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Reactive nitrogen and phosphorus removal from aquaculture wastewater effluents using polymer hydrogels

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Abstract

We have developed poly(allyl amine hydrochloride) (PAA · HCl) polymer hydrogels, that efficiently remove nitrate (NO_3^-), nitrite (NO_2^-), and orthophosphate (PO_4^{3-}) nutrient anions from the aquaculture wastewater. The hydrogels were prepared by chemically crosslinking linear PAA · HCl chains with epichlorohydrin (EPI). The anion binding capacity of the pH sensitive polymer gels was measured in standard solutions and studied as a function of gel synthesis parameters. Equilibrium NO_3–N, NO_2–N, and PO_4–P loading of 15, 1.6, and 17 mg/g of dry gel, respectively, were calculated from the measurement of decrease in anion concentration in aqueous solutions using UV–vis spectrophotometry. Batch experiments showed that nutrient concentrations in aquaculture wastewater effluents decreased with regard to PO_4–P by 98 + %, NO_3–N by 50 + % and NO_2–N by 85 + % within 3 h of reaction. The regeneration of the hydrogels was demonstrated by the release of bound nutrient anions upon washing the gels with a 1 N NaOH solution. These results have demonstrated that the hydrogels are appropriate materials for treating aquaculture wastewater effluents, and reducing the nutrient anion concentrations to levels, less than 10 mg/l NO_3–N, 0.08 mg/l NO_2–N, and 0.3 mg/l PO_4–P, suitable for discharge into natural surface waters.

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1. Introduction

Aquaculture is a rapidly expanding industry that requires quantities of water in the order 200–600 m^3/kg fish produced (Sauthier et al., 1998). Many aquaculture production facilities operate as 'flow through' or 'open' systems, thus releasing large quantities of nutrient rich water into another receiving body of water. Commercial recirculating or closed water systems are being developed to minimize the quantities of water used (Sauthier et al., 1998) and have achieved a certain degree of success. Given the increase in land prices, water shortages, and governmental regulation on the effluents from the aquaculture facilities, closed water systems will be needed if aquaculture is to continue growing (Stickney, 1994). Natural waters can be contaminated, and their quality decreased, by the discharge of nutrient anion pollutants such as nitrate, nitrite and phosphate. Most natural waters are nutrient limited. High NO_3^-, NO_2^-, and PO_4^{3-} concentrations are found in the wastewater discharge of recirculating and, to some extent, flow through aquaculture production systems.

At sufficiently high concentration levels nutrient anions could become toxic to fish or lead to the crash of a phytoplankton bloom resulting in the rapid growth of filamentous algae or undesirable macrophytes (Ng et al., 1993; Stickney, 1994; Sauthier et al., 1998). Algae blooms, (aquatic plant growth), produce unsightly areas, lower dissolved oxygen (DO) concentrations in the water and may lead to fish mortalities. High levels of nutrient anions lead to eutrophication of receiving water bodies resulting in the creation of marshland (Stickney, 1994). Productivity is the most conspicuous aspect of cultural eutrophication. It is accelerated by the runoff from the aquaculture waste discharges, rich in nutrients.

NO_2^- is highly toxic to certain species of fish. NO_2^- enters the bloodstream through the gill by the mechanism that normally transports chloride (Boyd and Tucker, 1998). After entering the bloodstream NO_2^- oxidizes the iron in the hemoglobin molecule from the ferrous state (Fe^{2+}) to the ferric state (Fe^{3+}). The resulting product, called methemoglobin, is incapable of reversibly binding with oxygen, so exposure to NO_2^- causes respiratory distress because of the loss in blood oxygen-carrying capacity. Other lesser effects contribute to NO_2^- toxicity as well. NO_3^- is the least toxic of the major inorganic nitrogen compounds. However, high levels NO_3^- can affect osmoregulation and oxygen transport, but toxic concentrations are much higher than for ammonia and NO_2^- (Zweig et al., 1999). Recommended inorganic nitrogen levels in ponds and tanks on a general basis are <0.15 mg/l for NO_2–N and <23 mg/l for NO_3–N (Zweig et al., 1999). The recommended release concentrations are <11 mg/l NO_3–N, 0.03 mg/l NO_2–N, and 2.6 mg/l PO_4–P. Interest in the impacts of wastewater discharge from the aquaculture production has not been limited to areas where cold-water facilities were releasing their wastewater into rivers, streams and lakes. Various states as well as the federal government have began developing effluent guidelines (Stickney, 1994).

The removal of dissolved nutrients requires the employment of more advanced technology, than primary and secondary treatment, such as ion exchange resins that will scrub the nutrients from the water. Inorganic and polymeric sorbents, such as clay minerals, zirconia, titania, polymeric ligand exchangers and activated alumina have been investigated as adsorbents of nutrients (especially PO_4^{3-}) in water (Urano and Tachikawa, 1991; Zhao and Sengupta, 1996, 1997). These conventional adsorbents, however, may not be feasible in practical wastewater treatment because their adsorption capacities are insufficient and the processes using these sorbents have not been fully developed. Some of the shortcomings with these sorbents can be summarized as follows:

- poor selectivity towards one anion over other competing anion species (i.e. poor PO_4^{3-} binding due to the presence of sulfate (SO_4^{2-}), chloride (Cl^-), bicarbonate (CO_3^{2-}), and dissolved organics);
- low capacity in the neutral pH range;
- insufficient regeneration;
- gradual loss in capacity due to dissolution of the sorbent or fouling by organic matter;
- long times to achieve desired anion removal.

