

**This is a self-archived version of an original article. This version may differ from the original in pagination and typographic details.**

**Author(s):** Kujala, Kukka; Pulkkinen, Jani; Vielma, Jouni

**Title:** Discharge management in fresh and brackish water RAS : Combined phosphorus removal by organic flocculants and nitrogen removal in woodchip reactors

**Year:** 2020

**Version:** Accepted version (Final draft)

**Copyright:** © 2020 Elsevier BV

**Rights:** CC BY-NC-ND 4.0

**Rights url:** <https://creativecommons.org/licenses/by-nc-nd/4.0/>

**Please cite the original version:**

Kujala, K., Pulkkinen, J., & Vielma, J. (2020). Discharge management in fresh and brackish water RAS : Combined phosphorus removal by organic flocculants and nitrogen removal in woodchip reactors. *Aquacultural Engineering*, 90, Article 102095.  
<https://doi.org/10.1016/j.aquaeng.2020.102095>

# Journal Pre-proof

Discharge management in fresh and brackish water RAS: combined phosphorus removal by organic flocculants and nitrogen removal in woodchip reactors

Kukka Kujala (Investigation) (Writing - review and editing), Jani Pulkkinen (Investigation) (Supervision) <ce:contributor-role>Writing – review and editing), Jouni Vielma <ce:contributor-role>Writing – original draft)



PII: S0144-8609(19)30230-4  
DOI: <https://doi.org/10.1016/j.aquaeng.2020.102095>  
Reference: AQUE 102095  
  
To appear in: *Aquacultural Engineering*  
  
Received Date: 19 December 2019  
Revised Date: 7 May 2020  
Accepted Date: 8 May 2020

Please cite this article as: Kujala K, Pulkkinen J, Vielma J, Discharge management in fresh and brackish water RAS: combined phosphorus removal by organic flocculants and nitrogen removal in woodchip reactors, *Aquacultural Engineering* (2020), doi: <https://doi.org/10.1016/j.aquaeng.2020.102095>

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2020 Published by Elsevier.

## Discharge management in fresh and brackish water RAS: combined phosphorus removal by organic flocculants and nitrogen removal in woodchip reactors

Kukka Kujala<sup>a</sup>, Jani Pulkkinen<sup>b</sup>, Jouni Vielma<sup>b,\*</sup> jouni.vielma@luke.fi

<sup>a</sup>Department of Biological and Environmental Science, University of Jyväskylä, P.O. Box 35, 40014 Jyväskylä, Finland

<sup>b</sup>Natural Resources Institute Finland (LUKE), , Survantie 9A, 40500 Jyväskylä, Finland

\*Corresponding author.

### Highlights

- Increased carbon concentration by organic flocculant use enhanced nitrate removal in the woodchip reactors
- Denitrification was incomplete in freshwater woodchip reactors
- Ammonia increase in woodchip reactors suggest dissimilatory nitrate removal to ammonia (DNRA)

### Abstract

The current study combined P and N removal using organic flocculant chemicals and woodchip bioreactors in both freshwater and brackish water (7 ppm) recirculating aquaculture systems (RAS). The use of carbon (C) containing flocculant chemicals in the process was hypothesized to further stimulate C-demanding N removal (denitrification) in bioreactors. The trial of combined P and N removal consisted of four treatments: freshwater and brackish water RAS with and without the addition of supernatant from flocculation process to the woodchip reactor. Duplicate woodchip reactors were used per treatment and the trial was run for six weeks. 56 % and 49 % of P was removed from fresh and brackish sludge water, respectively. The nitrate-N ( $\text{NO}_3\text{-N}$ ) removal rate was improved in the treatment when supernatant from flocculation process was used together with RAS discharge water when compared against the control. In brackish water RAS, the improvement was more pronounced (from 6.6 to 16.5 g  $\text{NO}_3\text{-N m}^{-3} \text{ d}^{-1}$ ) than in freshwater RAS (from 5.1 to 6.5  $\text{NO}_3\text{-N m}^{-3} \text{ d}^{-1}$ ). In the freshwater bioreactors using supernatant, N was largely discharged as a nitrite-N ( $\text{NO}_2\text{-N}$ ). High  $\text{NO}_2\text{-N}$  concentrations in freshwater reactors allude to incomplete denitrification reactions taking place. The results suggest that the organic flocculants did provide an additional C source for denitrification, which improved the N-removal process. However, in freshwater RAS this might have been partly due to untargeted processes such as DNRA (dissimilatory nitrate reduction to ammonium), and/or insufficient denitrification reactions taking place (excessive  $\text{NO}_2\text{-N}$  production).

Keywords: Woodchip bioreactor; Recirculating aquaculture, Wastewater, Flocculation

## 1. Introduction

Aquaculture in open systems such as net cages, raceways and ponds provide the vast majority of the global farmed fish but recirculating aquaculture systems (RAS) farming is a growing technology to raise fish for several reasons (e.g., Dalsgaard et al., 2013). Firstly, RAS farming allow better control over the production, such as temperatures for species outside their natural geographical regions. Secondly, due to intense water re-use, RAS farms can be located at places with more limited water supply than flow-through farming would require. Thirdly, RAS farming can have less environmental impact due to better control over escapees, parasites and nutrient discharges. Reduction in nutrient discharges is high on agenda in several countries, and due to limited amount of new and discharged water, efficient nutrient removal technologies can be utilized and far larger RAS farms can be established than would be possible by conventional technologies. Low nutrient discharges is the strongest argument for supporting expansion of RAS farming in Finland, where aquaculture is strictly governed by environmental legislation (Soininen et al., 2019).

