Water Workshop 2025

Advancing Methodologies for Assessing Protein–Microplastic Interactions in Simulated Intestinal Environments

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Microplastics

18. Septembar 2025. Novi Sad

Interreg Co-funded by the European Union
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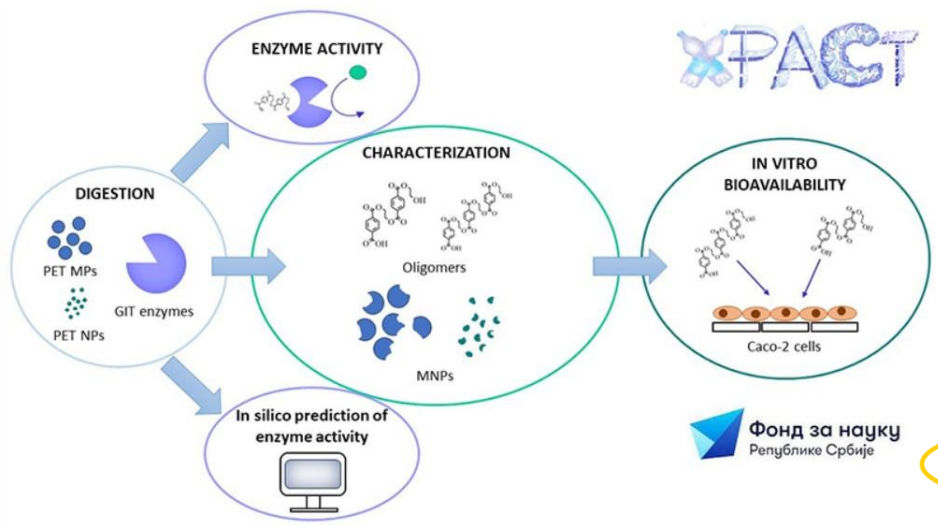
Introduction

EXploration of PETase side Activity of digestive enzymes of human gastrointestinal tract acting on micro- and nanoplastics: mode of action and products CharacTerization

Acronym: XPACT



Participating Scientific and Research Organizations (SROs):

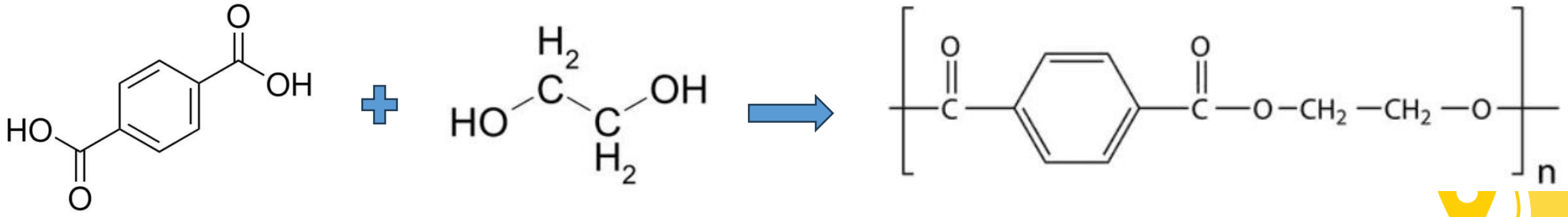
* University of Belgrade, Faculty of Chemistry (UBFC)
* Institute of Chemistry, Technology and Metallurgy (ICTM)

