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Research Article

Development, Characterization, and Evaluation of Hydrogels from Natural Resources for Ammonium Adsorption

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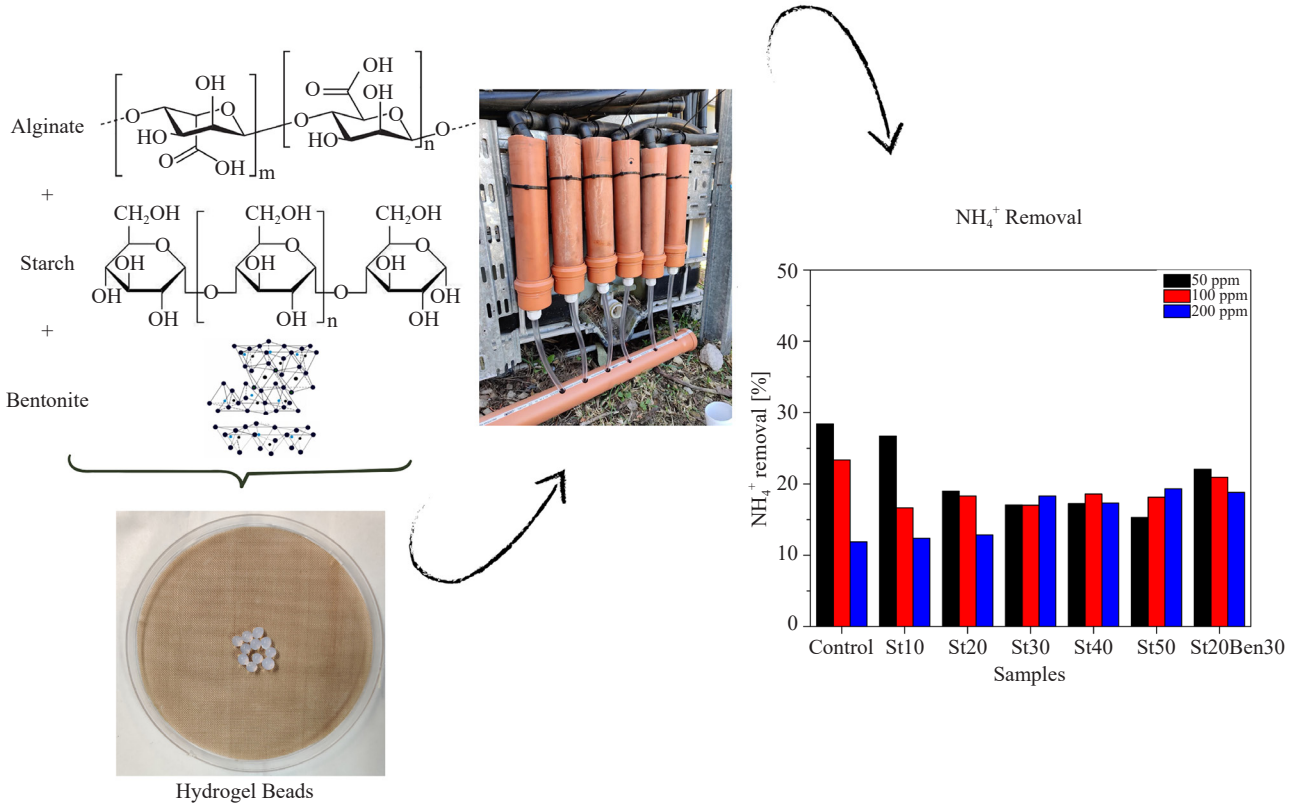

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Abstract: Due to its simplicity and low cost, biological nitrification in fixed bed biofilters followed by denitrification is one of the most applied approaches for the removal of ammonia from wastewater. However, traditional filling materials of biofilters, such as sand, gravel, and clay, have limited water adsorption capacities (10% to 25% by volume), which restricts the treatment capacity. Besides, the high weight of these materials increases construction labor. This study explores the potential of hydrogels as an alternative to conventional biofilter materials. Hydrogels can be formed from natural polymers like alginate and starch and possess high water retention properties and biodegradability, making them suitable for adsorbing contaminants and immobilizing microorganisms to enhance biological wastewater treatment processes. The incorporation of clay into the system is also investigated for its potential to improve mechanical and adsorption properties. The performance of these hydrogels for ammonium removal was evaluated using spectroscopic, gravimetric, and microscopic techniques in experimental-scale systems. The findings aim to provide insights into developing effective, eco-friendly solutions for traditional wastewater treatment systems.

***Keywords*:** hydrogel, alginate, starch, bentonite, ammonium adsorption

Nomenclature

FT-IR	Fourier-Transform Infrared
TGA	Thermogravimetric Analysis
SEM	Scanning Electron Microscopy
$S\%$	Swelling Ratio of the Hydrogel
w_t	Total Weight, Weight of the Swollen Gel Beads
w_o	Initial Weight, Weight of the Oven-Dried Gel Beads
$[NH_4^+]$	Ammonium Concentration
$[NH_4^+]^o$	Initial Ammonium Concentration
$\%REM_{NH_4^+}$	Ammonium Removal Percentage
Q_{max}	Maximum Adsorption Capacity
K	Langmuir Constant
q_e	Absorbed Amount at Equilibrium
C_e	Concentration of Adsorbate in Solution at Equilibrium

1. Introduction

Ammonium is present in urban sewage and in several industrial wastewaters.[1] Its discharge in water bodies, even in small concentrations, reduces oxygen concentration and favors algal growth, disrupting the natural ecosystem.

