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Assessment of Aquatic Environmental Parameters of In-Pond Aquaculture System

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Abstract

The In-pond raceway aquaculture (IPRA) was developed to increase production that is more controllable and efficient than pond culture system. This study was conducted on commercial scale in IPRA to characterize the water quality and microbial community study within IPRA farms. The IPRA covers an area of 2h of traditional pond with 10 raceways, each of 25m× 5m×2.5m of fish culture units. Where monitoring of water quality parameters and microbial abundancy had been studied between inlet and outlet of the raceways during 108 days of culture period. Average water quality parameters and total soluble solid (TSS) over the course of the study was not found to be significantly difference. Nitrite nitrogen, nitrate nitrogen, total ammonia nitrogen, total phosphorus, chlorophyll-a and chemical oxygen demand were higher in outlet than inlets of IPRA. However, average total nitrogen, soluble reactive phosphorus and total soluble solids concentrations were slightly higher in inlets of IPRA there was also insignificance difference of microbial abundance. Therefore, it can be concluded from this study that fishes in raceways consumed nearly all the feed offered and a negligible amount of feed was wasted. So, leftover feed settlement to the bottom of the pond became a concern of no issue, despite the raceway having intensive fish culture thus bringing non-significant differences in water quality parameters between the inlet and outlet of the IPRA system.

Keywords: Chemical oxygen demand, In- pond raceway system, Microbial abundancy, Total soluble solid

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Introduction

More than 30% of the total fish consumed worldwide is produced through aquaculture (FAO, 2018) and aquaculture is the primary means of boosting production to fulfill the growing need for high-quality protein for human consumption (Cheng et al., 2014; Boyd, 2003). Traditional and advanced agricultural practices run side by side, and productivity appears to increase at a slower pace. To meet the demands of the present food processing industry, intensive or high-density culture systems are the prominent option and it calls for a high level of technical and management expertise in order to produce fish in predictable volumes.

Water quality is one of the most neglected parts of pond management; at least until it has an impact on fish output but is evident to have a major effect on production, productivity, and quality. The aquaculture industry is continuing to expand in terms of diversity and intensity (Ahmed & Thompson, 2019), however, many problems such as polluted wastewater, poor product quality, and vulnerability to disease are associated with it. These problems not only translate to economic losses for farmers but also degrade the environment (Wu et al., 2018). Land and labor-intensive pond production can be efficient and profitable if production levels are increased through techniques that maintain adequate water quality (Boyd & Tucker, 1998; Brown et al., 2014).

The rapid expansion and intensification of aquaculture have led to major concerns over the last two decades (Wang et al., 2019), including the limitations of land and water resources, the disruption of aquatic ecosystems, frequent disease outbreaks, the unregulated discharges of wastewater, and the quality and safety of final products (Cao et al., 2007). Aquaculture effluent is the prime issue of water quality deterioration but an increase in yields with a low level of effluent can be achieved by the use of high-quality feeds (better utilized, resulting in less waste) in aquaculture per unit yield (Cho et al., 1994).

The In-pond raceway system (IPRS) was prescribed and developed at Auburn University to increase production by separating the culture system into functional units and by managing phytoplankton standing crop productivity at desired levels in the late 1990s (Masser, 2004; Brown et al., 2011, 2014). This system combines a number of intensification elements (physical, chemical, and microbial) into a single integrated unit that is more controllable and efficient than traditional pond culture (Brune et al., 2003; Brown et al., 2011). Despite the fact that pond production is generally land and labor-expensive and aquatic product quality is easily influenced by the condition of the water environment (Falconer et al., 2018; He et al., 2020), it may be economical and lucrative if output levels are improved using procedures that preserve acceptable water quality (Boyd & Tucker, 1998).

Materials and methods

Experimental Set-up

The experiment was conducted for 108 days (July 31th to 18th November, 2018) in Shanghai, China in the IPRA (in-pond raceway aquaculture) and the traditional pond culture system. The IPRA was installed in a 20000 m^2 traditional pond consisting of 10 (25m×5m×2.5m) raceways units as shown in Figure 1 (with fish stocking density was 32tail/m^3), which are interconnected to each other sharing a common walking way. The facility was rectangular and had three separate components: the airlift area, fish culturing area, and waste transferring area. The fish were confined in the culture area by two stainless-steel barriers (1.0× 1.0cm mesh) that were installed at the end of the water inflow and water outflow, separated by the stainless steel frame. Aeration was provided by the surface and bottom aeration system. Water flows from the east to the west of the raceway, passes through the purification area, and returns to the east of the raceway. Water depth was maintained.