Polymeric hydrogels are hydrophilic three-dimensional polymer networks that can absorb large amounts of water. The resulting water swollen polymer network is prevented from dissolving because of the presence of tie-points between the polymer chains such as crosslinks, physical chain entanglements, or crystalline regions. Poly(allyl amine), or, its analog HCl form (poly(allyl amine hydrochloride), PAA · HCl), is a water-soluble cationic polymer that can be chemically crosslinked to produce a highly water swollen hydrogel. PAA · HCl is a polymer having pendant primary amino groups (NH_2). When the PAA · HCl polymer, is placed in an anion-containing solution, counterions bind through electrostatic association to the pendant to the main chain protonated amine polyions (NH_3^+) and are trapped into the gel network. Thus PAA · HCl hydrogels selectively bind the nutrient anions into the polymer matrix permitting their subsequent removal from the contaminated system. Previous study performed in the batch mode indicated that the PAA · HCl hydrogels are capable of effectively removing PO_4^{3-} from the aquaculture wastewater (Kioussis et al., 1999; Kioussis, 1998). The focus of this study was the development of novel polyelectrolyte hydrogel materials for the removal of NO_3^- and NO_2^- as well as PO_4^{3-} from the aquaculture effluents.

The anion binding properties as well as the final microstructure of the PAA · HCl gels are influenced by the relative amounts of materials used in their synthesis. In this study the amounts of the reactants used to synthesize the hydrogel were varied in order to optimize the resulting hydrogel properties. To be effective in removing nutrient anions from the wastewater effluents, the rate of transport of the anions into the gels must be large enough so that efficient binding can be achieved. The transport process must also be reasonably insensitive to pH changes so that the anions can be bound from the aquaculture effluents of varying pH. Furthermore, the presence of particulates (suspended solids), organics, and counterions that are common fouling constituents of aquaculture system effluents should not limit the nutrient removal ability of the gels. The ultimate goal of this research will be to place the PAA · HCl gel into packed columns in commercial aquaculture systems with wastewater effluent being pumped over the column.

2. Materials and methods

2.1. Materials

PAA · HCl solid powder, sodium hydroxide (NaOH) (pellets, 97%), epichlorohydrin (EPI) 99 + % solution, potassium dihydrogen phosphate (KH_2PO_4) (99 + %), sodium nitrate ($NaNO_3$) (99 + %), and sodium nitrite ($NaNO_2$) (97 + %) were purchased from the Aldrich Chemical Company. All reagents were ACS grade and were used without further purification. Hybrid striped bass and tilapia fish tank wastewater from the Department of Biological Resources Engineering at the University of Maryland was used. Wastewater samples were collected directly from inside the fish tanks. The fish were grown in a pilot-scale recirculating water system (with circular fiberglass tanks, filters for solids removal, and biofilters) and were being fed commercial fish feed containing approximately 38% crude protein, 8% crude fat, and 5% crude fiber. This aquaculture wastewater effluent has an average pH of 7.70 and contains high levels of dissolved and particulate complex organics as well as other compounds such as ammonia (NH_3), PO_4–P (20 ± 12 mg/l), NO_3–N (19 ± 10 mg/l), NO_2–N (0.20 ± 0.01 mg/l), Cl^- (5 g/l), sulfate (SO_4^{2-}) (85 mg/l); $SO_4^{2-}/Cl^- = 0.017$. The average total ammonia nitrogen (TAN), total organic carbon (TOC), biological oxygen demand (BOD_5), alkalinity and DO content of the wastewater is 0.8, 21, 5.3, 110 and 6.2 mg/l, respectively. The aquaculture wastewater samples were filtered using Whatman qualitative filter circles (particle retention > 11 μm, porosity: medium) prior to the batch-binding experiments. Milli-Q reagent grade distilled water was used for washing the PAA · HCl gels, preparing standard/buffer aqueous solutions, and diluting samples prior to analysis.

2.2. Hydrogel preparation

The hydrogel networks were prepared by the aqueous reaction of 20% w/v PAA · HCl solution and EPI, which serves as the crosslinking agent. The PAA · HCl used in the synthesis of the anion binding hydrogels had average molecular weights of 9750 and 57 500 g/mol, including a hydrochloric acid (HCl) group ionically associated with amine (NH_2) groups on the polymer chain backbone. The PAA · HCl molecular weight used to make the gels depended on its availability by the supplier. Before crosslinking the polymer chains a portion of the HCl groups of PAA · HCl were neutralized with NaOH. This was done to provide 'free' NH_2 sites for the crosslinking reaction. EPI was subsequently added to react with the resulting free NH_2 sites and form crosslinks (tie-points) between neighboring PAA · HCl chains in the aqueous solution. The chemical crosslinking synthesis reaction steps are shown in Fig. 1 and explained in more detail elsewhere (Kioussis et al., 1999).

The anion binding properties as well as the final microstructure of the PAA · HCl gels are influenced by the relative amounts of reactants used in their synthesis. The relative amounts of NaOH and EPI were therefore independently varied during the synthesis reaction to determine their possible influence on the gels' final structure and anion binding capacity. For example, the crosslink density, which can affect both the mechanical properties and the anion transport through the hydrogel, was controlled via the number of free (neutralized) NH_2 sites available for crosslinking to occur (i.e. varying the amount of NaOH added) or via the amount of EPI added for the crosslinking reaction.

2.2.1. Variation in EPI content

The amount of NaOH and the PAA · HCl initial concentration (20% w/v solution) were kept unchanged, while the amount of EPI (crosslinker), was varied to produce gels having varying crosslink density. Gels were synthesized by adding 1.26, 1.57 (+25% EPI), 1.76 (+40% EPI) and 1.88 (+50% EPI) $\times 10^{-3}$ mol EPI/g PAA · HCl during the crosslinking reaction step.

