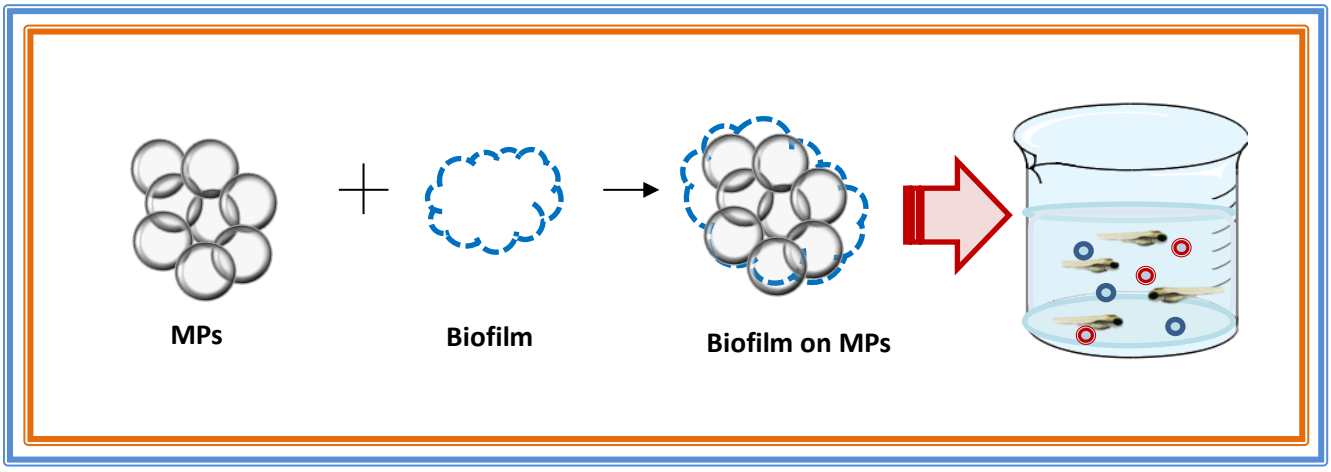
Procedure for biofilm formation on Microplastics for mesocosm experiments



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Abstract

The accumulation of Microplastics (MP) in the environment is continuous and persistent, posing a concern for potential adverse effects on biota. Since environmental MPs can carry considerable amounts of diverse substances (co-contaminants), the combined exposure to MPs and other contaminants represents one of the most important aspects. The presence of a biofilm on MPs surface can potentially change co-contaminant bioavailability to organisms. The aim of this technical report is to provide the method to create a biofilm on the microplastic surface in order to carry out mesocosm experiments useful to evaluate microplastic-associated biofilm influence on the bioavailability of contaminants to model organisms.

1. Introduction

Microplastics contamination has gained public concern and, in some instances, has even been considered a potential "planetary boundary threat" (Galloway and Lewis, 2016; Jahnke et al., 2017). In the aquatic environment, MPs are ubiquitously present in seas and rivers (Horton et al., 2017; Eriksen et al., 2017), offering several and constant routes of exposure for aquatic biota, and potentially to humans via ingestion (Boyle et al., 2020; Senathirajah et al., 2021).

Due to their chemical properties, MPs can adsorb persistent organic pollutants (POPs), such as polycyclic aromatic hydrocarbons (PAHs), either during the manufacturing process or from the environment contributing to MPs hazard effects (Gallo et al., 2018). Laboratory-based assessments have indicated that plastic-mediated transfer of POPs to the organisms can take place and that the mechanisms of how MPs interact with substances affect their bioaccessibility by organisms and consequent bioaccumulation and bioavailability (Trevisan et al., 2019).

