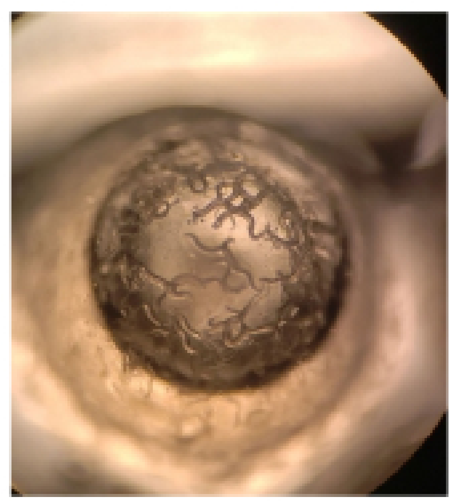
246

What scientific or technological advancements did you achieve as a result of the work described in Line 244?? (*Maximum 350 words*)

By the end of the fiscal year we had failed to reduce the time required for production and had not made any major improvements to the process used in the development of the encapsulating alginate balls themselves. However, we did identify that only one type of salt is required during the setting bath stage instead of two. Technological advancement was in identifying that xanthan gum meets the mechanical properties we are looking for, it currently defeats the manufacturing needs, and doesn't facilitate an improvement in the biological aspect of the balls. The real advancement was in laying the groundwork on the focus for the project in the future, as this year we obtained a lot of useful data even though the experiments were all failures. Such as:

- Increasing alginate viscosity reduced leakage.
- Xanthan gum stopped leakage completely when used at 1% concentration.
- Increasing xanthan gum decreased fracture, leakage and translucence of the balls.
- The use of xanthan gum in combination with alginate Protanal LF 120 yielded the best results for all tested options and had the best protreptics against syneresis.
- The relationship between pH and time in the salt baths and the impact on the alginate gel strength. Characterizing our alginate-calcium formulation by measuring changes to pH, use of a sequestrant, water hardness, presence of hydrocolloids, and water intake.
- The failed Zein coating experiments and their observable impact on drying time.



Nematode cut in half

calcium carbonate are significantly influenced by the pH of the solution. Consistent with all these salts, a lower pH resulted in a shorter and therefore faster gelation time. Calcium sulfate ($CaCl_2$) took no less then three days to gel, calcium carbonate took longer. Dicalcium phosphate gelled in a reasonable amount of time ranging between 5-20 hours depending on the pH. We also observed that calcium lactate and calcium chloride are insensitive to pH and will fully ionize in solution, causing these two solutions to react immediately with sodium alginate. We concluded that we should continue to use $Cacl_2$ as a result of these tests. Additionally, we identified that the first 20 minutes the balls were submerged in the $CaCl_2$ setting bath were when the balls absorbed the most water, which was critical for the life and longevity of the nematodes. These experiments did not lead to any overall improvements or changes, just helped us improve our understanding the established salt bath process.

Next, we examined potential modifications the formulation of the alginate to improve the water retention of the alginate as it solidifies. Overall, we experimented with six major formulations, which had their properties examined and compared to assess optimal formulation characteristics. These new compositions contained high amounts of additional ingredients, all of which had negative effects on the viability of nematodes in the final formulation. These ingredients included sugars sucrose and dextrose, glycerin, caster oil, and xanthan gum. We found all formulations incompatible for nematode based encapsulation due to insufficient moisture content. These tests provided new insight into how the ingredients will affect physical properties of the gel. We were able to observe that the use of a calcium sequestrant had a major influence on hardness. The greater the amount of sodium hexametaphosphate sequestering agent (SHMP) used, the lower the balls hardness. High amounts of SHMP chelate more calcium ions, leaving less to react with the alginate. It additionally decreased tackiness of the balls as SHMP increased. Hardness of the balls decreased as the pH decreased. However, the addition of xanthan gum resulted in an increase in hardness with a decreasing pH.

Due to the observed incompatibilities of nematodes with sugars and glycerin/glycol, we shifted focus to xanthan gum alone to increase the hardness and decrease syneresis of the alginate balls. A batch of 2.5% sodium alginate was modified to also contain 1% xanthan gum. When mixed viscosity increase3d to where it could not run through the pumping apparatus. The batch was diluted down to 1.25% sodium alginate and 1% xanthan gum. A second batch was created at 1.25% sodium alginate and 0.7% xanthan gum. The 0.7% xanthan gum changed the solutions mechanical properties, the balls formed were larger than normal but held their spherical shape better. The 1% batch was more difficult to extrude through the tooling head, causing the balls to be highly deformed and irregular.

Due to experiments with coatings in previous years we identified one which is used as a substitute for shellac. We conducted experiments with coating the balls in a Zein solution which is an alcohol-soluble protein, after we produced our own Zein solution we conducted several tests to identify how to best apply it to our alginate balls. We identified that it was best to be applied at a temperature between 30-40°C to facilitate the coating process. However, we were unable to get an even coating on the balls and observed that it was causing a large inconsistency in drying times as a result. We would like to investigate this further in the next fiscal year, once we identify a reliable means of coating with it.

242

What scientific or technological uncertainties ---could not be removed using standard practice? (*Maximum 350 words*)

Over the past several years we have successfully developed and patented a technology which enables us to provide nematodes to end users while avoiding the standard requirements for refrigeration or ice packs during shipping or storing. However, the development of this technology was done for the initial purpose of laboratory testing. We initially had to develop a method for encapsulating over a million nematodes per in a proprietary alginate ball which would provide a hospitable environment for six to twelve months. The spherification process we had developed was not conducive to manufacturing and was a tedious process; a three-day process that involved producing the balls, hardening their shells, and drying them to within tolerance of a 27% moisture content with large variations in nematode populations.

In the previous fiscal year, we had begun experimenting and adapting the spherical dripping machines used in the man-man caviar industry. We had achieved some limited success last year in proving that automating the production of our alginate balls is possible and as it greatly improved our ability to equalize the population of each ball. However, this work lead to a technological uncertainty, that in order to develop a process capable of large-scale manufacturing of our nematode alginate balls, the entire development process of the balls would have to be reexamined and modified to meet the conflicting needs of manufacturing vs. the laboratory needs they were initially developed for. Specifically, this meant modifications to our alginate formulation were required, as well as the production process used as the time it took was a production bottleneck. Additionally, we were unable to control the size of the balls during their formation process with a formulation which met our economic limitations.

We hypothesized, at the start of this year we would take a step back and prioritize any modifications made must assist in accelerating the overall process by eliminating steps without impacting the health of the nematodes. The process itself was to tedious to be practical for manufacturing. This was not limited to the process, or modifications to the machine, but even the nematode encapsulation formulation modifications made with respect to its hardness and synersis properties would need to be redeveloped.

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What work did you perform in the tax year to overcome the scientific or technological uncertainties described in Line 242?
(Summarize the systematic investigation or search) (*Maximum 700 words*)

We began our experiments this year by focusing on the production of the alginate balls. What we had developed to this point was the sodium alginate stock solution is extruded into droplets through a specialized tooling head, which become set by immersion in the calcium chloride solution. This generates the rounded shape. Second, the calcium ions penetrate deeper into the ball, reacting with the ungelded solution inside, solidifying the entire ball. Approximately 7.2% (w/w) calcium ions from the weight of the sodium alginate is stoichiometrically required for complete gelation, which takes approximately three days. We theorized, that we could improve this gel time by experimenting with different salt bath formulations, as the gelling time for dicalcium phosphate, calcium sulfate, and

WXA.

PROJECT #:3

PROJECT NAME

ALGINATE-BALL PROCESS IMPROVEMENT