



# Lab- and pilot-scale photo-biofilter performance with algal–bacterial beads in a recirculation aquaculture system for rearing rainbow trout

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## Abstract

Industrial aquaculture has proliferated due to increased world demand for fish and seafood. Aerobic bacterial biofilters typically perform the nitrogen abatement of wastewater. Recirculation aquaculture systems (RAS) require nitrifying microorganisms developed in the biofilter. Despite the advantages of these biofilters, there are disadvantages, such as the time needed to mature, decrease in oxygen concentration, accumulation of organic matter and difficulty of backflushing, among others. On the other hand, microalgae effectively eliminate nutrients-pollutants, consuming inorganic carbon, nitrogen, and phosphorus and balancing soluble oxygen, conditions not attributable to nitrifying biofilters. The current study used a photo-biofilter to determine the depuration capacity of an immobilized co-culture of microalga *Tetrademus dimorphus* and nitrifying bacteria isolated from a Salmon RAS. Bacteria from genera *Flavobacterium*, *Microbacterium*, *Raoultella*, *Sphingobacterium*, and *Pseudomonas* were identified. Biofilters were tested in sequential batch (lab-scale; 2.85 L) and continuous mode (pilot-plant scale; 120 L) attached to a RAS system for rearing rainbow trout. The algal–bacterial community structure was studied using 16S rRNA gene sequencing. Results showed that at typical loading rates, the algal–bacterial community could simultaneously remove ammonium, total ammonium nitrogen (TAN), nitrate and phosphate. Moreover, the system evaluated removed TAN daily, at an average of 1.18 kg per m<sup>3</sup> of beads.

**Keyword** Recirculation aquaculture system · Biofilter · Nitrifying bacteria · Microalgae · Mixotrophic · Microbial community

## Introduction

Recirculation aquaculture systems (RAS) can potentially reduce the environmental impacts of aquaculture compared to traditionally used open-flow systems. The latter requires an average of 30 m<sup>3</sup> of fresh water per year for each kilogram of farmed trout, while RAS fluctuate between 0.3 and 3 m<sup>3</sup> kg fish<sup>-1</sup> year<sup>-1</sup> allowing for 95.9–99.6% of water savings (Pedersen et al. 2012; Bregnballe 2015). The water treatment

step must eliminate nitrogenous compounds (ammonium, nitrite and, optionally, nitrate), whose accumulation causes toxicity, decreased body size, increased mortality, abnormal behaviours, and skeletal deformities in fish (Martins et al. 2009; Davidson et al. 2014; Ciji and Akhtar 2020). Additional equipment should also supply the system's oxygen consumed by fish and nitrogen abatement. Ammonium and nitrite are the most damaging compounds, comparatively. For this reason, biological nitrification treatment in "Nitrifying Biofilters" has been the most employed method in RAS. In addition to nitrifying biofilters, other processes such as reverse osmosis and electrochemical technology have been reported to control nutrient concentrations (Qin et al. 2005; Gupta et al. 2015). However, its high energy requirement is an unavoidable drawback so far.

Additionally, nitrifying biofilters have other significant drawbacks that include a decrease in the concentration of dissolved O<sub>2</sub>, the contribution of CO<sub>2</sub>, alkalinity consumption, difficulty in filter back-rinsing, accumulation of organic matter and presence of undesired microorganisms.

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Nitrifying bacteria are obligate autotrophs that use carbon dioxide as the primary carbon source and require oxygen to grow (Hagopian and Riley 1998). In RAS biofilters, this group accounts for roughly 15% of the total bacterial community (Roalkvam et al. 2020). The fast-growing heterotrophic bacteria dominate the rest of the biofilm (Schreier et al. 2010). An inoculum with the desired nitrifying species is needed to activate a nitrifying biofilter. The biofilter containing plastic carriers could be fed with inoculum by a closed loop, or the biofilter could use carriers from a mature filter (Gutierrez-Wing and Malone 2006; Delong and Losordo 2012). Biofilm development on the carriers is time-consuming since nitrifying bacteria grow slowly (Gutierrez-Wing and Malone 2006; Zhang et al. 2019).

Microalgae already constitute a real possibility for the removal of nutrients from urban (de-Bashan and Bashan 2010; Calicioglu and Demirer 2019), industrial (Bordel et al. 2009; Wu et al. 2017) and agricultural wastewater (Markou and Georgakakis 2011; Jayakumar et al. 2017). Algal photobioreactors (PBR) have been proposed for aquaculture facilities as additional units. However, the two main concerns are economic and practical feasibility (light supply, high retention times) (Ramli et al. 2020). The culture of microalgae in wastewater is fascinating since they can take up both N and P in their biomass, which could eventually be used in agriculture. Microalgae grown in RAS systems could also produce biomass for aquaculture feeding (Rawat et al. 2011), thereby contributing to the circular economy approaches. In addition to the efficiency in removing nutrients, these systems can significantly reduce costs related to oxygenation and CO<sub>2</sub> degassing (de Godos et al. 2009). The main limitations of using microalgae would be their culture systems, which require light-permeable surfaces and larger land areas (compared to nitrifying biofilters). In addition, another disadvantage of using algae in suspension is their unavoidable presence in the fishponds that would increase the turbidity, hindering fish feeding. Microalgal culture systems based on cell immobilization have attempted to overcome some previously discussed issues (He and Xue 2010; Pires et al. 2013). Cell encapsulation in a polymeric matrix and biofilm formation on a solid surface (Mallick 2002; Eroglu et al. 2015) are the standard methods for microalgal immobilization. Common polymeric matrixes are alginate and carrageenan. As with nitrifying bacteria, it is almost impossible to maintain a monoculture of microalgae in these processes. Far from being a problem for nutrient removal, several studies reported the advantages of using microalgal-bacterial consortia (Muñoz and Guieysse 2006; Subashchandrabose et al. 2011; Gonçalves et al. 2017; Sun et al. 2022). These consortia can occur naturally in the environment or be generated artificially by combining microorganisms that do not necessarily coexist for a specific purpose. Generally, photosynthetic microorganisms and heterotrophic bacteria constitute

the microalgae and bacteria consortiums for wastewater treatment (Gonçalves et al. 2017). Before full-scale biofilters are designed and built in RAS, tests must be performed on pilot-scale units to determine the efficiency and design accuracy. Such pilot-scale tests also allow for the evaluation of system operation, performance, and economics.

The main goal of this research was to develop a pilot-scale biofiltration system that could be used to perform tests involving the depuration of N and P from Salmon RAS water. The specific objectives of this project were to: 1) Design and build three lab-scale biofilters and a pilot-scale biofilter with the capability to monitor various parameters such as temperature, irradiance, and flow rate, and 2) Test and compare the performance of the biofilter units for removing nitrate and phosphate. The novelty of this study also lies in the fact that the algal–bacterial lab-scale biofilter tested for N and P depuration was successfully scaled to a pilot unit attached to a RAS system for rearing rainbow trout.

