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Extraction and identification of microplastic particles from secondary wastewater treatment plant (WWTP) effluent

A. Dyachenko,^a J. Mitchell^a and N. Arsem^{*b}

Although studies on microplastics in wastewater treatment plant (WWTP) treated effluent have recently been published, no standard method for sampling, identification and quantification of microplastics has been established, which complicates the comparison of results across various studies. This paper presents an attempt to optimize the Wet Peroxide Oxidation (WPO) digestion procedure applicable to wastewater effluent matrices and underscores unique challenges and interferences that analysts face when attempting to quantify microplastic particles in wastewater.

Introduction

Microplastics refer to tiny plastic pieces, typically less than 5 mm in every dimension,⁸ and can be generally categorized as fragments (breakdowns from large plastic products), microbeads (typically found in cosmetics), and fibers from synthetic clothes and fabrics. To better understand the prevalence of microplastics in wastewater, the development of an analytical method that allows accurate identification and quantification of microplastics is a key first step. However, unlike other pollutants, the lack of a clear definition and the varying size range and composition of microplastics make it difficult to develop methods and compare results across various studies. Furthermore, to be certain that microparticles, including microfibers, are indeed microplastics (*e.g.*, not cotton or cellulose fibers), a spectroscopic confirmation step for every particle may be needed. The small size of microplastics makes them bioavailable to thousands of species across nearly all trophic levels as they are often mistaken for food.² While further information on the impacts of this pollutant on the aquatic life and human health is emerging, indications are that microplastics present a risk as a result of physical blockages, inherent contaminants leaching from the microplastics, and chemical exposures from contaminants adsorbed onto plastic particles.⁷

We present a regional effort to develop a method to accurately identify and quantify microplastics in wastewater effluent. The study found that wastewater samples require special handling in order to remove inherent organic material-related interferences. While more work remains to be done, the major finding is that relying solely on microscopic visual identification of microparticles is inaccurate; each particle must be confirmed as plastic by a spectroscopic technique capable of tackling small particle sizes such as Microscopy-Fourier transform infrared microscopy (Micro-FTIR).

Experimental

East Bay Municipal Utility District's (EBMUD) main wastewater treatment plant (MWWTP), which serves approximately 680 000 people, provides secondary treatment for, on average, about 50 million gallons of wastewater per day. Samples for microplastic determination were collected after secondary treatment, prior to dechlorination and discharge to the bay (Fig. 1).

The sample collection setup was adapted from the sampling method reported by the San Francisco Estuary Institute (SFEI) in which the effluent flow was directed across a stack of 0.355 mm and 0.125 mm sieves at peak flow for two hours.¹ In this study, composite secondary effluent samples are collected over a stack of four 8" diameter stainless steel sieves (VWR) of various mesh sizes: 5 mm, 1 mm, 0.355 mm and 0.125 mm (Fig. 2). A 5 mm sieve is used to screen out larger particles and minimize clogging of smaller sieves; its content is discarded. A maximum flow rate of one gallon per minute was determined to not cause the sieves to clog and was selected for sample collection throughout this study. For this study, the effluent is collected over a period of 24 hours; a limited number of samples were collected as 2 hour composites at peak flow. Contents from 1 mm, 0.355 mm and 0.125 mm sieves are transferred, aided by a minimal amount of deionized H₂O, to 0.25 L glass collection jars sorted by sieve size and stored at ≤4 °C.

The extraction method was adapted from the National Oceanic and Atmospheric Administration's (NOAA) protocol

^aEast Bay Municipal Utility District (EBMUD), Laboratory Services Division, 2020 Wake Ave., Oakland, CA 94607, USA

^bBay Area Clean Water Agencies (BACWA) Microplastics Workgroup, USA. E-mail: nirmela.arsem@ebmud.com

Schematic of MWWTP Processes and Water Balance
3-yr Avg Daily Flows Used: Calendar Years 2011, 2012, 2013