Typical municipal wastewater treatment processes, coagulation and flocculation followed by mechanical treatment are common for RAS sludge treatment (e.g., van Rijn, 2013). Early studies on phosphorus (P) and organic matter removal by sludge thickening in RAS were published by Ebeling et al. (2003, 2005, 2006), followed later by Sharrer et al. (2009), Guerdat et al. (2013) and Zhang et al. (2014). In these studies, P removal efficiency has varied widely from 32 to 95 %. Coagulation, which is typically the first step in the sludge treatment, is an electrochemical process. Most suspended solids have a negative charge and by coagulation, particle surface charge is neutralized which destabilize the suspension and particles settle down (e.g., Wei et al., 2018). Coagulants are small inorganic molecules such as  $\text{AlCl}_3$ ,  $\text{Al}_2(\text{SO}_4)_3$ , or  $\text{FeCl}_3$ , or larger inorganic polymeric molecules such as poly-aluminum chloride (PAC), but they can also be organic polymers (e.g., Cheremisinoff, 2002). Flocculation, which is typically the second sludge treatment step, establishes chemical bridges between the settled particles and flocs, larger than achieved by coagulation alone, are formed. Flocculation aids are usually long chain inorganic or organic polymers and they can be further classified based on the molecular weight and on the electric charges to nonionic, cationic, anionic or ampholytic (Wei et al., 2018). Due to the potential risks of residual metal ions or the release of noxious polymeric monomers such as acrylamides into the target water, natural (organic) polymeric flocculants are being increasingly developed for municipal wastewater treatment (Lee et al., 2014). Acrylamide in sludge may become an issue also when global RAS production increases and ways to utilize sludge are considered. However, organic polymers as flocculant aid have not been studied in RAS environment before.

In addition to P removal requirements, nitrogen (N) removal from RAS effluent has become compulsory in several countries such as Denmark (Nielsen, 2012). Dedicated denitrification processes for effluent N control exist, e.g., single-sludge treatment (Suhr et al., 2014), upflow anoxic sludge bed reactor (Letelier-Gordo et al., 2019), and woodchip reactor (von Ahnen et al., 2018). Woodchip denitrification appears to offer affordable, technically simple and fairly stable N removal for RAS farms (Lepine et al., 2018).

The objective of this study was to assess whether carbon from the organic flocculant would aid the denitrification process in the woodchip reactor. Treatment efficiencies were studied at two salinities relevant for the northern Baltic Sea environment of the Finnish coastal aquaculture.

## 2. Material and methods

### 2.1 System description

Two trials were conducted. In the first series of trials, potato starch-based flocculants were screened by jar tests. In the second trial, the most promising flocculant treatment was combined with a woodchip reactor for combined P and N removal. Trials were conducted at the Natural Resources Institute Finland (LUKE), Laukaa fish farm.

To produce the waste streams, two laboratory scale RAS-units were used, one freshwater and one converted to brackish water (7 ppt salinity, Instant Ocean® Sea Salt, Spectrum Brands, Blacksburg, USA). The product has proprietary balanced composition is frequently used by those marine aquariums, which do not have access to sea water. Salt was added in the make-up water reservoir, the make-up water volume being 500 L per kg feed. The experimental RAS set-up has been described by Pulkkinen et al. (2018). In short, each RAS unit consists of a 500 L bottom drained plastic rearing tank (Arvo-Tec, Joroinen, Finland), a feed collector unit, a 24 cm swirl separator (Eco-Trap Collector1, Pentair Aquatic Eco-Systems, Minneapolis, USA), a drum filter with 60 µm filter panels (Hydrotech HDF501, Veolia, Paris, France), a 147 L fixed bed bioreactor, a 147 L moving bed bioreactor (Arvo-Tec, Joroinen, Finland), a trickling filter (Bio-Blok® 200, EXPO-NET Danmark A/S, Hjørring, Denmark) and a pump sump. In this particular trial, drum filters were by-passed to get more constant sludge from the swirl separators. pH was adjusted to 7.2 in the pump sump with diluted sodium hydroxide using an automated system (Prominent, Heidelberg, Germany).

During the trials, rainbow trout (*Oncorhynchus mykiss*) growing from an average of 281 to 410 grams were maintained in the RAS units. Fish were fed at a constant ratio of 100 grams per day. Periodically, individuals were removed to avoid too restricted feeding. Fish were fed with Orbit 929 (BioMar, Denmark) analyzed at Synlab accredited laboratory to contain 43.5 % protein, 33.6 % fat and 0.95 % P.

### 2.2 Flocculant jar tests

Initial jar test screening included the following flocculants based on potato starch (Chemigate Oy, Finland): PrimePHASE 3545 (high molecular weight, 2.5 meq g<sup>-1</sup>), PrimePHASE 3525 (average molecular weight, 2.5 meq g<sup>-1</sup>), PrimePHASE 3501 (low molecular weight, 2.5 meq g<sup>-1</sup>), and PrimePHASE 1501 (low molecular weight, 1.0 meq g<sup>-1</sup>). In the screening, polyaluminum chloride (PAC) PAX-XL100 (Kemira Oyj, Finland) was dosed at 50 mg active substance per L sludge. Al<sup>3+</sup> concentration of the product is 9.3 %, providing Al:P molar ratio 1.09 in freshwater and 0.85 in brackish water. PAC was mixed at 100 rpm for 1 min, followed by adding the flocculants, whereafter the sludge was mixed slowly at 20 rpm for 20 min using Lovibond ET 750 jar test apparatus. Finally, the sludge was left to settle for 20 min. The working solution was 10% of the commercial products for both PAC and the flocculants. Floc strength was visually observed and supernatant phosphate and turbidity measured. During jar tests, sludge contained on average (four daily samples) in the fresh water system: TSS 0.88 g L<sup>-1</sup>, tot-P 21.9 mg L<sup>-1</sup> and COD 1.24 g L<sup>-1</sup>, and in brackish water: TSS 0.95 g L<sup>-1</sup>, tot-P 25.5 mg L<sup>-1</sup> and COD 0.98 g L<sup>-1</sup>.

Due to the low floc strength achieved in all screening jar tests with cationic flocculants, series with a combination of two flocculants, anionic ( $-1.0 \text{ meq g}^{-1}$ ) high molecular weight PrimeBOND A0415 and cationic ( $2.5 \text{ meq g}^{-1}$ ) high molecular weight PrimePHASE 3545 was conducted. Twentyfour hour sludge from the swirl separators was collected, diluted to 10 L with water from the RAS units, and mixed with horizontal restaurant mixer. PAC at  $50 \text{ mg L}^{-1}$  sludge was mixed at 100 rpm for 1 min, followed by 5 min mixing with PrimeBOND and thereafter 20 min mixing with PrimePHASE for 20 min at 20 rpm. Finally, the sludge was allowed to sediment for 20 min. Both flocculants were added at 0, 10, 30, 50, 100, 200 or  $400 \text{ mg L}^{-1}$  sludge. For example at treatment 30, first PrimeBOND was added at  $30 \text{ mg L}^{-1}$  sludge and thereafter PrimePHASE was added at  $30 \text{ mg L}^{-1}$  sludge. Supernatant turbidity and phosphate-P were measured, and floc visually observed.

