Summary

Agricultural nonpoint source pollution such as nitrate and phosphorus loading in receiving waters is a major contributor to water quality concerns in South Dakota. The goal of this project was to develop low-cost technologies to remove pollutants from surface and subsurface agricultural drainage systems. This project aims at utilizing widely spread, abundant and low-in-cost agricultural materials, mainly polysaccharides, to improve the water quality. Polysaccharides are natural biopolymers used in food and pharmaceutical applications as thickeners and gelling agents. This project focuses on two polysaccharides namely alginate and iota-carrageenan. These are from marine algae and are being used in food products such as ice creams, chocolates and salad dressings. During the project period porous beads of size around 1 mm were prepared based on alginate. The data collected from laboratory experiments were used to evaluate the efficiency of the prepared beads in removing nitrate and phosphorus from the water. The project outcome will directly benefit the South Dakota producers by leading to an economical multi-function conservation method that has potential for in-field nutrient management and for edge-of-field treatment of surface runoff and subsurface drainage water**.** Agricultural producers will be able to incorporate our scientifically proven and environmentally friendly conservation practices in their operations.

Objectives

The main research objective is to ‘Prepare and characterize polysaccharide beads and to measure the beads capacity for nitrate and phosphorus removal’.

Project progress

Graduate student: Though funding for a PhD student was requested, there were some difficulties in finding a suitable candidate with the one-year support and thus we resorted to a MS student. Mrs Most Farzana Yesmin was recruited in the project during September 2017. Since joining, Farzana took active part in beads preparation and characterization along with measuring alginate beads capacity to capture nitrate and phosphate from water in addition to crediting graduate courses to meet the graduate school requirements for her MS degree. On June 8th, 2020 Farzana successfully defended her research.

Undergraduate students: Fanding Ceesay and Uday Mishra have assisted Farzana in beads preparation. Uday was predominantly supported from the SDSU Research/Scholarship Support Fund FY17 for the project entitled “Improving water quality to sustain agricultural ecosystems through novel use of biopolymers”. At this moment, Uday is pursuing his MS degree at the University of Saskatchewan, Canada.

The Co-PI move to San Diego: The co-PI, Dr. Laurent Ahiablame, started working at the University of California at Davis, from 1st January 2018, as a water quality and management advisor and county director of San Diego County. Dr. Ahiablame participated regularly in project meetings and helped in analyzing the data.

Beads preparation and characterization: Initially, solution concentrations of 1, 2 and 3% of alginate and iota-carrageenan were tested. Around 50 μL droplets were slowly added to the 100 mM $CaCl_2$ solution, and were left in the solution for 6, 12 and 24 hr for the bead formation. The $CaCl_2$ concentration increased up to 500 mM, and alginate and iota-carrageenan were mixed in 2:1, 1:1 and 1:2 ratio. The bead shape, drying time and water holding have been considered for determining the optimum $CaCl_2$ concentration and polysaccharides ratio. The results suggest that 100 mM $CaCl_2$ and 1:1 alginate:iota-carrageenan as the optimum mix. It was found that pure carrageenan beads were relatively soft compared to alginate beads and thus rest of the research was focused on alginate beads and most of the time was spent on how alginate beads would be useful to capture nitrate and phosphate. However, alginate-carrageenan beads could be useful as well, which need to tested as the future research. The beads before and after nutrient capture have been characterized with Fourier Transform Infrared Spectroscopy and Differential Scanning Calorimetry, which further provide proof for the presence of nutrient in the beads. **Fig. 1.** highlights the alginate beads and Fourier Transform Infrared Spectra from alginate and iota-carrageenan beads.

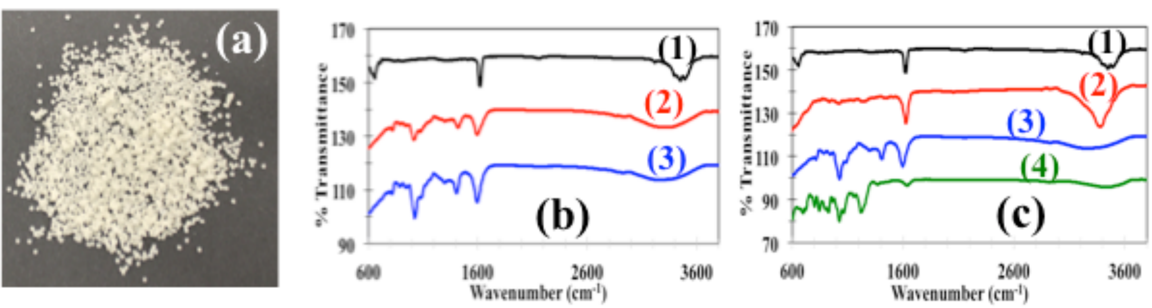

Fig. 1. **(a)** Alginate beads prepared from the 3% solution in the presence of 100 mL CaCl2, (**b**) FTIR spectra from (1) $CaCl_2$ powder, (2) alginate beads and (3) alginate powder, and (c) FTIR spectra from (1) $CaCl_2$ power, (2) 1:1 alginate:iota-carrageenan beads, (3) alginate powder and (4) iota-carrageenan powder. The characteristics peaks from the alginate and iota-carrageenan are present in the prepared beads.