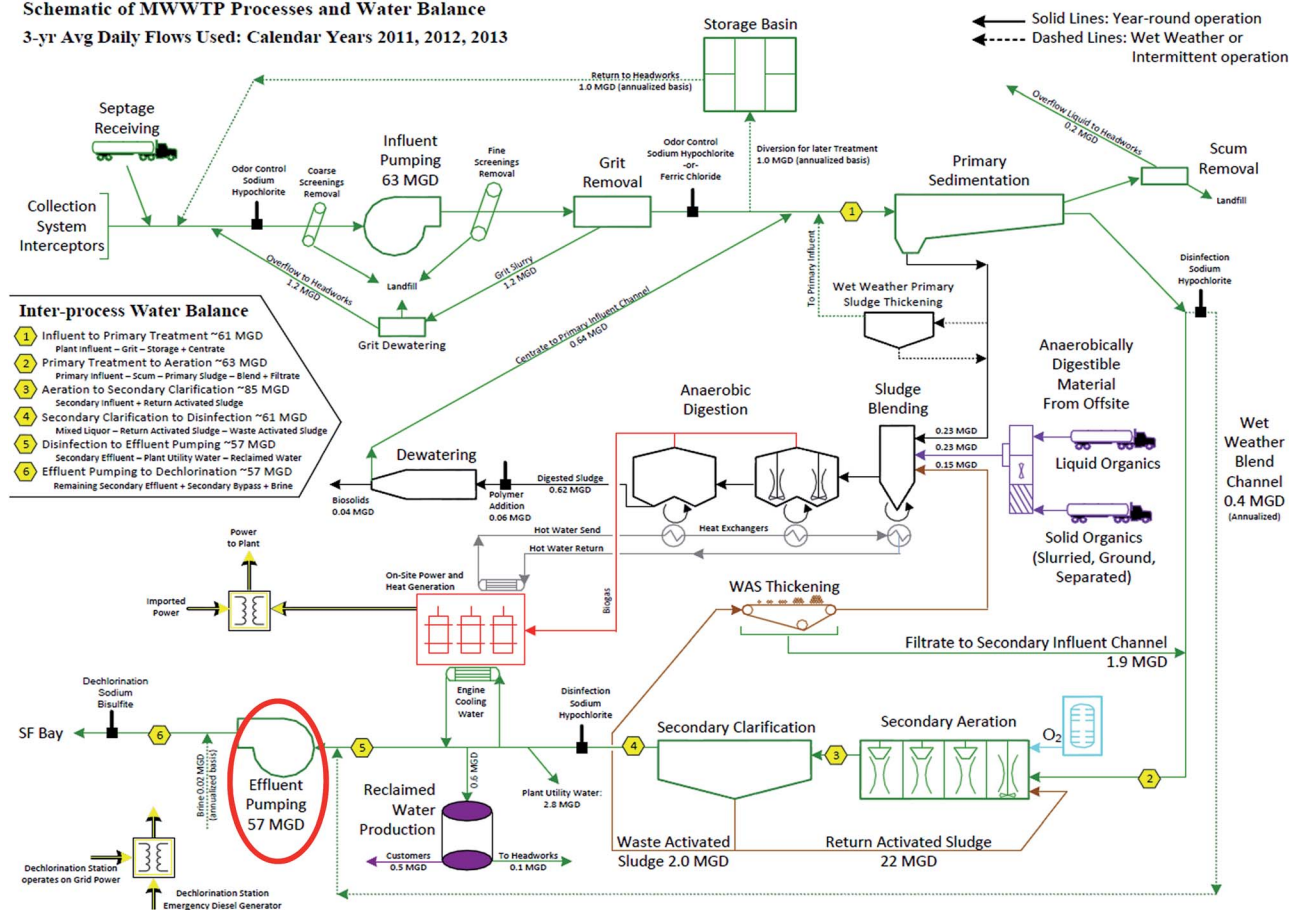


Fig. 1 Schematic representation of EBMUD's MWWTP processes.