## Materials and methods

### Natural aquaculture wastewater

A demonstration aquaculture recirculation system provided the wastewater (Department of Oceanography, Universidad de Concepción, Dichato, Bio-Bio, Chile). The facility reared *Oncorhynchus mykiss* (rainbow trout) under an intensive culture approach. For the optimization assays (described below), 0.3 m<sup>3</sup> of water leaving the fishponds was treated and stored at 3°C in a sealed container. Treatment consisted of 50 µm filtration and disinfection in a recirculated UV system for 12 h. The concentration of ammonium, nitrate and phosphate was checked daily; averaged values were 1.6 ± 0.5, 50 ± 0.4 and 7 ± 0.8 mg L<sup>-1</sup>, respectively.

### Algal–bacterial beads preparation

The inoculum was composed of the microalga *Tetrademus dimorphus* and nitrifying biomass obtained from the nitrification biofilter of the demonstrative aquaculture recirculation (Dichato, Bio-Bio, Chile). The microalgal strain was isolated from the fishponds using the standard serial-dilution technique (Button et al. 1993). The bacterial inoculum provided was cultured in agitated flasks (120 rpm) in a chamber at 10 °C using wastewater. Suspension of 0.5 g L<sup>-1</sup> (dry weight) of *T. dimorphus* and the bacterial biomass was mixed in 1:1 proportion and then cultured in batch mode using the aquaculture wastewater supplemented with NH<sub>4</sub>Cl up to 20 mg L<sup>-1</sup>. These cultures were carried out in 2 L agitated flasks with 0.75 L of working volume under agitation in an orbital shaker and an irradiance of 100 µmol

photons  $\text{m}^{-2} \text{s}^{-1}$ . After two consecutive batches, where the initial ammonium concentration ( $20 \text{ mg L}^{-1}$ ) was abated (approximately 15 days), biomass was concentrated by centrifugation from  $1.6$  to  $15 \text{ g L}^{-1}$ . This suspension was the feedstock for the formulation of microalgal-bacterial alginate beads. Food grade sodium alginate (W201502; Sigma-Aldrich) was weighed and dispersed slowly in cold distilled water to achieve a concentration of 2% w/v and then heated with continued stirring until a clear solution was obtained (approximately 30 min). It was then transferred to a container and allowed to stand for 24 h to eliminate air bubbles formed during homogenization. The algal-bacterial suspension and the alginate solution were mixed in 1:10 proportion. Then, the alginate solution was dropped into a 2 w/v% calcium chloride ( $\text{CaCl}_2$ ) solution to form alginate beads. The diameter of the discharge tip gives the size of the sphere. This study prepared spheres with a nominal diameter of 3 mm. Beads were allowed to harden for 5 h before use in the photobioreactors.

### Algal–bacterial genetic characterization

A genetic analysis assessed the algal–bacterial biomass grown. Microbial community analyses were based on 16S rRNA. Algal–bacterial biomass was suspended in PBS (phosphate buffered saline at  $\text{pH}=7.5$ ) and then diluted in series with a dilution factor of 10. One hundred  $\mu\text{L}$  of the algal–bacterial biomass solution was transferred to Tryptone-Casein Soy Agar (TSA) or Luria–Bertani Agar (LBA; Lennox) plates for incubation at  $18 \text{ }^\circ\text{C}$  for ten days. Isolated colonies were cultured under similar conditions three times consecutively. Then the biomass was transferred to a Tryptic Soy Broth (TSB) medium, cultured for four days, and harvested (centrifuged at 15,900 RCF for 15 min, followed by supernatant removal). DNA extraction followed the protocol of the EZNA bacterial DNA extraction kit (Omega Bio-Tek, USA). Total genomic DNA was quantified using an Epoch® micro-volume spectrophotometer system (BioTek Instruments, USA). DNA quality was verified in a UV transilluminator using agarose gel (1.0% w/v) stained with GelRed Nucleic Acid Gel Stain™ (Biotium, USA). DNA samples were stored at  $-20 \text{ }^\circ\text{C}$ . Specific markers for denitrification (*nriK* and *nirS*), nitrite oxidation (*nrxB*), ammonium oxidation (*amoA* and *cmx*) and nitrogen-fixing (*nifH*) were assayed (Touchdown-PCR; Don et al. 1991). The molecular classification of the isolated bacterial strains was based on the 16S rRNA gene sequences using the commercial kit SapphireAmp Fast PCR Master Mix (Takara Bio, Japan). The assay used 250 ng of DNA and 200 nM of each primer. PCR products were analyzed in a UV transilluminator using agarose gel (1.5% w/v) stained with GelRed Nucleic Acid Gel Stain (Biotium, USA). Likewise, PCR products were purified and sequenced by MACROGEN Inc. (Seoul, Korea)

using an ABI3730 ADN XL Analyser (Applied Biosystems). The platform Geneious R11 (Biomatters Ltd, NZ) processed the sequences. The bioinformatics pipeline included clustering, error-trimming, and taxonomic classification. The 16S rRNA gene sequences are available at GenBank (Benson et al. 2008), ENA (Leinonen et al. 2011) and DDBJ (Kaminuma et al. 2010).

### Optimization of operating parameters in lab-scale

An experimental design was carried out in a lab-scale photobioreactor (see details below) to optimize operating parameters. A Response Surface Design (Central Composite) was the model chosen to evaluate the effect of three factors (incident irradiance and air and liquid flow) with a total of 17 experiments (resumed in Table 1). The experiment response variables were the concentration of ammonium, nitrate, and phosphate, expressed in  $\text{mg L}^{-1}$  over time. The study considered a duplication of each sample.

### Lab-scale algal–bacterial biofilter

Lab-scale experiments required building three three-phase fluidized photobioreactors (Fig. 1). Each 3 L photobioreactor was custom manufactured using cylindrical methacrylate tubes (polymethyl methacrylate, PMMA), with external dimensions of 74 cm height and 9 cm diameter. The working liquid volume was 2.85 L. The setup included an air compressor, individual buffer

**Table 1** Experimental design for the optimization of the lab-scale biofilter operating conditions

ID code	Water flow ( $\text{L min}^{-1}$ )	Airflow ( $\text{L min}^{-1}$ )	Irradiance ( $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ )
N1	1	0.1	7
N2	5	0.1	7
N3	1	0.5	7
N4	5	0.5	7
N5	1	0.1	405
N6	5	0.1	405
N7	1	0.5	405
N8	5	0.5	405
N9	1	0.3	206
N10	5	0.3	206
N11	3	0.1	206
N12	3	0.5	206
N13	3	0.3	7
N14	3	0.3	405
N15	3	0.3	206
N16	3	0.3	206
N17	3	0.3	206

tanks, pumps and additional instrumentation for adjusting air and fluid inlet flow. Temperature control systems in buffer tanks kept the temperature at 10°C. Light-emitting diode (LED) string placed inside the transparent cylindrical vessel using a sealed tube with 2 cm of diameter supplied light to the photobioreactors.

