Biodegradable Chitin Based "Plastic" Utilized as a Plant Fertilizer

Brittany Rutt

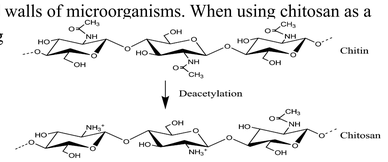
Hastings College, Hastings, NE, Department of Chemistry

Abstract

The accumulation of plastics in our landfills is a significant problem. Plastic does not easily break down, and when it eventually does, it produces toxic gases and microplastics which pollute our Earth. Additionally, the accumulation of food waste (e.g. crustacean shells) in our landfills produce an abundance of CO_2 gas which contributes to global warming. Being able to utilize these shells as a biodegradable alternative to plastic can cut down on waste production and reduce the amount of useful waste going into landfills. This project looks into how to extract chitin (a biopolymer) from different natural sources (such as crustacean shells) and use it to create a biodegradable "plastic" that is safe for the environment. The plastic will then be utilized as a fertilizer to see its potential effects on plants. This research will not only show how we can eliminate unnecessary waste, but how to use new methods to benefit our planet and plant life.

Introduction

Chitin is the second most abundant organic material on earth which makes it the perfect material to use for this type of experiment. Chitin is a long-chain polysaccharide that makes crustacean shells hard, almost armor-like in order to protect the sea creature from danger. This material can also be found in the exoskeletons of insects as well as in butterfly wings. Chitin is a linear, insoluble homopolymer of βP-1,4 linked N-acetylglucosamine, which is nature predominantly exists as an ordered crystalline microfibrils that is embedded in a matrix of proteins and minerals. There are repeating sugar units in a chain of chitin that are disaccharides with the monomers rotated 180 degrees relative to each other. The deacetylation of chitin then yields chitosan which is a water soluble heteropolymer of β(1,4)- linked GlcNAc (A-units) and D-glucosamine (GlcN or D-unit). Using chitin to industrially produce chitosan is an important application of this polymer. When looking at the amounts of chitin from crustacean shell waste that is available for exploitation, only a small part of the biopolymer is being utilized in this way with the most dominant application of chitin being used after chemical deacetylation. Chitosan also binds with heavy metals, hydrocarbons, debris and other toxins in water treatment, as well as binding to the cell walls of microorganisms. When using chitosan as a fertilizer, the plant will react to it as if it is being infested with pests (elicits an immune response from the plants). These plants then exhibit improved nutrient absorption, increased crop sizes and yields, and an increase in survival rates in severe growing conditions.



Methods

To start, obtain different sources of Chitin to use for this project. One source will be Mealworm feces, and the second will be from crustacean shells (lab grade shrimp shell flakes). The Mealworm feces will be obtained from a professor on campus in the Chemistry department while the shells will be obtained from places in/around town (Hastings Ne.).

The next step would be to crush the shells into flakes which will then be washed with distilled water a total of three times or until no contamination is present. This step however will be replaced with lab grade shrimp shell chitin power. Both sources will then soak in 2M HCl to remove excess minerals while stirring vigorously for 1-2 hours and then left at room temperature for up to 24 hours. Then both samples will get filtered and deproteinized with 2M NaOH to assist with deacetylation while the solution is boiled and stirred vigorously for about 12-24 hours. After this step is complete let the solution stand at room temperature for another 24 hours. Then the sample will get washed again with 50% NaOH and filtered.

After obtaining the Chitosan a control Chitosan will be introduced that was purchased online from a chemical supplier to test purity of Chitosan obtained in the lab. These three samples will then be used to create the biodegradable "plastic" sheets in which seeds will be placed by mixing the Chitosan with acetic acid. The plants will then be observed against plants without anything in them as a control to see if they work as a fertilizer and benefit the plants growth.

Research Question

Will chitosan samples derived from different sources of Chitin have different impacts on plant growth when used as a fertilizer?

Results

The experiment as a whole did not turn out as well as it could have. The production of chitosan from chitin based products may have been successful, and the production of chitosan 'plastic' was successful, however the fertilizer aspect of this experiment did not show any positive results. None of the plants with the experimental samples grew, whether this was due to the actual use of the samples or how well they were made is unknown. The plan was first to make cups to plant the seeds in, when this did not work the production of 'plastic' sheets were used instead for the chitosan samples and the other two, feces and chitin, were simply poured into each well of soil due to not solidifying into a solid form.

These numbers are the same for all three rows of each sample making the total number of 9 for each sample and a total of 3 samples for each number of wells.

Table 1

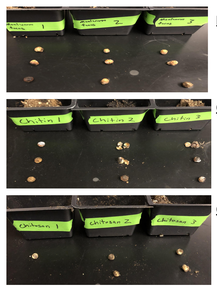
Well	Chitin	Vinegar added	MealWorm Feces	Vinegar added	Chitosan	Vinegar added
1	20mL	20mL	20mL	20mL	20mL	60mL
2	15mL	15mL	15mL	15mL	15mL	45mL
3	10mL	10mL	10mL	10mL	10mL	35mL

