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Cost-Effective Bioconversion of Banana Peel and Paper Pulp Residues into Polyhydroxybutyrate (PHB) Using *Bacillus Thuringiensis*

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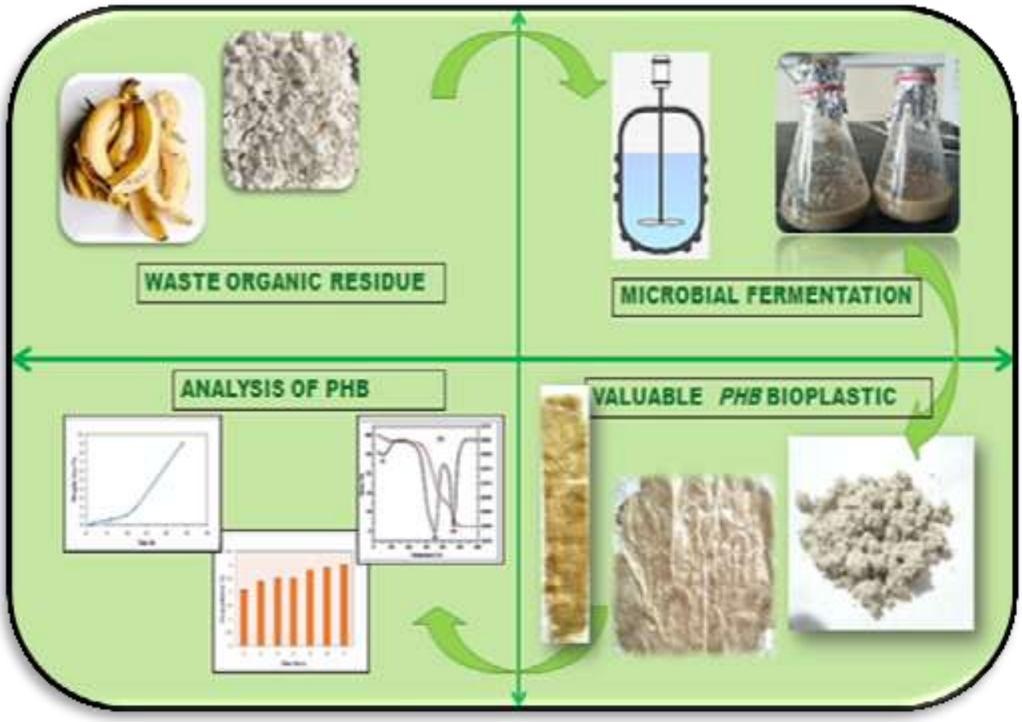
Abstract

Plastic pollution is a complex and challenging problem in the whole world. Accumulation of synthetic polymer is to becoming a major global concern due to its less biodegradation property. Bio-based polymer is a great alternative to synthetic polymer. PHB Bioplastic is plastic derived from organic waste obtained through household, industrial, and agricultural waste microbiological method. The present study involves the production a bio-degradable polymer viz. polyhydroxyburyate (PHB) by using *Bacillus thuringiensis*. Based on physical and mechanical investigations, this prepared PHB Bioplastic was identified to be of high tensile strength, normal elongation and slightly hard as related to that of starch based plastics. The permeability, solubility and biodegradability test of the prepared PHB Bioplastic from *Bacillus thuringiensis* was within the range of that of other starch bioplastics. All the properties of the PHB biopolymer composites produced by the waste banana peel and paper pulp residues were characterized and analysed.

Keywords: Biopolymer, Polyhydroxtbutryate (PHB), Agro-Industrial waste, Bacillus thuringiensis, Biodegradable Plastic.

Graphical Abstract



Introduction

Plastic pollution refers to the gathering of plastic waste in the environment, specifically in oceans, rivers, landfills, and other natural habitats. [1] It is a significant global environmental concern that poses detrimental to the ecosystem, wildlife, and human health. [2] Plastic pollution happens when plastic materials such as bottles, bags, straws, and packaging, are erroneously disposed of or not recycled. [3] As plastic does not dissolve, it remains in the water thereby hampering its purity. [4] An apple core thrown into the dirt can be consumed by a worm, which then excretes nutritious waste for new plants to grow on. [5] However, man-made items such as plastics, Styrofoam, rubber, and aluminum are defying this natural cycle that allows growth in our ecosystem. [6] While an apple can be recycled into new material within two months, a plastic bottle can take more than 400 years to decompose. Plastic waste chokes the normal cycles of our ecosystem, and it is critical to find ways to flush the durable material out without harming the environment even more. [5]