2.2.2. Variation in NaOH content

Initially, the aqueous PAA · HCl solutions were partially neutralized using NaOH to cleave off HCl groups associated with NH_2 groups. The pH of the gel formation reaction becomes more basic (pH 10.7–11.0) with increasing amount of NaOH added to the reaction medium thus increasing the number of free NH_2 sites available for crosslinking. Gels were synthesized with 20% w/v PAA · HCl solution, 1.26×10^{-3} mol EPI/g PAA · HCl, and 0.28, 0.30 (+7% NaOH), 0.36 (+30% NaOH), and 0.43 (+54% NaOH) g NaOH/g PAA · HCl. The time to gelation (network formation), upon the addition of EPI, decreased with increasing amount of NaOH. Gels synthesized with 0.43 g NaOH/g PAA · HCl gelled within 6–8 min after the addition of EPI to the stirred solution of NaOH and PAA · HCl. In

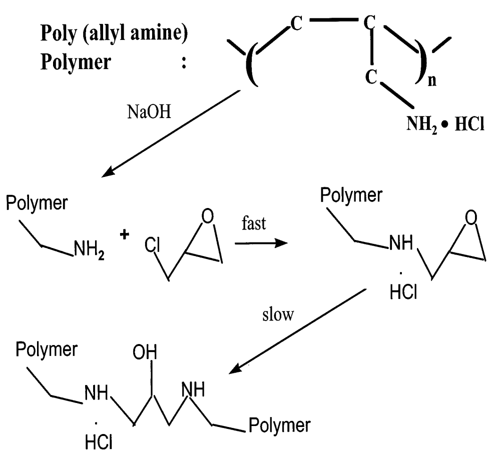

Fig. 1. Hydrogel synthesis reaction steps.

comparison, gels synthesized to 0.28 g $NaOH$/g PAA · HCl required more than 20 min to reach the gelation point after the addition of EPI to the stirred reaction medium.

2.3. Nutrient anion binding mechanism

The PAA · HCl hydrogels are pH sensitive (Kioussis et al., 1999; Rama Rao et al., 1999). At acidic pH's the primary (NH_2) and secondary (NH) amine groups present along the PAA chains and on crosslink junction units, respectively, become protonated, i.e. NH_3^+ and NH_2^+ state. The pK_a value of PAA · HCl is 9.67. Hence, at pH values lower than 9.67 the degree of swelling of PAA · HCl gels increases due to electrostatic repulsion (positive charge) of the chains within the network. At more alkaline pH's, in excess of pH 9.67, the NH_2 groups lose a hydrogen (become deprotonated) and exist in their uncharged NH and NH_2 forms. Hence the gel network collapses resulting in little or no binding since there is also no electrostatic attraction between the network and the nutrient anions. This charging and discharging of the network will affect the swelling behavior as well as the anion removal ability of the PAA · HCl gels. However, wastewater effluents typically exhibit a pH in the range 5.5–8.0, where the gels exhibit high anion binding capacity.

2.4. Anion concentration measurements

UV–vis spectrophotometry was used to measure the anion concentrations in the various samples. PO_4–P levels were measured using the Hach Company PhosVer 3 method (Hach, 1997) on a Hach DR/2010 UV spectrophotometer. The detection procedure used by the spectrophotometer is equivalent to USEPA method 365.2 and standard method 4500-P-e for natural water and wastewater, which is also known as the ascorbic acid Method. The NO_3–N and NO_2–N concentration levels were measured using the Hach Company NitraVer5 and NitriVer3 methods, respectively (Hach, 1997). The detection procedures used are the diazotation method, which is USEPA approved and the cadmium reduction method (Federal Register, 1979) for NO_2–N and NO_3–N, respectively. Aquaculture wastewater was filtered before any experimentation to reduce interference of particulates with the concentration measurements.

Error bars were calculated from the standard deviation of the pixel statistics and were plotted only when the uncertainty limits were larger than the size of the plotted data points. In all figures, the lines through the data points are guides to the eye.

2.5. Experiments in standard solutions

Nutrient uptake assays in batch reactors were carried out in ionic solutions prepared in deionized water since the composition of tap water varies considerably. This standardized system allowed us to study the NO_3^-, NO_2^-, and PO_4^{3-} uptake capacity of the gels without interference from other ions. The effect of varying the gel synthesis parameters (i.e. amount of NaOH) on the anion binding capacity of the gels was also evaluated in standard solutions.

Standard solutions of $NaNO_3$, $NaNO_2$ and KH_2PO_4 were prepared in deionized water. The concentrations of NO_3^-, NO_2^-, and PO_4^{3-} in the standard solutions were prepared to be similar to the nutrient anion concentrations characteristic of the aquaculture wastewater. Batch reactor sample volumes ranged from 40 to 80 ml and contained 0.005–0.01 g of ground dry PAA · HCl gel particles. Initially, the reactor was filled with the particular standard solution. A slurry of PAA · HCl gel particles was then added and the decrease of the anion concentration that occurred was measured at 30-min intervals. A number of identical batch experiments were also run simultaneously in order to supplement the sample volume extracted from the main reactor for the concentration measurements. The experiment stopped when the anion concentration in the batch reactor remained constant. At this time the gel had reached its saturation point. The final concentration was recorded and converted to an elemental basis.

