

# Marine microorganisms both biodegradable and non-biodegradable plastics are colonized

Charlotte Evans

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## ABSTRACT

Oceans are filled with plastic, which provides an ideal environment for bacterial growth. A critical component of evaluating the ecological effects and future of plastics in the marine environment is understanding biofouling mechanisms. In this study, we examined the various stages of plastic colonisation of polyester, Poly(3-Hydroxybutyrate-Co-3-Hydroxyvalerate) (PHBV), and polyolefin-based plastics, including standard low-density Polyethylene (PE), additives of PE with pro-oxidant (OXO), and Artificially Aged OXO (AA-OXO). We used a variety of methods, some of which were used for the first time on plastics, to combine measurements of physical surface properties of polymers (hydrophobicity and roughness) with biofilm

microbiological characterisation (cell counts, taxonomic composition, and heterotrophic activity). We were able to characterise the subsequent stages of primo-colonization, growth, and maturation of the biofilms thanks to our experimental setup using aquariums with naturally circulating seawater over a period of 6 weeks. The biodegradable AA-OXO and PHBV polymer types showed increased colonisation by active and particular bacteria compared to non-biodegradable polymers, which was one of the trends we highlighted between polymer types with various surface qualities and compositions (PE and OXO). The three stages of colonisation resulted in a succession of the bacterial population, with hydrocarbon clastic bacteria being extremely prevalent on all types of plastic. This study presents original data that offers fresh perspectives on how marine microorganisms colonise non-biodegradable and biodegradable plastics.

**Key Words:** *Bacterial growth; Primo-colonization; Biodegradable; Microbiological*

## INTRODUCTION

Due to its extremely slow degradability and the expanding amount of human trash, plastic has quickly surpassed all other pollutants in the oceans (80% of marine litter is made of plastic). Plastic waste gets broken down into little bits (5 mm) when it is released into the environment. More than 90% of the total counts of plastic debris at the sea's surface are made up of MPs. In the ocean, plastics almost instantly become coated with inorganic and organic material (known as the "conditioning layer"), which is then swiftly colonised by microorganisms that quickly develop a biofilm on their surfaces. Surface-associated bacterial colonies that are embedded in an exopolymeric material matrix are referred to as bacterial biofilms (EPS). These organic communities serve as a means of defence, a source of food, promote metabolic cooperation, and increase the likelihood of gene transfer between cells. The various stages of biofilm production in marine waters on man-made (glass, acrylic, and steel) or natural surfaces are well known.

First, "primo-colonization" refers to the pioneer bacteria occupying the surface through reversible attachment, where they engage with the conditioning film and create the first biofilm's first layer. Second, the "growth phase" encourages permanent attachment through proactive processes such as the production of pili, adhesion proteins, and EPS by secondary species, which lead to changes in the substratum's physical characteristics. Third, the "maturation phase" is characterised by a variety of competitive or cooperative interactions between cells, resulting in either further species recruitment or extinction. So far, only a small number of studies have so far detailed how biofilms develop on plastic in maritime environments. Early stages of the processes were seen on plastic bags or MPs made of Polyethylene (PE) over three weeks in seawater and sediments. There are two investigations that were conducted over a 6-month period on the longer-term biofilm growth on the surface of PE or PE Terephthalate (PET) in a maritime environment. Only one study, Mater-Bi N°014, which was done over the course of a month in a marine environment, has so far compared biofilm formation on PE with that observed on supposedly "biodegradable" plastics consisting of starch-based biopolymer and PET. As a result, population and community dynamics in these biofilms remain completely unknown. These studies were primarily

based on Scanning Electron Microscopy (SEM) observations and taxonomic identification, but none of them focused on bacterial abundance and activity. Additionally, it was shown that the hydrophobicity/hydrophilicity, structure, and roughness of the substrate are all important factors in the creation of a biofilm. These factors were never considered in research that focused on the marine environment. Polyethylene, Polypropylene (PP), and Polystyrene (PS) make up the majority of the plastic debris at sea level. PE is an extremely resistant substance because it contains stable aliphatic chains. A wide variety of possibly biodegradable plastics were created within the framework of sustainable development and divided into two main categories based on the mechanism of the biodegradation pathway: "hydro-biodegradable" and "OXO-biodegradable." The former are polymers made of polyolefin that have pro-oxidant ingredients (mostly PE) The addition speeds up the abiotic oxidation process by heat and/or UV light in the event that it is released into the environment, a condition that can be mimicked by artificially ageing. If the original OXO formulation is resistant to biodegradation, oxidative processes may be used to further biodegrade the oxidised AA-OXO. Numerous investigations on OXO pre-oxidized films revealed mineralization of between 12 and 24% after 90 days of incubation and 50% to 80% under half a year to a year and a half. Plastics that can be hydrolytically biodegraded make up hydro-biodegradable plastics. They consist of starch, cellulose, and, more frequently, polyesters such as Polyhydroxyalkanoates (PHA). PHAs, which are polyesters produced by bacteria for intracellular storage of carbon and energy, have drawn a lot of attention as potential replacements for standard plastics due to their mechanical similarities to several synthetic thermoplastics. Different bacteria have been demonstrated to degrade AA-OXO or PHA under various circumstances.

We described the biofilm colonisation phases on PE, OXO-degradable polymer with (AA-OXO) or without (OXO) artificial ageing, and Poly(3-Hydroxybutyrate-Co-3-Hydroxyvalerate) (PHBV) as a PHA representative in this study. Each type of polymer was incubated independently, and the evolution of each was tracked for six weeks in natural saltwater. Changes in bacterial diversity, heterotrophic activity, and polymer surface physical characteristics were used to describe the dynamics of bacterial biofilms.

Editorial Office, *Journal of Environmental Microbiology*, UK

Correspondence: Charlotte Evans, *Journal of Environmental Microbiology*, UK, Email: charlotteevans@gmail.com

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