A method to remove cellulose from rich organic samples to analyse microplastics

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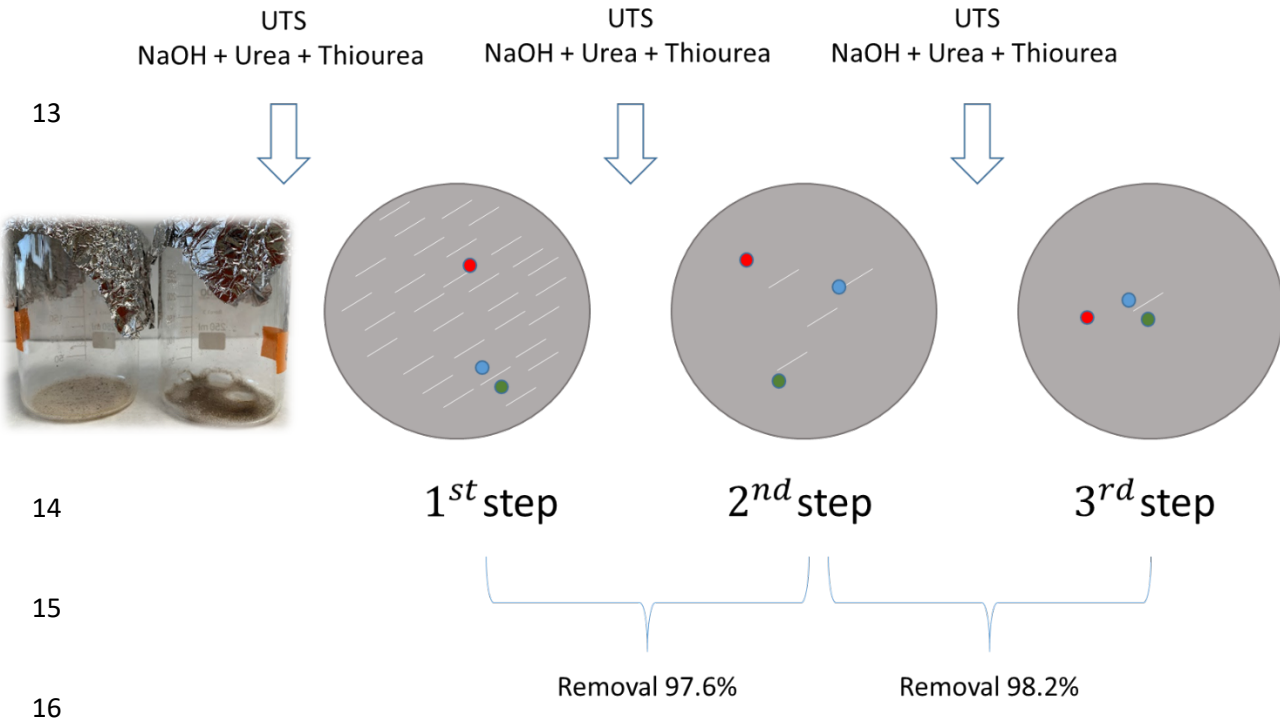
Graphical abstract



Highlights

- Cellulose in wastewater and sludge causes interference in microplastic analysis

- A new method for removal cellulose in the analysis of microplastics is reported

- The study covers the behaviour of samples of synthetic, wastewater and sludge samples

- Reducing the cellulose by 97.6% in a second treatment with the method proposed

Abstract

Knowing the amount of microplastics that currently reach wastewater is extremely important today. Furthermore, carrying out a good quantification and detection of the type of plastic provides valuable information. However, the wastewater is loaded, in addition to a high concentration of organic matter, with a high concentration of cellulose at the treatment plant influent, which seriously hinders detection, quantification and classification of microplastics. The abundance of cellulose materials makes them possible to become false positives for microplastics. Numerous studies on the analysis of microplastics in different matrices show how to remove organic matter from samples, but there are very few studies on the removal of cellulose, which is also found in the samples and hinders their analysis. This study offers a method that combines, for the analysis of microplastics, the already known advanced oxidation treatments for the elimination of organic matter with the novel cellulose removal treatment of the samples with the aim of reducing the

amount of cellulose in the influent samples of conventional wastewater treatment plants. To remove the cellulose, 40 mL of a solution of urea 8%, sodium hydroxide 8% and thiourea 6.5% (by weight) were added for every 100 mg of dry sample. The beakers were placed in the freezer at minus 20 ºC for 40 min and were then placed in agitation until they reached room temperature. After that, the samples were passed through a 53 µm mesh sieve. They were washed 15 times with 30 mL of ultra-pure water. The method is called UTS because of the acronym of its reagents (Urea/Thiourea/Sodium Hydroxide). By using the UTS method it is possible to reduce almost completely the cellulose residues from the influent sewage, and sludge samples by 97.6% in a second UTS treatment and 98.2% in a third UTS treatment. In all cases analysed, the microplastics were identified as high density polyethylene (HDPE) with correlation indices higher than 0.97, which shows that the treatment is harmless for this type of plastic material. The UTS method in combination with the WPO is an efficient and effective method for the analysis of microplastics in different matrices where cellulose and organic matter may cause possible interferences.

