1994) and *B. circulans* (173°C) (Roza *et al*., 1996). Gunaratne *et al*., 2004 and Desouky *et al*., 2007 showed that the melting point of PHB extracted from yeast ranged from 152.84°C to 168.33°C. Thermal degradation occurred around 230.07 and 269.90°C with a single weight loss step. Khan *et al*., 1995 determined the melting temperature of the polymer between 173°C and 176°C which showed a lower value compared to the present study. Pal and Paul (2002) results the melting temperature of PHB as 174.73°C while the thermal degradation at 250°C which were comparable to the results obtained in the present study. Thus characterization of PHB produced in the present study not only confirmed the purity of the product also showed the expected qualities of a plastic.

6 DEGRADATION OF POLY-ß-HYDROXYBUTYRATE AND POLYMER BLEND

6.1 Preamble

Polymeric materials (plastics) are now used in all sectors of life as very durable products with tailor-made properties. During the past decade the intense use of modern plastics, combined with their
enormous stability, had created serious problems with plastic waste, with the main problems being caused by plastic packaging. As possible alternative waste management strategies to landfilling, such as incineration or plastics recycling are not optimal and remain the subject of much controversy among scientists and the public. On the basis of these problems, an intensive research activities had been undertaken since the early 1990s to develop novel plastics which have performance comparable to that of conventional polymers, but are also susceptible to microbial degradation. The intention was that these materials would reduce waste disposal volume while undergoing degradation in a landfill, or treated in composting plants. These technologies offered a new approach to the management of plastic wastes. Moreover, when this waste management system is combined with the use of renewable resources to produce the polymers initially, it is likely that biodegradable plastics may simply become part of a natural cycle. Hence the present experiments on degradation of PHB that was produced as a part of present study itself.

6.2 Sources for PHB and PHB/PEG based film

The PHB was produced by solvent extraction method from the most potential strain A. eutrophus isolated from Vellar estuarine sediment. PHB/PEG blend was prepared by combining these two polymers using chloroform.

6.3 Preparation of polyester sheet

1.0 g of powdered PHB was dissolved in 100 ml of chloroform. The PHB solution was then poured (0.25 mm thickness) into an open, flat glass-tray and allowed to evaporate slowly at 28–30°C to form a film and used for degradation studies. The same procedure was followed to get the PHB/PEG blend. 50% of each polymer content was dissolved in chloroform and the blend was obtained through evaporation as per the method previously described by Kalnins et al., 1999.

6.4 Sediment samples tested for degradation

Sediment samples were collected from 1) Annankoil 2) Vellar estuary 3) Pitchvaram Mangroves 4) Ariyanguppam mangroves 5) Uppanar Estuary and 6) Salt pan of Marakkanam.

6.5 Degradation tests under various environment samples

The pre-weighed test pieces were buried into freshly collected soil samples (collected from 6 different environments) in wide-mouth jars. The mouth of the jars was kept open and incubated at three different temperatures under laboratory conditions (i.e.) 25,30 and 35°C. Moisture content of samples was maintained by adding sterile distilled water. Degradation of PHB was measured by the loss of weight after definite period of incubation as per the method described by Manna and Paul (2000). For each
temperarture 3 different jars (i.e) triplicates were maintained. 15 such sets were maintained for incubation and each set was assessed at an interval of 1 week. Based on the results the experiment was terminated after 10 weeks in most cases. % of degradation was estimated using the formula given below.

