

Identification and Quantification of Microplastics in Wastewater Using Focal Plane Array-Based Reflectance Micro-FT-IR Imaging

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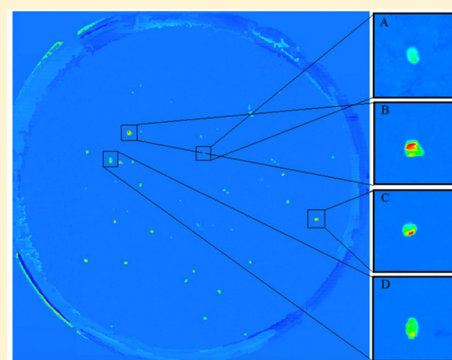
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S Supporting Information

ABSTRACT: Microplastics (<5 mm) have been documented in environmental samples on a global scale. While these pollutants may enter aquatic environments via wastewater treatment facilities, the abundance of microplastics in these matrices has not been investigated. Although efficient methods for the analysis of microplastics in sediment samples and marine organisms have been published, no methods have been developed for detecting these pollutants within organic-rich wastewater samples. In addition, there is no standardized method for analyzing microplastics isolated from environmental samples. In many cases, part of the identification protocol relies on visual selection before analysis, which is open to bias. In order to address this, a new method for the analysis of microplastics in wastewater was developed. A pretreatment step using 30% hydrogen peroxide (H₂O₂) was employed to remove biogenic material, and focal plane array (FPA)-based reflectance micro-Fourier-transform (FT-IR) imaging was shown to successfully image and identify different microplastic types (polyethylene, polypropylene, nylon-6, polyvinyl chloride, polystyrene). Microplastic-spiked wastewater samples were used to validate the methodology, resulting in a robust protocol which was nonselective and reproducible (the overall success identification rate was 98.33%). The use of FPA-based micro-FT-IR spectroscopy also provides a considerable reduction in analysis time compared with previous methods, since samples that could take several days to be mapped using a single-element detector can now be imaged in less than 9 h (circular filter with a diameter of 47 mm). This method for identifying and quantifying microplastics in wastewater is likely to provide an essential tool for further research into the pathways by which microplastics enter the environment.



Microplastic pollution is a topic of increasing concern to society.¹ The term “microplastics” was introduced approximately a decade ago to describe small synthetic plastic particles with an upper size limit of 5 mm.² This size category also includes microscopic fragments in the micrometer size range.^{2,3} A global survey of shorelines by Browne et al.⁴ found microplastic particles within all sampled locations. Moreover, Moore et al.⁵ estimated that two Californian rivers could transport over 2 billion small (<5 mm) plastic particles to the ocean over a 3 day period. Nowadays, industrial and domestic products have become two of the most rapidly growing sources of microplastic particles entering aquatic environments. They include polymeric fibers released by washing of synthetic clothing (Browne et al.⁴ found that >1900 microplastic fibers are released in a single wash of a single synthetic garment) and also hand, body, and facial cleansers.^{2,6,7} For example, the majority of facial cleansers list polyethylene as an ingredient, present in forms described as “microbeads”, “microbead formula”, or “microexfoliates”.⁸ This increased use of domestic products containing microplastic particles is particularly concerning as such particles will be found in the environment

with unknown consequences in the long-term.^{8–10} Other less-understood factors, such as plastic abrasion during dishwashing and the ability of plastic fragments to enter sewage systems during rainfall events, could also contribute to the presence of microplastics in water bodies.

If microplastics are entering rivers through wastewater treatment facilities, this may give rise to environmental and health concerns. Since plastics have been shown to harbor distinct microbial communities, there is a risk of introducing new and potentially pathogenic taxa from wastewater environments to freshwater (and subsequently, marine) habitats.^{11–13} In addition, the size and nature of these particles mean that they can enter food webs,^{14–20} particularly through filter feeders, and microplastic fragments have already been observed in the gut cavities of species such as *Mytilus edulis* and *Nephrops norvegicus*.^{21–23}

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In a review of freshwater microplastic studies by Eerkes-Medrano et al.,²⁴ the authors highlighted key areas that require further research, including the need for improved methodologies for detecting microplastics and identifying key microplastic transport pathways (which are likely to include wastewater).²⁵ However, despite several recent studies focusing on the presence of microplastics within freshwater environments,^{4,5,12,26–28} there is an absence of research into the presence of these contaminants in both municipal and industrial wastewater. In addition, Wagner et al.²⁹ gave particular emphasis to the importance of enhancing our knowledge of microplastic pollution within freshwater ecosystems, including the role that these environments are likely to play in microplastic transport.

Despite the potential adverse impacts of microplastics on both marine biota and human health, the methodology for isolating, detecting, identifying, and quantifying microplastics in environmental samples is still lacking in precision. Moreover, no standard operating protocol currently exists for detection of these rapidly emerging pollutants.³⁰

One of the fundamental challenges before the identification of microplastics is their separation from the initial matrix. In a review by Hildalgo-Ruz et al.,³⁰ in order to isolate microplastics from sand-based samples, most previously published studies rely on density separation using sodium chloride (NaCl), sodium iodide (NaI), or sodium polytungstate (SPT) solutions. However, plastics such as polyvinyl chloride (PVC) and polyethylene terephthalate (PET) have a density greater than saturated NaCl solution (the densities of PVC, PET, and saturated NaCl solution are 1.14–1.56, 1.32–1.41, and 1.2 g cm⁻³, respectively). Moreover, denser salt solutions (such as NaI or SPT) are frequently considered too expensive to be used for the bulk separation of large sample volumes.³¹ Alternative techniques such as elutriation^{31–33} have proven successful for isolating microplastics from marine sediments. However, for samples with a high concentration of organic matter, such as wastewater, the technique is far less useful, as naturally occurring particles within these matrices will exhibit densities similar to those of microplastics. Therefore, an alternate approach is needed to prepare these samples for analysis.

Cole et al.³⁴ have presented different ways in which organic matter in samples could be removed by acids, alkalis, and enzymatic digestion, with enzymatic digestion constituting the most suitable method for purifying plankton-based samples. In addition, Nuelle et al.³³ recently investigated the practicality of using several solvent types to remove organic matter from samples without damaging the polymer fragments. In this study, the treatment of sandy sediment retentates with 35% of hydrogen peroxide (H₂O₂) for 7 days removed the majority of biogenic organic matter on a membrane filter, making the samples easier to analyze by microscopy.

