Comparative Population Study of Aliphatic Polyesters-Degrading Microorganisms at 50 °C

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The populations of four aliphatic polyester-degrading microorganisms at 50 °C were demonstrated in vitro. The percentage of degraders to total microorganisms were: 5-86%, PHB; 3-49%, PCL; 0-87.5%, PBS; and 0-4.5%, PLA.

Several studies concerning biodegradation of aliphatic polyesters have centered at normal environmental condition.[1-8] In view of the potential use of biodegradable plastics in both developed and developing countries, this study aimed to determine the comparative population of degrading microorganisms of four commercially available biodegradable materials at 50 °C. Degrading microorganisms growing at 50 °C will be useful in compost seeding since they are known to play a leading role in compost fermentation.[9] The results can be utilized as baseline information for solid waste management systems concerning plastic refuse vis-a-vis composting mechanism.

The polymer samples used were: poly ([R]-3-hydroxybutanoate): poly (3-hydroxybutyrate) (PHB) from Mitsubishi Gas Chemicals (Mn = 1.45 x 10^5), poly (6-hydroxyhexanoate): poly (ε–caprolactone) (PCL, Tone) from Union Carbide Corporation (Mn = 6.73 x 10^4), poly (tetramethylene succinate): poly (butylene succinate) (PBS, Bionolle) from Showa Highpolymer (Mn = 3.5 x 10^4), and poly ([S]-2-hydroxypropionate): poly ([S]-lactate) (PLA Lacty) from Shimadzu Company Ltd. (Mn = 1.88 x 10^5). The chemical structures of the aliphatic polyesters were: {-O-CH(CH_3)-CH_2-CO-}$_n$, PHB; {-O-(CH_2)$_5$-CO-}$_n$, PCL; {-O-(CH_2)$_4$-O-CO-(CH_2)$_2$-CO-}$_n$, PBS; and {-O-CH(CH_3)-CO-}$_n$, PLA.

This report combines two studies conducted separately. For PHB and PCL, soil samples (8) were collected from different environments in Tsukuba whereas for PBS and PLA, samples (34) came from various Prefectures in Japan. Agar plates with emulsified polymer were prepared according to our previous report.[3] Polymer samples (2 g) were dissolved in 40 ml methylene chloride. The solution was emulsified with a homogenizer (10,000 rpm for 3 min) into the basal medium (1000 ml) that composed of the following: yeast extract (100 mg), $(NH_4)_2SO_4$ (1000 mg), $MgSO_4 \cdot 7H_2O$ (200 mg), NaCl (100 mg), $CaCl_2 \cdot 2H_2O$ (20 mg), $FeSO_4 \cdot 7H_2O$ (10 mg), $Na_2MoO_4 \cdot 2H_2O$ (0.5 mg), $Na_2WO_4 \cdot 2H_2O$ (0.5 mg), $MnSO_4$, (0.5 mg), K_2HPO_4 (1600 mg), and KH_2PO_4 (200 mg). Methylene chloride was evaporated in a rotary evaporator (15-20 min) at 40 °C. Agar (20 g) was then added to the emulsified medium (pH 7.2) and the mixture was sterilized at 121 °C (15 min). After autoclaving, the agar medium was poured into petri dishes (diameter, 90 mm). This method of preparing agar medium containing plastic specimen was applied in PBS, PLA, and PCL samples. PHB powder, however, was not dissolved in methylene chloride but directly mixed into the basal medium thru the homogenizer.

The total population of microorganisms and plastic-degraders in the samples were counted by the plate count and clear zone methods, respectively. A series of dilutions were prepared from 10^{-2} to 10^{-5} and an aliquot (0.2 ml) of the appropriate dilution was deposited and spread to the agar plates. The plates were sealed and incubated at 50 °C for 15 - 30 days. Newly appearing colonies and clear zones formed were counted periodically and were expressed as colony- and clear zones-forming unit per gram (CFU/g) of dry soil samples.