Meanwhile, there have been new ideas to help clean the large plastic soup either by reducing carbon dioxide release or using less material or energy to degrade plastic. Bio plastic is the best alternative to synthetic plastic which is made from fossil fuel and takes around 400 thousand years to degrade. [7] Bioplastics are defined as materials that are bio-based, biodegradable, or both; they can provide excellent biodegradability and can be used to help alleviate environmental problems [8] Various industries have shifted from non-biodegradable polymers to degradable polymers [9] Bio plastic has many advantages because it's cheaper, environmentally friendly, Biodegradable, Heat resistance, durable and Compatible.

Polyhydroxybutyrate (PHB) is a biodegradable polyester that is produced by certain bacteria as a form of energy storage and It is considered to be a promising alternative to traditional plastics because it is both biodegradable and biocompatible, meaning it can be broken down by natural processes and is not harmful to living organisms. [10] PHB polyhydroxybutryate and PHA polydroxyalkonate are both biodegradable polymers that are derived from renewable resources. Certain factors make PHB polymer stand out as a better option in certain applications. PHB has great mechanical properties, tensile strength, and more rigid structure, heat resistant to heat and allows it to maintain its structural integrity at higher temperatures, easier to process compared to PHA. [11]Ralstonia eutropha (also known as Cupriavidus necator), Alcaligenes spp., Azotobacter spp., Bacillus spp., Nocardia spp., Pseudomonas spp., and Rhizobium spp., with Ralstonia eutropha being the most extensively studied. [12] Banana peel, potato peel, and paper pulp are good sources for making bioplastics because they are waste products that are often discarded. By using this renewable polymer can reduce the amount of waste that ends up in landfills and reduce our dependence on fossil fuels. Overall, bioplastic made from biowaste is an innovative solution that has the potential to help us transition to a more sustainable and environmentally friendly future. [13]

Bacillus thuringiensis is a gram-positive, soil-dwelling organism, biological pesticide, spore-forming bacterium that produces insecticidal protein knows as delta-endotoxins or BT-toxin. [14]

Therefore, the aim of the present study was to produce Polyhydroxybutyrate (PHB) by a bacterial strain with a concentrate on its production from some low-cost agricultural wastes (fruit and vegetable peels).

1. Material And Methods

1.1. Inoculum development:

The bacterial Strain used during this was prior isolated and characterized from the *Bacillus thuringiensis* pesticide. It was preserved at low temperatures under standard conditions and was revived previously in this study. The pure colony of the culture was used throughout the study. The strain of *Bacillus thuringiensis* is cultured in a small volume of the nutrient broth medium to develop a healthy and active inoculum. This process helps to confirm an adequate quantity of active cells for the fermentation process.

1.2. Preparation of organic waste raw material

Bananas and potatoes were purchased from local market at Kamothe, Navi Mumbai, India. Further banana peels were removed and dipped in the sodium bicarbonate solution for 30 min prevention the browning of peel and stop the growth of microbial species. After 30 minutes, the banana peel was washed with ethanol and distilled water and squashed using a mortar and pestle until a uniform paste was obtained. The potato peel was removed with the help of a knife, and washed with ethanol and distilled water. After washing these peels are placed in a hot air oven at 50℃ for three days. Also, waste paper was collected from the scrap store and immersed in distilled water, and squashed using a sterile glass rod until it forms semi-solid consistency.

1.3.Extraction of Polyhydroxybutyrate (PHB)

Production of PHB polymer was performed with a microbial fermentation process. *Bacillus Thuringiensis* was cultured in nutrient broth supplemented with a mixture of banana peel, potato peel, and paper pulp as carbon sources for 7 days at 37℃ at 150 rpm in a rotary shaker. The culture broth with bio-waste sources was centrifuged at 5000 rpm for 15 minutes. The supernatant was discarded and the pellet was washed with acetone and methanol.

After centrifugation, the mixture of the sample with addition of turmeric, resin and moringa powder was poured on either silicon or silver foil paper which was placed in an oven at 130℃ for drying. Once the mixture is completely dried, scrap the powder from the silver foil paper and make a powder with mortar and pestle.