Principal Investigator (PI): Tanja Cirkovic Velickovic

Good neighbours creating **common future**

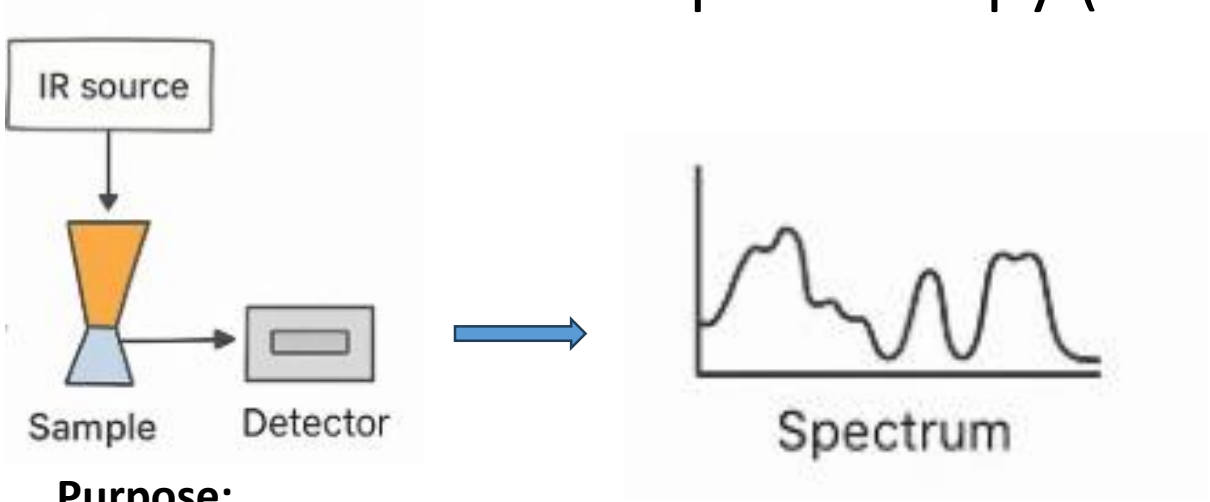
Introduction

- Microplastic size distribution: mesoplastics (5 mm - 25 mm), microplastics (1 µm - 5 mm), and nanoplastics (less than 1 µm).
- Polyethylene terephthalate - Terephthalic acid (TPA) + Ethylene glycol (EG)



- Enzymatic degradation of PET
- Hydrolases - esterases, lipases

Introduction - FTIR Spectroscopy (Fourier Transform Infrared Spectroscopy)



Purpose:

- Identify chemical bonds and functional groups in molecules
- Analyze solids, liquids, or gases

Principle:

- Molecules absorb infrared (IR) light at specific wavelengths
- Absorption causes vibrational transitions → "molecular fingerprint"

ATR-FTIR (Attenuated Total Reflectance)

- Specialized FTIR technique
- IR light reflects internally on the crystal in contact with the sample
- Measures the surface of the sample directly without complex preparation

How it Works:

- IR light passes through the ATR crystal into the sample
- Detector measures absorbed wavelengths
- Fourier Transform converts raw data to IR spectrum (absorbance vs. wavenumber)

Key Features:

- Peaks correspond to functional groups (e.g., O–H, C=O, C–H)
- Non-destructive, fast, minimal sample preparation
- Widely used in chemistry, materials, forensic science, and biochemistry