### 2.3 Combined P removal with woodchip denitrification

Based on jar tests, a flocculation procedure for P removal was selected and combined with woodchip reactor N removal. The trial consisted of four treatments: freshwater and brackish water RAS with and without P flocculation supernatant addition to the woodchip reactor. Duplicate woodchip reactors were used per treatment and the trial was run for six weeks.

At the beginning of the trial, two systems were stocked with 10.1 kg of rainbow trout each and fish were fed at a constant feed load of 100 grams per day. Twenty-four hour sludge from the swirl separators was collected two times per week, diluted to 10 L with water from the RAS units, and mixed using a horizontal restaurant mixer. This sludge batch was first mixed for 1 min at 100 rpm with PAC  $50 \text{ mg L}^{-1}$ . Thereafter PrimeBOND A0415 was added at  $100 \text{ mg L}^{-1}$  (weight of the product per liter sludge) and mixed at 40 rpm for 5 min followed by PrimePHASE 3545 added at  $100 \text{ mg L}^{-1}$  and mixed for an additional 20 min. Flocculant doses were selected on the basis of floc formation (visual observation) during jar tests. Moreover, doses above  $200 \text{ mg L}^{-1}$  increased turbidity while did not improve P removal in the supernatant. After 20 min sedimentation, supernatants were removed and stored in containers for consequent pumping into woodchip reactors.

RAS outflows were collected in 32 L containers and pumped to the woodchip reactors using peristaltic pumps (Ismatec SM1089, Cole-Parmer GmbH, Wertheim, Germany) (Fig 1). Supernatants were pumped from the storage containers to the woodchip reactors using peristaltic pumps (Watson Marlow 323, Spirax Sarco Engineering, Cheltenham, UK). Horizontal flow was used so that the inflow to the reactor was at the top of the reactor and the outflow at the bottom of the reactor. Reactors were filled above water level with sieved (5 mm) birch woodchips (*Betula pendula* and *Betula pubescens*). Average volume of one control reactor without the woodchips was 10.1 L and 13.7 L in the treatment reactors. Reactor volumes were different in the treatment group, which received two wastewater outflows so that the hydraulic retention time would be equal in all reactors. Empty bed contact time (EBCT = hydraulic retention time of the reactor without the woodchips) was set to 24 h in all of the reactors, and the hydraulic loading rate  $2.1 \text{ cm per hour}$ .

### 2.4 Water sampling and analyses

Sludge analyses were conducted using the following methods: total suspended solids, TSS (SFS-EN 872:2005), loss of ignition to calculate total volatile suspended solids (SFS-EN 872:2005, modif.), TOT-N (SFS-EN ISO 11905-1:1998), TOT-P (ISO 15923-1:2013) and COD (SFS 5504:1988). Inlet and outlet water of the woodchip reactors was analyzed once per week. Total ammonia nitrogen (TAN), nitrite, nitrate and orthophosphate were analyzed using a spectrophotometer (DS 3900, Hach, Loveland, USA). Alkalinity was analyzed by titration following standard method (ISO 9963-1:1994) (TitraLab AT1000, Hach, Loveland, USA). Turbidity was measured by Hach 2100Q turbidity meter.

## 2.5 Statistics

Effects of treatments on nitrate, nitrite and TAN removal rates were analyzed with two-way analysis of variance (two-way ANOVA), where salinity and supernatant treatment were fixed variables.

## 3. Results and discussion

### 3.1 Sludge characteristics

Solids were collected from the swirl separators and subsequently diluted to 100 L per kg feed, resembling drum filter sludge volume and TSS contents. Despite constant feeding of 100 g per day in fresh and brackish water, brackish water RAS sludge had more solids, organics, P and N in the six weekly samples during the combined P and N removal trial. Fish were weighed in the beginning and end of the trial, and in freshwater FCR was 0.79, compared to 1.01 in the brackish water. No feed wastage was observed during the trial, and the higher feed conversion ratio in brackish water suggest a metabolic load to fish due to salinity. However, the main emphasis of the trial was on combined P and N removal, and RAS as waste production units were not replicated and reasons for the different FCR are not clear.

Sludge mean TSS contents in the present study were 377-585 mg L<sup>-1</sup>, with a range on 200-920 mg L<sup>-1</sup> in individual daily samples (Table 1). In other RAS coagulation and flocculation studies, sludge TSS contents have varied widely between 80-1900 mg L<sup>-1</sup> as summarized in Table 2. The variation is most likely due to different solids collection systems, including micro screen and settler effluent and bead filter backwash water. Values of the present trial are in the lower range of the variation, resembling values of micro screen backwash 520-720 mg L<sup>-1</sup> in Summerfelt and Penne (2005). Systems include drum filter, where backwash starts automatically when the water level inside the drum elevates up to the level of electronic contactor. For this particular reason drum filters were by-passed for this trial, as daily variation in the TSS would have been larger if sludge were collected from the drum filters than by collecting the solids at the bottom of the swirl separators.

Mean tot-P of the sludge used for coagulation and flocculation was 14-18 mg L<sup>-1</sup>, with a range of 10-23 mg L<sup>-1</sup> in individual daily samples. Reported tot-P contents of sludge research literature vary widely from few to tens mg L<sup>-1</sup> (Table 2). In the studies cited in Table 2, Al:P molar ratio has not been provided, while in the present work, Al:P molar ratio was 1.09 in freshwater and 0.85 in brackish water.



Proportion of volatile SS of total SS in sludge was 82 and 87 % in freshwater and brackish water systems (Table 1), compared to 65 % in Suhr et al. (2015) and 56-74 % in Letelier-Gordo et al. (2015), thus confirming the fresh undigested state of the sludge used for P coagulation and flocculation in the present study.