Due to its low cost and high effectiveness, one of the most applied methods for removing ammonium from wastewater is biological nitrification and denitrification. This process consists of two stages. In the first stage, nitrifying bacteria oxidize ammonium to nitrate under aerobic conditions. In a subsequent stage commonly applied under anaerobic conditions, nitrate is reduced to gaseous nitrogen by denitrifying bacteria.

Nitrification can occur without the addition of external oxygen in trickling filters, vertical treatment wetlands, and sand filters, which are filled with particles forming a porous medium that allows wastewater to flow through vertically by gravity. In these systems, microorganisms adhere to the surface of the particles within, the bed, forming a biofilm where biological processes take place. The water flows through the packed medium and a thin film of liquid is retained on the surface of the particles, providing the necessary environment for microbial activity. Oxygen required for the aerobic processes is supplied passively from the air that occupies the interparticle spaces, allowing aerobic processes in the biofilm, including the oxidation of ammonia to nitrite and subsequently to nitrate.[2,3] In these systems, the specific surface area of the filling material plays a critical role in the performance of the system as it determines both the available surface for microbial colonization and the water retention capacity of the bed.[4] Optimizing this parameter often involves a trade-off, as small particles (such as coarse sand and small gravel applied in sand filters and treatment

wetlands) offer high surface area and improved water retention but also reduce bed permeability and increase the risk of clogging. Nitrogen removal efficiencies in sand filters with different particle sizes ranged between 45 and 67.5% with fine sand showing the higher removals.[5,6] On the other hand, coarse materials like large gravel or crushed rock, materials applied in trickling filters, enhance permeability and reduce the likelihood of clogging but provide lower specific surface area and water retention, thus achieving lower nitrogen removal.

In this context, hydrogels emerge as a promising alternative to conventional biofilter media. These polymeric matrices are capable of absorbing and retaining large amounts of water and contaminants within a small volume, while their porous structure provides a high specific surface area for microbial colonization. This makes hydrogels suitable for immobilizing biologically active substances and supporting living organisms such as yeasts, cells, and antibodies. Moreover, natural polymers (such as polysaccharides and proteins) are inherently biodegradable and biocompatible, which makes them excellent candidates for developing eco-friendly hydrogels.[7] In addition to enhancing the sustainability of the system, these materials may also help overcome the limitations to denitrification caused by the low availability of suitable carbon and electron donors in wastewater.[8]

Among natural polymers for forming hydrogels, alginates (polysaccharides obtained from certain species of marine brown algae) stand out: they are polyelectrolytes which have ionizable groups that can dissociate in polar solvents like water, so the polymeric chains release counterions into solution. This interesting property can be used to generate hydrogels through ionotropic gelation. Strong electrostatic interactions between alginate's ionized groups and small-sized divalent ions (e.g, Ca^{2+}) lead to the formation of gels with excellent properties.[9]

Another biopolymer that can be used to form gels is starch; it is one of the most abundant plant polysaccharides, and the most used hydrocolloid in the food industry.[10] Starch nanocomposite hydrogels have been evaluated as retainers of urea from contaminated waters with excellent results, and it has been shown that the incorporation of starch improves stability and permeability of alginate beads.[11,12] In particular, alginate/starch gels have been studied as encapsulators of bacteria of the *Pseudomonas* genus as biofertilizers and as urea encapsulations to be used in the slow release of this fertilizer.[13,14]

An important limitation of the hydrogels for their application at a large scale is their low mechanical stability. The incorporation of clay, a material that also has high adsorption capacity, may allow for improving the mechanical properties of the hydrogel while also improving the adsorption capacity. Clays are economical and abundant natural minerals in nature, consisting mainly of lamellar aluminosilicates. These nanomaterials are a viable alternative to be used in the adsorption of contaminants, including ammonia, since they have extraordinary versatility due to their physicochemical, textural, and mechanical properties, which allow easy manipulation.[15]

Hydrogels incorporating clays in their structure may allow more efficient nitrification and ammonia adsorption than with traditional materials. In this paper, the application of alginate hydrogels with different amounts of starch and clay for the removal of ammonium from wastewater in fixed-bed filters was studied. Different properties of the hydrogels were evaluated using spectroscopic, gravimetric, and microscopic techniques, and the removal of ammonium was analyzed in experimental scale systems fed with real wastewater. The findings aim to provide insights into developing effective, eco-friendly solutions for wastewater treatment in traditional systems.

2. Materials and methods

2.1 *Materials*

Sodium alginate, corn starch, and bentonite clay were purchased from Química Bolivar (Argentina), Ingredion (Argentina), and Minarco S.A. (Argentina), respectively. All other reagents (analytical grade) used in this study were supplied by Pueyrredon Pharmacy and were used without further purification. Ammonium chloride was purchased from Biopack (Argentina), and the ammonium solutions were prepared in distilled water.

2.2 *Hydrogel beads preparation*

The hydrogels were prepared as depicted in Figure 1. 2.5 g of alginate was dissolved in 100 mL of water. The solution was introduced dropwise by a peristaltic pump through a plastic tubing into a 500 mL of 0.2 M $CaCl_2$ solution. The beads were allowed to crosslink in Ca^{2+} in agitation at 250 rpm for 30 minutes. After that, the beads were washed

with bi-distilled water to remove unreacted $CaCl_2$ from the surface of the beads. The same procedure was followed for the incorporation of different amounts of corn starch (10, 20, 30, 40, and 50% in weight of added alginate) and bentonite clay (0 and 30% in weight in relation of added alginate) in order to obtain composite alginate hydrogel beads.