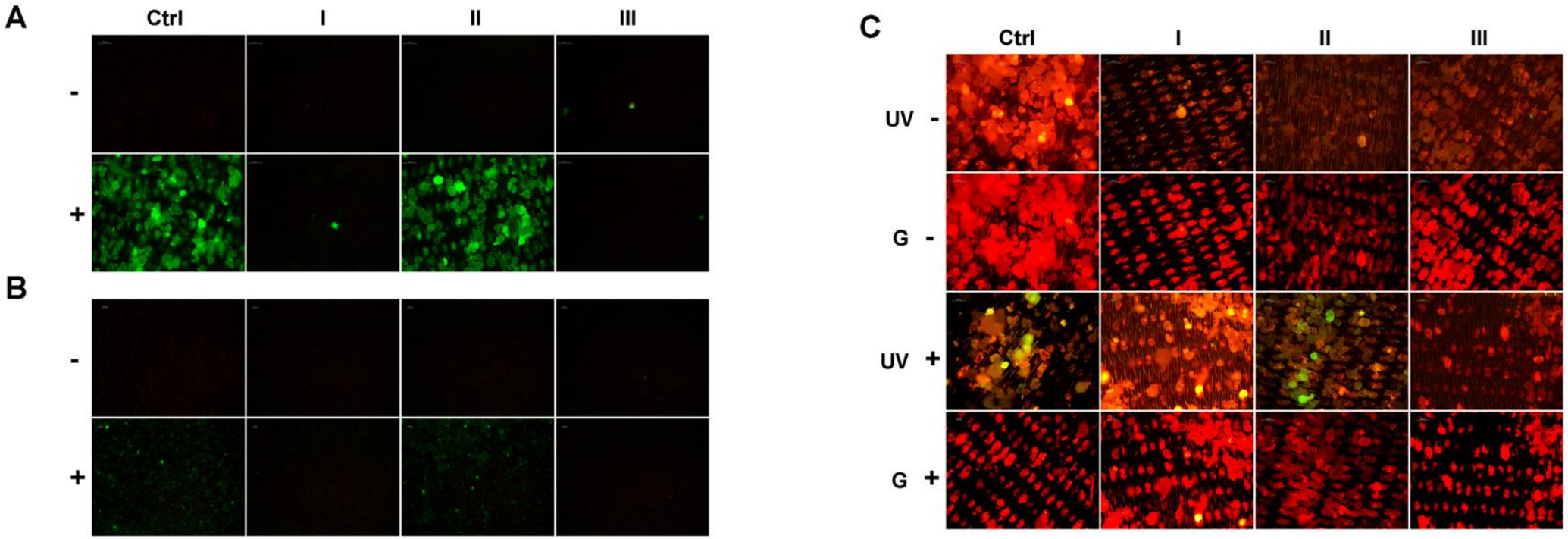


Figure 1. Fluorescence images of PET MPs with (Ctrl+) or without (Ctrl-) BSA-AF488 hard corona acquired with 10 x (A) and 4 x maginifcation (B), and after staining with Nile red (C) following treatment with three clean-up protocols: I (10% SDS + 15% H_2O_2); II (2 × 30% H_2O_2); III (15% H_2O_2 + 10% KOH). Images were acquired with 10× magnification after excitation at 340–380 nm (UV filter for BSA-AF488) and/or 527.5–552.5 nm (G – green filter for Nile Red).

