A method for reclaiming nutrients from aquacultural waste for use in soilless growth systems

Maha Ezziddine, Helge Liltved and Jan Morten Homme

ABSTRACT

The aim of this work was to develop a method that allows the recovery of nutrients from aquaculture sludge, not only to alleviate the disposal problem, but also to address the future scarcity of non-renewable fertilizers. This method includes two steps: Nutrient mobilization using aerobic digestion followed by solids precipitation using chitosan as the flocculant. The aerobic digestion experiments were conducted in aerated batch reactors, while a jar test apparatus was used to assess the capacity of chitosan to remove total suspended solids (TSS) and turbidity. During aerobic digestion, the concentration of soluble N (sum of NH_4-N, NO_2-N, NO_3-N) increased from 181 mg/L at the start to 890 mg/L after three weeks, and to 903 mg/L after four weeks and solids removal by chitosan flocculation. The corresponding concentrations for soluble P were 8.2 mg/L at start, 110 mg/L after three weeks of aerobic digestion, and 160 mg/L after four weeks of aerobic digestion and chitosan flocculation. Other macronutrients (K, Ca, Mg, S) and micronutrients (Fe, Mn, Zn, B, Cu, Mo) were mobilized to concentrations close to or higher than levels recommended for hydroponic growth of vegetables. Chitosan flocculation and precipitation using a dose of 15 mg/L resulted in a reduction of the turbidity by 96% from 156 to 6.5 FNU. After chitosan precipitation, 80% of the sludge could be reclaimed as a nutrient-rich clear phase, low in TSS and turbidity.

Key words | aerobic digestion, aquaculture sludge, chitosan, fertilizer, flocculation, mobilization

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INTRODUCTION

According to the Food and Agriculture Organization of the United Nations (FAO), the aquaculture industry is growing faster than other food production sectors. It is reported that by 2030 aquacultural production of fish and shellfish is expected to reach 109 million tonnes, which represents a growth rate of 37% over the 2016 production (FAO 2018). An increasing amount of this production will take place in semi-closed and closed systems, where effluent water and recirculating water is treated mechanically to remove solid waste (feed waste and feces). The collected sludge contains nutrients that may represent a pollution problem if inadequately disposed of but that sludge can also be utilized for fertilizing purposes after appropriate treatment. Thus, converting aquaculture waste into valuable fertilizer can not only alleviate the disposal problem, but also bring economic benefits and address the future scarcity of non-renewable fertilizers.

In Norway, the amount of waste from the production of Atlantic salmon (*Salmo salar*) is large and increasing. The life cycle of the salmon requires that the hatching and early growth phases take place in land-based freshwater farms to reach the smolt stage, after which the fish are ready to go into saltwater in the sea. After the freshwater phase, the smolt are transferred to floating cages in the sea. The annual amount of sludge from land-based smolt production in freshwater has been estimated to be approximately 11,000 tonnes dry weight (DW), (Aas & Åsgård 2017). In addition to solid waste, fish produce dissolved waste as urine and excretion products over the gills. In land-based systems, including systems with direct flow-through of water and systems with recirculated water, the feed waste and feces needs to be removed as fast as possible to prevent it from being broken down and suspended by the turbulence created by pumping and water transport. In systems with water recirculation, dissolution of particles into fine particles, colloids and dissolved compounds may impair water quality and pose a threat to fish health if not removed (Del Campo *et al.* 2010). Mechanical solids removal

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can be achieved by sedimentation, mechanical filtration or swirl separation. In recirculating aquacultural systems (RAS), The removal of dissolved compounds (e.g. ammonia and carbon dioxide) is also required, which means additional treatment such as biofiltration and aeration.

The sludge produced by solids removal systems is relatively low in solid content (approximately 1–2% DW), and must be treated by dewatering and stabilization before disposal or use (Wang *et al.* 2013). Without safe disposal or use, the sludge produced may cause detrimental environmental impacts, including pollution of surface water and ground water bodies, spreading of fish pathogenic microorganisms, and unpleasant odour from putrefaction (Bergheim *et al.* 1998). After proper treatment, land application is the most common way of disposing of sludge from aquaculture (Timmons *et al.* 2018). Land application is a valuable way of fertilizing crops (Goddek *et al.* 2016).

An alternative way to utilize the nutrients in aquacultural waste is to use the sludge from solids removal systems more directly in soilless growth systems for vegetables (hydroponic and aquaponic systems). However, nutrients associated with solids are not readily available for plant growth. Mobilizing nutrients to soluble forms can be achieved by various treatments, such as aerobic and anaerobic degradation, and then the nutrient-rich water can be used in soilless growth systems.

