December 27, 2024

https://bmcbiotechnol.biomedcentral.com/articles/10.1186/s12896-021-00733-3

- Conclusion states that "*Klebsiella oxytoca* ATCC 13182, *Klebsiella oxytoca* NBRC 102593 and *Klebsiella oxytoca* JCM 1665 may be some of the bacteria responsible for polystyrene biodegradation."
- "third group that had mealworms fed on polystyrene only had a percentage survival rate of 85% which was slightly lower than the percentage survival rates of both group 1 and group 2."
    - Note that group 1 and 2 had alternative food sources → corn starch and carrots in differing combinations per group
    - Note to research "quadrant streak method" as a means to obtain bacteria
    - Gram negative → what is this? Research
    - "The isolated bacteria were cultured in a medium with polystyrene as the carbon source and then plated on **polystyrene modified agar** so that those able to biodegrade the polymer could grow. Based on morphology, size and colour, 5 colonies were picked and subcultured until pure colonies were obtained. The five colonies from the *Tenebrio molitor's* gut were gram stained and all of them stained pink to red, hence, all were gram-negative."
        - Could use highlighted green part in our experiment? Would isolate potential bacteria.

Jan 8 2025

Idea brainstore, then do slides for SF club meeting

- At meeting, Martin and Evelyn want to start a sf project
    - Talk to Ms. Bajwa to help their CYSF registration ^

- Problem
    - Address a knowledge gap inciting by previous studies → what isn't very clear, how can we do an experiment that moves to clarify, or add info surrounding this knowledge gap?

Hypothesis
Procedure
Materials
Background research
    Sources, links/ideas
Safety declaration and initial project details → approved → access to the portal

- (Yang, et al., 2015): https://pubs.acs.org/doi/10.1021/acs.est.5b02661 ← ORIGINAL STUDY
    - Confirmed PS breakdown via mealworms
    - Part 2: https://pubs.acs.org/doi/10.1021/acs.est.5b02663?ref=recommended
- Mealworms
    - Nutritional sources

        - Carrots w
    - Chemicals in the worms
        - Many different things that people have hypthesized
            - Yang et al part 2 says one
            - 8 more potential
            - 12 more potential
                - Enzymes to enhance this process?
    - Feed to other animals
- Styrofoam
    - O chem structure (polymer), benzene ring, hydrocarbon backbone
        - C8h8 is eps
            - Styrene found naturally in foods: strawberries, alcohol
        - Break down? Inhibit? Structure → not detrimental to feed animals
- HBCD
- https://pubs.acs.org/doi/10.1021/acs.est.9b06501 no bioaccumulation of hbcd (toxic)

https://pmc.ncbi.nlm.nih.gov/articles/PMC11207799/

- radical oxidation reaction (autoxidation) using reactive oxygen species,
    - lignin by white rot fungi
    - low persistence of cycloalkyl compounds such as HBCD in the body suggest that benzene and cycloalkyl ring degradation occurs in mealworms
    - PS chain scission occurs in the mealworm's body, most likely in the gut [16,17,20,26]
    - suggests that the benzene ring is decomposed by quinonization via a phenolic intermediate.
    - EPS is an alternative nutrient to carbohydrates because it is composed only of C and H elements, but it cannot be a protein source because it does not contain N.

Issue plastic pollution

- Mealworms eat plastic pollution
- Not that effective → what to do?
    - Isolate gut bacteria
    - Alternative benefits of waste + beetles/dead mealworms
        - HBCD

    - **autoxidation**, chemical groups
        - Benzene carbon carbon rings (cleave)
            - Depolymerization
            - Cannot confirm → suspect it with the FT IR tech
                - py-GC/MS analysis
                    - Confirms peaks of α,β-unsaturated ketones
                        - indicating that the benzene ring was decomposed by quinonization via a phenolic intermediate

        - This was the same mechanism as a yellowing reaction of PS caused by the ring-opening reaction of the benzene ring.
    - Wavenumbers → form of measurement?
    - Alpha beta ketones? What is this → research this

https://www.innovatechlabs.com/newsroom/1882/interpreting-analyzing-ftir-results/ ← interpret FT-IR results