Water quality measurement

Water samples for physicochemical analysis were collected fortnightly before 8 am throughout the duration of the experiment in an integrated manner with the help of water Plexiglass water collector from inflow and outflow of raceway and subsequently transferred to polyethylene sampling bottle then immediately placed in sample cooler box and transported to the laboratory for further processing. Water temperature, pH, and dissolved oxygen were measured in situ using YSI556 meter (YSI Company, 1725 Branner Lane, Huangquan, Ohio, USA). The water sample was filtered (Whatman GF/C glass fiber0.45µm pore size) and analyzed by NO_2-N spectrophotometry; NO_3-N by Sulfamic acid ultraviolet spectrophotometry method; NH_3-N by Nesslerisation calorimetry method; total nitrogen (TN) by Alkaline potassium persulfate digestion UV spectrophotometry method; total phosphate by Ammonium molybdate spectrophotometry method; and orthophosphate phosphorus (PO_4^{3}-P) by Phosphomolybdenum Blue Spectrophotometry method; Total Suspended Solids (TSS) mg/L determined according to APHA (2005).

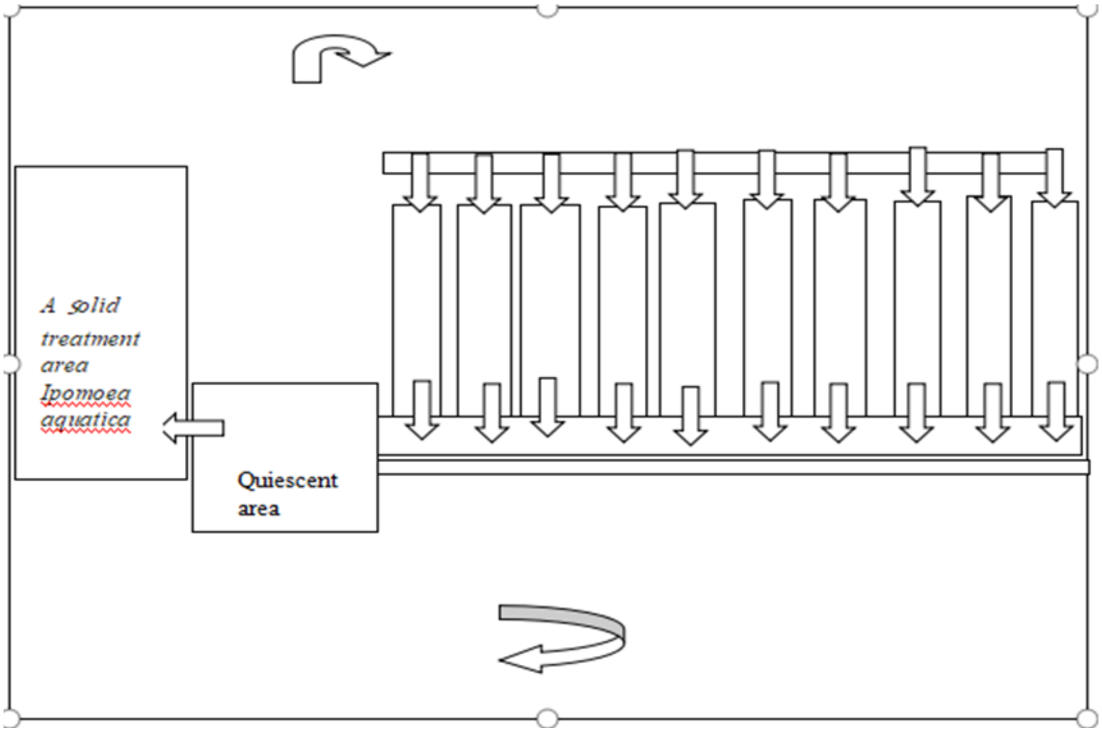

Figure 1: A schematic layout of the IPRA showing the direction of water flow throughout the pond and the raceways including water inflow (I), and water outflow (O)

Microbial community composition

Water samples were collected from cells of IPRA, filtered through a 0.2 µm polycarbonate filter with a diameter of 47 mm (Millipore, Billerica, MA, USA), and then stored in liquid nitrogen for subsequent DNA extraction while transporting and processing.

Microbial communities were analyzed by high-throughput pyrosequencing using MiSeq technology (Illumina, USA). Genomic DNA was extracted and purified from filtrate, using the DNA Kit (Omega Biotechnology, Norcross, GA, USA). Nucleotide concentration was checked using a NanoDrop 2000 spectrophotometer (ThermoFisher Scientific, Wilmington, USA) and DNA quality was checked by 1% agarose gel electrophoresis. The purified genomic DNA (20 ng/ml) was sent to Shanghai Major Biopharmaceutical Technology Co., Ltd. (China), and the MiSeq benchtop sequencer (Illumina, USA) was used for 16 S rRNA gene-based amplicon sequencing. The bacterial 16 S rRNA gene was analyzed by polymerase chain reaction (PCR), and 338 f (5'-actcctacgggagcagcag-3') and 806 r (5'-GGACTACHVGGGTWTCTAAT-3') were used as bacterial primers (Qian et al., 2017).

