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Removal of microplastics from secondary wastewater treatment plant effluent by coagulation/flocculation with iron, aluminum and polyamine-based chemicals

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ABSTRACT

Microplastic (MP) removal by coagulation/flocculation followed by settling was studied in a secondary wastewater treatment plant (WWTP) effluent matrix. MP concentration in size range <10 µm in wastewater is currently unknown due to the exclusion of this size range in many studies and due to difficulties in microplastic quantification. WWTP effluent samples were spiked with a known amount of polystyrene spheres of two different sizes 1 µm and 6.3 µm. The samples were treated with inorganic and organic coagulants typically used in WWTPs, i.e., ferric chloride, polyaluminum chloride, and polyamine. The effect of pH was studied with ferric chloride by changing the pH from 7.3 to 6.5. In this study, MP removal was monitored using flow cytometry. The role of chemicals in MP removal at WWTPs has not been in the focus of previously reported MP studies. Our results showed that all tested coagulants enhanced the removal of MPs with dosages applicable to tertiary treatment. The highest removal efficiency obtained was 99.4%, and ferric chloride and polyaluminum chloride were more efficient than polyamine. Performances of ferric chloride and polyaluminum chloride were close to each other, with a statistically significant difference at a certain dosage range. Our findings suggest that chemical coagulation plays a key role in the removal of MPs, and the process can be optimized by selecting the right coagulant and pH.

Keywords:

Microplastic

Removal

Coagulation/flocculation

Wastewater treatment

Flow cytometry

Abbreviations:

Chemical Oxygen Demand, COD

Dissolved organic carbon, DOC

Flow cytometry, FCM

Microplastic particle, MP

Polyethylene, PE

Polystyrene, PS

Suspended solids, SS

Wastewater, WW

Wastewater treatment plant, WWTP

1 Introduction

Microplastics (MPs) are plastic particles smaller than 5 mm in size (Arthur et al., 2009). They exist in various shapes and materials, and they can be classified as primary and secondary MPs, according to source. Primary MPs are produced in a small size on purpose, whereas secondary MPs are the result of weathering, degradation, or the break down of larger plastic particles. For instance, tire wear and textile fibers are secondary MPs. Primary MPs include pellets used in industry and microbeads in cosmetic products. (Alimi et al., 2018)

Several studies report a high microplastic (MP) removal of 90–98 % at wastewater treatment plants (WWTPs) (Talvitie et al., 2017a; Ziajahromi et al., 2017; Murphy et al., 2016; Magnusson & Noren, 2014; Lares et al., 2018; Carr et al., 2016), and it is clear that WWTPs contribute to the MP load in freshwater (Alimi et al., 2018; Simon et al., 2018; Lares et al., 2018; Ziajahromi et al., 2017; Talvitie et al., 2017a; Talvitie et al., 2017b; Mintenig et al., 2017). Due to the large water volumes handled, WWTPs are suggested as point sources of MPs (Lares et al., 2018; Talvitie et al., 2017a; Ziajahromi et al., 2017). However, some studies point out that the relative significance of WWTPs as the MP source is difficult to estimate, as the total emission estimates are highly uncertain (Simon et al., 2018; Lassen et al., 2015; Sundt et al., 2014; Magnusson & Noren, 2014, Alimi et al., 2018). In an aquatic environment, various organisms have been observed to ingest MPs and suffer adverse effects caused by both MPs themselves and chemical substances in MPs (Anbumani & Kakkar, 2018; Wang et al., 2018). In addition, MPs are reported to be transferred within the food chain (Setälä et al., 2014). MPs are also suspected to have a negative impact on humans (Wang et al., 2018), who might be exposed to MPs through drinking water (Koelmans et al., 2019) or food (Wright and Kelly, 2017).

At WWTPs, it is reported that pre- and primary treatments are the most efficient stages in MP removal (Murphy et al., 2016; Lares et al., 2018; Carr et al., 2016). However, the MP studies conducted at WWTPs have focused on the MP concentration and MP classification, not on the effect of the operational characteristics of the treatment processes on removal. Thus, details on coagulation and flocculation chemicals, dosages, and operational conditions are excluded in these studies. The role of chemicals in MP removal in synthetic wastewater has previously been studied outside of WWTPs (Table 1). Larue et al. (2003)

compared coagulation/flocculation and electrocoagulation in MP removal, whereas Perren et al. (2018) studied MP removal by electrocoagulation followed by settling. Both of these studies report above 90% removal after treatment with ferric chloride, ferrous sulfate, or electrocoagulation with iron electrodes. In addition, there are two recent studies about iron and aluminum coagulants in MP removal in a synthetic drinking water matrix that reports maximums of 17% and 36% MP removal after ferric chloride and aluminum chloride treatments, respectively (Ma et al., 2019a; Ma et al., 2019b). In all of these studies, MP quantification was done gravimetrically except for Perren et al. (2018), who counted the MPs after filtration.

