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Reactive Phosphorus Removal from Aquaculture and Poultry Productions Systems Using Polymeric Hydrogels

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This work reports on the features of a sorption processes for the ultimate removal and recovery of reactive phosphorus from aquaculture and poultry production wastewater effluents. The sorbent used was a cross-linked polyamine (PAA·HCl) polymeric hydrogel. The PAA·HCl hydrogels were prepared by chemically cross-linking aqueous solutions of linear PAA·HCl chains with epichlorohydrin (EPI). The phosphorus binding capacity of the gels was measured in standard aqueous solutions as a function of ionic strength. Equilibrium PO_4^{3-}, loadings of 100 mg anion/g gel were obtained. The regeneration ability of the gels was demonstrated by release of the bound phosphorus anions upon washing with 1−2 M NaOH solution, providing opportunities to recover and reuse the gel over multiple cycles. The ionic polyamine gels have been demonstrated to be appropriate materials for treating poultry and aquaculture wastewater effluents. Upon treatment, phosphorus anion concentrations were reduced to levels suitable for discharge into natural surface waters.

Introduction

Aquatic ecosystems are often limited in the availability of two nutrients: phosphorus (P) and nitrogen (N) (*1*−*4*). Limited nutrient availability prevents the excessive growth of algae blooms that produce unsightly areas, lowers dissolved oxygen (DO) concentrations in the water, and may lead to fish kills (*1*). Methods that will effectively remove nutrient anion pollutants even at extremely low concentrations from wastewater effluents are a major environmental need. This work describes an approach that introduces novel cross-linked cationic polymeric materials into the contaminated aqueous slurry. The polymer materials we have developed selectively bind the pollutant anions into the polymer matrix, permitting their subsequent removal from the contaminated system. The polymer containing the bound pollutant anion can either be regenerated or incinerated. The treated system can then be safely discharged into natural waters or sent for further treatment for use as a municipal water supply.

Pfiesteria outbreaks have occurred in estuarine waterways from Delaware and Maryland to North Carolina. Pfiesteria, a toxic organism, attacks and kills fish in a matter of minutes (*1*). This organism has also been shown to affect humans. Symptoms range from skin rashes, to respiratory problems, and memory loss. The vast majority of outbreaks have occurred in waterways that have high nutrient levels. It is for these reasons that recently the Water Environment Research Foundation (WERF) prepared a detailed report that overviewed the present state of technology and identified high priority research needs pertaining to nutrient removal (*5*).

Aquaculture is a rapidly expanding industry that requires quantities of water in the order of 200−600 m^3 per kg fish produced (*6*). 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. At sufficiently high concentration levels, nutrient anions can become toxic to fish or lead to the crash of a phytoplankton bloom resulting in the rapid growth of filamentous algae or undesirable macrophytes (*6*−*8*). Productivity is the most conspicuous aspect of cultural eutrophication. It is accelerated by the runoff from aquaculture waste discharges, rich in nutrients.

Poultry litter, also referred to as broiler litter as it is generated primarily in broiler production, has recently been the focus of attention as a source of excessive phosphorus (P) levels in agricultural soils to which it is applied as fertilizer. The litter is removed from chicken houses in the form of a dry cake and is stored in piles either under a roof or in the open or is spread directly onto the farm fields in its dry form. Poultry litter has a nitrogen (N) content of 2−4 wt % and is commonly applied to farm fields as a N source for crops. Broiler litter contains up to 4% P, which is far in excess of the crop P requirement (most plants require: 0.05−1.0% P) (*9, 10*). Broiler litter is usually removed from the houses at a frequency often less than once per year. It contains the accumulated components of chicken excrement. 1/4 of the total N produced is lost in the form of ammonia (NH_3) due to continuous vaporization over the life of the litter (*9*). P tends to accumulate because it cannot assume a volatile form. Excess P and N applied to the soil may cause nutrient runoff and potential contamination to surface and groundwater sources. Nutrient management regulations have recently been issued which will require broiler litter and other P containing manures to no longer be applied to fields whose P levels exceed regulations.