### Pilot-scale algal–bacterial biofilter

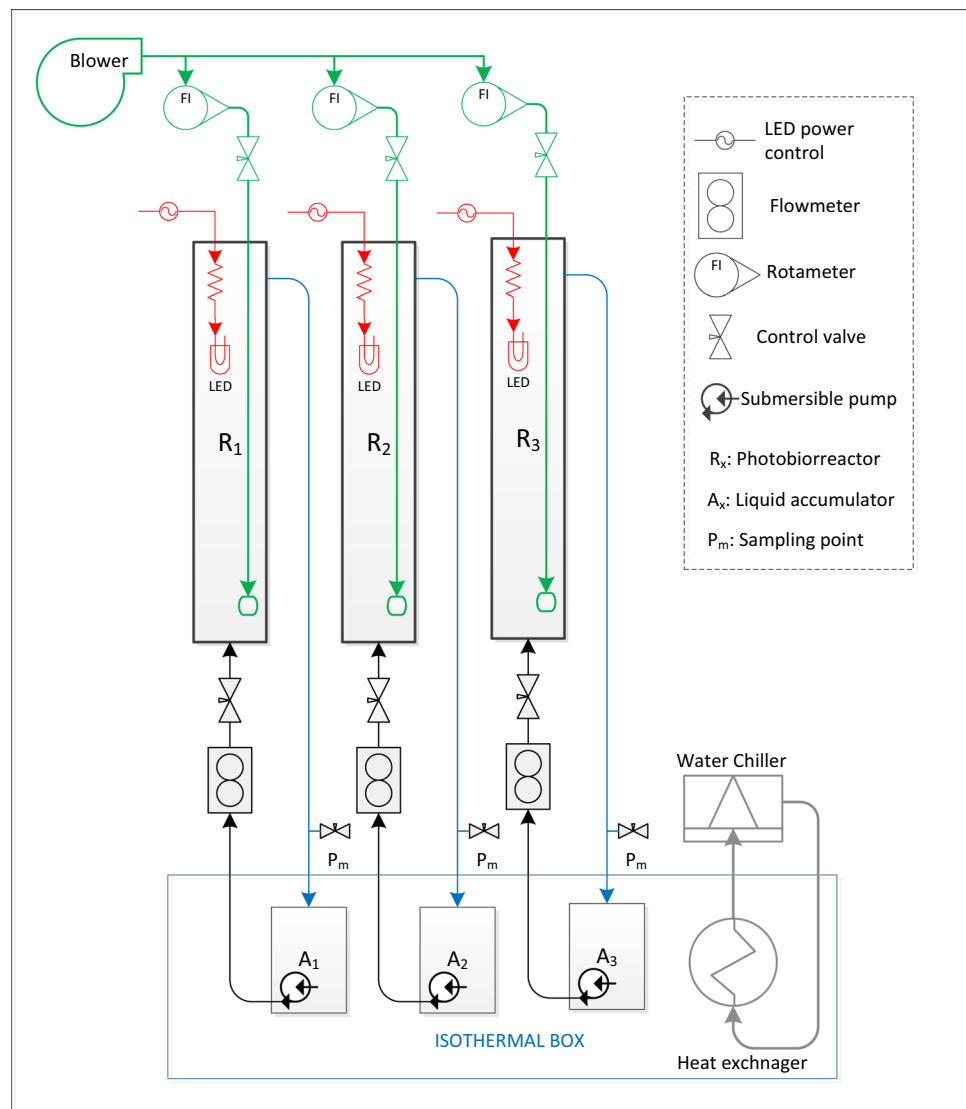
Pilot-scale biofilters were built, installed, and operated in a RAS line of an aquaculture facility at Dichato (Bio-Bio, Chile) for rearing rainbow trout using commercial polyvinyl chloride (PVC) pipes and accessories (Fig. 2). The facility operates as a typical RAS system with auxiliary equipment for water recirculation (e.g., sand filter, air blowers, submerged nitrifying biofilter, etc.). The pilot-scale algal–bacterial biofilter used pumped wastewater from a fishpond with fish weighing 100 and 200 g during operation. The average fish density was

25 kg m<sup>-3</sup>, and the fed rate was 1 to 1.5% of their daily body weight. The protocol included daily environmental parameters checking. Table 2 summarises lab- and pilot-scale biofilter dimensions and alginate beads-related parameters. Vessels 0.95 m high and 0.4 m wide had a working volume of 120 L. Air flowed at 6 L min<sup>-1</sup> (0.3 L min<sup>-1</sup> through 20 sparger holes). Inlet flow (water recirculation) was 3 L min<sup>-1</sup>, and 40 min of hydraulic retention time. Irradiance was kept at 200 μmol photons m<sup>-2</sup> s<sup>-1</sup> using four fluorescent lamps (T8 Tuxline F25W/30"/840) located outside the vessel (Fig. 2). The study protocol included the analysis of ammonium, nitrate and phosphate by duplicating each sample.

### Chemical analysis

Standard analytical procedures determined the nutrient concentrations. The nitrate and phosphate quantification

**Fig. 1** Scheme of the lab-scale algal–bacterial biofilter (3 L-photobioreactor) system for the optimization experiments





**Fig. 2** Photograph of the pilot-scale algal–bacterial biofilter

protocols were 4500-P and 4500-N from APHA (APHA 2012) using a Biotek Epoch™ microplate spectrophotometer. Ammonium was quantified using Nessler Reagent ( $K_2HgI_4$ ) according to a modified version of the ASTM D1426-08 method (ASTM 2008). Additionally, nutrient removal measurements for batch assays followed the equation (Eq. 1):

$$DR = \frac{(C_{inlet} - C_{outlet})}{t} \quad (1)$$

$C_{inlet}$  and  $C_{outlet}$  are inlet and outlet concentrations ( $mg L^{-1}$ ) of a particular nutrient.  $t$  represents treatment time (hours) (the experimental design time setup was 5 h).

For continuous systems, the depuration rate ( $mg L^{-1} day^{-1}$ ) equation is:

$$DR = \frac{Q}{V} (C_{inlet} - C_{outlet}) \quad (2)$$

**Table 2** Parameters of lab-scale and pilot plant algal–bacterial biofilters

Parameter	Units	Lab-Scale biofilter	Pilot-scale biofilter
Diameter	m	0.09	0.4
Height *	m	0.47	0.95
Diameter/Height ratio	-	0.2	0.4
Volume*	L	2.85	120
Number of beads	-	$24 \times 10^3$	$10^6$
Total volume of beads	L	0.36	15
Beads density	$g L^{-1}$	8,421	8,333

(\*) working volume

Surface and volumetric depuration rates were calculated from Eqs. 3 and 4, respectively:

$$SDR = \frac{Q}{A_{beads}} (C_{inlet} - C_{outlet}) \quad (3)$$

$$VDR = \frac{Q}{V_{beads}} (C_{inlet} - C_{outlet}) \quad (4)$$

where  $C_{inlet}$  and  $C_{outlet}$  are inlet and outlet concentrations of a particular nutrient, respectively;  $Q$  is inlet liquid flow;  $A_{beads}$  is the total surface area of alginate beads;  $V$  is the working liquid volume in the biofilter;  $V_{beads}$  is the total volume of alginate beads;  $A_{beads}$  and  $V_{beads}$  assumed that the bead shape was spherical (3 mm in diameter).  $SDR$  and  $VDR$  were calculated in  $mg m^{-2} day^{-1}$  and  $mg L^{-1} day^{-1}$ , respectively.