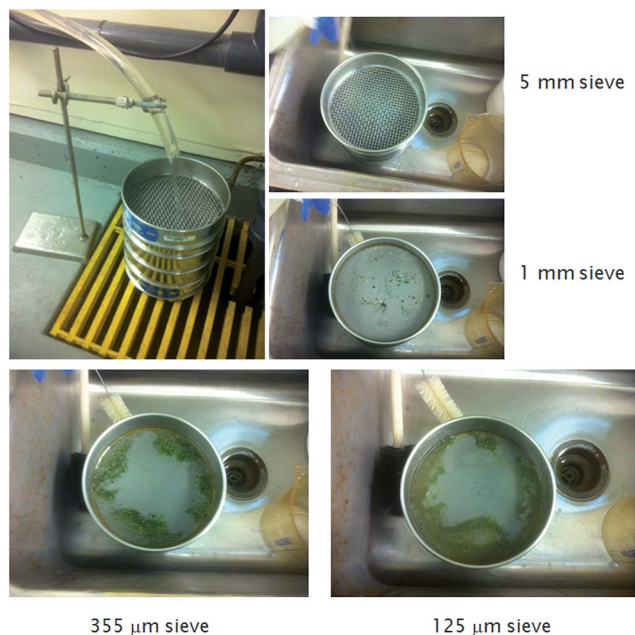


Fig. 2 Sample collection setup.

published in July, 2015.⁴ In Catalytic Wet Peroxidation Oxidation (CWPO), hydroxyl radicals generated upon decomposition of hydrogen peroxide, oxidize the majority of the natural organic matter to carboxylic acids, aldehydes, CO₂ and H₂O. The presence of a catalyst (FeSO₄) allows for rapid digestion of organic matter under mild conditions. Other extraction methods have been considered and rejected due to their potential to degrade microplastics: (a) centrifugation may lead to compacting, deforming and breaking down of microplastic particles, (b) microwave digestion is likely to lead to decomposition of microplastics and (c) the effects of enzymatic digestion on infrared spectra have not been fully studied. In this study, content from 0.25 L sample jars is transferred to 0.5 L beakers and is subjected to catalytic Wet Peroxide Oxidation (WPO) in accordance with "Recommendations for quantifying synthetic particles in waters and sediments".⁴

40 mL of 0.05 M FeSO₄ and 80 mL 30% H₂O₂ are added to each sample, and the solution is heated at 70 °C while stirring for 30 minutes or until the reaction is complete (Fig. 3). An additional amount of 30% H₂O₂ is added if digestion of organic matter is incomplete. The extract is filtered using a Buchner funnel (vacuum filtration) and collected onto a 90 mm 0.8 µm membrane filter (Pall Corp., Cat. no. 60112). The vacuum is drawn through a membrane filter for an additional five minutes

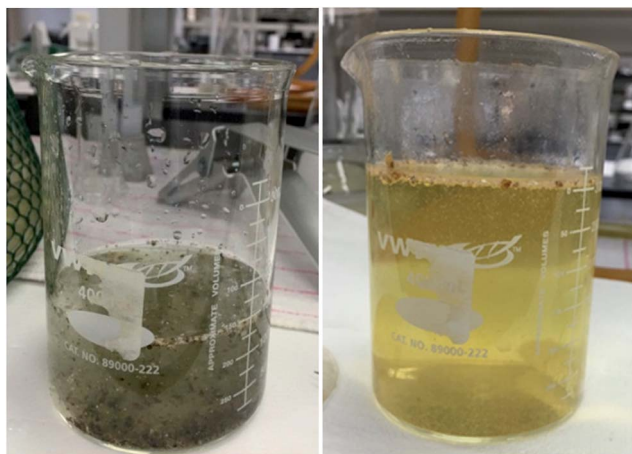


Fig. 3 Composite wastewater effluent sample collected from a 0.355 mm sieve before (left) and after WPO (right).

to dry the extract. The membrane filter is examined under a dissecting stereomicroscope (Olympus SZ61 stereomicroscope, a 45 \times objective combined with a SC100 digital camera and Olympus Stream 2.1 Imaging Software). The initial performance evaluation of the method was conducted using a blank aqueous sample fortified with a known amount of polystyrene beads (200 μm , Sigma-Aldrich, Cat. no. 16435). The extraction resulted in 87% recovery of the beads as confirmed microscopically.

Results and discussion

Large amounts of interferences were found to be present in effluent samples after several hours of continuous digestion.