Later alginate beads were prepared with a variety of divalent and trivalent cations such as Fe^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} and Sr^{2+}, and Fe^{3+} and Al^{3+} (**Fig. 2**) so as to understand the role of charge balancing cation in capturing the nutrients from contaminated water.

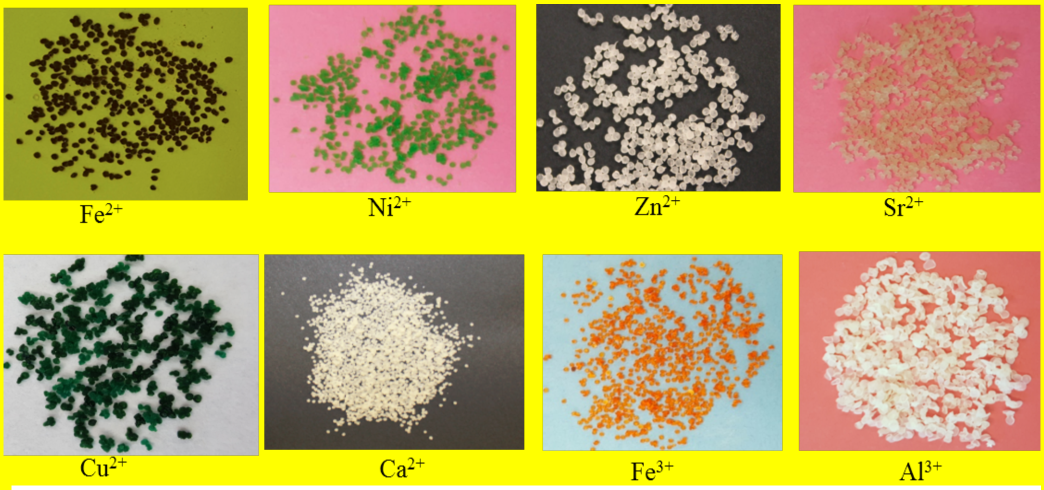
Fe^{2+} Ni^{2+} Zn^{2+} Sr^{2+}
Cu^{2+} Ca^{2+} Fe^{3+} Al^{3+}

Fig. 2. Alginate beads prepared from the 3% solution in the presence of 100 mL (a) FeCl2, (**b**) $NiCl_2$, (c) $FeCl_3$ and (d) $SrCl_2$.

Phosphate removal: The initial screening showed that aluminum, iron and zinc cross-linked alginate beads can absorb detectable amount of phosphate at all concentrations and results are presented in Figure 3 through 7. The phosphate absorption increases over time and alginate-Al^{3+} beads could absorb up to (94±0.1) % of phosphate in 24 hours. Similarly, alginate-Fe^{2+} and alginate-Zn^{2+}

beads could absorb up to (93.6 ± 1.4) % and (40 ±0.2) % of phosphate, respectively, in 24 hours. The order of absorption efficiency is found to be alginate-Al^{3+} > alginate-Fe^{2+} > alginate-Zn^{2+}. Overall, the type of ionic cross-linker appears to dictate the amount of phosphate that could be absorbed by the alginate beads.

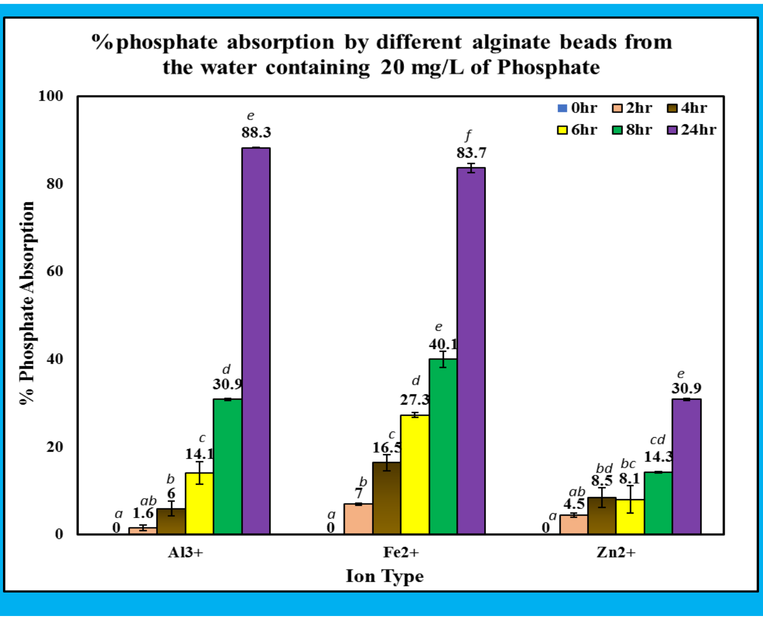

Fig. 3. Percent of phosphate absorption by different alginate beads using 20 mg/L of standard phosphate water. Each bar represents mean ± SD. For each ion type, means share same letters indicate statistically insignificant difference, while different letters indicate significantly different from each other ($p < 0.05$).