Monsees *et al.* (2017) compared nutrient mobilization during anaerobic and aerobic digestion of aquacultural sludge. They obtained higher amounts of soluble nutrients such as phosphorus (P) and potassium (K) after aerobic treatment than anaerobic treatment. Likewise, Delaide *et al.* (2018) showed that aerobic digestion has some ability to solubilize nutrients from aquacultural sludge by allowing an increase in soluble macro- and micronutrients of 10–60%. Delaide *et al.* (2018) and Monsees *et al.* (2017) used traditional aerobic digestion for the mobilization of nutrients. Recently, more advanced techniques for nutrient recovery from domestic wastewater sludge have been developed (Xu *et al.* 2018a, 2018b). Such techniques may be applied in the aquaculture industry in the future for more efficient nutrient recovery.

Once nutrients are solubilized in the water phase, solids need to be removed in order to produce a good quality clear phase, in compliance with water quality guidelines for hydroponic and aquaponic systems. Techniques to remove solids from the treated aquacultural sludge include flocculation and sedimentation. Several studies have been conducted using metal-based coagulants and synthetic organic polymers for flocculation and settling of solids (Siah *et al.* 2014). Inorganic metal salts, such as ferric and aluminium salts, are the most commonly used flocculants due to their low cost and ease of use (Zhang *et al.* 2014). The metal salts hydrolyse and combine with suspended solids to produce settlable flocs, but also react with dissolved phosphate to produce insoluble iron phosphate or aluminum phosphate, thereby removing the valuable phosphorus from the solution (Ge *et al.* 2017). Al flocculants may also leave Al residuals in the clear phase, with a potentially toxic effect on plants (Yang *et al.* 2016). With the intention of using the dissolved nutrients in the sludge for plant growth, alternative flocculants, which do not react with soluble phosphorus, should be used.

Chitosan is a natural cationic polymer made from shrimp and crab shells that has been shown to be an excellent turbidity remover (Yang *et al.* 2016). Cationic polymers may carry out the dual functions of coagulation and flocculation. The effectiveness of chitosan as a coagulant and flocculant can be explained by its long chain structure with abundant free amino groups that are protonated in neutral and acidic mediums, as they impart high charge density. The positive charges neutralize the negative charges on particle surfaces and bridge the destabilized particles into aggregates (Yang *et al.* 2016).

In wastewater treatment, chitosan has mainly been used as a flocculant for the removal of different types of dissolved and undissolved compounds, including suspended solids, heavy metals, humic acid, dyes, algae, and bacteria (Lee *et al.* 2014; Yang *et al.* 2016). Improved solids settling characteristics have been demonstrated in various effluents such as sewage, food processing wastewater, aquacultural wastewater, manure wastewater and dye wastewater (No & Meyers 2000; Yang *et al.* 2016). The solids removal efficiency of various synthetic cationic polymers has been evaluated for the treatment of dilute sludge in aquaculture (backwash water from microscreen filters) (Ebeling *et al.* 2005). However, no such studies have been found where chitosan has been used as the flocculant. Preliminary trials in our laboratory of chitosan flocculation of sludge from aquaculture have revealed high removal of suspended solids and turbidity, but marginal removal of soluble phosphorous (data not shown). Chitosan is also known as a metal chelator (Zhang *et al.* 2016), and regarded as nontoxic, with no health concerns (Yang *et al.* 2016).

The objective of this study was to propose a holistic method for the treatment and use of aquacultural sludge for fertilizing purposes in soilless growth systems. The method includes solubilization of nutrients by aerobic digestion and polishing of the nutrient-rich solution by the use of the biopolymer chitosan as the flocculant.

MATERIALS AND METHODS

The aquaponic facility and sludge collection

Sludge was collected from swirl separators and filter backwash water at an aquaponic research facility at the Norwegian Institute of Bioeconomy Research (Landvik, Norway). The aquaponic facility is a closed system based on RAS technology connected to tanks for the production of vegetables; it consists of two separate identical test units (unit A and unit B). Figure 1 shows a flowchart of one of the two units. The total water volume of each unit is 16 m^3, divided into four fish tanks with a volume of 0.63 m^3 each, and two plant compartments with a volume of 3 m^3 each. Outside each fish tank, a swirl separator is mounted to collect uneaten fish feed and feces directly from the outgoing water. A pumping sump for each unit provides water to the fish tanks, plant compartments and water treatment system. The water treatment system consists of aeration, heating/cooling, a combined particle and biofilter (Polygeyser Bead Filter, New Orleans, USA), swirl separators and a pH-control unit (Figure 1).

