Quantitative analysis of microplastics in wastewater from treatment plant by visual identification and FT-IR imaging using H$_2$O$_2$ and FeSO$_4$: A case study

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Abstract. Plastic is a commonly used and perhaps unavoidable material due to its multifaceted nature. Plastic wastes do not degrade easily and hence present as a major threat to the environment. Plastics of particle size less than 5mm are universally considered as microplastics. The present study investigates the identification and quantification of microplastics. The sample was collected from the wastewater treatment plant of the Kalasalingam University campus as a bulk sample. The sample was prepared using Hydrogen peroxide and Iron II sulfate to oxidize the organic matter. Filtration was carried out in a set of filter papers arranged in series with decreasing pore size. Sediments were collected and analyzed using FTIR imaging. The surface of the paper was analyzed using 40X dissecting microscope for visual identification. Further, SEM analysis with EDS mapping was performed to study the material composition. Eight different types of microplastics (MPs) were identified and sizes measured. The particle size varied from 10 - 20 micron.

1. Introduction

Nowadays, usage of plastics has become intensified in the day to day life. The quantity of plastics used per year is greater than 240 million tonnes per year across the world (Thompson et al., 2004). India generates about 26000 tons of plastic waste per day. Degradation of plastic is a very slow process, eventually, the degraded plastic enters the aquatic environment. The degradation of plastic particles like scrubbers, items of baggage, polymer fragments, pellets in cosmetics, industrial pellets, etc., are the reason for microplastics formation (Fendall et al., 2009; Hidalgorus et al., 2012; Browne et al., 2011). After degradation, the plastic particle can be divided as microplastics, macroplastics, mesoplastics as per their size (Fendall et al., 2009; Browne et al., 2010). Microplastics size range is <5 mm in the global level. Wastewater treatment plant is considered as the main source which expels the microplastics into the environment (Minteing et al., 2017). It is very important to understand the major source of microplastics to handle environmental problems (Browne et al., 2011). The intake of microplastics by marine living organisms is a significant threat nowadays (Cole et al., 2010). The microplastics forms the probability of commencing the pathogenic taxa from wastewater to the marine habitats (Harrison et al., 2014; Zettler et al., 2013).

The separation of microplastics can be considered as primary microplastics and secondary microplastics. Primary microplastics are plastic pellets, scrubbers in cosmetics, toiletries and fibers released from fabrics. The photo-oxidative breakdown of larger plastic particles are called secondary microplastics (Minteing et al., 2017). The main obstacle for the identification of microplastics is its separation. Different types of separation processes such as density separation, bulk separation are usually performed. But the successful isolation of microplastics is achieved through elutriation method, but this method is suitable only for marine samples (nuelle et al., 2014; Claessens et al., 2013). However, the elutriation process is far less useful for the sample with a high concentration of organic compounds and microorganisms.
In FT-IR analysis, we can study both absorbance and transmittance of microplastics. The popular use of FT-IR is mainly due to its high-resolution images and time reduction in the analysis of microplastics (Minteing et al., 2017; Talvite et al., 2017). The usage of microscope in association with FT-IR spectroscopy is called micro FT-IR which can identify very small particle because of its high spatial resolution (Koenig et al., 2001). Transmission or reflectance mode is used for analysis of plastic particle in micro FT-IR (Ojeda et al., 2009).

2. Objective of the present study

The objective of present study is to identify and classify microplastics from the sample collected from the wastewater treatment plant, and also to quantify the microplastics based on their numbers and types present. The sample is pretreated with H₂O₂ and iron sulfate to remove organic matter, in case of identification FT-IR is used to identify the types and number of microplastics present in the sample followed by Scanning Electron Microscope analysis to determine the thickness of microplastic.

3. Experimental work

3.1 Collection of sample

The study starts with waste water sample collection. The sample was collected from Kalasalingam University Sewage Treatment Plant. The University is located in southern Tamil Nadu in the Virudhunagar district with more than 8000 students both the day scholar and hostellers 600 staff members. The recycled water from the treatment plant is used for the gardening purpose. The sewage treatment plant follows the norms of Pollution Control Board norms and helps to save the habitat. The waste water samples were collected from three stages of the treatment plant, ie, after the screening(raw water), aeration tank(aerated water), recycled wastewater(treated water). Two litres of sample at each stage of the treatment plant was collected manually. Telescopic sampling pole was used to collect the sample from each stage of the wastewater treatment plant. To protect the sample from airborne microbes and bacterias, the collected samples were stored in an LDPE (Tagg et al., 2015). To avoid the development of microorganisms in the water sample, it was kept in a freezer until the further usage of the sample.