### Statistical analysis

Statistical analyses used the multivariate statistical software package Statgraphics Centurion XIV (USA). The experimental results were the average values of the two independent samples ( $N = 2$ ) and their standard deviation. Normality and homogeneity analysis used the Kolmogorov–Smirnov and Levene tests. A three-factor ANOVA analysis was performed for the operating condition optimization.

### Results

The consortium algal–bacterial (biomass) came from a non-axenic isolated microalgal culture and bacterial biomass extracted from a mature biofilter grown on a plastic carrier (Kaldness, K1). This biomass forms a dense biofilm on the plastic support as the biofilter works for more than 12 months. The *Materials and Methods* section indicated that algal and bacterial inoculum was mixed and co-cultured. Then, two batches arranged sequentially operate for 15 days (each) using outlet pond water supplemented with an ammonium concentration of  $20 mg L^{-1}$ . The biomass was harvested by centrifugation from an initial concentration of  $1.6 g L^{-1}$  (in the broth) to  $15 g L^{-1}$ .

The culture condition simultaneously considered bacterial and microalgal growth (See *Materials and Methods* section). Characterizing any RAS biofilter in bacterial communities is essential for understanding biofilter performance. The biofilter's stability and efficiency depend on the dynamic balance of the bacterial groups that should be adapted to the specific conditions of every RAS (Schreier et al. 2010). In the present study a source of irradiance and the presence of microalgae may also impact bacterial diversity. Consequently, the study included measuring the bacterial composition after the microalgal–bacterial



co-culture. Results showed at least twelve viable heterotrophic aerobic bacterial strains, recoverable in culture using TSA, and five likely heterotrophic aerobic bacterial strains, recoverable in the culture at 18 °C using LBA. Only ten bacterial strains from the isolated ones showed the presence of genetic markers (at least one) associated with the denitrification process (*nriK* and *nirS*), nitrite oxidation (*nxrB*), ammonium oxidation (*amoA* and *cmx*), or nitrogen fixation (*nifH*). Table 3 lists the classification and characterization of isolated bacterial strains.

The study on optimizing the operating conditions of algal–bacterial biofilters relied on previous tests. Accordingly, earlier runs in batch mode defined the accurate sampling time for the optimization experiments. As shown in Fig. 3A, differences in depletion rates for each nutrient compromised the sampling time based on ammonium concentration. In other words, the removal of almost all ammonium took 5 h. The removal rate for nitrate was steady after the first hour, whereas the phosphate removal rate slope slightly decreased after 5 h. The experimental time (24 h) was insufficient to remove nitrate and phosphate altogether.

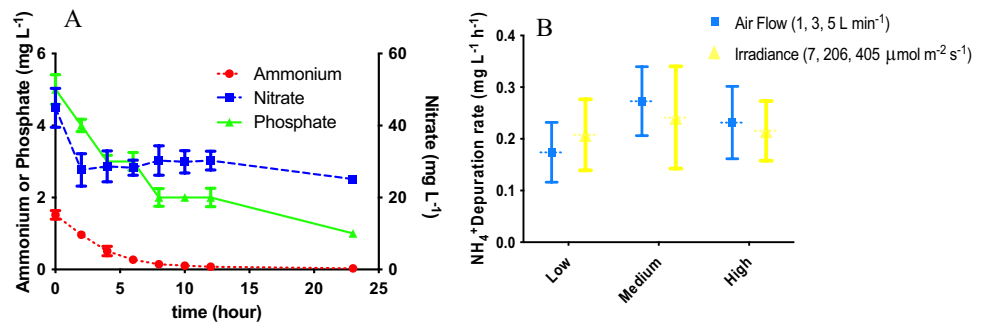
Additionally, the medium condition combination of airflow and irradiance performed well for removing ammonium, according to Fig. 3B.

Ammonium depuration rate ( $\text{mg L}^{-1} \text{h}^{-1}$ ) was the response variable for the experimental design. Table 1 shows the experimental design for optimizing the lab-scale biofilter operating conditions. A bubble column reactor of 2.85 L with airflow rate from 0.1 to 0.5  $\text{L min}^{-1}$  implied a volume of air/volume of liquid/time ratio (typically volume/volume/minute) of 0.035, 0.105 and 0.176 vvm for low, medium and high flow rates, respectively. These are typical values for a bubble column reactor with suspended microalgae (Sánchez Mirón et al. 2000). On the other hand, bubble column reactors with continuous water flow are not typical in microalgal processes, and fluid dynamics depend only on bubbling. In our system, we imposed liquid flow rates of 1, 3 and 5  $\text{L min}^{-1}$  (low, medium and high, respectively), achieving hydraulic retention times of 2.85, 0.95 and 0.57 min, respectively. These values were comparatively lower than those of tubular photobioreactors. Tubular PBRs, for instance, keep liquid rates from 0.25 to 0.5  $\text{m s}^{-1}$  in the solar collector (Acién Fernández et al.

**Table 3** Classification and characterization of isolated bacterial strains

Strain isolated	Cand. Species	Identity (%)	Coverage (%)	GenBank reference	<i>nriK</i>	<i>nirS</i>	<i>nifH</i>	<i>cmx</i>	<i>nxrB</i>	<i>amoA</i>
<i>Flavobacterium</i> sp. FDM-3	<i>Flavobacterium johnsoniae</i>	99.53	62.31	<a href="#">MF737174.1</a>	■		■			■
<i>Flavobacterium</i> sp. FDM-5	<i>Flavobacterium quisquiliarum</i>	99.92	99.48	<a href="#">NR_158092.1</a>		■	■		■	■
<i>Microbacterium</i> sp. FDM-6	<i>Microbacterium natoriense</i>	99.10	99.70	<a href="#">MT367754.1</a>					■	■
<i>Flavobacterium</i> sp. FDM-10	<i>Flavobacterium quisquiliarum</i>	100	100	<a href="#">NR_158092.1</a>				■		
<i>Raoultella</i> sp. FDM-11	<i>Raoultella terrigena</i>	99.78	100	<a href="#">LR131271.1</a>		■				■
<i>Pseudomonas</i> sp. FDM-13	<i>Pseudomonas kunmingensis</i>	100	100	<a href="#">MZ338617.1</a>	■					■
<i>Pseudomonas</i> sp. FDM-14	<i>Pseudomonas reinekei</i>	97.08	100	<a href="#">KC790314.1</a>	■	■	■		■	■
<i>Flavobacterium</i> sp. FDM-15	<i>Flavobacterium johnsoniae</i>	99.85	100	<a href="#">MF737174.1</a>		■		■	■	
<i>Sphingobacterium</i> sp. FDM-16	<i>Sphingobacterium mizutaii</i>	99.93	100	<a href="#">JF899285.1</a>		■				■
<i>Pseudomonas</i> sp. FDM-17	<i>Pseudomonas stutzeri</i>	99.93	100	<a href="#">CP073105.1</a>	■	■				■

**Fig. 3** Operation in lab-scale biofilter (batch mode): **A**) Concentration of ammonium, nitrate and phosphate and **B**) Effect of operating conditions (airflow and irradiance) on ammonium depuration rate at 5 h of operation. Error bars represent standard deviations ( $N=2$ )



1999; Molina et al. 2001) resulting, for 9 cm diameter tubes, in a water flow of around 190 L min<sup>-1</sup>. In tubular photobioreactors, a turbulent flow regime (high flow rates) is required in the tubes for the cells to be equally exposed to light. The only source of turbulence is the liquid flow (its velocity). In our experiments, water and air flow upward mixed, requiring much less flow to homogenates beads. Based on literature, the limiting, optimal and saturating irradiance of the microalgal strain were 7, 206 and 406  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , respectively (Macintyre et al. 2002).