Finally, a final extension of 10.0 minutes was performed at 72°C. The PCR product was extracted from a 2% agarose gel and further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union, CA, USA). 16S rDNA gene cloning and sequencing were performed by MajorBio-pharm Biotechnology Co. Ltd. (Shanghai, China). PCR reaction products of the correct size were randomly selected and the sequenced 16 S rDNA genes were analyzed using the search tool of the National Center for

Biotechnology Information (NCBI) to identify the closest match.

Statistical analysis

Results were shown as mean with standard deviation. Statistical analyses were performed using SPSS Base 16.0 statistical software (IBM, Armonk, NY, United States). The differences between the inlets and outlets of IPRA were evaluated by one-way analysis of variance (ANOVA) followed by Duncan's multiple comparison tests when differences were found using the ANOVA. Statistically significant differences were determined at $P < 0.05$ for all analyses.

Results and Discussion

Water quality of inlet and outlets of In-pond raceway aquaculture system

Nitrite Nitrogen, Nitrate-Nitrogen, Total Ammonia Nitrogen, Total Nitrogen, Soluble Reactive Phosphorus, Total Phosphorus, Chlorophyll-a, Chemical Oxygen Demand, and Total Soluble Solid mean value obtained during the experimental period were presented in Table 1. The results of this study showed that there were no significant differences in all water quality parameters between inlets and outlets in the IPRA system.

Table 1. Water quality variables (mean±SD) of water samples collected from the Inlets and Outlets of an in-pond raceway aquaculture (IPRA)

Variable	Inlet	Outlet
Nitrite Nitrogen (mg/L)	1.4±0.71	1.43±0.68
Nitrate nitrogen (mg/L)	1.72±0.68	1.76±0.75
Total ammonia nitrogen (mg/L)	0.81±0.35	0.84±0.29
Total Nitrogen (mg/L)	5.00±2.79	4.99±2.79
Soluble reactive phosphorus (mg/L)	3.50±1.60	3.48±1.58
Total phosphorus (mg/L)	4.41±2.23	4.48±2.09
Chlorophyll-a (mg/L)	272.70±138.63	286.89±157.32
Chemical oxygen demand (mg/L)	8.90±4.10	9.67±4.98
Total soluble solid (mg/L)	75.40±53.90	66.42±39

The water quality measure produces the baseline knowledge for the growth of the culture specimen. The quality of water gets deteriorated when it passes through the fish culture area (Sidoruk & Cymes, 2018). All the water quality parameters measured and documented in this study were found to have non-significant differences between inlets and outlets water samples in the IPRA system. Total Nitrogen and Soluble Reactive Phosphorus showed slightly lower concentrations, while NO_2^--N, NO_3^-N, TAN, TP, Chlorophyll-a, and TSS were observed to have the higher value. In this study, a higher concentration of Chlorophyll-a was observed in the outlets of IPRA which might be due to the fish stocking inside the cell, used in the experiment that produced a higher amount of excreta and leftover feeds calling for increased levels of nitrogenous content and then the Chlorophyll-a. In line with these findings, Brown et al. (2012) reported a peak concentration of chlorophyll-a as 949 µg/L in IPRS with stocking densities of 12,000–30,000 fish/raceway and stated that this value may increase with increasing stocking density of fishes. Increases in the nitrogen contents in the experimental ponds were probably caused by the accumulation of residual feeds and feces in the ponds (Zhang et al., 2019). The higher SRP concentration was documented in an outlet in this study which is in agreement with Li et al. (2019) and this difference between the inlet and outlet in IPRA might be due to the reason that the flowing water increases the release of SRP from the particulate phosphorus in the sediment.

Nitrite-N and TAN were lower concentration in inlet while they were higher in outlets, mainly because of fish respiratory and metabolic wastes. Concentration of TAN and Nitrite-N were the lowest at the water inflow point indicating nitrification (Brown et al., 2011). Nitrogen is rapidly lost through denitrification and ammonia volatilization (Boyd, 1985) and phosphorus is not absorbed by the pond mud so it was high in outlet.TSS was documented low in outlet due to low feed residue in system (Mutea et al., 2021).

Water quality parameters of various forms of Nitrogen in inlets and outlets

There was no significant variation (p>0.05) in the concentration of Nitrite Nitrogen, Nitrate Nitrogen, Ammonia Nitrogen and Total Nitrogen between different points of IPRA, basically at inlets and outlets, in this study. The concentration trend of various forms of nitrogen (NO^{-}_{2}, NH_{3}, NO^{-}_{3} and TN) in inlet and outlet of the IPRA system during the experimental period was shown in Figure 2and displayed a gradual rising trend up to October but a drop down in November. The Nitrite Nitrogen concentration was observed to range from 0.63±0.23mg/L and 2.52±0.43mg/L in the inlet. Similarly, the concentration of Nitrate Nitrogen was observed to be 1.29±0.67 mg/L to 2.75±0.39 mg/L in outlets of IPRA. The concentration of Nitrate Nitrogen in the outlet was at its lowest in August and September. The concentration of highest value of Ammonia Nitrogen was observed 1.35±0.21 mg/L, and lowest value (0.40±0.12) mg/L was recorded in the inlet of IPRA in July. The highest value of Total Nitrogen was9.58±0.14 mg/L, and then fell sharply in November. The lowest concentration (0.75±0.30 mg/L) was recorded in the inlet. The tendency of fluctuation was recorded almost similar in both inlet and outlet.