Table 1 Previous studies on microplastic (MP) removal by chemicals.

Reference	Sample matrix	MP size,	MP material	MP concentration,	Coagulants/electrodes
Larue et al. (2003)	tap water	0.7–1.58	Latex	2400–2700	ferric chloride, ferrous sulfate, or iron electrodes
Perren et al. (2018)	industrial fresh water + NaCl	300–355	PE	100	iron electrodes
Ma et al. (2019a)	ultrapure water + humic acids + kaolin	<500	PE	100*	aluminum chloride, ferric chloride, polyacrylamide
Ma et al. (2019b)	ultrapure water + humic acids + kaolin	<500	PE	100*	ferric chloride, polyacrylamide

*Calculated from polyethylene (PE) mass of 0.1 g assuming sample volume 1 L.

The present study focused on investigating commonly used inorganic and organic coagulants to remove MPs in wastewater treatment. The aim was to study the effect of ferric chloride, polyaluminum chloride, and polyamine on the removal of MPs less than ten micrometers in a real municipal wastewater matrix using flow cytometry (FCM). Based on the literature, MP removal by chemicals has not been previously studied in a municipal wastewater matrix (Alimi et al., 2018; Cai et al., 2018; Larue et al., 2003; Perren et al., 2018; Ma et al., 2019a; Ma et al., 2019b). In addition, the reviewed literature does not report MP removal or MP aggregation with polyamine. The difference between these chemicals and the effect of dosage and pH were studied. MP removal was studied by spiking secondary WWTP effluent with a known number of polystyrene (PS) spheres, which are typically used when studying MP or nanoplastic aggregation (Alimi et al., 2018; Cai et al., 2018). The MPs were 1 µm and 6.3 µm in diameter. These particles are smaller than 20 µm, a size range excluded in many wastewater (WW) studies (Lares et al., 2018; Sun et al., 2019), and thus there is a lack of analytical methods for studying MPs this small in the WW matrix. However, this size range of MPs has been studied in other matrices. For instance, MP aggregation in different water matrices (Alimi et al., 2018; Cai et al., 2018), ingestion of MP by planktonic organisms (Setälä et al., 2014), and MP concentration in bottled water (Ossmann et al., 2018) have focused on small MPs. The original MP concentration in this study was 22000 times smaller than that used in a previous removal study on a similar particle size range (Larue et al., 2003). The samples were treated by coagulation/flocculation, followed by settling. The MP concentration was recorded with FCM, which is a promising tool for MP detection and quantification (Long et al., 2017; Woods et al., 2018; Sgier et al., 2016). This method has not been previously reported to be utilized in a wastewater matrix, and so this was the aim of this study (Sun et al., 2019). The benefit of flow cytometry in an MP study is that it allows for relatively quick quantification of the smallest MPs even nanoplastics while at the same time distinguishing them from other same-sized particles in the sample (Long et al., 2017). Typically, studies at WWTPs utilize FTIR or Raman spectroscopy, of which FTIR has a size range limitation of 10–20 µm, and Raman spectroscopy is time-consuming (Silva et al., 2018; Ribeiro-Claro et al., 2017; Käßler et al., 2016; Sun et al., 2019). Turbidity and COD were measured to support the coagulation/flocculation treatment results from flow cytometry. In addition,

the zeta potential was measured to study the changes in electrostatic interactive forces and thus the removal mechanism of MPs (Li et al., 2018; Cai et al., 2018; Gillberg et al., 2003: 171).

2 Materials and methods

2.1 Materials and chemicals

The study was conducted with spherical polystyrene (PS) particles that were 1 μm and 6.3 μm in diameter. The 1 μm MPs were red and fluorescent (ThermoFisher, R0100), and the 6.3 μm MPs were yellow and non-fluorescent (Polysciences, 15716-5). Both particles were delivered as a ready suspension. These sizes were chosen for this study to minimize the effect of settling without the help of coagulation/flocculation treatment, thus aiming at studying only the effect of coagulants on MP removal. The particle sizes for the study were selected from commercially available particle products smaller than 10 μm .

Chemicals used in the study were typical wastewater treatment chemicals. Two coagulants—ferric chloride (Kemira PIX-111, 13.8% \pm 0.4% Fe^{3+}) and polyaluminum chloride (Kemira PAX-XL100, 9.3% \pm 0.3% Al^{3+})—and one cationic polymer polyamine product (Kemira Superfloc® C-577, total solids 40–52%) were used. The coagulant metal dosage varied between 0.017 and 1.4 mmol/L, and the polyamine dosage varied between 0.25 and 46.4 mg/L as product. Polyamine is denoted hereafter as C-577. The same metal dosage was used for ferric chloride and polyaluminum chloride. These dosage ranges are similar to those studied previously in tertiary and quaternary treatment applications and are commonly used in WW treatment processes (Langer et al., 2017; Santoro et al., 2013; Ulliman et al., 2018; Gillberg et al., 2003; Talvitie et al., 2017b; HSY, 2019).