### 3.2 Phosphate removal in jar tests

During the screening, phosphate removal efficiency with PAC and most flocculants was 70-80 %, solids sedimented well in 20 min and supernatant turbidity was low at appr. 2-5 FTU in the most efficient treatments. However, floc formation with the tested anionic and cationic flocculants was not satisfactory under our conditions. Under practical RAS conditions, sedimentation may be a too lengthy process and instead of sedimentation, solids may need to be rapidly removed with belt or other filter type, which would require large intact flocs. Of the potato starch based flocculants screened in the present trial, only introducing PrimeBOND A0415, a cationic high molecular weight flocculants mainly for paper manufacturing, provided intact flocs and reasonably good phosphate removal (Table 3). In fresh and brackish water, P removal efficiencies were 73-84 and 75-84 %, respectively. In perhaps the first thorough coagulation study on RAS effluent, Ebeling et al. (2003) achieved 80-90 % soluble reactive P removal. They studied alum and ferric chloride at different concentrations, also varying the mixing speeds and time, by jar tests. Polyacrylamide products gave very high reactive P removal efficiencies of 92-95 % in jar tests by Ebeling et al. (2005). In Ebeling et al. (2006), alum and various flocculant polymers were studied first in jar tests followed by a trial using commercial size belt filters. Highest doses of both alum and polymer resulted in best reactive phosphate reduction of 80 %, and tot-P reduction was at highest 93 % with the belt filter. Sharrer et al. (2009) mixed drum filter sludge with alum, ferric chloride, or hydrated lime in combination with polyacrylamide polymer and led the sludge into geotextile (Geotube) solids and nutrient capture. In that study, tot-P removal efficiency varied from 47 to 77%. Finally, a study by Zhang et al. (2014) may be one of the only P coagulation/flocculation studies in marine RAS and they achieved tot-P removal efficiency above 85 % in jar tests using  $\text{FeCl}_3$  and polymeric aluminum sulfate as coagulants. In summary, reduction in sludge phosphate in our study was within the range of published studies.

### 3.3 Phosphorus balance in the woodchip reactors

In the present setup, majority of P removal took place in the sludge thickening, which was not replicated but instead produced the supernatant for the replicated woodchip reactors. After one week of operation, all treatments discharged more P than received in the inlet indicating P release from the woodchip bed (Figure 2). P release from the woodchips was most pronounced in the freshwater control, where clearly negative P balance continued for the entire six week trial. P release from the woodchip material has also been reported by von Ahnen et al. (2016). In their study, the initial reactor effluent concentrations were very high at  $47 \text{ mg L}^{-1}$  but declined rapidly within the first few days. In the present study, initial woodchip reactor effluent P concentrations were around  $6\text{-}8 \text{ mg L}^{-1}$  and thus the initial flushing was much more moderate than in the pilot-scale trial of von Ahnen et al. (2016). Healy et al. (2012) reported elevated phosphate levels in pine woodchip reactor effluents for up to several months compared to steady-state levels. Although P

release could be diminished by flushing the woodchip in advance, observation by Healy et al. (2012) suggests it may be quite difficult to do in practice. In the present study, all other reactors but freshwater control remained close to P neutrality with removal rates from -8 % to 17 % by the end of the trial. Based on our data, we cannot suggest possible causes of such a large difference between freshwater control and other treatment groups.

### 3.4 Influence of organic flocculation on denitrification performance and nitrogen compounds in the woodchip reactors

The flocculant addition increased the carbon inflow concentration to the woodchip reactors (Table 4). In the control reactors, outflow had higher carbon concentration than inflow, indicating that carbon leached from the woodchips more than was used in the denitrification process. In the treatment groups, flocculant addition increased the carbon inflow and it was used in the woodchip reactors.

The average inflow  $\text{NO}_3\text{-N}$  to woodchip reactors during the six week trial was 47-49  $\text{mg L}^{-1}$  in freshwater and 42-44  $\text{mg L}^{-1}$  in brackish water. Make-up water use was 500  $\text{L kg}^{-1}$  feed during the trial and observed nitrate levels are in line with previous studies using our systems (Pulkkinen et al., 2018).

Woodchip reactors reduced effluent nitrate-N levels with removal rates varying from 6.3 to 15.9  $\text{g NO}_3\text{-N/m}^3\text{/day}$  between the treatments (Table 4). The nitrate removal rates were not statistically different between the two salinities (two-way ANOVA,  $p=0.46$ ), but the flocculation addition affected the removal rates (two-way ANOVA,  $p<0.001$ ). The removal rates are within the range of observed removal rates in RAS (Lepine et al. 2016, 2018 and von Ahnen et al. 2016, 2018). As hypothesized, extra carbon provided through the flocculation process improved removal nitrate in the woodchip reactors. This would be in accordance with the central role of carbon donor in denitrification (van Rijn et al., 2006), but would also suggest that at least under our conditions, birch woodchip reactors were carbon limited. Nitrate removal rates were not statistically different between the brackish water and freshwater (two-way ANOVA,  $p=0.46$ ), but the flocculation addition affected the removal rates (two-way ANOVA,  $p<0.001$ ). Salinity above 10 ppt has been shown to decrease denitrification (von Ahnen et al., 2019; Dinçer & Kargi 1999), and our result of no salinity effect at 7 ppt is in line with these studies.

Flocculation increased the woodchip reactor inlet TAN levels (Figure 3). The increase in freshwater was from the control 0.36-0.45 to treatment 0.78-1.16  $\text{mg L}^{-1}$ , and in the brackish water, the increase was from control 0.66-0.87 to treatment 1.17-2.71  $\text{mg L}^{-1}$ . Control TAN levels are in the normal range for our systems (Pulkkinen et al al., 2018). According to the manufacturer, flocculant contains no nitrogen, and the increase in TAN must be due to processes during the coagulation and flocculation or during the supernatant storage. We did not analyze the TAN content of the supernatant immediately after the flocculation to differentiate between these two alternatives. The TAN removal rates were affected by the salinity ( $P<0.001$ ) but not the flocculation treatment ( $P=0.34$ ). TAN levels did not change in the brackish water reactors, thus suggesting no nitrification in

anoxic conditions. On the contrary, TAN levels increased by the passage through the freshwater reactors, suggesting nitrate reduction to TAN via DNRA (Figure 4). This would be in line with Krom et al. (2014). It is unclear why DNRA would benefit from the flocculant or the flocculation process, but could be due additional carbon with the flocculant, since DNRA is favored by increased C/N ratio (von Ahnen et al. 2019).

Nitrite-N in the control inlet water was low as the nitrification of the two RAS was stable throughout the study (Figure 5). In line with observations by von Ahnen et al. (2016), a distinct  $\text{NO}_2\text{-N}$  peak after start-up was observed in the outlet of all treatments. A reason for temporarily increased  $\text{NO}_2$  may lie in a shift from denitrification fueled first by the most readily available carbon to carbon present in woodchips in less available form, as suggested by von Ahnen et al. (2016). Nitrite removal rates were affected by the salinity ( $P < 0.001$ ) and the flocculation treatment ( $P < 0.001$ ). Nitrite accumulation can take place at high pH (Glass & Silverstein 1998), but our flocculation supernatant was slightly acidic, thus it is unclear why the supernatant caused elevated nitrite accumulation.