**Formula used**

\[
\left\{ \frac{\text{Weight of the initial film} - \text{Weight of the film after degradation}}{\text{Weight of the initial film}} \right\} \times 100
\]

6.6 **Isolation of PHB-degrading bacteria**

PHB-degrading bacteria were isolated from the degraded PHB sheets following washing and dilution plating on mineral base medium (Malik and Claus, 1978) supplemented with 0.1% (w/v) powdered PHB. The PHB-degrading isolates were confirmed by the formation of clear zone around the growth. The organisms were purified by dilution-streaking and maintained on the same medium. The isolates were identified up to genus level based on their colony morphology, micro morphological and physiochemical characteristics.
6.7 RESULTS AND DISCUSSION

Fig. 39 PHB used for degradation study

Fig. 40 PHB/PEG blend

Totally 1329 strains were isolated on modified mineral medium where PHB incorporated as a substrate. Based on the clear zone formed around the colony 176 isolates were selected as the positive polymer degrading strains.
6.7.1 Degradation in the soil collected from Annan koil

Complete degradation had occurred on 48 days of incubation at 30°C. The other two temperature extended their incubation by the next 24 hrs. 41% was the reduction of blend in this soil. 5.1% of strains were identified as the potential degraders.

![Fig. 42 Estimation of PHB degradation in the soil of Annan koil](image1)

![Fig. 43 Estimation of PHB/PEG blend degradation in the soil of Annan koil](image2)
6.7.2 Degradation in the soil collected from Vellar estuary

100% degradation of pure PHB was observed on 49 days at 30°C.

Fig. 44 Estimation of PHB degradation in the soil of Vellar estuary

7.7% was the estimation of the degraders among the total number of strains isolated. In this environment blend was reduced up to 40% at 30°C.
6.7.3 Degradation in the soil of Pitchavaram mangrove

Degradation process demanded 40 days for the complete degradation of pure PHB at 30°C. 7.9% of degraders were isolated. 14% was the difference between the degraders and non-degraders.

Fig. 45 Estimation of PHB/PEG blend degradation in the soil of Vellar estuary

Fig. 46 Estimation of PHB degradation in the soil of Pitchavaram mangrove
Only 37% of degradation was estimated with the blend at 30°C. The rest of the polymer content was remained more than one month.

**Fig. 47** Estimation of PHB/PEG blend degradation in the soil of Pitchavaram mangrove

**Fig. 48** Degradation of PHB in the soil from Ariyankuppam estuary
When the pure PHB needed, 52 days of incubation to degrade completely at 30°C, only 50% of blend was reduced by the 3.4% of the degrading members. The potential members made 16 days delay in degradation when compared to the leading degrading system.

![Fig. 49 Estimation of PHB/PEG blend degradation in the soil of Ariyankuppam estuary](image)

### 6.7.4 Degradation in the soil collected from Uppanar estuary

7.3% of the degrading members consumed total polymer content with 42 days of incubation. 40°C was made complete degradation on 45 days and 49 days were utilized to degrade completely at 30°C. Nearly one week extension of incubation was observed when compared to the leading degrading system.
While testing the blend 37% of reduction was observed at 30°C. There was no complete degradation of the blend until the 12th week of incubation.
6.7.5 Degradation in the soil collected from Salt pan

In salt pan soil also 30°C was influenced the complete degradation of PHB within 47 days of incubation. But 25°C and 35°C ranges, crossed more than 50 days for the complete degradation.

![Figure 51: Estimation of PHB degradation in the soil of Salt pan](image)

At 30°C 11 days more of incubation was needed when compared to the fast degrading system (i.e.) Pitchavaram mangroves. Regarding degradation of blend, only 40% had reduced under 30°C.

![Figure 52: Estimation of PHB/PEG blend degradation in the soil collected from Salt pan](image)

The results of the present study thus demonstrated that the degradation of PHB under laboratory condition is a process which resulted in the complete degradation at all the environmental soil samples.
tested. Maximum rate of degradation was occurred with the soil collected from Pitchavaram mangroves. Manna and Paul (2000) measured the maximum degradation of nearly 45% weight losses in municipal sewage sludge after 200 days of incubation. This was followed by the degradation in soils of saline, clay and latarite types. However, degradation of polymer was comparatively lower in water and compost. Irrespective of environments in which samples were buried, incubation temperature influenced the degradation to a greater extent. Scherer (1996) stated that PHB is one of the representative microbial polymers that degrades readily in the environments such as sewage, soil, and waste water treatment facility where prokaryotic as well as eukaryotic microorganisms are congregated. This was found to be true in the present study also.