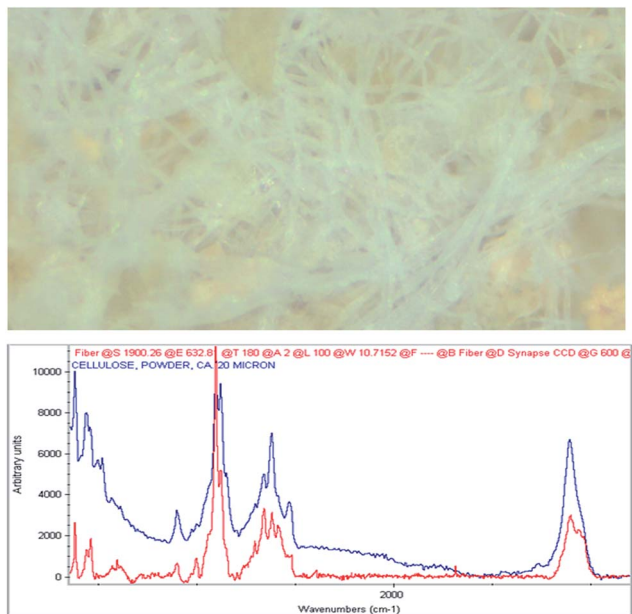


Fig. 4 Cellulose fiber present in wastewater effluent extracts and the corresponding FTIR spectra.

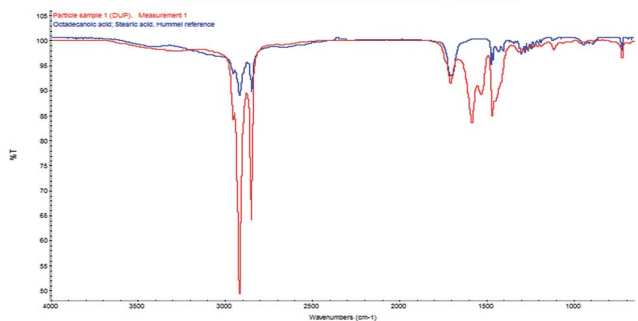
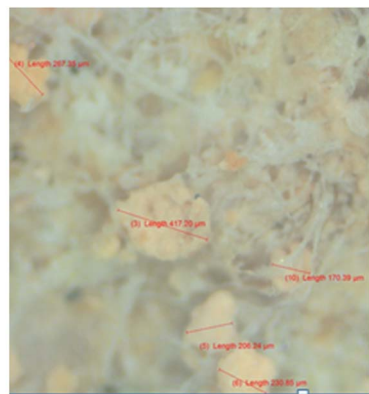


Fig. 5 Brown organic contaminant particles present in wastewater effluent extracts and the corresponding FTIR spectra.

The adapted catalytic WPO method was not designed for a wastewater matrix and an additional clean-up step is necessary for robust microplastic identification. Density separation with saturated sodium chloride solution was not effective in separating the interferences. A recent study suggested that for samples with a high concentration of organic matter such as wastewater, density separation is not as useful due to the fact that many naturally occurring particles exhibit densities similar to microplastics.⁵



Fig. 6 Non-microplastics after WPO digestion.

Cellulose fiber was found to be a major interferent as determined microscopically (Fig. 4). Isolated cellulose fibers were submitted for Raman spectroscopic confirmation (Evans Analytical Group, Sunnyvale, CA).

The Raman measurements were performed with a J-Y spectrometer ("LabRam") equipped with a "BX40 Olympus" microscope using the backscattering geometry. A HeNe laser (632.8 nm wavelength) and a 600 gr per mm grating were used in these measurements. The baseline corrected spectrum matched the reference spectrum of cellulose.

The second major source of interference was determined to be fatty acids typically found in secondary wastewater effluent and not digested by the catalytic WPO process (Fig. 5).



Fig. 7 Extracts collected in the membrane filter after continuous (left) vs. sequential WPO (right).

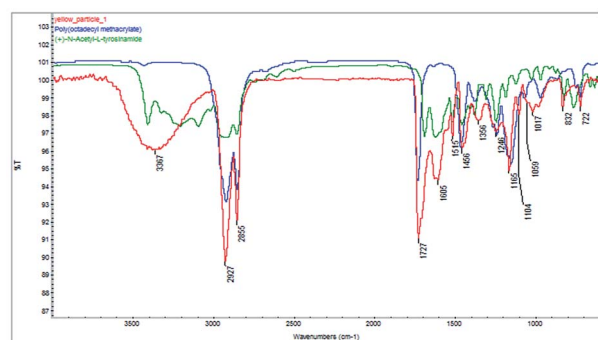


Fig. 8 Yellow "Honeycomb" like particle and the corresponding FTIR spectra.