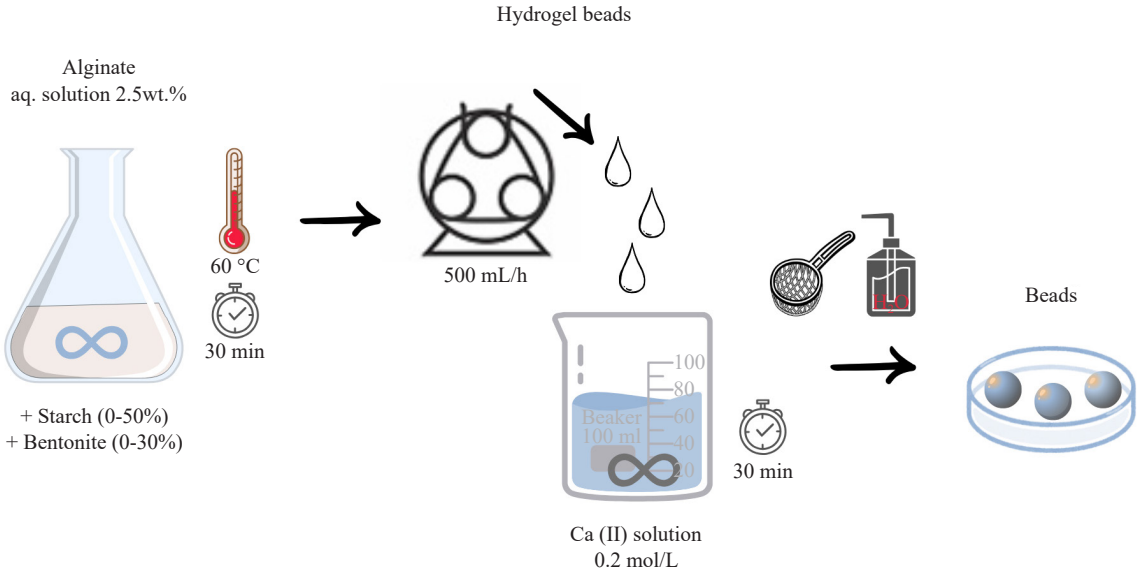


Figure 1. Scheme of hydrogel beads preparation

Gel beads were named as shown in Table 1. For certain assays, the beads were freeze-dried at -50 °C using lyophilization equipment.

Table 1. Hydrogel sample names and compositions, all crosslinked in 0.2 M $CaCl_2$ solution and agitated at 250 rpm for 30 minutes

Sample name	Composition of the polymeric solution		
	Alginate (wt.%)	Starch (wt.% relative to alginate)	Bentonite (wt.% relative to alginate)
Control	2.5	0	0
St10	2.5	10	0
St20	2.5	20	0
St30	2.5	30	0
St40	2.5	40	0
St50	2.5	50	0
St20Ben30	2.5	20	30

2.3 *Hydrogel beads characterization*

Fourier Transform Infrared (FT-IR) spectra were obtained in a Thermo Scientific Nicolet 6700 spectrometer, which had a resolution of 4 cm^{-1}. Measurements were carried out from 400 to 4,000 cm^{-1}. Freeze-dried samples were used with

the intent of avoiding interference related to aqueous phase absorption bands.

Thermal Gravimetric Analysis (TGA) with a TA Q500 v20.13 (New Castle, DE) instrument was performed. Measurements were performed under N_2 flow using approximately 15 mg of oven-dried sample and heating it from 25 °C to 900 °C at a rate of 10 °C/min. The internal structure of the gels was analyzed via Scanning Electron Microscopy (SEM), using a Sigma 300 Zeiss equipment (from LAMARX-UNC-Argentina). Samples were previously swollen for 24 hours in distilled water, then frozen, cryo-fractured, and finally lyophilized. Hydrogel beads that had been freeze-dried and cryofractured were employed, as this technique of removing moisture preserves the hydrogel's porous structure. Imaging was performed after coating the samples with a thin layer of gold. The swelling behavior was studied by measuring the percentage of water uptake of the gel beads via the immersion method. Samples of dried gel beads were allowed to swell in distilled water at room temperature. After 48 hours, the swollen gel beads were weighed again. The swelling ratios (S%) of the gel beads were calculated as:

$$S\% = \frac{w_t - w_o}{w_o} \times 100 \tag{1}$$

Where w_t is the weight of the swollen beads after 48 hours, and w_o is the initial weight of the beads. The beads were used in both states, the fresh state (as prepared) and the dried state (after freeze-drying).

2.4 *Ammonium adsorption*

Initially, 1 g samples of fresh hydrogel were immersed in 10 mL of NH_4^+ solution in distilled water (at concentrations of 50 ppm, 100 ppm, and 200 ppm) and kept under gentle agitation for 24 hours. For each of the ammonium concentrations, 10 mL of solution without hydrogel was used as a blank. Finally, the material was removed, and the remaining ammonium concentrations in the solutions were measured.