The anion binding capacity (mg anion bound per g dry gel) of the PAA · HCl gel used in the experiment, for each anion, was calculated from the experimental data using the following equation:

$$\frac{\text{Amount of } A^- \text{ bound (mg)}}{\text{gel (g)}} = \frac{(C_0 - C_f)V_s}{m_0} \tag{1}$$

where, C_0, initial anion concentration (mg/l); C_f, final anion concentration (mg/l); V_s, reactor volume (l); and m_0, amount of dry gel used in the experiment (g). The anion binding capacity values calculated were converted to an NO_3–N, NO_2–N, and PO_4–P basis. The dependence of the total anion binding capacity of the PAA · HCl hydrogels on their chemical composition was determined.

2.6. Experiments in aquaculture effluents

These experiments were designed to evaluate the effect of various inorganic and organic constituents present in aquaculture wastewater on the nutrient anion removal ability of the PAA · HCl gels. Proper sample collection, preservation and storage were critical for accurate testing. Wastewater samples were vacuum filtered immediately upon collection, to remove particulates larger than 11 μm, and stored at 4°C if immediate analysis was not possible. The initial NO_3^-, NO_2^-, and PO_4^{3-} concentrations were measured before testing. Batch reactors with vol. 40–100 ml of sample wastewater containing 0.1–1.1 g of ground dry gel particles were used. The decrease in NO_3^-, NO_2^-, and PO_4^{3-} concentrations was monitored at 30-min intervals. The nutrient concentrations stopped decreasing when the gel reached its saturation point. The final nutrient anion concentrations were recorded and converted to an NO_3–N, NO_2–N, and PO_4–P basis. The aquaculture wastewater sample was emptied out of the reactor and subsequently filtered to remove the saturated PAA · HCl gel. The experiment was repeated using a fresh supply of the same type of PAA · HCl gel in the batch reactor.

Using the anion binding capacity values, obtained from the experiments in standard solutions, prepared with deionized water, the theoretical amount of PAA · HCl gel required for complete removal of the nutrient anions from three 100 ml wastewater samples was calculated. From these experiments, the average anion binding capacity and the percent of average efficiency for anion removal in aquaculture wastewater of the PAA · HCl gel were determined.

2.7. Regeneration

Experiments were performed to determine whether the PAA · HCl hydrogels could be regenerated after having bound the nutrient anions from the aquaculture wastewater. Approximately 0.1 g of anion saturated PAA · HCl gel was treated with 5 ml of 1 N NaOH solution for 1 h after the anion removal experiment in wastewater. The gel was then vacuum filtered to remove the waste solution consisting of Na_3PO_4, $NaNO_3$, $NaNO_2$, and NaCl that were formed during the treatment. The gel was re-washed with deionized water to remove any residual Na_3PO_4, $NaNO_3$, $NaNO_2$, and NaCl precipitated solids that remained. The gel was then air-dried in an oven to prepare it for reuse in a new anion removal experiment. The above procedure thus describes a 'regeneration cycle' that involves an anion removal experiment followed by a release experiment and a second removal experiment.

3. Results

3.1. Hydrogel anion binding capacity measurements

The effectiveness of the PAA · HCl hydrogels in removing NO_3^- and NO_2^- from the wastewater was initially investigated in $NaNO_3$ and $NaNO_2$ aqueous standard solutions. Fig. 2 shows the NO_3–N concentration decrease with time upon the addition of 0.01 g PAA · HCl gel to a batch reactor containing 40-ml $NaNO_3$ solution. The hydrogel reached its saturation point after 2.5 h of reaction time and the NO_3–N concentration remained constant at 1.5 mg/l. More than 70% of NO_3–N initially presented was removed. The average binding capacity in $NaNO_3$ solution was found to be 15 ± 6 mg NO_3–N/g gel on an NO_3–N basis, depending on the chemical composition of the gel used and the initial NO_3–N concentration (ionic strength). Fig. 3 shows the NO_2–N concentration decrease with time, after the addition of PAA · HCl gel particles to a batch reactor filled with $NaNO_2$ aqueous standard solution. The NO_2–N concentration stopped decreasing and remained constant after 2 h of reaction time. The final NO_2–N concentration in the reactor was 0.05 mg/l. The gel removed more than 80% of NO_2–N initially present in the $NaNO_2$ sample. The average NO_2–N binding capacity was found to be 1.6 ± 0.5 mg NO_2–N/g gel depending on the synthesis of the gel used and the initial NO_2–N concentration.

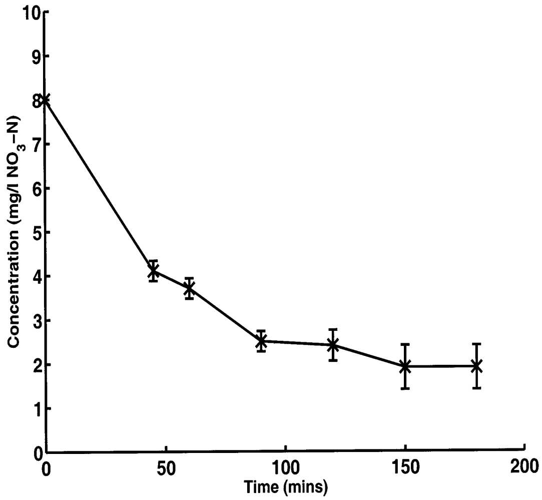

Fig. 2. Measurement of the NO_3–N binding capacity of gel in $NaNO_3$ standard solution. PAA · HCl concentration, 20% w/v; PAA · HCl $M_n = 9750$ g/mol; EPI, 1.57×10^{-3} mol/g PAA · HCl; NaOH, 0.28 g NaOH/g PAA · HCl.