A representative particle of the contamination was transferred to an infrared transmitting substrate and examined by using a FTIR spectrometer (Thermo Nicolet 6700) combined with a FTIR Continuum microscope in transmission mode (EAG, Sunnyvale, CA). The material is a mixture of a fatty acid similar to stearic acid (prominent bands at ~ 2919 , 2851 , 1707 , 1467 and 723 cm^{-1}) and a fatty acid salt similar to magnesium stearate (prominent bands at ~ 2919 , 2851 , 1585 , 1467 , and 723 cm^{-1}). The band at ~ 1531 cm^{-1} in Fig. 5 was neither typical of stearic acid nor typical of magnesium stearate and possibly represents the presence of a second stearate species such as lead stearate, aluminum stearate or iron stearate.

In order to test the effectiveness of the catalytic WPO procedure, common non-plastic contaminants such as human hair, cotton clothing fibers, and cigarette filter and toilet paper fragments were subjected to the digestion protocol.

None of these potential contaminants were digested by the catalytic WPO technique (Fig. 6).

The digestion procedure was further optimized by introducing a sequential WPO. In sequential WPO, the extract, after a single digestion cycle, is filtered through a 0.125 mm sieve which is then rinsed with hexane (HPLC grade, VWR) three times followed by a rinse with deionized H_2O . The content from the sieve is then transferred back into the beaker for a second

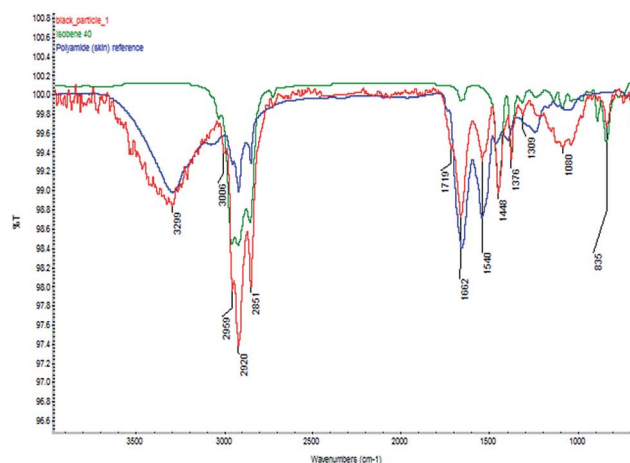
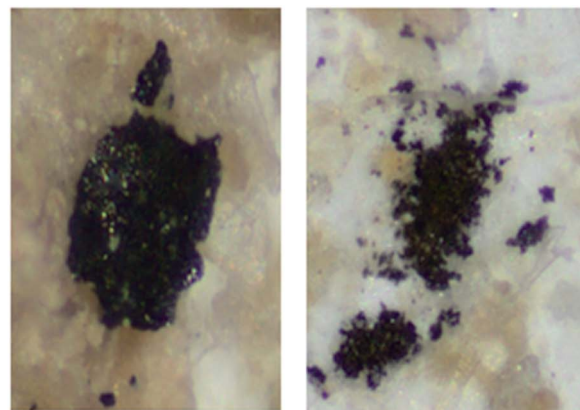


Fig. 9 Representative abundant "black" particle and the corresponding FTIR spectra.

WPO digestion. Typically, 3–6 digestions are necessary to remove most cellulose and other organic material interferents (Fig. 7).

Most commonly observed, suspected microplastic particles (0.355 mm sieve size) were isolated with micro-forceps (VWR Cat. no. 100497-620) and submitted for micro-FTIR confirmation (Evans Analytical Group, Sunnyvale, CA). Individual particles were isolated to avoid contribution of spectral interferences from the membrane filter.

The particle in Fig. 8 was identified as a polyacrylate similar to poly(octadecyl methacrylate) and amide and/or trace aromatic species by comparison with library references. In particular, the prominent vibrations at 2927, 2855, 1727, 1456, 1246, 1165, 1104 and 724 cm^{-1} exhibit excellent overlap with those of the poly(octadecyl methacrylate) reference, supporting the proposed assignment. Likewise, the peaks at 1605, 1515, 1017 and 832 cm^{-1} are likely due to an amide and/or trace aromatic species.