We have developed polyamine polymers which can be cross-linked to form three-dimensional hydrophilic polymer networks that are able to absorb large amounts of water. The resulting water-swollen polymer network is prevented from dissolving due to the presence of tie-points between the polymer chains (i.e., cross-links) and is characterized by high mechanical strength. Anions can be selectively and reversibly bound into the polymer matrix permitting their subsequent removal from the contaminated system. To be effective in removing pollutant anions from 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 wastewaters of varying pH. Furthermore, the presence of particulates (suspended solids), organics, and counterions which are common fouling constituents of wastewaters, should not limit the anion removal ability of the gels.

Materials and Methods

Materials Used. Poly(allylamine) hydrochloride (PAA·HCl) solid powder (25 g), sodium hydroxide (NaOH) (pellets, 97%), sodium nitrate ($NaNO_3$) (99+ %), and sodium nitrite ($NaNO_2$) (97+%) were purchased from Aldrich Chemical Co. The cross-linking agent purchased from Aldrich, epichlorohydrin 99+ % solution (EPI), was used to produce the hydrogel network. All reagents were ACS grade and were used without further purification. The PhosVer3, NitraVer5, reagents used to

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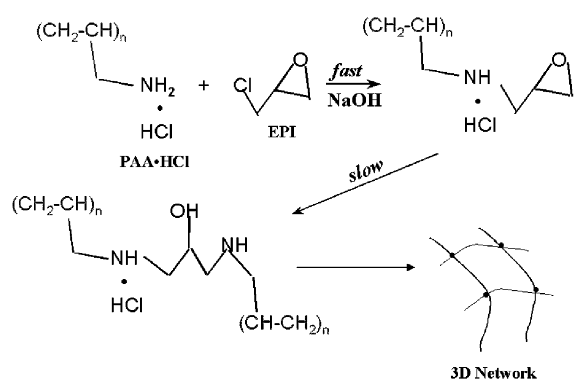

FIGURE 1. PAA·HCl Hydrogel synthesis by chemical cross-linking with EPI cross-linker.

perform the UV−vis concentration measurements were purchased from the Hach Company. Milli-Q reagent grade distilled water was used for washing the PAA·HCl gels, preparing standard aqueous solutions, and diluting samples prior to analysis.

Hydrogel Preparation. The randomly cross-linked poly(allylamine) hydrochloride (PAA·HCl) networks were prepared by the aqueous post polymerization reaction of 20% w/v solution of linear PAA·HCl chains and EPI as the cross-linking agent. The PAA·HCl used in the synthesis of the gels had an average molecular weight of 15 000 g/mol, including a hydrochloric acid (HCl) group ionically associated with amine (NH_2) groups on the polymer chain backbone. Before cross-linking 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 cross-linking reaction. Cross-linker was subsequently added to react with the resulting free NH_2 sites and form cross-links (tie points) between neighboring PAA·HCl chains in the aqueous solution. The chemical cross-linking reaction steps are shown schematically in Figure 1. A typical batch of hydrogel using EPI as the cross-linking agent was synthesized as follows: 10 mL of 20% w/v solution of PAA·HCl were mixed with 0.72 g of NaOH solid powder (0.36 g NaOH/g PAA·HCl) until the NaOH dissolved. Approximately 83% of the total NH_2·HCl groups were neutralized (on a molar basis). When the temperature of the solution dropped below 27 °C (the dissolution of NaOH is exothermic), 0.196 mL of EPI was added (1.26×10^{-3} moles EPI/g PAA·HCl). EPI reacted with the free NH_2 groups produced from the neutralization of HCl by NaOH. Typically, 28.3% of the neutralized free NH_2 groups were cross-linked (on a molar basis). The PAA·HCl, NaOH, EPI reaction mixture was stirred for 6 to 20 min depending on the amount of NaOH or EPI that was added. The reaction mixture was poured into a Petri dish to set into a gel slab before the sol−gel transition point was reached. Upon curing for an additional 18−24 h, the gel slab was washed with portions of deionized water to remove residual NaCl (produced from the neutralization of HCl by NaOH), unreacted PAA·HCl and EPI. Finally, the washed hydrogel slab was air-dried in an oven at 50 °C. The final water content of the dry gels, as determined by thermogravimetric analysis, was 5 to 8 wt %. The dried gel slab was ground into small pieces for use in the anion binding experiments.

