Investigating the properties of modified PHB

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Abstract

The issue of pollution is jeopardising our health. Plastics are often dumped in landfills or irresponsibly discarded in waterways (Parker et al, 2019). Biopolymers play a huge role in reducing the reliance on non-degradable plastics. However, there are several physical drawbacks to the use of biopolymers, such as high production cost, thermal instability, and poor mechanical properties, due to secondary crystallisation and slow nucleation rate, which limit its competition with traditional plastics in industrial and biomedical applications. Therefore, this study aims to come up with a cost-efficient and environmentally friendly method to synthesise biopolymers. This study mainly consists of 3 stages. Firstly, a biopolymer, Polyhydroxybutyrate (PHB) was synthesized using *Alcaligenes Eutrophus.* Surface modification was then conducted on the PHB to improve its tensile strength. A tensile strength test was then conducted to determine the new tensile strength of the PHB. The modified PHB was found to have the higher tensile strength.

Introduction

There has been a growing concern about the issue of pollution. Pollution is causing many deaths each year, more than war and all other forms of violence combined. (Fuller et al., 2017). One such pollutant is plastic. Plastic is introduced to the naturally occurring environment as a result of human activities, causing much damage to the environment every year, leading to devastating impacts on wildlife. The far-reaching effects of plastic pollution may even affect humans, as studies have demonstrated plastics' tendency to absorb persistent, bioaccumulative, and toxic substances. The constituents of plastics, as well as the chemicals and metals they absorb, can travel into the bodies of animals such as marine organisms upon consumption where they may concentrate and climb the food chain, ultimately into humans(Seltenrich, 2015). Burning to dispose of plastic may also release toxic gases into the environment which may affect the human respiratory system when ingested, resulting in unimaginable consequences.

Over the years, several techniques have been developed to design biodegradable plastic, and much biodegradable plastic has been synthesized (Lenz & Marchessault, 2005). However, most of these techniques are either expensive, energy-demanding, environmentally unfriendly, thermally unstable, or possess undesirable mechanical properties. This study investigates a method that would be more cost-efficient and more environmentally friendly to synthesize and modify PHB such that it has desirable properties, thus allowing it to replace the non-biodegradable polystyrene.

Objectives and Hypotheses

This study aims to:

1. Investigate the biosynthesis of PHB from *Alcaligenes Eutrophus*
2. Conduct surface modification of extracted PHB
3. Compare the properties of the modified PHB with other non-bio-degradable polymers

This study hypotheses that:

1. *Alcaligenes Eutrophus* is able to produce PHB
2. The modified PHB will have a higher tensile strength than the unmodified PHB and remain biodegradable

Methodology

Preparation of samples and medium (agar) for synthesis of PHB from Alcaligenes Eutrophus

The modified agar was incubated at 30°C for 48 hours. Pure cultures of morphologically distinct colonies were grown in modified agar plates. The constituents of modified agar were: Beef extract (0.3%), Peptone (0.5%), Sodium Chloride (0.8%), Glucose (1%) and Agar(1.5%). 2% glucose, sucrose, and fructose were added into PHB producing media as carbon sources and the selected isolates were grown in it.

Figure 1: Modified agar plates containing bacterial cultures

Preparation of samples and medium (broth) for synthesis of PHB from Alcaligenes Eutrophus

The modified broth was incubated at 30°C for 48 hours. Pure cultures of morphologically distinct colonies were grown in modified broth. The constituents of modified broth were: Beef extract (0.3%), Peptone (0.5%), Sodium Chloride (0.8%), Glucose (1%). 2% glucose, sucrose, and fructose were added into PHB producing media as carbon sources and the selected isolates were grown in it.

