



# The characterization of Biofilm formed on microplastics

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## Introduction

MPs (1-1000  $\mu\text{m}$ ) enter the aquatic environment; adsorption of different inorganic and organic substances, followed by colonization of various microorganisms – **biofouling**

## Materials & Methods

- **PE MPs**, extracted from a facial scrub
- Incubation of MPs in **freshwater** (Glinscica River) for 15 weeks ( $23 \pm 2$  °C, 125 rpm, light/dark cycle 16h/8h)
- Every week a new freshwater was added
- **Week 13 and 15: biofilm characterization**

## Biofilm characterization

### Mass of biofilm per mass of MPsB

Measurement of mass prior and after digestion of MPs with biofilm (MPsB) by Fenton reaction.

### Extracellular polymer substances (EPS)

(excreted by microorganisms for establishing structural stability of biofilm)  
Determined spectrophotometrically using phenol sulphuric acid.

### Chlorophyll *a* content

(chlorophyll pigment indicate that microalgae are likely to be present in the biofilm)  
Determined spectrophotometrically after the extraction with 95% ethanol.

### Urease activity

(indispensable enzymes in numerous organisms, therefore the determination of urease activity indicate the activity of microorganisms in biofilm)  
Determined indirectly by hydrolysis of urea to ammonia.

## Results

Table 1: Biofilm characteristics at week 13 and 15.

Method	Week 13	Week 15
Mass of biofilm per mass of MPsB (%)	$34 \pm 8$	$43 \pm 6$
EPS ( $\text{mg}/\text{g}_{\text{biofilm}}$ )	$0.29 \pm 0.05$	$0.59 \pm 0.10$
Chlorophyll <i>a</i> ( $\text{mg}/\text{g}_{\text{biofilm}}$ )	$0.18 \pm 0.03$	$0.37 \pm 0.11$
Urease activity ( $\text{mg}_{\text{hydrolysed N}}/\text{g}_{\text{biofilm}}$ )	$0.28 \pm 0.01$	$0.95 \pm 0.27$

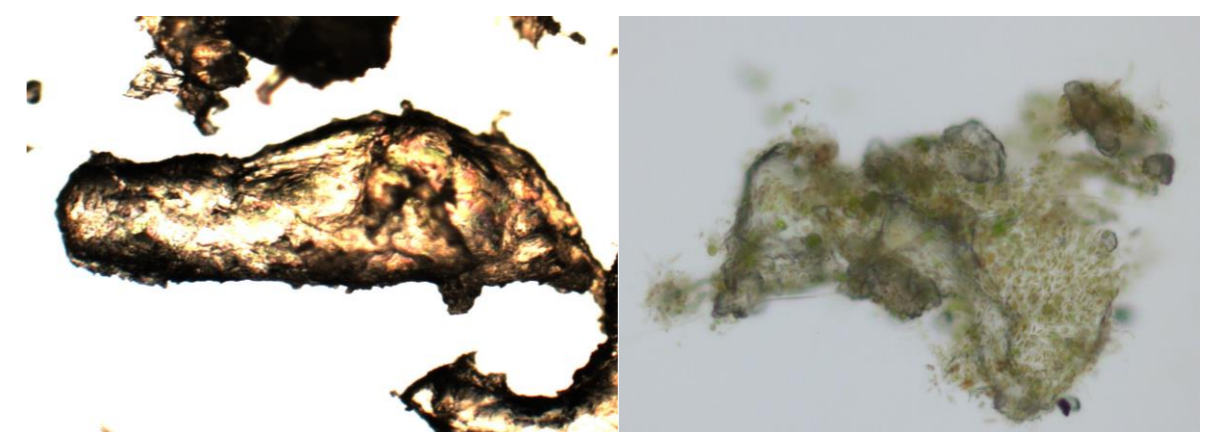


Figure 1: PE MPs (left) and PE MPs with biofilm (right).

## Conclusions

- The amount of biofilm on microplastics increased within the two weeks, consequently the amount of EPS, chlorophyll *a* content and urease activity also increased.
- We observed **changes of MPs behaviour**; the density of particles significantly increased with time and they started to slowly sink and the end of the experiment, the majority of the particles settle to the bottom.

More about our research:



<https://planterastics.fkkt.uni-lj.si/>