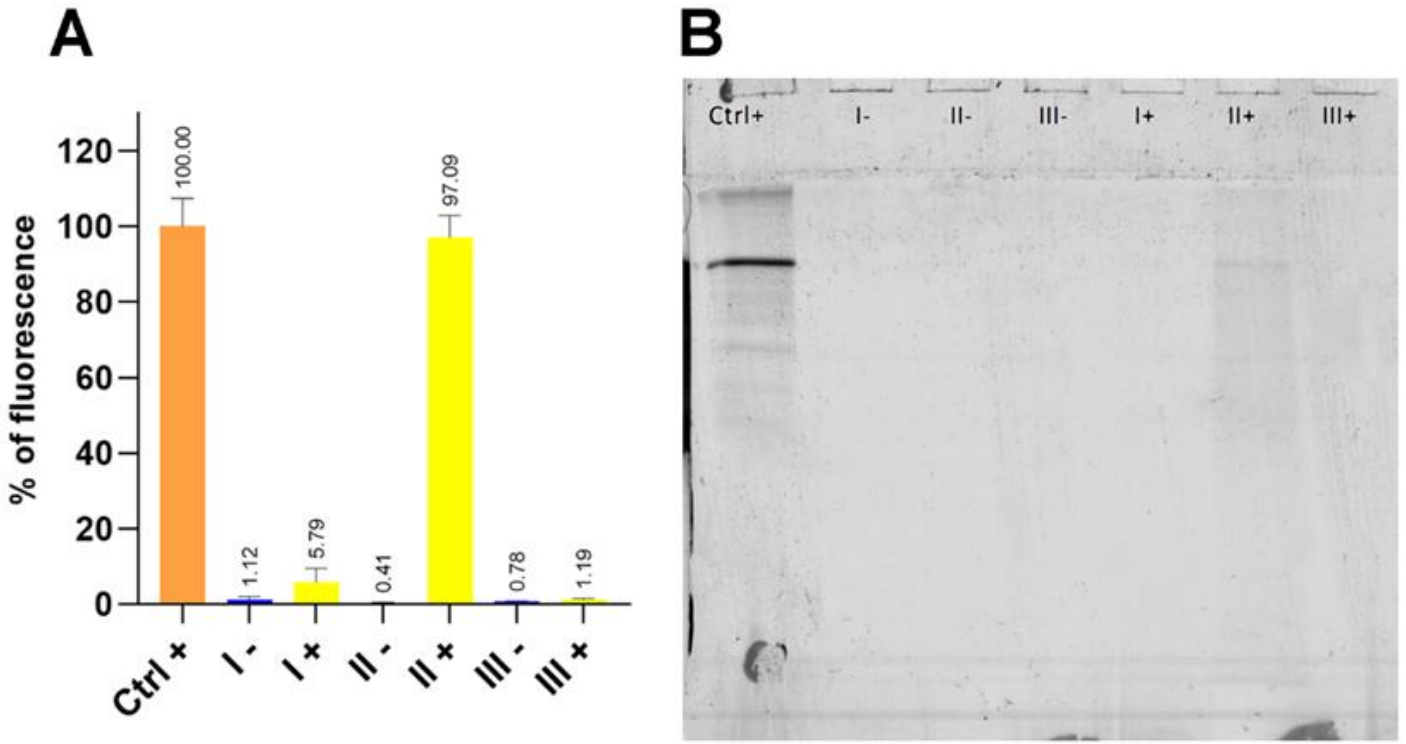
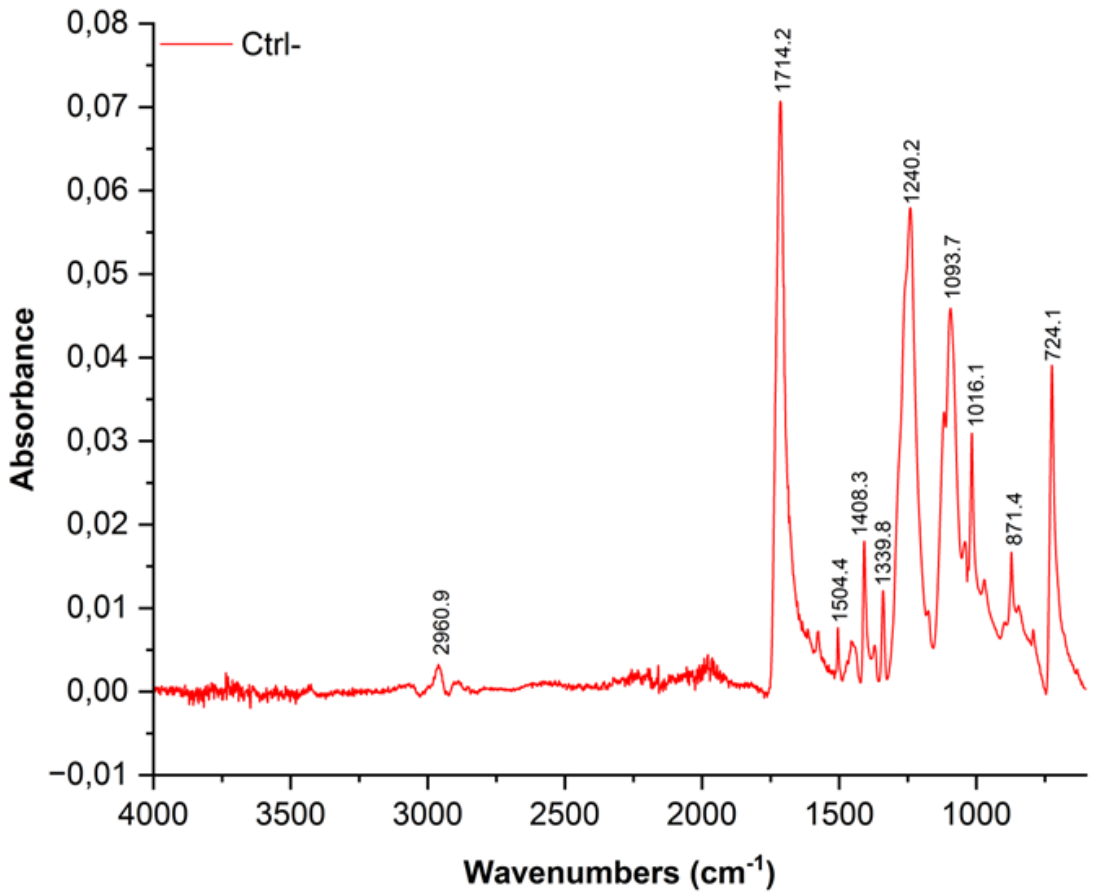


Figure 2. Fluorescence intensity (%) (A), and fluorescent image of the gel after reducing SDS-PAGE (B) of PET MPs with (+) or without (-) BSA-AF488 hard corona following treatment with three clean-up protocols: I (10% SDS + 15% H_2O_2); II (2 × 30% H_2O_2); III (15% H_2O_2 + 10% KOH). Ctrl+ - Positive control (PET MPs with BSA-AF488 hard corona without treatment. The fluorescence intensity was normalized to the mass of the weighed PET MP particles. 30 µL of each sample was loaded into each well.