Ammonium removal was determined from the quotient between the remaining ammonium concentration and the ammonium concentration of the blank (initial concentration) according to Equation 2, and represents the ammonium adsorption capacity of the material.

$$\%REM_{NH_4^+} = \left(1 - \frac{\left[NH_4^+\right]}{\left[NH_4^+\right]_o}\right) \times 100 \tag{2}$$

Ammonium concentration was determined with a Hanna HI98191 portable meter with a selective ion exchange measurement probe. In operation, the device detects and reports the ammonium concentration through released ammonia, so the solution must be made alkaline to promote the complete transformation of ammonium to ammonia. To do this, 10 µL of 10 $molL^{-1}$ sodium hydroxide (NaOH) solution is added for each milliliter of sample before placing the probe. All experiments were conducted in triplicate.

2.5 *Ammonium removal from wastewater*

Experimental biofilters, 30 cm in height, were constructed using 63 mm diameter drain pipes and filled with 1.5 L of St20Ben30, control hydrogel, or sand as a traditional filling material (Figure 2). Caps with a plastic mesh to avoid material loss were set on the bottom of the columns. Each biofilter consisted of a small water pump controlled by a custom-made panel that regulated the water pumps' power supply, allowing, in this way, pulsed feeding of the systems. Ten pulses of about 50 mL of sewage water were fed to each column daily, representing a hydraulic loading of 0.16 m per day.

Figure 2. Lab-made column of the biofilter system

On these systems, ammonium concentration at the inlet and outlet was determined as previously explained. Nitrate concentration was determined using a standard procedure which consists of measuring absorbance at 220 nm and 275 nm in acid conditions (the latter to remove possible interferences).[16]

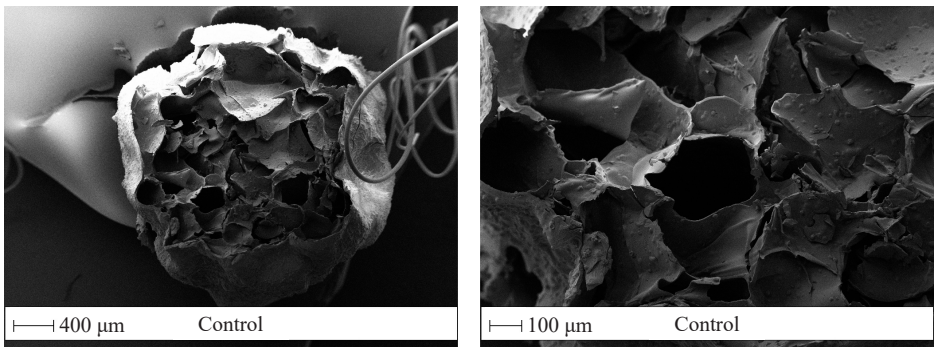
3. Results

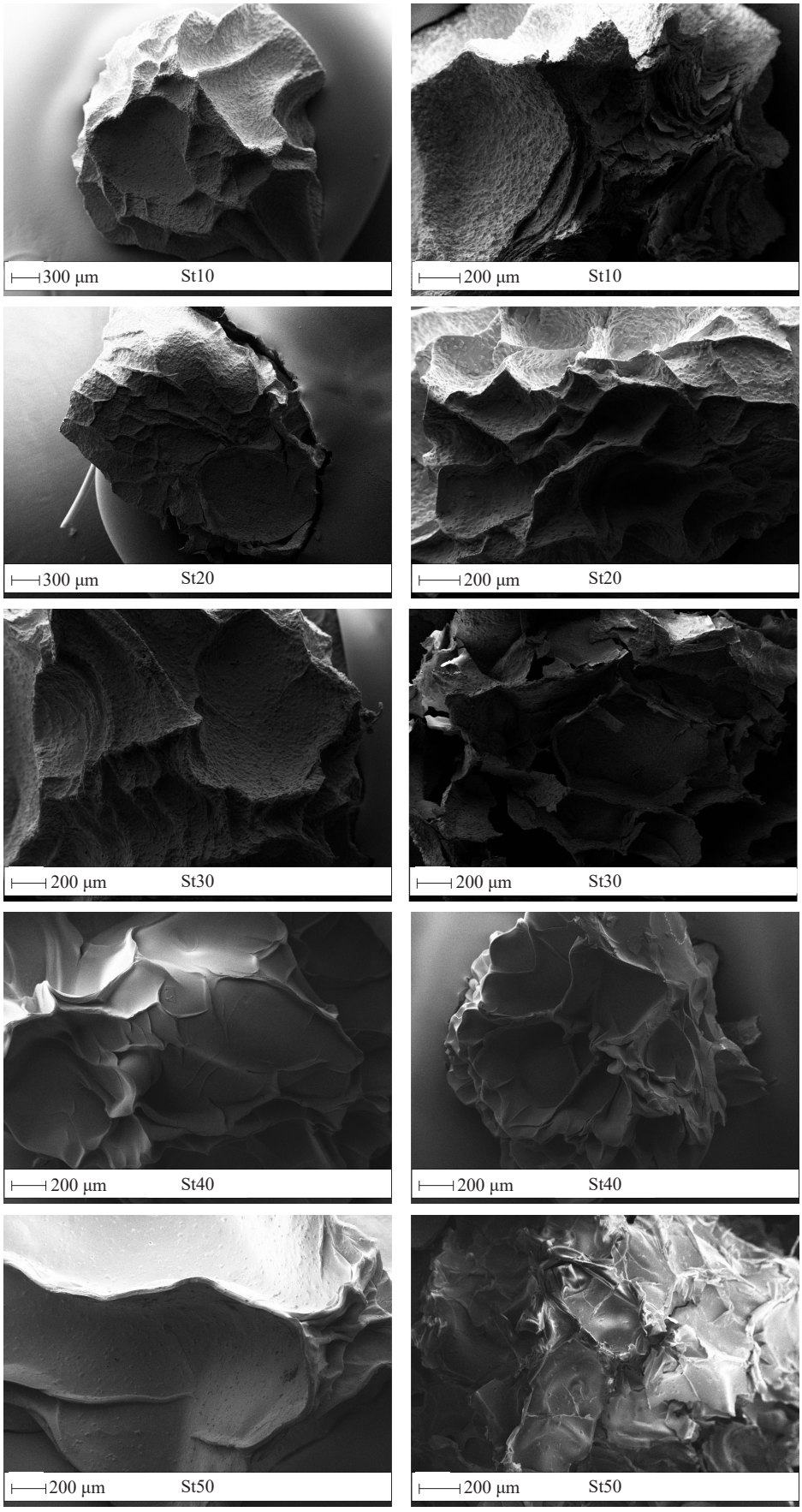
3.1 *Morphological and physical-chemical characterization*