In addition to this complex process, several studies have demonstrated that the surface of MPs acts as an anthropogenic substrate for phylogenetically and functionally distinct communities of microorganisms called "biofilm" or "epiplastic community" (Reisser et al., 2014; Zettler et al., 2013). Epiplastic microbiota appears to play a key role in the fate and ecological impacts of plastic pollution, and in the last years, microbiologists are investigating these communities present on MPs surfaces. This organic layer can act as a reservoir for pollutants, affecting the adsorption of chemicals for organisms ingesting MPs with unpredictable effects on the co-contaminants ecotoxicity (Rummel et al., 2017; Flemming et al., 1995). Due to the biofilm sorption characteristics and ability to degrade organic chemicals (Writer et al., 2011; Wen et al., 2015), the presence of biofilm on microplastic surfaces can influence the plastic-mediated transfer of pollutants to organisms. Although several studies have suggested that a contaminant transfer in the organism can occur (Chua et al., 2014; Rochman et al., 2014; Browne et al., 2013; Gaylor et al., 2012), it is still unclear how biofilms interact with plastic-associated chemicals and consequently their bioavailability for organisms ingesting MPs (Rummel et al., 2017). Therefore, the aim of this technical report is to provide the method to create a biofilm on microplastic surface in order to carry out mesocosm experiments useful to evaluate microplastic-associated biofilm influence on the bioavailability of contaminants in model organisms.

2. Material and Methods

2.1 Experiment timeline

The experiment requires careful time management. The biofilm takes 6 days to grow on MP. Contaminants should then be equilibrated with MPs for 24 h, and finally, organisms (i.e., zebrafish at 72 hpf) can be exposed to contaminants for 24 h i.e., until 96 hpf. In total, the experiment takes 8 days (Figure 1).

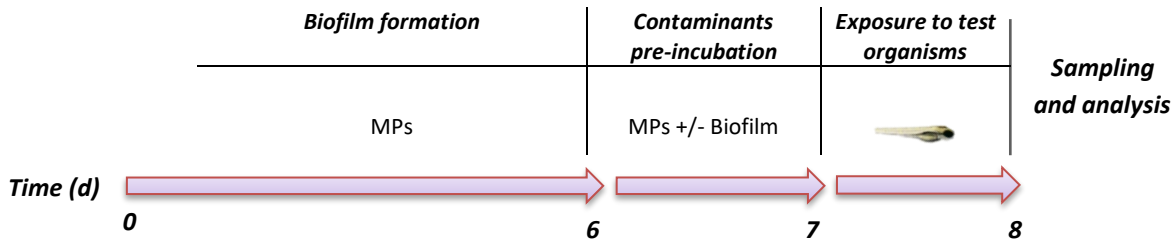

Figure 1: Experimental design and timeline featuring the experimental steps to assess the bioavailability of contaminants from MPs to test organisms with and without (+/-, respectively) the presence of a biofilm. Time "*day 0*" indicates the start of biofilm formation which for 6 days. contaminants have been equilibrated with MPs for 24 h, and finally, test organisms can be exposed for 24 h to MPs. In total the experimental steps took 8 days, at the end of which individuals can be collected.

2.2 Biofilm formation

For biofilm formation (figure 2), 20 mg of MPs (~250 µm) were weighted and added directly into clear 20 mL borosilicate vials with a screw top containing water (20 mL). The water used for this step was collected from the water reservoir of organisms (i.e. zebrafish) recirculating system. Prior collection, bioballs (the substrate of the biofilter of the water system) in the box were agitated using acid washed beaker. Water was collected and filtered two times through two filters of different sizes (125 µm and 100 µm) to avoid larger detritus and then pipetted into each of the experimental vials. To protect the biofilm-forming treatments from light and prevent algal growth, vials were covered with aluminum foil and then incubated at 27 - 29 °C for 6 days. After incubation with media, MPs were collected by filtration (100 µm Celltric® filters) and transferred into new vials containing 20 mL of filtered water media. Of the vials used in the biofilm formation step, some vials can be used as control MPs with biofilm for the following test organisms exposures, while other vials can be subjected to pre-incubation with co-contaminants.