It is generally assumed that denitrification process in woodchip reactor is not carbon limited. However, if a woodchip reactor benefits from additional carbon, it could be supplied externally as methanol and acetic acid. Alternatively, increased carbon could be achieved by the remaining organic flocculant of the P removal process, similarly to presented in the current study. Taking into account the costs of flocculant and methanol and their carbon contents, carbon in the flocculant used in the present study is about 3.6 times more expensive than carbon in methanol. However the cost of methanol use would increase due to a dosing system needed. For more detailed economic analyses, investments and all variable should be determined for a RAS farm.

#### 4. Conclusions

- Using the selected anionic and cationic potato starch-based flocculants, strong floc formation was found only by a combination of two flocculants.
- Flocculant addition increased the soluble carbon concentration in the inflows of the treatment reactors as measured by sCOD.
- Increased carbon concentration enhanced nitrate removal in the woodchip reactors.
- In the freshwater woodchip reactors, denitrification was incomplete as nitrite concentration increased in the control and treatment reactors.
- In the treatment reactors, dissimilatory nitrate removal to ammonia (DNRA) was observed, as ammonia increased in the outflows.
- Removal of P as the first step, followed by denitrification as the second step, is a simple strategy for removal of both nutrients because P solubilization from the sludge during denitrification is prevented. However, prolonged P release from freshwater reactors and incomplete denitrification indicate that the process is not ready for commercial use and further optimization of the process is needed.

Author statement Kujala et al.

**Kukka Kujala:** Investigation, Writing - Review & Editing. **Jani Pulkkinen:** Investigation, Supervision, Writing – Review & Editing. **Jouni Vielma:** Writing – Original Draft

### Declaration of interests

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

### Acknowledgements

Authors would like to thank all the technicians involved in daily management of the experiment at Laukaa fish farm. This work resulted from the BONUS CLEANAQ project and was supported by BONUS (Art 185), funded jointly by the EU and national funding institutions of Finland (Academy of Finland), Sweden (Vinnova) and Denmark (Innovation Fund Denmark IFD).

### Reference

Cheremisinoff, N.P., 2002. Handbook of water and wastewater treatment technologies, first ed. Elsevier

Dalsgaard, J., Lund, I., Thorarinsdottir, R., Drengstig, A., Arvonen, K., Pedersen, P.B., 2013. Farming different species in RAS in Nordic countries: Current status and future perspectives. Aquac. Eng. 53, 2-13. <https://doi.org/10.1016/j.aquaeng.2012.11.008>

Dinçer, A.R., Kargi, F. 1999. Salt inhibition of nitrification and denitrification in saline wastewater. Environ. Technol. 20, 1147-1153. <https://doi.org/10.1080/09593332008616912>

Ebeling, J.M., Sibrell, P.L., Ogden, S., Summerfelt, S.T., 2003. Evaluation of chemical coagulation–flocculation aids for the removal of phosphorus from recirculating aquaculture effluent. Aquac. Eng. 29: 23-42. [https://doi.org/10.1016/S0144-8609\(03\)00029-3](https://doi.org/10.1016/S0144-8609(03)00029-3)

Ebeling, J.M., Rishel, K.L., Sibrell, P.L., 2005. Screening and evaluation of polymers as flocculation aids for the treatment of aquacultural effluents. Aquac. Eng. 33, 235-249. <https://doi.org/10.1016/j.aquaeng.2005.02.001>

Ebeling, J.M., Welsh, C.F., Rishel, K.L., 2006. Performance evaluation of an inclined belt filter using coagulation/flocculation aids for the removal of suspended solids and phosphorus from microscreen backwash effluent. Aquac. Eng. 35, 61-77. <https://doi.org/10.1016/j.aquaeng.2005.08.006>

Glass, C., Silverstein, J. 1998. Denitrification kinetics of high nitrate concentration water: pH effect on inhibition and nitrite accumulation. *Wat. Res.* 32, 831-839. [https://doi.org/10.1016/S0043-1354\(97\)00260-1](https://doi.org/10.1016/S0043-1354(97)00260-1)

Guerdat, T.C., Losordo, T.M., Delong, D.P., Jones, R.D., 2013. An evaluation of solid waste capture from recirculating aquaculture systems using a geotextile bag system with a flocculant-aid. *Aquac. Eng.* 54, 1-8. <https://doi.org/10.1016/j.aquaeng.2012.10.001>

Healy, M.G., Ibrahim, T.G., Lanigan, G.J., Serrenho, A.J., Fento, O., 2012. Nitrate removal rate, efficiency and pollution swapping potential of different organic carbon media in laboratory denitrification bioreactors. *Ecological Engineering* 40, 198-209. <https://doi.org/10.1016/j.ecoleng.2011.12.010>

Krom, M.D., Ben David, A., Ingall, E.D., Benning, L.G., Clerici, S., Bottrell, S., Davies, C., Potts, N.J., Mortimer, R.J.G., van Rijn, J. 2014. Bacterially mediated removal of phosphorus and cycling of nitrate and sulfate in the waste stream of a “zero-discharge” recirculating mariculture. *Wat. Res.* 56, 109-121. <https://doi.org/10.1016/j.watres.2014.02.049>

Lee, C.S., Robinson, J., Chong, M.F., 2014. A review on application of flocculants in wastewater treatment. *Process Safety and Environmental Protection* 92, 489-508. <https://doi.org/10.1016/j.psep.2014.04.010>

Lepine, C., Christianson, L., Sharrer, K., Summerfelt, S., 2016. Optimizing hydraulic retention times in denitrifying woodchip bioreactors treating recirculating aquaculture system wastewater. *Journal of Environmental Quality* 45, 813-821. <https://doi.org/10.2134/jeq2015.05.0242>

Lepine, C., Christianson, L., Davidson, J., Summerfelt, S., 2018. Woodchip bioreactors as treatment for recirculating aquaculture systems' wastewater: A cost assessment of nitrogen removal. *Aquac. Eng.* 83, 85-92. <https://doi.org/10.1016/j.aquaeng.2018.09.001>

Letelier-Gordo, C.O., Dalsgaard, J., Suhr, K.I., Ekmann, K.S., Pedersen, P.B., 2015. Reducing the dietary protein:energy (P:E) ratio changes solubilization and fermentation of rainbow trout (*Oncorhynchus mykiss*) faeces. *Aquac. Eng.* 66, 22-29. <https://doi.org/10.1016/j.aquaeng.2015.02.002>