Keywords: Cellulose; Detection; Microplastics; Sludge; Wastewater

1. Introduction

The elimination of microplastics (MPs) in the environment is a current topic of study. Environmental pollution by MPs is a growing problem, and the problem is expected to persist for hundreds of years (Ivar, 2021). Microplastics are plastic particles smaller than 5 mm (Franco et al., 2021). These MPs are generating a global environmental problem that has an impact not only on the environment as such, but also on the food chain in particular. According to WHO (2019), global plastics production has increased almost exponentially since the 1950s. Taking into account population growth and current plastic consumption and waste, plastic production is expected to double by 2025 and triple by 2050 (FAO, 2017).

As the manufacture and use of plastics has steadily increased over the decades, the occurrence of MPs in the environment has also intensified and these new contaminants are now commonly found in rivers, lakes, and coasts (Carr et al., 2016; McCormick et al., 2014). According to Feng et al. (2020) fibers were the most frequently observed form in surface water and sediments. It is known that one of the largest inputs of MPs into the environment is from wastewater treatment plants (WWTPs) (Turan et al., 2021). Many authors have presented results on the detection and quantification of MPs in WWTPs effluent (Dyachenko et al., 2017; Franco et al., 2020; Xu et al., 2019). Other authors present research on the percentage of MPs removal in WWTPs (Gies et al., 2018; Franco et al., 2021). Mechanical, chemical, and biological treatment processes removed up to 99% of the MPs entering a WWTPs (Ziajahromi et al., 2016). After treatment, the removed MPs were primarily transferred to the sludge phase

87 (Ngo et al., 2019). However, there are few authors talking about the
88 problems that we encounter for the detection, quantification, and
89 identification of these pollutants in both the influent of the waterline and
90 the sludge line of conventional WWTPs.

91 Additionally, there is not a standardised method for the analysis of
92 MPs. Numerous authors have proposed methods but none have yet been
93 approved. This makes it difficult to compare methodologies and to
94 corroborate that MPs analysis is being carried out correctly. Cunsolo et al.,
95 (2021) have optimised sample preparation for FTIR-based MPs analysis
96 in wastewater and sludge samples: multiple digestions, however, it uses
97 quite a few reagents and some that could be considered aggressive by other
98 authors for microplastic analysis (Al-Azzawi, et al., 2020). The importance
99 of a correct detection, quantification, and identification of MPs where no
100 other factors are involved, is due to the current circular economy trend in
101 WWTPs (Neczaj et al., 2018). The recognition of sludge as a resource, the
102 use of sewage sludge as a source of energy and resource recovery is a good
103 alternative for its management considering the requirements of the
104 legislation and the principles of the circular economy, for this it is
105 necessary to know all the damages that MPs could cause in the
106 environment with a correct detection, quantification and identification
107 (Gherghel et al., 2019).

108 Similarly, with regard to water, in order to avoid environmental
109 pollution from insufficiently treated wastewater discharged into the
110 environment or subsequently reused in the environment, the European
111 Union has approved a regulation "Regulation (EU) 2020/741 of the

112 european parliament and of the council of 25 may 2020 concerning
113 minimum requirements for water reuse" where, in Annex II, it is stated that
114 depending on the results of the risk assessment there would be additional
115 requirements. Therefore, the importance of knowing the quantity and type
116 of MPs in reclaimed water prior to its use is emphasised.

117 In the influent of WWTPs, in addition to already known organic
118 matter, there is a high concentration of cellulose which makes the normal
119 process of identification and detection of MPs difficult. Cellulose is the
120 most abundant natural biopolymer on planet Earth (Peng et al., 2020).
121 Hurley et al. (2018) proposed a validation of a method for the extraction
122 of microplastics from complex and organic-rich environmental matrices.
123 With the proposed treatment and various protocols, they were able to
124 eliminate between 57-67% of organic material in sludge and 35-68% of
125 soil organic matter, but reported problems with cellulosic and chitinous
126 material being resistant to KOH and NaOH treatment (Hurley et al. 2018).
127 Olsen et al. (2020) demonstrated the need for a method for cellulose
128 removal in wastewater samples, because without the possibility of
129 spectroscopic techniques, it is not possible to discern cellulose fibers from
130 microplastic fibers, even after commonly used digestion procedures. These
131 same authors (Olsen et al., 2020) tested a removal method for seawater
132 matrices containing a lower amount of cellulose fibers than influent
133 samples or sewage sludge. Lares et al. (2018) also reported that the
134 cellulose present in the samples can lead to inadequate separation during
135 the density separation step commonly applied in wastewater sample
136 treatments to isolate MPs due to the density of cellulose, which is very

similar to that of certain plastic polymers. Ivleva (2021) in a review of current analytical methods, recommends avoiding misidentification errors and strongly recommends including the cellulose spectrum, which is named as a typical matrix component. These statements demonstrate the need to remove cellulose before starting the detection, quantification and identification process.

The abundance of cellulose materials makes them possible to become "false positives" for microplastics. The elimination of cellulose from rich organic samples could offer more significant and real values of the amount of MPs entering the treatment plant and being removed before any treatment. The aim of the present study is the optimisation of cellulose removal in water and sludge samples, improving the detection, quantification, and identification of MPs.

2. Materials and methods

This section will refer to sampling, reagents and chemical products used, sample processing, method validation and sample characterization.