Anion Detection. UV−vis spectrophotometry was used to measure anion concentrations in the various samples. PO_4^{3-} levels were measured using the Hach Company PhosVer 3 method (*12*) 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. In this method, PO_4^{3-} reacts with molybdate in an acid medium to produce a phosphomolybdate complex. Ascorbic acid then reduces the complex, giving an intense molybdenum blue color. NO_3^- concentration levels in poultry litter were measured using the Hach Company NitraVer5 method (*12*). The detection procedures used was the Cadmium Reduction method (*13*). Cadmium metal reduces NO_3^- present in the sample to NO_2^-. The NO_2^- ion reacts in an acid medium with sulfanilic acid ($NH_2C_6H_4SO_3H$) to form an intermediate diazonium salt. The salt couples to gentisic acid (2,5-dihydroxybenzoic acid, $C_7H_6O_4$) to form an amber-colored product whose intensity is measured by the spectrophotometer. All wastewater samples were filtered before any experimentation to reduce interference of particulates with the concentration measurements.

Total Phosphorus. The Acid Persulfate Digestion Method (*12*) was used to measure the total phosphorus (TP) content of the poultry litter wastewater samples. The procedure is equivalent to USEPA Method 365.2 and Standard Method 4500-P B,5 & P E for water and wastewater. The following experimental procedure was used: 25 mL of the sample was measured into a 50 mL Erlenmeyer flask. The contents of one potassium persulfate ($K_2S_2O_8$) Powder Pillow were added. The flask was swirled to mix and 2 mL of 5.25 N sulfuric acid solution was added. The flask was then placed on a hot plate and allowed to boil gently for 30 min in a water bath. The acid combined with heating causes hydrolysis of condensed inorganic forms of P. The acid, heating, and persulfate causes organic P to convert to PO_4^{3-}. The sample was subsequently cooled to room temperature, and 2 mL of 5.0 N NaOH solution was added. The sample was then poured into a 25 mL graduated cylinder and brought back up to volume and analyzed for PO_4^{3-}. The results of this test at this point included the organic PO_4^{3-} plus the ortho PO_4^{3-} and the acid-hydrolyzable (condensed) PO_4^{3-} (*12*).

Phosphorus Batch Binding Experiments. Batch reactor sample volumes ranged from 40 to 100 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 gel particles was then added, and the decrease of the anion concentration that occurred was monitored using UV−vis spectrophotometry. The experiment stopped when the anion concentration in the batch reactor remained constant. At this time the gel had reached its saturation point. The anion binding capacity (mg anion bound/g dry gel) of the PAA·HCl gel used in the experiment, for each anion, was calculated from experimental data.

Regeneration Experiments. Experiments were performed to determine whether the PAA·HCl gels could be regenerated after having bound anions from wastewater samples. Approximately 0.1 g of wet anion saturated gel was recovered by vacuum filtration following a 2 h batchwise anion removal experiment from a 100 mL wastewater sample. The recovered gel was subsequently treated with 5 mL of 1 N NaOH solution for 1 h. The gel was then vacuum filtered again to remove the waste regenerant solvent consisting of Na_3PO_4, NaCl, and unreacted NaOH that were produced during the treatment. The gel was further washed with distilled water to remove any residual solids that remained. The gel was then dried to prepare it for reuse in a new anion removal experiment. The above procedure thus describes a "regeneration cycle" that involves a (2 h) anion removal step, followed by gel treatment with NaOH solution for anion release, and steps to remove residual precipitated solids formed during the regeneration. This procedure was repeated several times in order to determine the operational lifetime of the gels.

Wastewater Characteristics

Aquaculture Wastewater. Experiments were designed to evaluate the effect of various inorganic and organic constituents present in aquaculture wastewater on the anion removal ability of the PAA·HCl gels. 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 (NH_3), PO_4^{3-}, NO_3^-, NO_2^-, Cl^- (5 g/L), S^{2-}, SO_4^{2-}; $SO_4^{2-}/Cl^- = 0.017$. The average total ammonia nitrogen (TAN), total organic carbon (TOC), biological oxygen demand (BOD_5), alkalinity, and dissolved oxygen (DO) content of the wastewater is 0.8 mg/L, 21 mg/L, 5.3 mg/L, 110 mg/L, and 6.2 mg/L, respectively. Proper sample collection, preservation, and storage were critical for accurate testing. The aquaculture wastewater samples were filtered using Whatman Qualitative filter circles (particle retention > 11 μm, porosity: medium) prior to the batch-binding experiments. The initial target anion concentrations were measured before testing. Batch reactors with volumes of 40−500 mL of sample wastewater containing 0.05−4.0 g of ground dry gel particles were used. The decrease in phosphage concentration was monitored via UV−vis spectrophotometry at timed intervals. When the anion concentrations stopped decreasing, the gel had reached its saturation point and the final anion concentrations were recorded. Subsequently, the aquaculture wastewater sample was emptied out of the reactor and vacuum filtered to remove the anion-saturated PAA·HCl gel. The experiment was repeated using a fresh supply of the same type of PAA·HCl gel in the batch reactor.