The WW used in the experiments was secondary WWTP effluent taken from a municipal WWTP in Finland. The treatment process is based on an activated sludge process with primary co-precipitation of phosphorus by the addition of ferrous sulfate. In 2017, the population equivalent of the plant was approximately 320000. In the same year, 97% of organic material (BOD7), 97% of suspended solids (SS), 95% of total phosphorus (P-tot), and 73% of total nitrogen (N-tot) were removed during the treatment process. Experiments were conducted between June 2018 April 2019. The chemical oxygen demand (COD) in the effluent used was 24–49 mg/L, the suspended solids (SS) were 3–25 mg/L, and the pH was 6.8–7.5 (Table S1).

2.2 Microplastic removal experiments

2.2.1 Sample preparation

For the MP removal experiments with 1 μm MP, the MP product was diluted twice with ultrapure water (Millipore, Milli-Q, Q-POD). Then 500 mL of this prepared microplastic stock solution was mixed with 9500 mL of WW. This sample solution is called the original sample. Then 1-L samples were taken for coagulation/flocculation experiments.

To compare the coagulation/flocculation process with and without added MPs, some samples were also prepared without adding MPs. These samples were prepared by taking 9500 mL of WW and mixing 500 mL of ultrapure water into it. Then 1-L samples were taken for coagulation/flocculation experiments.

The samples with 6.3 μm MP were prepared by mixing 500 mL of ultrapure water (Millipore, Milli-Q, Q-POD) and 9500 mL of WW, and then taking 1-L samples that were spiked with 259 μL of the 6.3 μm MP product.

2.2.2 Coagulation/flocculation experiments

The coagulation and flocculation experiments were conducted in a Kemira flocculator using 1-L beakers and a mixer. The fast mixing speed of 400 rpm was continued for 0.5 min, followed by a slow mixing speed of 40 rpm for 20 min. After this, the samples were left to settle for 30 minutes. The selected amounts of coagulation/flocculation chemical and the pre-defined amounts of 1 mol/L NaOH for pH adjustment were dosed to the samples simultaneously at the beginning of the fast mixing. The pH in the experiments was 7.0–7.5 or 6.5–6.8. After settling, approximately 0.1-L samples were taken for analysis using a glass pipette, 3 cm below the surface. The tip of the used glass pipette was cut to allow big flocs to enter the pipette. The samples were taken for further analysis of the MP particle concentration measurement as counts by flow cytometry and analysis of turbidity and COD. The effect of pH on MP removal was studied with 1 µm samples and ferric chloride. The samples were prepared and treated similarly as described earlier, but the pH during treatment was adjusted to 6.5 instead of 7.3. As a reference for all the samples, some samples were mixed and let settle similarly as other samples but without the coagulant addition. These are called settled samples.

2.3 Measurements

MP concentration measurements as particle counts were conducted with a flow cytometer (Sysmex-Partec CyFlow® SL). To avoid additional settling in the case of the 6.3 µm samples, the samples for flow cytometry were taken while mixing. The results were analyzed using an application developed at Kemira Oyj as well as Microsoft Excel. Flow cytometry was used to measure the concentration of all particles in the samples as well as the particle size. The MPs added to the samples are visible in a certain area on a flow cytogram, which allows distinguishing them from other particles present in the samples (Figure S1).

Turbidity and COD were measured to support the interpretation of coagulation/flocculation treatment results from the flow cytometry. Turbidity was measured with a HACH 2100AN turbidity meter, and COD was determined with a HACH Lange DR 3900 portable photometer using suitable quick test cuvettes (HACH Lange) and an oven (HACH Lange HT2005). Both turbidity and COD were measured from samples without filtration.

Zeta potential measurements were conducted to estimate the change in electrostatic interactions between particles due to coagulant addition (Li et al., 2018; Cai et al., 2018) and to study the MP removal mechanism. The smaller these interactions are, the less stable the particles, and the easier they typically aggregate (Cai et al., 2018). Zeta potential was measured with a Zetasizer Nano-ZS (Malvern Instruments Ltd.).

2.4 Microplastic removal efficiency calculation

Microplastic removal efficiency due to chemical treatment was calculated using the flow cytometry results. MP removal after coagulation/flocculation was calculated by comparing samples to the settled sample (Equation 1).

$$\% = \frac{(\quad) - (\quad)}{(\quad)} \times 100\%, \quad (1)$$

where Counts(Settled) are the MP-induced counts from settled sample without coagulant and Counts(Sample) are the MP-induced counts from a sample treated with coagulant.