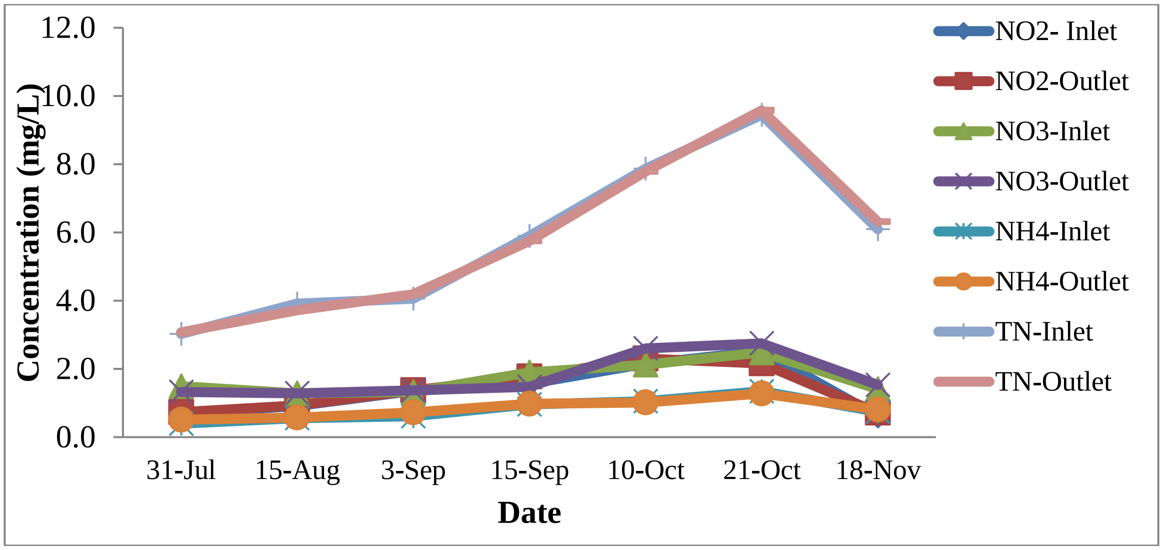

Figure 2. Water quality parameters of Inlet and outlet in IPRA system of various forms of nitrogen (NO^{-}_{2}, NH_{3}, NO^{-}_{3} and TN) measured over experimental period

The mean total ammonia nitrogen was found slightly higher in the outlet than that in the inlet of IPRA, stating the fact that the water quality of the pond was better in terms of TAN in the outlets as its concentration is less than 1mg/L as in an unpolluted water body. High ammonia can arise due to overfeeding with protein-rich feed that ultimately can decay to liberate toxic ammonia gas (Abedin et al., 2017). While nitrates are the final products of the aerobic decomposition of organic nitrogen compounds that are produced from oxidation and reduced to ammonia by bacterial action. The nitrate level (1.76±0.75 mg/L) documented in the outlet represents completely oxidized organic matter in the pond water (Dey et al., 2021). However, if oxygen is present in the water body, the ammonia is oxidized to nitrite (NO_2). The Nitrite nitrogen concentration was documented to be higher than its recommended concentration (less than 0.1 mg/L) but was at par with Abedin et al. (2017), i.e., up to 2 mg/L. Similarly, Nitrite-N and TAN concentrations were lower in the inlet suggesting the facts of accumulation and build-up of respiratory and metabolic wastes of fish as well as the leftover feed particles. Furthermore, the lower concentration of TAN and Nitrite-N at the water inflow point indicates the process of nitrification in the culture area and at the outlet points (Brown et al., 2011). Boyd & Tucker (1985) suggested that nitrogen is rapidly lost through denitrification and ammonia volatilization and phosphorus is not absorbed by the pond mud so it was high in the outlet. Total nitrogen and Phosphorus were reported to accumulate and increase with the extension of the culture period (Seo& Boyd, 2001) in the pond culture and the present findings are in line with this statement. Total Suspended Solids were documented lower in the outlet, which might be due to low feed residue in the system (Mutea et al., 2021).

Orthophosphate and Total phosphorus in inlets and outlets

The Orthophosphate (mg/L) concentration trend was observed comparable in both inlet and outlet of IPRA (Figure 3). Inlet and outlet Orthophosphate concentration decreased initially till mid-August, then showed the spiking trend till October with the highest concentration of 5.57±0.21 mg/L in the inlet but experienced the decreasing trend in November. The lowest concentration of Orthophosphate was recorded 1.57±0.02 mg/L in the inlet of IPRA.