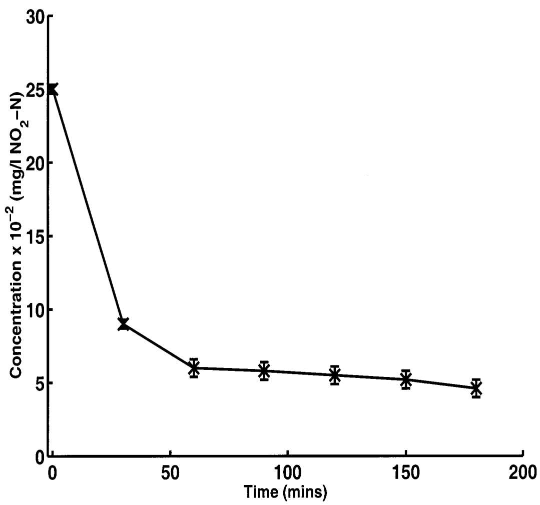

Fig. 3. Measurement of the NO_2–N binding capacity of gel in $NaNO_2$ standard solution. PAA · HCl concentration, 20% w/v; PAA · HCl $M_n = 9750$ g/mol; EPI, 1.57×10^{-3} mol/g PAA · HCl; NaOH, 0.28 g NaOH/g PAA · HCl.

The effectiveness of the PAA · HCl gels in removing PO_4–P has been previously reported (Kioussis et al., 1999). PO_4–P removal of more than 99% was achieved in KH_2PO_4 buffer solutions at pH values between 5.5 and 8.0. An average binding capacity of 17 ± 3 mg PO_4–P/g gel was measured in the buffer solutions.

It must be noted that if a larger amount of PAA · HCl gel was added to the batch reactor nearly complete anion removal would be achieved. However, the aim of these experiments was to calculate the NO_3–N and NO_2–N binding capacities of the gel and not to achieve maximum removal of nutrient anions. Hence, the PAA · HCl gel amounts used were small enough for the gel to reach its saturation point before complete removal of nutrient anions from the standard solutions. Optimization of nutrient anion removal was performed in aquaculture effluents.

3.2. Effect of synthesis parameters on binding capacity

The effect of PAA · HCl gel synthesis parameters, such as the amount of NaOH, on the anion binding capacity was determined. Gels were prepared with varying amount of NaOH and were used in batch reactors containing the various standard solutions. The amount of NaOH added to PAA · HCl during the synthesis reaction was determined to affect the anion removal performance of the resulting gel. Figs. 4 and 5 show the change in the gel's anion binding capacity with increasing amount of NaOH. In all the cases an increase in the amount of NaOH from 0.28 to 0.43 g NaOH/g PAA · HCl (+54%) added during the synthesis of the gel lead to a $30 \pm 10\%$ drop of its anion binding capacity.

Experiments were carried out to determine the effect of the degree of crosslinking on the PAA · HCl gel anion binding ability. By varying the amount of EPI, it was possible to prepare gels with varying crosslink density; and hence, networks that were either loosely crosslinked or more densely crosslinked. Fig. 6 shows that a 50% increase in the EPI content of the gels caused a $6 \pm 5\%$ change in their anion

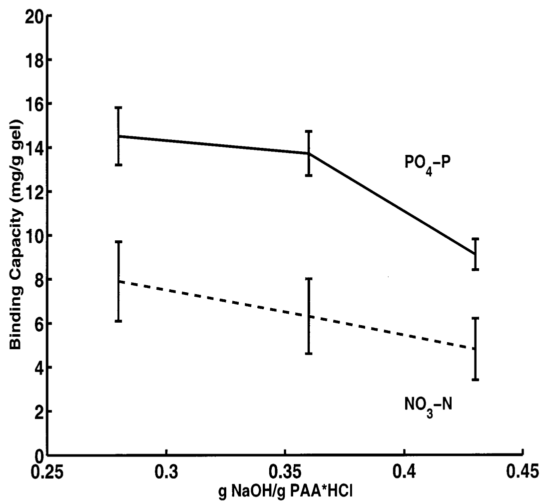

Fig. 4. Effect of NaOH amount on gel NO_3–N and PO_4–P binding capacity in standard solutions. PAA · HCl concentration, 20% w/v; PAA · HCl $M_n = 57\,500$ g/mol; EPI, 1.26×10^{-3} mol/g PAA · HCl.

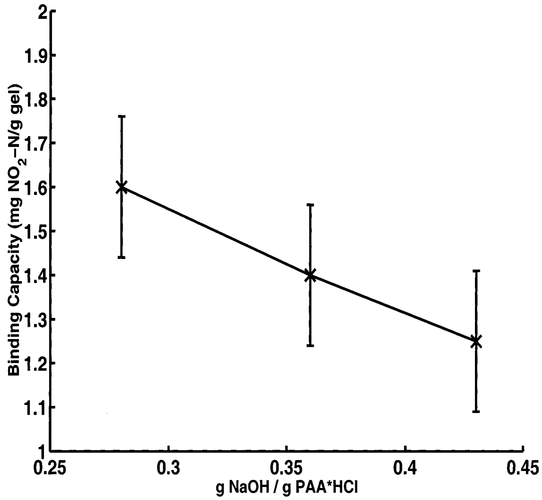

Fig. 5. Effect of NaOH amount on NO_2–N binding capacity in standard solution. PAA · HCl concentration, 20% w/v; PAA · HCl $M_n = 9750$ g/mol; EPI 1.26×10^{-3} mol/g PAA HCl.