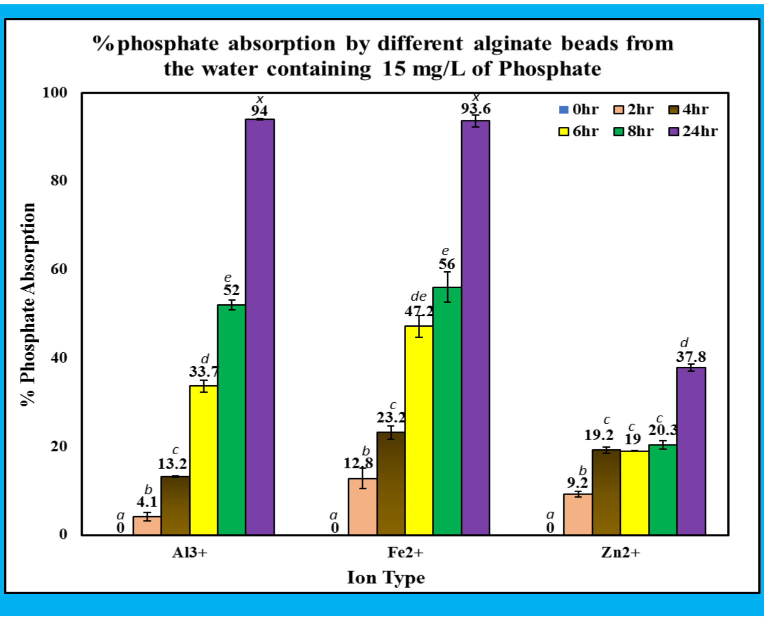

Fig. 4. Percent of phosphate absorption by different alginate beads using 15 mg/L of standard phosphate water. Each bar represents mean ± SD. For each ion type, means share same letters indicate statistically insignificant difference, while different letters indicate significantly different from each other ($p < 0.05$).

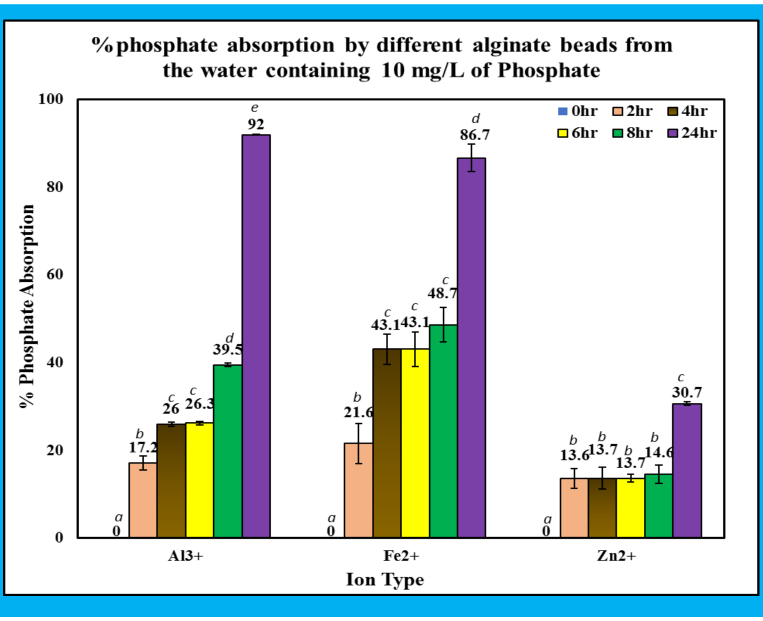

Fig. 5. Percent of phosphate absorption by different alginate beads using 10 mg/L of standard phosphate water. Each bar represents mean ± SD. For each ion type, means share same letters indicate statistically insignificant difference, while different letters indicate significantly different from each other ($p < 0.05$).