Autoxidation is: a slow chemical reaction where an organic substance reacts with oxygen without heat, light, or enzymes to start the process. Over time, the oxidation causes the substance to break down and its properties change.
Steps:

Steps

1. **Initiation :** A molecule loses an electron, forming a free radical. Happens spontaneously due to oxygen exposure, UV radiation, , or chemicals reactions.
2. Propagation – The free radical reacts with oxygen, forming peroxides or hydroperoxides, which cause more reactions and break down the material further. This step propagates — like a chain reaction.
3. Termination – The reaction stops when two radicals combine, or when the material is fully broken down.

In Polystyrene;

- Oxygen slowly reacts with the plastic, breaking down the benzene rings (hexagon with circle) and forming quinones and ketones.
- This can cause molecular weight reduction — observed in the study, plastic yellowing and brittleness
    - study found that autoxidation was happening inside the mealworms'
    - This means oxygen was breaking down the PS' benzene rings, leading to 'quinonization' and forming 'α,β-unsaturated ketones', which are seen in plastic yellowing.
    - Autoxidation plays a big role in the mealworms' ability to degrade the Ps

FT-IR

- FT-IR spectrometers measure how much infrared light a sample absorbs, and then generate a spectrum based on the functional groups in the material.

FT-IR (fourier transform infrared) spectroscopy: a lab technique using IR light to determine a material's composition.

- Spectrometer measures how much IR is absorbed or transmitted (depending on machine) at a given wavelength, by shining all wavelengths at it and using FFT to separate the wavelengths (watch 3b1b's video on fourier transform).

- Specific peaks indicate specific functional groups

What has already been proved? (i.e. a no-go for us)

- Consuming only EPS will increase mortality long-term
- The mealworms are indeed depolymerizing and breaking down the benzene rings of styrene, as proved through FT-IR tests
- The mealworms are safe to feed to other animals
- The strains of bacteria that consume EPS do exist, can be isolated, and can be used to break down EPS.

Ps degrade in mealworms

- Combo of
    - Bacteria strains
    - Enzymes that initiate autoxidation (degradation mechanisms)
        - Products shown in FT-IR study → reflective of process that breaks down plastic (yellow, decrease molecular weight), make food smell bad after long time
        - How to increase/decrease/manage autoxidation?
            - Ao stabilizers mitigate rate

Antioxidants slow autoxidation

- Find the opposite? Oxidant?

Mealworm

- Keep them alive?
- Carrots and

Expanded Polystyrene Foam

- Ways to degrade

References & Studies

1. **Yang, Y. et al. (2015)**

    - Found that *Tenebrio molitor* can digest plastic and produce oxidized intermediates.
    - DOI: 10.1021/es504038a
2. **Brandon, A. M. et al. (2018**
    - Investigated gut microbiota's role in plastic degradation.
    - PMC11207799

1. Zhang, Y., et al. (2015)
    - explored the impact of antibiotic ingestion on the gut microbiota of *a different bug's* larvae. The larvae were fed diets containing antibiotics like ciprofloxacin, which significantly altered their gut bacterial communities and affected their development.
    - https://www.mdp
    - Ciprofloxacin was identified i.com/2075-4450/13/9/838
    -
2. Lou, Y., et al.2023.
    - examined the roles of mealworms and their gut microbiota in polyethylene (not polystyrene)degradation. Mealworms were fed diets supplemented with antibiotics like gentamicin to suppress gut bacteria, allowing the study of the larvae's innate degradation capabilities.
    - https://link.springer.com/article/10.1007/s10924-023-02843-9
    -
3. Kafil, M., et al. 2013.
    - investigated the effects of eliminating gut symbionts in *different bug (like zhang et al 2015)* using antibiotics..
    - https://academic.oup.com/jinsectscience/article/13/1/99/1079633
    - Oxford Academic
4. Moghadam, N.N., et al. (2023).
    - explored how antibiotic ingestion altered the gut microbiota composition in *bug* and its effect on insecticide susceptibility.
    - https://link.springer.com/article/10.1007/s10123-024-00507-9
    -

Jan 9

CYSF portal basic info

Manipulated variable: Biodiversity of mealworm gut bacteria

- This is controlled by using **antibiotic treatment** to sterilize some of the gut in one group while keeping the other group normal. → WAS CHANGED TO *reducing* gut biodiversity in one group, cannot fully STERILIZE

Responding variable: Indicators of autoxidation in the digested expanded polystyrene foam.