Following separation of microplastics (and other particulate matter) from environmental samples, the majority of previously published studies have relied on a visual sorting step to select putative plastic particles for further analysis.³⁰ This approach is open to bias since, even when using a microscope, visually differentiating between microplastics and other extracted organic and inorganic matter of similar size is very difficult. In addition, this approach also requires a considerable amount of researcher time and effort. Spectroscopic approaches can provide a more reliable tool for the identification of the plastic fragments. Techniques employed for the identification of

microplastics have included, for example, sequential pyrolysis-gas chromatography coupled to mass spectrometry (pyrolysis GC/MS),³⁵ Raman spectroscopy^{23,36,37} and, most commonly, Fourier-transform infrared (FT-IR) spectroscopy.^{2,38–42} In comparison with visual sorting using a microscope, FT-IR spectroscopy has been shown to detect a significantly higher number of microplastics within environmental samples.⁴³

FT-IR spectroscopy is a desirable technique for the identification of microplastics for several reasons, including efficiency of cost, reliability, and ease of use. Additionally, infrared spectroscopy is nondestructive^{44–47} and the functional groups of different plastic types have already been established.^{48–54} Because of these attributes, FT-IR analyses have been successfully used for identifying microplastics in both sediment and water samples.^{2,38–40,42}

FT-IR microspectroscopy (micro-FT-IR) is a tool that combines FT-IR spectroscopy with microscopy. By using micro-FT-IR, infrared bands can now be identified and compared with increasingly smaller samples due to the improvement in spatial resolution.⁵⁵ Micro-FT-IR is particularly useful as it requires little sample preparation and can be used to identify microplastics directly on membrane filters.⁴¹ Micro-FT-IR analyses of plastics can be performed in either transmission or reflectance mode. Transmission mode gives high quality spectra but requires infrared-transparent substrates.^{56,57} In contrast, analyses in reflectance mode enable the rapid analysis of thick and opaque samples and are therefore highly suitable for detecting microplastics in environmental samples.⁵⁶

A previous study by Harrison et al.⁴¹ eliminated the need for visual sorting of microplastics by introducing a chemical mapping technique using reflectance micro-FT-IR spectroscopy. In this study, the measurements were performed manually or by semiautomatic molecular mapping of polyethylene fragments within sediment retentates. The manually collected spectra were obtained from randomly selected locations on the sample surface, whereas 3 × 3 mm molecular micrographs were obtained at intervals of 150 μm, using an aperture size of 15 × 15 μm. Unfortunately, with this point by point-based mapping approach, scanning an entire membrane filter would take an implausible amount of time and the use of smaller apertures would further increase the scanning time.

Recent advances in micro-FT-IR detectors now allow for the simultaneous acquisition of a higher number of spatially resolved spectra ($n \times n$ pixels) where each pixel in the detector array provides an independent infrared spectrum. This technique utilizes focal plane array (FPA) detectors and, by simultaneously acquiring thousands of spectra within minutes,^{58,59} FPA detectors could provide information about the identification of even smaller microplastic fragments on larger surface areas, without the need for the visual preselection of particles for analysis, at faster times, and without compromising spatial resolution.

In this study, we present a novel method for the analysis of microplastics in wastewater samples using FPA-based reflectance micro-FT-IR imaging and H₂O₂ pretreatment. We evaluate the effectiveness of a 30% H₂O₂ preparation step to remove the organic matter from wastewater, including the efficiency of this method at aiding sample filtration, and the effects of H₂O₂ on the FT-IR spectra of 5 plastic types commonly recorded in microplastic studies.³⁰ In addition, we present, to our knowledge, the first application and experimental validation of a FPA-based reflectance micro-FT-IR protocol for the identification and quantification of

microplastics within complex and organic-rich wastewater samples.

■ EXPERIMENTAL SECTION

Sampling. Wastewater was collected from the Severn Trent Water treatment facility in Derby, UK. Ten liters of sample wastewater was collected from the surface (top 5 cm) of the activated aerobic biological stage using a telescopic sampling pole (Telescoop, Waterra Ltd., Solihull, UK). This sample was transported back to the laboratory in a sterilized 10 L Nalgene carboy LDPE container (Thermo Scientific, UK). In order to minimize any changes in the wastewater composition during transport and storage, the sample was kept aerobic by maintaining a constant air flow. 25 mm syringe-type filters with a 0.2 μm cellulose-acetate membrane (VWR, Leicestershire, England) were placed on the air-in and air-out lines to prevent any contamination from the air.

H₂O₂ Pretreatment. To examine the effects of a H₂O₂ pretreatment, 45 mL centrifuge tubes (VWR, UK) were filled with wastewater and centrifuged at 2038g for 2 min. The pellets were immersed in 15 mL of 30% H₂O₂ (Prolabo, BDH, UK), briefly shaken, and stored for 3, 5, or 7 days. Control samples consisting of wastewater pellets were immersed in 15 mL of ultra high quality (UHQ) water for 7 days. Each treated pellet was vacuum-filtered using a Millipore vacuum filtering assembly (Millipore Corporation, Billerica, MA, USA), through 47 mm Isopore polycarbonate membrane filters with a pore size of 0.2 μm at -40 kpa. The time required to filter each treated pellet was recorded, the dry mass (mL^{-1}) of the wastewater samples was measured before and after the treatment, and photographs of the membrane filters were obtained to assess the extent to which H₂O₂ exposure removed organic matter from the sample surfaces. These experiments were performed in triplicate.

The exposure times and concentration of H₂O₂ employed in our study were based on previous research by Nuelle et al.³³ These authors demonstrated that using either 30% or 35% H₂O₂ successfully eliminated the majority of biogenic material from membrane filter surfaces within 7 days of exposure.

Effects of H₂O₂ on the FT-IR Spectrum of Microplastics. The plastic types employed in this study were polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS), nylon-6 (all Goodfellow Cambridge Ltd., Huntingdon, England) and polyethylene terephthalate (PET) (Costcutter Montgomery Q4 Natural Water plastic bottle). With the exception of PET (~ 1 mm), all microplastics were ~ 150 – 250 μm in size. A small amount (~ 0.01 g) of microplastics was applied to individual 2-ml sterile tubes (Fisher Scientific, Waltham, MA, USA) containing 1 mL of 30% H₂O₂. These were briefly shaken and left for a period of 3, 5, or 7 days, with a 7-day UHQ water control included. The peroxide was subsequently removed using a Pasteur pipet while retaining the microplastics. The plastics were manually rinsed in 1 mL of 80% (v/v) ethanol.¹¹ Once dry, the plastics were analyzed using a PerkinElmer FT-IR spectroscopy equipped with a Specac Golden Gate attenuated total reflectance (ATR) accessory, with 50 coadded scans carried out for each experimental replicate at a spectral resolution of 4 cm^{-1} and a wavenumber range of 4000 cm^{-1} –650 cm^{-1} . This process was repeated in triplicate.