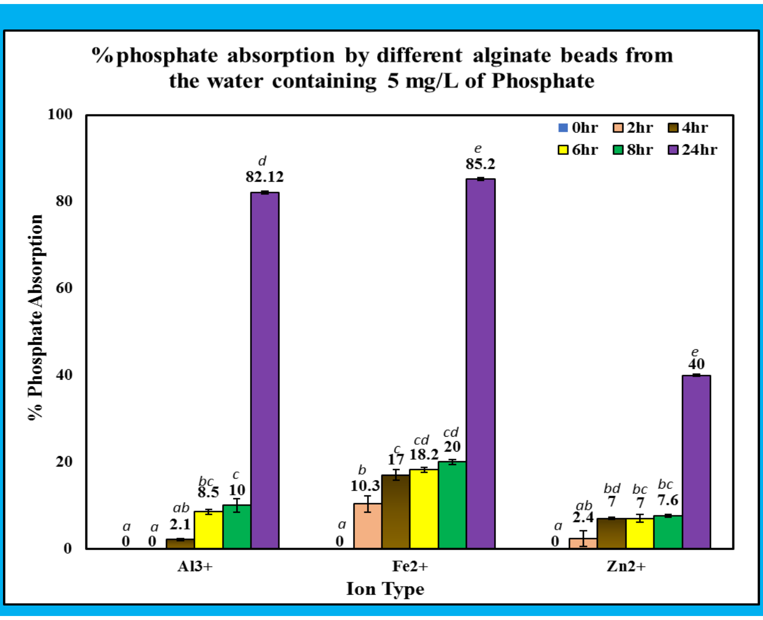

Fig. 6. Percent of phosphate absorption by different alginate beads using 5 mg/L of standard phosphate water. Each bar represents mean ± SD. For each ion type, means share same letters indicate statistically insignificant difference, while different letters indicate significantly different from each other ($p < 0.05$).

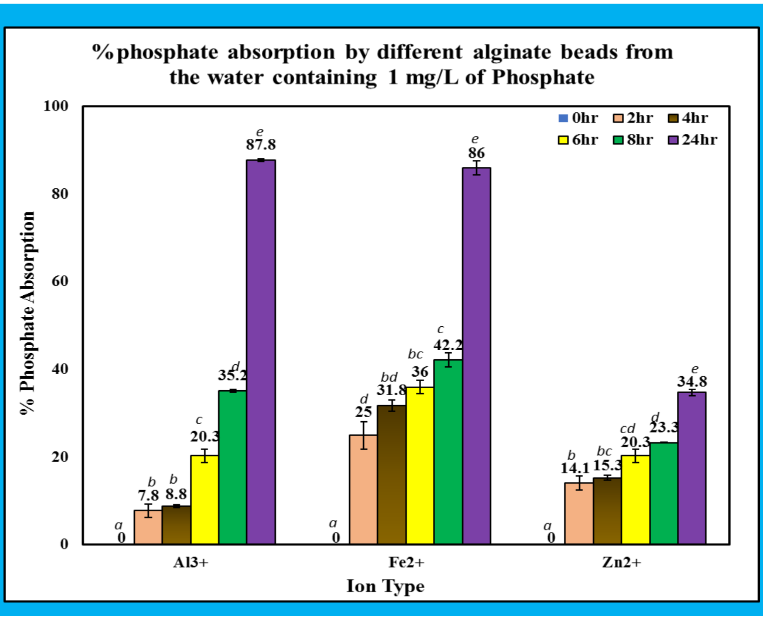

Fig. 7. Percent of phosphate absorption by different alginate beads using 1 mg/L of standard phosphate water. Each bar represents mean ± SD. For each ion type, means share same letters indicate statistically insignificant difference, while different letters indicate significantly different from each other ($p < 0.05$).

Nitrate removal: Although nitrate absorption has been carried for 24 hours, maximum absorption for most of the beads has been observed at 6 hours and results are presented in Figure 8 to 10. A maximum (33.6 ±1.9) % of absorption is observed by alginate-Fe^{3+} beads (Figure 8). The alginate-Al^{3+}, alginate-Fe^{2+}, alginate-Cu^{2+}, alginate-Sr^{2+}, alginate-Ni^{2+} and alginate-Zn^{2+} beads absorbed up to (25.0 ±1.35)%, (25.9± 0.6)%, (22.6 ± 0.65)%, (23.4 ±1.3)%, (12.3 ± 0.25)% and (10.4 ± 0.04)%, respectively. Overall, the peak nitrate absorption was obtained at 6 hours and gradual decrease of nitrate absorption was detected afterwards presumably due to slow release of absorbed nitrate.

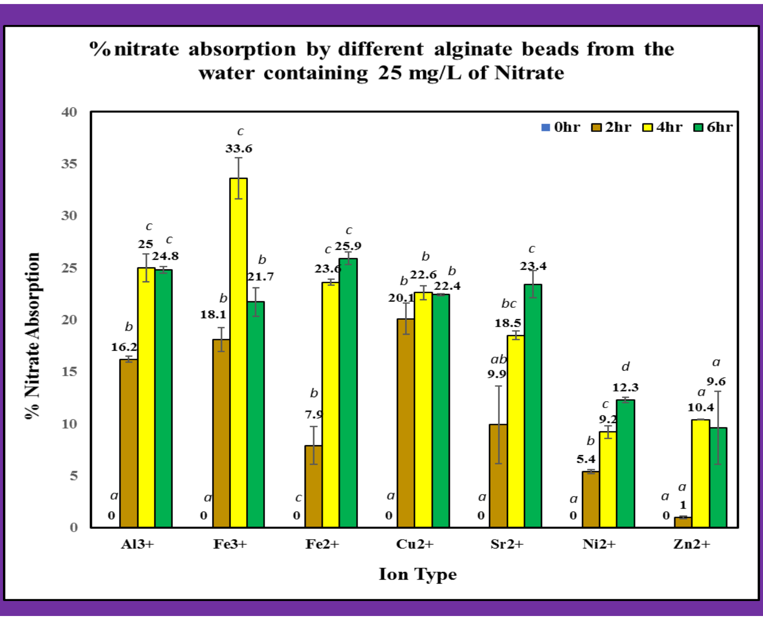

Fig. 8. Percent of nitrate absorption by different alginate beads using 25 mg/L of standard nitrate water. Each bar represents mean ± SD. For each ion type, means share same letters indicate statistically insignificant difference, while different letters indicate significantly different from each other ($p < 0.05$).