2.5 *Quality control and contamination control*

To study the repeatability of the results, experiments with some dosage points were repeated two or three times. The variation of these replicates is reported in the graphs in the Results section. To avoid contamination, glassware was mostly used in the experiments instead of plastic. A glass pipette was rinsed with tap water and deionized water after taking each sample. In addition, a cotton laboratory coat was worn. Several blanks were prepared to detect possible airborne contamination. The microplastics studied were standard particles, which are probably not present in the laboratory environment.

2.6 *Statistical methods*

To compare the results of treatment with ferric chloride and polyaluminum chloride, a t-test was performed in Excel. The test was performed as a two-sample test assuming equal variances. The test result would show any significant difference between these result sets. The results indicate a 95% confidence interval, as the α -value used was 0.05. If the p-value obtained from the test is less than α (i.e., less than 0.05), the difference is said to be probably significant. If the p-value is smaller than 0.01, the difference is highly significant (Dean, J., 1999). In addition, Pearson's correlation coefficients were calculated. Two variables were estimated to correlate with each other when the Pearson's correlation coefficient was between 1 and 0.7. If the coefficient was between 0.7 and 0, they were considered to correlate little or not at all.

3 **Results and discussion**

3.1 *Microplastic quantification*

MP removal was detected after coagulation/flocculation treatment with flow cytometry (Long et al., 2017). The coagulation/flocculation experiments were conducted with PS spheres sizes of 1 μm and 6.3 μm . For 1 μm MP, the original concentration used was 182×10^6 MP/L, which corresponds to approximately 0.1 mg/L. This is over 22000 times smaller than the concentration of 2400–2700 mg/L used in a previous study of MP removal by coagulation/flocculation that used a similar particle size range (Larue et al., 2003). For the 6.3 μm MP, the original concentration was 50×10^6 MP/L, which corresponds to 6.7 mg/L. This is over 350 times smaller than that used by Larue et al. (2003).

The correlation between MP concentration and detected counts in flow cytometry was linear for both MPs in a wide concentration range. The limit of detection (LOD) was calculated as an average of several samples without MP plus three times and the limit of quantification (LOQ) plus ten times the standard deviation of the samples without MP (AOAC, 2002). For the 1 μm MP, the LOD was 650 counts/mL, and the limit of quantification (LOQ) was 1793 counts/mL. These correspond to MP concentrations of 0.5×10^6 MP/L (2.7×10^{-4} g/L) and 1.6×10^6 MP/L (9.0×10^{-4} mg/L) for the LOD and LOQ, respectively (Table 2). This is close to the previously reported LOQ of 0.5×10^6 MP/L for 2 μm PS MPs in a seawater matrix with FCM (Long et al., 2017).

Table 2 Limit of detection (LOD) and limit of quantification (LOQ) in flow cytometry (FC) in this study for both studied microplastic (MP) particles compared to the LOQ in the study by Larue et al. (2003).

Limit	FC	result,	MP/L,	mg/L	Maximum	measurable
LOD 1 μm MP		650	0.5	2.7×10^{-7}		99.4
LOQ 1 μm MP		1793	1.6	9.0×10^{-7}		98.3
LOD 6.3 μm MP		8929	13.2	1.8		76.7
LOQ 6.3 μm MP		24285	39.1	5.3		36.2
LOQ 2 μm MP (Larue et al.,		-	0.5	-		-

When compared by mass, the LOQ for 1 μm samples in this study is below the earlier reported MP concentration in treated municipal WW: 4.2×10^{-3} mg/L (Simon et al., 2018). This value included only the MPs larger than 10 μm and thus might underestimate the real MP concentration. Based on this comparison, the method in this study is able to quantify realistic mass concentrations of MP in wastewater.

For the 6.3 μm MP, the LOD was 8929 counts/mL, and the LOQ was 24485 counts/mL. These correspond to MP concentrations of 13.2×10^6 MP/L (1.8 mg/L) and 39.1×10^6 MP/L (5.3 mg/L) for the LOD and LOQ, respectively (Table 2). The LOD and LOQ were higher for the 6.3 μm MP than for the 1 μm MP due to a single settled 6.3 μm sample that had high counts and is used here for comparison. With replicate samples, the LOD and LOQ could probably be decreased.