The concentration of Total phosphorus (mg/L) during the complete experimental period is depicted in Figure 3. The trend was similar in both inlet and outlet. The concentration of Total phosphorus was observed to increase till October with the peak value of 7.69±0.19 mg/L in the inlet, then showed a solid decreasing trend during November. However, the lowest concentration (2.08±0.08 mg/L) was recorded in the inlet of IPRA in July.

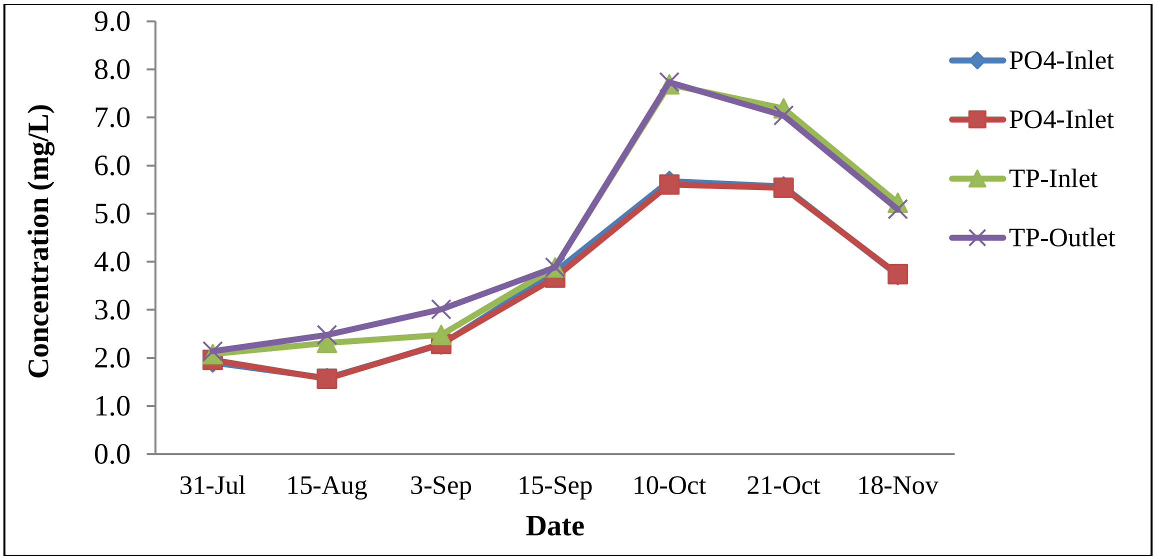

Figure 3. Water quality parameters of Inlet and outlet in IPRAsystem, $PO^{3-}_4$and TP were measured over experimental period

Chlorophyll-a, Chemical Oxygen Demand and Total Suspended solids in inlets and outlets

The mean concentration of Chlorophyll-a (mg/L) was observed 458.12±120.9 mg/L in the outlet water sample and 101.70±23.22 mg/L in the inlet sample of the IPRA system during the experimental period but the inlet and outlet concentrations were found to follow the similar trend. The concentration of Chlorophyll-a was documented depressed in the 3rd, 5th, and 7th samplings (on 3rd September, 10th October, and 18th November) and spiked at 1st, 2nd, 4th and 6th samplings (on July, August, 15th September and 21st October) as depicted in Figure 4.

The concentration of Chemical Oxygen Demand (mg/L) of the inlet and outlet of the IPRA followed a similar trend during the experimental period and is shown in Figure 4. The concentration of Chemical Oxygen Demand was found to fluctuate between 16.82±3.29 mg/L and 3.54±1.6) mg/L in the inlet. It was observed that Chemical oxygen demand declined till August and then, showed a rising trend up to October before going down in November again.

Total Suspended solids (mg/L) of the inlet and outlet of the IPRA system were documented to range between 104.62±44.90 mg/L on 15 August in the inlet and 38.33±40.70 mg/L in July in the outlet during the experimental period and is shown in Figure 4. The Total Suspended solids concentration was found to undulate during the complete experimental period but interestingly, both inlet and outlet concentrations followed the comparable trend. The Total Suspended solids concentration was found lowest in September in the inlet and in October in the outlet of IPRA.