1.4.Formulation of PHB Bioplastic sheets

Sample PHB Bioplastic film was prepared by dissolving 5 grams of dried PHB powder in 5 ml distilled water, 5 ml plasticizer (glycerol), 5 ml vinegar, and 1 ml lanolin. This formulated mixture was poured onto a silver foil paper and was dried in a hot air oven. The biofilm layer was scraped off from the surface of silver foil.

1.5. Characterization of PHB Bioplastic composites

1.5.1. Physical properties

The density of composites was measured by calibrated pycnometer and distilled water was used as the immersing liquid with three repetitions. The sample was weighed before putting into pycnometer that contains liquid. After that, the sample in the pycnometer was weighed (m) and the density (ρ) was calculated by the equation: $\rho = m/V$

1.5.2. Thermal properties

DSC analysis coupled with TGA was performed in TA-Instrument for determination of T_g and T_m. The weight of the specimen was in the range of 10–25mg and the temperature increased by 5 °C per minute starting from 30 °C up to 600 °C.

1.5.3. Mechanical properties

Tensile tests were performed using the CMT-10 Computer Control Electronic Universal Testing Machine. Tensile strength (TS) and elongation were determined from the stress-strain curves, estimated from force-distance data obtained for the different and a strain rate of 2 mm/min at room temperature. Also the durability of PHB sheet was confirmed by puncture test by adding weights gradually until it reaches its breaking point. All mechanical testing of PHB Bioplastic sample was conditional according to the standard method of ASTM-D638-14.

1.5.4. Permeability and solubility tests

The permeability tests performed by micro-perforated films using the Dynamic Accumulation method enable the measuring of the Oxygen Transmission Rate (OTR) and the Water Vapour Transmission Rate (WVTR) to determine the shelf life of PHB bioplastic sample.

PHB bioplastic sample was immersed in water for 2 days to check solubility % and water absorption % for 60 min. Water absorption (Ab) was calculated as follows:

$$Ab = \frac{(W1 - W0 + Wsol)}{W0} \times 100$$

Where, *W1* is weight of PHB sample containing water, *W0* is weight of dried PHB sample; *Wsol* is weight of water soluble residuals.

1.5.5. Soil burial biodegradation test

PHB sheet sample was incorporated in soil for soil biodegradation study for 7 days. Degradation percentage of PHB sheet sample was checked for 1 week with 24 h time interval.

$$Biodegradability\,\% = \frac{(Mi - Mf)}{Mi} \times 100$$

Where, *Mi* is the initial mass of PHB sample, *Mf* is final mass of PHB sample after time interval.

2. Result

2.1. Extraction of Polyhydroxybutyrate (PHB)

The bacterial strain of *Bacillus thuringenesis* was isolated and confirmed from a particular pesticide using sudan black staining for PHB production. The isolated colony of *Bacillus thuringenesis* carried out fermentation process of a waste Banana peel and paper pulp residues for 7 days in a rotary shaker for Polyhydroxybutyrate (PHB) production. The sodium hypochlorite method was selected for the extraction of PHB. After PHB extraction, turmeric, resin, and moringa powder were added to the fermented sample for shelf life, elasticity, and antibacterial activity respectively. Further the PHB undergoes drying to form PHB granules polymer in a hot air oven (Figure 1).