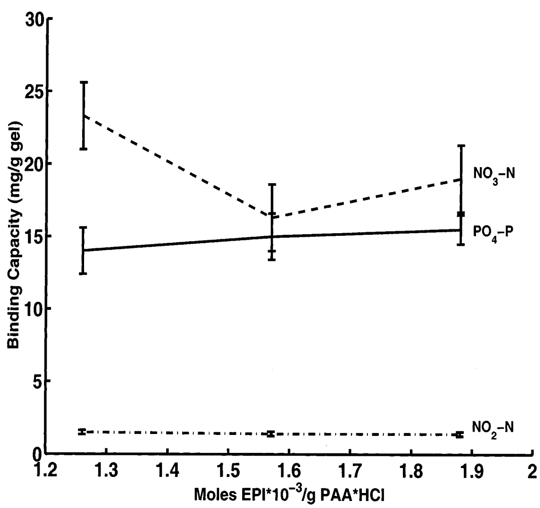

Fig. 6. Effect of EPI amount on gel NO_3–N and PO_4–P binding capacity in standard solutions. PAA · HCl concentration, 20% w/v; PAA · HCl $M_n = 9750$ g/mol; NaOH, 0.28 g NaOH/g PAA · HCl.

binding capacity (depending on the type of anion). This change is within the limits of experimental error and thus it can be concluded that the EPI content does not influence the anion binding capacity.

The effect of molecular weight was not investigated in this study. However, Kioussis et al. (1999) showed that gels prepared with low molecular weight

PAA · HCl had 5–10% higher PO_4–P binding capacity than gels synthesized with high molecular weight PAA · HCl (57 500 g/mol).

3.3. Nutrient removal from aquaculture wastewater

Experiments were conducted using aquaculture production system wastewater to determine the effectiveness of the PAA · HCl hydrogels in removing nutrient anions. Kioussis et al. (1999) have previously shown that within 2 h of batch reaction time the PAA · HCl gels are capable of binding more than 95% of PO_4–P initially in the aquaculture wastewater (see Fig. 7). Binding values up to 15.3 mg PO_4–P/g gel have been obtained in wastewater samples depending on the chemical composition of the gel used.

The aquaculture wastewater samples contained high concentrations of NO_3–N (19 ± 10 mg/l), NO_2–N (0.20 ± 0.01 mg/l), and PO_4–P (20 ± 12 mg/l). Batch experimentation showed that the PAA · HCl gels concurrently bind PO_4–P, NO_2–N, and NO_3–N from the waste effluents as shown in Figs. 8 and 9. The PAA · HCl gels have a higher affinity towards the PO_4^{3-} anion due to its trivalent ionic charge as compared with NO_3^- and NO_2^-, which are monovalent anions. In this experiment, more than 98% of PO_4–P, 85% of NO_2–N, and 53% of NO_3–N initially present were removed. Figs. 8 and 9 show that the PO_4–P and NO_2–N concentrations decreased to 0.3 and 0.08 mg/l, respectively, within 3 h of batch reaction time. The NO_3–N concentration stopped decreasing after 4 h and remained constant at 10 mg/l. Experiments were also performed with unfiltered wastewater. Similar nutrient anion removal was observed in the unfiltered wastewater samples. However, sample aliquots used for measuring the anion concentrations were filtered to

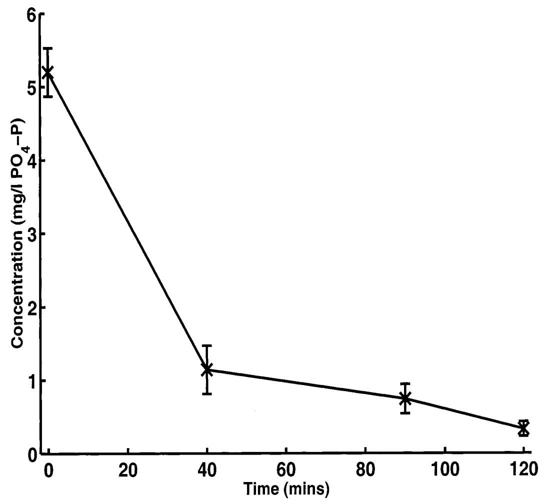

Fig. 7. Decrease in PO_4–P concentration in aquaculture wastewater, pH 7.60 ± 0.50. PAA · HCl concentration, 20% w/w; NaOH, 0.28 g NaOH, 0.28g NaOH/g PAA · HCl; EPI, 1.26×10^{-3} mol/g PAA · HCl.

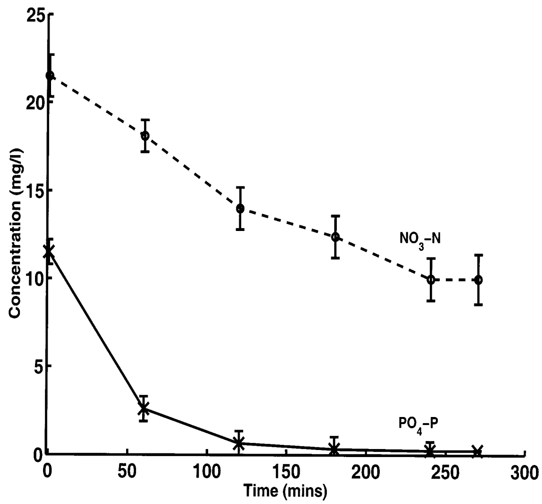

Fig. 8. Concurrent decrease in NO_3–N and PO_4–P concentrations in aquaculture wastewater, pH 7.97 ± 0.50. PAA · HCl concentration, 20% w/v; PAA · HCl $M_n = 57\ 500$ g/mol; NaOH, 0.28 g NaOH/g PAA · HCl; EPI, 1.26×10^{-3} mol/g PAA · HCl.