During the experimental period, the fish tanks were stocked with brown trout (*Salmo trutta*) and salmon (*Salmo salar*). There was a total of 530 brown trout with an average weight of 105 g each in unit A, and 260 salmon with an average weight of 340 g each in unit B. The daily amount of feed given was 280 g for each unit, distributed evenly to each tank by automatic feeders. The plant growing compartments were stocked with lettuce (*Lactuca sativa* L., Batavia-type, cv. 'Partition') in a deep-water floating raft system. The plant compartments were operated in a continuous production mode with weekly harvesting of lettuce heads with an average weight of approximately 150 g. The harvesting took place seven weeks after seeding. The production weight ratio of fish biomass to lettuce biomass was 1:7.

The total sludge production from the aquaponic facility (from unit A and unit B) was approximately 20 L per day. The sludge consisted of backwash water from the two bead filters, and drainage from the eight swirl separators. The sludge from unit A and unit B was transferred and mixed in a common aerated tank, from which sludge for this study was collected. The daily amount of makeup water supplied to the system was only to compensate for evaporation and transpiration losses, and the amount of sludge taken out from filters and swirl separators.

Batch aerobic digestion

The sludge was transferred to duplicate 25 L polyethylene batch reactors covered with a lid to prevent evaporation; they were equipped with diffusors which provided aeration at a rate of 20 L min^{-1}. After four weeks of aerobic digestion, the experiment was terminated, and chitosan precipitation was applied for the removal of solids and analysis for characterization of the clear phase. During the experimental period, the pH was monitored daily and maintained at 7.0 ± 0.3 using small amounts of a dilute NaOH and HCl solutions. In addition, the oxygen concentration was measured regularly. A volume of 250 ml was sampled weekly from each reactor for determination of total suspended solids (TSS), volatile suspended solids (VSS), chemical oxygen demand (COD), soluble chemical oxygen demand (SCOD), total nitrogen (TN), total phosphorus (TP) and soluble nutrients

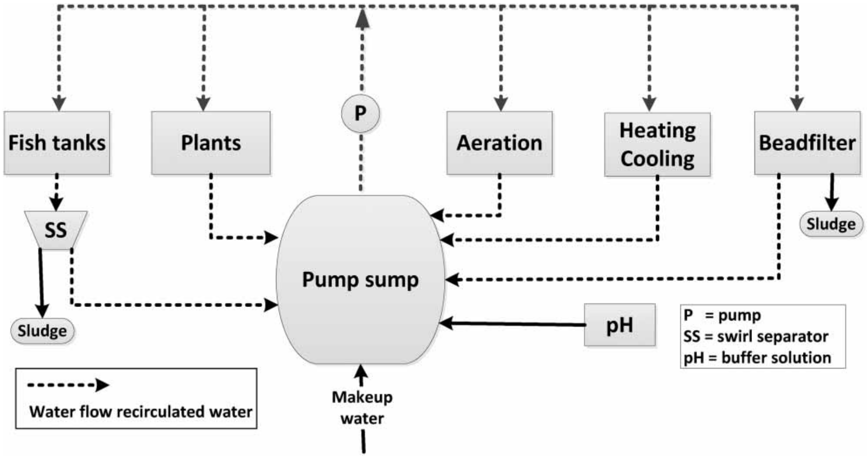

Figure 1 | Flowchart of one of the two identical units of the aquaponic research facility, showing water flows, water treatment and sludge removal sites. The facility co-produced fish and vegetables with 100% recirculation of water.

(ammonium (NH_4-N), nitrite (NO_2-N), nitrate (NO_3-N), P, K, copper (Cu), zinc (Zn), sulfur (S), manganese (Mn), magnesium (Mg), calcium (Ca), molybdenum (Mo), boron (B), iron (Fe)). Samples for soluble nutrients and SCOD were centrifuged at 4,500 rpm for 3 min and filtered through membrane filters with pore openings of 0.45 μm before analysis.

Solids separation

To remove residual particles from the clear phase after aerobic digestion, jar tests with flocculation and sedimentation were performed. A commercial chitosan (Kitoflokk High from Teta Vannrensing AS, Norway) was used as the coagulant at different concentrations (15, 25, and 40 mg/L), and compared to the performance of a synthetic cationic polyacrylamide polymer for sludge dewatering (Superfloc C-496 HMW from Kemira AB, Sweden). The molecular weight of the Kitoflokk High was 161 kD, and the degree of deacetylation was 80%. Chitosan stock solution (0.25%) was prepared by mixing 0.25 g chitosan powder with 100 mL distilled water, and then adding drops of a 30% HCl solution under continuous stirring until the chitosan was dissolved. Superfloc C-496 HMW stock solution (0.25%) was prepared by adding 0.25 g of powder to 100 mL distilled water under continuous stirring until the polymer was dissolved.