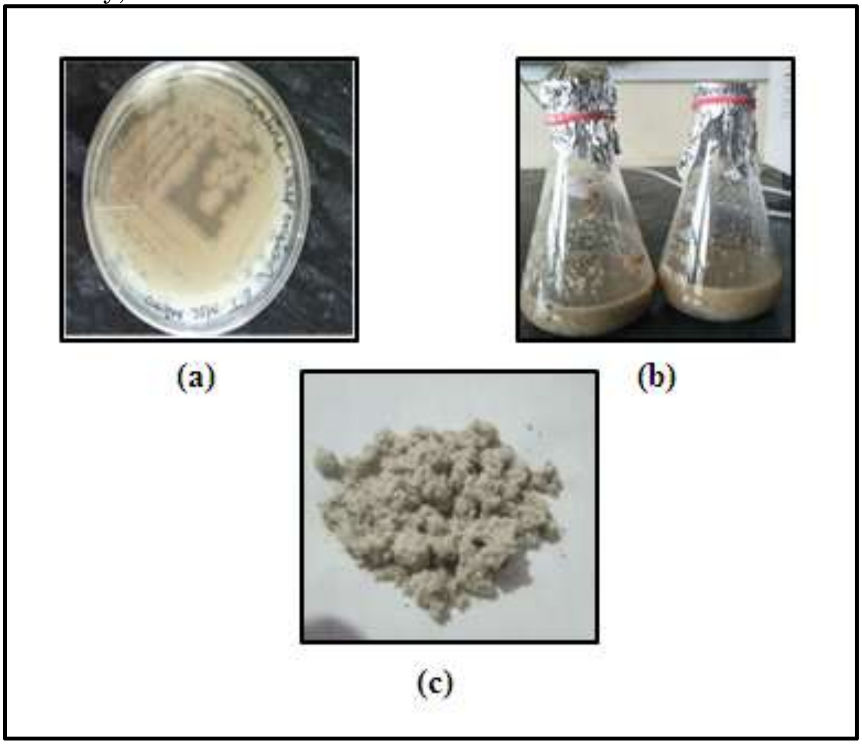


Figure 1: Stages of PHB production (a) Isolated *Bacillus thuringenesis* (b) Fermented broth of waste residue (c) Extracted PHB granules.

2.2.Formulation of PHB Bioplastic sheets

PHB granules produced undergo formulation of PHB Bioplastic polymer sheets by addition of glycerol (plasticizer), lanolin and vinegar with its perfect consistency. PHB Bioplastic composites were characterized for different properties and tests.

Figure 2: Morphology of PHB Bioplastic polymer sheets

2.3. PHB Bioplastic Characterization

2.3.1. Physical and thermal properties

Table 1 shows physical properties of prepared PHB bioplastic sample such as density, the glass transition temperature (Tg) and the melting temperature (Tm) which is within the range of Bioplastic and petroleum plastics available in the market.

Table 1: Physical properties of prepared PHB sample

Properties	PHB Sample
Physical properties	
Density (g/cm^3)	1.21
T_g (°C)	2
T_m (°C)	170

Thermogravimetric analysis (TGA) of starch bioplastic and PHB bioplastic composite decomposition graphs is shown in 3. Bioplastics has a 2-step process mechanism of decompositions. In the first step, the moisture of starch bioplastic is evaporated at 50–150 °C. In this stage, the very light volatile matter compounds (vinegar) are lost, and the thermal decomposition process occurs due to evaporation of the water. The thermal decomposition of Bioplastics occurred in the second stage between 250-500 °C. In the graph, the mass of glycerol starts to evaporate at 250 °C and entirely evaporate at 500 °C.