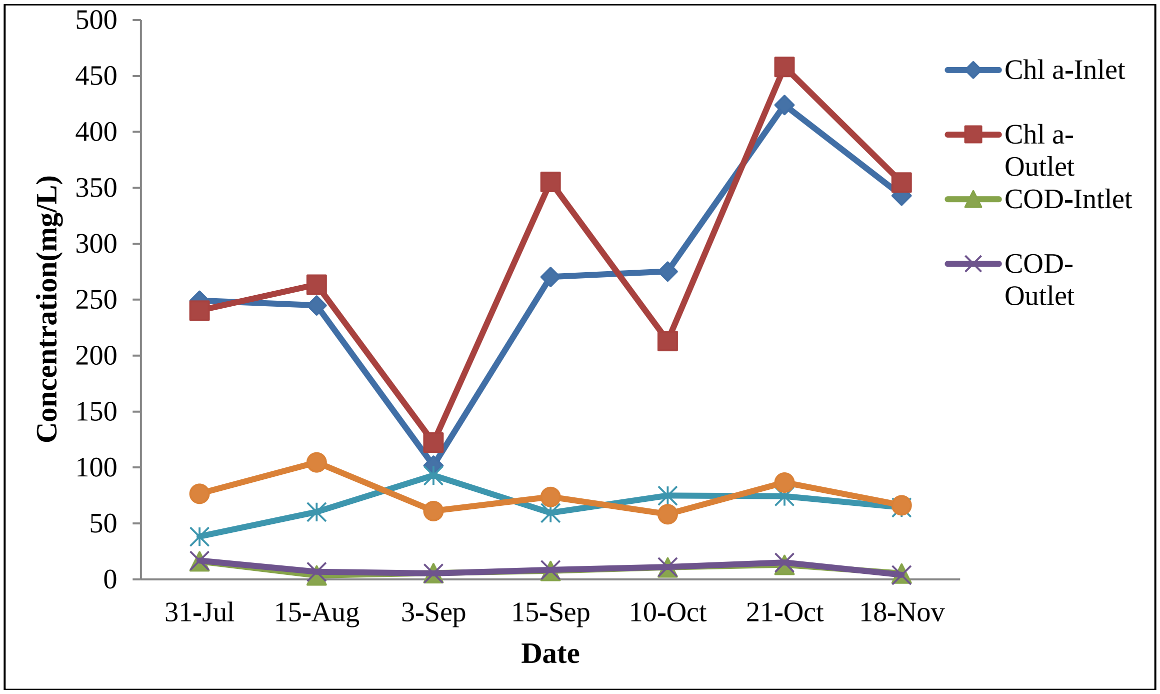

Figure 4. Water quality parameters of Inlet and outlet in IPRA system, Chlo-a, CODand TSS were measured over experimental period

A higher amount of excreta and leftover feeds from the cells, calling for increased levels of nitrogenous content and then the Chlorophyll-a might be the reason for a higher concentration of Chlorophyll-a in the outlet of IPRA. Brown et al. (2012) reported comparable findings of a peak concentration of chlorophyll-a as 949 µg/L in IPRS with stocking densities of 12,000–

30,000 fish/raceway and stated that this value may increase with increasing stocking density of fishes. COD is a measure of the oxygen equivalent of the organic matter in a water sample that is susceptible to oxidation by a strong chemical oxidant so it is widely used as a measure of the susceptibility to oxidation of the organic and inorganic materials present in water bodies. The COD was found lower in the inlet than that at the outlet of IPRA, which might be because of the lower organic matter content in water because COD in water has a positive relationship with the concentration of organic matter (Tian et al., 2009). Suspended solids have the character of settling in the bottom of water bodies if the water velocity is low. The mean concentration of Total suspended Solids was found lower in the outlet in this study because heavier suspended solids might have settled out at the bottom of a water body over a period of time (Dey et al., 2021).

Distribution of microbial communities at the phylum level in the IPRA system

The microbial communities were dominated by *Proteobacteria*, *Cyanobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Chloroflexi* in both Inlets and Outlets (Figure 5) and their relative abundances in water mass are shown in Figure 6. *Fusobacteria*, *Fermicutes*, *Verrucomicrobia*, *Plantomycetes*, *Patesibacteria*, *Acidobacteria*, *Deinococcus*, etc. are observed as the minor microbial community in the water body.

Microbial communities are complex in nature, and environmental factors shape the structure and function of microbial communities (Allison and Martiny, 2008; Fuhrman et al., 2015). Chlorophyll-a, total nitrogen (TN), PO^{3-}_{4}, Total phosphate, chemical oxygen demand (Zhang et al., 2014), and feed sources provided in ponds are the principal determinants and influence the succession of microbial communities (Xiong et al., 2014; Qin et al., 2016).

Microbial community found in Inlet and outlet of IPRA

The microbial communities were dominated by *Cyanobacteria*, *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, and *Chloroflexi* in both inlets and outlets of IPRA systems. The major factors affecting the compositional dynamics of microbial communities differ depending on time and space (Dang & Chen, 2017). The composition of bacterial communities in the aquaculture environment can serve as an indicator to reflect the ecological status of the ecosystem (Zhao et al., 2020). Excessive nutrients in the aquaculture environment can change the dynamics of bacterial communities, which are closely related to the state of stocking species and are also affected by external environmental factors (Xue et al., 2017).