Sludge samples were transferred to 1 L beakers and placed in a semi-automatic jar-test device (Kemira Kemwater, Helsingborg, Sweden). Coagulant was added during rapid mixing, and the pH was adjusted to 6.0 ± 0.2 by adding drops of a dilute HCl or NaOH solution. After 60 seconds of rapid mixing (400 rpm), followed by 10 min flocculation under slow stirring (30 rpm) and 20 min sedimentation, water samples were carefully siphoned from the clear phase for analysis of turbidity and TSS.

Analytical methods

TSS, VSS, color, turbidity, pH and oxygen were measured in the laboratory of University of Agder according to Norwegian and European Standards. TSS and VSS were measured using pre-weighed 1.6 μm Whatman GF/A glass fiber filters. Color was determined using a calibrated Hach DR3900 spectrophotometer (Loveland, CO, USA), turbidity using a calibrated Hach 2100Q turbidimeter (Loveland, CO, USA), pH using a calibrated Jenway 3150 instrument (Cole-Parmer, UK), and oxygen concentration using a calibrated Hach HQ40d instrument (Loveland, CO, USA). Soluble nutrients (P, K, Cu, Zn, S, Mn, Mg, Ca, Mo, B, Fe) were measured using inductively coupled plasma optical emission spectrometry (ICP-OES) according to accredited standards by the LMI laboratory (Sweden). NH_4^+, NO_3^- and NO_2^- were determined using a QuAAtro continuous segmented flow autoanalyzer. For TN and TP, samples were dissolved by nitric acid microwave extraction and analysed using ICP-OES according to European Standards (DIN EN ISO 11885) at the Eurofins laboratory, Norway.

Statistics

The aerobic digestion experiments and the polymer flocculation experiments were repeated twice. Mean values with standard deviations are presented. Where standard deviation bars are not shown on the graphs, they do not extend beyond the dimensions of the symbols.

RESULTS AND DISCUSSION

Sludge characterization

The sludge used in this study was a mixture of filter backwash water and solids from the swirl separators of unit A and unit B in the aquaponic facility. As shown in Table 1, the sludge was a dilute suspension with a solids content below 1%, mainly composed of organic matter with TSS and VSS concentrations of 8.7 g/L and 8.5 g/L, respectively. The total COD concentration was 6,000 mg/L, with a minor part of 147 mg/L in soluble form. Most of the essential nutrients N and P were associated with solids, even though the TSS only comprised 0.87% of the sludge (8.7 g/L). For nitrogen, the dissolved compounds (NH_4-N, NO_2-N and NO_3-N) comprised approximately 26% of the total N, with the majority in reduced form as NH_4^+. For phosphorus, only 6% of the total P was in soluble form.

The suspended solids in recirculating systems are generated from feed waste, feces, and bacteria. Aquaculture solids in recirculating systems are characterized by great size variation from centimetres (cm) to microns (μm). The size variability of suspended solids is due to the tendency of feed waste and feces to break down in the water column by water turbulence, fish motion and pumping (Timmons *et al.* 2018). Chen *et al.* (1993) reported that 90% of the total particles in RAS are less than 30 μm. With good feeding practice and operation, the feed waste can be reduced, and most of the waste can be taken directly out of the water flow from the fish tanks by mechanical solids removal, preventing it from being broken down and suspended by turbulence and pumping.

Table 1 | Characteristics of the aquacultural sludge mixture collected in duplicate from swirl separators and filter backwash water

Parameter	Mean values	Standard deviations
pH	7.9	± 0.01
TSS (g L^{-1})	8.7	± 0.1
VSS (g L^{-1})	8.5	± 0.35
COD (mg L^{-1})	6,000	± 800
SCOD (mg L^{-1})	147	± 5
Total P (mg L^{-1})	135	± 5
Total N (mg L^{-1})	705	± 15
Soluble nutrients		
B (mg L^{-1})	0.14	± 0.01
Ca (mg L^{-1})	53.9	± 0.75
Cu (mg L^{-1})	0.03	± 0.01
K (mg L^{-1})	62.5	± 0.5
Mg (mg L^{-1})	21.9	± 0.20
Mn (mg L^{-1})	0.24	± 0.01
Mo (mg L^{-1})	0.01	± 0.003
NH_4-N (mg L^{-1})	181	± 2
NO_2-N (mg L^{-1})	0.03	± 0
NO_3-N (mg L^{-1})	0.10	± 0
P (mg L^{-1})	8.2	± 0.25
S (mg L^{-1})	26	± 1
Zn (mg L^{-1})	1.65	± 0.05

