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Progress Report

2021 South Dakota Nutrient Research and Education Council Invited Proposals

Progress Report Title:	Draft Final Report - Due December 1, 2021
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Application Title:	Synthetic nodules for nitrogen nutrition in cereal crops
Application ID:	1328
Review Deadline:	12/1/2021 11:59 PM

Draft Final Report - Due December 1, 2021

Project Information

	Start Date	End Date
Start and End Dates of Funding:	1/1/2021	12/31/2021

Title of Project: Synthetic nodules for nitrogen nutrition in cereal crops

Project Description:

Nitrogen is a major limiting nutrient for crop production. The major source of nitrogen for cereal crops is the addition of ammonia and or nitrate fertilizer. Unfortunately, continued use of nitrogen fertilizers is not sustainable due to their loss from agricultural fields and increasing cost. Combined use of biological nitrogen fixation (BNF) and fertilizer nitrogen is a viable and sustainable alternative. A major example of BNF is the conversion of atmospheric nitrogen into ammonia in root nodules of legume crops. The nodules provide an oxygen-reduced, nutrient rich environment suitable for high-efficient nitrogen fixation. However, cereal crops such as maize, wheat and rice are non-legumes and do not have the required capacity to produce the nodules and fix nitrogen. This project focuses on developing synthetic nodules wherein nitrogen-fixing bacteria (H. seropedicae Z152) will be encapsulated in polysaccharide beads that provide an oxygen- reduced environment along with carbon nutrition for nitrogen fixation. These beads would be biodegradable and can be directly applied as fertilizer that offer a sustainable source of nitrogen for non-legume crops such as maize. This form of nitrogen is expected to be less susceptible to leaching and be available continuously to the plant.

Publications: None to date.

Project title: Synthetic nodules for nitrogen nutrition in cereal crops

Overview: Nitrogen is a major limiting nutrient for crop production. The major source of nitrogen for cereal crops is the addition of ammonia and or nitrate fertilizer. Unfortunately, continued use of nitrogen fertilizers is not sustainable due to their loss from agricultural fields and increasing cost. Combined use of biological nitrogen fixation (BNF) and fertilizer nitrogen is a viable and sustainable alternative. A major example of BNF is the conversion of atmospheric nitrogen into ammonia in root nodules of legume crops. The nodules provide an oxygen-reduced, nutrient rich environment suitable for high-efficient nitrogen fixation. However, cereal crops such as maize, wheat and rice are non-legumes and do not have the required capacity to produce the nodules and fix nitrogen. This project focuses on developing synthetic nodules wherein nitrogen-fixing bacteria (*H. seropedicae Z152)* will be encapsulated in polysaccharide beads that provide an oxygen- reduced environment along with carbon nutrition for nitrogen fixation. These beads would be biodegradable and can be directly applied as fertilizer that offer a sustainable source of nitrogen for non-legume crops such as maize. This form of nitrogen is expected to be less susceptible to leaching and be available continuously to the plant.

Research objectives:
(**1**) Evaluate polysaccharide beads in the presence of various divalent and trivalent cations to produce beads with desirable properties as synthetic nodules: (a) Preparation of beads with different combinations of polysaccharides and cations, (b) Evaluation of bead physical properties for suitability as synthetic nodules, (c) Evaluation of oxygen concentration, pore size, bead structural stability, and (d) Evaluation of bacterial viability and retention, and biodegradability of beads.
(**2**) Evaluate the impact of energy source and biofilm substrate on the rate and amount of nitrogen fixed: (a) Prepare beads with different carbon sources and evaluate bacterial viability and N fixation, (b) Prepare beads with 2D material and metal organic framework substrates and evaluate bacterial viability and N-fixation, and (c) Select the most desirable combination of polysaccharide, cation, carbon source, and biofilm substrate and evaluate bacterial viability and N fixation.

Fig. 1. Alginate beads in the presence of various cations.

Accomplishments: We chose alginate as the initial polysaccharide and prepared beads in the presence of 8 cations, Ca^{2+}, Ni^{2+}, Zn^{2+}, Sr^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+} and Al^{3+} (**Fig. 1**). Among these Fe^{2+} and Fe^{3+} were discontinued as they were readily oxidized in the air. Beads were prepared with the other six cations with alginate as polysaccharide and Glucose, Arabinose and Dextrose (at 0.2, 0.4, 0.6, 0.8 and 1.0% of total volume) as carbohydrate source. This also aided us to further screen the cations as Cu^{2+} readily oxidized the arabinose. Evaluation of the oxygen concentrations in the micropores of beads was performed on the six cationic combinations with indigo-carmine method modified with CHEMetrics kit and color was measured with NIX Pro color sensor (**Fig. 2**). The oxygen concentrations are found to be 1.97, 1.84, 2.64, 5.46, 5.89 and 2.54 (ppb) for Ca^{+2}, Zn^{+2}, Sr^{+2}, Cu^{+2}, Ni^{+2} and Al^{+3}, respectively.

Fig. 2. Color measurement to quantify oxygen concentration in the beads.

Initially, we have had difficulty to procure *H. seropedicae Z67*, however we arranged different strain in *H. seropedicae Z152* and received the shipment during the 1st week of November, and started preparing working stock, and assessing bacterial maturity to load within the beads. However, we evaluated *Azospirillum brasilense*, another nitrogen fixing bacteria, encapsulation capacity within the beads. The Ca^{2+} and Sr^{2+}cation beads are found to be efficient to encapsulate 72% and 42.18% of, respectively, while other combinations resulted relatively low encapsulation. We have initiated experiments to understand the root cause for the observed smaller encapsulation amount.

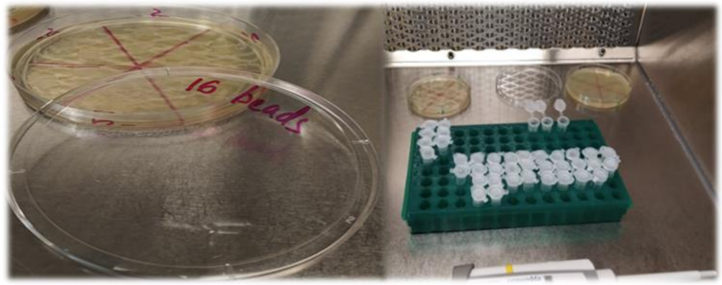

Fig. 3. Measurement of bacterial encapsulation.

We got the preliminary data on biodegradability of alginate beads in soil which shows half-life of beads around 120 days. We are currently working on evaluating the biodegradability in more controlled environment. Meanwhile, we are working on assessing the growth curve, and encapsulation ability of *H. seropedicae Z152* on alginate and iota-carrageenan beads. Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) to analyze the pore size and beads structural stability has been going on parallelly.

Research that needs to be completed: The dry beads are taking longer time than we expected to biodegrade. On the other hand, wet beads, those without drying, could degrade within 48 hours. This suggests that dry beads take longer durations to completely biodegrade, and we are continuing the experiments to find out the optimum time. Parallelly, we initiated the work on assessing the Nitrogenase activity by encapsulated bacteria on a culture and encapsulated beads through acetylene reduction assay. This helps us to estimate and analyze the rate and amount of nitrogen fixed by each bead. Later, we need to evaluate the role of 2D material and metal organic framework substrates on improving the Nitrogenase activity. In this regard, we sincerely request no-cost extension until December 2022.