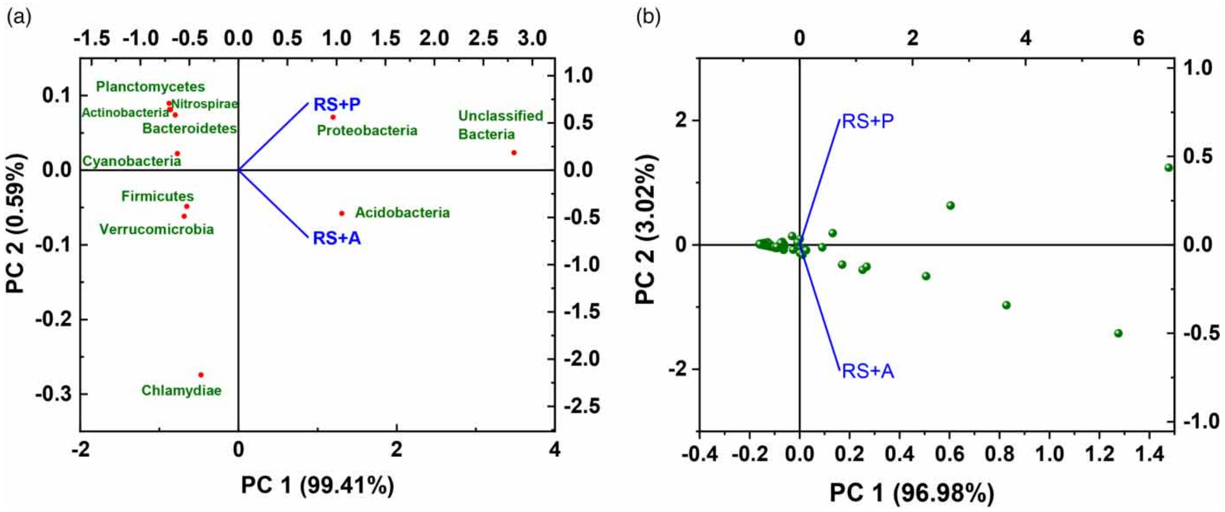


Figure 8 | A PCA plot for comparative reactor samples: (a) phylum level and (b) genus level.

RS + A samples were 213 and 317, respectively. RS + A had a greater Shannon–Wiener diversity index as well as Fisher alpha diversity index in comparison to the RS + P system. This result was mainly attributed to the coexistence of heterotrophic and autotrophic denitrifiers as well as PAOs in the reactor when nitrate and phosphate concentration was high.

3.5.3. Comparative principal component analysis plot

The results of microbial diversity at the phylum and genus level were shown using Bray–Curtis dissimilarity as principle component analysis (PCA) plots. Both the RS + P and RS + A did not show much overlap, but both were positively correlated in terms of microbial diversity at both phylum and genus levels and showed difference in abundance. At the phylum level, the first and second principal components (PC1 and PC2) explained 99.41 and 0.59% of total variation, whereas at the genus level it was seen to be 96.98 and 3.02%, respectively, as shown in Figure 8(a) and 8(b). A variety of factors including climatic shifts, changes in pH, and temperature as well as differences in nitrogen and phosphate levels could be contributing elements to these differences in abundance levels.

4. CONCLUSION

Eutrophic water bodies are usually characterized by excess nutrients especially N and P, organics, and suspended solid from sewage. Hence, rejuvenation should first address the removal of these nutrients. This study has explored different methodologies to address the issue and obtained results that demonstrate the use of a bio-carrier in addition to phytoremediation and aeration enhances the efficiency of biodegradation and nutrient removal while also eliminating the existing toxic and rigorous treatment protocols. The rice straw (bio-carrier) used as an excellent ecological floating bed substrate has various advantages such as greater natural microbial adhesion anchoring, which is made possible by its increased specific surface area. Furthermore, rice straw can support a range of microorganisms that help plants develop and remove nutrients, according to high-throughput gene sequencing. The coupling of rice straw and *C. indica* (RS + P) and rice straw and aeration (RS + A) showed improved performance by removing COD, NH_4^+-N,NO_3^-N, and P by 81–82%, 80–85%, 78–86%, and 61–69%, respectively. Therefore, the current study provides a low-cost clean-up option for eutrophic water bodies, designed to treat and increase social as well as cultural aspects of any aquatic ecosystem.

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AUTHOR CONTRIBUTIONS

S.N. contributed to investigation, data curation, analysis, methodology, software, writing original draft. D.K. contributed to data curation and analysis. A.K. contributed to conceptualization, data curation, methodology, resources, writing, review, editing, and supervision.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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