Clear zone formations by degrading microorganisms were first observed after 1 to 5 days in PHB and PCL plates, after 3 to 13 days in PBS plates, and after 21 to 24 days in PLA plates. These degraders excrete extracellular enzymes which diffuse through the agar, degrade the polymer to water soluble materials, and produce a zone of clearing. Colony counts of microorganisms in PHB and PCL plates did not vary greatly at 50 °C (Figure 1). The total microbial count was found equally to be in the range of 0.1 to 9.0 x 10^5 CFU/g on both agar plates. PHB-decomposers were 2.0 x 10^3 to 1.8 x 10^5 CFU/g and PCL-

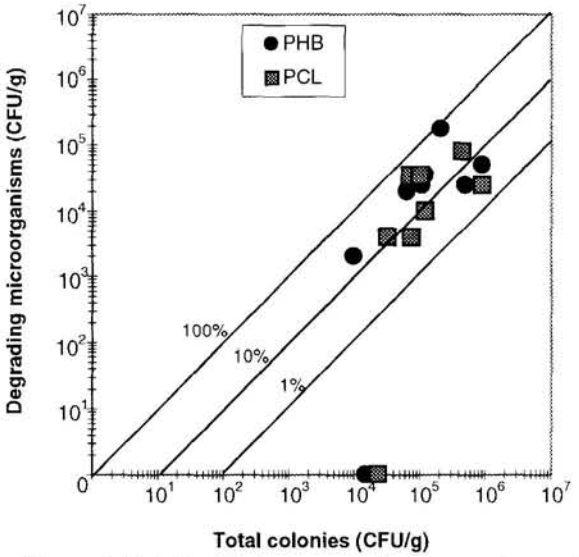

Figure 1. Relationship between total microorganisms and PHB- and PCL-degraders cultured at 50 °C for 15 days.

degraders were 0.4 to 3.5 x 10^4 CFU/g. The percentage of PHB- and PCL-decomposers to the total colonies were 5 - 86% and 3 - 49%, respectively. Only one (forest soil) out of eight soil samples did not form any clear zone on both plates. This might suggests that the type of soil and or temperature influenced the activity or growth of plastic-decomposers. A parallel study at 30 °C showed that the PHB-degrading microorganisms were 3.5 x 10^4 to 1.8 x 10^7 CFU/g and for PCL were 1.5 x 10^4 to 3.6 x 10^6

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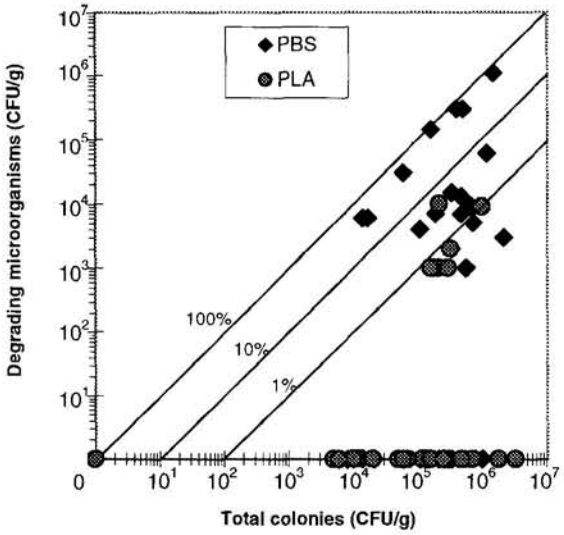

Figure 2. Relationship between total microorganisms and polyester-degraders cultured at 50 °C for 15 days in PBS plates and for 30 days in PLA plates.

CFU/g. Decrease in population is expected since microorganisms especially bacteria and fungi cannot tolerate high temperature condition for their vegetative development and their capacities for catalyzing chemical reactions is poor or entirely lacking.[10]

Figure 2 compared the population of PBS- and PLA-degraders. From the 34 samples collected, PBS- and PLA-degraders were found in 19 (56%) and 7 (21%) samples, respectively. The total microorganisms in PBS plates were 3.0 x 10^4 to 1.1 x 10^7 CFU/g and in PLA plates were 2.5 x 10^4 to 1.7 x 10^7 CFU/g. Total degraders were 0 - 5.5 x 10^6 CFU/g for PBS versus 0 - 5.5 x 10^6 CFU/g for PLA. The percentage of degraders relative to the total population of microorganisms were 0 - 87.5% and 0 - 4.5% on PBS and PLA plates, respectively. This low percentage of PLA degraders is affirmed in our previous study[5] wherein only 0.04% PLA degraders were noted. The limited distribution and the presence of small population of PLA degraders as well as its slow degrading activity in most environmental samples merit much attention and further study to better understand its biodegradability and its application in composting process. Regardless of soil samples used, the state of distribution of polyester-degraders at 50 °C in decreasing order was: PCL = PHB > PBS > PLA.

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