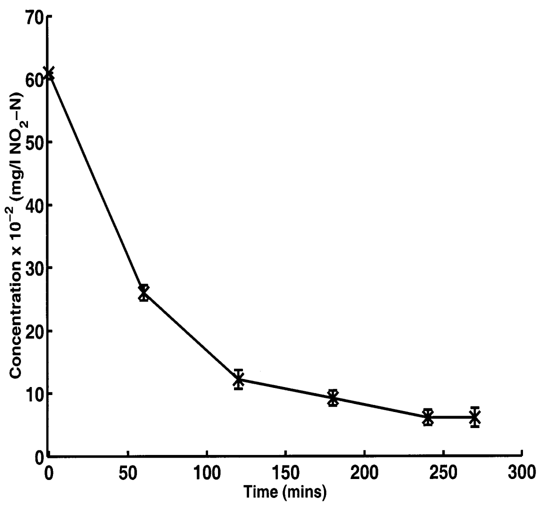

Fig. 9. Decrease in NO_2–N concentration in aquaculture wastewater, pH 7.97 ± 0.50. PAA · HCl concentration, 20% w/v; PAA · HCl $M_n = 57\ 500$ g/mol; NaOH, 0.28 g NaOH/g PAA · HCl; EPI, 1.26×10^{-3} mol/g PAA · HCl.

remove larger particulates that could interfere with the spectrophotometric detection methods. Thus, samples were filtered before use in the batch binding experiments in order to expedite the sampling time (and reduce costs). The gels selectively bound PO_4^{3-} over NO_2^- and NO_3^-. The experimental results therefore demonstrated that the PAA · HCl gels are capable of concurrently removing these nutrients from the aquaculture wastewater.

The competition of other counteranions such as Cl^-, SO_4^{2-}, and S^{2-} for NH_3^+ groups was also investigated. During the batch experiments the concentration of Cl^- in the reactors increased with time since Cl^- anions ionically associated with NH_3^+ groups on the polymer chains were exchanged for nutrient anions that became ionically bound to NH_3^+. Preliminary experiments have shown that the gels have a high affinity for SO_4^{2-} anions. These preliminary results have indicated that the competitive binding of SO_4^{2-} anion prevails over the binding of NO_3^- and NO_2^- by the gel. This is probably due to the divalent ionic charge of the SO_4^{2-} anion versus the monovalent anions. The S^{2-} anion's competition effect was negligible since its concentration in the aquaculture wastewater was extremely low.

3.4. Hydrogel removal efficiency in aquaculture wastewater

The aquaculture wastewater contained high levels of particulates of size <11 μm, dissolved organics, and counterions. Experiments were performed to determine how these fouling agents effect the nutrient anion removal ability of the PAA · HCl gels.

The theoretical amount of gel required for complete removal of PO_4^{3-}, NO_2^-, and NO_3^- from the aquaculture wastewater was calculated based on average binding capacity values of 17 mg PO_4–P/g gel, 1.6 mg NO_2–N/g gel, and 15 mg NO_3–N/g gel, (determined from the experiments in the standard solutions) and the nutrient anion mass content of the sample. This amount of PAA · HCl gel was used to remove nutrient anions from the aquaculture effluents. Batch experiments were carried out as previously described. Comparison to the anion binding capacity of the gels in standard solutions to their binding capacity measured in the aquaculture effluents resulted in a calculation of percent of average removal efficiency values which are presented in Table 1. The 10–72% (depending on the anion) decrease in nutrient anion binding by the gels in aquaculture wastewater is due to counterions (i.e. SO_4^{2-}) as well as other fouling factors that are presented in the wastewater and can potentially clog the gel or interact with the PAA · HCl NH_3^+ groups.

Table 1
Gel binding efficiency in aquaculture wastewater[a]

Nutrient	Average binding capacity (mg/g gel), aquaculture wastewater	Efficiency (%)
PO_4–P	15	90 ± 8
NO_3–N	6	33 ± 17
NO_2–N	0.6	28 ± 7

[a] PAA · HCl concentration, 20% w/v; PAA · HCl $M_n = 57\,500$ g/mol; NaOH, 0.24 g NaOH/g PAA · HCl; EPI, 1.76×10^{-3} mol/g PAA · HCl.

Table 2
Gel regeneration in aquaculture wastewater[a]

Nutrient	Removal (%)	
	Cycle 1	Cycle 2
PO_4–P	98	80
NO_3–N	53	28
NO_2–N	73	50

[a] Initial PAA · HCl concentration, 20% w/v; PAA · HCl $M_n = 57\,500$ g/mol; NaOH, 0.28 g NaOH/g PAA · HCl; EPI, 1.26×10^{-3} mol/g PAA · HCl.

3.5. Gel regeneration

Kioussis et al. (1999) have demonstrated that the PAA · HCl gels can be regenerated after having bound PO_4^{3-} from the aquaculture effluents, and their removal efficiency can be retained for at least five binding/washing cycles upon treatment with 1 N NaOH. Preliminary results from the regeneration studies for NO_3^- and NO_2^- showed the ability of the PAA · HCl gels to be regenerated for the reuse in removing nutrient anions from the aquaculture wastewater. Data for two regeneration cycles presented in Table 2 indicates that the gels can be reused to remove these nutrient anions from the aquaculture wastewater.

4. Discussion

It was possible to determine the NO_3^- and NO_2^- binding capacity of the PAA · HCl gels and the effect of synthesis parameters on the gel anion removal ability from the studies conducted in controlled environments containing only the target anion (i.e. standard solutions). The ionic strength of the anion standard solutions was determined to influence the anion removal ability of the gels. Higher anion binding capacities were achieved in standard solutions of higher ionic strength.