Letelier-Gordo, C.O., Herreros, M.M., 2019. Denitrifying granules in a marine Upflow Anoxic Sludge Bed (UASB) reactor. *Aquac. Eng.* 84, 41-49. <https://doi.org/10.1016/j.aquaeng.2018.12.002>

Nielsen, R. 2012. Introducing individual transferable quotas on nitrogen in Danish fresh water aquaculture: Production and profitability gains. *Ecological Economics* 75, 83-90. <https://doi.org/10.1016/j.ecolecon.2012.01.002>

Pulkkinen, J.T., Kiuru, T., Aalto, S.L., Koskela, J., Vielma, J., 2018. Startup and effects of relative water renewal rate on water quality and growth of rainbow trout (*Oncorhynchus mykiss*) in a unique RAS research platform. *Aquac. Eng.* 82, 38-45. <https://doi.org/10.1016/j.aquaeng.2018.06.003>

Sharrer, M.J., Rishel, K., Summerfelt, S., 2009. Evaluation of geotextile filtration applying coagulant and flocculant amendments for aquaculture biosolids dewatering and phosphorus removal. *Aquac. Eng.* 40, 1-10. <https://doi.org/10.1016/j.aquaeng.2008.10.001>

Soininen, N., Belinskij, A., Similä, J., Kortet, R., 2019. Too important to fail? Evaluating legal adaptive capacity for increasing coastal and marine aquaculture production in EU-Finland. *Marine Policy* 110, in press. <https://doi.org/10.1016/j.marpol.2019.04.002>

Suhr, K.I., Pedersen, L.F., Nielsen, J.L. 2014. End-of-pipe single-sludge denitrification in pilot-scale recirculating aquaculture systems. *Aquac. Eng.* 62, 28-35. <https://doi.org/10.1016/j.aquaeng.2014.06.002>

Suhr, K.I., Letelier-Gordo, C.O., Lund, I., 2015. Anaerobic digestion of solid waste in RAS: effect of reactor type on the biochemical acidogenic potential (BAP) and assessment of the biochemical methane potential (BMP) by a batch assay. *Aquac. Eng.* 65, 65-71. <https://doi.org/10.1016/j.aquaeng.2014.12.005>

Summerfelt, R.C., Penne, C.R., 2005. Solids removal in a recirculating aquaculture system where the majority of flow bypasses the microscreen filter. *Aquac. Eng.* 33, 214-224. <https://doi.org/10.1016/j.aquaeng.2005.02.003>

van Rijn, J., 2013. Waste treatment in recirculating aquaculture systems. *Aquac. Eng.* 53, 49-56. <https://doi.org/10.1016/j.aquaeng.2012.11.010>

van Rijn, J., Tal, Y., Schreier, H.J., 2006. Denitrification in recirculating systems: Theory and applications. *Aquac. Eng.* 34, 364-376. <https://doi.org/10.1016/j.aquaeng.2005.04.004>

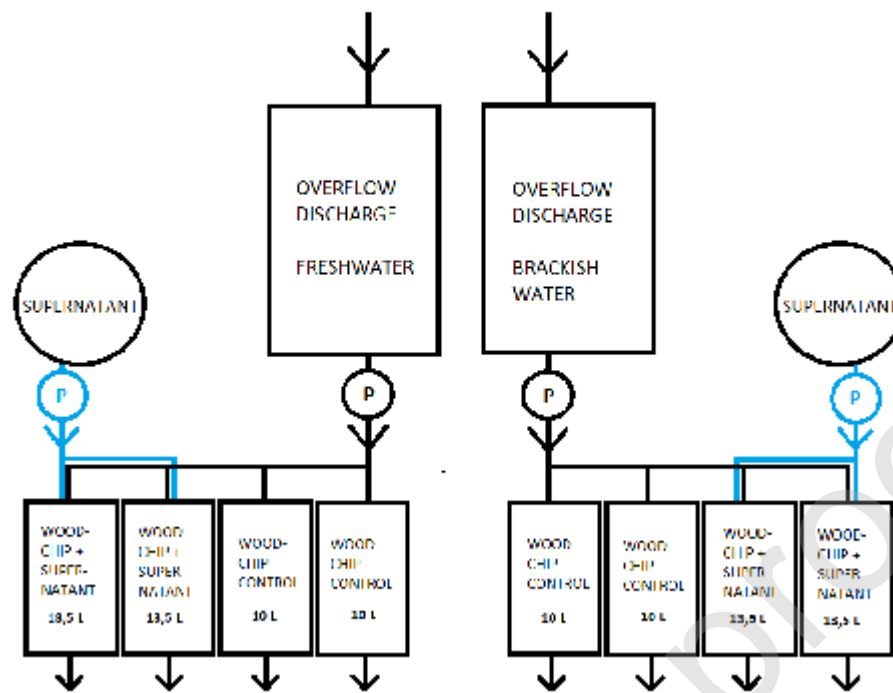
von Ahnen, M., Pedersen, P.B., Dalsgaard, J., 2016. Start-up performance of a woodchip bioreactor operated end-of-pipe at a commercial fish farm—A case study. *Aquac. Eng.* 74, 96-104. <https://doi.org/10.1016/j.aquaeng.2016.07.002>

von Ahnen, M., Pedersen P.B., Dalsgaard, J., 2018. Performance of full-scale woodchip bioreactors treating effluents from commercial RAS. *Aquac. Eng.* 83, 130-137. <https://doi.org/10.1016/j.aquaeng.2018.10.004>

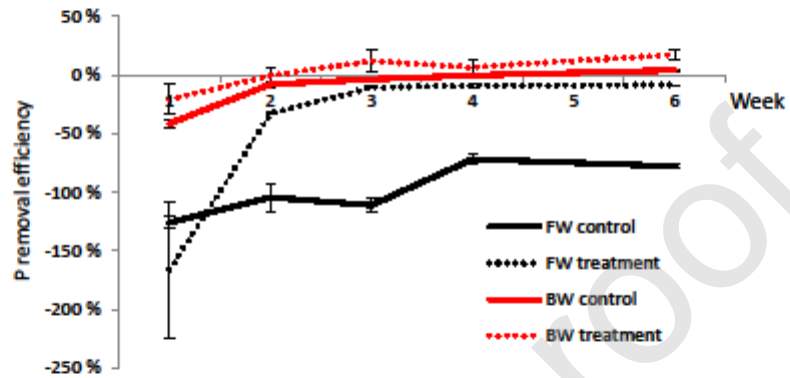
von Ahnen, M., Aalto, S.L., Suurnäkki, S., Tirola, M., Pedersen, P.B., 2019. Salinity affects nitrate removal and microbial composition of denitrifying woodchip bioreactors treating recirculating aquaculture system effluents. *Aquaculture* 504, 182-189. <https://doi.org/10.1016/j.aquaculture.2019.01.068>