Table 4 summarizes the results from the ANOVA analysis. This analysis explored the influence of A, B and C factors on the response variable (ammonium depuration rate). Only airflow and irradiance influence the response variable ( $P$ -value < 0.05). Based on F-ratio values, we can conclude that the airflow had a more substantial impact on the response variable than irradiance (9.28 vs 6.62). Figure 3B shows the dependence of the ammonium depuration rates against the airflow and the irradiance. The medium irradiance and airflow conditions achieved the maximum values.

The lab-scale biofilter system was scaled up to 120 L. The pilot-scale biofilter operated in a steady-state mode for 20 days at 3 L min<sup>-1</sup> of wastewater. The hydraulic retention time was 40 min, higher than the hydraulic retention in lab-scale system (Table 2). The pilot-scale biofilter considered a similar density value of beads to the lab-scale biofilter. The aspect ratio was modified, resulting in a less slender biofilter (Fig. 2). Pipes and fitting were PVC standard water supply

items. For bubbling, an inlet airflow of 6 L min<sup>-1</sup> flowed through 20 holes homogeneously distributed, resulting in a flow of 0.3 L min<sup>-1</sup> per inlet hole. The setting of the inlet airflow and irradiance considered those operating conditions where the lab-scale reactor achieved the maximum ammonium depuration rate (Fig. 3B). Figure 4 shows the evolution of inlet and outlet concentrations of ammonium (Fig. 4A), nitrate (Fig. 4B) and phosphate (Fig. 4C). For example, an initial ammonium concentration of 4 mg L<sup>-1</sup> shown in Fig. 4A was reduced to 0.3 mg L<sup>-1</sup>, estimating an average ammonium depuration rate of 0.1 mg L<sup>-1</sup> min<sup>-1</sup> (148.5 mg L<sup>-1</sup> day<sup>-1</sup>). Table 5 summarizes the average depuration rates at operation parameters.

The algal–bacterial systems have a distinct advantage on removing nitrate and phosphate because microalgae fix it into their biomass. Our system consumed 78% and 64% of the nitrate and phosphate, achieving removal rates of 284.7 and 165.5 mg L<sup>-1</sup> day<sup>-1</sup>, respectively.

## Discussion

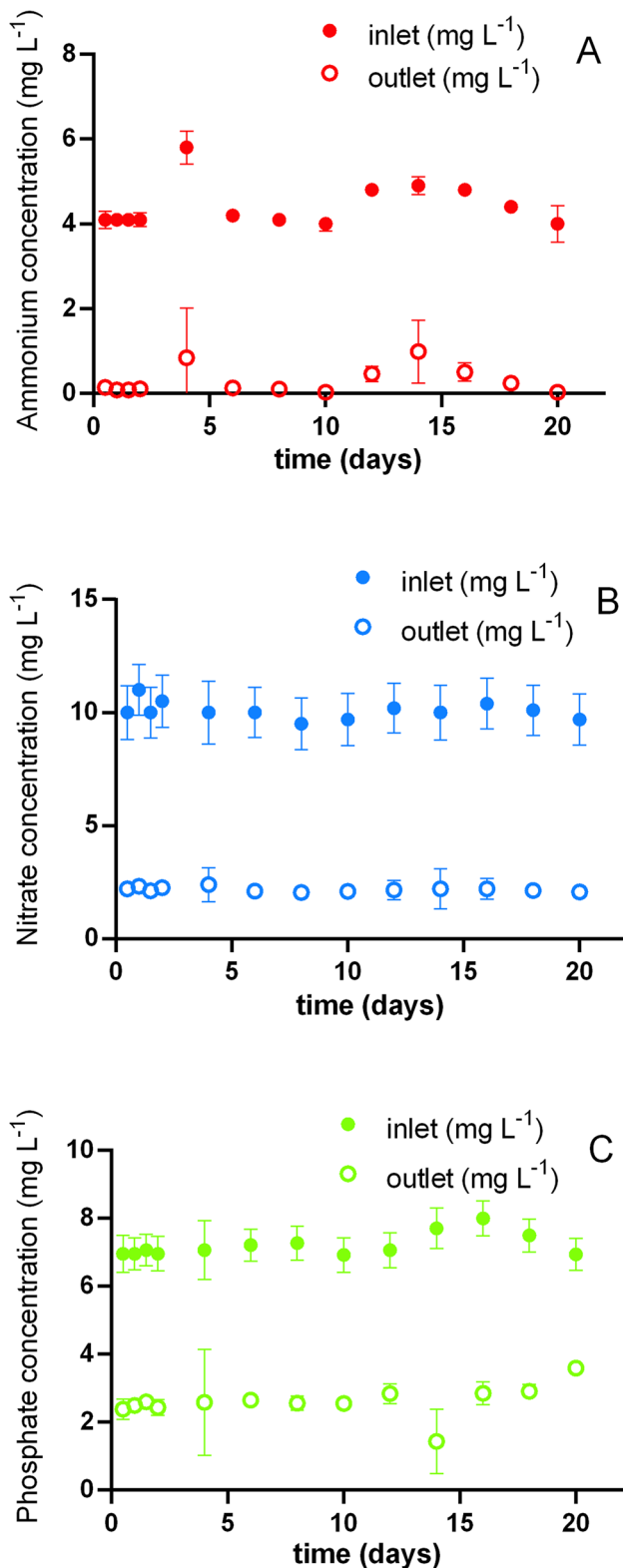
A wide variety of commercial biofilters are available elsewhere for aquaculture facilities running at different operation conditions, enabling comparisons within them (Malone and Pfeiffer 2006). Bacterial biofilters have two approaches, suspended growth and fixed film. Although the first is a lesser standard, microbial floc systems were proposed to produce tilapia and marine shrimp (McIntosh

**Table 4** ANOVA analysis of experiments in the lab-scale optimization

Source	Sum of squares	dF	Mean Sum of squares	F-ratio*	P-Value
A: Water Flow	0.0163808	2	0.0081904	1.11	0.3656
B: Air Flow	0.136382	2	0.0681908	9.28	0.0053
C: Irradiance	0.0973543	2	0.0486772	6.62	0.0147
RESIDUALS	0.0734847	10	0.00734847		
TOTAL (Corrected)	0.370819	16			

(\* ) F-ratio is based on mean residual error square; dF: degrees of freedom

P-value < 0.05



**Fig. 4** Evolution of **A)** ammonium, **B)** nitrate, and **C)** phosphate concentrations in the pilot-scale biofilter operating as a continuous system with natural rainbow trout wastewater. Error bars represent standard deviations ( $N=2$ )

2001; Browdy et al. 2009). A bacterial biofilm is formed either in a moving or fixed carrier. The floating bead filters used in this study typically deplete at 140 to 350 mg L<sup>-1</sup> day<sup>-1</sup> (Malone and Beecher 2000). Microbead or fluidized sand biofilters have good volumetric deputation rates (200–1000 mg L<sup>-1</sup> day<sup>-1</sup> (Timmons et al. 2006); however, they are energy-intensive reactors and high-cost operation systems (Timmons et al. 2006). The floating bead bio-reactor uses plastic carriers (e.g., Kaldness K1) to avoid clogging. The plastic media have a common surface area of 250–500 m<sup>2</sup> m<sup>-3</sup>. The bacterial biomass film covering the plastic carrier usually achieves deputation rates from 200 to 400 mg L<sup>-1</sup> day<sup>-1</sup> (Rusten et al. 2006). These deputation rates are higher than in this study; however, researchers studied and optimized bacterial systems for decades, which is still not the case with our biological system. In response, we could increase the deputation rates by expanding the number of beads per unit of volume though we should find an optimum value for irradiance for pilot-scale biofilter.