2.1. Sampling

Wastewater and sludge samples were collected in the influent of an urban WWTP located in Medina Sidonia, a Spanish municipality situated in Cadiz, Andalusia. According to the Spanish National Institute of Statistics (SNIS, 2021), the municipality had 11,773 inhabitants in 2020,

159 but the plant is capable of treating the wastewater of a population of 17,599
160 equivalent inhabitants and can process a flow rate of up to 2,223 m^3 d^{-1}.

161 There are two treatment lines: water and sludge. As at the majority of
162 the existing WWTPs in Spain, the water line of the plant consists of
163 primary and secondary treatment made up of the following units: a)
164 Primary treatment with a coarse and fine screening unit and a de-gritting
165 and degreasing system and b) The secondary treatment consists in an
166 extended aeration biological reactor and secondary settling.

167 The effluent from Medina Sidonia WWTP discharges into the Satillo
168 stream and complies with European regulations (Directive 91/271/EEC)
169 and its transposition into Spanish law (Royal Decree-Law 11/1995), which
170 establishes the regulations applicable to the treatment of municipal
171 wastewater.

172 The WWTP also has a sludge line, which consists of thickening the
173 sludge before it is dewatered to a dryness of more than 60%. The sludge
174 line is equipped with a deodorisation system using activated carbon
175 filtration. The sludge from this sewage treatment plant is collected by a
176 soil amendment company.

177 The characteristics of the urban wastewater of the WWTP of Medina
178 Sidonia have been collected in several publications by the authors (Egea-
179 Corbacho et al., 2019a, 2019b, 2019c). The specific characteristics of these
180 samples are shown in Table 1.

181 Table 1. Characterization of the Medina Sidonia WWTP.

	pH (pH units)	Conductivity (µS cm^{-1})	TOC (mg L^{-1})	NT (mg L^{-1})	COD (mg L^{-1})
Influent	7.67 (±0.15)	1465 (±352)	137.71 (±63)	51.10 (±12.19)	597.92 (±112.31)
Effluent	7.79 (±0.15)	1048 (±9)	20.14 (±2.57)	3.75 (±0.55)	166.99 (±71.31)

2.2. Reagents and chemical products used

The pure urea pearls, extra-pure sodium hydroxide and extra-pure sodium chloride were provided by Scharlau (Barcelona, Spain). Iron II sulphate 7-hydrate purissimum, hydrogen peroxide 30% v/v and thiourea were supplied by Panreac (Barcelona, Spain). Filters (0.8 µm polycarbonate filters PC membrane 47 mm) were purchased from IsoporeTM (Darmstadt, Germany).

2.3. Sample processing and method validation

Two litres of untreated wastewater were filtered through three stainless sieves of 1000, 355 and 100 µm. From previous experience with the different WWTP samples that have been taken, most of the cellulose accumulates on the 100 µm sieve. Therefore, the 100 µm sieve was chosen to carry out the experiments of this study. The solid fraction was collected with ultrapure water into a beaker. The same sample was prepared in triplicate and was left to dry in the oven at 70 ºC. The samples were covered with aluminium foil to avoid external contamination.

To validate the method, three replicates of synthetic samples were made with a known amount of cellulose and MPs, in ultra-pure water. In this way, the effectiveness of the method could be known. This allows the efficacy of the UTS treatment to be determined at each stage by taking into

account the percentage of cellulose removal. Furthermore, by knowing the type and quantity of MPs added, it could be determined whether they are affected by the different UTS treatment steps. For the analysis, beakers with a known amount of cellulose and MPs were weighed. Subsequently, they were dried at 70 °C for 24 h. Once the sample had been tempered, it was weighed again to determine the dry weight of previous cellulose.

In the case of the sludge, 16 g were weighed and then passed through the 355 and 100 µm sieve. The 100 µm sieve was also used for the study. This fraction was collected in a beaker and placed in the oven at 70 °C. As with the water samples, they were covered with aluminium foil to avoid external contamination. The samples were prepared in triplicate.

To remove the cellulose, 40 mL of a solution of urea 8%, sodium hydroxide 8% and thiourea 6.5% (by weight) were added for every 100 mg of dry sample. The method is called UTS because of the acronym of its reagents (Urea/Thiourea/Sodium Hydroxide). The beakers were placed in the freezer at minus 20 ºC for 40 min and were then placed in agitation until they reached room temperature. After that, the samples were passed through a 53 µm mesh sieve. They were washed 15 times with 30 mL of ultra-pure water. Finally, the samples were recovered in the same beakers and the previous drying procedure was repeated. This method has been adapted from Olsen et al. (2020).

Once the samples were dry, the wet peroxide oxidation (WPO) of the organic matter was performed. According to Masura et al. (2015), in order to oxidise organic matter, 20 mL of 0.05 M iron sulphate solution and 20

mL of 30% v/v hydrogen peroxide were added to the samples and stirred at 200 rpm and 75 ºC for 30 min. Then they were passed through the sieve, washed with ultra-pure water and left to dry again. The whole procedure could be repeated from 1 to 3 times depending on the cellulose and organic matter content. The synthetic samples were treated in the same way as the wastewater samples.

In the specific case of sewage sludge, the process was carried out in reverse, starting the process with the WPO method previously described. After that, the samples were filtered through 0.53 µm mesh sieve to remove excess reagents, collecting the samples in the same way as described for wastewater. This was followed by UTS treatament described above.