3.2 Sample pretreatment

Based on volume reduction method, the two litre samples were reduced to 1 litre and proceed with the further processes. Pretreatment process of the sample was carried out to eliminate the microorganism and organic matter present in the sample. Raw, aerated and treated wastewater may contain organic matter and living microorganisms. To eradicate these impurities, the sample was treated using Hydrogen peroxide (H₂O₂) and Iron II sulfate. Procedure given by Marta Simon et al.(2018) was followed.

The quantity of chemicals added to the sample as follows:

i) Raw wastewater: Hydrogen peroxide (H₂O₂) – 250g/l and Iron II sulfate - 2.5 g/l is added.
ii) Aerated wastewater: Hydrogen peroxide (H₂O₂) – 180g/l and Iron II sulfate - 1.8g/l is added.
iii) Treated wastewater: Hydrogen peroxide (H₂O₂) – 125g/l and Iron II sulfate - 1.25g/l is added. The samples are kept idle at room temperature for 7 days period.
After seven days, the pretreated samples are ready for filtration. A filtering media setup was created for filtering the sample. Three different filter paper sizes were used for the filtering process. Filter paper pore size ranges of 240 micron, 120 micron, 110 micron are used. Once filtration is done, the filter paper is allowed to dry at room temperature to get rid of the moisture content present in the retained sediment. Then the dried sample retained on the filter paper was collected and analyzed by FT-IR spectroscopy.

3.3 Fourier transform infrared imaging spectroscopy (FT – IR) analysis

FT-IR spectroscopy is one of the popular techniques widely used for polymer identification. This technique is reliable, accurate and straightforward in the identification of micro-plastics. To differentiate the plastics and natural materials, the distinct band pattern with certain highly specific infrared spectral techniques are used. The main concept is that, in the electromagnetic spectrum, most of the molecules absorb light in the infrared region (Crawford et al., 2017) for polymer plastics. The results obtained from FT-IR is used for polymer type and particle number identification.

3.4 Visual identification

Micro-plastics have two characteristics, namely, physical and chemical. So, identification of micro-plastics requires more than one method for reliable results. Therefore, combination of two analytical methods is used widely. For physical analysis, microscopy is used. In this study, dissecting microscope with a magnification of 40X and scanning electron microscope is used. To make sure the presence of adhesive polymers in the filter paper, the filter paper is examined under the dissecting microscope and the length of the polymer fibers are measured.

3.5. Scanning electron microscope/energy dispersive x-ray spectroscopy mapping

A scanning electron microscope can provide the high-resolution images. The resolution images of the surface texture can differentiate the microplastics from other organic matters (Cooper et al., 2010). SEM can also analyze the particular selected location of the sample. Likewise, EDS is used to find the elemental composition of the sample (Vainello et al., 2013). To identify the carbon dominating plastics from inorganic particle, elemental composition analysis is very useful. The composition of metals and polymers can also be found by using this method.

4. Results and Discussion

4.1 Fourier transform infrared imaging spectroscopy (FT – IR) analysis

Figure 1, shows the result of FT-IR imaging for the screened sample (S1). From the FT-IR analysis, the dry sample shows the presence of microplastics namely polystyrene, polypropylene, polyethylene terephthalate, polyvinyl chloride, PELD, PEHD. The type of polymers are found out by using their wavelength range and corresponding frequency value. The particle number has also been determined. It is also found that, S1 (screened sample 1), A3 (aerated sample 3), and T1 (Treated water 1) samples has the presence of polymer.