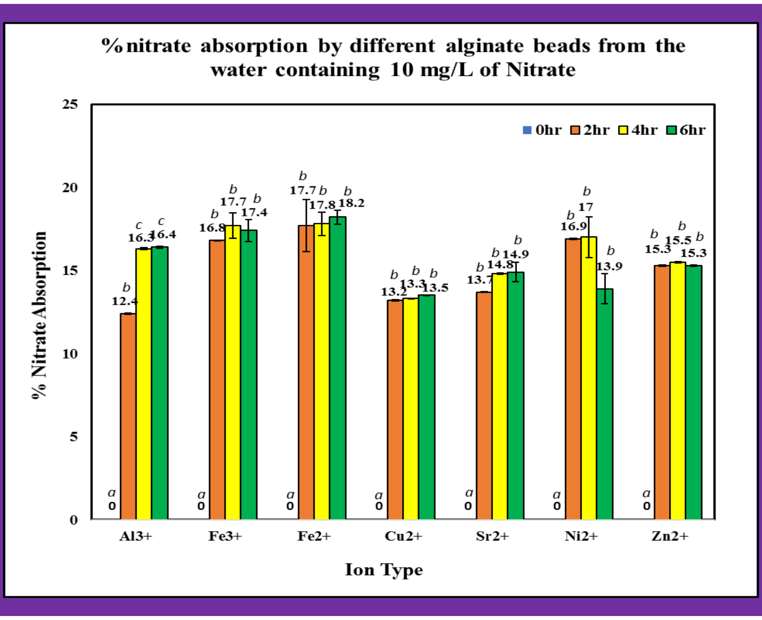

Fig. 9. Percent of nitrate absorption by different alginate beads using 10 mg/L of standard nitrate water. Each bar represents mean ± SD. For each ion type, means share same letters indicate statistically insignificant difference, while different letters indicate significantly different from each other ($p < 0.05$).

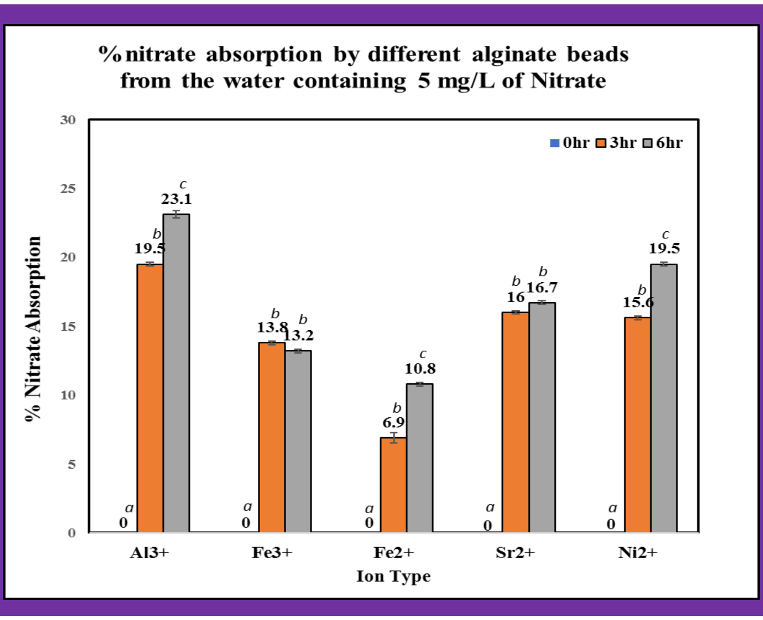

Fig. 10. Percent of nitrate absorption by different alginate beads using 5 mg/L of standard nitrate water. Each bar represents mean ± SD. For each ion type, means share same letters indicate statistically insignificant difference, while different letters indicate significantly different from each other ($p < 0.05$).

Correlation between concentration of nitrate or phosphate standard solution and maximum absorption by alginate beads: Three different concentrations of nitrate standard solution (5, 10, and 25 mg/L) and five different concentration of phosphate standard solution (1, 5, 10, 15, and 20mg/L) have been used in this research to observe the efficiency of alginate beads in terms of their absorption. Pearson correlation coefficient (r) was estimated to find the correlation between concentration of nitrate or phosphate solution and maximum absorption of different alginate beads (Table 1). No significant linear association was found between the concentration of nitrate or phosphate solution and beads absorption except Ferric alginate beads showing strong positive correlation (p -value < 0.05). However, it appears that maximum absorption of nitrate or phosphate by alginate beads will not significantly change with the changing level of concentration of nitrate or phosphate solution from lower to higher.