- The level of **plastic oxidation** as measured by **FT-IR spectroscopy** (carbonyl, hydroxyl, and alkane peak intensities).

Name:

Correlating Autoxidation Trends of Expanded Polystyrene Foam in Tenebrio Molitor Larvae with Gut Bacterial Biodiversity.

Problem:

How might the biodiversity of mealworm gut bacteria correlate with the rate of autoxidation of expanded polystyrene foam in *Tenebrio molitor* larvae?

Brief Description:

Studies have revealed that mealworms (*Tenebrio molitor* larvae) can degrade EPS via autoxidation and gut bacteria. We aim to determine the source of EPS oxidation by comparing FT-IR spectra of plastic residues in normal vs. antibiotically-treated larvae.

Feb 1

purpose:

This study aims to identify the mechanisms for expanded polystyrene (EPS) autoxidation in *Tenbrio molitor* larvae. Potential processes that have been determined in past studies suggest internal autoxidation through intrinsic means, or microbially within the gut microbiota. Via mealworm gut bacteria inhibition by antibiotic treatment, we will analyze the EPS residues using Fourier Transform Infrared (FT-IR) spectroscopy, and assess the peak intensity of oxidation byproducts in larvae with unmodified, and modified bacterial activity. The results of this study will provide insight on potential plastic waste management strategies.

Description:

Expanded polystyrene (EPS) is a widely-used plastic and synthetic polymer of styrene resistant to degradation, and as such is a significant contributor to plastic pollution. Previous studies have demonstrated that *Tenebrio molitor* (mealworms) can consume and degrade EPS, the evidence of such being internal autoxidative processes detected via Fourier Transform Infrared (FT-IR) spectroscopy (Yang et al., 2015) (Natakani et al., 2024). However, the mechanistic basis of this degradation remains unresolved—specifically, whether oxidation is mediated by methods within the mealworm's digestive system or by its gut microbiota. Three cases have been hypothesized:

mealworm gut conditions cause autoxidation, mealworm gut bacteria produce oxidative enzymes that create radicals necessary for autoxidation, or autoxidation is independent of bacterial processes within the gut microbiota.

Clarification on the role of gut bacteria in EPS degradation will be determined by the comparison of oxidation levels in both normal mealworms and those where the gut biodiversity has been decreased, the latter treated with antibiotics to reduce bacterial activity. EPS residues from both groups will be analyzed using FT-IR to identify plastic autoxidation reaction byproducts such as carbonyls and hydroxyls, as well as alpha and beta ketones. Groups with a higher intensity of autoxidation byproducts reveal the extent to which autoxidation is reliant on that samples' processes. If autoxidation continues in antibiotically-inhibited mealworms, it could indicate autoxidation being an intrinsic process within the host. Alternatively, if oxidation is significantly reduced or absent, it would suggest that bacteria is contrastingly responsible for autoxidation and EPS breakdown.

-

Where will this take place:

A sterile home space with regulated air pressure and temperature. Larvae frass sample and the undigested EPS control sample will be taken to a local lab for FT-IR analysis. Potential spaces include the University of Calgary or Mount Royal University.

https://pmc.ncbi.nlm.nih.gov/articles/PMC11207799/#sec4-polymers-16-01632

https://link.springer.com/article/10.1007/s10924-023-02843-9

EMAIL ARC (character building)

Jan 16:

Draft to Mr. Li:

Opportunity for Lab Use for Science Fair

Hello Mr. Li,

We are two grade 12 students interested in participating in the Calgary Youth Science Fair (CYSF) this year. However, our project requires the use of a FT-IR spectrometer and supervision in an accredited lab to gain access to pharmaceuticals. As such, our project has not

yet received approval by CYSF board members until an accredited lab is able to host our project.