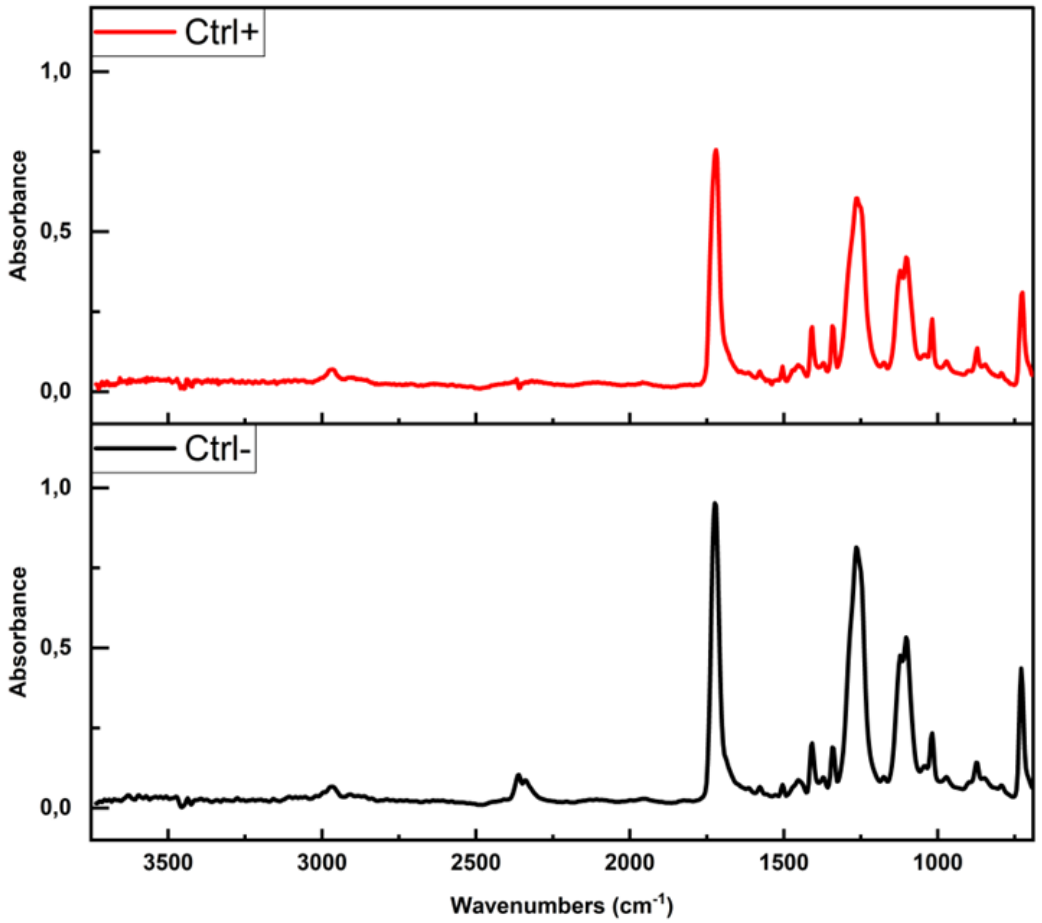


Figure 3. ATR-FTIR spectra of PET MPs with BSA-AF488 hard corona compared to untreated PET MPs.