The abundance of *Proteobacteria* in both inlets and outlets might be due to its high level of metabolic diversity related to carbon, nitrogen and sulfur cycling (Kersters et al., 2006). The accumulation of harmful ammonia and nitrite in the water column is a serious issue in intensive aquaculture since it not only reduces water quality but also causes different diseases and even death in fish (Qin et al., 2016). In this study, *Bacteroidetes* were observed enriched in water samples of both inlets and outlets which might be associated with ammonia concentration as described by Jiang et al. (2016) and Qin et al. (2016). Surprisingly, a higher abundance of *Actinobacteria* in the samples were observed in spite of higher concentration of both the nitrate and ammonium which is against a report that demonstrates both the nitrate and ammonium concentrations negatively affect the abundance of *Actinobacteria* (Philippot et al., 2009*).*In line with this observation, Tian et al. (2009), Qin et al. (2016), and Sun et al. (2021) reported that environmental factors such as Temperature, DO, nitrite, nitrate, ammonium, total nitrogen, and total phosphorus influence the composition of bacterial communities in pond water.

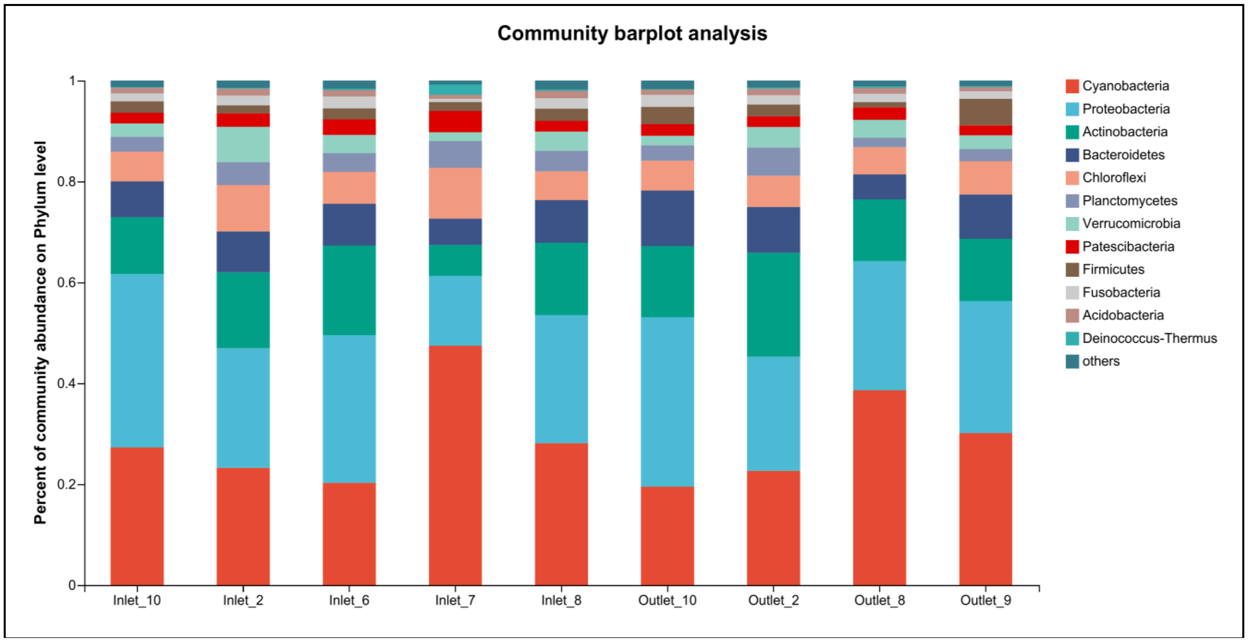

Figure 5. Classification of bacterial community in In- pond raceway system

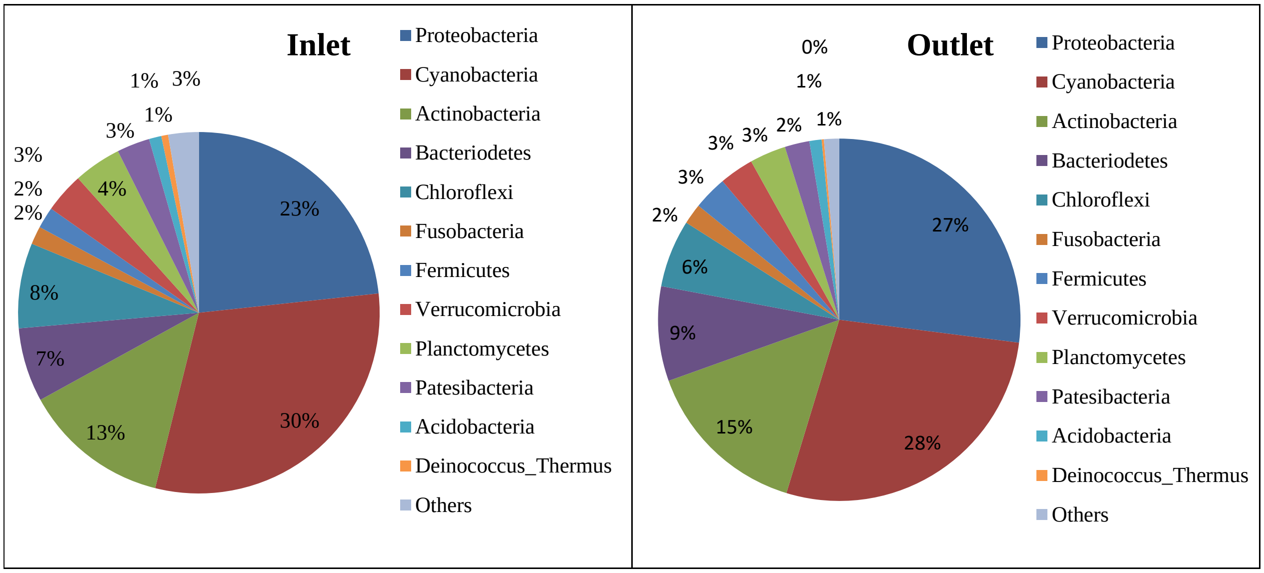

Figure 6. Phylum wise composition of microbial communityfound in Inlet and outlet of IPRA System

McLellan et al. (2010) described the bacterial diversity in the influent from a municipal wastewater treatment plant and reported that *Proteobacteria* were the most abundant phylum in the influent sample, which is consistent with this finding. However, the clustering of bacterial phyla in the present study is consistent with the findings of Wei et al (2015) who documented *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* as the main phyla in the surface water samples in sewage treatment plants. Wei et al. (2015) showed that the dominant phyla were *Proteobacteria* (20.28-67.89%), *Bacteroidetes* (3.85–16.14%), *Acidobacteria* (19.78 –53.59%), and *Cyanobacteria* (0.68 to 2.6%) in surface water samples, while our results revealed that *Proteobacteria* (27.0%) and

Cyano-bacteria (28.0%) were the most abundant phylum followed By *Bacteroidetes* (9.0%), *Actinobacteria* (15.0%) and others. These findings are in accordance with Qin (2016) who reported very similar microbial compositions at different pond depths, and no significant differences between the four dominant phyla (*Proteobacteria*, *Cyanobacteria*, *Bacteroidetes*, and *Actinobacteria*) were observed. Comparable results of the microbial compositions of four different water layers were also described by Fan et al. (2015) in intensive GIFT tilapia (*Oreochromis niloticus*) ponds. This phenomenon may be due to the similarity of water conditions in the inlets and outlet of IPRA. But, Qian et al. (2011) described that the bacterial abundance and composition may vary according to the depth of the water surface and documented *Cyanobacteria* domination at the upper layers (20 and 50 m) and *Proteobacteria* in the deeper layers (200 and 1500 m) in the Red Sea.