Aerobic digestion

After three weeks of aerobic digestion, the TSS and VSS in the raw sludge were reduced by 31.6% and 48.5%, respectively (Figure 2). The reductions in TSS and VSS were modest and in line with those reported from domestic wastewater treatment. It has been stated that solids reduction during aerobic stabilization is not expected to be higher than 40%, even after a long digestion time (Foladori *et al.* 2010). As shown in Figure 3, the average content of total COD was reduced from 6,000 mg/L to 3,000 mg/L after three weeks of aerobic digestion, which indicates that organic solids (VSS) were solubilized and oxidized. According to the literature, 60–70% of the oxidized organic matter can be converted into new cellular biomass (Foladori *et al.* 2010). When all biodegradable organic matter is depleted, die-off and bacterial decay will generate new soluble matter which can be used for metabolism, generating new biomass (Schultz *et al.* 1999; Foladori *et al.* 2010). The slight observed increase in TSS and VSS on day 21 may be explained by bacterial growth induced by release of metabolites by bacterial decay towards the end of the period (Figure 2). This was supported by a pH drop after 15 days of aerobic digestion (data not shown) which indicated high endogenous metabolic activity with a high alkalinity demand (Bailey 2008).

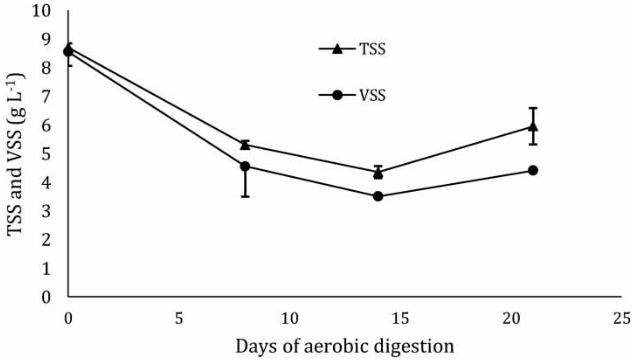

Figure 2 | Concentrations of total suspended solids (TSS) and volatile suspended solids (VSS) during aerobic digestion. Bars represent standard deviations.

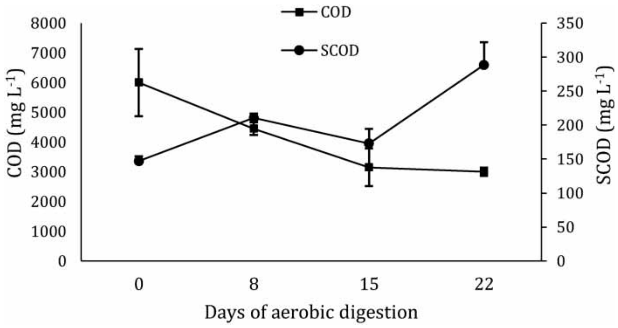

Figure 3 | Concentrations of chemical oxygen demand (COD) and soluble chemical oxygen demand (SCOD) during aerobic digestion. Bars represent standard deviations.

The initial level of SCOD comprised only 2.5% of the total COD and increased from 147 mg/L to 288 mg/L during the three weeks of aerobic digestion (Figure 3). This increase can be explained by the accumulation of non-degradable soluble organic compounds in the reactors. It is known that by increasing sludge retention time, the inert and endogenous residue fraction will accumulate due to biomass decay (Foladori *et al.* 2010). As much as 20–25% of cell material is composed of inert inorganic and organic compounds that are not biodegradable (Bailey 2008). It has also been reported that when most of the biomass has been broken down after aerobic stabilization, a high concentration of SCOD can remain in the sludge (Foladori *et al.* 2010), which supports the increase in SCOD observed in our study. The data show that raw sludge from aquaculture can be digested by solubilizing some of the organic solids and oxidizing organic matter, and simultaneously releasing nutrients bound to solids.

Solids separation after aerobic digestion

For the removal of particles after aerobic digestion, chitosan (Kitoflokk High) was used as the coagulant, and compared to the performance of a synthetic polymer (Superfloc C-496 HMW). Results of the jar tests are shown in Figure 4(a) and 4(b). Compared to the synthetic coagulant, chitosan showed better TSS and turbidity removal at lower dosages. By using a dose of 15 mg/L chitosan, reductions of TSS and turbidity by 91% and 96%, respectively, were achieved. The same dosage of the synthetic coagulant resulted in higher TSS and turbidity concentrations. Even higher dosages of the synthetic polymer (up to 40 mg/L) did not improve the treatment efficiency beyond the results of 15 mg/L chitosan. Increasing the chitosan dose above a threshold limit did not increase the treatment efficiency. The lower dosage of chitosan required compared to the synthetic polymer compensated for part of the higher cost of chitosan. Costs of bulk qualities obtained from suppliers indicate 4.50 €/kg for the polyacrylamide (Superfloc C-496 HMW) and 25 €/kg for the chitosan (Kitoflokk High). Therefore chitosan still is more expensive, even if the required dosage is one-third of the dosage of the polyacrylamide.

