## Effect of biofilm on the physicochemical characteristics and physiognomy of biodegradable microplastics

Prasanth Babu Ramesh<sup>\*1</sup>, Sarthak Saxena<sup>1</sup>, Sumit Saxena<sup>2</sup>, and Shobha Shukla<sup>†2</sup>

<sup>1</sup>IITB Monash Research Academy – Indian Institute of Technology Bombay, Mumbai, Maharashtra, 400076, India

<sup>2</sup>Nanostructures Engineering and Modeling Laboratory, Department of Metallurgical Engineering and Materials Science – Indian Institute of Technology Bombay, Mumbai, Maharashtra, 400076, India

## Abstract

Nearly 4.5 trillion cigarette butts flicked to the ground each year worldwide. This most littered trash item is composed of biodegradable plastic called cellulose acetate releases large influx of microplastics and other harmful chemicals into the waterways. Although cellulose acetate is biodegradable in 18 months under compostable conditions, it remains unknown of its biodegradation potential in natural waters. Formation of bacterial biofilm is an essential mechanism that determines the rate of biodegradation and bioremediation of any plastic waste at given environment. Hence in this study, synthesised cellulose acetate was used as substrate for bacterial biofilm formation of Escherichia coli and Pseudomonas aeruginosa with different artificial water types such as artificial seawater (ASW), synthetic freshwater (SFW) and potable water. It aimed to understand the effect of bacterial growth dynamics on the physiognomy and physicochemical parameters of biodegradable microplastics. Cellulose acetate films were synthesised using a solvent casting technique. These films are subjected to bacterial inoculation of exponential phase E.coli and P.aeruginosa strains at different water composed broth medium. Biofilm formed observed at the weekly time interval with samplings to reveal the changes in substrate physiology, biofilm structural moieties and its influencing factors. The formed biofilms were isolated from the film substrates using an ultrasonic oscillometer. Scanning electron microscopy (SEM) and Atomic force microscopy (AFM) of film substrates and biofilms provided the spatial arrangement of biofilms and its structural abrasions. Biofilm characterisation involved biomass determination and crystal violet staining followed by observation in confocal laser scanning microscopy (CLSM). In addition, zeta potential and hydrophobicity analysis of the films used to understand the effect of biofilm on the film substrate. This work highlights structural and functional attributes of the bacterial biofilm interactions that can be manoeuvred to better remediate the environmental pollutants.

**Keywords:** Cigarette butts, cellulose acetate, microplastic, biofilm, Escheriachia coli, Pseudomonas aeruginosa, natural waters

\*Speaker

<sup>&</sup>lt;sup>†</sup>Corresponding author: sshukla@iitb.ac.in