The control samples (without starch and bentonite) are colorless and translucent beads. The resulting material turns white and slightly opaque when starch is added to the mixture used to make the hydrogels, and this change in appearance gets more pronounced as the starch concentration of the composition rises. The addition of bentonite to the initial aqueous dispersion gives the beads a brownish hue, confirming the presence of the filler in the material.

As for the freeze-dried beads, the hydrogels containing starch were able to maintain their spherical shape when exposed to the drying procedure in contrast to the control sample. The material's uniform starch dispersion, which fills the interstitial gaps in the hydrogel structure and lessens shrinkage during drying, is the cause of this phenomena.[17]

The effect of adding starch or bentonite to the material on the internal and external morphology of the hydrogels was analyzed through SEM microscopy. The images from the microscopy tests for each of the examined samples are displayed in Figure 3.

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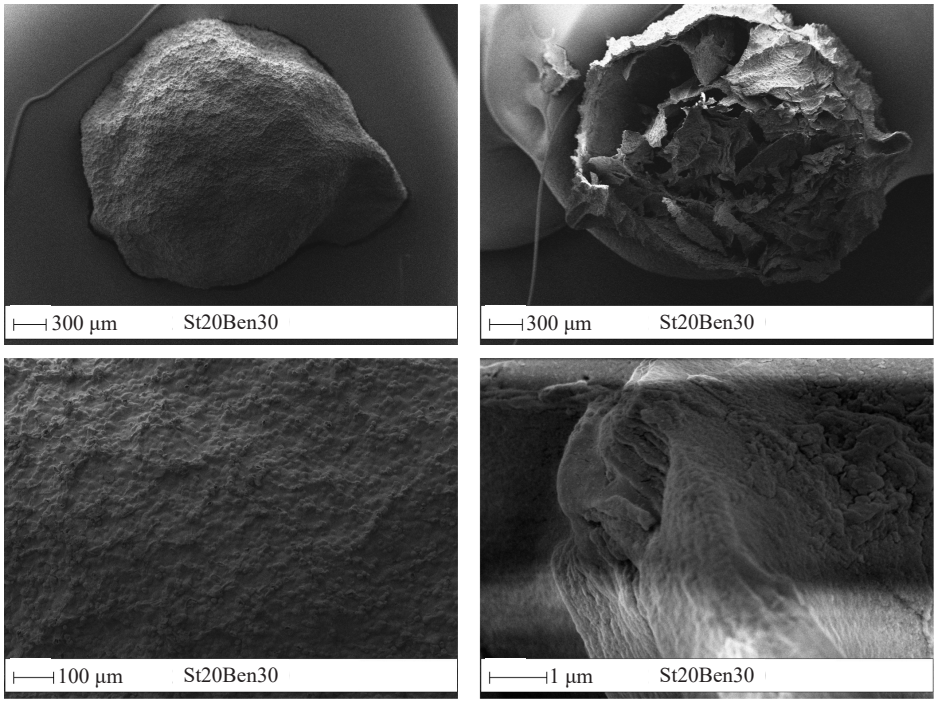

Figure 3. Hydrogel beads SEM microscopy images. Two images from each sample, except for St20/Ben30

After freeze-drying, the samples largely retained their surface roughness and their form. It was easy to see the hydrogel's open interior structure with interconnecting pores (shown in more detail in Figure 3). A closer look reveals a more granular surface and agglomerates on the walls of the interior structure, linked to the presence of bentonite in the material in samples St20Ben30.