Figure 1. FT-IR result showing wavelength Vs absorption
### Table 1. Polymers in the screened sample

| S.No | Frequency (cm⁻¹) | Polymer type | Abbreviation                  |
|------|-----------------|--------------|-------------------------------|
| 1    | 2920.23         | PEHD         | Polyethylene high density     |
| 2    | 2920.23         | PELD         | Polyethylene low density      |
| 3    | 2920.23         | PP           | Polypropylene                 |
| 4    | 2850.93         | PEHD         | Polyethylene high density     |
| 5    | 2850.93         | PELD         | Polyethylene low density      |
| 6    | 2850.93         | PP           | Polypropylene                 |

### Table 2. Polymers in the aerated sample

| S.No | Frequency (cm⁻¹) | Polymer type | Abbreviation                  |
|------|-----------------|--------------|-------------------------------|
| 1    | 1064.71         | PET          | Polyethylene terephthalate    |
| 2    | 1064.71         | PEHD         | Polyethylene high density     |
| 3    | 1064.71         | PELD         | Polyethylene low density      |
| 4    | 1064.71         | PVC          | Polyvinyl chloride            |
| 5    | 1064.71         | PP           | Polypropylene                 |
| 6    | 1064.71         | PTFE         | Polytetrafluoroethylene       |
| 7    | 605.65          | PS           | Polystyrene                   |
| 8    | 605.65          | PTFE         | Polytetrafluoroethylene       |

### Table 3. Polymers in the treated sample

| S.No | Frequency (cm⁻¹) | Polymer type | Abbreviation                  |
|------|-----------------|--------------|-------------------------------|
| 1    | 1078.21         | PET          | Polyethylene terephthalate    |
| 2    | 1078.21         | PVC          | Polyvinyl chloride            |
| 3    | 1078.21         | PTFE         | Polytetrafluoroethylene       |
| 4    | 605.65          | PS           | Polystyrene                   |

### 4.2 Visual identification

With the help of the dissecting microscope, the adhesive polymer present in the filter paper is found and the length of the polymer is measured using MOTIC software.

![Microscopic image of fiber length](image)

### Figure 2. Microscopic image of fiber length(S1, A3, T1)

### 4.3 Scanning electron microscope analysis

Due to the good magnification, the scanning electron microscope generates the high-resolution images. It can even measure very small feature of the sample. The thickness of the polymer present in the filter paper is measured likewise. The results are given in Tables 4 to 6

### Table 4. Dimension of polymer in the screened sample

| S.No | Sample | Length (mm) | Thickness (µm) |
|------|--------|-------------|----------------|
| 1    | S1     | 16.172      | 100            |
| 2    | S2     | 10.041      | 100            |
| 3    | S3     | 5.280       | 100            |
Table 5. Dimension of polymer in the aerated sample

| S.No | Sample | Length (mm) | Thickness (µm) |
|------|--------|-------------|----------------|
| 1    | A1     | 8.973       | 100            |
| 2    | A2     | 4.501       | 100            |
| 3    | A3     | 3.245       | 20             |

Table 6. Dimension of polymer in the treated sample

| S.No | Sample | Length (mm) | Thickness (µm) |
|------|--------|-------------|----------------|
| 1    | T1     | 7.694       | 20             |
| 2    | T2     | 3.556       | 20             |
| 3    | T3     | 3.170       | 10             |

4.4 EDS mapping

Based on the analysis of EDS report we found several compounds like C, O, Al, P, Co, Fe, etc., the compounds like iron and cobalt can the trace of chemicals used in pre-treatment process and SEM analysis. The presence of carbon and oxygen in the sample is found to be <= 80%, the trace of is not found because hydrogen has no core electron, the signal from hydrogen and the signal from valance electron overlap each other. Therefore the existence of H compound is not found in elemental identification by EDS mapping. Although we have 80% presence of carbon and oxygen in the sample, we can perceive the existence of polymer.

Figure 3. EDS mapping

5 Conclusion

The presence of microplastics in wastewater is found based on the particle number and types of the polymer. PEHD, PELD, PP are found three in numbers and PVC, PET, PTFE, PS are found two in numbers. By the approach of \( \text{H}_2\text{O}_2 \) pre-treatment allows the oxidation of organic matter present in the sample. Polymer type is determined based on the result obtained from FT-IR analysis. The thickness of the polymer and the existence of the polymer is studied with the help of SEM analysis and EDS mapping.

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