Due to its high volatility, a part of NH<sub>3</sub> can be removed abiotically. The rate of ammonia volatilization is known to be pH-dependent. For pH < 8 the proportion of NH<sub>3</sub> is less than 10% (Eshchar et al. 2006). Since, rainbow trout ponds were maintained at a pH around 7, most of the ammonia would be in its ionized form (NH<sub>4</sub><sup>+</sup>). The range of pH for bacteria in moving bed bioreactors is similar to microalgal cultures (Shitu et al. 2022). Therefore, there are no advantages of one system over the other in this aspect. For both systems most of the ammoniacal nitrogen is removed biotically. For instance, in sparged microalgal cultures with pH in the range of 7–8, volatilization was shown to be under 5% (Yu et al. 2022).

The literature describes only a few examples of immobilized algae systems for RAS. Ramli et al. (2017) compared the performance of bubbling column photobioreactors with suspended *Stigeoclonium nanum* or *S. nanum* immobilized in alginate beads. The main advantage of this species is that its filamentous appearance allows it to adapt well to immobilized systems. Their results showed higher TAN removal in immobilized alginate beads than in free suspension cultures. Notwithstanding, the ammonium deputation rate was less than 1 mg L<sup>-1</sup> day<sup>-1</sup> in their best scenario (Ramli et al. 2017). Although limited studies refer to the use of microalgae in RAS facilities, they propose integrating microalgal cultures (suspended or immobilized) as an additional treatment unit (Ramli et al. 2020). The additional unit could be incorporated alone or with a nitrification biofilter. In the first case, the ammonium tolerance of the selected microalgal species is critical. Accordingly, these systems' high retention times and light supply are the two main issues, introducing a meaningful impact on production costs. For example, production costs



**Table 5** Average depuration rates from the pilot-scale biofilter

	DR <sup>1</sup> (mg L <sup>-1</sup> day <sup>-1</sup> )	VDR <sup>2</sup> (mg L <sup>-1</sup> day <sup>-1</sup> )	SDR <sup>3</sup> (mg m <sup>-2</sup> day <sup>-1</sup> )
Ammonium	148.5 ± 10.1	1188 ± 81.2	605.9 ± 0.029
Nitrate	284.7 ± 11.9	2277 ± 0.07	1161 ± 48.6
Phosphate	165.5 ± 23.6	1324 ± 184	675 ± 94.1

<sup>1</sup> liquid volume estimated from Eq. 2

<sup>2</sup> biomedica volume estimated from Eq. 3

<sup>3</sup> biomedica surface estimated from Eq. 4

could grow 20 to 30% if only microalgae assimilate all the ammonia-N generated in a tilapia RAS facility (Ramli et al. 2020). Although these systems are not yet fully optimized, microalgae introduce other vital advantages.

The microbial communities from RAS biofilters with different fish species vary significantly in their composition from one another (Sugita et al. 2005). However, the type of water (freshwater or marine) or specific RAS operating conditions (e.g., installation of UV lamps and ozonizers) leads to unique microbial flora development (Schreier et al. 2010). A nitrification biofilter was originally the source of the bacterial biomass; consequently, it was expected to find *Nitrosomonas*, *Nitrosococcus* and *Nitrospira*. Nonetheless, *Flavobacterium* and *Sphingobacterium* were identified along with the heterotrophic bacteria (e.g. *Pseudomonas stutzeri*), also commonly found in RAS systems. Contrary to expected, the analysis did not show the heterotrophic denitrifiers since anaerobic conditions were incompatible with photosynthetic oxygen produced within the algal–bacterial beads.

Ramli et al. (2017) reported significant differences in the composition of bacterial communities between a conventional RAS system and a RAS supplemented with an algal pond as an additional treatment unit. The authors concluded that the bacteria contributing to those differences were mainly heterotrophic. This additional unit contributed to stabilized nitrite concentrations after any system perturbation.

Our pilot-scale biofilter reached 78% and 64% of the nitrate and phosphate consumption, meaning depuration rates of 284.7 and 165.5 mg L<sup>-1</sup> day<sup>-1</sup>, respectively. These values are high compared to systems that use immobilized microalgae for treating wastewater (tertiary/polishing treatment) (Taziki et al. 2015). However, different strains, operational conditions and immobilization techniques led to considerable differences in the depuration rates. Mallick and Rai (1994) compared N-nitrate and N-nitrite depuration rates of two photosynthetic cells: *Anabaena doliolum* and *Chlorella vulgaris*. The comparison between suspended cells and cells immobilized in chitosan, agar, alginate, and carrageenan showed the best results for chitosan biomedica. In this case, *A. doliolum* and *C. vulgaris* reached nitrate depuration rates of 3.66 and 2.86 µg mg<sup>-1</sup> h<sup>-1</sup> (based on dry biomass), respectively. Our study reached nitrate depuration rates of 86.2 µg mg<sup>-1</sup> h<sup>-1</sup> (based on dry biomass). The

inhibition effect of high ammonium concentration or other inhibitor constituents of the wastewater could explain these differences. Algal–bacterial beads used in this study performed well in nitrate removal, even if we compared them to suspended microalgal cultures. For example, the best results found for *C. vulgaris* and *Neochloris oleoabundans* are 103.3 and 150.74 mg L<sup>-1</sup> day<sup>-1</sup>, respectively (Li et al. 2008; Hulatt et al. 2012).

Unlike nitrate, microalgae actively store phosphate (Powell et al. 2009). They face low concentrations of phosphate in their natural environment. Therefore, microalgae must store a large amount of phosphate whenever it becomes available (Solovchenko et al. 2019). However, high concentrations of phosphate could inhibit growth. For instance, more than 150 mg L<sup>-1</sup> and 24 mg L<sup>-1</sup> inhibited growth of the green microalga *Chlorella* (Li et al. 2018)) and the dinoflagellate *Peridinium reticulatum* (Gallardo-Rodríguez et al. 2009), respectively. Phosphate uptake rates strongly depend on the strain and operational conditions. To illustrate, suspended *C. vulgaris* achieved a phosphate depuration rate of 62 mg L<sup>-1</sup> day<sup>-1</sup> (Singh et al. 2018), but phosphate depuration rates by *C. vulgaris* decreased to 2 mg L<sup>-1</sup> day<sup>-1</sup> for wastewater (Lavrinovics et al. 2021). Other species showed values even lower, as Lavrinovics et al. (2021) reported.

