Supplementary Information (SI) for Environmental Science: Advances.

Wastewater-induced Microplastic Biofouling in Freshwater: Role of Particle Size and Flow Rate

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1. Synthetic Water Preparation

Table S1: Preparation of Synthetic Hard Reconstituted Water (1 L)

Step	Component	Quantity	Notes
Initial Solution	Deionized Water	900 mL	Added to a cleaned Glass Bottle.
	Magnesium Sulphate ($MgSO_4$)	120 mg	
	Sodium Bicarbonate ($NaHCO_3$)	192 mg	
	Potassium Chloride (KCl)	80 mg	
			Aerate overnight to ensure complete dissolution
Calcium Solution	Calcium Sulphate Dihydrate ($CaSO_4 \cdot 2H_2O$)	120 mg	Dissolved in 100 mL deionized water
Combination	Combined Solutions	1 L total	Mix thoroughly to obtain homogeneous solution

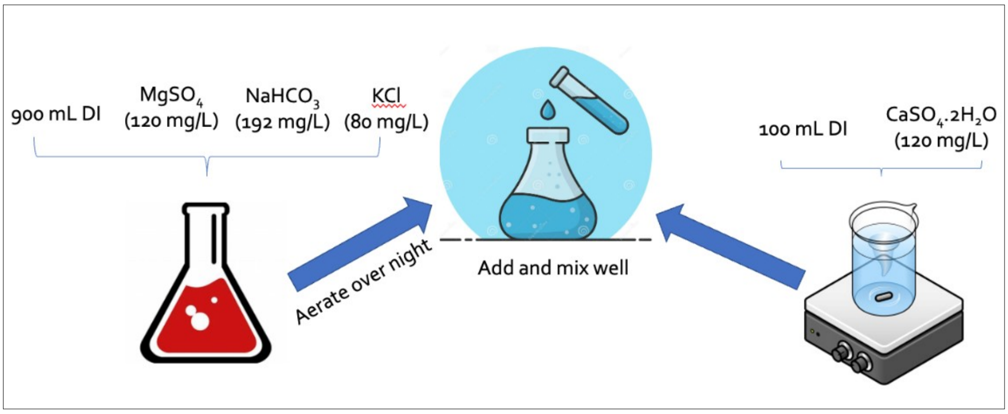

Figure S1: The preparation process of 1 L synthetic water based on the USEPA 2002 method.

Table S2: Nutrient Composition for Microbial Growth

Nutrient Source	Compound	Concentration	Purpose	References
Carbon Source	Glucose ($C_6H_{12}O_6$)	1000 mg/L	Provides carbon for microbial growth	[1]
Nitrogen Source	Ammonium Chloride (NH_4Cl)	100 mg/L	Supplies ammonium ions (NH_4^+)	
Phosphorus Source	Diammonium Phosphate ($(NH_4)_2HPO_4$)	10 mg/L	Provides phosphate ions (PO_4^{3-}) for microbial growth	[2]
C/N/P Ratio	100/10/1	-	Balances growth and inhibits excessive EPS production	[3]
Natural Organic Matter (NOM)	Suwannee River NOM	2 mg/L	Introduces complex organic compounds	[4]

NOM: Natural Organic Matter; EPS: Extracellular Polymeric substances

2. Microplastics Characteristics

PE microbeads of two distinct sizes—small (180-200 µm) and larger (3-4 mm)—were purchased from Cospheric LLC (Somis, CA, USA). The microbeads were selected based on their high purity and availability in the desired size ranges.

