## **Evaluation of PHB Biodegradation in Japanese Landfill Soils**

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Polyhydroxybutyrate (PHB) is a biodegradable polymer synthesized and degraded by microorganisms and is widely regarded as an environmentally friendly alternative to conventional plastics. However, its fate under landfill conditions, especially in alkaline soils, remains poorly understood. In Japan, incineration has been the predominant municipal waste treatment method since the 1970s, and the resulting incineration ash accounts for approximately 70% of landfill waste. Therefore, landfill soils are often highly alkaline, yet studies on PHB degradation under such conditions are limited. This study aimed to evaluate the biodegradability of PHB in operational landfill soil and to elucidate the effects of elevated pH and associated microbial communities with its degradation. Soil samples were collected from the Nishi-Iburi Regional Union Final Disposal Site in Muroran, Hokkaido, and uncontaminated campus soil (pH 7.0) from the Muroran Institute of Technology was used as a control. PHB films were buried in both soils, and their weight loss was measured periodically. As a result, delayed PHB biodegradation was observed in the landfill soil. In the university soil, fragmentation of the films progressed by day 28, and substantial degradation was evident by day 49. In contrast, in the landfill soil, although fragmentation was observed in some films, little degradation occurred overall. Specifically, on day 49, the residual PHB content had decreased to 32.5% in the control soil, whereas it remained at 63.8% in the landfill soil. Microbial counts also differed substantially: bacterial colonies were 4.0×108 CFU/g in the control versus  $1.0 \times 10^7$  CFU/g in landfill soil, while fungal counts decreased from  $2.0 \times 10^6$  CFU/g to  $7.0 \times 10^3$ CFU/g, corresponding to 40-fold and 300-fold reductions, respectively. Microbial community analysis and PHB-specific isolation revealed actinomycetes (Actinomadura, Kitasatospora, Streptomyces), and spore-forming bacteria (Priestia). These taxa are known for their resilience in harsh environments, particularly through spore formation, suggesting adaptation to alkaline stress. These results suggest that although landfill soils harbor microorganisms adapted to the highly alkaline conditions caused by incineration ash, such alkalinity may suppress both the viable cell numbers and the degradation activity of PHB-degrading bacteria. Furthermore, according to the literature, incineration ash may contain heavy metals, and their effects, such as inducing oxidative stress, should also be considered.

Our findings provide new insights into the post-disposal fate of biodegradable plastics and contribute to improving evaluation and management strategies for landfill environments. Understanding the interactions between environmental conditions, microbial communities, and PHB degradation is essential for promoting the effective use of biodegradable plastics in sustainable waste management.

Keywords: polyhydroxybutyrate (PHB), landfill, biodegradation, soil pH, incineration ash