3.2 Microplastic removal efficiency

For 1 μm MP containing samples, when the original sample was settled there was no MP removal, and MP could not be separated by settling only. This is expected as it is typical for particles of this size to be colloidal. All tested chemicals enhanced MP removal compared to the settled samples. With ferric chloride, the minimum MP concentrations after coagulation/flocculation were below the LOD, corresponding to a removal efficiency above 99.4%. With polyaluminum chloride, the maximum removal obtained was 98.2% (Figure 1). Larue et al. (2003) also reported high, 90% latex MP removal with ferric chloride with an iron concentration of 1 mmol/L (pH 6). In the present study, the required dosage for 90% MP removal at pH 7.3 was 0.37 mmol/L for iron and 0.16 mmol/L for aluminum. Perren et al. (2018) also reported a high removal of 99.2% with electrocoagulation with iron electrodes (pH 7.5), but their study was conducted with relatively large PE MPs over 300 μm in diameter. In contradiction to these two previous studies and this study, Ma et al. (2019a; 2019b) reported relatively low MP removal for <500 μm PE MPs—only 17% and 36% after 2 mmol/L Fe^{3+} (pH 8) and 15 mmol/L Al^{3+} (pH 7) treatments, respectively. This could be due to the different mixing conditions. Ma et al. (2019a; 2019b) had relatively fast mixing throughout the treatment: 14 minutes at 100 rpm compared to 10 minutes and 1 hour at 60 rpm used by Larue et al. (2003) and Perren et al. (2018), respectively. It was also faster than the slow mixing of 40 rpm used in this study. It might be that the 100 rpm mixing speed used by Ma et al. (2019a; 2019b) was too high and prevented the floc growth or broke the formed flocs. The settling time of 30 minutes was the same as used in this study, but the MP material was different. Some MPs like PE could float (Talvitie, 2018), which can reduce removal by settling. Perren et al. (2018) studied the same material MPs as Ma et al. (2019a; 2019b). However, neither Perren et al. (2018) nor Ma et al. (2019a; 2019b) mention floating. Perren et al. (2018) used a much longer settling time of 16 hours in their study than Ma et al. (2019a; 2019b). Thus, the low removal efficiencies reported by Ma et al. (2019a; 2019b) might also be partly explained by the relatively short settling time. The relatively pure sample matrix made of DI water and mimicking drinking water (Ma et al., 2019a; 2019b) could have also affected the removal result,

though according to Ma et al. (2019b), the turbidity and humic acid content had little effect on the MP removal. The difference in pH cannot fully explain the different results because all of the studies—Ma et al. (2019ab), Larue et al. (2003) and Perren et al. (2018), as well as this study—covered at least the pH range of 6–8, where the optimum results were reported.

The removal of turbidity and COD logically increased when the dosage of ferric chloride and polyaluminum chloride was increased (Figure S2). This was expected as these chemicals can remove particles and dissolved substances, both of which affect turbidity and COD (Gillberg et al., 2003). Even though the addition of MP to the samples did not have an effect on the sample turbidity or COD, the removal of both turbidity and COD correlated well with the removal of MP and removal of all counts. Thus, the turbidity and COD results support the flow cytometry results (Table 3). The turbidity decreased from 2.5 NTU in the original sample to 2 NTU by settling, and further below 1 NTU by chemical treatment with both chemicals. COD decreased from 32 mg/L to 28 mg/L by settling, and further below 20 mg/L by both chemicals. The removal of turbidity, COD and all counts were similar in samples with and without added MPs (Figures S3, S4).