Table 1: Pearson correlation coefficients (r) between beads absorption and concentration of Nitrate or Phosphate standard solution and their corresponding p -values

Absorption	Concentration	
	Concentration of Nitrate	Concentration of Phosphate
Al^{3+}-Alginate	r = 0.47, p -value=0.68	r = 0.46, p -value =0.42
Fe^{2+}-Alginate	r = 0.96, p -value =0.17	r = 0.15, p -value =0.8
Zn^{2+}-Alginate	r= 0.11, p -value = 0.93	r = -0.37, p -value =0.54
Fe^{3+}-Alginate	r = 0.99, p -value =0.04	
Cu^{2+}-Alginate	r = 0.92, p -value=0.25	
Sr^{2+}-Alginate	r = 0.9, p -value =0.28	
Ni^{2+}-Alginate	r = -0.99, p -value =0.07	

FTIR spectroscopy of the alginate beads before and after treatment with nutrients: The FTIR absorption of sodium-alginate powder, different ionic cross-linked beads, corresponding beads before and after nitrate and phosphate adsorption were evaluated to identify the shifts in vibrational bands, if any. As a representative, the major FTIR absorptions of sodium-alginate powder, alginate cross-linked beads before and after treatment with nitrate and phosphate are highlighted in Table 2 and the corresponding spectra are highlighted in Figures 11. The major vibrations of sodium-alginate are observed at 1018, 1295-1326, 1404, 1604, 1245-2360 cm^{-1} that corresponds to -C-OH stretching, -C-O-stretching, asymmetric -C-O- stretching, and -C-O-O- stretching, respectively. However, some additional bands are also observed at 1342, 1512-1543, 1651, 1682-1697, 2916-1931, 3595-3641, 3734, and 3826-3857 cm^{-1} after each type of ionic cross-linking. In addition, shifting of absorption peaks before and after treatment with nitrate and phosphate are also noticed. Mainly, in the case of aluminum-alginate beads, additional absorption peaks are observed at 1527, 1697, 2854, and 3610 cm^{-1} that correspond to -C-O- stretching. The shifts of absorption peaks of 1527, 1697 cm^{-1} are observed after treatment of aluminum-alginate beads with nitrate and phosphate which corresponds to symmetric and asymmetric of -C-O- stretching.

Similar results are obtained with Fe^{2+}, Fe^{3+}, Zn^{2+}, Cu^{2+}, Ni^{2+} and Sr^{2+}ions cross-linking beads with more or less obvious shifts of absorption peaks after treatment with nitrate and phosphate.

Table 2: The major FTIR absorption (cm^{-1}) of sodium-alginate powder, aluminum-alginate beads before and after nitrate and phosphate removal.

Na-Alginate powder	Al^{3+}-Alginate beads	Al^{3+}-Alginate beads-Nitrate	Al^{3+}-Alginate beads-Phosphate
*	663	663	663
1018	1018	1018	1018
1295-1326	*	*	*
*	1342	1342	1342
1404	*	*	*
*	1512-1543	1527	1527
1604	*	*	*
*	1651	1651	1651
*	1682-1697	1697	1697
2345-2360	2345	2345	2345
*	*	2854	2854-2869
*	2916-2931	2916	2916
*	3595-3641	3610	3610
*	3734	3734	3734
*	3826-3857	3841	3841-3857

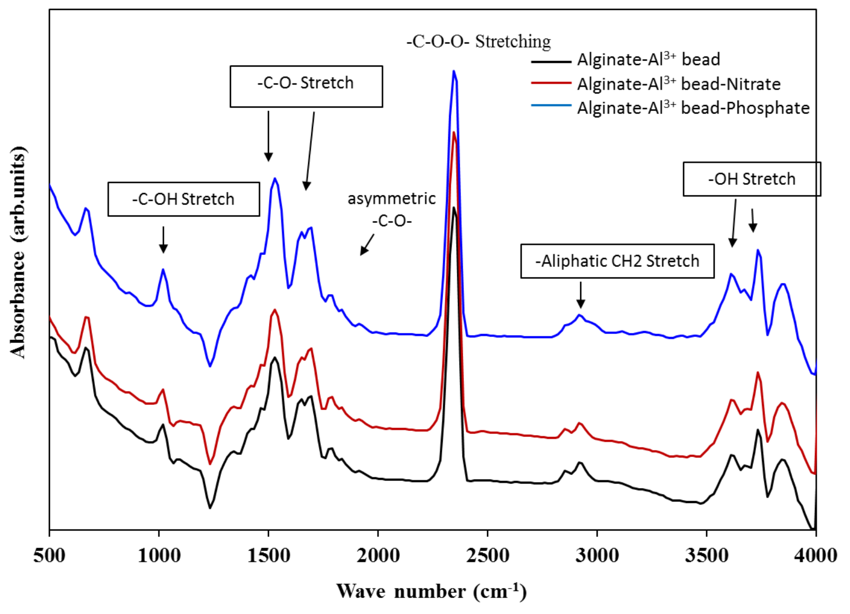

Fig. 10. The FTIR spectra of aluminum-alginate beads before and after capture of nitrate and phosphate from water.