Briefly, our project aims to determine the source of expanded polystyrene oxidation in mealworms by comparing FT-IR spectra of plastic residues in mealworm frass in normal vs. antibiotically-treated larvae.

Studies have demonstrated that mealworms can consume and degrade EPS, the evidence of such being internal autoxidative processes detected via FT-IR spectroscopy. However, the basis of this degradation remains unresolved—specifically, whether oxidation is mediated by methods within the mealworm's digestive system or by its gut microbiota. Three cases have been hypothesized: mealworm gut conditions cause autoxidation, mealworm gut bacteria produce enzymes that create radicals necessary for autoxidation, or autoxidation is independent of bacterial processes within the gut microbiota. We plan to analyze the EPS residues of the larvae using FT-IR spectroscopy, and assess the peak intensity of oxidation byproducts in larvae frass with unmodified, and modified bacterial activity.

Noting this, we have no previous experience or clear understanding of how to use the FT-IR spectrometer. We just know that you can use the technology to identify chemical groups in a sample such as carbonyls and hydroxyls, which are byproducts of plastic oxidation.

Several studies have pointed to antibiotics like ampicillin as a viable method of reducing gut biodiversity in mealworms.

For this project, we would require access to ampicillin (or ampicillin capsules), a lab supervisor, and assistance in using and interpreting the results of the FT-IR spectrometer.

We would deeply appreciate any opportunity from U of C to complete this project.

Thank you so much,

Ziyu Li and Zipeng Li

Draft to mr Lovallo:

Opportunity for Lab Use for Science Fair

Hello Mr. Lovallo,

We are two grade 12 students interested in participating in the Calgary Youth Science Fair (CYSF) this year. However, our project requires the use of a FT-IR spectrometer and supervision in an accredited lab to gain access to pharmaceuticals. As such, our project has not yet received approval by CYSF board members until an accredited lab is able to host our

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Briefly, our project aims to determine the source of expanded polystyrene oxidation in mealworms by comparing FT-IR spectra of plastic residues in mealworm frass in normal vs. antibiotically-treated larvae.

Studies have demonstrated that mealworms can consume and degrade EPS, the evidence of such being internal autoxidative processes detected via FT-IR spectroscopy. However, the basis of this degradation remains unresolved—specifically, whether oxidation is mediated by methods within the mealworm's digestive system or by its gut microbiota. Three cases have been hypothesized: mealworm gut conditions cause autoxidation, mealworm gut bacteria produce enzymes that create radicals necessary for autoxidation, or autoxidation is independent of bacterial processes within the gut microbiota. We plan to analyze the EPS residues of the larvae using FT-IR spectroscopy, and assess the peak intensity of oxidation byproducts in larvae frass with unmodified, and modified bacterial activity.

Noting this, we have no previous experience or clear understanding of how to use the FT-IR spectrometer. We just know that you can use the technology to identify chemical groups in a sample such as carbonyls and hydroxyls, which are byproducts of plastic oxidation.

Several studies have pointed to antibiotics like ampicillin as a viable method of reducing gut biodiversity in mealworms.

For this project, we would require access to ampicillin (or ampicillin capsules), a lab supervisor, and assistance in using and interpreting the results of the FT-IR spectrometer.

We would deeply appreciate any opportunity from MRU to complete this project.

Thank you so much,

Ziyu Li and Zipeng Li

<u>Emails:</u>

Hello Mr. Pohorelic,

We are basing our choice of ampicillin off of previous studies on the impact of antibiotics on the gut microbiomes of insects. Some have shown ciprofloxacin to be most effective in reducing the biodiversity of insects' guts, but its potentially harmful effects in the environment coupled with ampicillin's ease of access is what drove our decision.