Wei, H., Gao, B., Ren, J., Li, A., Yang, H., 2018. Coagulation/flocculation in dewatering of sludge: a review. *Water Research* 143, 608-631. <https://doi.org/10.1016/j.watres.2018.07.029>

Zhang, X., Hu, J., Spanjers, H., van Lier, J.B, 2014. Performance of inorganic coagulants in treatment of backwash waters from a brackish aquaculture recirculation system and digestibility of salty sludge. *Aquac. Eng.* 61, 9-16. <https://doi.org/10.1016/j.aquaeng.2014.05.005>

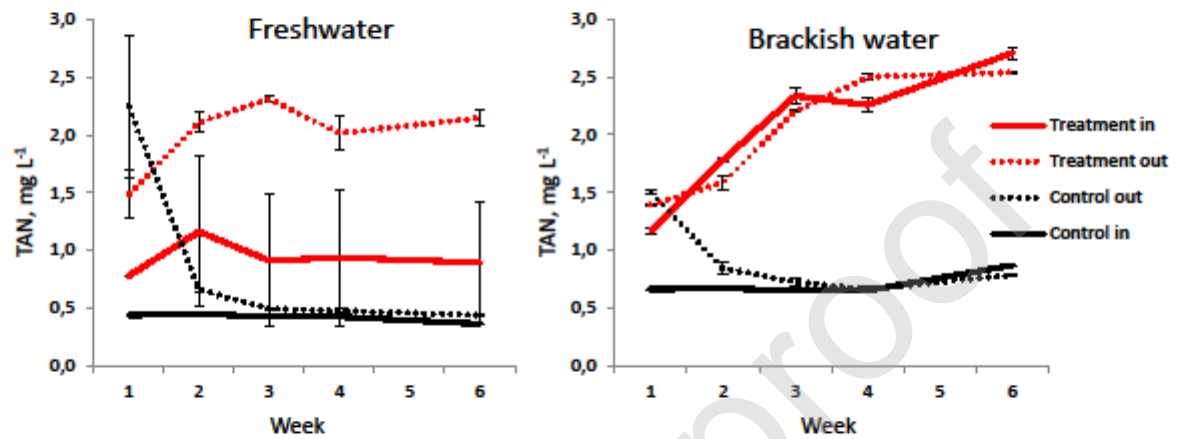


**Figure 1.** Schematic diagram of the experimental set-up. P = peristaltic pump.

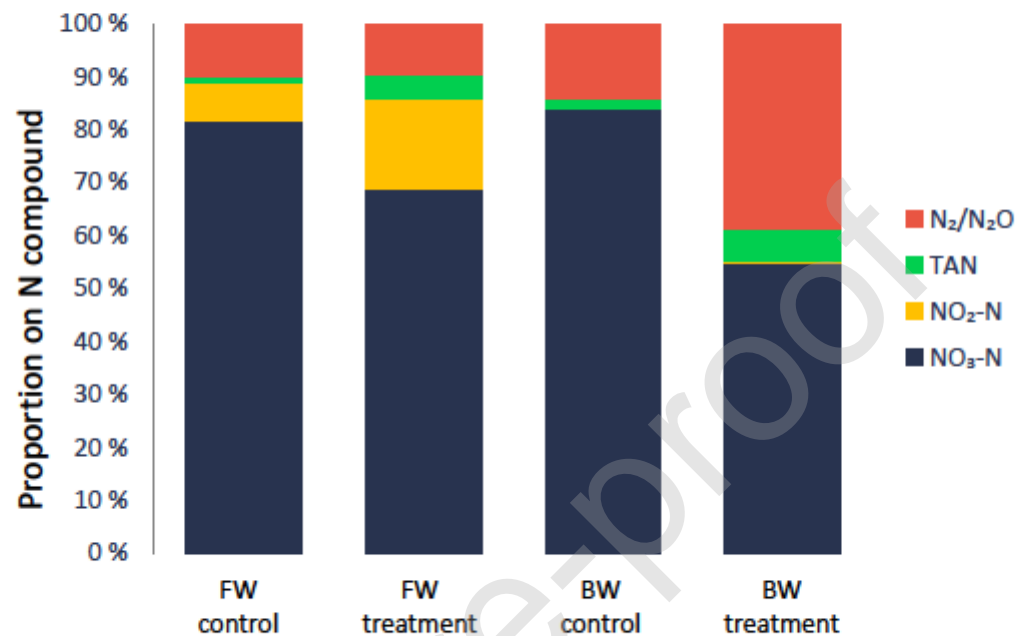


**Figure 2.** Phosphate-P removal in the woodchip bioreactors fed with freshwater (FW) and brackish water (BW) RAS overflow discharge (Control) or RAS overflow discharge with supernatant from the sludge coagulation (polyaluminum chloride) and flocculation process (PrimeBOND A0415 and PrimePHASE ; Treatment) during the six weeks study. Data is mean  $\pm$  SD, n=2.

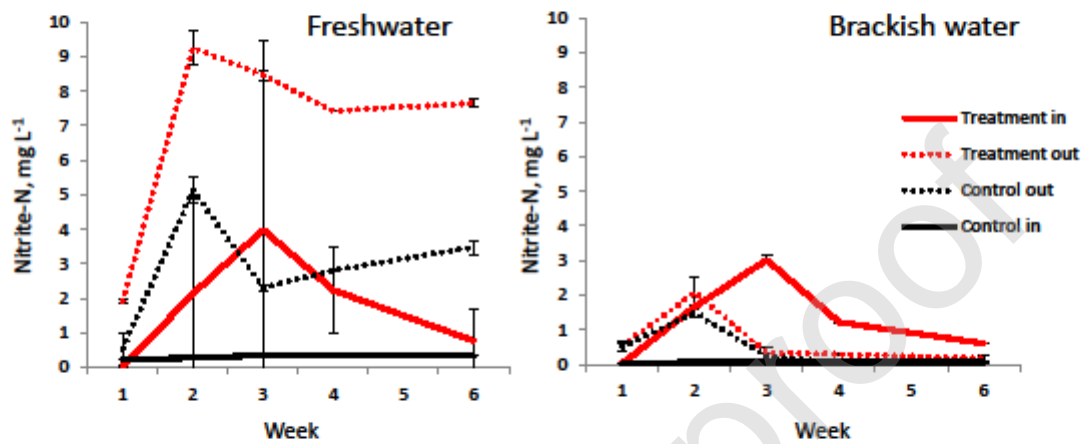




**Figure 3.** Total ammonia nitrogen (TAN) in the inlet and outlet of woodchip bioreactors fed with freshwater (Figure A) and brackish water (Figure B) RAS overflow discharge (Control) or RAS overflow discharge with supernatant from the sludge coagulation (polyaluminum chloride) and flocculation process (PrimeBOND A0415 and PrimePHASE ; Treatment) during the six weeks study. Data is mean  $\pm$  SD, n=2.