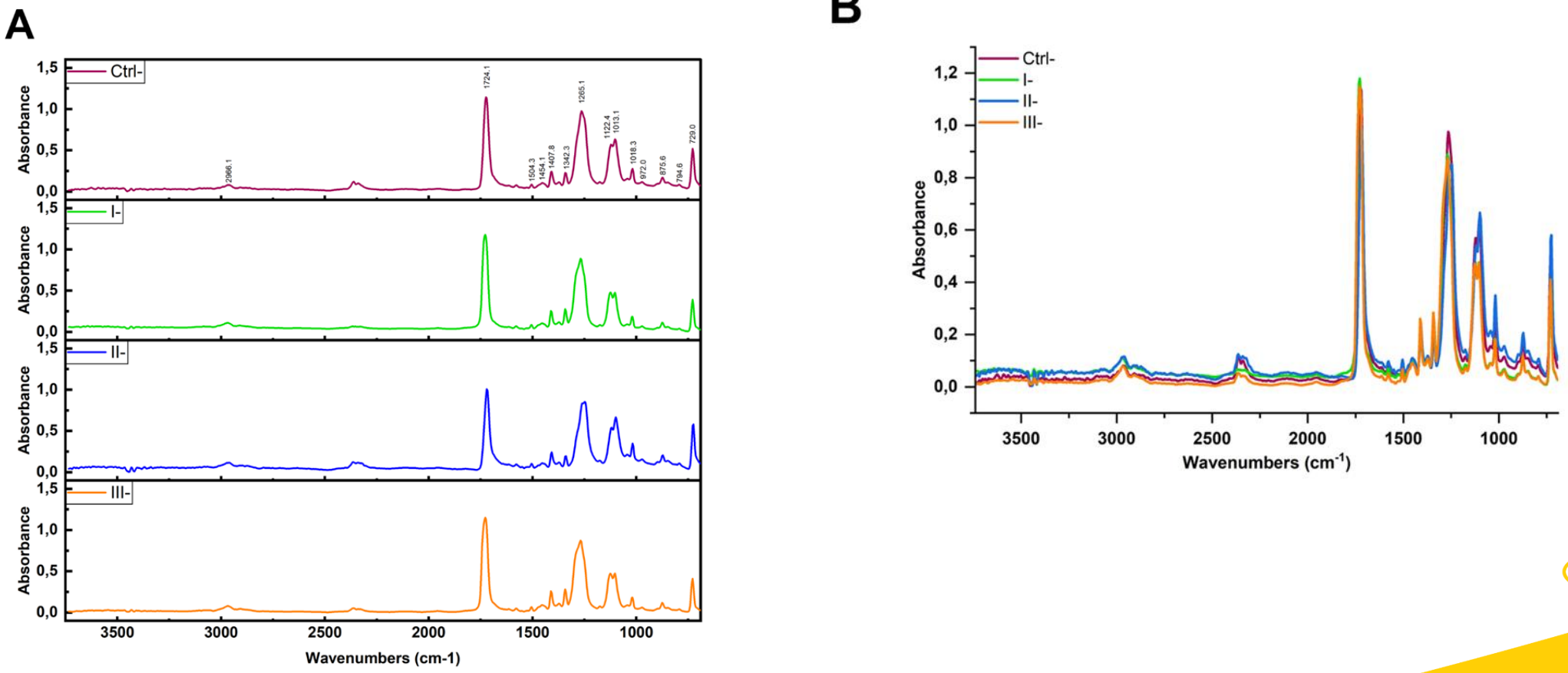


Figure 4. ATR-FTIR spectra (A) and overlaid spectra (B) of PET MPs incubated without BSA-AF488 and treated with different clean-up protocols: I-(10% SDS + 15% H_2O_2), II- (2 × 15% H_2O_2), and III- (15% H_2O_2 + 10% KOH), compared to untreated PET MPs (Ctrl-).