In summary, for wastewater, the UTS treatment of removal cellulose is carried out first followed by WPO whereas for sludge samples the method is in reverse. This is due to the high amount of organic matter in the sludge, which would make the first UTS treatment more difficult and less effective.

Once the process was finished, density separation was carried out to isolate MPs from the rest of the particles retained within the samples and that might remain in the medium. For this purpose, 20 mL of 5 M sodium chloride solution was added for each 20 mL of sample. They were left in the decantation funnel overnight. The decanted material was discarded and the supernatant was filtered with vacuum filtration equipment through 0.8 µm polycarbonate filters. Once the samples were filtered, the filters were

left to dry for 2 h at 40 ºC and observed under a Carl Zeiss Axio Imager M1m binocular magnifying lens for the quantification of the MPs. FTIR analysis was carried out after each step in order to check changes in the estructure and composition of the MPs in the blank samples. Fig. 1 shows an overview of the analysis process in wastewater samples, the treatment for sludge is the same but the UTS and WPO steps are reversed.

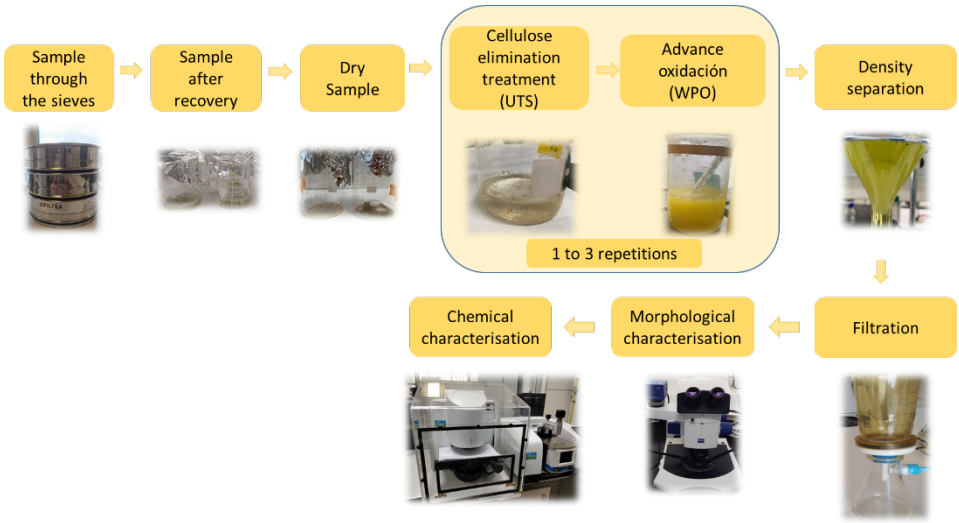


Fig. 1. Overview of the analysis process in wastewater samples. Sludge samples have the UTS and WPO treatments at reverse.

2.4. Sample characterisation

After the treatment of the samples, the microparticles were differentiated according to their morphological and chemical characteristics. It was necessary to perform an initial count of the microparticles and differentiate them according to their morphological characteristics. However, in this step it was not possible to confirm that they were polymers, therefore they are called microparticles. To determinate whether the particles are plastics or not, it is required to

perform a second analysis of their chemical characteristics and distinguish between microparticles and MPs.

2.4.1. Morphological characterisation

In order to monitor the effect of each of the UTS and WPO treatment replicates on the samples, photographs were taken of each of them. For this purpose, it was use a Carl Zeiss Axio Imager M1m optical microscope.

2.4.2. Chemical characterisation

Chemical characterisation was performed according to spectroscopic methods used to identify the types of polymers in the collected samples employing a PerkinElmer Spectrum 100 Fourier Transform Infrared Spectroscope (ATR-FTIR) in total attenuated reflection mode, in order to control whether the MPs added to the synthetic samples had been degraded during the different UTS and WPO treatment replicates.

To determine the composition of the MPs, the particles were exposed to infrared radiation (Sun et al., 2019), generating a particle-specific spectrum based on the chemical bonds between the atoms. The resulting spectrum was analysed using the characteristic spectrum compared to the polymer spectrum library of the reference. This library had previously been created with the pure polymer used as a reference.

3. Results and discussion

The proposed method is an enzyme-free digestion for the dissolution of cellulose and chitin. Based on existing methods to dissolve cellulosic materials (Hu et al., 2007; Jin et al., 2007; Yan et al., 2007; Olsen et al.,

297 2020). The three synthetic replicates of cellulose and microplastic samples
298 were tested with different repetitions of the same UTS treatment to remove
299 cellulose, with one repetition for replicate 1 (R1), two repetitions for
300 replicate 2 (R2) and three repetitions for replicate 3 (R3). R1 was
301 processed with a UTS treatment and WPO, R2 was treated with UTS and
302 WPO twice, and finally, R3 was pre-treated three times with UTS and
303 WPO. After each UTS and WPO treatment step, the samples were dried
304 and weighed to record the cellulose removal, by weight, for each sample.
305 A known weight of cellulose and 15 visual MPs between 1000 and 500
306 µm were added to the control cellulose samples (R1, R2 and R3). Table 2
307 shows the percentages of cellulose removal for each of the replicates for
308 the different UTS and WPO treatment steps and repetitions.