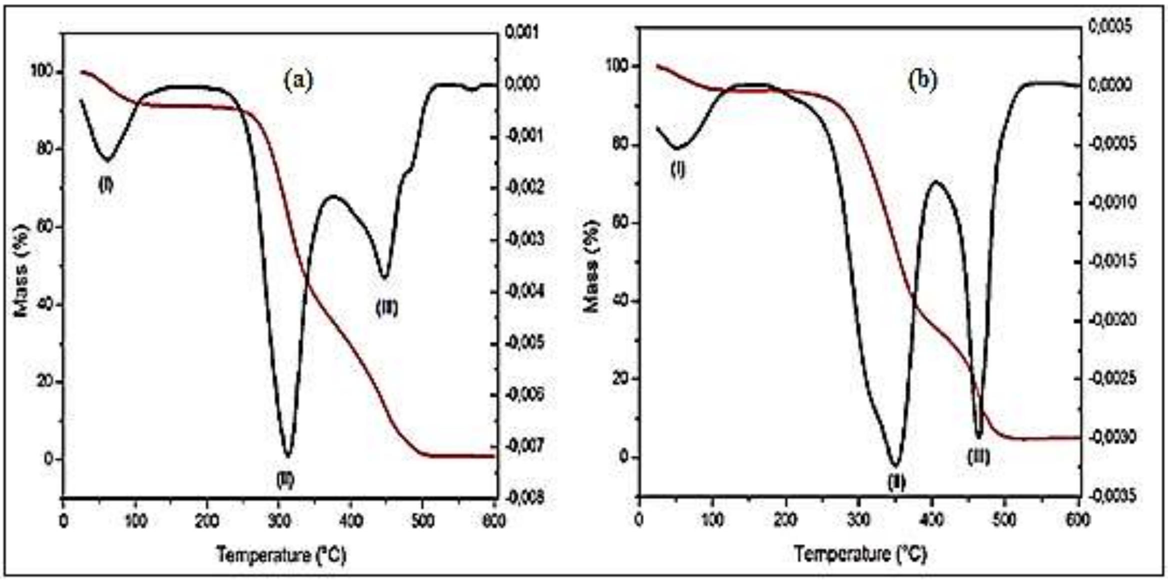


Figure 3: Thermogravimetric analysis (TGA) of (a) Starch Bioplastic (b) PHB Bioplastic sample

2.3.2. Mechanical properties

The tensile strength in the bioplastics was only affected by the plasticizer, showing a decreasing effect in the tensile strength with increasing glycerol concentration (Eq. 18). The normal tensile strength (26 MPa) was recorded for prepared PHB Bioplastic with 30% of glycerol.

The film stiffness (Young's Modulus) was influenced by the concentrations of starch of waste residues and glycerol and the interaction between them (**Young's Modulus equation**). The higher the starch concentration and the lower the plasticizer concentration, the higher was the stiffness of the material. The bioplastics produced with 20% glycerol showed an antiplasticizer effect, which was characterized by ruptures and the non-formation of a continuous matrix.

Young's Modulus = 106.87 – 0.0348 X_2^2 **+0.1564** X_1X_2

Where, X_1 is the starch concentration and X_2 is the glycerol concentration.

In the determination of the percentage of elongation of the plasticized PHB Bioplastic with glycerol, the influence of starch of waste residues and glycerol was evaluated by regression analysis. The percentage of elongation of the biopolymers was determined by an equation as follows:

Elongation % = 29.5500 + 5.3200 X_1 – 0.5280 X_2 -0.3181 X_1^2

Where, X_1 is the starch concentration and X_2 is the glycerol concentration.

The percent elongation of the bioplastics was influenced by both the starch and glycerol. On the effect of glycerol, this study found that the lower the concentration, the greater the elongation of the bioplastics. (Table 2)

Table 2: Mechanical properties of prepared PHB sample

Properties	PHB Sample
Mechanical properties	
Tensile strength (M pa)	26
Young's modulus (G pa)	3.5
Elongation %	6