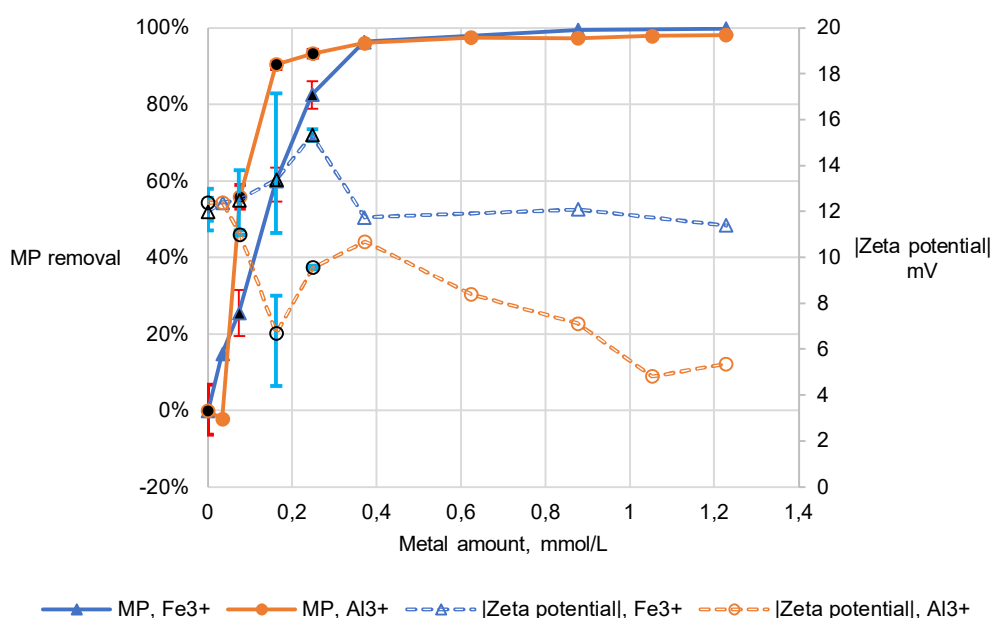


Figure 1 Comparison of ferric chloride and polyaluminum chloride treatments in effluent samples containing 1 μm microplastics. Four replicate settled samples were made. Samples marked with black are the averages of 2–3 replicates. The red lines indicate the highest and lowest microplastic removal values, and the light blue lines indicate the highest and lowest absolute values of the zeta potential. When more particles were created than removed during treatment, negative microplastic removal was recorded. The zeta potential of all the samples is negative

The zeta potential of the settled samples was -12.4 mV ($n=4$) at pH 7.3. This is in line with the previously reported negative zeta potential of PE MPs with a similar pH (Ma et al., 2019a; Ma et al., 2019b; Cai et al., 2018; Larue et al., 2003). For ferric chloride and polyaluminum chloride at all metal dosages of 0.017–1.4 mmol/L, the zeta potential stayed negative. The absolute values of the zeta potential are presented in Figure 1. This is contrary to the previously reported charge inversion of PS nanoplastic spheres when 0.1 or 1.0 mmol/L ferric chloride treatment was used (Cai et al., 2018). They reached a high positive zeta potential of 39.6 mV. However, the MP removal efficiency results are in line with Cai et al. (2018), who also reported an obvious MP aggregation. This was explained by the electrostatically heterogeneous surface (Cai et al., 2018). In the case of

ferric chloride, the absolute value of the zeta potential correlated poorly with removal of all counts (Pearson's $r=-0.38$) and not at all with MP removal (Pearson's $r=-0.18$) (Table 3). This indicates that the removal mechanism is for instance sweep coagulation or a combination of sweep coagulation and charge neutralization. For polyaluminum chloride, the same correlations were stronger—Pearson's $r=-0.81$ and -0.79 , respectively—which indicates that removal may occur due to charge neutralization.

Table 3 Pearson's correlations between microplastic (MP) removal or all counts removal and other parameters: turbidity removal, chemical oxygen demand (COD) removal, absolute value of zeta potential, and all particles removal. All of the measured samples were included in the comparison. Colors: $\pm(1-0.7)$ white, $\pm(0.7-0.51)$ light grey, $\pm(0.5-0)$ dark grey.

Sample	Comparison point and chemical	Turbidity removal, %	COD removal, %	Zeta potential , mV	All particles removal, %
1 μm	MP removal, polyaluminum chloride	0.97	0.75	-0.79	0.93
1 μm	MP removal, ferric chloride	0.92	0.93	-0.18	0.74
1 μm	MP removal, polyamine	-0.91	0.17	-0.93	0.55
6 μm	MP removal, ferric chloride	0.99	0.89	0.52	0.07
1 μm	all counts removal, polyaluminum chloride	0.92	0.88	-0.81	1
1 μm	all counts removal, ferric chloride	0.86	0.90	-0.38	1
1 μm	all counts removal, polyamine	-0.43	0.30	-0.66	1
6 μm	all counts removal, ferric chloride	0.17	0.51	-0.65	1

For both ferric chloride and polyaluminum chloride, MP removal was greater than the removal of all particles (Figure S5). This means that the MPs are removed more efficiently than other particles in the samples. The formation of new particles is seen as negative removal of all counts with low dosages of ferric chloride. This is explained by the formation of hydroxide particles in the sample matrix due to the chemical addition (Duan & Gregory, 2002; Gillberg et al., 2003). The formation of hydroxide particles relates to turbidity removal, which logically increases only after the removal of all counts is positive at a metal dosage of 0.16 mmol/L (Figure S1; Figure S5). The MP removal reached a plateau with both ferric chloride and polyaluminum chloride at a metal dosage of around 0.25 mmol/L. The largest difference in MP removal efficiency between these two coagulants seemed to be for metal dosages between 0.07 and 0.25 mmol/L (Figure 1), where the difference was statistically probably significant ($P=0.048<0.05$, $n=14$).

Compared to ferric chloride and polyaluminum chloride, C-577 had lower performance in MP removal, the maximum removal being 65% (Figure 2). The difference between C-577 and polyaluminum chloride was statistically significant ($P=0.03<0.05$, $n=20$), but not when compared to ferric chloride ($P=0.23>0.05$, $n=20$). Opposite to the other tested chemicals, C-577 largely increased the turbidity at all dosages, whereas COD was not removed at any of the dosages, despite the over-60% MP removal. It was not measured how the addition of C-577 affected the COD content of the samples. According to the FCM

results, it seems that both MP and other particles (all counts) were removed, but the turbidity results are contradictory as turbidity was still increased.