2.1. Microscopic Characteristics

2.1.1. Optical microscopy

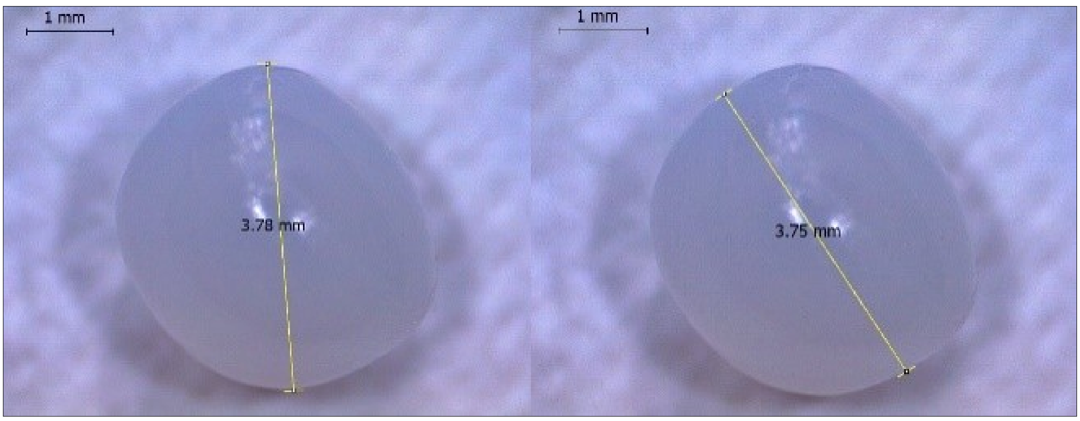

Figure S2: Optical microscopic images of polyethylene microbead (3-4mm), showing size distribution.

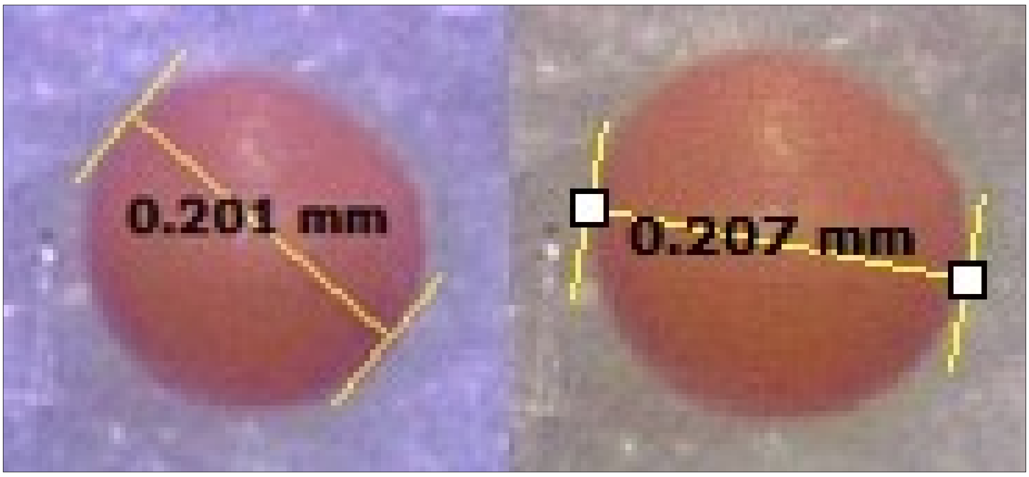

Figure S3: Optical microscopic images of polyethylene microbead (180-200 µm), showing size distribution.