In order to assess the potential for H₂O₂ exposure to affect the FT-IR spectra of each plastic type, spectral data were analyzed using the statistical package PRIMER (v. 6.1.13) with the PERMANOVA+ add-on (v. 1.0.3).^{60–62} A Euclidean

similarity matrix was constructed using normalized absorbance data (4000–650 cm^{-1}). A 2-way permutational multivariate analysis of variance (PERMANOVA)⁶¹ was performed using “plastic type” and “time of exposure” (to H₂O₂) as the factors (Type III sums of squares, 9999 permutations of residuals under a reduced model).

Initial FPA-Based Micro-FT-IR Validation. Five different microplastics (PE, PP, PVC, PS and nylon-6) were applied to a small preselected area of microscope slide (10 fragments per plastic type). This area was then imaged using a PerkinElmer Spotlight micro-FT-IR spectroscopy equipped with a mercury–cadmium–telluride focal plane array (FPA) detector (consisting of 16 gold-wired infrared detector elements). An aperture size of 25 μm \times 25 μm was used, meaning that each pixel sampled a 25 μm \times 25 μm area on the sample plane, with 2 coadded scans per pixel and a spectral resolution of 16 cm^{-1} . No transformation or postprocessing of the spectra were carried out. To distinguish microplastics from naturally occurring particles in the false-color images, 3 characteristic regions of absorbance were selected for each plastic type, with a positive identification requiring each of these bands to be present. A table showing the characteristic FT-IR peaks for each plastic type is shown in Table S1 (Supporting Information).

To validate the instrumental parameters established by this initial experiment, a second analysis was undertaken where the positions of the microplastic particles were unknown. False-color imaging was then performed using the same characteristic absorbance ranges as previously determined.

Analysis of Microplastics in Polymer-Spiked Wastewater. Ten particles of PE, PP, PS or PVC were added into a 45-ml centrifuge tube (VWR, Leicestershire, England) containing wastewater from the activated biological stage of treatment (see ‘Sampling’). The spiked wastewater samples were centrifuged at 2038g for 2 min and the effluent was stored for filtration. The pellet was immersed in 15 mL of 30% H₂O₂ for 7 days. The pellet and effluent were then filtered using a Millipore vacuum filtering assembly as described above. The tubes used for storing the pellet and effluent were rinsed using UHQ water to ensure no visible debris were left within the tubes. Membrane filters were fully air-dried after vacuum filtration. Each membrane filter was visually imaged using the camera attachment of the micro-FT-IR instrument. For reasons of ease and speed (to avoid scanning redundant areas), each filter scan was split into 5 regions. Each region was imaged using the same parameters (25- μm aperture size, resolution of 16 cm^{-1} and 2 coadded scans). Once imaged, each region was analyzed for each plastic type and all regions were combined to construct a false-color image showing each plastic type present on the filter surface. This entire validation study was repeated in triplicate. Negative controls were established using UHQ water only, and were then prepared identically to other sample types (involving storage, H₂O₂ treatment, filtration and micro-FT-IR analysis). A control wastewater sample with no spiked microplastics was also included in this study.

■ RESULTS AND DISCUSSION

H₂O₂ Pretreatment. In this study, the main rationale for applying a H₂O₂ pretreatment step was to facilitate filtration and to improve the micro-FT-IR imaging of microplastics within wastewater samples by removing organic matter that would otherwise accumulate on the membrane filter surface.

As mentioned by Hidalgo-Ruz et al.,³⁰ the majority of previous research into the extraction of microplastics from

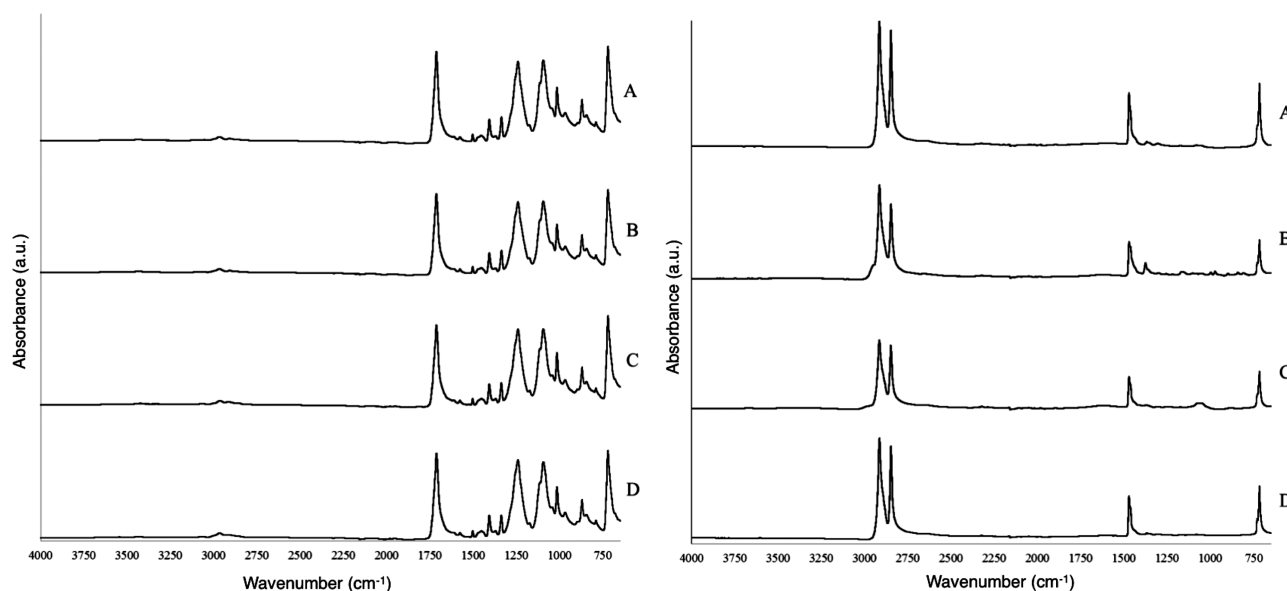


Figure 1. ATR-FT-IR spectra of polyethylene terephthalate (left) and polyethylene (right) subjected to H_2O_2 for a range of time points up to 7 days. A: 7 days; B: 5 days; C: 3 days; D: control.