When increasing the C-577 dosage, due to agglomeration the average particle size increased with a decrease in all counts. The total surface area of the particles in the samples was calculated from the average particle size and all counts. This was noted to increase along with higher dosages. The increase in surface area correlated strongly with the increase in turbidity (Pearson's $r=0.91$), indicating that the increase in particle size and thus in surface area was responsible for the increased turbidity. This interpretation shows that the flow cytometry results and turbidity results are in line and support each other. The removal of turbidity, COD and all counts were similar in samples with and without added MPs (Figure S6).

For C-577, a charge inversion from negative to positive was observed with increasing dosage (Figure 2). Differing from the other two chemicals, an interesting strong correlation between the absolute value of the zeta potential and the MP removal was recorded for C-577: the MP removal increased when the absolute value of the zeta potential was decreased (Pearson's $r=-0.93$) (Figure 2). This indicates that the removal mechanism is charge neutralization (Gillberg et al., 2003: 171).

Particle size was thought to increase because C-577 adsorbs onto the negatively charged colloidal material in the sample.

These tiny aggregates do not form large enough flocs that could settle in the selected settling time, but instead they remain in the sample solution. The zeta potential results support this thinking, as the zeta potential approaches zero when the turbidity remains high. So up to a certain dosage, the absolute value of the zeta potential is decreased, while at the same time the mean particle size is increased, indicating that flocculation takes place in the system. This is supported by the flow cytometry, as fewer MPs are measured, and the most efficient removal of MPs is measured at the same dosage. The settling of the formed flocs can be further improved by a longer settling time or by the addition of a high molecular weight flocculant, for example, polyacrylamide. At a C-577 dosage of 31 mg/L, the zeta potential is -0.3 mV; and at the slightly higher dosage of 34.8 mg/L, charge inversion took place and the zeta potential was +3.5 mV. The charge inversion is seen in Figure 2 as an increase in the absolute value of the zeta potential. Simultaneously, the efficiency of MP removal decreases. This is explained by the increased repulsion between the particles, which prevents them from attaching to larger flocs.

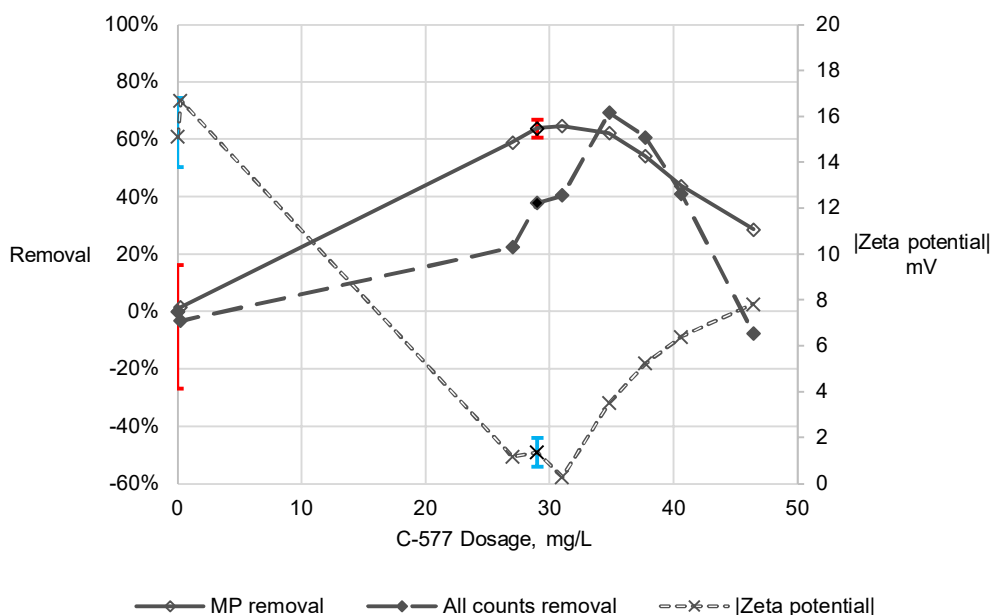


Figure 2 C-577 treatment of the effluent samples containing 1 μm microplastics. Microplastic removal of 64% was reached with a large dosage of 29 mg/L of C-577, which is the average of the two replicates. Red lines indicate the highest and lowest values for microplastic removal, and the light blue lines indicate the highest and lowest absolute values of the zeta potential. The zeta potential is negative below dosages of 34.8 mg/L and positive at this dosage and higher.

