Biodegradability of LDPE film by *Bacillus* bacteria Activated by Plasma Treatment

Sang Hye Ji[1], Seungil Park[1] and Seungryul Yoo[2]

[1]*Plasma Bio Research Division, Institute of Plasma Technology, Korea Institute of Fusion Energy, 37 Dongjangsan-ro, Gunsan city, Jeollabuk-do 54004, Korea*
[2]*Convergence Technology Research Division, Institute of Plasma Technology, Korea Institute of Fusion Energy, 37 Dongjangsan-ro, Gunsan city, Jeollabuk-do 54004, Korea*

Abstract: Environmental pollution caused by plastic waste is becoming an increasingly ecological threat worldwide. Plastic biodegradation using microorganisms is an environmentally friendly and sustainable method without side effects. In this study, *Bacillus safensis*, a plastic-degrading microorganism, was treated with plasma to improve the decomposition efficiency of LDPE, and the possibility of PE decomposition was verified through various analysis methods. Plasma technology, which activates plastic-degrading bacteria, is an effective helper for bacteria to degrade plastics, opening up the possibility of a new degradation method.

Keywords: plastic biodegradation, microorganisms, plasma, LDPE

1. Introduction

Worldwide, more than 6.3 million tons of plastic waste is emitted each year, of which less than 10% of the plastic is recycled, and the rest is thrown away as it is. As such, most of the waste plastic is landfilled, and the final products resulting from this are methane and carbon dioxide, which can cause other environmental pollution. Among plastics, polyethylene (PE) is an artificial polymer used in many aspects of human life and is widely used because it is lightweight and durable. However, PE is one of the serious environmental pollutants because it is highly resistant to biodegradation due to various factors such as high tensile strength, hydrophobicity, absence of functional groups and long carbon chains [1]. Decomposit-ion of non-degradable LDPE using microorganisms is an environmentally friendly biodegradation method that does not leave toxic residues after decomposition, and active research using this method is being conducted. Some researchers have been working to improve the microbial decomposition of plastics through a physical and chemical pre-treatment process [2, 3]. Other researchers are working to increase the degradation efficiency by enhancing the production of plastic-degrading enzymes from exogenous microorganisms [4-5]. Plasma has physical parameters such as photons (UV/VIS), heat, electric field, and charged particles (electrons, ions) and chemical factors such as various reactive oxygen species and nitrogen species. Therefore, plasma can give a wide range of effects (from inactivation to activation) on the target sample depending on how use the parameters. In particular, most studies in which plasma is applied to microorganisms are inactivation studies for the purpose of sterilization. However, the poten-tial of plasma in activating beneficial microorganisms has rarely been investigated. In this study, plasma played a significant role as a potential tool to improve plastic degradation efficiency by enhancing the vitality and functional activity of plastic-degrading bacteria.

2. Materials and methods

Sample collection and bacterial strain isolation. Waste plastic samples were collected from various sites including a wasted disposal in Jeollabuk-do, Korea.

Screening of PE-degrading bacteria. For isolation of PE-degrading bacteria, a minimal salts medium (MSM) containing polyethylene powder was prepared (on a per L): 0.5g of K_2HPO_4, 0.04 g of KH_2PO_4, 0.1 g of NaCl, 0.002 g of $CaCl_2 \cdot 2H_2O$, 0.2 g of $(NH_4)_2SO_4$, 0.02 g of $MgSO_4 \cdot 7H_2O$, 0.001g of $FeSO_4$ and 0.01g of $MnSO_4$.

Molecular identification of PE-degrading isolates and whole genome sequencing. Bacterial isolates were partially identified by the analysis of 16S rDNA sequence and then the complete genome sequence of strain *B.safensis* BS-10L was deposited in GenBank under the accession number is CP115172.

PE-degrading bacteria growth monitoring. In order to confirm in real time whether the bacteria use LDPE as an energy source as they grow, the growth rate was monitored in real time.

Plasma device. Surface dielectric barrier discharge (sDBD) plasma device with a burst pulse type high voltage inverter was used. Figure 1 shows a schematic drawing of plasma treatment apparatus.