Experiments showed that higher amounts of NaOH added during the synthesis reaction caused a decrease in the anion binding capacity of the gel. The NaOH is used to partially neutralize the NH_2 groups of the PAA · HCl chains, by cleaving off HCl groups, to enable crosslinking of the chains. By varying this parameter during synthesis, the number of neutralized NH_2 per PAA chain available for crosslinking was altered. As the pH of the reaction is increased, by neutralizing more HCl groups with NaOH, more free NH_2 sites are produced. The average number of neutralized NH_2 groups available for crosslinking per PAA chain increased by 33% upon increasing the amount of NaOH from 0.28 to 0.43 g NaOH/g PAA · HCl (+54%). This resulted in an increase in the number of crosslinks in the polymer network structure since there are free NH_2 sites per PAA chain available for crosslinking. However, the probability of occurrence of neighboring free NH_2 sites on the PAA chains also increases, which in turn may cause the EPI to react with two NH_2 groups on the same chain forming loops instead of crosslinks (Kofinas and Cohen, 1997). The structure and anion removal characteristics of the resulting gel are negatively influenced. The gel is not uniformly crosslinked and hence does not have the expected mechanical integrity of a more uniformly crosslinked network. This results in break-up of the gel during the batch experiment.

Another factor that must be considered when varying the amount of NaOH during the gel forming reaction is the duration of the reaction itself. Gels prepared using 0.28-g NaOH/g PAA · HCl react for more than 20 min before the reaction reaches the gel point. Gels synthesized with 0.43 g NaOH/g PAA · HCl would typically react for an average of 7 min. This significant reduction in reaction time has an effect on the homogeneity of the crosslinking reaction. In contrast to model networks, with constant length of network chains between crosslinks, real networks exhibit a wide distribution of chain lengths between network junction points (Lindemann et al., 1997). Furthermore, a variety of network defects, such as dangling ends, elastically ineffective loops, and crosslink agglomerations, are known to occur. These defects change the effective crosslinking density and topological microstructure of the networks and thus influence their swelling behavior and mechanical properties. Therefore, PAA · HCl gels synthesized with relatively small reaction times until gelation (larger NaOH and/or EPI contents) have an increasing amount of crosslink inhomogeneities present in the polymer matrix, which ultimately have a negative effect on the mechanical properties and subsequently the anion binding capacity and of the gel.

The EPI content of the PAA · HCl gels was determined to have negligible effect on the rate of transport of the anions into the gel network and therefore did not influence their anion binding capacity. This was due to the fact that the mesh size of the network (the space between two adjacent crosslink units) was orders of magnitude larger than the size of the NO_3^-, NO_2^-, and PO_4^{3-} nutrient anions. However, the crosslinking density and uniformity of the gels is directly related to their EPI content. Increasing the amount of EPI in the gel synthesis causes the resulting gel to be more tightly crosslinked. This reduces its swelling in water, thus improving its mechanical integrity. It can also reduce the binding capacity of the gel by using up a larger number of neutralized NH_2 sites for forming crosslinks, instead of binding nutrient anions.

The ultimate goal of this study is to perform nutrient anion removal from the wastewater using a continuous-flow process. In this process the PAA · HCl hydrogel will be placed in a packed column. Aquaculture wastewater effluent can then be pumped over the column containing the gel as packing medium. Alternatively, the process can be set-up as a fluidized bed. The input and output anion concentration will be monitored and the percent of anion removal in the presence of fouling agents will be determined. The packed columns will be sized based on the nutrient anion removal requirements of the specific aquaculture system. The anion removal rates in the continuous-flow mode are expected to be faster than in batchwise operation. This is because there is negligible startup time for continuous processes and the wastewater is continuously coming into contact with a new supply of gel as it flows through the packing medium.

The packed column can be integrated into a recirculating aquaculture production system to maintain low levels of nutrients in the fish tanks. A typical recirculating aquaculture production system consists of 10 m^3 recirculating tanks with stocking density of 60 kg fish per m^3 and two tank exchanges per h. In this kind of system the effluent flowrate will be 20 000 l/h. The system can be setup so that 25% of the effluent flowrate (5000 l/h) is passed through the packed column. Based on average nutrient concentrations of 18 mg/l NO_3–N, 0.2 mg/l NO_2–N, and 13 mg/l PO_4–P, 115 kg of dry PAA · HCl gel are required to completely scrub the nutrients from the water for a 12-h operation (assuming no startup time for the process, and the nutrient concentrations remain constant). Two columns will be used, each one operating for 12-h (115 kg gel per column) while the other column is being regenerated.

5. Conclusions

The PAA · HCl hydrogels exhibited efficient nutrient removal in both anion standard solutions and aquaculture wastewater originating from the recirculating aquacultural production systems. The relative amounts of NaOH and EPI used during the synthesis reaction of the PAA · HCl gels were independently varied to determine their possible influence on the anion binding capacity of the gels. The experimental results indicated that the binding capacity of the PAA · HCl gels decreased with increasing amount of NaOH. The amount of EPI was determined to have a negligible effect on the anion binding capacity of the gels. The results for anion removal from the wastewater suggest that the binding capacity of the PAA · HCl gels decreased by 10–70% depending on the nutrient. This decrease was attributed to other counterions competing for binding sites, particulates, and dissolved organics present in the aquaculture effluents. Upto 98+% PO_4–P, 85+% NO_2–N, and 53+% NO_3–N removal has been achieved from the wastewater in a batch reactor. Thus, it has been demonstrated that the crosslinked PAA · HCl polymeric hydrogels are the appropriate materials for removing nutrient anions from the aquaculture wastewater effluents, resulting in the reduction of nutrient anion concentrations to levels suitable for discharge into natural surface waters.

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