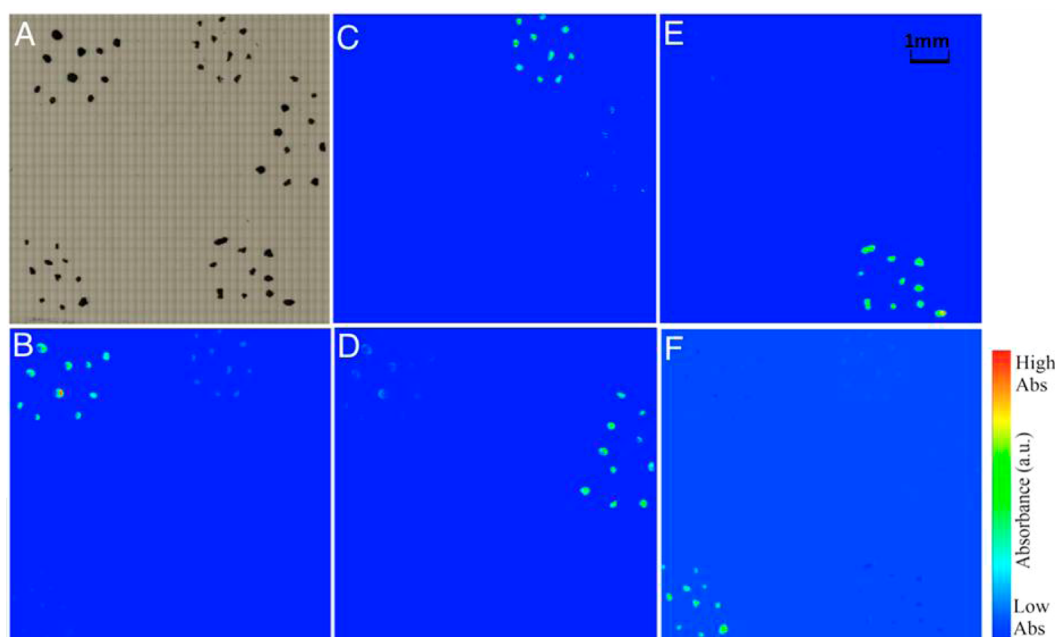


Figure 2. False color images of microplastics in known positions on a glass slide achieved using a PerkinElmer micro-FT-IR spectroscope with a FPA-based detector. Each map displays a particular polymer type; A: Visible image; B: PP; C: PVC; D: Nylon; E: PS; F: PE.

environmental samples has been concerned with sandy sediments. These types of samples propose relatively few challenges to the successful isolation of microplastics, since sands have a greater density than any plastic type.³⁰ However, Nuelle et al.³³ found membrane filters to retain a considerable amount of biogenic organic matter following the elutriation and filtration of microplastics from marine sediments, with a H_2O_2 digestion step employed as a solution to this issue. While the authors observed that the majority of the plastic particles remained unchanged in size (apart from a slight change in the size of polyethylene and polypropylene) following peroxide treatment,³³ the ability of H_2O_2 to affect the infrared spectra of microplastics was not characterized. Therefore, our study used a

7-day exposure assay to determine the impacts of H_2O_2 exposure on the FT-IR spectra of 5 different plastic types.

The efficiency of filtration was dramatically improved with use of a 30% H_2O_2 pretreatment step. Without H_2O_2 pretreatment, filtration of the centrifuged wastewater pellet (originating from a total volume of 45 mL) required a mean duration of 88.87 s ($n = 3$) at -40 kpa. After 3, 5, and 7 days of H_2O_2 pretreatment, the mean durations required for filtration were 28.38, 22.64, and 18.16 s, respectively ($n = 3$). Overall, the mean reductions in the time required for filtration (compared with control samples) were 60.49 s after 3 days, 66.23 s after 5 days, and 70.71 s after 7 days of pretreatment. These reductions in filtration time were additionally accompanied by visual evidence for a reduction in the amount of biogenic matter on

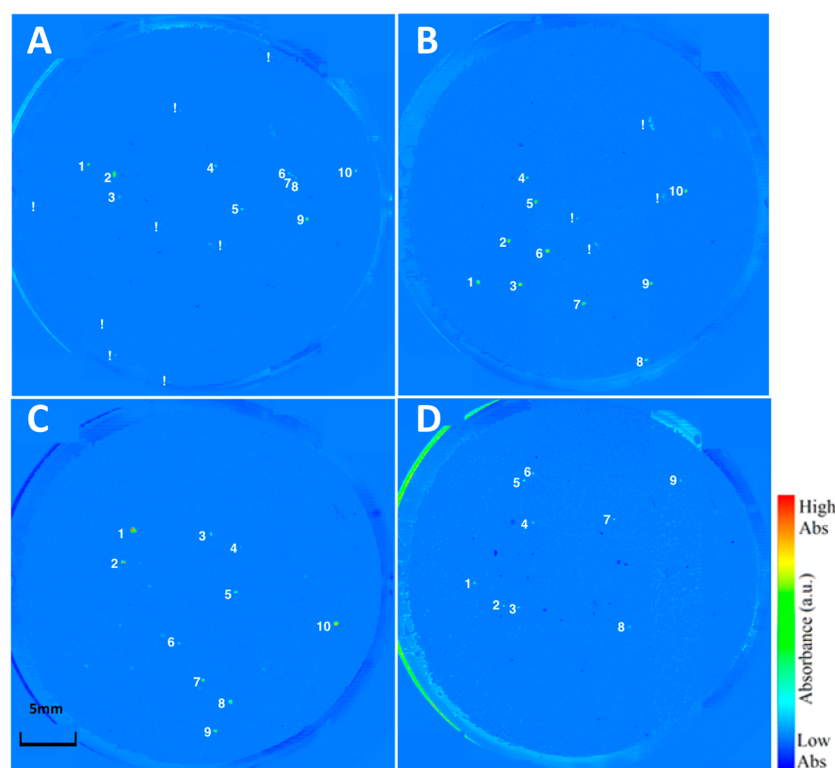


Figure 3. False color images of entire membrane filters achieved using micro-FT-IR imaging with a FPA-based detector. Each map displays a particular polymer type; A: PE; B: PP; C: PS; D: PVC. Correctly identified spiked microplastics are denoted with a number 1–10 (except in PVC where only 1–9 could be identified). Additional microplastics present in the wastewater confirmed as the correct polymer type are denoted by “!”.

the filter surface (See Figure S1 in the Supporting Information). Dry organic matter content (100 mL^{-1}) was reduced from 0.0528 g (control) to 0.0147 g after 3 days, 0.0125 g after 5 days, and 0.0090 g after 7 days of exposure to H_2O_2 (equivalent to a 83% overall reduction in organic matter content). Taken collectively, these results demonstrate that a H_2O_2 pretreatment step of approximately 7 days is optimal for the filtration of microplastics within wastewater samples. These data are in general agreement with those of Nuelle et al.,³³ who found that a 7 day postfiltration treatment using a 35% H_2O_2 solution was suitable for preparing microplastic samples isolated from sediments that primarily consisted of silicate. However, postfiltration treatments are inappropriate for the preparation of plastic samples originating from organic-rich wastewater, due to the rapid buildup of biogenic matter on the membrane filters during filtration.

