Monitoring of biofilm growth on the microplastic surface

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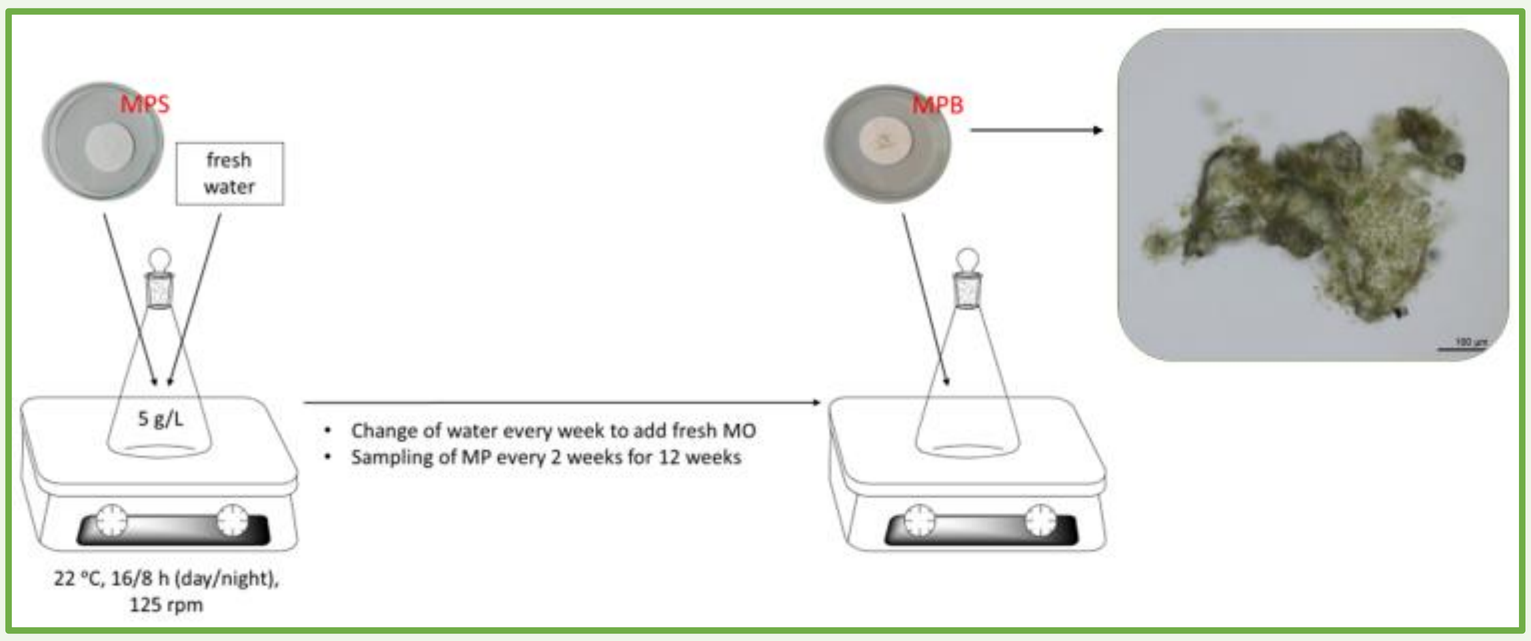
Introduction and aim

In the aquatic environment, microplastics coexist with microorganisms that attach to their surface and form a biofilm - this process is often referred to as biofouling. Biofilm has a significant impact on the properties of microplastics, but its growth on the surface of microplastics is not well understood. In this study, the formation of a biofilm on floating polyethylene (PE) microplastics (MP) was monitored under simulated laboratory conditions. Microplastics (extracted from a cosmetic product, of irregular shapes and size around 100 µm) were incubated in natural stream water for 12 weeks, and the stream water was replaced every week to add new nutrients and microorganisms (MO). Every two weeks, the microplastics were examined and the biofilm was characterized.