309 Table 2. Monitoring of the mass loss of the synthetic samples after each step of the
310 treatment procedure.

	First repetition		**Second repetition**		**Third repetition**	
	% elimination w/ UTS	% elimination w/ WPO	% elimination w/ UTS	% elimination w/ WPO	% elimination w/ UTS	% elimination w/ WPO
R1	27.3	*	-	-	-	-
R2	26.3	40.7	97.6	*	-	-
R3	28.6	41.9	97.3	97.5	98.2	*

311 * The treatment was carried out but not weighed as it had been filtered directly for
312 analysis.

313 As shown in Table 2, for the synthetic samples (cellulose, MPs, and
314 ultra-pure water), with a single repetition of the complete treatment (UTS
315 and WPO), an average of 41.3% (± 0.8) was obtained. After the second
316 treatment replicate step UTS and WPO, a cellulose removal of 97.5 –

97.6% was observed, so that two replicates were accepted as the optimal number of replicates to successfully remove most of the cellulose present in the samples. The trade-off between time invested, reagent consumption and treatment efficacy indicates that the third repetition is not really necessary. This can be skipped as it does not provide much variation on the results in cellulose removal treatment. After the end of the treatment for each of the synthetics replicates, a count of the MPs present in each replicate was made to ensure that there had been no losses during the different stages. Corradini et al. (2019) show how organic matter affected the recovery rate of each polymer. With the proposed method adapted from Olsen et al. (2020), a large part of these interferences by organic matter in the samples and by cellulose in particular in the samples from the different treatment stages of the wastewater treatment plants would be eliminated.

Other potential methods for organic matter removal are derived from existing studies that extract MPs from biota. Acid digests, such as hydrochloric acid (HCl) and nitric acid (HNO_3), have been shown to be very effective in destroying organic matter, but they also affect MPs particles, leading to degradation and melting (Hurley et al., 2018). Because many of the processes are aggressive and can degrade MPs, it is necessary to know the amount of MPs and the type of polymer (High Density Polyethylene) in the synthetic sample. Therefore, a count was made after each of the stages to which the sample was subjected. The number of MPs counted in replicate 1 was 19, in replicate 2 was 17 and in replicate 3 was 16. The increase in MPs quantified with respect to the initial number may

be due to their fragmentation into smaller particles by mechanical actions during treatment.

Different stages of the wastewater treatment plant have a high amount of organic matter and cellulose. These stages can be the water influent, the biological reactor or the sludge line. The removal of this organic matter and cellulose is crucial for proper detection, identification and quantification. Further research on techniques and/or methods that allow the separation of MPs from organic matter is certainly a challenge, but is of great importance to reduce further microplastic contamination when reused water or sludge is used for soil improvement (Sol et al., 2020).

Referring to the above-mentioned, the removal of natural organic matter (NOM) becomes necessary. Different studies show that, in addition to the already known organic load in the wastewater, approximately 35% of the suspended solids in the influent originated from toilet paper (Ruiken et al., 2013). In wastewater, they have estimated a consumption of about 10 kg y^{-1} of toilet paper per person (Ruiken et al., 2013). These same authors give the example of Waternet in Amsterdam, with a population of 1,200,000 people who are connected to Waternet's WWTPs. This population discharge 12,000 to 15,000 t y^{-1} of toilet paper. The total mass of suspended solids measured in the influent is 32,000 t y^{-1}. This indicates that approximately 40% of the influent suspended solids could be cellulose derived from toilet paper and wipes (Ruiken et al., 2013).

Furthermore, primary sludge from sewage treatment plants has been reported to contain a considerable amount of cellulose, about 20%, based

on suspended solids (Honda et al., 2002). Cipolletta et al. (2019) stated that the treated sludge had an average of 87% cellulose, hemicellulose and lignin content. Ruiken et al. (2013) show values for the cellulose fraction of 32% and 38% of the organic mass.

The removal of natural organic matter in water and sludge samples from WWTPs must be improved by dissolving cellulose. Many authors use WPO treatment to remove the organic matter present in this type of sample (Franco et al., 2020; Magni et al., 2019). However, applying this type of treatment to samples with a high amount of cellulose is not sufficient to remove it and, when performing the last step of separation by density of the microparticles of interest, the cellulose in suspension forms a matrix that traps these microparticles, preventing them from being separated from the rest of the solution by density and, therefore, hindering their recovery and subsequent study by microscopy and FTIR. The objective is to analyse, quantify and identify MPs in wastewater or sludge samples after a pre-treatment step that serves to ensure the subsequent extraction of impurity-free MPs. By using the UTS method it is possible to reduce almost completely the cellulose residues of the input and sluge samples.

In order to verify that the method worked, it was decided that it should be carried out on a sample of raw sewage. Three replicates of the wastewater sample were subjected equally to different numbers of replicates of the cellulose removal treatment: the first sample was subjected to only one WPO step (Fig. 2a). The second sample was subjected to one repetition of the complete treatment (WPO + UTS) (Fig.

2b), while the third sample was subjected to three repetitions (3 WPO + 3 UTS) (Fig. 2c). The aim was to observe the effect of the different treatment steps on the samples and to compare them with each other. It can be seen how the different treatment repetitions on the sample remove cellulose and other organic matter residues, facilitating the subsequent quantification and classification by shape of the microparticles present. Although three repetitions of the treatment were carried out for the last replicate, the results obtained with the synthetic samples of cellulose and MPs allow us to determine, with only two repetitions, that the elimination of cellulose would be sufficient. In heavily loaded samples a third repetition may be necessary.