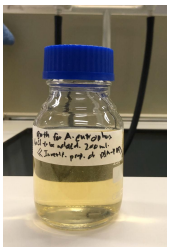


Figure 2: Broth samples post-autoclaving

Extraction of PHB from Alcaligenes Eutrophus

After 48 h incubation at 37°C, 10 mL of culture was taken and centrifuged at 9500 rpm for 15 min. The supernatant was discarded and the pellet was treated with 10 mL of 20% sodium hypochlorite (Loba) and the mixture was incubated at 30°C for 2 h. After incubation, the mixture was centrifuged at 5000 rpm for 15 min and left to evaporate to dryness to extract the PHB and discard the supernatant.

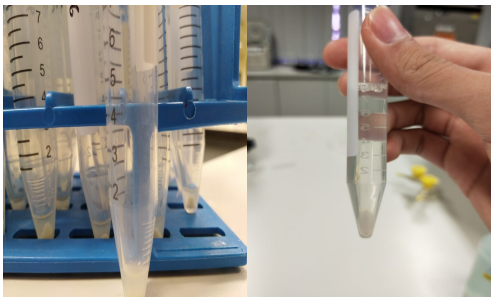


Figure 3: Broth samples post-centrifuging

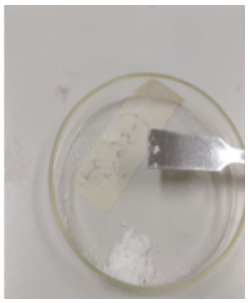


Figure 4: Dried PHB

Sudan staining of the extracted PHB from Alcaligenes Eutrophus

Detection for PHB production was employed by using lipophilic stain Sudan Black B. Stain was prepared by dissolution of 0.3 gm powdered stain in 100 ml of 70% ethanol. For microscopic studies, smears of colonies were heat-fixed on clean, grease-free glass slides, followed by staining with 0.3% solution of the Sudan Black B. After leaving the slides undisturbed for 15 minutes, immersion in xylene and counterstaining with safranin (5% w/v in sterile distilled water) was performed. Cells appearing blue-black under microscope were accredited as PHB positive strains.

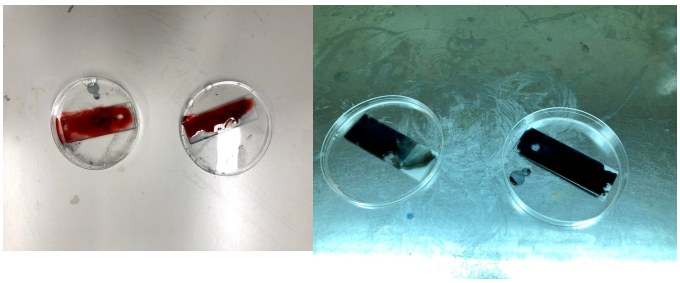


Figure 5: Broth samples stained with Sudan Black

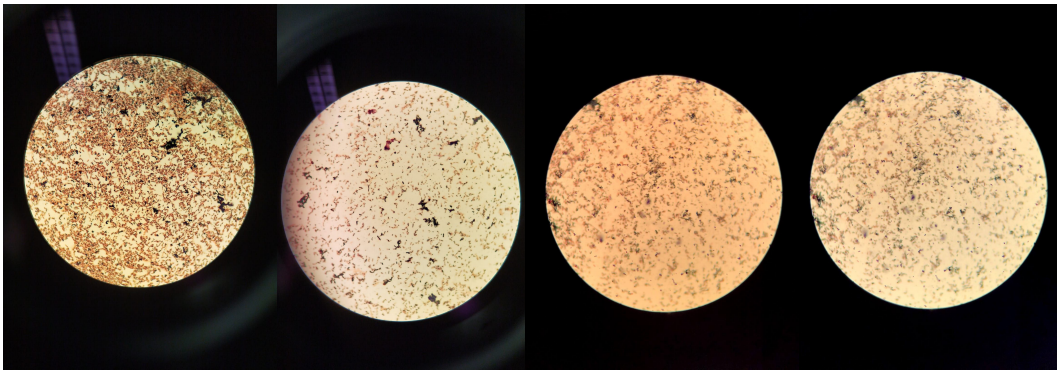


Figure 6: Microscopy of a sample stained with Sudan Black

Modification of the extracted PHB

The PHB pellets were mixed with acetic acid and heated to boiling in a covered beaker under constant stirring until the sample was dissolved entirely (typically ~40 to 60 minutes). A polymer solution with a concentration of 0.05 g/ml of PHB in acetic acid was used to prepare all