Poultry Waste. Poultry production facility broiler litter waste was used for the P binding experiments. Broiler litter includes all floor-type birds such as broilers, pullets, floor layers, and bedding material (e.g. wood shavings or peanut hulls used to absorb liquids). Chemical analysis of the manure varies due to moisture, temperature (more N will be lost at higher temperatures), amount and kind of bedding, amount of soil picked up while a house is cleaned, number of batches consecutively reared, and conditions under which the manure was stored and handled prior to spreading. The average nutrient composition of broiler litter on a fresh weight basis is as follows: 19.7% moisture, 3.1% total N, 2.8% P_2O_5, 2.0% K_2O, 1.8% Ca, 0.4% Mg, and 0.4% S as well as other inorganics (e.g. Cu (332 mg/L), Fe (1950 mg/L), etc.). For our experimental purposes, samples were made by dissolving 40 g of the solid litter in 200 mL of deionized water, resulting in a 20 wt % solution. The samples were left to dissolve over a 24 h period. The resulting mixtures had a high content of suspended solids, particulates, and inorganics (i.e., Zn, Mg, P_2O_5). The mixture was vacuum filtered to remove particulates larger than 15 μm, and the initial total phosphorus (TP) and nitrate-nitrogen (NO_3−N) concentration levels were determined. The initial concentrations measured were 115 ± 15 mg/L TP and 79 ± 6 mg/L NO_3−N. Batch experiments were run for 4 h in 50−200 mL reactors with 1−2 g of ground dry gel particles, respectively. The final TP and NO_3−N were measured after the 4 h. The % TP and NO_3−N removal was calculated from the initial and final concentrations.

Results and Discussion

Hydrogel Anion Removal Properties. The PAA·HCl hydrogel networks contain amine groups on each repeat unit. Amines are nucleophiles and can act as a Bronsted-Lowry base by accepting a proton (H^+) from a proton acid (i.e. HCl). An amine can abstract an H^+ from water, giving an ammonium ion (NH_3^+) and an hydroxide (OH^-) anion. The gels are pH sensitive. At acidic pH's the primary (NH_2) and secondary (NH) amine groups present on cross-link junction units and along the PAA chains respectively become protonated, i.e., NH_3^+ and NH_2^+. The pK_a value of PAA·HCl is 9.67 (*11*). 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. The attracted negative anions may form clusters around NH_3^+ and NH_2^+ sites in the network. At more alkaline pH's, in excess of pH 9.67, the NH_3^+ and NH_2^+ groups lose a hydrogen (become deprotonated) and exist in their uncharged NH_2 and NH_3 forms. In this state the gel network collapses resulting in little or no anion binding, since there is also no electrostatic attraction between the network and the 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 of 5.0−8.0 where the gels exhibit high anion binding capacity.

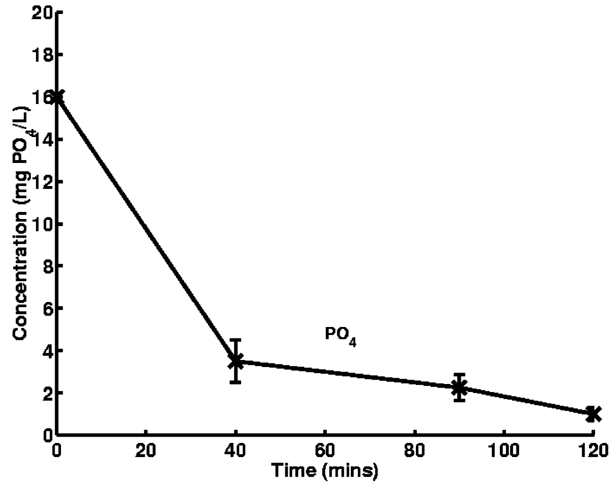

FIGURE 2. PO_4^{3-} binding from aquaculture wastewater using PAA·HCl hydrogels.