Pore diameter and external wall thickness of all samples were determined using ImageJ. For the asterisk marked samples, more precise measurements could not be taken as the interconnected structure cannot be seen with clarity. For these samples, pore size was estimated from the relief generated by the presence of pores underneath the surface. Results from the image analysis are presented in Table 2. They can be considered macroporous hydrogels having closed pores.[18]

Table 2. Hydrogel pore diameter and wall thickness measurements from SEM image analysis

Sample name	Average pore diameter (µm)	Average external wall thickness (µm)
Control	340 ± 63	20 ± 4
St10*	142 ± 54	22 ± 6
St20	274 ± 82	36 ± 5
St30	335 ± 87	35 ± 12
St40	367 ± 25	28 ± 13
St50*	230 ± 93	43 ± 7
St20Ben30	380 ± 136	40 ± 17

As for the FT-IR spectra depicted in Figure 4, all hydrogel samples exhibit an absorption band between 3,500 and 3,000 cm^{-1}, attributed to the stretching of hydroxyl groups that are more closely related to alginate. Since hydroxyl groups and Ca^{2+} ions work together to generate the three-dimensional gel structure, the absorption band in the hydrogels is less intense than in the raw material. Absorption peaks at approximately 1,600 cm^{-1} and 1,415 cm^{-1} were seen in all samples, corresponding to the carbonyl groups of alginate.[19]

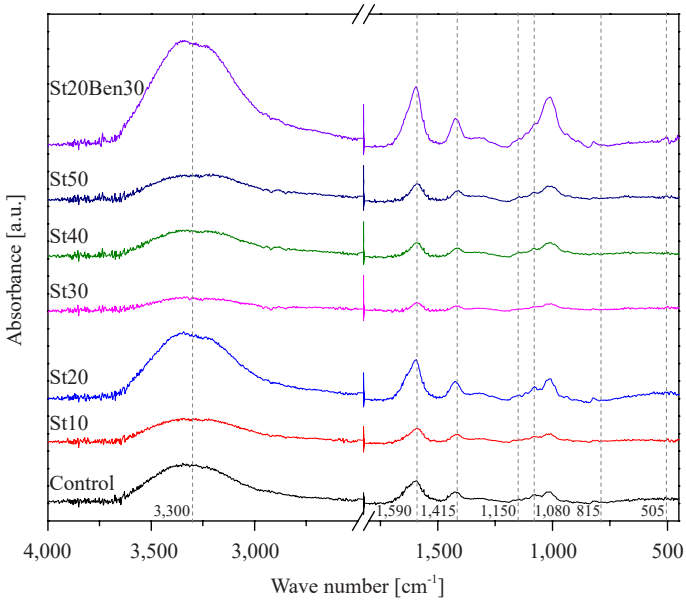

Figure 4. FTIR spectra for alginate beads (control), alginate with starch and composite beads

Furthermore, the spectrometry of the starch hydrogel samples revealed overlaps between 1,080 cm^{-1} and 1,005 cm^{-1} and absorption peaks at about 1,150 cm^{-1}, which are indicative of the functional groups of both starch and alginate.[20]

Lastly, the hydrogel sample reinforced with both starch and bentonite showed absorption peaks at about 815 cm^{-1} and 505 cm^{-1}, respectively, which corresponded to the stretching of the Si-O and Al-O-Si groups, and a minor peak at around 1,115 cm^{-1}, which was linked to the longitudinal stretching of the Si-O group. These absorption peaks, along with the ones mentioned previously, confirm the presence of the reinforcements on the material.[21]

Given that all of the hydrogels were created using the same concentration of Ca^{2+} ions in the gelation solution, the samples had swelling degrees of comparable magnitudes (between 60 and 70%) as expected.[22]

3.2 *Ammonium adsorption*

Ammonium adsorption capacity was determined for all hydrogel formulations, using solutions with different concentrations of the compound, as seen in Figure 5.

The hydrogel samples made from alginate without composite material (Control) or composites with a low starch load (St10 with 10% starch) demonstrated a higher capacity for ammonium removal from solutions with a low concentration of ammonia. Due to the saturation of the absorption capacity, the percentage of removed ammonium was lower with solutions of higher concentrations of ammonia. The hydrogels with higher starch content and with bentonite, primarily St20Ben30, exhibited similar ammonia removal under all the analyzed ammonia concentrations.

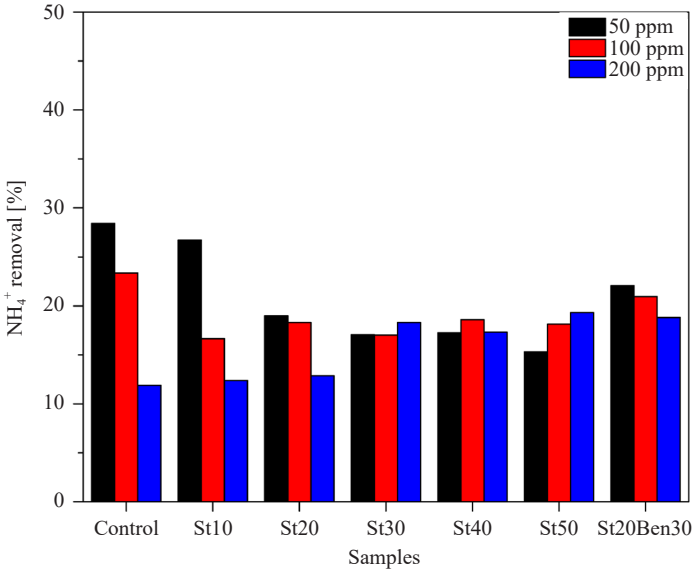

Figure 5. Percentage of ammonium removed with different hydrogels from with 50, 100, and 200 ppm of ammonium

Langmuir isothermal equation is one of the most applied equations to model the adsorption process. Once linearized, the equation has the following functional form:

$$\frac{1}{q_e} = \frac{1}{KC_eQ_{max}} + \frac{1}{Q_{max}} \tag{3}$$

q_e is the amount adsorbed at equilibrium (expressed as mg NH_4^+/L), Q_{max} is the maximum adsorption capacity of the adsorbent (mg NH_4^+/g of hydrogel), C_e is the concentration of the remaining adsorbate in the solution at equilibrium (mg NH_4^+/L) and K is the Langmuir constant (L/g of hydrogel). Results shown in Figure 6 were used to obtain the maximum adsorption capacity of different hydrogels, presented in Table 3.