test films. Approximately 4.5–5 ml of polymer solution – previously brought to the required casting temperature – was poured on a pre-heated glass slide (70 mm X 35 mm) maintained at the required casting temperature (80 °C). Films were obtained after complete evaporation of the solvent (Fig. 12c). Samples were dried for 6 minutes to ensure complete removal of solvent. The prepared film samples were stored at room temperature for 24 hours prior to characterization.

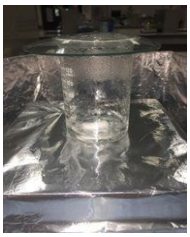


Figure 7: Modification of PHB by boiling with acetic acid

Figure 8: Casted PHB after drying for 24h

Synthesis of PLA Method 1

Test-tubes were filled 1/5 full with lactic acid. 5 drops of hydrochloric acid and two anti-bumping granules were then added. Test-test holders were put around the top of the test-tube and the tube was heated. The mixture was allowed to gently boil and the tube was stirred to mix the contents.

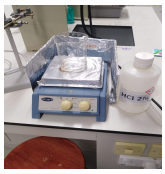


Figure 9: Mixture of lactic acid and hydrochloric acid

Synthesis of PLA Method 2

Lactic acid was dehydrated to produce oligomers at temperatures of 160 °C for 2 hours. The product gas was collected by distillation and then washed with cold water, separated by filtration and dried overnight. It was then mixed with Stannous octoate (Sn(Cl)2) at 140 °C.

Figure 10: Dehydration of lactic acid

Figure 11: Dried sample after mixing with $SnCl_2$

Results and Discussion

Failures and modifications

The extraction of bacterial samples required the inoculation loop to be heated until red-hot, which runs the risk of causing thermal decomposition of the PHB produced. Agar samples that were reused over time also promoted less bacterial growth as compared to fresh broth cultures. These resulted in a lower mass yield percentage of PHB at the end of the experiment. Therefore, it was concluded that agar was a less efficient method of growing PHB as compared to broth, and the setup was modified to accommodate broth cultures instead.

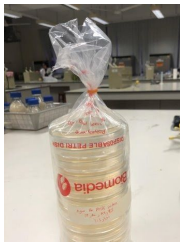


Figure 12: Modified agar plate samples

While synthesizing PLA, it was expected that the PLA mixture would yield a yellowish membrane after about 10 or 15 minutes due to the reaction between lactic acid and hydrochloric acid. However, the product failed to solidify and remained clear and colourless. It was concluded that the concentration of lactic acid was too low to facilitate a reaction. The concentration of the lactic acid was thus increased from 0.5 M to 1M. However, the modified setup failed to yield a visible reaction. It was then concluded that the underlying issue was that such a reaction was too slow and thus inefficient and impractical to carry out.

Figure 13: Mixture of 1.0M of lactic acid with hydrochloric acid

A new method was thus selected for the synthesis of PLA. However, the acid thermally oxidized in oxygen while heated as the experiment was not conducted in an inert atmosphere. Due to safety reasons, the laboratory could not allow for the use of N2 gas during heating.

Figure 13: PLA sample during heating

As such, bacterial synthesis of PLA via E. Coli with lactic acid as the monomer was considered. However, due to the circuit breaker, no further experiments involving bacterial synthesis could be conducted. The project was thus modified to instead focus on the modification of bacteria-synthesised PHB to meet the time constraints.

