CHAPTER IV

CONCLUSION

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PHA synthesized by *P. putida* PGA1 from saponified palm kernel oil (SPKO) was inherently degradable in the river water of Kayu Ara in Selangor. A maximum weight loss of 71.3% was achieved in 86 days at 28°C. The PHA film appeared to have been degraded by a combination of biological, physical and chemical reactions. Among these three, biodegradation was significant in the degradation of PHA. The PHA film surface became translucent and highly blemished with many irregular holes and pits after 28 days' incubation whereas the PHA incubated for 28 days in sterile condition was still transparent and had no detectable morphological changes (Run 2, Figure 3.3). The PHA film in the unsterilized river water was completely disintegrated in 86 days with a weight loss of 71.3%, whereas the PHA film incubated in the sterilized river water only reach a 27.2% weight loss (Run 3). 52.4% and more of the CO_2 from degradation depending on the period of incubation of PHA came from biodegradation (Run 2, Figure 3.7). Similar trend of the river water DOC (Figure 3.12) with the growth pattern of the microorganisms (Figure 3.11) showed that the microbial activities on PHA caused the released of soluble organic products into the river water (Run 3). Water current also played an important role in speeding up the degradation of PHA film. Many irregular pits and blemished areas were visible on the PHA film surface after incubation for 6 days in stirred river water (Figure 3.15*b*). In the unstirred river water, however, very little blemishing effect and very few pits and lesions were visible on the PHA film surface after incubation for 15 days (Figure 3.4*c*) to 21 days (Figure 3.4*d*). Chemical reactions from the unknown chemical substances present in the river water might have caused the 10.6% weight loss in PHA after 28 days' incubation in sterile unstirred river water (Run 2). Table 4.1 gives a summary of the

weight change in the PHA and the respective controls for all the 3 runs conducted.

Table 4.1 - Weight loss of PHA and PHB for all the runs at their respective full incubation period in river water at 28°C

Experiment	Reactor	PHA/PHB*	Microbe	% weight loss	Incubation period (days)
Run 1 without stirring (Trial run)	Test 1	+	+	-1.7	10
	Control 1	-	+	-	10
	Control 2	+	-	-3.6	10
	PHB positive control	+*	+	37.2	10
Run 2 without stirring (Short-term run)	Test 5	+	+	-5.8	28
	Control 1	-	+	-	28
	Control 2	+	-	10.6	28
	Control 3	-	-	-	28
	PHB positive control	+*	+	64.5	28
Run 3 with stirring (Extended run)	Test 5	+	+	71.3	86
	Control 1	-	+	-	86
	Control 2	+	-	27.2	86
	Control 3	-	-	-	86
	PHA positive control	+*	+	100	86
	PHB sterile control	+*	-	12.8	86

Biodegradation might have occurred throughout the entire PHA film and predominantly removed C8 monomers from the film within 6 days. The rate of PHA monomer hydrolysis was

related to the size of the monomers. The smaller monomers were preferentially hydrolyzed compared to the larger monomers. Hence, C8 was completely hydrolyzed first, followed by C10, C12 and C14 in succession. The product(s) of PHA degradation was soluble organic compound(s) which was acidic that reduced the pH of the river water from pH 7.48 initially to pH 4.69 after 86 days' incubation of PHA in the river water. Microorganisms in the river water could utilize PHA for growth. Hence, the sigmoidal growth pattern typical of a batch culture was observed.

Even though biodegradation might occur throughout the entire PHA film, it might be more effective on the film surfaces, which were directly exposed to the microorganisms. The degradation might start from the film surface by forming surface lesions (pits) and then proceeded sideways and inwards, forming wider and deeper holes. As the holes and pits converged in all directions, three-dimensional "cross-linked tunnels" were formed throughout the film. As the "tunnels" continued to degrade, holes were formed on the PHA film and finally, the entire film disintegrated.

Modifications of the ASTM standard test method (D5209-91) and additional analysis were necessary to obtain more specific and accurate results for the carbon balance analysis to verify the extent of the PHA biodegradation. Sterile conditions, additional control reactors and other analysis such as scanning electron microscopy (SEM), infrared red (IR) spectroscopy and gas chromatography (GC) were carried out. They acted as additional data not only to verify the PHA biodegradation, but also carried information to analyze the biodegradation mechanism of PHA. The proposed carbon balance equation for PHA biodegradation (PHA + O_2 $\longrightarrow$

residual PHA + intermediate products + new biomass + CO_2) could not be applied. Firstly, the DOC, which represents the biomass could not be obtained because the microbial activities reduced the sample DOC during the extended storage period. Secondly, an accurate and precise value for the CO_2 from the biodegradation process of PHA could not be obtained due to the non-uniformity of the rate of air supplied to the test reactors and control reactors. It is suggested that a computerized automatic airflow adjuster could be fixed to the inlet air supplied to every individual reactor to maintain a constant airflow. Then, carbon balance can be used to verify the extent of the PHA biodegradation from GC, DOC and CO_2 analysis.

Some suggestions for improvements and further studies:

i)   Similar experiment with well monitored aeration rate and stirrer to provide a uniform air supply and stirring rate in order to obtain a carbon balance equation.

ii)   Method to verify that the PHA depolymerase can diffuse into the entire PHA film matrix and degrade the bulk of the film.

iii)   Biodegradability test in other natural water such as university pond, coastal water, activated sludge from municipal-sewage treatment plant, lake water, etc.

iv)   Isolation and identification of the PHA-degrading microorganisms in the water tested.

v)   Well controlled tests to distinguish among biological, physical or chemical degradation of PHA.

vi)   To study the molecular weight change during the degradation process of PHA by gel permeation chromatography.