CHAPTER FIVE

CONCLUSION

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Polyhydroxyalkanoates produced by *Pseudomonas putida* PGA1 using saponified palm kernel oil (SPKO) as the carbon source was found to be degraded in garden soil in both natural and enclosed environment through physical, chemical and biological activities.

The moisture content of the soil from natural and enclosed environment was approximately around 20 % at day 0 and remained the same until day 90. This suggests that the condition of the soil did not undergo much changes in terms of water content and the condition is suitable for microbial activity.

Study of the pH of the garden soil in the natural and enclosed environment showed that the soil was in acidic condition, that is pH 6.5 at day 0 and changed to 6.0 at day 90 in both the environments. The change of pH could be due to the accumulation of the hydroxyalkanoic acids in the soil during the degradation of the PHA.

The PHA was found to decrease in its gross weight after burying it in the garden soil under natural environment for 11, 45 and 90 days. PHA buried under enclosed environment for 90 days was found to have lower

gross weight loss compared to the gross weight loss of PHA buried for the same period of time but under natural environment.

As a way to indicate biodegradability of the PHA, the carbon dioxide evolution test was conducted under the enclosed environment experiment. The percentage of CO_2 evolved after 90 days for the test was 35.9% and 20.2% for the control. Control experiment was carried out to obtain the evolution of CO_2 by microbes not due to PHA degradation but by other activities. The percentage of CO_2 evolved by PHA degradation was obtained by deducting the percentage of CO_2 evolved in the control from the percentage of CO_2 evolved in the test and the value obtained was 15.7%. This result supported the result of loss in the gross weight. CO_2 evolution is due to the mineralisation of PHA due to biological (microbial activities) and non biological (chemical and physical) forces.

Studies of film surface morphology using electron microscope and phase contrast microscope showed that there was physical degradation of the PHA film during soil burial. The fractured surface with cracks and holes indicated that there was some degradation process occurring to the surface of the film. The surface of the PHA buried for 11, 45 and 90 days were observed to be rough in structure compared to the PHA before burial. The morphological alterations of the surface are possibly due to the

activities of the microorganisms in the soil and also the environmental factor such as hydrolysis.

The biochemical analysis carried out were the infrared spectrophotometry (IR) and gas chromatography (GC). IR showed no loss in the functional groups of the polymer even after 90 days of burial time. This showed that the degradation of the PHA did not effect the chemical composition of its functional groups. Therefore, the remaining PHA did not change into some other compound even after the degradation process unless parts that were already converted to CO_2 and water which were then released to the environment.

Analysis of GC showed a tendency for the longer chain monomers (C10, C12 and C14) to be shortened more rapidly than the shorter chain monomers (C6 and C8). Probably the microbes in the soil could easily attack the longer monomers due to their length thus shortening the length. This would then increase the proportion of the shorter monomers while at same time the proportion of the longer monomers was decreased.

From studies conducted, it is concluded that PHA produced by *P.putida* using SPKO as the carbon source degraded in garden soil under both natural and enclosed environments. Biodegradation is indicated by the evolution of carbon dioxide There was only partial degradation during

the study period of 90 days. A longer soil burial period might produce clearer degradation patterns.

Suggestions for future studies:

In order to strengthened the study of PHA degradation in garden soil, the scope of the study should be elaborated in future.

In this research, due to limitation of time, studies of microbial population of the soil around the buried PHA samples cannot be carried out with proper controls. This study would help to provide supporting evidence of the degradation of the polymer in garden soil. Study of weight loss carried out in this experiment was based only on one sample per time period of burial. Thus in future, several PHA films should be buried at the same time, and at each sampling period, at least two films should be analysed in order to show that the degradation profile is reproducible. Another way is to repeat the degradation experiment several times. Difference in molecular weight of the PHA could be analysed using Gas Permeation Chromatography (GPC) It could not be carried out in this study because of some technical problems This is one way to indicate the degradation of the polymer at molecular level.

Studies mentioned above, if could be done, would make this study of the degradation of PHA in soil more conclusive.