Synthesis of PHB - FTIR

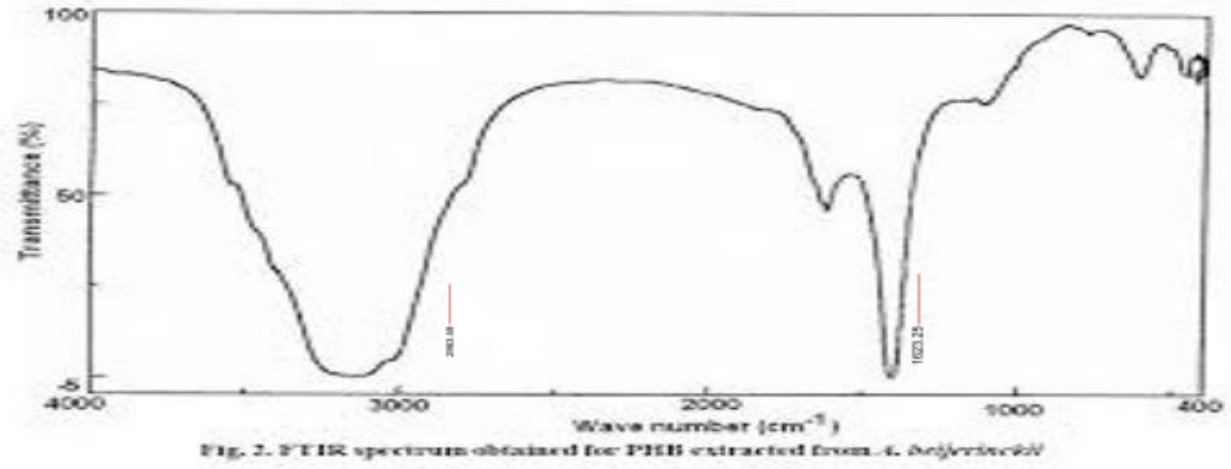
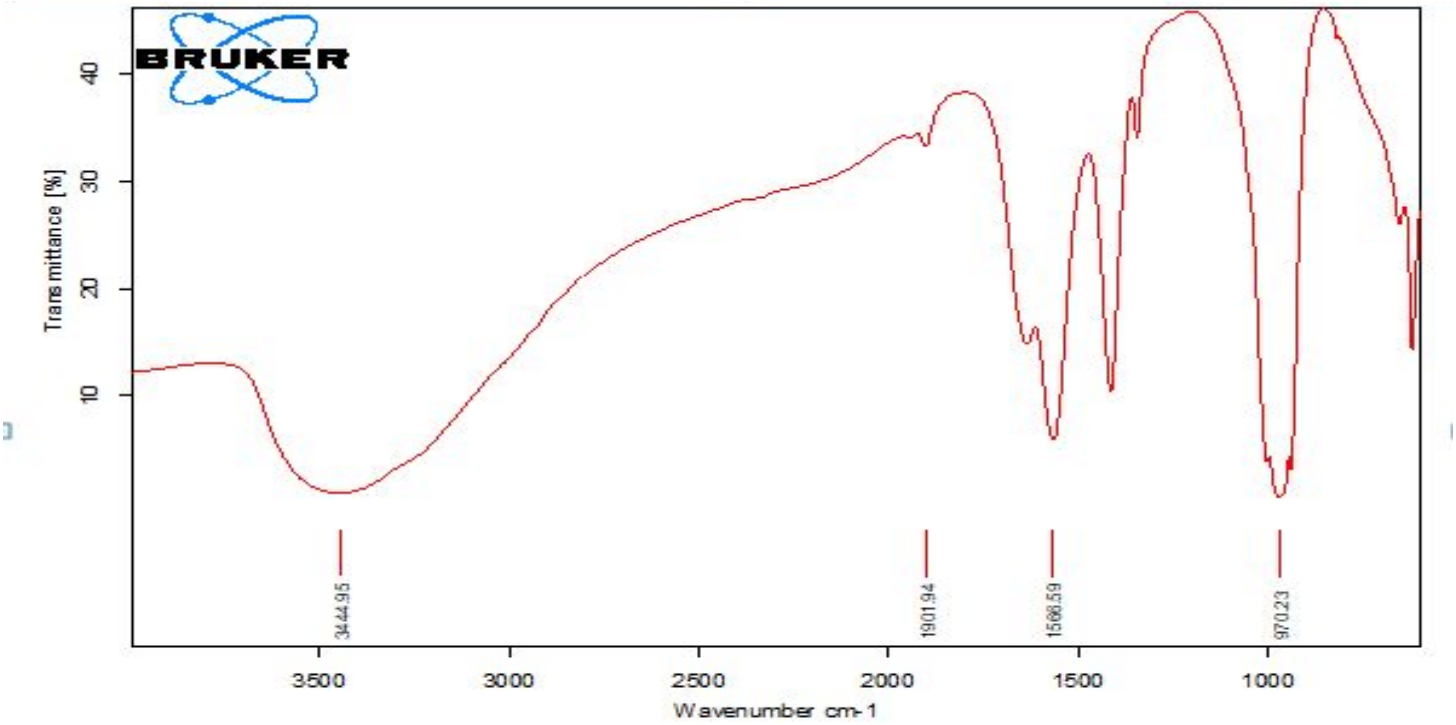


