Biodegradable plastic from fish scales (*Epinephelus coioides*) using fermented bacteria (*Staphylococcus aureus*)

Abstract

A large amount of the domestic and industrial waste produced by humans ends up in the environment and the oceans. The manufacturing of plastic has increased because of the world population growth-related rapid developments in urbanization and industrialization. Living creatures and the environment are both at risk from this garbage (fish byproducts & plastics). Plastic takes hundreds of years to break down, plastic fragments can persist in the earth and oceans, which can threat the life. On the other hand, waste management problems have arisen because of fish processing industry which are producing enormous volumes of byproducts (guts, bones, scales, heads, and fins). Because of fish scales which are more flexible, light- weight, and comparatively clear than other materials, they can be utilized to create bioplastics. In this Project, fish scales were fermented by *Staphylococcus* bacteria to make bioplastic, which has several beneficial characteristics including heat tolerance, smooth texture, transparency, soft touch, high tensile strength, and can be degradable in two days. In conclusion, the production of bioplastic using fermented bacteria is extremely economical, environmentally benign, and sustainable, all of which align with world Vision.

Introduction

The increasing demand for fish products (guts, bones, skin, heads, and fins) has resulted in massive production volumes from the fish processing sector. Almost sixty percent of the weight is lost during the canning process; this weight is usually gathered and dumped in landfills or waterways. Fish wastes were used to produce bioplastics through several research, and these have since supplanted plastic. Chitin and chitosan, which are found in fish waste, are essential to produce bioplastics.

To obtain raw materials that are renewable and biodegradable, it is therefore possible to adequately manage the massive waste that is generated during the processing of seafood. The ability of fish proteins to form networks, as well as their flexibility, elasticity, and strong oxygen barrier, are critical properties that facilitate the formation of films. It has been more popular recently to create biodegradable plastics from natural polymers including proteins, starch, and cellulose (Pablo, et al., 2008) (Shirai et al., 2013).

In 2019, Maria and her colleagues succeeded in creating a homogenous, thin, and transparent biopolymer film using the myofibrillar and gelatin proteins found in king weakfish. It is also elastic and flexible. Moreover, bioplastic made from fish scales was attempted and produced by Arunagiri et al. in 2021; it degraded in two weeks. It can be used as a wrapper for candies.

Additionally, fish scales were used by Varna et al., 2019 to produce bioplastic. Also, the product that was formed as bioplastic from fish scales was by Kabeer et al., 2021 is strong, flexible and translucent. It gets biodegrades within four to six weeks and it does not require industrial composters to break it down. To reduce pollution and increase environmental safety, fish waste is utilized to make bioplastics. According to Magera et al. (2013) and other researcher, compostable plastic is biodegradable and derived from bio-based sources.

Prior research employed fish scales to synthesize bioplastic by chemical means; although not entirely organic and natural, this bioplastic can still be classified as bioplastic. Among all the studies we conducted, there was one where "fermentation" was used, but no information was provided regarding the bacteria or the procedure they employed for the bacterium, or even the media or chemicals used.

Kasmuri in 2018 stated that the bioplastic from eggshells, which was used by the Julfa student company at the University of Technology and Applied Sciences, took a maximum of two weeks to produce by the use of chemicals, however, the texture of the raw plastic was rough and the eggshell texture was still present.

In this research, it was possible to successfully produce bioplastics with all the required qualities using fish scales by fermented bacteria. It was found that these components can be recycled to create bioplastics. In order to save the environment in the modern world, it was necessary to identify alternative solutions that are suitable, safe, workable and sustainable. The bioplastic from this study broke down within two days and had a smooth texture, very strong, elastic, soft and flexible. Fish scales were selected over shrimp scales, because the shrimps are seasonal and thus not always available, however, fish scales are available around the year and everywhere (Siddeek, et al., 1999). Using fermented bacteria in such research, is a sustainable way and environmentally friendly alternative to traditional petroleum-based plastics. It reduces the reliance on fossil fuels or chemicals and results in products that are biodegradable, which reduces the impact on the environment. Also, the product was soft and smooth in texture compared to bioplastic without bacteria.