Link 1

Link 2

We plan to have 3 trials of 2 x 25 mealworms, one treated with antibiotics and the other not. They will be stored in plastic containers with one portion having no substrate to facilitate easier frass collection. The food will be frozen for at least 48 hours prior to being given, and the mealworms to be treated with ampicillin will have the antibiotic solution mixed into the wheat bran and water gel. The frass will be collected daily over a course of 7 days to be analyzed by FT-IR.

Thank you so much,

Zipeng and Ziyu

—

Hi Mr. Pohorelic,

Yes, we were trying to sanitize the wheat bran with the freezer, but an autoclave does seem to work better and will also moisturize the food potentially allowing for the removal of water gel.

Is it possible that we come into the lab somewhere around mid-February on Mondays and Wednesdays? It is most ideal that we finish collecting the data by early March to meet CYSF deadlines.

To ensure daily observation, the mealworms can be left at our house, with all the required bran being sterilized and antibiotically treated at once. We would require lab use three times: once to add antibiotics to the autoclaved wheat bran, and twice more to analyze the frass samples using the FT-IR spectrometer. Another thing to note is that CYSF requires a lab supervisor while using the lab.

And about the last email, the second link is not working and should be linking here.

When would be possible times to use your teaching lab?

Thank you so much,

Zipeng and Ziyu

Mr. Pohorelic's email:

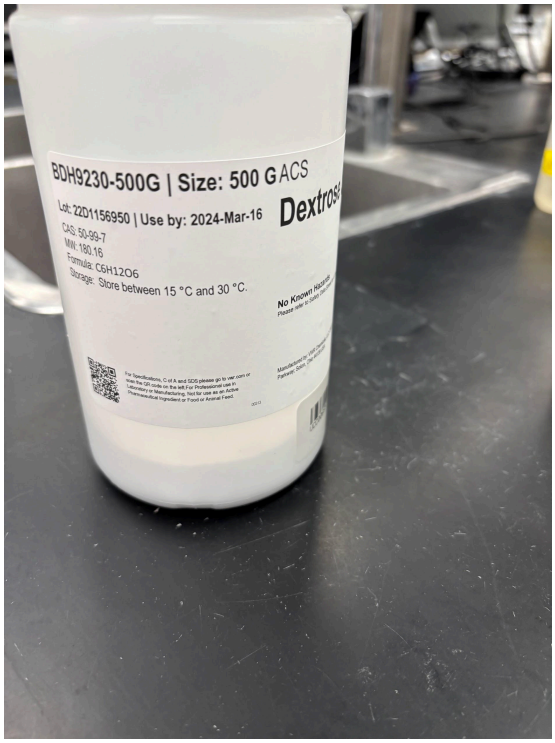
I imagine that the reason CYSF requires a lab supervisor and a lab in the first place is that the use of the antibiotics can only be done in the lab. You would not be able to take the meal worm food that has been treated with antibiotic home. It would have to stay in the lab for disposal afterwards through our waste handling processes. Also, it might take a week or 2 to get the antibiotics ordered and for it to arrive.