A large number of black particles as shown in Fig. 9 are observed in extracts originating from 0.355 mm and 0.125 mm sieves. They appear to be fragments of larger particles that break down easily upon contact with forceps. A small portion of the sample (Fig. 9) was transferred to an infrared transmitting substrate and examined using a Thermo-Nicolet 6700 FTIR

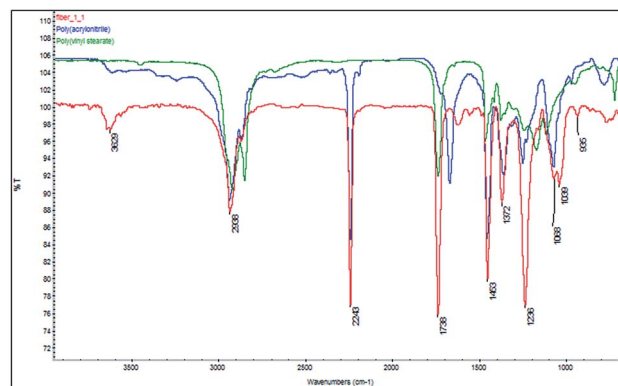
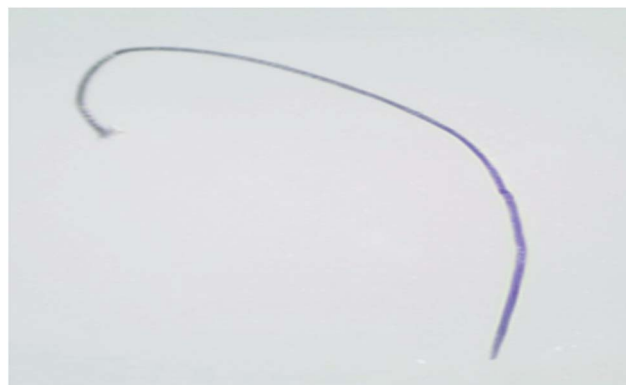


Fig. 11 Representative blue fiber confirmed as polyacrylic with corresponding FTIR spectra.

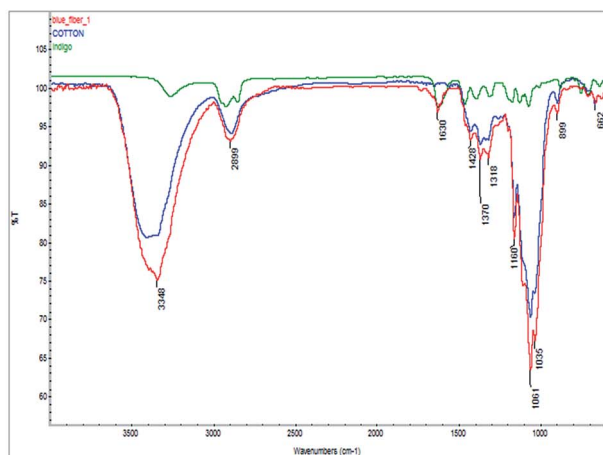
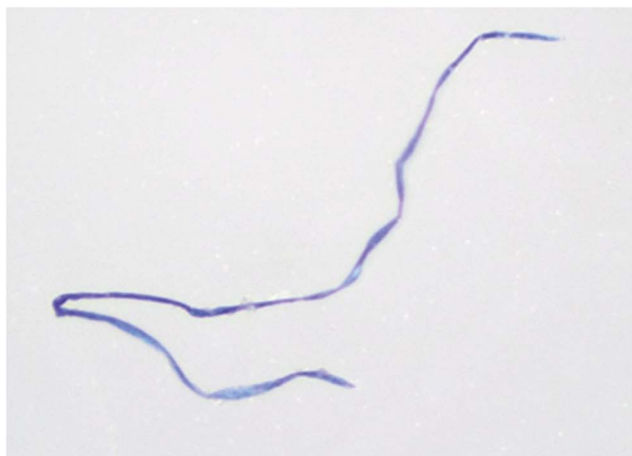


Fig. 10 Representative fiber particle confirmed as cotton with the corresponding FTIR spectra.