100% degradation of pure PHB by *A. chroococcum* MAL-201 strain which degraded in all the natural samples incubated under laboratory conditions was reported by Scherer (1996). Variations in the extent of degradation as evident from percentage weight loss might be due to variation of soil type, qualitative and quantitative differences of the P (3HB)-degrading microbiota and the incubation temperature. The highest rate of degradation observed at 30°C might be due to increased microbial activity at this temperature, as it might be the optimum temperature for their growth and activity. Earlier studies of Mergarert *et al.*, 1992, 1993 and 1994a,b have also demonstrated that P(3HB) are degradable in various soils and composts under laboratory conditions and also in natural waters (Mergarert *et al.*, 1994b).

When analyzing the degradation of PHB/PEG blend more than 20-25% of the polymer contents were retained at the soil itself and they were stable to the microbial attack and latter the PHB content present in the blend might have helped to degrade to some level. When compared to the pure PHB, with blend 50% or even lesser degradation was observed. There was no complete degradation recorded in the blend. 30°C was found as the optimum temperature for both pure and blended PHB. Kim *et al.*, 2000 made an experiment of degradation of PHB along with the synthetic non degrading polymers like Sky-Green and Mater-Bi by using fungal isolates. They did biodegradation of PHB, Sky Green and Mater-Bi by the soil burial experiment in the laboratory, in which the temperature and humidity were optimized. Degradation occurs at 25-55 days. In the activated sludge soil PHB degraded 68.9% in 25 days at 28°C and 98.9% at 37°C (four of the PHB films disappeared almost completely after degradation at 37°C for 25 days, however one of the five PHB films left some measurable remains so that the average weight loss was 98.9%), while in the farm soil they degraded 41.3% at 28°C and 68.8% at 37°C. Thus, PHB degraded at the fastest rate at 37°C in the activated sludge soil. On the contrary, in the forest soil and sandy soil the weight loss was <10.0% in 25 days. Degradation activity was found to be low in these types of soil. At 60°C in the activated sludge soil PHB films degraded only 30.5%, which was much lower compared to 28
or 37°C. Degradation of SG, synthetic polyester, was slower than that of PHB. In 25 days, the degradation of PHB in the activated sludge soil at 37°C was nearly completed. In 55 days, SG was degraded by 77.5% at 28°C and 69.1% at 37°C in the activated sludge soil. In the farm soil it was degraded by 64.6% at 28°C and 51.3% at 37°C. It was interesting to note that PHB degraded more at 37°C than at 28°C, while SG degraded more at 28°C than at 37°C. MB degraded slower than PHB, too. In the activated sludge soil MB degraded 65.0% at 28°C and 60.0% at 37°C in 55 days, while in the farm soil it degraded 27.1 and 24.4%, respectively. For PHB the extent of degradation at 60°C was far smaller than at 28°C and at 37°C, regardless of the soil type. However, the extent of degradation of MB at 60°C was higher than at 28°C or at 37°C. Savenkova et al., 2000 made an experiment on biodegradation characteristics of PHB based films. They found the mass loss of pure PHB, PHB/LAP (33% W/W) and BM film samples for 30 days. Complete degradation of pure PHB film occurred after 30 days, but the PHB/LAP samples with 33% of LAP showed only half mass loss after 30 days exposure in soil. Because of its chemical structure it might be stable to microbial degradation. It could also be possible that during the formation of a PHB modified system, laprol encapsulated partially the PHB component and thus retarded biodegradation of the blend. An average lifetime of the PHB/LAP film was 6-8 months. The present study along with other studies showed that pure PHB easily degradable compared to blended plastics.

Even for PHB-PEG blend the time taken for degradation was comparatively lesser. Thus the present study showed that the PHB produced by A. eutrophus was easily degradable in different marine environments.