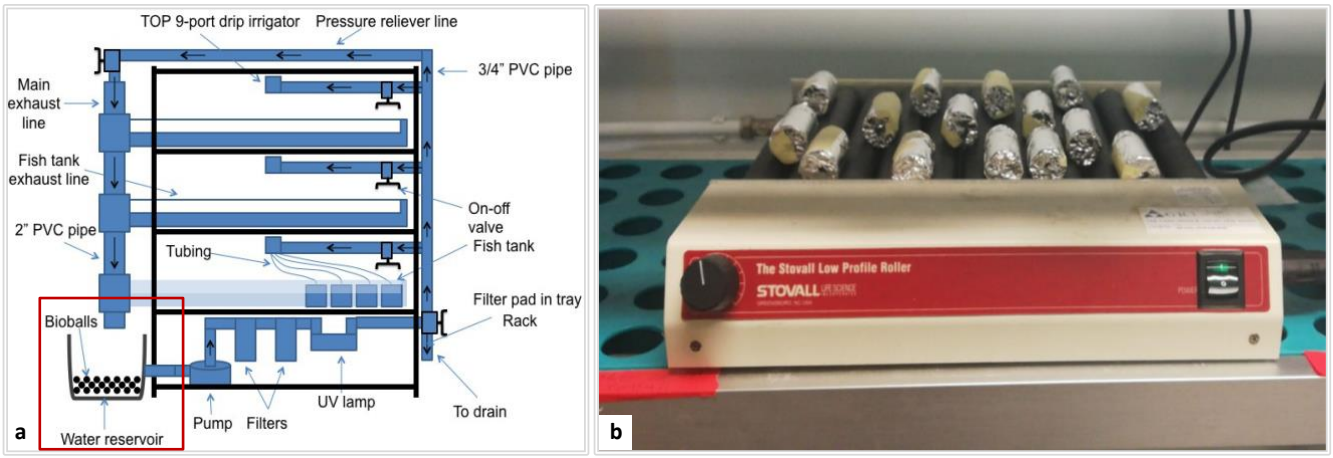

Figure 2: Biofilm formation phases: (a) Box from which water has been collect to form biofilm; (b) Vials on the roller covered with aluminum foil in incubation for 6 days

2.3 Contaminants pre-incubation

Contaminants have been pre-incubated with MPs for 24 h before exposure to zebrafish. Pre-incubation concerned three treatment groups including: 1) MPs without biofilm (MPs), 2) MPs with biofilm (MPs + BF, figure 3) and 3) contaminants controls (without MPs). The adsorption of contaminants on MPs was obtained in the following way:

1. Weigh 20 mg MPs into each vials. Add 20 mL of filtered water. Spike with contaminants.
2. Collect MPs with biofilm attached by gently pouring/pipetting the solution containing the MPs through a 100 µm celltric filter. Back wash into a new 20 mL borosilicate vial with 20 mL filtered water. Spike with contaminants
3. Pipette 20 mL of filtered zebrafish water into each borosilicate vials and spike with contaminants

Finally, after 24 h incubation, aqueous and MPs phases can be collected and used for exposure to test organisms.

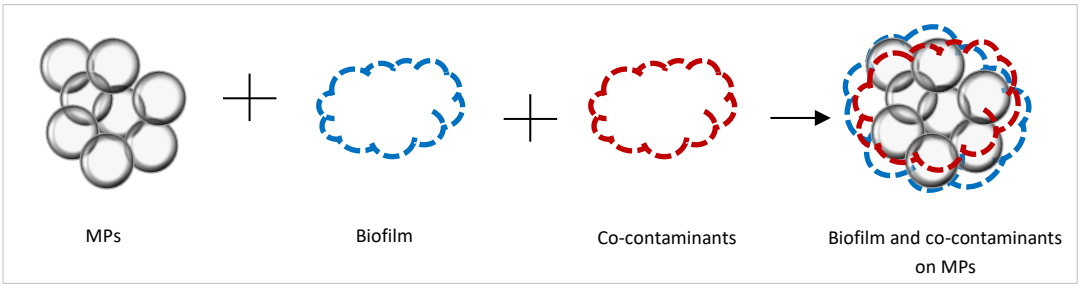

Figure 3: Schematic representation of biofilm and co-contaminants.

