Influence of Plasticizers and Arsenic on the Microbial Degradation of Polyhydroxybutyrate (PHB)

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Polyhydroxybutyrate (PHB) is a biodegradable polyester that has drawn increasing attention as a sustainable alternative to conventional plastics. Although PHB is widely recognized for its biodegradability, its persistence in certain environments has raised concerns about its actual environmental impact. In particular, the role of plasticizers commonly added to bioplastic formulations remains poorly understood regarding biodegradation. In recent years, the detection of the heavy metal arsenic in soil has become a serious contamination problem. Because arsenic is highly cytotoxic, even trace amounts can be harmful to humans, animals, plants, and microorganisms. Therefore, when decomposing PHB products in soil, the impact of the heavy metal arsenic on decomposition must be taken into consideration. Until now, there has been little research on the effect of the heavy metal arsenic on PHB-decomposing bacteria, and it has not yet been fully elucidated.

In this study, we examined the effect of phthalate esters and the heavy metal arsenic on PHB degradation using *Ralstonia* sp. C1. The bacterium was cultivated in LB medium at 30 °C for 18 h, washed to remove residual nutrients, and subsequently transferred to a defined medium containing 0.5% (w/v) polyhydroxybutyrate (PHB) as the sole carbon source. Plasticizers were applied at concentrations ranging from 50 to 1000 μg/L. The heavy metal arsenic was applied at concentrations ranging from 5 to 40 mg/L.

Cultures were incubated aerobically at 30°C for 96 hours with shaking, and samples were collected every 24 hours. Residual PHB was measured using HPLC. In addition, to investigate whether arsenic affects the growth of the strains, arsenic and the strains were added to MS medium, then cultured at 30°C for 96 hours, with OD_{600} measurements every 24 hours.

Under all tested conditions about additives, over 50 percent of the PHB was degraded within the first 24 hours, and more than 98 percent degradation was observed by the end of the incubation period. The degradation rates were comparable regardless of the presence or absence of additives.

Additionally, no evidence was found that *Ralstonia* sp. C1 metabolized the additives. These results suggest that phthalate plasticizers neither inhibit the microbial degradation of PHB nor are utilized by strain C1. The tested conditions about arsenic, like the results of the additive experiment, over 50% of PHB was decomposed within 24 hours, and almost all of it was decomposed after the end of the cultivation. No change in the PHB decomposition rate was observed depending on the arsenic concentration added. Furthermore, the OD_{600} experiment results showed that arsenic did not affect the growth of the strain. These results suggest that Ralstonia sp. C1 is likely not affected by the cytotoxicity of arsenic at concentrations ranging from 5 to 40 mg/L.

This study provides the first clear evidence that environmental isolates can effectively degrade PHB containing such additives. Furthermore, it was suggested that the environmental isolates could efficiently degrade PHB even in the presence of arsenic at concentrations as high as 40 mg/L. The findings offer valuable insights into the environmental behavior of bioplastics and support the continued development and recycling of additive-containing biodegradable materials for commercial use.

Keywords: PHB, Additives, arsenic, Biodegradation, *Ralstonia* sp. C1