After consecutive repetitions of the treatment, it is possible to observe the gradual disappearance of the white fibres corresponding to cellulose, while the microparticles of interest for the analysis remain practically unaltered. The existence of cellulose in wastewater samples makes the analysis of MPs as difficult as large amounts of organic matter can be. Authors such as Lavoy et al. (2021) argue that large concentrations of organic matter can make it impossible to study wastewater and MPs at certain stages in WWTPs because, if it is not correctly removed, it can lead to incorrect counts of the amount of MPs present, either because the organic matter adheres to them, modifying their density and thus affecting the separation by density of the treatment, or because it obscures the MPs themselves when performing the visual count or because it interferes with the analysis by infrared spectroscopy. Fig. 2 shows the wastewater sample

and the same sample after repeated treatments under an optical microscope. A decrease in cellulose is observed in the samples.

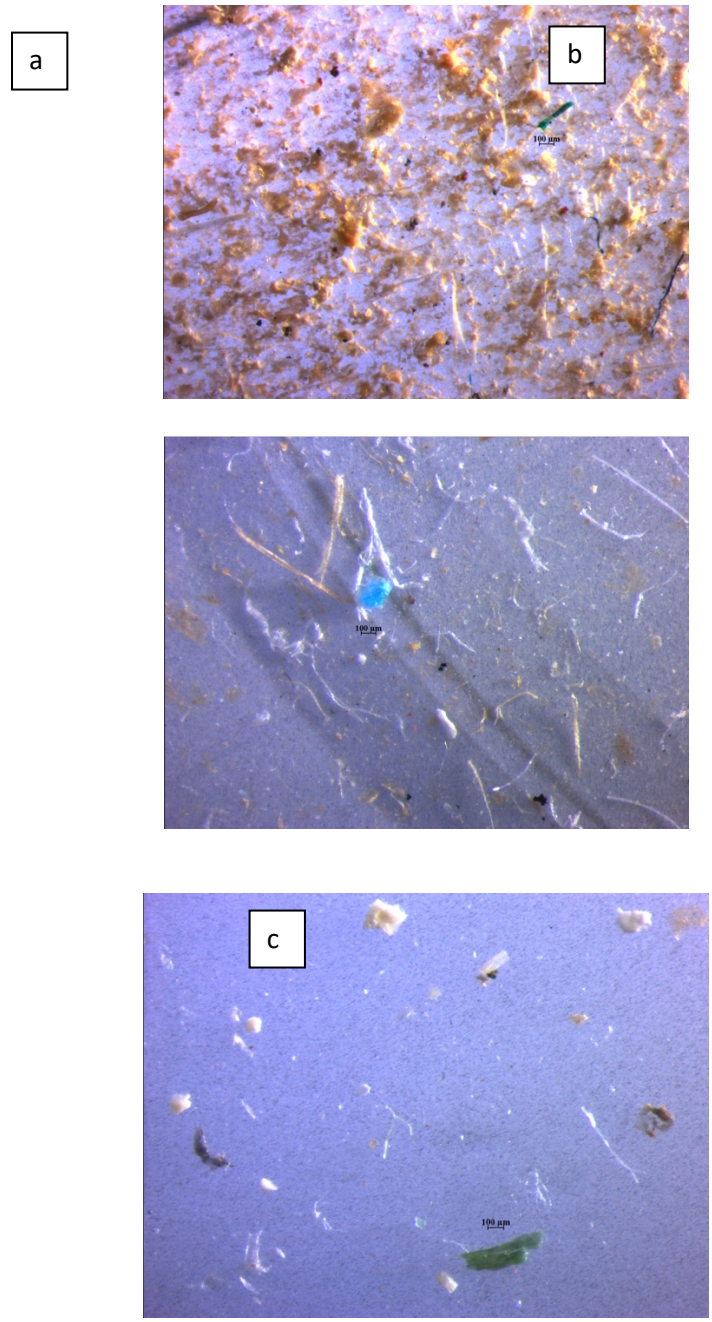


Fig. 2. Wastewater sample without treatment to remove cellulose, only with WPO (a), the same sample after the first UTS treatment and first WPO (b), the same simple after third UTS treatment and third WPO (c) under optical microscope.

As with the water line, the procedure was corroborated for a sludge sample. For the sludge, the first sample was treated with only one WPO (Fig. 3a), the second sample with WPO + UTS (Fig. 3b), the third sample with two cycles of WPO + UTS (Fig. 3c) and finally, the fourth sample with three cycles of WPO + UTS (Figure 4d).

Fig. 3 shows how the cellulose is decreasing in the sample, facilitating the analysis for the detection, counting and subsequent identification of MPs. In the case of sludge, it can be seen that 2 treatments of WPO + UTS could be sufficient (Fig. 3c), with a third treatment being recommended in order to obtain a clean sample (Fig. 3d).