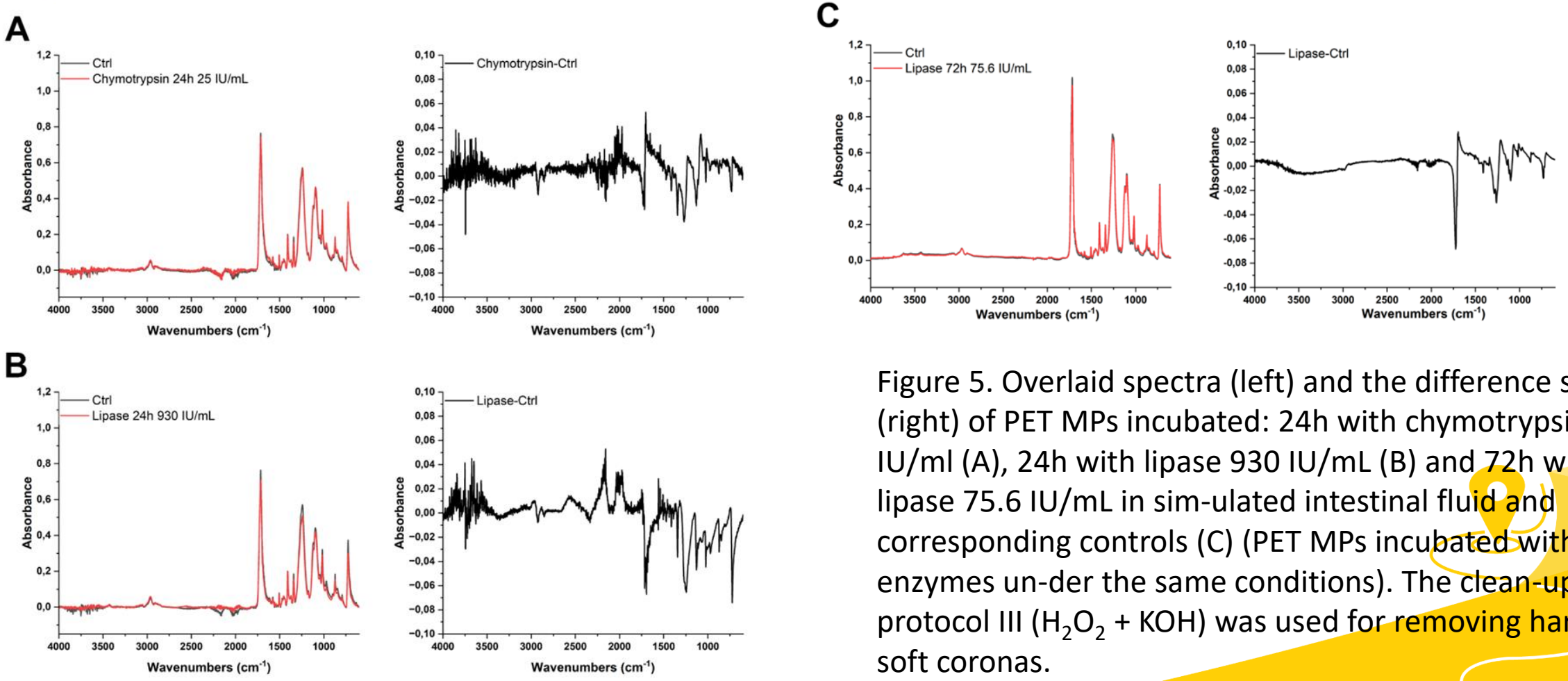
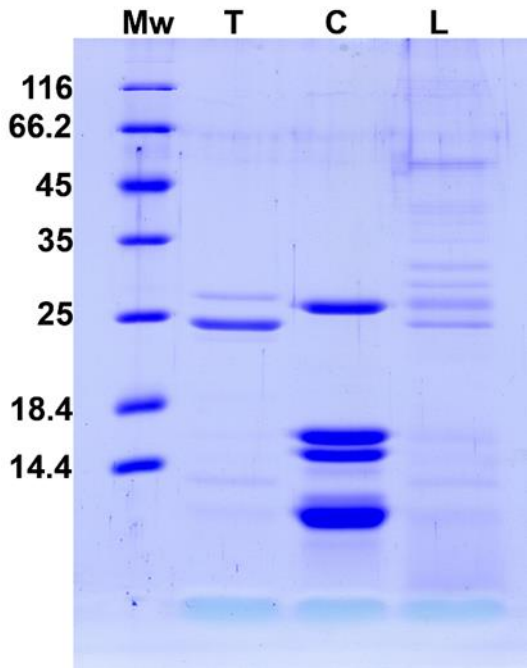


Figure 5. Overlaid spectra (left) and the difference spectra (right) of PET MPs incubated: 24h with chymotrypsin 25 IU/ml (A), 24h with lipase 930 IU/mL (B) and 72h with lipase 75.6 IU/mL in sim-ulated intestinal fluid and corresponding controls (C) (PET MPs incubated without enzymes un-der the same conditions). The clean-up protocol III (H_2O_2 + KOH) was used for removing hard and soft coronas.