Color removal by chitosan and the synthetic coagulant was also evaluated. As shown in Figure 5, chitosan exhibited better color removal at all dosages than the synthetic polymer. A dose of 15 mg/L of chitosan allowed a reduction in color of 44.5%. With the same dose of the synthetic coagulant, the color was only reduced by 13%, leaving a yellowish color in the treated water. A yellow color in water is normally associated with dissolved organic compounds and can be difficult to completely remove using polymers. Even the highest dosage of chitosan did not achieve a better color removal efficiency than 49.4%. For the purpose of using the treated water in aquaponic systems, this may not be a problem since the literature indicates that the presence of dissolved organic matter in the nutrient solution at low concentrations promotes plant growth and nutrient uptake and consequently leads to higher yields (Goddek *et al.* 2016).

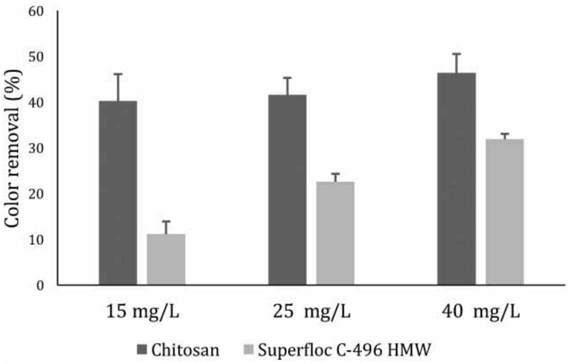

Figure 5 | Color content in the clear phase after treatment with increasing doses of chitosan and Superfloc C-496 HMW. Bars represent standard deviations.

Other benefits of chitosan include the absence of toxicity, which is an issue because residues of synthetic cationic polymers (polyacrylamides) may have toxic effects on aquatic animals (Bolto & Gregory 2007). It has also been found that cationic polyacrylamides may accumulate in sludge from wastewater treatment and inhibit microbial degradation during anaerobic fermentation (Liu *et al.* 2019).

Soluble nutrient concentrations after aerobic digestion and after solids separation

The study showed that aerobic digestion of the sludge was able to mobilize the majority of essential nutrients from particles associated with soluble forms (Table 2). The soluble micro- and macronutrients in raw sludge before aerobic

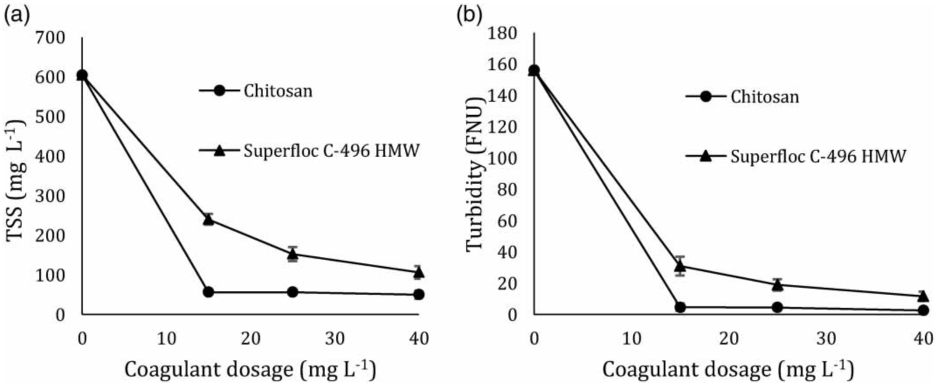

Figure 4 | Concentrations of TSS (a) and turbidity (b) in the clear phase after treatment with increasing doses of chitosan and Superfloc C-496 HMW. Bars represent standard deviations.

Table 2 | Concentrations of soluble macro- and micronutrients, and metals, in raw sludge, after aerobic digestion, and after aerobic digestion and chitosan precipitation compared to levels in a standard nutrient solution for hydroponic growth of lettuce (Libia & Gómez-Merino 2012).