Figure 13: FTIR spectrum graph for sample PHB



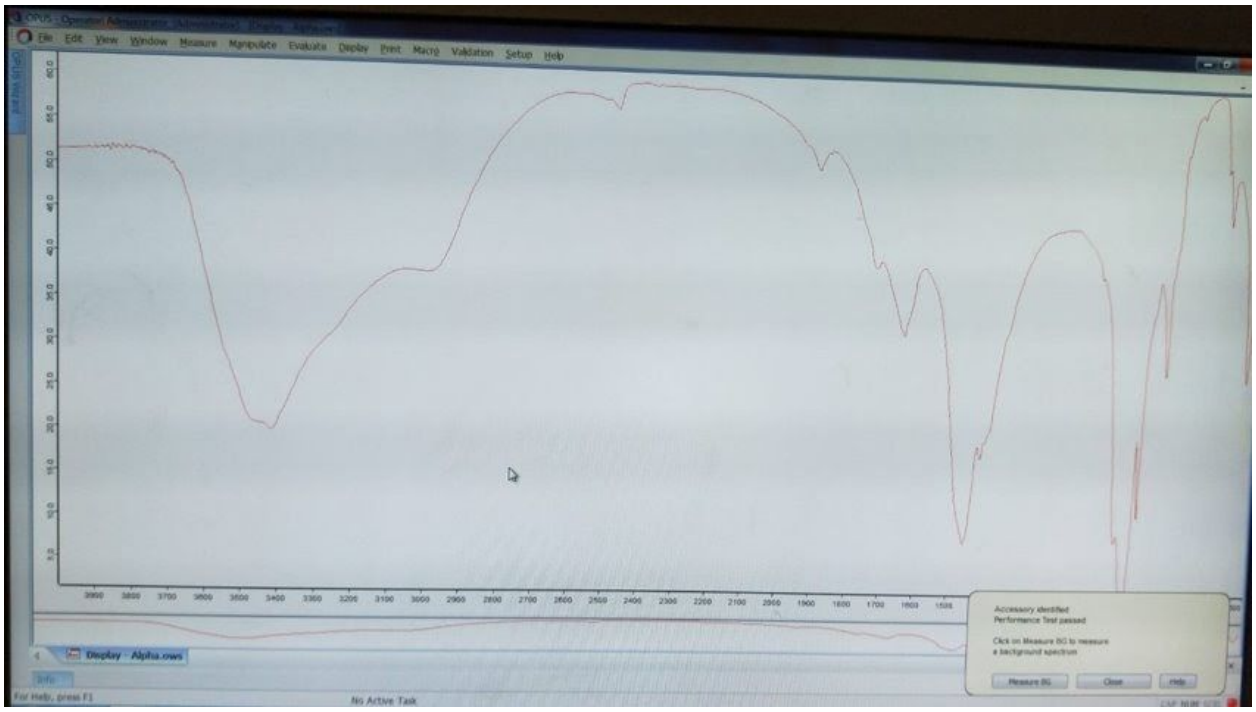


Figure 15: FTIR spectrum graph for modified PHB

The Fourier-transform infrared spectroscopy (FTIR) spectra of PHB from bacteria in Figure 15 was compared against that of modified PHB in Figure 16. In Figure 15, at point 3432.5, there is indication of O-H stretching. At point 2563.5, there is a slight peak indicating C-H stretching. At point 1623.25, there is a peak indicating C=O stretch. With the graph for obtained PHB similar to the expected FTIR graph for PHB, this study has successfully extracted PHB through bacterial synthesis. On the other hand, in Figure 16, at point 1723.64, there is a peak indicating C=O stretching. Due to poor FTIR transmission, there are a