Phosphate Binding from Aquaculture Wastewater Effluent. Experiments were conducted using aquaculture production system wastewater to determine the effectiveness of the PAA·HCl hydrogels in removing nutrient anions. Batch experimentation showed that the PAA·HCl gels bind PO_4^{3-} from the waste effluents. In this experiment, more than 98% of PO_4^{3-} initially present was removed (see Figure 2). 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-} from aquaculture wastewater was calculated based on average binding capacity values of 100 mg PO_4^{3-}/g gel (determined from experimentation with standard phosphate solutions using 20% w/v PAA·HCl gels in DI water) 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 of the anion binding capacity of the gels in standard solutions to their binding capacity measured in the aquaculture effluents resulted in a calculation of a % phosphorus average removal efficiency of 90 ± 8%, which is remarkable given the amount of counterions as well as other fouling factors that are present in the wastewater which can potentially clog the gel or interact with the PAA·HCl NH_3^+ groups.

Phosphate Binding from Poultry Broiler Litter. The ability of the PAA·HCl gel to treat poultry broiler litter waste

TABLE 1. TP and NO_3-N Removal from 20% Poultry Broiler Litter Solution[a]

broiler litter batch	final TP (mg/L)	removal %	final NO_3-N (mg/L)	removal %
1	10	91	62	21
2	15	87	62	21
3	17	86	68	14
4	8	92	71	11

[a] Initial P concentration = 115 ± 15 mg/L, initial NO_3-N = 79 ± 6 mg/L.

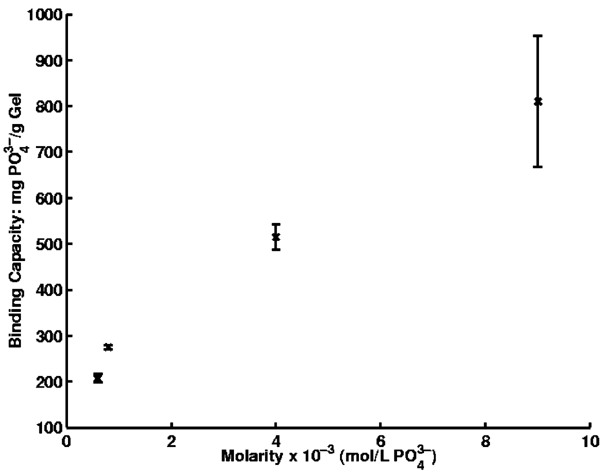

FIGURE 3. Effect of ionic strength on gel PO_4^{3-} binding capacity. Experiments performed in KH_2PO_4 standard solutions.

was investigated by batchwise removal studies in 20% poultry litter waste solutions. Even though the waste solutions were filtered to remove most of the suspended solids (larger than 15 μm), the filtered solutions still contained very high concentrations of particulates, dissolved organics, and counterions. The experimental results are shown in Table 1. An average of 88% of TP and 16.8% of NO_3−N initially present in the waste solution was removed in 4 h of batch reaction time. The average TP and NO_3−N binding capacity was 12 ± 6 mg TP/g gel and 4 ± 1 mg NO_3−N/g gel. Fouling agents, such as complexed metal cations and particulates, penetrated into the gel networks and inhibited available NH_2 sites from binding P and NO_3−N. The gel displayed excellent selectivity for P over N. This was probably due to the higher valence (ionic charge) of the P anions in solution.

Poultry litter is commonly applied to farm fields as a N source for crops since it contains 2−4% N by weight. However, when the litter is applied on the basis of its ability to supply N, the P level is far in excess of the crop P requirement. By treating the poultry litter with the P selective PAA·HCl gel, the P content was reduced to levels (0.4% TP) matching crop P requirements (see Table 1). At the same time the N content remains high (3.3% N) retaining the litters value as an excellent nutrient source for crops.