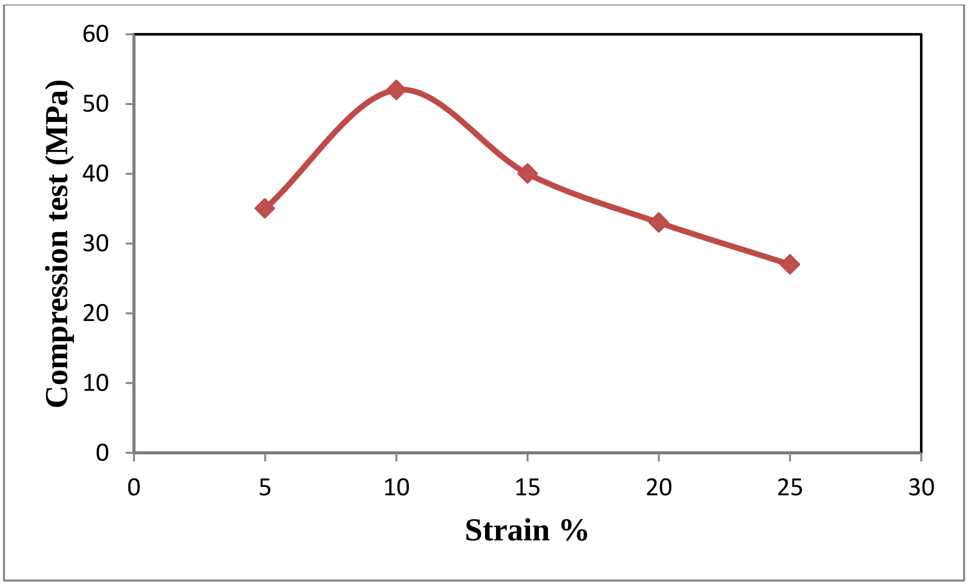


Figure 4: Compression test of PHB Bioplastic sample

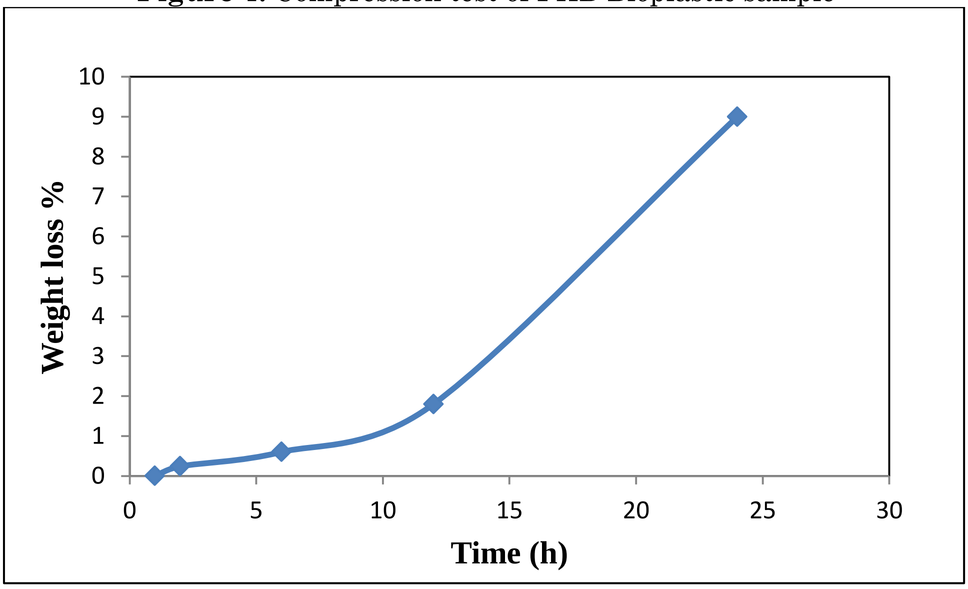


Figure 5: Puncture test of PHB Bioplastic sample

2.3.3. Permeability and solubility tests

Permeability:

The Oxygen Permeability was found to be 12.6 (cm3 mm/m2 day Kpa) while the Water Vapour Permeability (WVP) was found to be 22.4 (g mm/m2 day Kpa) for PHB Bioplastic sample with 840 Pa.s Viscosity.

Solubility:

The result for solubility of the PHB Bioplastic was found to be 22 %. This result was interpreted based on concentration of starch as compared to that of glycerol (plasticizer) used to produce the PHB Bioplastic. Figure 6 shows Water absorption % v/s immersion time of PHB Bioplastic sample.