In addition to the improvement in filtration time, it is also important that the FT-IR spectra of microplastics are unchanged as a consequence of the H_2O_2 pretreatment step. It was found that up to 7 day exposures to 30% H_2O_2 had no significant impact on the FT-IR spectra of the polymer types employed in our study (2-way PERMANOVA: pseudo- $F_{6,56} = 0.715$, $P = 0.658$, 9935 unique permutations). This finding demonstrates that H_2O_2 pretreatment can be used to purify microplastic samples without resulting in substantial alterations to polymer chemistry, enabling the reliable identification (and quantification) of these contaminants within environmental samples. Representative spectra of two types of microplastics over the course of 7 days of H_2O_2 treatment are shown in Figure 1. This supports our finding that the IR absorbance peaks of microplastics (including key bands selected for polymer identification; see Table S1, Supporting Information)

are unchanged by up to 7 days of exposure to H_2O_2 , with little to no alterations in peak positions or intensities across the entire examined wavenumber range ($4000\text{--}650 \text{ cm}^{-1}$).

Recent work has investigated the possibility of using enzymatic digestion, and this may offer an improvement to the use of H_2O_2 , since Cole et al.³⁴ found enzymatic digestion to remove 97% of organic matter in a plankton-rich sample. However, before such a digestion step could be combined with the method presented in this study, it would be necessary to test the effects of enzymatic treatment on the IR spectra of microplastics. The duration required for sample filtration could also be reduced by microwave digestion.⁶³ However, care would need to be taken to avoid melting or degrading the plastics, and any changes to spectral properties would also need to be investigated.

Initial FPA-Based Micro-FT-IR Validation. Figure 2 shows micro-FT-IR chemical images of the 5 microplastic types (PVC, PA, PS, PP, and PE) in known positions. All 50 microplastic particles were successfully identified, with the wavenumber regions used for identification (Table S1, Supporting Information) providing reliable results for all polymer types. The absorbance scale on the right approximately indicates the abundance of molecules absorbing the infrared radiation on a specific area. In cases where microplastic particles are discernible from the background in more than one image, the particle was identified as whichever polymer type that exhibited a greater relative absorbance signal. For example, in Figure 2B, in a map of PP, PVC is also registering a faint signal. However, the comparable signal in Figure 2C (the PVC map) is more intense for these same microplastics, and as such, these particles were identified as PVC despite their faint appearance in the PP map.

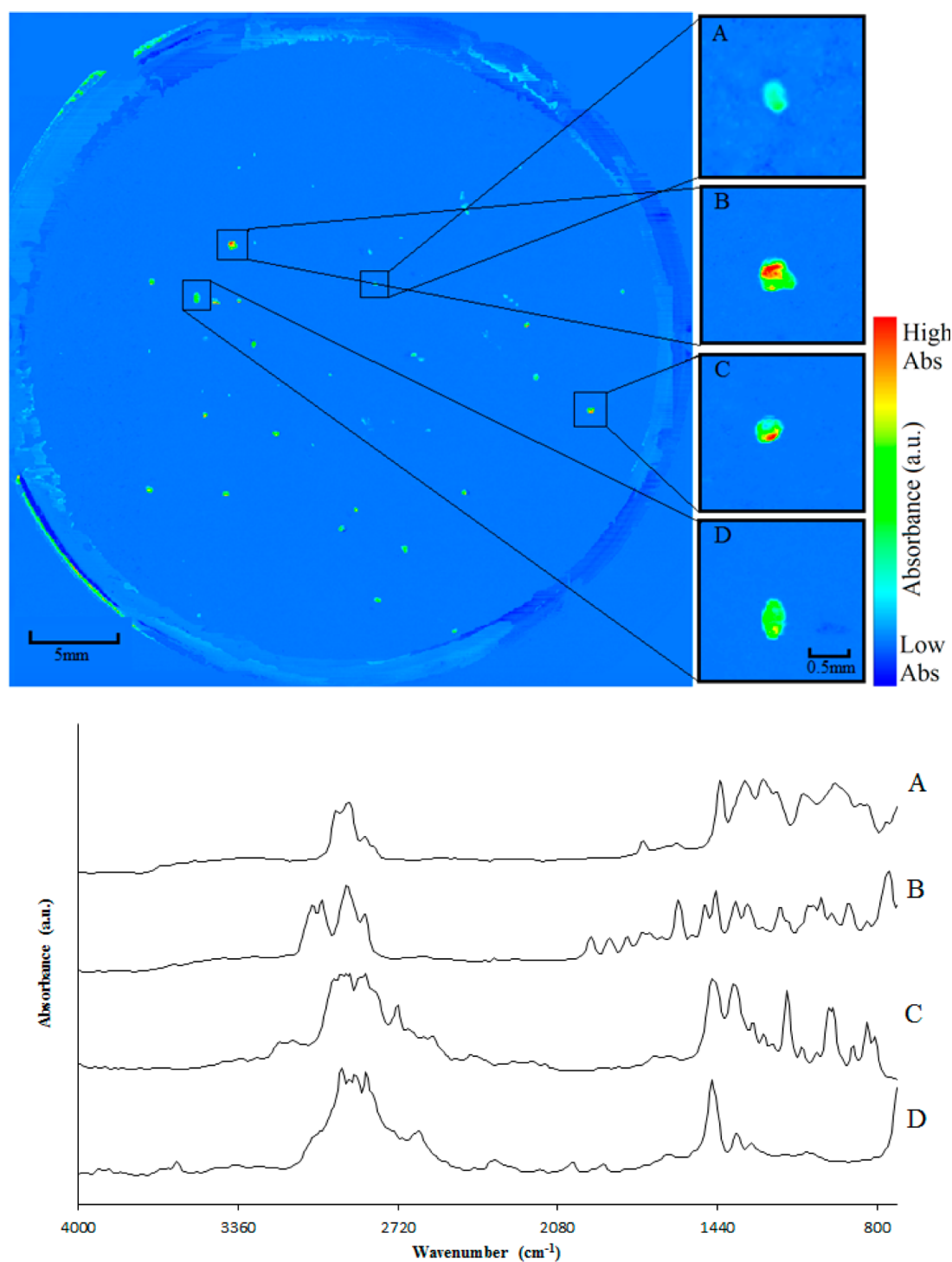


Figure 4. Combined and overlapped false-color images showing 4 different microplastic types. Four fragments of each polymer type have been selected and magnified (above). FT-IR spectra of selected and magnified microplastic fragments; these spectra are typical of plastic fragments mapped using these parameters (below). A: PVC; B: PS; C: PP; D: PE.

In order to validate the instrumental parameters established above, a second analysis was undertaken in which the microplastic particles were unknown. All 50 microplastic particles were again successfully identified, showing that it is still possible to map these 5 types of microplastics with 100% accuracy. The false-color image showing the same 50 microplastics present in Figure 2, but in unknown locations, is presented in the Supporting Information (Figure S2).

It is important to highlight that the microplastics used in this study were of a roughly spherical shape. Irregularly shaped fragments observed in the false-color images could have arisen due to the amalgamation of several particles. Indeed, accounting for this possibility is important for the successful quantification of microplastic fragments. Therefore, when a high degree of accuracy is required, visual (microscopic) investigation of the detected fragments may occasionally be necessary.