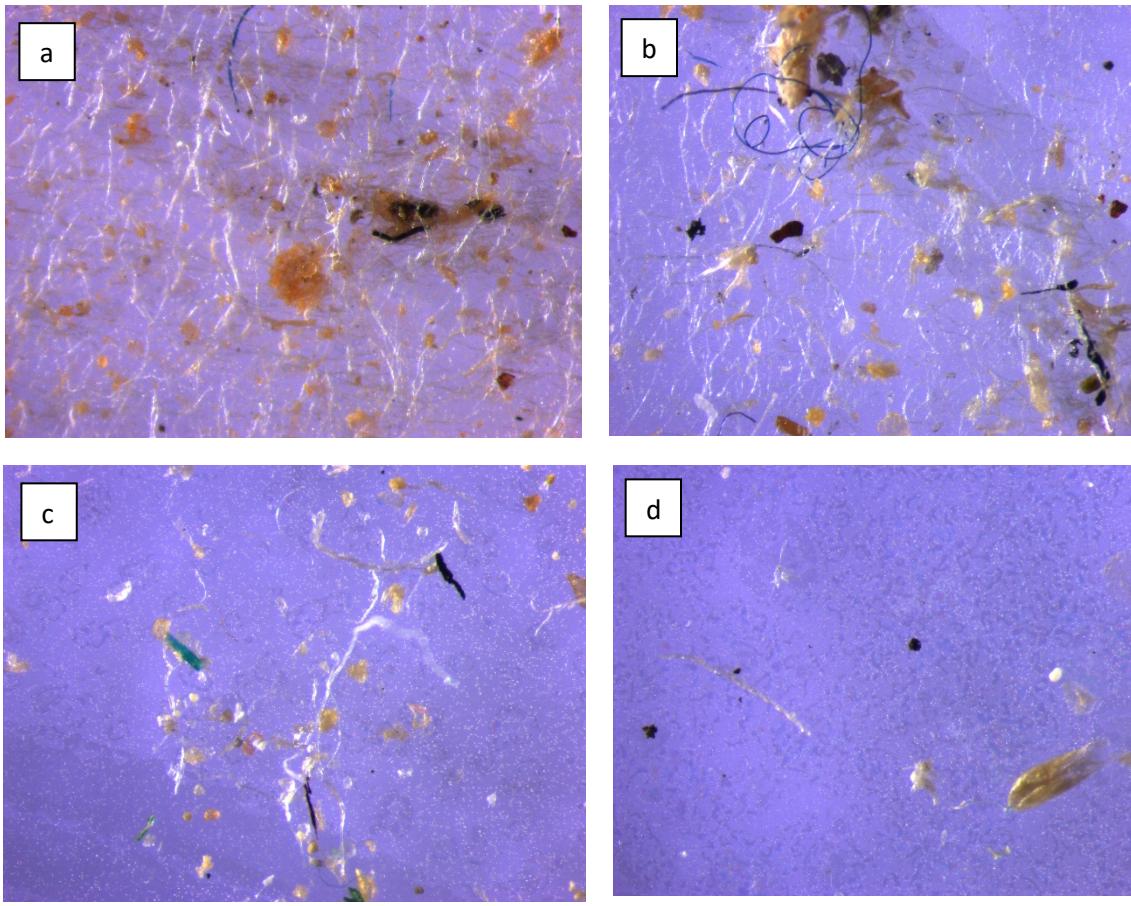


Fig. 3. Sludge sample without treatment to remove cellulose, only with WPO (a), the second replica of sample with WPO + UTS (b), the third replica of sample with two cycles of WPO + UTS (c) and the fourth replica of sample with three cycles of WPO + UTS (d) under optical microscope.

Finally, to verify that the treatment of the samples does not imply a degradation of the plastic materials and that the cellulose does not interfere with their identification, the MPs of the three synthetic cellulose samples were analysed by FTIR after treatment. Fig. 4 shows the spectra obtained by ATR (Attenuated Total Reflectance) after analysing the MPs and comparing them with a reference library. In all cases analysed, the MPs were identified as high density polyethylene (HDPE) with correlation indices higher than 0.97, which shows that the treatment is harmless for this type of plastic material.

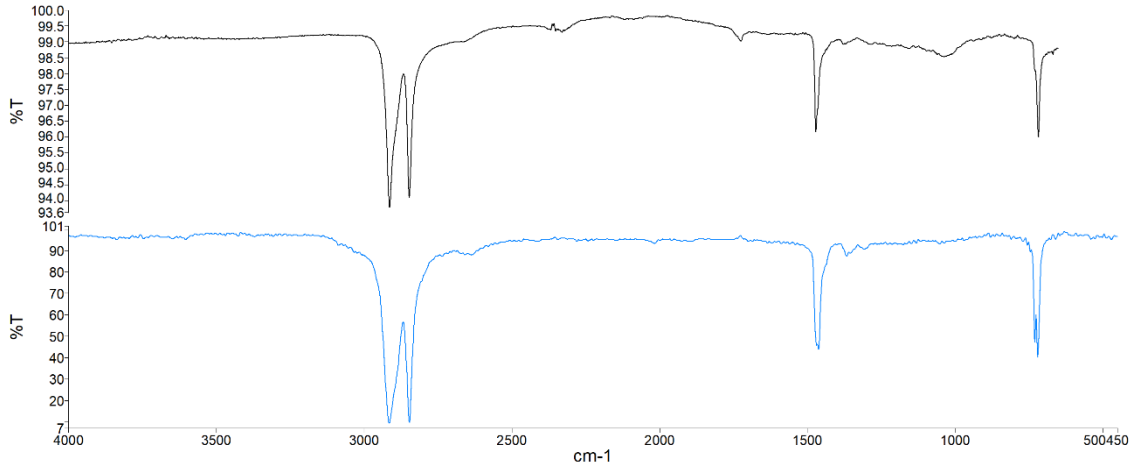


Fig. 4. Microplastic spectra aquired with FTIR-ATR from synthetic cellulose samples. Microplastic sample from R1 (—) and Reference espectre HDPE, High Density Poly-Ethylene (—).