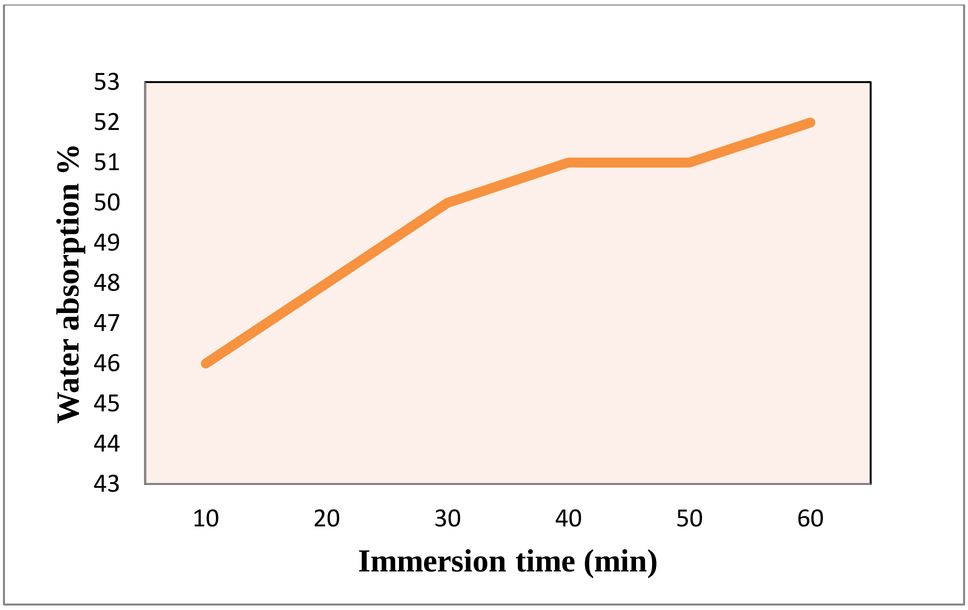


Figure 6: Water absorption % v/s immersion time

2.3.4. Soil burial biodegradation test

The results of the biodegradability test using Soil Burial Test method are shown in Figure 7. It can be seen that the longer the burial time, the higher the weight loss of Bioplastic that means the higher the degradation of Bioplastic occurred. The percentage of degradation was increasing each day until 3 % on day 7. The rate and mechanism of biodegradation of plastic materials are strongly influenced by temperature, oxygen, humidity and microbial conditions of Prepared PHB Bioplastic sample.

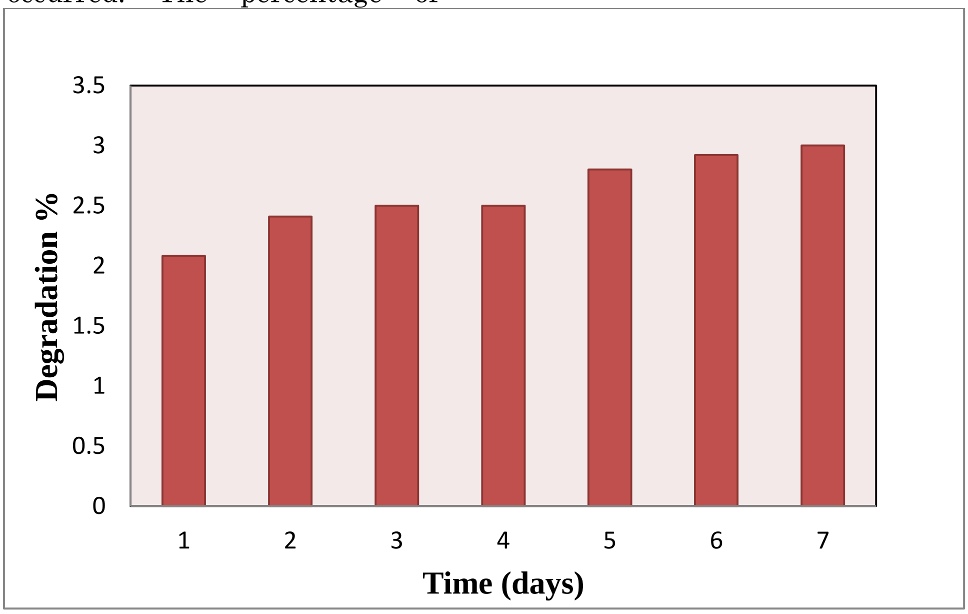


Figure 7: Soil Burial test Method

Conclusion

Polyhydroxyburtyate (PHB) has been considered as a commercially important product because of uses as biodegradable thermoplastics. In present study, production of PHB from waste banana peel and paper pulp by using *Bacillius thurengenesis* was studied. In this work, physical as well as mechanical properties were studied for the tensile strength, elongation, density, Tm and Tg of the prepared PHB Bioplastic sample which was up to the range of already available bioplastics. DSC-TGA study reveals the increased thermo- plasticity and thermo-degradability of the prepared PHB Bioplastic sample than that of the starch based standard bioplastic sample. Also, soil burial biodegradability test influenced the better use PHB bioplastics replacing non-biodegradable petroleum plastics. To deal with ever-increasing plastic pollution, PHB from the waste organic residue using microbial fermentation is the better alternative.

Discussion

The present study was designed for the production of effective polyhrdroxybutrayte (PHB) producing strain from *Bacillius thurengenesis* is ideal position for its possible application along with banana peel and paper pulp waste residue as source for fabrication of PHB Bioplastic composite. Researches focusing on biopolymer producing microorganisms for developing bio-degradable plastic. Production of PHB and utilization as precursor for bioplastic can be made more common so as to overcome the problem associated with petroleum–derived plastic. The production of PHB can maximize in future by including Biodegradable polymers and mico-based methods which will reduce the dependency on non- renewable sources. Thus, Bio-based methods or technologies are equally important for sustainable environment and development of our society.

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