Analysis of Microplastics in Polymer-Spiked Wastewater. Figure 3 shows the false color images of individual microplastic types on an entire 47 mm membrane filter. Scans of these filters can be completed in under 9 h with one scan per pixel and in under 16 h with 2 scans per pixel. While nylon-6 was initially included in the spiked samples, recognizable spectra of this plastic type were unattainable using reflectance micro-FT-IR spectroscopy, and identifying these particles against a complex organic background was impossible (due to either impurities on the plastic surface or because microscopic analysis of these specific microspheres revealed a rough topography, that could have affected the analysis by scattering the reflected infrared light).⁴¹ Analyses in transmission mode⁶⁴ or ATR spectroscopy³⁹ could facilitate the identification of these types of microplastics by enabling the detection of absorbance bands that cannot be discerned by reflectance FT-IR microspectroscopy.

Figure 4 shows all of the imaged plastic types combined into a single image, with representative FT-IR spectra collected from individual microplastic fragments. Taken collectively, Figures 3 and 4 demonstrate that combining a H₂O₂ pretreatment step with FPA-based reflectance micro-FT-IR imaging is highly suitable for the detection of PVC, PS, PP, and PE microplastics within organic-rich wastewater samples.

On occasion, the mixture of biogenic matter (with a small amount of nonorganic debris) on the polycarbonate membrane filter gave rise to background spectra that exhibited a limited degree of overlap with some of the IR spectral peaks used for the identification of microplastics (Table S1, Supporting Information). However, as the identification of each polymer relied on the presence of three characteristic peaks, the microplastics tested could be successfully identified. In wastewater samples, it is expected that some compounds can be adsorbed to or associated with the plastic particles (and/or plastic-associated biofilms), but as long as the H₂O₂ pretreatment is able to remove most of the biogenic matter and provided that characteristic peaks for each polymer (as shown in Table S1, Supporting Information) are present in the spectra, the fragments should be correctly identified.

Despite the ability of the present method to detect microplastics in wastewater, there were some unexpected results in the full filter scans. For example, filter scans consistently identified more than the total of 10 PE particles which were added to the wastewater samples (Figure 3). Fragmentation of the spiked particles seemed unlikely because Nuelle et al.³³ did not report any fragmentation of PE particles treated with 35% H₂O₂ for up to 7 days. Moreover, a single fragment with a spectrum consistent with polyethylene was identified in a wastewater sample with no spiked microplastics, which indicates that microplastics may have been present in the wastewater matrix. It is also unlikely that these microplastics originated from air-borne contamination or as a result of sample preparation, since no evidence for microplastic contamination was evident in the negative control (water only) samples. Polyethylene microbeads are a common ingredient in many toiletries, particularly facial washes,^{2,6,8} and therefore, it is not unexpected to identify microplastics such as these in wastewater samples.

There are two main issues that should be considered when applying this method to real environmental samples. The first is the volume of the sample being analyzed. In our study, 45 mL of wastewater was used for each experimental replicate, and it would be expected that higher amounts of organic matter will

require higher volumes of hydrogen peroxide. The second issue to consider is the need for further experimental data on the ability of this method to identify microplastics that are weathered and/or have been exposed to organic-rich matrices for prolonged periods of time. As mentioned before, certain compounds in wastewater are likely to become adsorbed to plastic surfaces (and/or plastic-associated biofilms), but as long as the H₂O₂ pretreatment is able to remove most of the biogenic matter, successful identification of distinct polymer types is possible. Indeed, some of the detected polymer fragments were suspected to be present in the wastewater samples already prior to the spiking step (Figure 3), suggesting that our method is likely to provide a useful analytical platform for the analysis of field-collected samples.

Refractive error has previously been shown to represent a source of some uncertainty when interpreting the reflectance micro-FT-IR spectra of irregularly shaped materials.^{41,65–68} Therefore, the identification of microplastics must be based on specific regions from the polymeric functional groups that are easily recognizable and insensitive to variation in sample morphology.^{41,69} Where necessary, the identification of a specific fragment could be complemented by the use of ATR-FT-IR or analyses in transmission mode.^{41,64}

In addition to FT-IR spectroscopy, Raman spectroscopy has been previously used to detect microplastics in environmental samples.^{34,36,37} While this technique does offer some benefits over FT-IR, including its ability to identify microplastics down to 500 nm in size,³⁶ FT-IR spectroscopy has been shown to provide faster analysis of the samples.³⁴ It was also observed that Raman spectroscopy can suffer from interferences due to additives (such as colorants) or contaminants (including microalgae).^{34,37} However, Raman spectroscopy is a useful alternative to FT-IR spectroscopy when the analysis of wet samples is desirable or when complementary spectral profiles are required. Pyrolysis GC/MS has also been applied to simultaneously identify polymer types of microplastic particles and associated organic plastic additives.³⁵ Unlike FT-IR spectroscopy, pyrolysis GC/MS is a destructive analytical technique and cannot provide information on the sizes and shapes of polymer microfragments. However, pyrolysis GC/MS permits the analysis of inorganic plastic additives (such as plasticizers and antioxidants),³⁵ which can be difficult to detect using infrared spectroscopy.

Despite the challenges involved in detecting and quantifying petrochemical microplastics in complex organic matrices such as wastewater, the methodological approach developed in this study provides a highly promising starting point for the further analysis of microplastics in these environments. In fact, a combination of H₂O₂ pretreatment with FPA-based reflectance micro-FT-IR imaging was found to achieve an overall success rate of 98.33%, based on the number of correctly identified microplastic fragments (Table 1). The success of this methodology means that the abundance of microplastics in wastewater can now be examined to further our understanding of how microplastics enter the natural environment. Wastewater treatment plants are likely to represent an important source of microplastics in rivers and subsequently marine environments. Therefore, research into the role of the wastewater treatment process as a microplastic transport pathway is crucial to understanding the abundance and fate of microplastics in the environment.

Table 1. Results of Validation Experiment^a

microplastic	replicate no.	no. fragments spiked	no. fragments successfully identified	no. spiked fragments successfully identified
polyethylene	1	10	12	10
	2	10	17	10
	3	10	12	10
polypropylene	1	10	10	10
	2	10	14	10
	3	10	9	9
polyvinyl chloride	1	10	10	10
	2	10	9	9
	3	10	18	10
polystyrene	1	10	10	10
	2	10	10	10
	3	10	10	10
total		120	131	118
success rate (%)				98.33

^a118 of the 120 artificially spiked microplastic fragments were correctly identified, resulting in a success rate of 98.33%.