few missing speculated peaks in the diagram. However, lack of a peak that is not displayed in the graphs in Figures 15 and 16 show that the modification does not involve chemical modification and instead relies on physical modification.

Mass yield of synthesized PHB

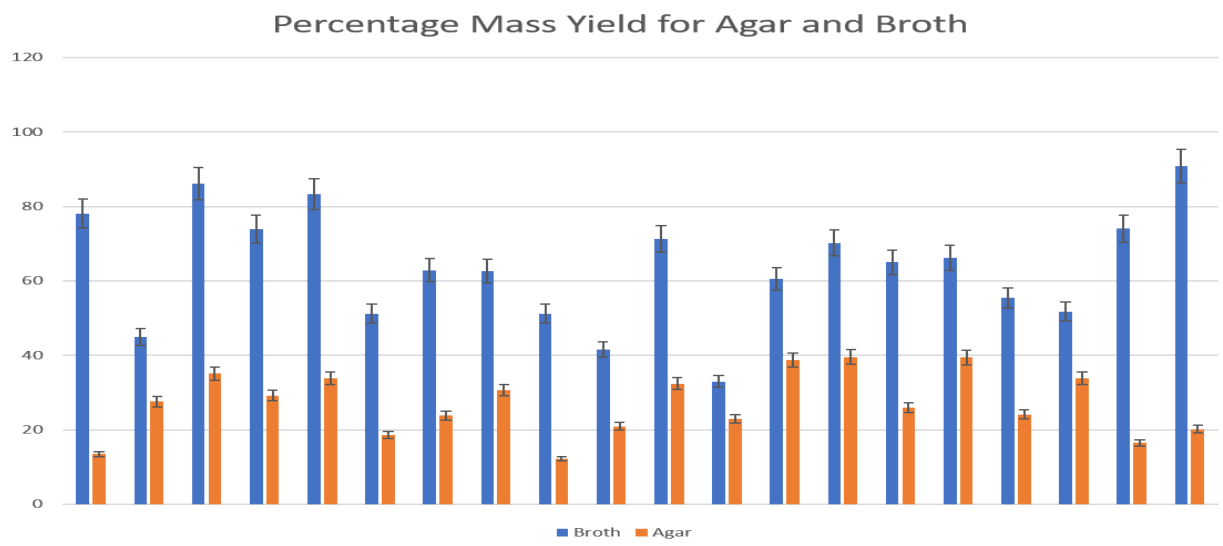


Figure 16: Mass yield percentages for bacteria grown in agar and broth media

Figure 16 shows the percentage mass yield for agar and broth prepared cultures. In the chart, the average mass yield for PHB extracted from agar samples was 26.95% while the average mass yield for PHB extracted from a broth medium was 63.7%. It can thus be concluded that there is a higher average mass yield for arterial samples grown in a broth medium rather than an agar medium.