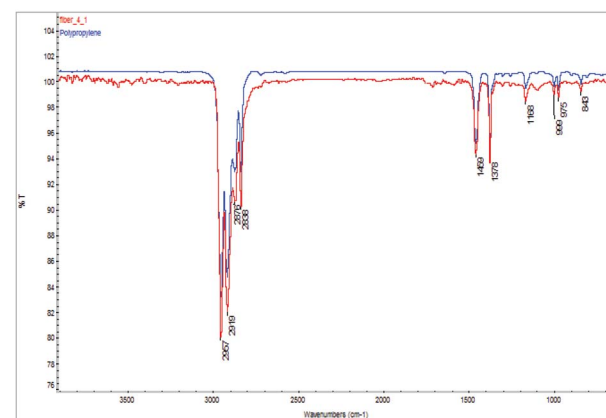


Fig. 12 Representative "Pink" fiber confirmed as polypropylene with corresponding FTIR spectra.

spectrometer equipped with a Continuum microscope in transmission mode. The analytical spot size was approximately 100 microns \times 100 microns. OMNIC 8.0 software was used to perform data analysis. The sample is identified as a mixture of the poly(styrene-isoprene) copolymer, a polyamide similar to human skin, possible trace additional aromatic species and an organic acid by comparison with library references. In particular, the prominent vibrations at 2930, 2851, 1448, 1376, 1309, 1080 and 835 cm^{-1} exhibit excellent overlap with those of the Isobene 40 reference, supporting the proposed assignment. The peaks at 3299, 1662 and 1540 cm^{-1} indicate the presence of a polyamide similar to skin, whereas the peak at 3006 cm^{-1} may be due to an additional aromatic species (or a double bond) and the peak at 1719 may be due to an organic acid.

The particle in Fig. 10 was analyzed by FTIR and the results are provided in the accompanying spectrum. The sample is identified as cellulose similar to cotton and possibly indigo on comparison with library references. In particular, the prominent vibrations at 3348, 2899, 1428, 1370, 1318, 1160, 1061, 1035 and 899 cm^{-1} exhibit excellent overlap with those of the cotton reference, supporting the proposed assignment. The peak at 1630 cm^{-1} may be due to indigo.

The particle in Fig. 11 was identified as a Acrilan fiber composed of mostly polyacrylonitrile and an acrylic resin possibly similar to poly(vinyl acetate) on comparison with

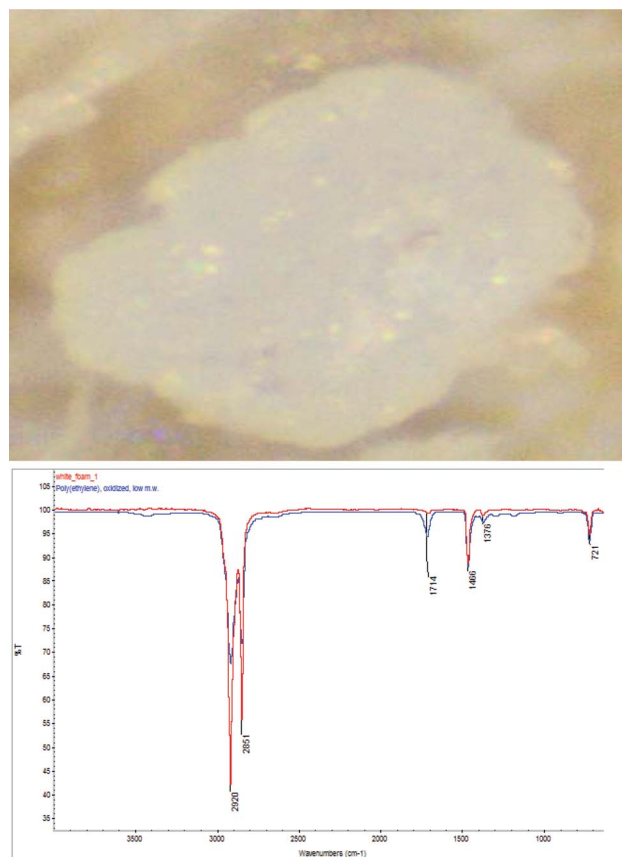


Fig. 13 Foam particle confirmed as polyethylene with the corresponding FTIR spectra.