In previous research used eggshells & prawn shells, which were not used by us, since eggshells showed in the texture of the product, and for the prawns is not always available, since prawns availability is seasonal.

Material & Method

In preparation of fish scales

Fish scales were soaked in pure Acetic Acid (vinegar) for 2 days. This process was added to get rid of the fish smell which was not available in any of the previous works.

In bioplastic production

Equal amounts of socked fish scales were distributed in 6 agar plates which contain *Staphylococcus aureus* bacteria and placed in an anaerobic jar in an incubator at a temperature of 37°C for 3 months. This bacterium and the process was not mentioned in any of the papers that we came through. Using *Staphylococcus* bacteria for fermentation of fish scales was available in the lab. The duration depends on the amount of the fish scales used. Here we used 200g of fish scales, which took approximately 3 months to be fermented.

Furthermore, the mixture was spread (thin/ thick layers-depend on the use of that plastic) on a mold allowed to dry at room temperature for 48 hours. This mold was designed by us in the lab.

Moreover, the obtained bioplastic can be degraded within 2 days. Unlike other bioplastic products in other research papers which take from one till six months (Cheung & Not, 2024).

In this project, we used fermented fish scales by *Staphylococcus aureus* bacteria to produce biodegradable plastic.

Preparation of pure culture of *Staphylococcus aureus*

A bacterial colony was taken and inoculated in Nutrient broth. The inoculum was kept in an incubator for 24 hours at a temperature of 37°C.

Preparation of fish scales

Fish scales were initially purchased straight from markets. To remove the impurities, the scales were washed and cleaned by distilling water. Grams of fish scales were weighed. After that, the mixture was soaked in pure Acetic Acid (vinegar) for few days to remove the undesired smell of the fish. The scales were dried in a hot air oven for 24 hours. Then it was grinded by the grinder till it became small pieces. NaOH was used in soaking the grinded fish scales for few days.

Bioplastic production

Equal amounts of socked fish scales were distributed in agar plates which contain *Staphylococcus aureus* bacteria and placed in an anaerobic jar in an incubator at a temperature of 37°C for 3 months. After fermentation, the fermented fish scales were mixed with distilled water and boiled with the addition of gelatin, glycerol as a plasticizer, and Corn starch with algae oil (coloring agent). The mixture was spread (thin/ thick layers-depend on the use of that plastic) on a mold allowed to dry at room temperature for 48 hours.

Physical tests:

- **Microbiology test:** The product after fermentation was tested for the availability of bacteria, and it showed clean empty agar plate.
- **Solubility:** The product was tested by dissolving it in distilled water for one day at room temperature (25 °C), and it was soluble.
- **High Tensile strength***:* The product was tested, and the strength was 32.4 Mpa.
- **Quick degradation in soil:** The bioplastic product was tested by burying small piece 2x2 cm^2 in soil for 2 days. Surprisingly, the product was able to be degraded in less than 48 hours.
- **Combustion:** The product was tested by burning a small piece (2x2 cm^2) of bioplastic by using a matchstick, and it was not showing any combustion.
- **pH measurement:** The product was tested by dissolving small piece in the distilled water and then measured by the pH parameter. It was almost Neutral (6.8).
- **Effect of heat:** The bioplastic product was tested by being placed in difference places; exposed to sunlight for 2 days and placed it in oven at different temperature (100 °C, 450 °C and 600 °C). The product started to be affected slightly at 450°C, and more at 600°C.

This product can be used in different area:

- As computer mouse pad.
- As Butter paper for backing purposes
- As candy wrapper
- As A4 size file and as A4 size grocery bags
- As compost holder for agricultural purposes.

It can be used as drink cups, but not advisable, because it is soluble. Although it is soluble within 24 hours.