2.1.2. Scanning Electron Microscopy

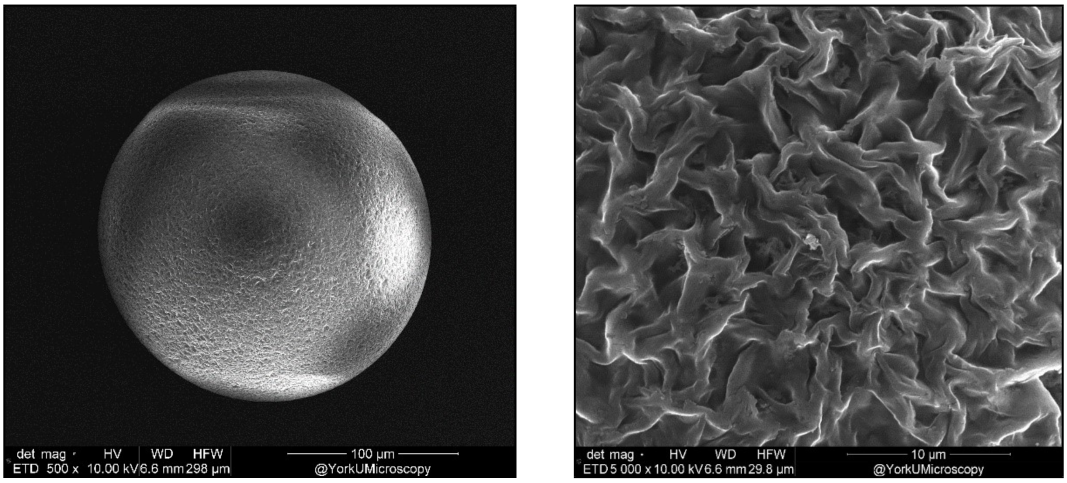

Figure S4: SEM images of polyethylene microbead (180-200 µm), showing size distribution and surface morphology.

2.2. Spectroscopic Characteristics

The Fourier Transform Infrared (FTIR) spectra of the polyethylene (PE) microbeads confirm their high purity and chemical stability. Key absorption peaks characteristic of PE, including the CH_2 asymmetric stretching at 2914 cm^{-1}, CH_2 symmetric stretching at 2847 cm^{-1}, the bending vibrations of CH_2 at 1472 cm^{-1} and the rocking motions at 719 cm^{-1}. These stable peak positions and intensities shows that the microbeads used in our experiments were of high purity PE.

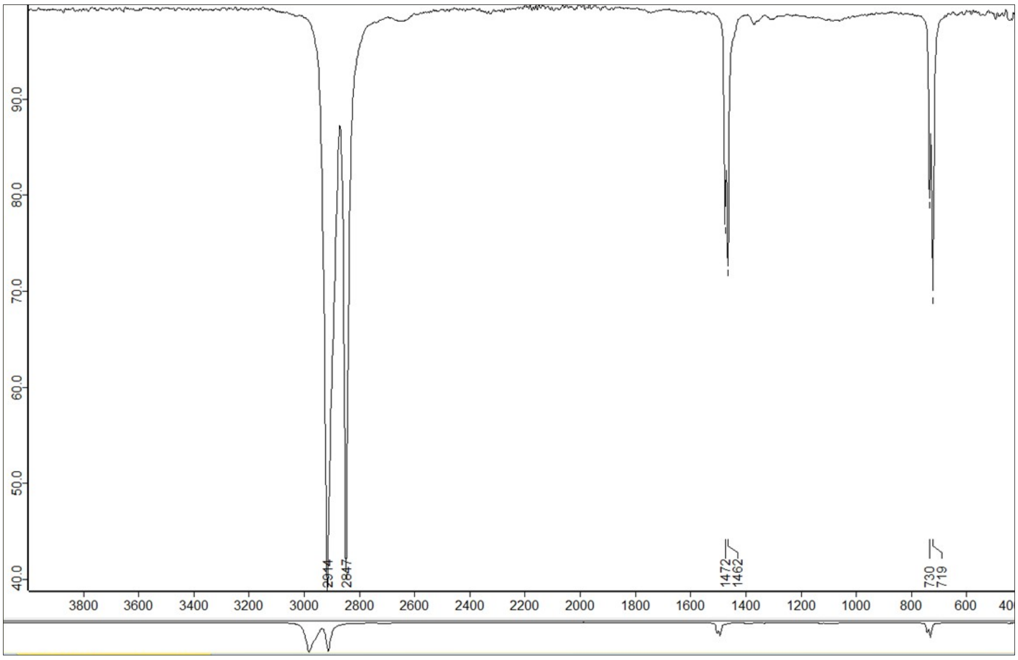

Figure S5: FTIR spectra for polyethylene microbead (180-200 µm).