Thermal properties of alginate beads: The melting behavior of ion cross-linked alginate beads before and after treatment with nutrients are analyzed by the differential scanning calorimetry (DSC). The results clearly indicate that there is change in melting behavior of alginate beads after absorbing nitrate and phosphate.

The enthalpy for alginate powder is 209±1.6 J/g and changed after cross-linking with cations. Furthermore, it increased significantly after absorption of nitrate and phosphate (Table 3 to Table 10). The enthalpy of alginate-Al^{3+} beads is 307.4±6.5 J/g before absorption of nitrate or phosphate, and it changes 281.0±6.4 J/g and 445.1±0.1 J/g after absorption of nitrate and phosphate, respectively (Table 3).

Table 3: DSC of Aluminum alginate beads before and after absorption of nitrate and phosphate

Sample type	Onset Temperature(^{0}C)	Peak Temperature(^{0}C)	Enthalpy(J/g)	End Temperature(^{0}C)
Alginate	83.9 ± 1.0^{a}	139.2 ± 1.4^{a}	209.9 ± 1.6^{a}	198.9 ± 0.8^{a}
+Al^{3+}	32.3 ± 1.2^{b}	95.9 ± 2.2^{b}	307.4 ± 6.5^{b}	144.9 ± 0.8^{b}
+ Al^{3+} + NO_3^-	49.8 ± 3.0^{c}	105.5 ± 2.6^{c}	281.0 ± 6.4^{c}	184.3 ± 0.3^{a}
+ Al^{3+} + PO_4^{3-}	31.1 ± 0.1^{b}	104.0 ± 0.9^{c}	445.1 ± 0.1^{d}	189.7 ± 7.5^{a}

Each value represents mean ±SD. Means share same letters in each column indicate statistically insignificant difference, while different letter indicates significantly differs from each other ($p < 0.05$).

The enthalpy for alginate-Fe^{2+} is found to be 277.4±0.4 J/g, and it significantly increases to 313.6±5.4 and 372.1±0.8 J/g after absorbing nitrates and phosphates, respectively (Table 4). Similarly, a significant change in the enthalpy of alginate-Zn^{2+} beads (153.6 ± 6.8 J/g) is also observed after capturing nitrate (202.0 ± 1.7 J/g) and phosphate (207.1 ± 7.4) (Table 5).

Table 4: DSC of Ferrous alginate beads before and after absorption of nitrates and phosphates

Sample type	Onset Temperature(^{0}C)	Peak Temperature(^{0}C)	Enthalpy(J/g)	End Temperature(^{0}C)
Alginate	83.9 ± 1.0^{a}	139.2 ± 1.4^{a}	209.9 ± 1.6^{a}	198.9 ± 0.8^{a}
+Fe^{2+}	30.1 ± 1.0^{b}	95.9 ± 0.1^{b}	277.4 ± 0.4^{b}	170.0 ± 1.8^{b}
+Fe^{2+} + NO_3^-	31.2 ± 0.8^{b}	106.0 ± 1.2^{c}	313.6 ± 5.4^{c}	172.0 ± 1.0^{b}
+Fe^{2+} + PO_4^{3-}	31.3 ± 1.0^{b}	106.4 ± 0.8^{c}	372.1 ± 0.8^{d}	182.1 ± 0.2^{c}

Each value represents mean ±SD. Means share same letters in each column indicate statistically insignificant difference, while different letter indicates significantly differs from each other ($p < 0.05$).

Table 5: DSC of Zinc alginate beads before and after absorption of nitrates and phosphates

Sample type	Onset Temperature(^{0}C)	Peak Temperature(^{0}C)	Enthalpy(j/g)	End Temperature(^{0}C)
Alginate	83.9 ± 1.0^{a}	139.2 ± 1.4^{a}	209.9 ± 1.6^{a}	198.9 ± 0.8^{a}
+Zn^{2+}	33.4 ± 0.7^{b}	85.5 ± 7.1^{bc}	153.6 ± 6.8^{b}	128.1 ± 3.8^{b}
+ Zn^{2+}+ NO_3^-	40.42 ± 5.5^{b}	106.1 ± 3.9^{d}	202.0 ± 1.7^{a}	177.4 ± 1.2^{c}
+Zn^{2+} + PO_4^{3-}	36.4 ± 0.5^{b}	98.8 ± 3.6^{bcd}	207.1 ± 7.4^{a}	159.0 ± 4.0^{d}

Each value represents mean ±SD. Means share same letters in each column indicate statistically insignificant difference, while different letter indicates significantly differs from each other ($p < 0.05$).
The enthalpy of Ferric alginate beads has significantly changed from (185.1 ± 5.0) to

(296.1 ± 7.8) J/g after treating with nitrates (Table 6). Similarly, enthalpy is fluctuated for alginate beads prepared using copper, strontium and nickel ions before and after treatment with nitrates.