Conclusion

The water quality is the mirror of the pond ecosystem and provides vital information for the growth and production of fish and aquatic specimens. All the water quality parameters measured and analyzed in this study were found to have non-significant differences between the inlet and outlet water samples in the IPRA system. Total Nitrogen (TN) and Soluble Reactive Phosphorus (SRP) were at lower concentrations, while NO_2^--N, NO_3^-N, TAN, TP, Chlorophyll-a, COD, and TSS were observed to have higher concentrations in the outlet of the IPRA system. In this study, a higher concentration of Chlorophyll-a was observed in the outlet of IPRA which might be due to the higher stocking density used in the experiment that produces a higher amount of excreta and leftover feeds calling for increased levels of nitrogenous content and then the Chlorophyll-a. Similarly, Chemical Oxygen Demand (COD), a measure of the oxygen equivalent of the organic matter in a water sample, was found lower in the inlet than that at the outlet of the IPRA system and displayed a positive relationship with the concentration of organic matter. Likewise, the mean concentration of total suspended solids was found lower in the outlet in this study because heavier suspended solids might have settled out at the bottom of a water body over a period of time.

The bacterial community did not differ significantly between inlets and outlets of the IPRA system with a higher abundance of *Cyanobacteria*, *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Chloroflexi*. The *Cyanobacteria* and *Chlorflexi* were non-significantly higher in the pond inlets while *Proteobacteria*, *Actinobacteria*, and *Bacteriodetes* were rich in pond outlets. These results suggest that the modulation of IPRA system management might influence the community structure and thus might effectively change water quality. This finding could thus open a promising working space for the sustainable and healthy culture of grass carp.

Therefore, it can be concluded from this study that fishes in IPRA consumed nearly all the feed offered and a negligible amount of feed was wasted. So, leftover feed settlement to the bottom of the pond became a concern of no issue, despite having high stocking density in the IPRA thus bringing non-significant differences in water quality parameters between the inlet and outlet of the IPRA system.

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