Experimental setup and biofilm characterization



The amount of developed biofilm

- *Subtraction of mass of MPB before and after digestion by Fenton oxidation*

Extracellular polymer substances (EPS)

- *Spectrophotometric determination using phenol-sulfuric acid reaction (with glucose as the standard)*

Chlorophyll *a*

- *Spectrophotometric determination after extraction with 95 % ethanol*

Results

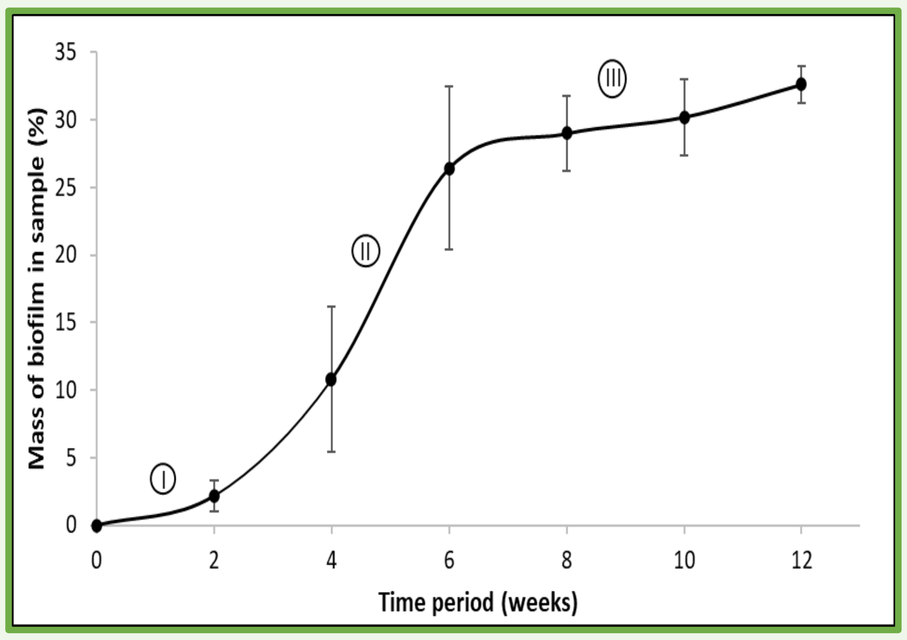


Fig. 1: The amount of developed biofilm. In phase I (lag phase) the initial layer of biofilm was formed on MP, followed by phase II (exponential growth phase), from week 2 to week 6 (µ2 = 0.700 /week) and phase III (stationary phase), from week 6 to week 12 (µ3 = 0.045 /week).

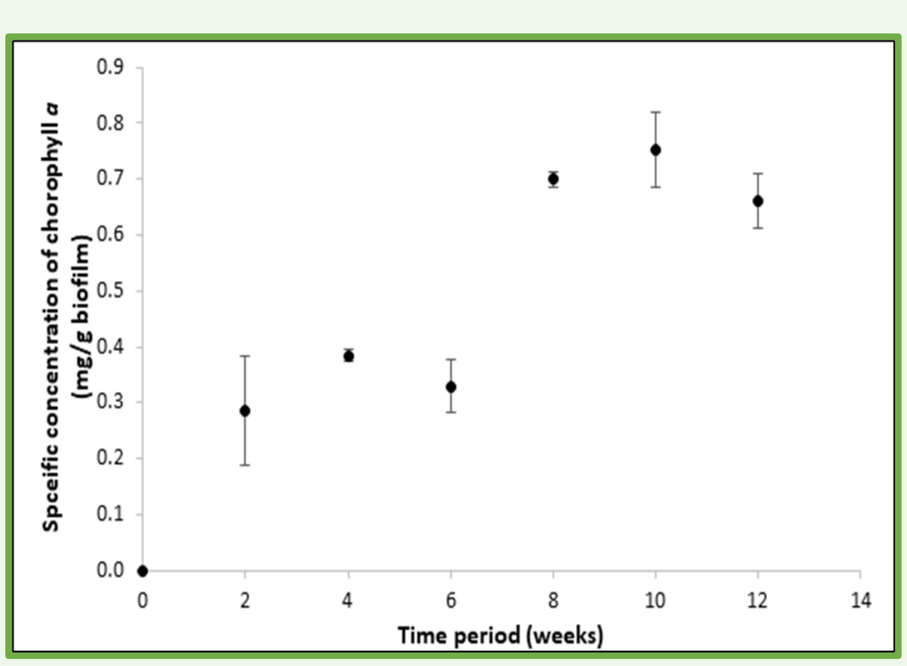


Fig. 2: The specific concentration of chlorophyll *a* in biofilm.