Tensile Strength of PHB

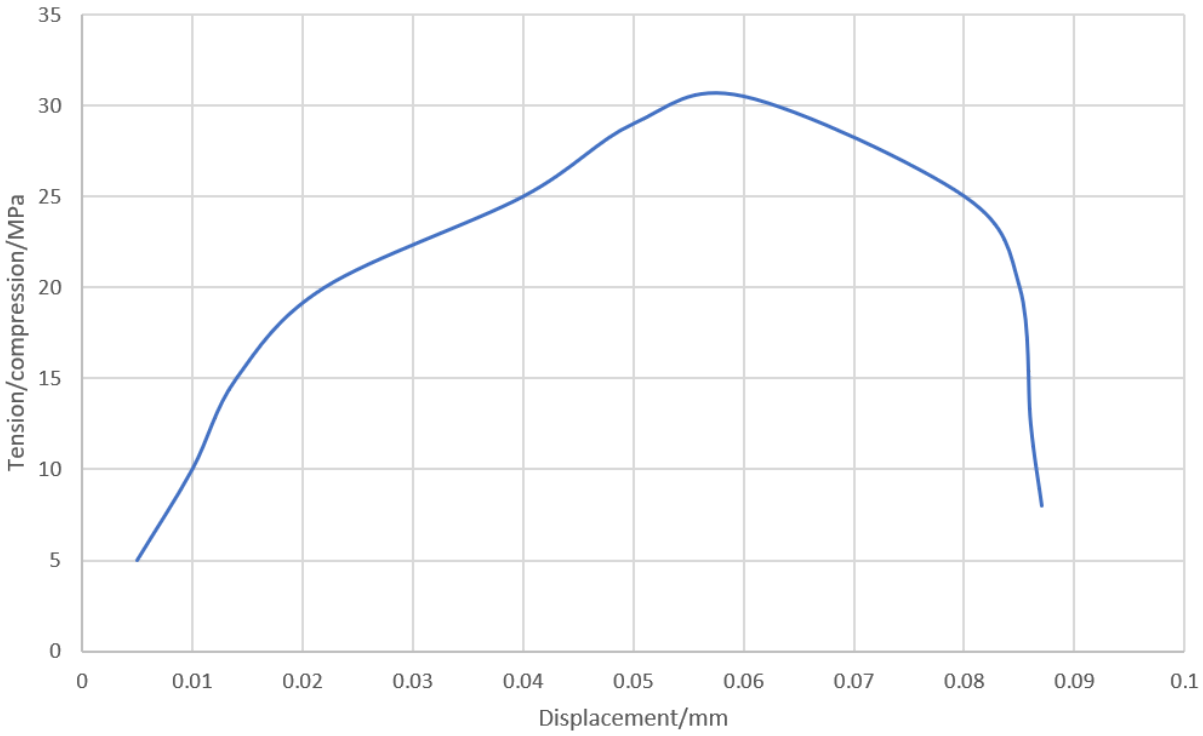


Figure 17: Tensile Strength of Modified PHB

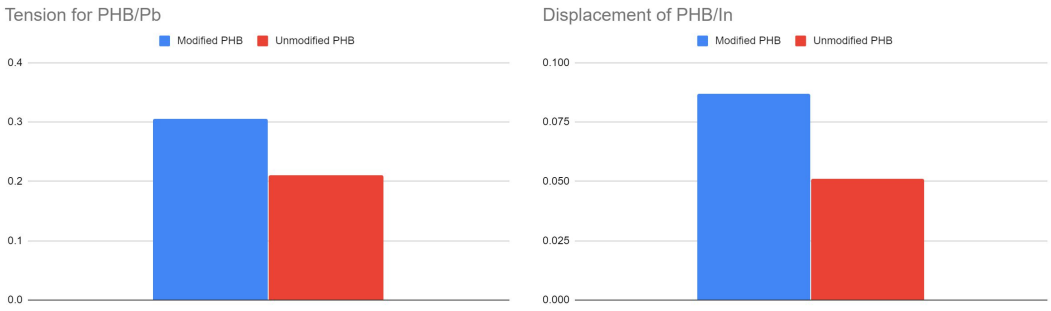


Figure 18: Tension and Displacement values for PHB

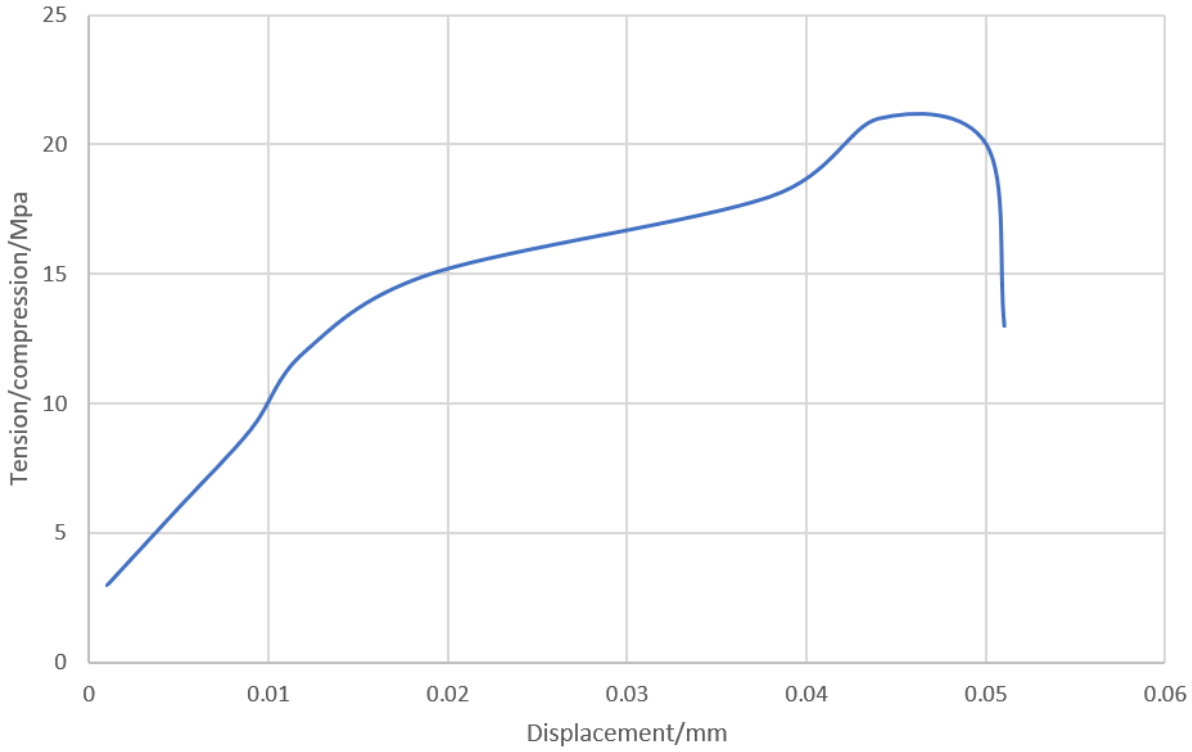


Figure 19: Tensile Strength of non-Modified PHB

As seen in Figure 18, there are higher values for tension and displacement for the modified PHB as compared to the unmodified PHB, with the modified PHB having a maximum tension value of 0.305Pb as compared to a value of 0.21Pb for the unmodified PHb, as well as a maximum displacement value of 0.087 In for the modified PHB as compared to 0.0501In for that of the unmodified PHB. It can thus be concluded that the PHB that underwent modification has increased tensile strength.

Conclusion

The PHB that was treated with acetic acid was found to have improved properties over the unmodified sample. Future experiments could be done to further determine the properties of the modified PHB formed, such as thermal conductivity and ductility and identify if the modification process comprises other physical properties. Also, the temperature for modification of PHB and

the acetic acid to PHB ratio should be increased further for increased effectiveness in modification of PHB.

References

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