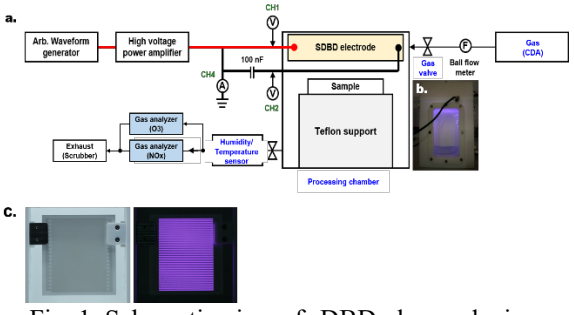

Fig. 1. Schematic view of sDBD plasma device

Cell viability and change of protein. Confocal Laser Scanning Microscopy (Olympus, USA) was observed to compare cell activity by plasma treatment using a Live/Dead Assay Kit (Thermo Fisher Scientific, USA).

Bacterial ATP (adenosine triphosphate) levels were compared for cell viability using the BacTiter-Glo™ Microbial Cell Viability Assay Reagent (Promega, USA). Bacterial biomass estimation and EPS measurement. The density of microorganisms attached to the LDPE surface was confirmed through the protein content of the microorganisms. The extracellular polymeric material (EPS) content was measured through the microbial biofilm formed on the LDPE surface.
LDPE surface change analysis. Changes on the surface of the LDPE film due to the action of microorganisms activated by the plasma treatment were investigated with a SEM, FTIR, AFM and XPS analysis.

3. Results and discussion

Isolation and identification of PE-degrading bacteria and whole genome characterization.

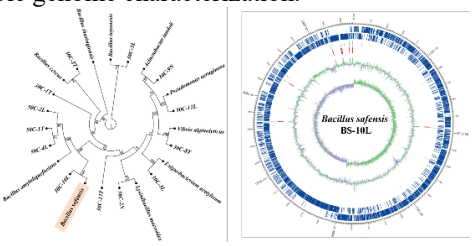

Fig. 2. Phylogenetic tree and genome circular map of *B.safensis* BS-10L.

Plasma enhances population and vitality of *B.safensis* BS-10L. Bacterial cell populations were accelerated by plasma treatment. In particular, the concentration of bacterial cells exposed to 3 min plasma was confirmed to be an optimal condition for microbial activation. Plasma treatment not only increased the bacterial cell population, but also increased cell vitality as cell viability increased.

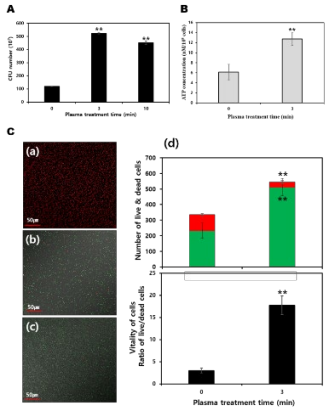

Fig. 3. Bacterial vitality after plasma treatment. (A) CFU number of bacteria after plasma treatment. (B) Quantification of the ATP contents in bacteria cells. (C) CLSM images of *B.safensis* BS-10L

Biofilm formation and LDPE degradation by *B. safensis* BS-10L. The result of confirming the morphological change of the LDPE surface by microorganisms through SEM. Cracks and small holes began to be observed on the surface of the LDPE film 60 days after inoculation with microorganisms. As a result of XPS (X-ray Photoelectron Spectroscopy) analysis, it is assumed that the oxidation reaction increased as a result of the strong C=O bond in the film inoculated with the plasma-treated microorganism.

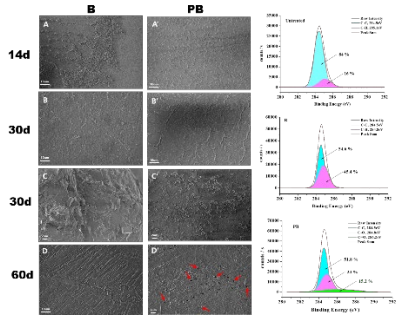

Fig. 4. Bacterial degradation of LDPE film

3. Conclusion

In this study, *B.safensis* BS-10L is a novel microorganism capable of degrading LDPE, and its LDPE degrading ability was verified. In particular, we newly obtained that the plasma treatment increased the activity of PE decomposing bacteria and improved the decomposition efficiency. It was confirmed that *B.safensis* BS-10L changed the hydrophobicity of the LDPE surface to form a microbial biofilm, thereby generating C-O bonds. It was confirmed that the oxidation reaction increased through the result that a stronger C=O bond was generated in the film inoculated with the plasma-treated microorganism. The results of this study suggest that plasma serves as a trigger to increase the LDPE biodegradation efficiency of *B.safensis* BS-10L and it shows a major cause of environmental pollution can play a positive role in plastic waste management.

4. References

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