2.4 Evaluation of biofilm on microplastics

In order to evaluate the presence of a biofilm on MPs surface, formation of biofilm on MPs has been evaluated by crystal violet an metagenomic analysis. MPs with biofilm have been fixed in NBF and stained with crystal violet in order to stain and differentiate microbial cells. For metagenomic anlaysis, DNA from microplastic particles was extracted with the Powersoil DNA Isolation kit (Mo Bio Laboratories, Inc., Carlsbad, CA, United States) according to the manufacturer's recommendations. To prepare the samples for metagenome sequencing library, the total nucleic acid extractions were quantified using a Qubit HS (high sensitivity) dsDNA kit. The library preparation was achieved using the Swift Biosciences Accel-NGS 2S Plus DNA Kit, as per the manufacturer's instructions. Using a Covaris S220 ultrasonicator, fragments were sheared to ~ 450 bp, prior to adding barcodes using the Swift Biosciences 2S dual indexing kit. The subsequent libraries were checked for fragment length using a Bioanalyser DNA 7500 chip and quantification was performed using a picrogreen fluorescence assay. Libraries were then standardized to 4 nM as verified by qPCR (Kapa Biosystems Library Quantification kit, Applied Biosciences). An equimolar pool of all samples was generated and sequenced on Illumina HiSeq 2500 rapid runs (10-11 pM: V2 rapid sequencing chemistry), yielding reads of 251 bp paired-end sequences.

The metagenomic sequence analyses were done using Shotgun sequencing reads, which were assessed for quality, and adapters were removed using the Trim Galore package (v0.6.4_dev). Low-quality ends were removed from sequences with a phred score lower than 20. Paired reads were merged using PandaSeq (v2.11), using default settings. The resulting contigs were randomly sub-sampled, which resulted in 2.2 million reads per sample. Metagenome merged reads were de-novo assembled by passing the merged reads into megahit (v1.2.9), thus generating meta-contigs. Taxonomic identification of the adapter trimmed reads was performed by comparison to the Maxikraken2 database 38, using the Kraken2 package (v2.0.8_beta) and visualized as Sankey plots using the Pavian Package (Breitwieser, 2016) in R (v1.2.0). Gene annotation of the Meta-contigs was conducted using Prokka (1.13), and these were examined for functional identification using Microbe annotator (v2.0.5) (Seemann, 2014; Ruiz-Perez et al., 2020).

3. Conclusions

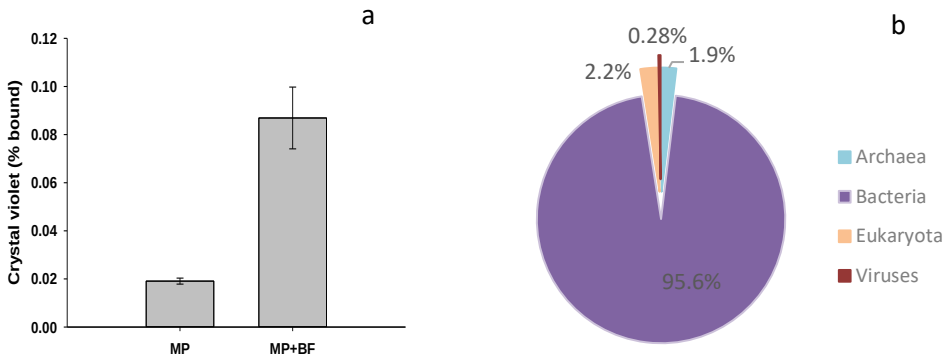

Figure 4: Evaluation of biofilm composition

The evaluation of biofilm on MPs through staining with Crystal violet analysis showed microbial growth on MPs (figure 4a). Taxonomic assignment of shotgun metagenomic merged reads revealed that the biofilm on the MPs was mainly composed of Bacteria (95.6 %), whereas only a small fraction of reads were associated with Eukaryota (2.2 %), Archaea (1.9 %), and Viruses (0.2 %) (figure 4b). The conclusions of this technical reports highlight that the methods used for biofilm formation can create realistic conditions in mesocosm experiments useful for understanding biofilm influence on contaminants bioavailability to model organisms.

4. References

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