**Figure 4.** Average percentages of different N compounds in the outflows of woodchip bioreactors fed with freshwater (FW) and brackish water (BW) RAS overflow discharge (Control) or RAS overflow discharge with supernatant from the sludge coagulation (polyaluminum chloride) and flocculation process (PrimeBOND A0415 and PrimePHASE ; Treatment) during the six weeks study. Woodchip reactors were run in duplicates.



**Figure 5.** Nitrite-nitrogen ( $\text{NO}_2\text{-N}$ ) in the inlet and outlet of woodchip bioreactors fed with freshwater (Figure A) and brackish water (Figure B) RAS overflow discharge (Control) or RAS overflow discharge with supernatant from the sludge coagulation (polyaluminum chloride) and flocculation process (PrimeBOND A0415 and PrimePHASE ; Treatment) during the six weeks study. Data is mean  $\pm$  SD,  $n=2$ .

**Table 1.** Characteristics of sludge produced by rainbow trout in fresh and brackish water RAS. Data is based on pooled weekly samples from the six-week study. Data is presented as mean  $\pm$  SD,  $n=6$ .

	TSS, $\text{mg L}^{-1}$	TVSS, $\text{mg L}^{-1}$	Tot-P, $\text{mg L}^{-1}$	COD, $\text{mg L}^{-1}$	Tot-N, $\text{mg L}^{-1}$
Fresh water RAS	$377 \pm 147$	$310 \pm 115$	$14 \pm 3$	$512 \pm 71$	$59 \pm 4$
Brackish water RAS	$585 \pm 168$	$510 \pm 148$	$18 \pm 3$	$857 \pm 105$	$70 \pm 12$

**Table 2.** A summary of sludge properties, phosphorus (P) removal efficiencies, scale of the study and coagulation and flocculation chemicals in studies conducted at RAS.

Reference	Sludge source	TSS, mg L <sup>-1</sup>	P or PO <sub>4</sub> -P	P red., %	Scale	Chemicals
Ebeling et al. (2003)	Sedimentation tank overflow	78	3-20	80-90	Jar tests	Alum and ferric chloride
Ebeling et al. (2005)	Microscreen backwash	1015	6-22	92-95	Jar tests	Polyacrylamides
Ebeling et al. (2006)	Microscreen backwash	1015		80	Jar test and belt filter	Alum and Polyacrylamides
Sharrer et al. (2009)	Drum filter backwash and settler solids	1500-1900	34-42	47-77	Geotextile	Alum, ferric chloride and lime
Guerdat et al. (2013)	Drum filter backwash and swirl separator, fresh water	1176	28	32	Geotextile	Polyacrylamide
Guerdat et al. (2013)	Bead filter backwash, brackish water	1489	54	42	Geotextile	Polyacrylamide
Zhang et al. (2014)	Drum filter backwash, fresh and brackish water	108	10-12	95	Jar tests	FeCl <sub>3</sub> and polymeric aluminum sulfate

**Table 3.** Phosphate removal efficiency (%) and turbidity in the sludge supernatant by using 50 mg L<sup>-1</sup> PAC and graded levels of flocculants PrimeBOND A0415 and PrimePHASE 3545 in jar tests. Data is mean ± SD, n=2.

Flocculant, mg L <sup>-1</sup>	Fresh water		Brackish water	
	PO <sub>4</sub> removal (%)	Turbidity	PO <sub>4</sub> removal (%)	Turbidity
10	83.8 ± 0.3	4.7 ± 0.7	83.6 ± 0.6	5.0 ± 0.6
30	73.1 ± 2.2	4.7 ± 0.3	74.7 ± 3.2	9.5 ± 2.1
50	76.1 ± 5.2	5.0 ± 0.4	75.6 ± 5.1	11.6 ± 3.2
100	82.0 ± 0.1	6.1 ± 0.4	78.9 ± 0.7	11.8 ± 0.4
200	80.8 ± 0.6	7.6 ± 1.0	79.0 ± 0.4	14.8 ± 0.2
400	78.6 ± 2.3	12.9 ± 2.1	78.9 ± 0.8	20.2 ± 1.6

**Table 4.** Removal rates of nitrogen products by the woodchip reactors and the average sCOD concentrations into the inflows (n=1) and from the outflows (n=2) of bioreactors during the six weeks study. Control = RAS overflow discharge; Treatment = RAS overflow discharge with supernatant from the sludge coagulation (polyaluminum chloride) and flocculation process (PrimeBOND A0415 and PrimePHASE).

Treatment	NO <sub>3</sub> -N g m <sup>-3</sup> d <sup>-1</sup>	TAN g m <sup>-3</sup> d <sup>-1</sup>	NO <sub>2</sub> -N g m <sup>-3</sup> d <sup>-1</sup>	sCOD (g d <sup>-1</sup> )		
				In	Out	Used
Freshwater treatment	13.80 ± 2.43	-1.19 ± 0.37	-24.13 ± 10.52	1.19	0.69 ± 0.02	0.50 ± 0.02
Freshwater control	8.30 ±	-0.19 ±	-4.29 ±	0.28	0.46 ± 0.00	-0.18 ± 0.01

	1.26	0.12	2.15			
	15.92 ±	0.06 ±	4.05 ±			
Brackish water treatment	0.47	0.19	4.58	1.92	0.85 ± 0.00	1.07 ± 0.03
	6.30 ±	-0.05 ±	-0.80 ±			
Brackish water control	8.54	0.10	1.20	0.42	0.52 ± 0.03	-0.10 ± 0.01