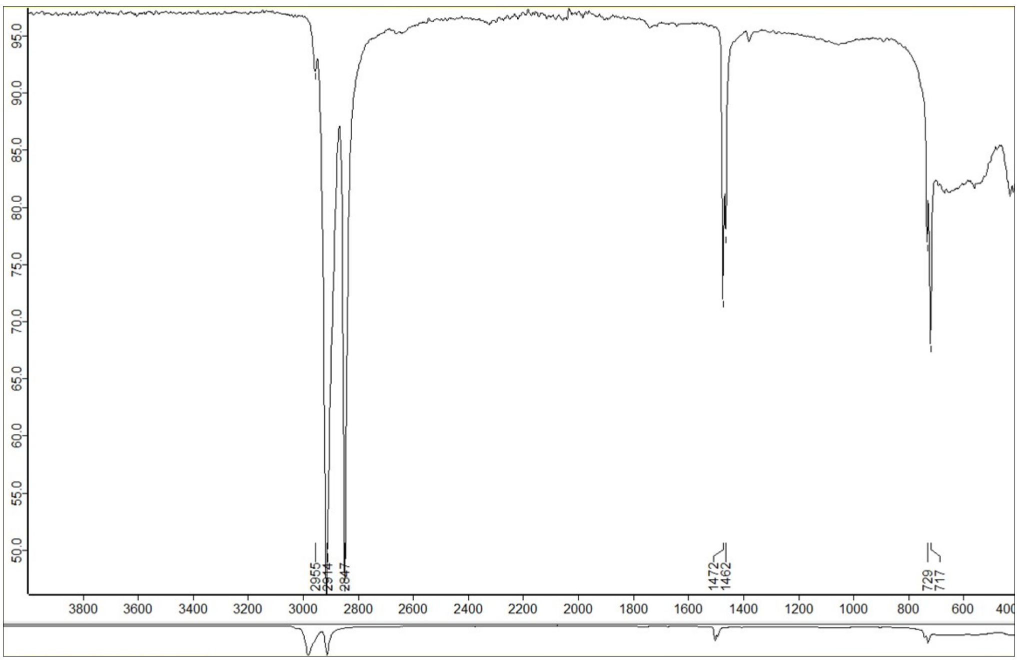

Figure S6: Figure S5: FTIR spectra for polyethylene microbead (3-4mm).

3. Hydrodynamic calculations

Given the cylindrical flow channel dimensions:

Diameter = 0.076 m; Height = 0.08 m

Cross-sectional area = $\pi*(d/2)^2 = 3.14*(0.076/2)^2 = 0.004545\ m^2$

3.1. Average Flow velocity (υ):

$$(\upsilon) = Q/A$$

- 65 ml/min

  Flow velocity (υ)= Q/A = $(6.5\times10^{-5}\ m^3/s) / (0.004545\ m^2) = 0.238\ m/s$

- 50 ml/min

  Flow velocity (υ)= Q/A = $(5\times10^{-5}\ m^3/s) / (0.004545\ m^2) = 0.11\ m/s$

- 35 ml/min.

  Flow velocity (υ)= Q/A = $(5\times10^{-5}\ m^3/s) / (0.004545\ m^2) = 0.077\ m/s$

The selected flow rates and corresponding average flow velocities were found to be within a range for mimicking typical outflow conditions in nearly stagnant freshwater bodies, such as lakes. Similar hydrodynamic conditions can be generated by wastewater treatment plant discharges, further validating the suitability of the chosen parameters for simulating such environments.

References

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3 I. Rahman, 2013.
4 W. Liu, S. A. Andrews, M. I. Stefan and J. R. Bolton, *Water Res.*, 2003, **37**, 3697–3703.