Each plant was watered every other day during the week (MWF) with 30mL of water measured out using a graduated cylinder. The brand of pea plant seeds as well as the soil that was used during this experiment were kept consistent. The pea seeds were Alaska Peas from the company Carolina and the soil was Professional Potting Mix with moisture pro crystals. Starting out after the seeds were planted, from day 0 to day 3 nothing occurred due to the seeds having a sprouting period of 5-7 days. On the fourth day there was noticeable growth on the chitin and feces samples that was possibly due to any NaOH that was still in the samples before mixing with the soil. The chitosan samples showed that the plastic sheet pieces that were added started to breakdown and form a goop like consistency. On day 6 the control plants started to slightly sprout and the chitosan seeds were seemingly being pushed out of the dirt by the chitosan pieces breaking down and leaving the seeds to sit on top of the dirt. By day 9 the control plants looked healthy and were growing at a steady rate. The other plants still have not shown any signs of growth. Day 10 showed less growth on top of the soil for the chitin and feces samples indicating that the excess NaOH was slowly being washed out of the soil. From day 11 to day 22 there was no growth present in any of the experimental samples. The control plants kept growing at a decent pace with the tallest standing at about 7 inches tall by the end of the experiment and the shortest being around 5 ½ inches tall. The next step would be to dig up all of the seeds that did not grow and see if any of them split while trying to grow or if the seeds had died during the experiment due to the pH imbalances and that is why no plants were produced. Towards the end of the experiment, around day 15, the production of mold is observed in the chitosan wells. The mold appears to be growing on the goop that formed from the chitosan breaking down which makes sense due to the goop being very moist and with some of it sitting on the surface of the soil being exposed to the air in the green house. Due to the results of this experiment I cannot prove nor disprove my research question about whether Chitin could be utilized as a fertilizer.

Table 2

	Mealworm Frass	Chitin	Chitosan
1)	1) 10.23	1) 10.45	1) 8.08
	2) 10.38	2) 10.42	2) 8.41
	3) 10.22	3) 10.43	3) 7.62
2)	1) 9.96	1) 10.42	1) 8.50
	2) 10.06	2) 10.46	2) 8.32
	3) 10.28	3) 10.51	3) 7.69
3)	1) 9.80	1) 10.40	1) 8.46
	2) 10.03	2) 10.49	2) 8.24
	3) 10.23	3) 10.42	3) 8.12

Average Control pH: 6.50



Frass:
- Black spots
- Not split
- Discolored
- White flakes

Chitin:
- Black spots
- Split
- Discolored
- White flakes
- Falling apart

Chitosan:
- No growth
- No rot
- No discoloration
- Still intact

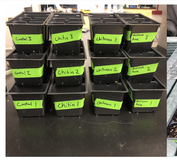


Figure 1: This is the setup that was used for each of the three samples to keep which ones were which apart from each other. The sides of each are also labeled with 1, 2, or 3.

Figure 2: This is a picture of all of the wells from day one with the control being the three in the very back, the chitin samples, the mealworm feces samples, and lastly the chitosan samples being in the front.

Figure 3: This is a picture of the control pea plants on the last day of the experiment (Day 22)

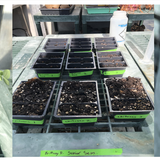


Figure 4: This is a picture of the chitin, mealworm feces, and chitosan samples on the last day of the experiment (Day 22)

Figure 5: This is a picture of the goop that was forming from the breakdown of the chitosan pieces on day 12 of the experiment. The production of goop is seen in each well with the dirt being almost packed down tightly.

Discussion

The data that was found for this experiment was useful when looking into plastic alternatives, however there were a few things that did slow down or hinder the process. The deacetylation process took a full week to complete which then made it harder to possibly repeat this experiment if need be due to the lack of time from the start of the project. If the project was started earlier, there could have been a possible second trial that could have helped figure out what might have gone wrong the first time through. To start, the sheets did not form properly for each of the three things being tested. The chitosan was the only one that formed the sheets, the mealworm feces and chitin had to be poured directly into the plant wells as they would not set up as a solid. Another thing that went wrong with this experiment is that none of the experimental plants grew, only the control ones. We can see that something was done correctly since the controls did grow. The next step would be to figure out why the other pea plants did not. One of the reasons why they did not grow would be due to the pH of the product being added to the soil. All of the samples were too acidic for the seeds to grow to the point that they killed the seeds before they could sprout. Another reason would be that somewhere during the process I was not successful in extracting the chitosan from the chitin based products. Ways that this experiment could be improved would be to start ahead of time to allow for extra time if needed to run the experiment a second time. Testing the pH of the samples before adding them to the soil is another example of what could have been done to ensure the project was successful.

Acknowledgements

- Advisor: Dr. Kevin Robb
- Dr. Neil Heckman for providing the Mealworm frass
- The Biology department for the use of the green house
- The Chemistry department

Literature Cited

- Insiya, et al. "Shellworks." *Shellworks*, 2018, www.theshellworks.com/.
- "Manufacturing a Solution to Planet-Clogging Plastics." *Wyss Institute*, 25 Aug. 2017, wyss.harvard.edu/news/manufacturing-a-solution-to-planet-clogging-plastics/.
- Drahl, Carmen. "How Seafood Shells Could Help Solve the Plastic Waste Problem." *Science News*, 8 Aug. 2019, www.sciencenews.org/article/seafood-shells-chitin-plastic-food-waste.
- Aguirre, Mary Joy, and Polymer Solutions News Team. "Biodegradable Plastic From Crab Shells." *Material Analysis & Materials Testing Lab*, 18 June 2014, www.polymersolutions.com/blog/biodegradable-plastic-from-crab-shells/.
- Quimno, Marie Jenine. "Chitin In Lobster Shells As A Biodegradable Plastic." *Mandaue City Comprehensive National High School - Senior High Department (Science and Technology, Engineering and Mathematics)*, Academia, 2016, www.academia.edu/38521331/Chitin_In_Lobster_Shells_As_A_Biodegradable_Plastic.
- Ingunn A. Hoell , Gustav Vaaje-Kolstad & Vincent G.H. Eijsink (2010) Structure and function of enzymes acting on chitin and chitosan, Biotechnology and Genetic Engineering Reviews, 27:1, 331-366, DOI: 10.1080/02648725.2010.10648156
- "Chitosan / Chitin: Made in the USA with Eco-Friendly Processes." *Tidal Vision*, tidalvisionusa.com/chitosan/.