CONCLUSIONS

The combination of H₂O₂ pretreatment with FPA-based reflectance micro-FT-IR imaging is an effective approach for the accurate and semiautomated detection of microplastics in aqueous samples with high levels of biogenic organic matter, including wastewater. The methods developed in this paper enabled the successful recovery, identification, and quantification of microplastics in such a medium and offer a significant improvement over spectroscopic analyses based on the use of a single-element detector. In particular, while analyses using a single-element detector are limited to point-by-point measurements involving small and predefined regions on a sample surface, the FPA-based imaging of entire membrane filters leads to more robust results that are subject to minimal analytical bias. By developing and validating a method for the isolation and detection of microplastics within wastewater, this study has provided an important tool that can now be applied for improving our understanding of the transport of microplastics from freshwater habitats to marine environments and the role that wastewater treatment plants play in this process. In addition to analyses of plastic pollution within wastewater, this method could also be applied to determine the abundance of microplastics in other organic-rich environments, such as peat soils.

ASSOCIATED CONTENT

Supporting Information

Key characteristic FT-IR spectrum peaks for micro-FT-IR chemical imaging of microplastics (Table S1), improvement of filtration times and organic matter removal by H₂O₂ treatment (Figure S1), and false color images of microplastics in unknown positions on a glass slide (Figure S2). The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.5b00495.

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Notes

The authors declare no competing financial interest.

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REFERENCES

- (1) Katsnelson, A. *Proc. Natl. Acad. Sci. U.S.A.* **2015**, *112*, 5547–5549.
- (2) Thompson, R. C.; Olson, Y.; Mitchell, R. P.; Davis, A.; Rowland, S. J.; John, A. W. G.; McGonigle, D.; Russell, A. E. *Science* **2004**, *304*, 838.
- (3) Galgani, F.; Hanke, G.; Werner, S.; Oosterbaan, L.; Nilsson, P.; Fleet, D.; Kinsey, S.; Thompson, R.; Van Franeker, J.; Vlachogianni, T.; Scoullou, M.; Veiga, J. M.; Palatinus, A.; Matiddi, M.; Maes, T.; Korpinen, S.; Budziak, A.; Leslie, H.; Gago, J.; Liebezeit, G. *Guidance on Monitoring of Marine Litter in European Seas*; European Commission - MSFD Technical Subgroup on Marine Litter, Report EUR 26113 EN; Publications Office of the European Union: Luxembourg, 2013.
- (4) Browne, M. A.; Crump, P.; Niven, S. J.; Teuten, E.; Tonkin, A.; Galloway, T.; Thompson, R. *Environ. Sci. Technol.* **2011**, *45*, 9175–9179.
- (5) Moore, C.; Lattin, G.; Zellers, A. J. *Integr. Coastal Zone Manage.* **2011**, *11*, 65–73.
- (6) Derraik, J. G. B. *Mar. Pollut. Bull.* **2002**, *44*, 842–852.
- (7) Leslie, H. A. *Review of Microplastics in Cosmetics*; Report R14/29; IVM Institute for Environmental Studies: Amsterdam, 2014.
- (8) Fendall, L. S.; Sewell, M. A. *Mar. Pollut. Bull.* **2009**, *58*, 1225–1228.
- (9) Cole, M.; Lindeque, P.; Halsband, C.; Galloway, T. S. *Mar. Pollut. Bull.* **2011**, *62*, 2588–2597.
- (10) Gregory, M. R. *Mar. Pollut. Bull.* **1996**, *32*, 867–871.
- (11) Harrison, J. P.; Schratzberger, M.; Sapp, M.; Osborn, A. M. *BMC Microbiol.* **2014**, *14*, 232.
- (12) McCormick, A.; Hoellein, T. J.; Mason, S. A.; Schlupe, J.; Kelly, J. J. *Environ. Sci. Technol.* **2014**, *48*, 11863–11871.
- (13) Zettler, E. R.; Mincer, T. J.; Amaral-Zettler, L. A. *Environ. Sci. Technol.* **2013**, *47*, 7137–7146.
- (14) Boerger, C. M.; Lattin, G. L.; Moore, S. L.; Moore, C. J. *Mar. Pollut. Bull.* **2010**, *60*, 2275–2278.
- (15) Cole, M.; Lindeque, P.; Fileman, E.; Halsband, C.; Goodhead, R.; Moger, J.; Galloway, T. S. *Environ. Sci. Technol.* **2013**, *47*, 6646–6655.
- (16) Dantas, D. V.; Barletta, M.; da Costa, M. F. *Environ. Sci. Pollut. Res.* **2012**, *19*, 600–606.
- (17) Davison, P.; Asch, R. G. *Mar. Ecol.: Prog. Ser.* **2011**, *432*, 173–180.
- (18) Graham, E. R.; Thompson, J. T. J. *Exp. Mar. Biol. Ecol.* **2009**, *368*, 22–29.
- (19) Possatto, F. E.; Barletta, M. R.; Costa, M. F.; do Sul, J. A. I.; Dantas, D. V. *Mar. Pollut. Bull.* **2011**, *62*, 1098–1102.
- (20) Ramos, J. A. A.; Barletta, M.; Costa, M. F. *Aquat. Biol.* **2012**, *17*, 29–34.
- (21) Browne, M. A.; Dissanayake, A.; Galloway, T. S.; Lowe, D. M.; Thompson, R. C. *Environ. Sci. Technol.* **2008**, *42*, 5026–5031.
- (22) Farrell, P.; Nelson, K. *Environ. Pollut.* **2013**, *177*, 1–3.
- (23) Murray, F.; Cowie, P. R. *Mar. Pollut. Bull.* **2011**, *62*, 1207–1217.
- (24) Eerkes-Medrano, D.; Thompson, R. C.; Aldridge, D. C. *Water Res.* **2015**, *75*, 63–82.