## Conclusions

We demonstrated the improvement in the depuration rates using a biofilter with immobilized algal–bacterial beads. The ammonium depuration rates of the pilot-scale continuous photo-biofilter were comparable to industrial bacterial biofilters, but our photo-biofilters could reduce operational costs and recycle exhausted algal–bacterial biomass. The primary concern is the beads' mechanical and physical stability and durability. Further investigation should predict the life span of beads to determine the harvesting time of biofilters biomedica. The algal–bacterial biomass from our biofilters is a valuable by-product of the aquaculture facilities, unlike typical plastic biomedica. Microalgae capture different forms of nitrogen and phosphorus, unlike bacteria of traditional nitrification–denitrification systems. Accordingly, the obvious application for our algal–bacterial biomass is

the production of biofertilizers and biostimulants. Ongoing studies also include transforming biomass into new applications like biomaterials.

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**Data availability** Data will be available from the corresponding author on request.

## Declarations

**Conflict of interest** The authors declare no conflict of interest.

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## References

- Acien-Fernández FG, García Camacho F, Chisti Y (1999) Photobioreactors: Light regime, mass transfer, and scaleup. *Prog Ind Microbiol* 35:231–247
- APHA (2012) Standard methods for the examination of water & wastewater, 22nd edn. American Public Health Association, Washington DC
- ASTM (2008) Standard test methods for ammonia nitrogen in water ASTM D1426–08. ASTM International, West Conshohocken
- Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL (2008) GenBank. *Nucleic Acids Res* 36:25–30
- Bordel S, Guieysse B, Muñoz R (2009) Mechanistic model for the reclamation of industrial wastewaters using algal–bacterial photobioreactors. *Environ Sci Technol* 43:3200–3207
- Button DK, Schut F, Quang P, Martin R, Robertson BR (1993) Viability and isolation of typical marine oligotrophic bacteria by dilution culture: theory, procedures and initial results. *Appl Environ Microbiol* 59:881–891
- Bregnballe J (2015) A guide to recirculation aquaculture. Food and Agriculture Organization of the United Nations (FAO) and EUROFISH International Organisation. <https://www.fao.org/3/i4626e/i4626e.pdf>
- Browdy CL, Venero JA, Stokes AD, Leffler J (2009) Superintensive bio-floc production technologies for marine shrimp *Litopenaeus vannamei*: Technical challenges and opportunities. In: Burnell G, Allan G (Eds), *New Technologies in Aquaculture*, Woodhead Publishing, Cambridge, pp 1010–1028
- Calicioglu O, Demirer GN (2019) Carbon-to-nitrogen and substrate-to-inoculum ratio adjustments can improve co-digestion performance of microalgal biomass obtained from domestic wastewater treatment. *Environ Technol* 40:614–624
- Ciji A, Akhtar MS (2020) Nitrite implications and its management strategies in aquaculture: a review. *Rev Aquac* 12:878–908
- Davidson J, Good C, Welsh C, Summerfelt ST (2014) Comparing the effects of high vs. low nitrate on the health, performance, and welfare of juvenile rainbow trout *Oncorhynchus mykiss* within water recirculation aquaculture systems. *Aquac Eng* 59:30–40
- de-Bashan LE, Bashan Y (2010) Immobilized microalgae for removing pollutants: a review of practical aspects. *Bioresour Technol* 101:1611–27
- de Godos I, González C, Becares E, García-Encina PA, Muñoz R (2009) Simultaneous nutrients and carbon removal during pre-treated swine slurry degradation in a tubular biofilm photobioreactor. *Appl Microbiol Biotechnol* 82:187–194
- Delong DP, Losordo TM (2012) How to start a biofilter, SRAC Publication N° 4502. [http://aquaculture.ca.uky.edu/sites/aquaculture.ca.uky.edu/files/srac\\_4502\\_how\\_to\\_start\\_a\\_biofilter.pdf](http://aquaculture.ca.uky.edu/sites/aquaculture.ca.uky.edu/files/srac_4502_how_to_start_a_biofilter.pdf)
- Eroglu E, Smith SM, Raston CL (2015) Application of various immobilization techniques for algal bioprocesses. In: Moheimani N, McHenry M, de Boer K, Bahri P (Eds) *Biomass and biofuels from microalgae*. Springer, Cham, pp 19–24
- Eshchar M, Lahav O, Mozes N, Peduel A, Ron B (2006) Intensive fish culture at high ammonium and low pH. *Aquaculture* 255:301–313
- Gallardo-Rodríguez JJ, Sánchez-Mirón A, Cerón-García MC, Belarbi EH, García-Camacho F, Chisti Y, Molina-Grima E (2009) Macro-nutrients requirements of the dinoflagellate *Protoceratium reticulatum*. *Harmful Algae* 8:239–246
- Gonçalves AL, Pires JCM, Simões M (2017) A review on the use of microalgal consortia for wastewater treatment. *Algal Res* 24:403–415
- Gupta VK, Sadeh H, Yari M, ShahryariGhoshekandi R, Maazinejad B, Chahardori M (2015) Removal of ammonium ions from wastewater: A short review in development of efficient methods. *Glob J Environ Sci Manag* 1:149–158
- Gutierrez-Wing MT, Malone RF (2006) Biological filters in aquaculture: Trends and research directions for freshwater and marine applications. *Aquac Eng* 34:163–171
- Hagopian DS, Riley JG (1998) A closer look at the bacteriology of nitrification. *Aquac Eng* 18:223–244
- He S, Xue G (2010) Algal-based immobilization process to treat the effluent from a secondary wastewater treatment plant (WWTP). *J Hazard Mater* 178:895–899
- Hulatt CJ, Lakaniemi AN, Puhakka JA, Thomas DN (2012) Energy demands of nitrogen supply in mass cultivation of two commercially important microalgal species, *Chlorella vulgaris* and *Dunaliella tertiolecta*. *BioEnergy Res* 5:669–684
- Jayakumar S, Yusoff MM, Rahim MHA, Maniam GP, Govindan N (2017) The prospect of microalgal biodiesel using agro-industrial and industrial wastes in Malaysia. *Renew Sustain Energy Rev* 72:33–47
- Kaminuma E, Mashima J, Kodama Y, Gojibori T, Ogasawara O, Okubo K, Takagi T, Nakamura Y (2010) DDBJ launches a new archive database with analytical tools for next-generation sequence data. *Nucleic Acids Res* 38:33–38
- Lavrinovics A, Murby F, Ziverte E, Mežule L, Juhna T (2021) Increasing phosphorus uptake efficiency by phosphorus-starved microalgae for municipal wastewater post-treatment. *Microorganisms* 9:1598