Discussion

Fish scales were selected over shrimp scales because ethe last are seasonal and thus not always available, however, fish scales are available around the year and everywhere (Siddeek, *et al*., 1999). The bioplastic from eggshells, which was used by the Julfa student company at the University of Technology and Applied Sciences, Muscat, Sultanate of Oman took a maximum of two weeks to produce using chemicals, and it took the same when the chemicals were used in fish scales. But, with the fermented bacteria, it took approximately 3 months. It was noticed from the texture and color of fermented fish scales bioplastic was white, smooth and thin film compared to the non-fermented fish scales bioplastic, which was tough, white and thick film. Also, fermented fish scales bioplastic was free almost from scale texture but, the non-fermented fish scales bioplastic has scale texture. That was because bacteria used fish scales, which caused the scales to deteriorate and make rubber look. On the other hand, the eggshell bioplastic was white light, almost rough and the eggshell texture was still present (Kasmuri, 2018). However, in shrimp shell bioplastic film the structure was mainly random sheets with brown smooth surfaces (J V Setiawan et al., 2022).

The water solubility of bioplastic films was entirely dependent on polymer structure and intermolecular bonds (Kaewprachu and Rawdkuen, 2014). Glycerol is hydrophilic in nature and is used as a plasticizer to ensure bioplastic solubility. In addition, its hydrophilic nature increases the mobility of the peptide chains and contributes to solubility (Ekrami and Emam-Djomeh, 2014).

The determination of bioplastic thickness is important as well to estimate the barrier properties of a packaging system(Durai *et al* .,2008). The observed thicknesses of the bioplastic sheets obtained was less than 0.1cm but the unfermented bioplastic was thicker than fermented bioplastic.

The Tensile strength test determined the quality of bioplastics (Dasumiati et al., 2019) in this project both (fermented, and unfermented) were high tensile strength about 32.4 Mpa. Bioplastics should have sufficient tensile strength to ensure their integrity when used as packaging materials(Harnkarnsujarit et al., 2021).

The biodegradability of plastic in the soil was determined using a biodegradability test. After two days, only a few crumbs remained and proved the biodegradation of the film. Another bioplastic made from shrimp scales degraded in 2 weeks (Ismail *et al.,* 2023) was due to the properties of fish scales, which contain biodegradable compounds such as glycerin, gelatin, and starch which have chemical properties that make them biodegradable. For example, glycerin is an alcohol that can be degraded by microorganisms in the environment. Gelatin and starch are natural polymers that can be hydrolyzed by enzymes found in living organisms (Patwary *et al*., 2020) .

To observe the effect of heat on the bioplastic, two methods were used: The first method was: microwave, at 100°C both fermented, non-fermented were not effect, at 450°c: Both fermented, non-fermented were started to melt, at 600°C: Both fermented, non-fermented were combust. The second method was to expose the bioplastic to sunlight outside the lab, the Fermented bioplastic was not affected, while the non-fermented bioplastic was affected in texture (tough). On the other hand, other bioplastic made from fish wastes used TGA (Thermogravimetric analysis) to determine the nature and thermal stability of bioplastic components. TGA curves of the bioplastic films showed thermal stability in the range of 250°C–330°C (Surya *et al.,* 2021). This indicates that the temperature above 300°C bioplastic has been exhausted when heated.

Conclusion

Plastic is a product that is widely used in our daily lives due to its affordability and practicality. However, because plastic is non-renewable and difficult to degrade, it poses a hazard to the environment. Fish scales were successfully used to create bioplastics with all the desired properties. It was discovered that the bioplastics made from these materials were recycled. The bioplastic from this project was degraded in 2 days, and had a smooth texture, soluble, strong, and flexible, to protect the environment nowadays, it was a must to find other options that are appropriate, safe, practical, and affordable. Therefore, there should be a greater demand for production of bioplastics to ensure a bright and sustainable future.

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