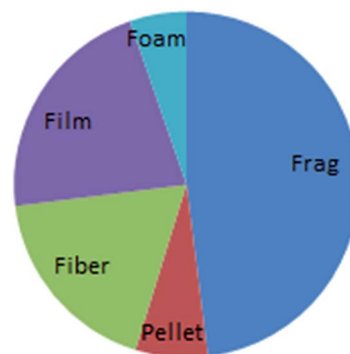


Fig. 14 An example of the particle type distribution in extracts obtained from a 24 hour sampling event.

library references. In particular, the prominent vibrations at 3529, 2938, 2243, 1453, 1372, 1236 and 1068 cm^{-1} exhibit overlap with those of the polyacrylonitrile reference, supporting the proposed assignment. Likewise, the peak at 1738 cm^{-1} indicates the presence of an acrylic resin.

The particle in Fig. 12 was identified as polypropylene possibly containing an organic acid on comparison with library references. In particular, the prominent vibrations at 2957, 2919, 2876, 2838, 1459, 1378, 1168, 999, 975 and 843 cm^{-1} exhibit excellent overlap with those of the polypropylene reference, supporting the proposed assignment.

The microparticle is identified as polyethylene possibly containing an organic acid on comparison with library references. In particular, the prominent vibrations at 2920, 2851, 1466, 1376 and 721 cm^{-1} exhibit excellent overlap with those of the polyethylene reference, supporting the proposed assignment. Note that the peak at 1714 cm^{-1} may be due to oxidation or may indicate the presence of an organic acid (Fig. 13).

Additionally, several film-like particles were confirmed as polyethylene of various densities during a vendor's instrument demonstration of iN-10 MX FTIR in Attenuated Total Reflectance (ATR) mode (Thermo Fisher Scientific, San Jose, CA).

Conclusion

A preliminary attempt to count and categorize the type of microparticles extracted from secondary wastewater effluent has been made and results were extrapolated using WWTP's average daily flow rates. Early findings reveal significant discrepancy in the microparticle count in extracts obtained from 2 hour sampling at peak flow and 24 hour composite sampling events. Out of the five monthly 24 hour sampling events, a maximum concentration of 0.09 microparticles per gallon was observed compared to 0.64 microparticles per gallon for a single 2 hour sampling event at peak flow. The vast majority of the microparticles could be categorized as fragments with pellets (or beads) appearing to contribute less than 10% of the overall microparticle count (Fig. 14). This result is consistent with recent studies that reported a low contribution of microbeads to the overall microparticle count.^{3,6} Visual microscopic identification alone is not sufficient in confirming

microplastic presence. For example, visually similar “blue” fibers were confirmed by micro-FTIR as cotton-indigo and polyacrylic respectively (Fig. 10 and 11).

Some particles are prone to fragmentation which may prevent accurate counting and categorization (Fig. 9).

Sequential WPO digestion leads to cleaner extracts with significantly reduced amounts of major interferents such as cellulose and fatty acids (Fig. 7). Matrix spike recovery study with reference microplastic standards is recommended to establish acceptance criteria.

Many microplastic particles are not homogeneous with traces of other compounds present. It may be necessary to analyze each particle at 2–3 different spots for positive confirmation. Advanced ‘tandem’ techniques such as Microscopy-FTIR allow analysts to focus a narrow IR beam on a selected spot of a microparticle. This technology is considerably more expensive than a traditional FTIR instrument and may be out of reach for many wastewater laboratories.

This is a limited scope feasibility study with the goal of developing a method for extracting and identifying microplastic particles utilizing tools and equipment readily available in a wastewater laboratory setting. Only a small number of suspected microplastic particles have been spectroscopically confirmed due to the high cost of analysis as wastewater laboratories do not normally possess FTIR or Raman spectrometers. Furthermore, microplastics have only been identified by using available, standard libraries. For a more accurate confirmation, obtaining spectra from reference microplastic standards may be necessary. Gravimetric analysis was outside the scope of this study and would require fully addressing the non-homogeneous composition of many microplastic particles before any meaningful extrapolations can be reported. A broader scope, inter-laboratory study involving wastewater samples from various locations would aid in developing a reproducible, standardized

method. A collaborative effort in creating an imaging database of confirmed microplastic particles could enhance method robustness by minimizing the need of costly micro-FTIR confirmation of every suspected microplastic particle.

Acknowledgements

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