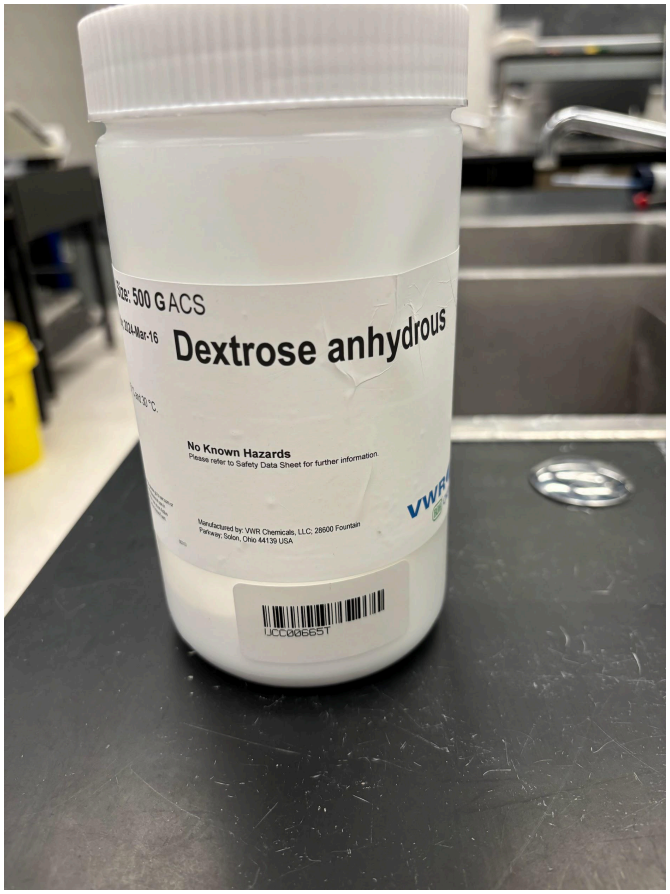
You will also need to arrange the time and Technical assistance with Michelle Forgeron to make use of the FT-IR spectrometer after you collect the samples for testing. That equipment is over in Chemistry in another building. All I can do is help with lab space to make the media, autoclave, grow the worms and supply the antibiotic which would have to remain here. The lab for this work is available 9-5 Mondays and Wednesdays and I can act as the lab supervisor for the work done in my lab.

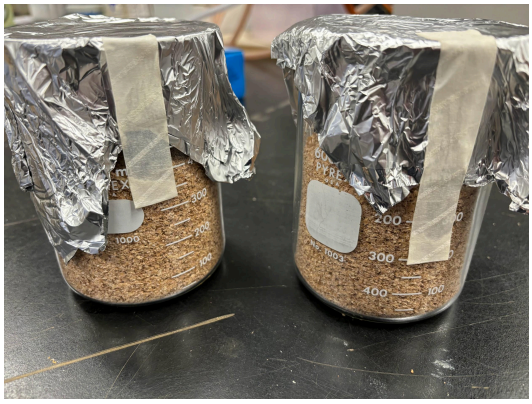
Feb 9, 2025

- Lab and ft-ir secured with u of c, ethics form approved by cysf
- Details, procedure, and materials

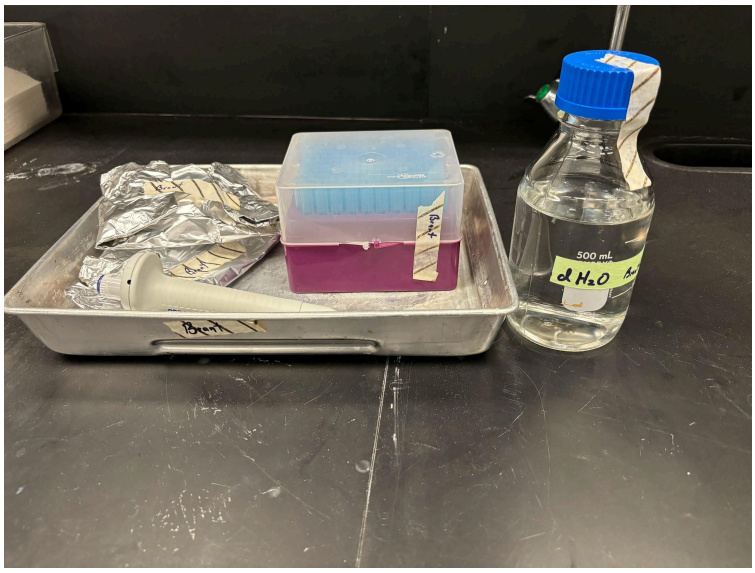
Feb 12:

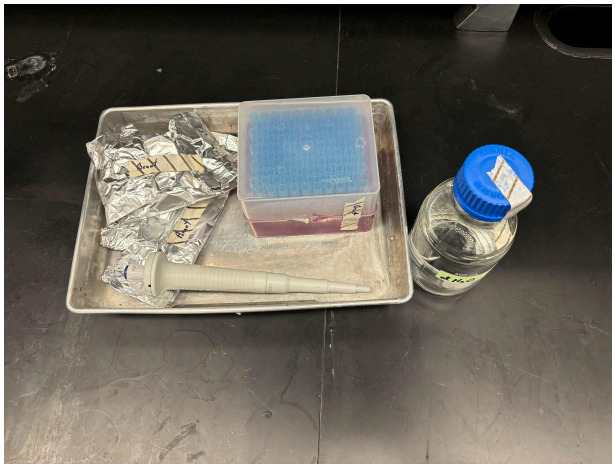
- Bought wheat bran, mealworms, containers
- Select 120 mealworms out of 200 based on physical health using chopsticks
- 

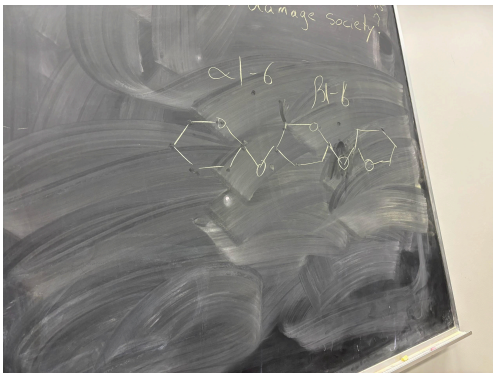

Dextrose anhydrous
No Known Hazards
Please refer to Safety Data Sheet for further information.
Manufactured by: VWR Chemicals, LLC; 28600 Fountain Parkway; Solon, Ohio 44139 USA




STERIS
DO NOT TOUCH!!
AUTOCLAVE
STAFF ONLY!!


500 mL
dH2O




α1-6
β1-6


ROGERS
Wheat Bran
Son de blé
450 g
ALL NATURAL WHEAT BRAN
Very high fibre - No additives
SON DE BLÉ TOUT NATUREL
Très riche en fibres - Sans additifs

Feb 13

- Project start → containers sprayed and wiped with ethanol solution
- Mealworms placed in with wheat bran and water and glucose and (ampicillin)

Feb 18

- Ps blocks cut in morning
- Mealworms transfered — PS, paper towels, water, and (ampicillin) now in containers

Feb 19, feb 20,

- Water topped off

Feb 21

- Mealworms killed, ps disposed, paper towels shaken off, frass trasnfered to tiny little plastic tubes, to be analyzed later

Rest of images linked here:

https://drive.google.com/drive/folders/1-OSZ0s6ZizMHfEnTCp_bl9-rn7OaCDd0?usp=sharing

March 11 — frass samples analyzed on march 10

//
March 18, 2025

- Observations, analysis, graphs

Things that should be seen in the e1-3 ftir scans

- **Reduced hydroperoxide formation**, indicated by a weaker peak at **~3550 cm^{-1}**, suggesting decreased oxidative chain scission. Lower, but slope is on decline to peak at 3250
- **Diminished alcohol and phenol production**, reflected by reduced intensity in the broad **3250 cm^{-1}** region. SEEN VISUALLY CLEARLY
- **Less pronounced α,β-unsaturated ketone formation**, shown by a weaker peak at **~1670 cm^{-1}**, indicating less benzene ring modification. All lower but at different amounts
- **Retention of PS structural integrity**, evidenced by a stronger **1490 cm^{-1} benzene ring stretch**, implying reduced degradation. All lower, but at different levels
- Research
- NEED MEASURE TO QUANTIFY??

- How were these responding variables determined? Where did these numbers come from? How do we know that these make sense
- Review how ftir chart(?) work

→ research first :(

Last week! 17-21

Todo:

- Do logbook
    - Add links, entries
- Citations
    - Into apa format
    - Add other links (video, mann u test)
- Edit SoE and applications
- Add, format, figures (numbers, add more figures)
- Banner picture
- Presentation
    - Script
    - Presentation
    - Recording