Table 3. Maximum adsorption capacity calculated from the Langmuir model

Sample	Maximum adsorption capacity Q_{max} (mg/g)	Langmuir constant (L/g)	R^2
Control	0.277	0.0289	0.9924
St10	0.329	0.0241	0.866
St20Ben30	1.566	0.00185	0.9998

In comparison to the hydrogel made of starch and bentonite (St20Ben30), the neat hydrogel (Control) and the low starch hydrogel (St10) both displayed a low Q_{max} which is comparable to the adsorption capacity of gravel (0.76 mg/g).[23] The addition of bentonite increased the adsorption capacity of the hydrogels. This is due to the high adsorption capacity of the clay bentonite (5.85 mg/g).[24] The resulting adsorption capacities of the hydrogels are comparable to other hydrogels, such as those modified with aluminum salts (2.4 mg/g).[25]

In order to compare the performance and efficiency of the hydrogel against other ammonium adsorbents, a measure of cost-effectiveness can be established from the maximum adsorption capacity and the market price of each material.

Table 4 displays the cost-effectiveness of different absorbents in similar conditions, considering St20Ben30's calculated Q_{max} and an estimated price of 0.238 USD/kg.

Table 4. Ammonium adsorbents Q_{max}, estimated price, and cost-effectiveness

Material	Maximum adsorption capacity Q_{max} (mg/g)	Estimated price (USD/kg)	Cost-effectiveness (mg/USD)
Bentonite[24]	5.85	0.23	25,434
Natural zeolite	5.22[26]	0.12[27]	43,500
Biochar	5.38[28]	0.23[29]	23,391
St20Ben30[30]	1.566	0.238	6,580

3.3 *Test in biofilter systems*

Samples of the inlet and outlet wastewater were collected weekly, and the first samples were taken one week after the systems were put into service. Nitrate and ammonium were quantified on each sample as described in materials and methods. Systems monitoring was carried out for four weeks, until the water samples from the column filled with Control hydrogel presented several hydrogel fragments, indicating that the material was disintegrating. The results were presented in Figure 6, shown below.

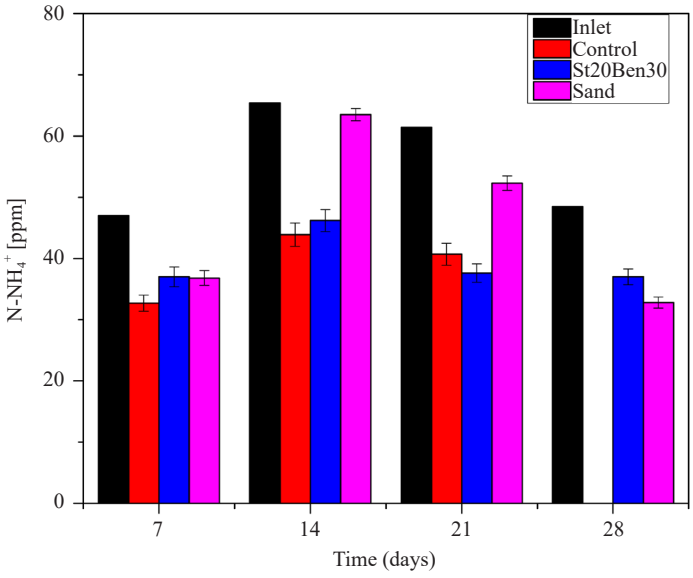

Figure 6. Ammonium concentration at the inlet (black) and the outlet (colored) with different samples at 7, 14, 21, and 28 days

Both adsorption on the filler particles and biological activity, such as nitrification, can remove ammonium from biofilters. It is anticipated that the adsorption process would be primarily responsible for the removal during the first few weeks, since bacteria must grow in the biofilter for the biological process to take place.

As nitrifying bacteria have a slow growth rate,[31] ammonia removal during the first weeks is expected to be produced by adsorption on the filling material. Hydrogels have an advantage over conventional materials in that their adsorption keeps the ammonium concentration in wastewater low when the bacterial community is still developing.

Considering that St20Ben30 hydrogel absorbed all of the ammonium fed with the wastewater, it would take roughly 20 days to attain saturation, according to the results of the maximum adsorption capacity (shown in Table 3) and the average ammonium levels at the system's input. On the other hand, sand has a substantially lower adsorption capacity than hydrogels (about 0.05 $mg \cdot cm^{-3}$)[32] saturation of this material would be achieved on the first day of system operation. In accordance with this, a clear difference in ammonia removal between the columns filled with hydrogels and those filled with sand was observed at 7, 14, and 21 days, demonstrating the alginate hydrogels' advantage over traditional biofilter filling materials in terms of ammonium adsorption.

Similar elimination was seen in the hydrogel and stone columns in the samples collected on day 28, indicating that the nitrifying bacteria in both materials were active. In this scenario, hydrogels have the advantage of having higher hydraulic conductivity, which enables the systems to be fed with higher flow rates per unit area even after bacteria growth on the filling material that may cause system fouling.