	Before aerobic digestion		**After aerobic digestion (3 weeks)**		**After aerobic digestion (4 weeks) and chitosan flocculation**		**Standard nutrient solution**
	Mean	**St.dev.**	**Mean**	**St.dev.**	**Mean**	**St.dev.**	
Macronutrients							
N, mg L^{-1}	181	± 2.8	890	± 16.3	903	± 15.5	186
P, mg L^{-1}	8.2	± 0.35	110	± 0	160	± 0	40
K, mg L^{-1}	62.5	± 0.70	110	± 0	130	± 0	156
Ca, mg L^{-1}	53.8	± 1.06	170	± 14.1	269	± 3.5	168
Mg, mg L^{-1}	21.9	± 0.28	44.5	± 3.5	66.8	± 1.13	42.0
S, mg L^{-1}	26.0	± 1.4	70.5	± 3.5	80.5	± 0.70	60.0
Micronutrients							
Zn, μg L^{-1}	1,650	± 71	1,750	± 70	1,050	± 71	100
B, μg L^{-1}	140	± 14	270	± 35	3,050	± 70	450
Cu, μg L^{-1}	30	± 21	49	± 15	38	± 4	50
Fe, μg L^{-1}	1,000	± 0	1,300	± 140	1,900	± 0	2,000
Mn, μg L^{-1}	240	± 14	205	± 21	330	± 0	200
Mo, μg L^{-1}	12	± 4.9	31	± 18	13	± 1.4	40
Other metals							
Al, μg L^{-1}	<9	± 0	62.5	± 19	15.5	± 9	–
Cd, μg L^{-1}	3	± 0.10	4.2	± 1.2	2.2	± 1.1	–
Ni, μg L^{-1}	5.7	± 1.0	12.5	± 2.1	15	± 5.6	–

digestion, after three weeks of aerobic digestion, and after four weeks of aerobic digestion and chitosan precipitation are shown. The concentrations of dissolved N increased from 181 mg/L before aerobic digestion to 890 mg/L after three weeks of aerobic digestion, and to 903 mg/L after four weeks of aerobic digestion and chitosan precipitation. The corresponding numbers for dissolved P were 8.2 mg/L before aerobic digestion, and 110 mg/L and 160 mg/L after three and four weeks of aerobic digestion, respectively. This implied 84% mobilization of N and 63% mobilization of P after three weeks when related to the total N and total P concentrations. The corresponding mobilizations after four weeks were 79% of N and 78% of P. Increases in TN and TP occurred from the start to the end of the four-week period, which were attributed to a volume decrease of approximately 15% due to evaporation and the volume decrease caused by sampling. The high percentages of mobilization indicate low amounts of undissolved N and P in the residual TSS and VSS. In untreated aquacultural sludge, the N content has been measured as 2.9% of TSS (Maillard *et al.* 2005). After three weeks, the TSS concentration was 5,950 mg/L (Figure 2), which corresponds to an undissolved N amount of 172 mg/L when assuming an N-content of 2.9% of solids. This value corresponded reasonably well to the difference between the TN concentration of 1,050 mg/L and the soluble N concentration of 890 mg/L after three weeks of aerobic digestion, which was 160 mg/L.

There are limited studies in the literature when it comes to mobilization of nutrients from aquacultural waste by aerobic digestion. From published studies, low degrees of mineral solubilization were found (Monsees *et al.* 2017; Delaide *et al.* 2018). Monsees *et al.* (2017) investigated the nutrient mobilization from aquacultural sludge collected from a clarifier during a 14 day aerobic treatment period. No total rise in soluble N-compounds was found, while an increase of dissolved P from 9.4 to 29.7 mg/L was found. These findings are in contrast to our results, which showed the following increases in N and P: 4.4- and 9.8-fold after two weeks of aerobic digestion (data not shown), 4.9- and 13.4-fold increases in N and P after three weeks of aerobic digestion, and 5.0- and 19.5-fold increases after four weeks of aerobic digestion and solids separation (chitosan flocculation) (Table 2). In the study by Monsees *et al.* (2017), low pH may have restricted bacterial activity and thereby solubilization of nutrients. The pH was not controlled and dropped from 6.2 to 5.3 during the aerobic digestion period, while we controlled the pH to 7.0 ± 0.3. Because N is a key component in proteins, the increase of dissolved N in the sludge during aerobic digestion is expected to be caused by the hydrolysis of fish feed proteins by extracellular enzymes excreted by microorganisms (Schultz *et al.* 1999).

In our study, all other dissolved macro- and micronutrients increased during aerobic digestion, most of them to concentrations higher than mineral levels recommended for hydroponic growth systems (Table 2). The concentrations of K, Ca, Mg, S and B rose by factors of 1.7, 3.2, 2.0, 2.7, and 1.6, respectively after three weeks of aerobic digestion, and by factors of 2.1, 5.0, 3.1, 3.1 and 21.8 after four weeks of aerobic digestion and chitosan flocculation (Table 2). The strong increase in boron concentration from week three to week four of aerobic digestion was unexpected. It may have been related to leakage from the chitosan added for flocculation. It is known that the marine environment is relatively rich in boron compared to the terrestrial environment (Carrano *et al.* 2009). A high concentration of boron in the final nutrient solution was regarded as positive since boron is an essential element for plant growth.