- Leinonen R, Akhtar R, Birney E, Bower L, Cerdano-Tárraga A, Cheng Y, Cleland I, Faruque N, Goodgame N, Gibson R, Hoad G, Jang M, Pakseresht N, Plaister S, Radhakrishnan R, Reddy K, Sobhany S, Ten Hoopen P, Vaughan R, Zalunin V, Cochrane G (2011) The European nucleotide archive. *Nucleic Acids Res* 39:28–31
- Li Y, Horsman M, Wang B, Wu N, Lan CQ (2008) Effects of nitrogen sources on cell growth and lipid accumulation of green alga *Neochloris oleoabundans*. *Appl Microbiol Biotechnol* 81:629–636
- Li Q, Fu L, Wang Y, Zhou D, Rittmann BE (2018) Excessive phosphorus caused inhibition and cell damage during heterotrophic growth of *Chlorella regularis*. *Bioresour Technol* 268:266–270
- Macintyre HL, Kana TM, Anning T, Geider RJ (2002) Photoacclimation of photosynthesis irradiance response curves and photosynthetic pigments in microalgae and cyanobacteria. *J Phycol* 38:17–38
- Mallick N (2002) Biotechnological potential of immobilized algae for wastewater N, P and metal removal: A review. *Biometals* 15:377–390
- Mallick N, Rai LC (1994) Removal of inorganic ions from wastewaters by immobilized microalgae. *World J Microbiol Biotechnol* 10:493–443
- Malone RF, Beecher LE (2000) Use of floating bead filters to recondition recirculating waters in warmwater aquaculture production systems. *Aquac Eng* 22:57–73
- Malone RF, Pfeiffer TJ (2006) Rating fixed film nitrifying biofilters used in recirculating aquaculture systems. *Aquac Eng* 34:389–402
- Markou G, Georgakakis D (2011) Cultivation of filamentous cyanobacteria (blue-green algae) in agro-industrial wastes and wastewaters: A review. *Appl Energy* 88:3389–3401
- Martins CIM, Pistrin MG, Ende SSW, Eding EH, Verreth JAJ (2009) The accumulation of substances in Recirculating Aquaculture Systems (RAS) affects embryonic and larval development in common carp *Cyprinus carpio*. *Aquaculture* 291:65–73
- McIntosh RP (2001) High rate bacterial systems for culturing shrimp. In: *Proceedings from the Aquacultural Engineering Society's Second Issues Forum*, Ithaca, NRAES-157, pp 117–129
- Molina E, Fernández J, Acién FGG, Chisti Y (2001) Tubular photobioreactor design for algal cultures. *J Biotechnol* 92:113–131
- Muñoz R, Guieysse B (2006) Algal–bacterial processes for the treatment of hazardous contaminants: A review. *Water Res* 40:2799–2815
- Pedersen LF, Suhr KI, Dalsgaard J, Pedersen PB, Arvin E (2012) Effects of feed loading on nitrogen balances and fish performance in replicated recirculating aquaculture systems. *Aquaculture* 338–341:237–245
- Pires JCM, Alvim-Ferraz MCM, Martins FG, Simões M (2013) Wastewater treatment to enhance the economic viability of microalgae culture. *Environ Sci Pollut Res* 20:5096–5105
- Powell N, Shilton A, Chisti Y, Pratt S (2009) Towards a luxury uptake process via microalgae – Defining the polyphosphate dynamics. *Water Res* 43:4207–4213
- Qin G, Liu CCK, Richman NH, Moncur JET (2005) Aquaculture wastewater treatment and reuse by wind-driven reverse osmosis membrane technology: a pilot study on Coconut Island. *Hawaii Aquac Eng* 32:365–378
- Ramli NM, Verdegem MCJ, Yusoff FM, Zulkifely MK, Verreth JAJ (2017) Removal of ammonium and nitrate in recirculating aquaculture systems by the epiphyte *Stigeoclonium nanum* immobilized in alginate beads. *Aquac Environ Interact* 9:213–222
- Ramli NM, Verreth JAJ, Yusoff FM, Nurulhuda K, Nagao N, Verdegem MCJ (2020) Integration of algae to improve nitrogenous waste management in recirculating aquaculture systems: a review. *Front Bioeng Biotechnol* 8:1004
- Rawat I, Ranjith Kumar R, Mutanda T, Bux F (2011) Dual role of microalgae: Phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. *Appl Energy* 88:3411–3424
- Roalkvam I, Drønen K, Dahle H, Wergeland HI (2020) Comparison of active biofilm carriers and commercially available inoculum for activation of biofilters in marine recirculating aquaculture systems (RAS). *Aquaculture* 514:734480
- Rusten B, Eikebrokk B, Ulgenes Y, Lygren E (2006) Design and operations of the Kaldnes moving bed biofilm reactors. *Aquac Eng* 34:322–331
- Sánchez Mirón A, García Camacho F, Contreras Gómez A, Molina Grima E, Chisti Y (2000) Bubble-column and airlift photobioreactors for algal culture. *AIChE J* 46:1872–1887
- Schreier HJ, Mirzoyan N, Saito K (2010) Microbial diversity of biological filters in recirculating aquaculture systems. *Curr Opin Biotechnol* 21:318–325
- Shitu A, Liu G, Muhammad AI, Zhang Y, Tadda MA, Qi W, Liu D, Ye Z, Zhu S (2022) Recent advances in application of moving bed bioreactors for wastewater treatment from recirculating aquaculture systems: A review. *Aquac Fish* 3:244–258
- Singh D, Nedbal L, Ebenhöf O (2018) Modelling phosphorus uptake in microalgae. *Biochem Soc Trans* 46:483–490
- Solovchenko AE, Ismagulova TT, Lukyanov AA, Vasilieva SG, Konyukhov IV, Pogosyan SI, Lobakova ES, Gorelova OA (2019) Luxury phosphorus uptake in microalgae. *J Appl Phycol* 31:2755–2770
- Subashchandrabose SR, Ramakrishnan B, Megharaj M, Venkateswarlu K, Naidu R (2011) Consortia of cyanobacteria/microalgae and bacteria: biotechnological potential. *Biotech Adv* 29:896–907
- Sun X, Li X, Tang S, Lin K, Zhao T, Chen X (2022) A review on algal-bacterial symbiosis system for aquaculture tail water treatment. *Sci Total Environ* 847:157620
- Taziki M, Ahmadzadeh H, Murry MA, Lyon SR (2015) Nitrate and nitrite removal from wastewater using algae. *Curr Biotechnol* 4:426–440
- Timmons MB, Holder JL, Ebeling JM (2006) Application of microbead biological filters. *Aquac Eng* 34:332–343
- Wu JY, Lay CH, Chen CC, Wu SY (2017) Lipid accumulating microalgae cultivation in textile wastewater: Environmental parameters optimization. *J Taiwan Inst Chem Eng* 79:1–6
- Yu H, Kim J, Rhee C, Shin J, Shin SG, Lee C (2022) Effects of different pH control strategies on microalgae cultivation and nutrient removal from anaerobic digestion effluent. *Microorganisms* 10:357
- Zhang M, Yu M, Wang Y, He C, Pang J, Wu J (2019) Operational optimization of a three-stage nitrification moving bed biofilm reactor (NMBBR) by obtaining enriched nitrifying bacteria: Nitrifying performance, microbial community, and kinetic parameters. *Sci Total Environ* 697:134101

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