It is important to keep in mind that nitrification does not mean that nitrogen is removed from wastewater; rather, it only involves the conversion of one nitrogen form (ammonium) to another (nitrate), which is still soluble in the wastewater and poses environmental hazards. When the next stage of denitrification is completed, which entails reducing nitrate into gaseous nitrogen that escapes from the water, nitrogen is finally removed. Nitrate concentration was in all analyzed samples below 1.5 ppm $N\text{-}NO_3^-$ (data not shown), suggesting the presence of active denitrifying bacteria on the biofilters.

3.4 *Hydrogel stability*

When evaluating hydrogels as a potential substitute for traditional biofilter fillers, one issue was how long they would last when in contact with wastewater. This breakdown is thought to have been brought on by both biological activity and the water's ionic makeup. Despite having less hydraulic conductivity than when the experiments started, the columns filled with St20Ben30 hydrogel and sand infill continued to operate properly on day 28th.

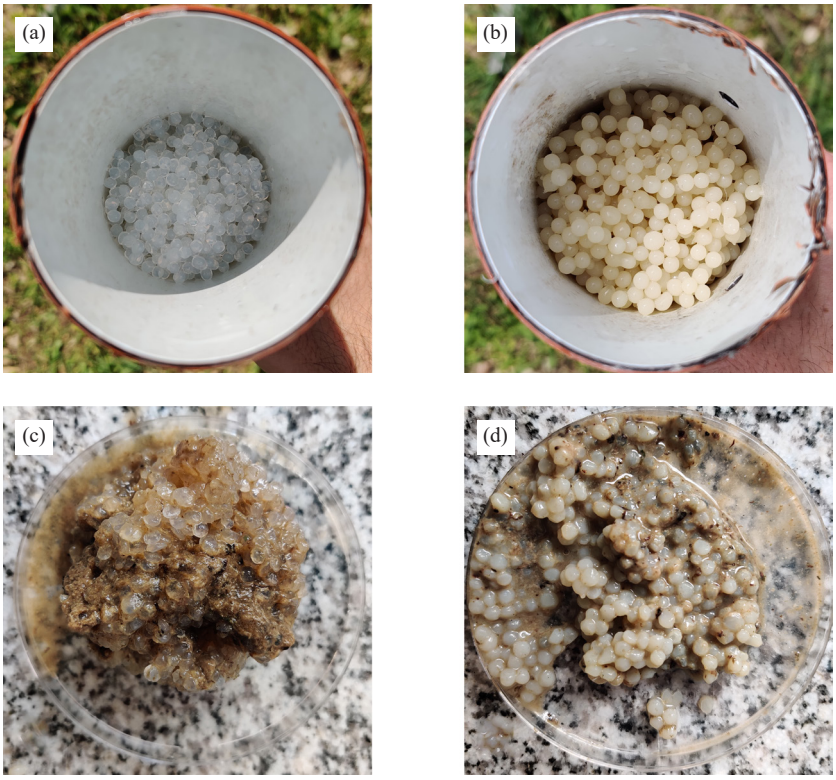

Figure 7. Control hydrogel beads at day 1 (a) and day 28 (c), St20Ben30 hydrogel beads at day 1 (b) and day 28 (d)

Following the removal of the columns from the circulation system, the material was taken out and photographed. The state of the hydrogels before and after the field tests can be seen in Figure 7.

The pictures depict the materials' condition following a 28-day exposure to wastewater. As expected, the control hydrogel was severely damaged, primarily in the lower portion of the column, where the material had been totally disintegrated. Remarkably, St20Ben30 hydrogel had retained its functional integrity.

It has to be noted that the hydrogel's decomposition may be used in favor of the system's operation. Denitrifying bacteria that convert nitrates to gaseous nitrogen (the anaerobic stage of denitrification) may use the degraded material as a source of carbon. In this way, the limitations often observed for nitrogen species removal from wastewater with low levels of soluble carbon sources would be lessened. Further work has to explore this effect and to control the extent of hydrogel degradation in order to extend the material lifespan.

4. Conclusions

Several formulations of alginate hydrogels made of starch and bentonite were effectively produced. TGA, FTIR, SEM, and other common laboratory methods and tests in materials science and engineering were used to characterize the hydrogels.

Laboratory studies conducted under controlled conditions were used to assess the hydrogels' applicability. It was shown that alginate hydrogels can effectively adsorb ammonium, but their chelating agents can remove calcium from the structure, making them vulnerable to degradation.

According to field tests, the hydrogels are effective at removing ammonium, showing high ammonium adsorption during the first weeks of operation. This may help to achieve high ammonium removal efficiencies, even during the first weeks of operation when nitrifying bacteria are not yet fully developed. The incorporation of bentonite into the hydrogel increased the lifespan of the material.

While these hydrogels favour water retention without reducing the bed's permeability and increasing the risk of clogging, they are still sensitive to the presence of other ions and contaminants that accelerate the material's degradation. There are improvements to be made in reducing the cost of the hydrogels (such as reusing $CaCl_2$ solution) and optimizing the synthesis process, but further work is needed to increase material stability and cost-effectiveness to make these materials suitable substitutes for traditional filling materials.

Acknowledgments

Author contribution

Justo Dietrich: Conceptualization, formal analysis, investigation, data curation, methodology, writing–original draft, writing–review;

Sebastián Bonanni: Methodology, data curation, project administration, supervision, writing–review;

Jimena González: Methodology, data curation, project administration, supervision, writing–review.

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Conflict of interest

The authors declare no conflict of interest.

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