Effect of Ionic Strength on Anion Binding. The amount of phosphate anions the gels are able to bind per gram of dry gel from a particular aquaculture or poultry broiler litter effluent depends on the ionic strength of the effluent. To demonstrate such effect of ionic strength on the cationic gel's anion binding capacity, the phosphorus binding of a polymer hydrogel was studied in aqueous KH_2PO_4 pH 7.0 standard solutions of varying anion concentration. The experimental results obtained via batch experimentation with the PO_4^{3-} anion aqueous solutions are shown in Figure 3. An 90% increase of solution ionic strength causes a 75% increase in the PO_4^{3-} binding capacity of the gel. The increase is due

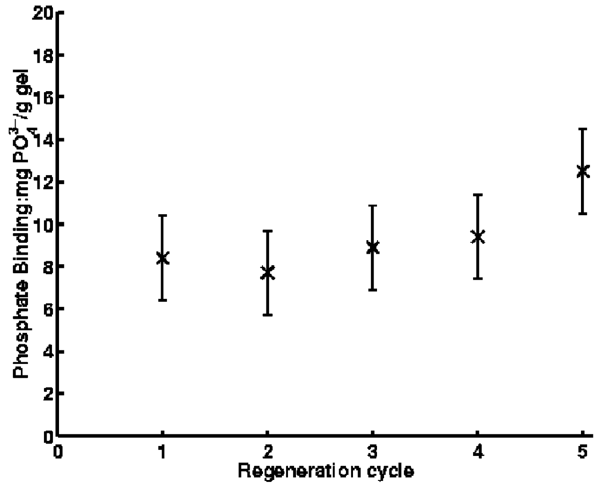

FIGURE 4. PAA·HCl hydrogel regeneration cycles in aquaculture wastewater.

to the higher anion concentration gradient between the surrounding liquid medium and the initially dry polymer matrix, which increases the flux of PO_4^{3-} anions into the cationic polymer matrix, and therefore the anion binding capacity. The aquaculture and poultry wastewater effluents that were tested contain low concentrations of phosphorus, as indicated in Figure 2, and thus the ability of the gel to bind phosphorus should be substantially higher in wastewater effluents containing higher nutrient concentrations than the ones used in this study.

Hydrogel Regeneration. The PAA·HCl gels can be regenerated after having bound PO_4^{3-} from aquaculture effluents, and their removal efficiency can be retained for at least five adsorption/desorption cycles (or regeneration cycles) upon treatment with 1 N NaOH solution (Figure 4) without siginificant change in the gel's phosphorus binding capacity. The data indicate that the gels can be reused over multiple cycles for the removal of phosphorus from aquaculture wastewater.

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 cross-link units) was orders of magnitude larger than the size of the PO_4^{3-} anion. However, the cross-linking 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 cross-linked. This reduces its swelling in water, thus improving its mechanical integrity. Regeneration of the hydrogel results in concentration of the wastewater effluent by a factor of 100, i.e., if 100 mL of wastewater are treated using the PAA·HCl gels, the regenerated PO_4^{3-} effluent upon treatment with NaOH will have a volume of 1 mL.

There are various commercial ion-exchange resins that claim to remove nutrient anions from wastewaters; however, most of them cannot be easily regenerated, they suffer from bacterial growth (bio fouling), or they break down after extended use. Many exhibit selectivity problems, i.e., they might bind the specific anion very well in one medium but not in another that contains specific counterions that the resin is more selective toward. The proposed anion binding hydrogels appear to be an improved technology that does not suffer from these disadvantages. The proposed polymer materials selectively bind phosphorus into the polymer matrix, permitting its subsequent removal from the contaminated source of pollution. The polymer containing the bound phosphorus can either be regenerated for reuse or incinerated. The treated system can then be safely discharged into natural waters or sent for further treatment for use as a municipal water supply. In some cases the phosphorus saturated polymer can be employed as a fertilizer.

The PAA·HCl hydrogels presented in this report exhibit efficient anion removal in both anion standard solutions and wastewater originating from recirculating aquacultural production systems and poultry broiler litter production. The experimental results for anion removal from wastewater effluents suggest that the PAA·HCl gels were not significantly fouled by either the organic constituents or the counterions present in the wastewater effluents. Up to 99+% PO_4^{3-} removal has been achieved from the wastewater effluents. Thus, it has been demonstrated that the novel cross-linked PAA·HCl polymeric hydrogels are appropriate materials for removing nutrient anions from aquaculture and poultry broiler litter wastewater effluents, resulting in the reduction of reactive P concentrations to levels suitable for discharge into natural surface waters. The ultimate goal of this research will be to produce a continuous process where the PAA·HCl gel will be placed into packed columns in commercial aquaculture systems with wastewater effluent being pumped over the column.

Acknowledgments

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