ENZYME	ACTIVITY	
Lipase from porcine pancreas, cat.no. L3126-100G, pcode 1003455012, Source SLCN3698	28 U/mg	
Pepsin (XPACT), cat.no. P7012-1G, Source SLCR2106	1418 ± 43 U/mg	
	LIPASE ACTIVITY	**TRYPSIN ACTIVITY**
Pancreatin (IMPTOX), cat.no. P1750-100G, Pcode 1003351803, source SLCK2763 (solubility in water 7 mg/ml)	25.62 U/mg	1.13 ± 0.07 U/mg
Pancreatin, cat.no. P7545-100G, lot 0000344645, 0000324607 (solubility in water 20 mg/ml)	96.8 U/mg	6.99 ± 0.58 U/mg

Figure 6. SDS-PAGE of digestive enzymes used in the experiments on 14% polyacrylamide gel. Mw – molecular weight marker; T – trypsin; C – chymotrypsin; L-lipase.

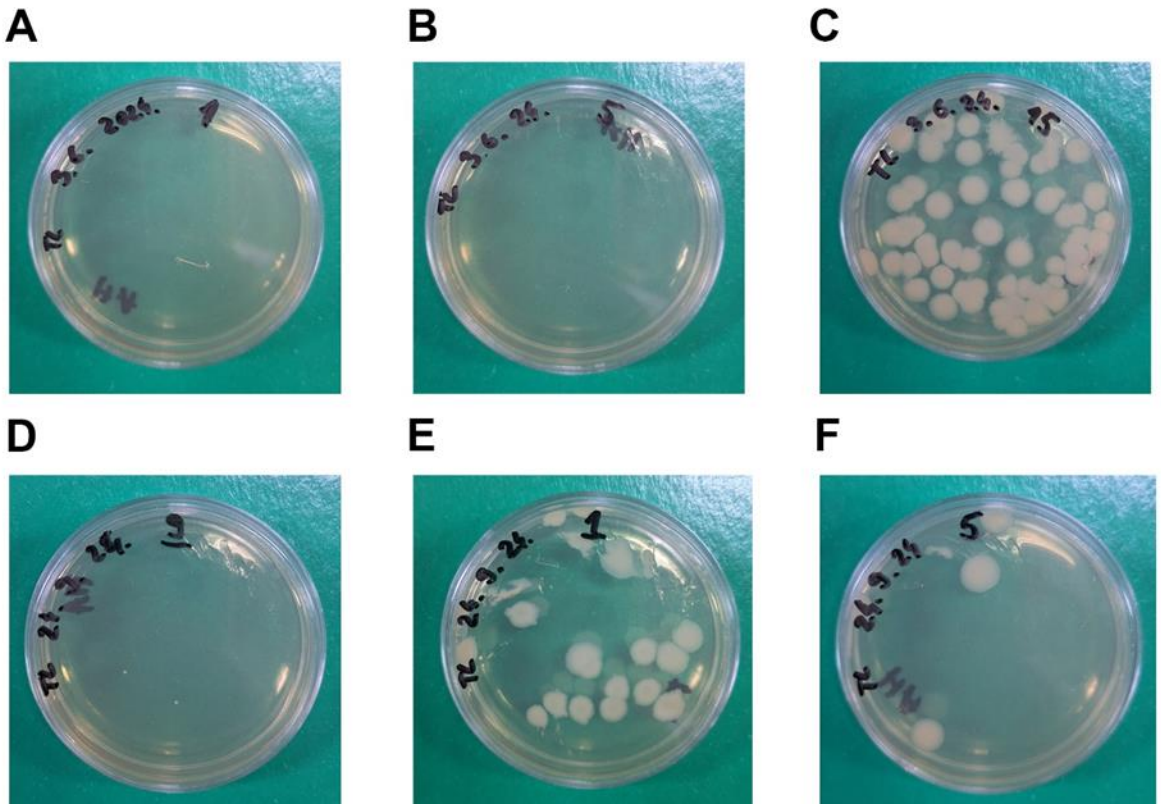


Figure 7. Testing of microbial growth for PET MP samples incubated with intestinal enzymes. A – SIF after 24 h; B – 25 U/mL chymotrypsin after 24 h; C – 2000 U/mL lipase after 24 h; D – SIF after 72 h; E – 50 U/mL chymotrypsin after 72 h; F – 200 U/mL trypsin after 72h.

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Thank you!

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