Authors such as Lavoy et at. (2021) have also observed the possibility that certain polymers may degrade when subjected to treatments with prolonged digestion or with strong acids or alkalis. The treatment used on

461 these samples avoids long exposure times of the MPs to the reagents used,
462 thus reducing their potential degradation, as shown in Fig. 4.

463 Once it was known that the method did not affect the synthetic HDPE
464 polymer that was added to the pure water samples, wastewater and sludge
465 samples also treated with the method were analyzed. Table 3 shows some
466 of the particles analyzed, where organic matter, polyethylene (PET),
467 HDPE, polypropylene (PP), polyethylene (PP) and polyvinyl chloride
468 (PVC) have been detected. All the polymers had a correlation higher than
469 0.91 with respect to those of the library used. Fig. 5 shows the spectra for
470 the different particles analyzed.

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472

473

474 Table 3. Particles analyzed in one of the wastewater samples after application of the
475 method.

Search Score	Search Best Hit Description	Polymer
0,980613	poly(ethylene terephthalate)	PET
0,954811	high density poly- ethylene	HDPE
0,835022	ricinoleic acid	--
0,918259	polypropylene	PP
0,93204	polyethylene	PE
0,903578	polyvinyl chloride	PVC

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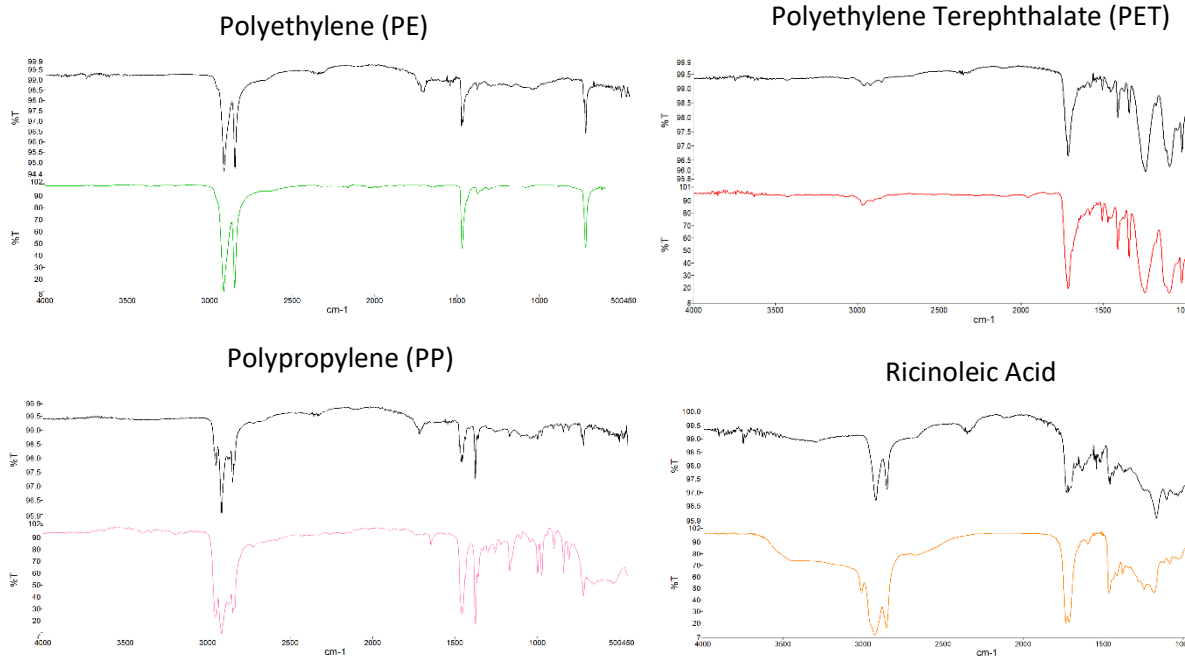

Fig. 5. Microplastic spectra aquired with FTIR-ATR from real samples. Microplastic sample (—) and Reference espectre PolyEthylene (—), PolyEthylene Terephthalate (—), Polypropylene (—) and Ricinoleic Acid (—),

Applying the method to all samples the authors have found a high correlation in more than a dozen different polymers. By detecting different polymers with high correlation, it can be stated that the proposed method facilitates the analysis of these polymers.

4. Conclusions

The correct and efficient removal of microplastics in different environmental matrices, and in particular in wastewater and sludge, depends on the method used. Organic and cellulose impurities make the detection, quantification and identification of microplastics difficult.

- Good visual pre-counting and obtaining a sample with a minimum of impurities facilitates the processes for the analysis of microplastics.
- The UTS method in combination with the WPO is proposed as a method for the analysis of microplastics in different matrices where cellulose and organic matter may cause possible interferences. Almost complete elimination of the cellulose present in the samples was achieved from the second repetition of the treatment.
- The establishment of a standardized methodology for the extraction of microplastics in different matrices is essential for efficient and reliable detection, quantification and identification. Include cellulose removal combined with organic matter removal.

There are not many studies on the amount of cellulosic matter in wastewater and sludge. A study on quantity and how it affects COD is launched as future lines of research.

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