Table 6: DSC of Ferric alginate beads before and after absorption of nitrates and phosphates

Sample type	Onset Temperature(^{0}C)	Peak Temperature(^{0}C)	Enthalpy(J/g)	End Temperature(^{0}C)
Alginate	83.9 ± 1.0^a	139.2 ± 1.4^a	209.9 ± 1.6^a	198.9 ± 0.8^a
+Fe^{3+}	31.9 ± 0.8^b	92.8 ± 2.5^b	185.1 ± 5.0^b	136.0 ± 1.9^b
+Fe^{3+} + NO_3^-	25.9 ± 2.7^b	107.9 ± 3.4^c	296.1 ± 7.8^c	168.2 ± 0.3^c

Each value represents mean ±SD. Means share same letters in each column indicate statistically insignificant difference, while different letter indicates significantly differs from each other ($p < 0.05$).

Summary and Conclusions:

This study successfully establishes the proof-of-concept that alginate beads could aid in water treatment technologies. More comparative large-scale studies, however, need to be conducted to further the research concept. Indeed, these provide a strong evidence to use a specific ionic cross-linker of alginate to develop a useful tool to remove nitrate and phosphate from contaminated water. The outcome could be extended to other polysaccharides such as cellulose, iota-carrageenan, kappa-carrageenan and gellan, to name a few.

Indeed, this research opens up new opportunities, mainly: (1) polysaccharide beads loaded with nutrients could be re-applied to the filed as fertilizer, adding value to farmers, and (2) metal ions in the beads could further add value to crops as metal ions deficiency in foods is a growing health problem due to depleted soils leading to grains deprived of essential nutrients. However, further studies involving following are in need:

- Experiments needs to be conducted using field water
- Research needs to be carried to enhance the nitrate absorption and holding capacity of different beads.
- These beads could be made as a large-scale filter and installed near the water streams wherein the agriculture run-off adds-in so that the nutrient contamination to the water streams could be reduced substantially.

Products

Invited talks and Conference presentations: Research findings based on this project were delivered/presented at the following locations/meetings/conferences:

1. Biodegradable biopolymers as novel tools for capturing water nutrients at the School of Food Science and Technology, Dalian Polytechnic University, Dalian, China
2. Biopolymers for encapsulation and delivery of nutrients and flavors at the School of Perfume and Aroma Technology, Shanghai Institute of Technology, Shanghai, China.
3. The 9th Annual Avera/South Dakota State University Research Symposium. Average Health and South Dakota State University, Brookings, SD, Oct 25, 2017. Biodegradable biopolymers as safe alternatives for water purification. Yesmin, M.-F., Mishra, U., Ahiablame, L., Janaswamy, S.
4. Eastern South Dakota Water Conference, Eastern South Dakota Water, Brookings, SD, Nov 8, 2017. Bioinspired materials for the development of renewable water treatment technologies. Yesmin, M.-F., Mishra, U., Ahiablame, L., Janaswamy, S.

5. It's All About Science Festival, Sanford Research Center, Sioux Falls, SD, June 9, 2018. Biodegradable Biopolymers for Renewable Water Treatment Technologies. Yesmin, M.-F., Ahiablame, L., Janaswamy, S.
6. 4th NDSU Annual Conference on Food for Health, NDSU, Fargo, ND, July 8-11, 2018. Biodegradable Biopolymers for Renewable Water Treatment Technologies. Yesmin, M.-F., Ahiablame, L., Janaswamy, S.
7. American Society of Agricultural and Biological Engineers (ABASE) Annual International Meeting, Detroit, MI, July 29-August 1, 2018. Bioinspired Materials for Developing Water Treatment Technologies. Yesmin, M.-F., Ahiablame, L., Janaswamy, S.

News Releases: During October 2017, there were several news releases about the research proposed in this project. A few of them are listed here:

- Cleaning up with carbs by The Brookings Register
- Researchers might be able to recover nutrients from run-off by Pure Pierre Politics
- Recycling fertilizer? South Dakota State researchers test ways to reduce and reuse runoff from farms by Tri-state Neighbor, Globe Gazette & Fertilizerworks.com
- Researchers explore complex carbohydrates to protect water quality by SDSU.

Invention Disclosure: One disclosure entitled "Carbohydrates for extracting and reuse of water nutrients" (T-00442) has been filed with SDSU during January 2018.

Conclusions

The PI thanks profoundly the South Dakota Nutrient Research and Education Council for the award to his group. The PI sincerely believes that the project laid strong foundation toward using polysaccharide beads for water treatment applications. The project was very productive and gained opportunity to train next generation of scientists and the outcome will be highly useful for farmers as alluded by the news releases and informal discussions at the presentations during conferences/meetings with scientists, farmers and general public. The outcome has potential for in-field nutrient management and for edge-of-field treatment of surface runoff and subsurface drainage water but large-scale studies are in need, and toward this end PI is actively scouting for the grant dollars.