The results show that aerobic digestion has a great potential for solubilizing nutrients from aquacultural sludge. It has been suggested that divalent cations bound to polysaccharides are released during aerobic digestion by the destruction of flocs and solubilization of biopolymers (Novak *et al.* 2003). After four weeks of aerobic digestion and chitosan flocculation, approximately 80% of the sludge could be reclaimed as a clear phase rich in dissolved nutrients as shown in Table 2. The chitosan did not remove the macro- or micronutrients previously dissolved by aerobic digestion, except for a 39% reduction in Zn concentration, and modest declines in Cu and Mo concentrations (Table 2). However, the level of Zn was still 10 times higher than the recommended value for lettuce growth in hydroponic systems, while the Cu concentration was slightly below the recommended level (Table 2). For N and P, the final concentrations were 4.8 and 4.0 times higher than recommended concentrations for lettuce growth.

Al and cadmium (Cd) were detected in the sludge after three weeks of aerobic digestion at concentrations of 62.5 μg/L and 4.2 μg/L, respectively (Table 2). Al is toxic to plants even at low concentrations – it inhibits plant cell growth and division (Imadi *et al.* 2016). Cd is a heavy metal with high toxicity to crops. It is easily absorbed by plant roots and alters the cellular, molecular, biochemical, and physiological mechanisms of plants and thus affects plant growth and morphology (Shanmugaraj *et al.* 2019). The literature does not give limits for concentrations of Al and Cd in nutrient solutions for hydroponic systems, but it is argued that Al in ionic form with +3 charge, which is considered the soluble form, is harmful to plants even at micromolar concentrations, and that Cd is phytotoxic at even lower concentrations (Imadi *et al.* 2016; Shanmugaraj *et al.* 2019). After chitosan precipitation, the concentrations of Al and Cd were reduced to 15.5 μg/L and 2.2 μg/L, respectively, which correspond to 75% and 48% removal efficiencies.

Chitosan is considered to be a good metal chelator (Zhang *et al.* 2016), and the ability to remove Cd is consistent with the results reported by Bailey *et al.* (1999), who found that chitosan has high specificity for heavy metals of environmental concern (e.g. lead, mercury, cadmium and chromium). Interestingly, the chitosan treatment did not reduce the concentrations of metals essential for plant growth, including Mn and Fe (Table 2). Such limited removal of some metals by chitosan flocculation was explained by Guibal (2004), who argued that the interaction of metal ions with dissolved chitosan did not lead to the formation of settleable flocs. The metal-chitosan complex remained in solution. He further pointed out that due to the poor removal capacity by coagulation and flocculation, chitosan is mostly used to chelate metal ions in a variety of solid forms, such as beads, flakes and membranes (Guibal 2004).

CONCLUSIONS

This study shows that aerobic digestion followed by solids precipitation using chitosan as the flocculant is a promising method for mobilizing and reusing nutrients in sludge from aquaculture. The resulting nutrient solution can be used as fertilizer in soilless growth systems. During aerobic digestion, the concentrations of soluble N increased from 181 mg/L at the start to 890 mg/L after three weeks of aerobic digestion, and to 903 mg/L after four weeks of aerobic digestion and solids removal by chitosan precipitation. The corresponding concentrations for dissolved P were 8.2 mg/L at start and 110 mg/L after three weeks, then 160 mg/L after four weeks of aerobic digestion and chitosan treatment. This implied approximately 80%

mobilization of the total N and P content in the sludge. Other macro- and micronutrients were also mobilized to concentrations close to or higher than levels recommended for hydroponic growth of vegetables. K, Ca, Mg, S and B showed rises in concentration by factors of 1.7, 3.2, 2.0, 2.7, and 1.6, respectively after three weeks of aerobic digestion, and by factors of 2.1, 5.0, 3.1, 3.1 and 21.8 after four weeks of aerobic digestion and chitosan flocculation. For removal of solids and color after aerobic digestion, the biopolymer chitosan proved to be a good alternative. After coagulation, flocculation and sedimentation, the turbidity was reduced to an average level of 6.5 FNU. The chitosan did not remove the macro- or micronutrients previously dissolved by aerobic digestion, except for a 39% reduction in Zn concentration, and modest declines in Cu and Mo concentrations. Another positive effect of chitosan was the removal of potentially phytotoxic metals, including Al and Cd removals of 75% and 48%, respectively. After aerobic digestion and chitosan treatment, approximately 80% of the raw sludge could be reclaimed as a nutrient-rich clear phase to be supplied to recirculating hydroponic or aquaponic systems. The effect of the reclaimed nutrient solution on plant growth in such systems should be further studied.

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