- (25) Lechner, A.; Ramler, D. *Environ. Pollut.* **2015**, *200*, 159–160.
- (26) Castañeda, R. A.; Avlijas, S.; Simard, M. A.; Ricciardi, A. *Can. J. Fish. Aquat. Sci.* **2014**, *71*, 1767–1771.
- (27) Eriksen, M.; Mason, S.; Wilson, S.; Box, C.; Zellers, A.; Edwards, W.; Farley, H.; Amato, S. *Mar. Pollut. Bull.* **2013**, *77*, 177–182.
- (28) Imhof, H. K.; Ivleva, N. P.; Schmid, J.; Niessner, R.; Laforsch, C. *Curr. Biol.* **2013**, *23*, R867–R868.
- (29) Wagner, M.; Scherer, C.; Alvarez-Muñoz, D.; Brennholt, N.; Bourrain, X.; Buchinger, S.; Fries, E.; Grosbois, C.; Klasmeier, J.; Marti, T.; Rodriguez-Mozaz, S.; Urbatzka, R.; Vethaak, A.; Winther-Nielsen, M.; Reifferscheid, G. *Environ. Sci. Eur.* **2014**, *26*, 12.
- (30) Hidalgo-Ruz, V.; Gutow, L.; Thompson, R. C.; Thiel, M. *Environ. Sci. Technol.* **2012**, *46*, 3060–3075.
- (31) Claessens, M.; Van Cauwenberghe, L.; Vandegehuchte, M. B.; Janssen, C. R. *Mar. Pollut. Bull.* **2013**, *70*, 227–233.
- (32) Imhof, H. K.; Schmid, J.; Niessner, R.; Ivleva, N. P.; Laforsch, C. *Limnol. Oceanogr. Methods* **2012**, *10*, 524–537.
- (33) Nuelle, M.-T.; Dekiff, J. H.; Remy, D.; Fries, E. *Environ. Pollut.* **2014**, *184*, 161–169.
- (34) Cole, M.; Webb, H.; Lindeque, P. K.; Fileman, E. S.; Halsband, C.; Galloway, T. S. *Sci. Rep.* **2014**, *4*; DOI: 10.1038/srep04528.
- (35) Fries, E.; Dekiff, J. H.; Willmeyer, J.; Nuelle, M.-T.; Ebert, M.; Remy, D. *Environ. Sci.: Processes Impacts* **2013**, *15*, 1949–1956.
- (36) Fischer, D.; Kaeppler, A.; Eichhorn, K.-J. *Am. Lab.* **2015**, *47*, 32–34.
- (37) Van Cauwenberghe, L.; Vanreusel, A.; Mees, J.; Janssen, C. R. *Environ. Pollut.* **2013**, *182*, 495–499.
- (38) Frias, J.; Otero, V.; Sobral, P. *Mar. Environ. Res.* **2014**, *95*, 89–95.
- (39) Reddy, M. S.; Basha, S.; Adimurthy, S.; Ramachandriah, G. *Estuarine, Coastal Shelf Sci.* **2006**, *68*, 656–660.
- (40) Gregory, M. R. N. Z. *J. Mar. Freshwater Res.* **1978**, *12*, 399–414.
- (41) Harrison, J. P.; Ojeda, J. J.; Romero-Gonzalez, M. E. *Sci. Total Environ.* **2012**, *416*, 455–463.
- (42) Vianello, A.; Boldrin, A.; Guerriero, P.; Moschino, V.; Rella, R.; Sturaro, A.; Da Ros, L. *Estuarine, Coastal Shelf Sci.* **2013**, *130*, 54–61.
- (43) Song, Y. K.; Hong, S. H.; Jang, M.; Han, G. M.; Rani, M.; Lee, J.; Shim, W. J. *Mar. Pollut. Bull.* **2015**, *93*, 202–209.
- (44) Nakamoto, K. *Infrared and Raman Spectra of Inorganic and Coordination Compounds. Part B*, 5th ed.; John Wiley & Sons: New York, USA, 1997.
- (45) Skoog, D. A.; Leary, J. J. *Principles of Instrumental Analysis*; Saunders College Publishing: Philadelphia, 1992.
- (46) Smith, B. C. *Fundamentals of Fourier Transform Infrared Spectroscopy*; CRC Press: Boca Raton, FL, 1996.
- (47) Wade, L. G. *Organic Chemistry*; Prentice-Hall: Upper Saddle River, NJ, 1995.
- (48) Chalmers, J. M.; Everall, N. J. *TrAC, Trends Anal. Chem.* **1996**, *15*, 18–25.
- (49) Coates, J. *Interpretation of Infrared Spectra, A Practical Approach*; John Wiley & Sons, Ltd.: Hoboken, 2006.
- (50) Evanson, K. W.; Urban, M. W. *J. Appl. Polym. Sci.* **1991**, *42*, 2287–2296.
- (51) Mansur, H. S.; Oréface, R. L.; Mansur, A. A. *Polymer* **2004**, *45*, 7193–7202.
- (52) Milstein, O.; Gersonde, R.; Huttermann, A.; Frund, R.; Feine, H.; Ludermann, H.; Chen, M.-J.; Meister, J. *J. Environ. Polym. Degrad.* **1994**, *2*, 137–152.
- (53) Siesler, H. J. *Polym. Sci. Polym. Lett. Ed.* **1979**, *17*, 453–458.
- (54) Weiland, M. L.; Daro, A.; David, C. *Polym. Degrad. Stab.* **1995**, *48*, 275–289.
- (55) Koenig, J. L.; Wang, S.-Q.; Bhargava, R. *Anal. Chem.* **2001**, *73*, 360A–369 A.
- (56) Ojeda, J. J.; Romero-Gonzalez, M. E.; Banwart, S. A. *Anal. Chem.* **2009**, *81*, 6467–6473.
- (57) Wenning, M.; Seiler, H.; Scherer, S. *Appl. Environ. Microbiol.* **2002**, *68*, 4717–4721.
- (58) Agilent Technologies. *FTIR chemical imaging using focal plane array-based systems*; Appl. Note SI-2645; Agilent Technologies: Santa Clara, CA, 2011.
- (59) Bhargava, R.; Wang, S.-Q.; Koenig, J. L. In *Liquid Chromatography/FTIR Microspectroscopy/Microwave Assisted Synthesis*; Springer: Berlin, Heidelberg, 2003; Vol. 163, pp 137–191.
- (60) Anderson, M. J. *Austral Ecol.* **2001**, *26*, 32–46.
- (61) Anderson, M. J.; Gorley, R. N.; Clarke, K. R. *PERMANOVA+ for PRIMER: Guide to software and statistical methods*; PRIMER-E Ltd.: Plymouth, UK, 2008.
- (62) Clarke, K. R.; Gorley, R. N. *PRIMER v6: User Manual/Tutorial*; PRIMER-E Ltd.: Plymouth, UK, 2006.
- (63) Nakashima, S.; Sturgeon, R. E.; Willie, S. N.; Berman, S. S. *Analyst* **1988**, *113*, 159–163.
- (64) Löder, M.; Kuczera, M.; Mintenig, S.; Lorenz, C.; Gerdt, G. *Environ. Chem.* **2015**, DOI: 10.1071/EN14205.
- (65) Bassan, P.; Byrne, H. J.; Bonnier, F.; Lee, J.; Dumas, P.; Gardner, P. *Analyst* **2009**, *134*, 1586–1593.
- (66) Budevskva, B. O. *Vib. Spectrosc.* **2000**, *24*, 37–45.
- (67) Davis, B. J.; Carney, P. S.; Bhargava, R. *Anal. Chem.* **2010**, *82*, 3487–3499.
- (68) Földes, E.; Szabo, Z.; Janecska, A.; Nagy, G.; Pukanszky, B. *Macromol. Symp.* **2003**, *202*, 97–116.
- (69) Cooper, D. A.; Corcoran, P. L. *Mar. Pollut. Bull.* **2010**, *60*, 650–654.