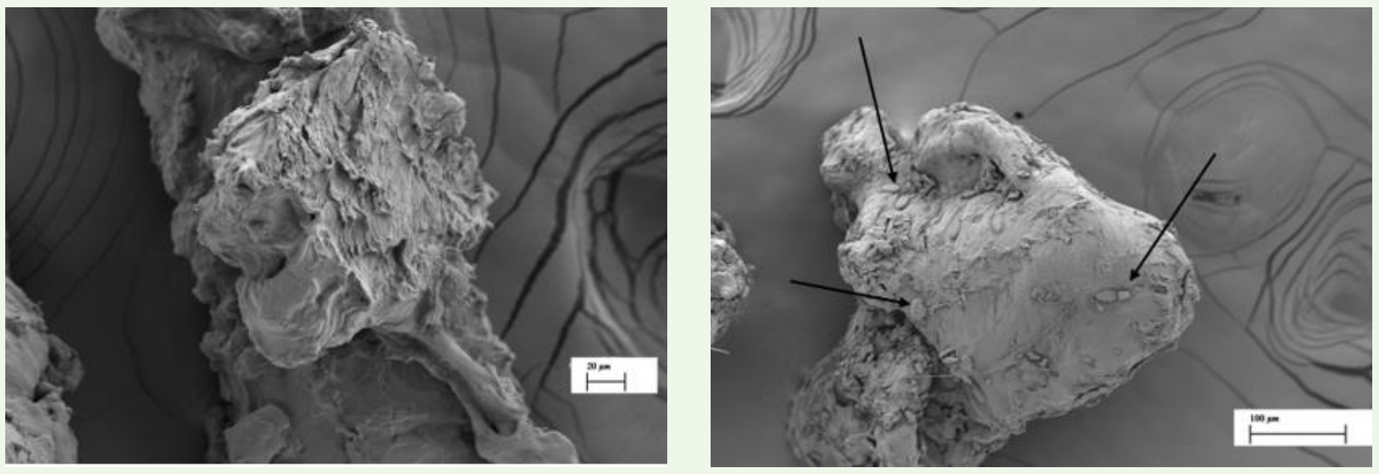


Fig. 3: FE-SEM image of MPS (left) and MPB (right). MPS is in the form of fragments, as it is irregularly shaped and has sharp edges, and on MPB we can confirm the presence of oval-shaped microorganisms (indicated by arrows).

Table 1: The specific concentration of EPS in biofilm.

Time period (weeks)	Specific concentration of EPS (mg/g biofilm)
0	0.00 ± 0.00
2	0.56 ± 0.97
4	1.06 ± 0.78
6	0.47 ± 0.08
8	0.49 ± 0.11
10	0.64 ± 0.39
12	0.47 ± 0.08

Conclusions

The growth rate of the biofilm corresponded to the common bacterial growth curve (Fig. 1). At the end of the experiment the mass of the biofilm reached 32.6 ± 1.4 % of the mass of the MPB. The EPS content (Table 1) was proportional to the amount of biofilm throughout the experiment and had a constant specific concentration - at week 12, the concentration was 0.47 ± 0.08 mg/g biofilm. The EPS are an important component of any biofilm as they provide better adhesion of microorganisms and stability of the biofilm. The concentration of chorophyll *a* (Fig. 2) increased with time, reaching a maximum value of 0.752 ± 0.068 mg/g biofilm. The presence of chlorophyll *a* indicated the presence of photosynthetic organisms (e.g., green algae). Our results shed light on the important interactions between microorganisms and microplastics and show the trend of various parameters related to the development of a biofilm on microplastics.