3.3 Effect of pH

In both pH 7.3 and 6.5, MP removal efficiency increased with increasing coagulant dosage as soon as a high enough dosage was applied. At a lower pH at the lowest applied dosage of 0.07 mmol/L Fe^{3+} , the MPs were not removed, but a negative removal was recorded (Figure 3). This is explained by the formation of new disturbing particles, e.g., hydroxides, which are not settled and this explanation is supported by the simultaneously increasing turbidity (Figure S7). At the same dosage, at a higher pH, MP removal was 26% with no changes in turbidity. At higher dosages of 0.16 and 0.25 mmol/L Fe^{3+} , MP removal was significantly more efficient in lower pH ($P=0.04 < 0.05$, $n=9$). As with pH 7.3, the zeta potential of the settled sample was negative (-13.6, $n=2$) and stayed negative at all metal dosages from 0.074 to 0.25 mmol/L at the lower pH 6.5. The absolute values of the zeta potential are presented in Figure 3.

3.4 Effect of microplastic size

Similarly to 1 μm MPs, an MP removal above 76.7% (LOD) was also observed for 6.3 μm MPs. For the larger MPs, a lower coagulant dosage was needed than for the smaller MPs (Figure 4). It was clear that unlike the 1 μm samples, the addition of 6.3 μm MP to the samples increased the turbidity. The turbidity of the settled 6.3 μm sample was 7.4 NTU compared to 2 NTU of the settled sample without MP. COD was also increased—it was 33 mg/L in the settled 6.3 μm sample compared to the COD of 32 mg/L of the settled sample without MP. After chemical treatment of the 6.3 μm samples, the turbidity decreased logically with increasing dosage, reaching a minimum of 1.3 NTU at the highest coagulant dosage (Figure S8). The turbidity and COD removal correlated strongly with the MP removal and poorly with the all counts removal (Table 3). Thus, the MPs were largely responsible for the change in these variables. This confirms the MP removal by coagulants.

Like the 1 μm MP samples, the zeta potential was also relatively stable for samples with 6.3 μm MP. It remained between -15.3 and -12.8 mV in the settled sample and at all coagulant dosages at pH 7.3 without charge inversion. The absolute value of the zeta potential correlated poorly with both MP removal and removal of all counts (Table 3). It might also be that the different original concentration of the smaller and larger MPs affects the results.

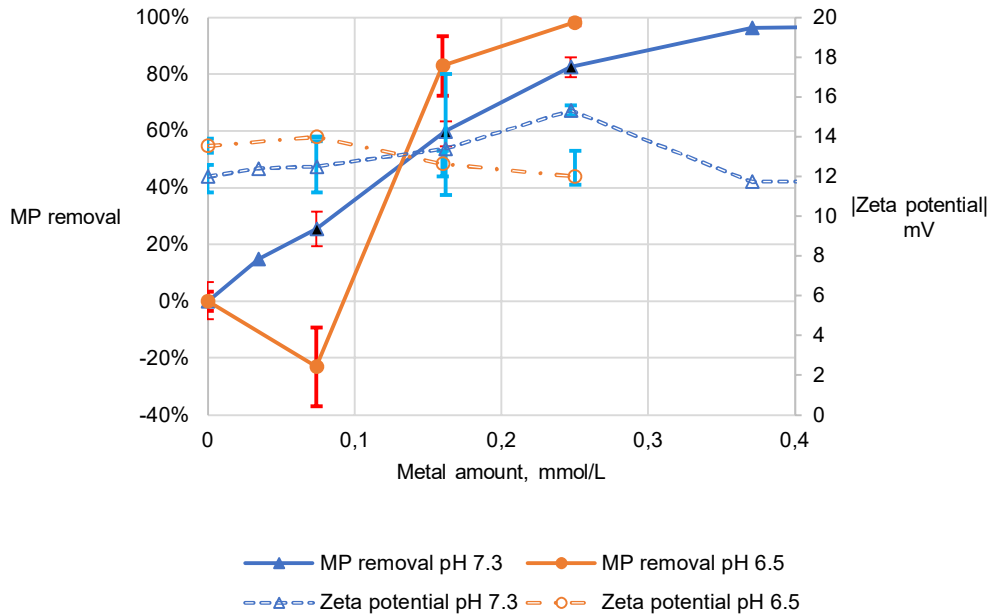


Figure 3 The 1 μm microplastic removal and absolute value of the zeta potential of samples after treatment with ferric chloride at pH 7.3 and 6.5. Samples marked with black are averages of two or three samples. The red lines indicate the highest and lowest for microplastic removal, and the light blue lines indicate the highest and lowest absolute values of the zeta potential. For clarity, the two highest dosages at pH 7.3, already shown in Figure 1, were left out of the graph. The zeta potential of all the samples is negative.

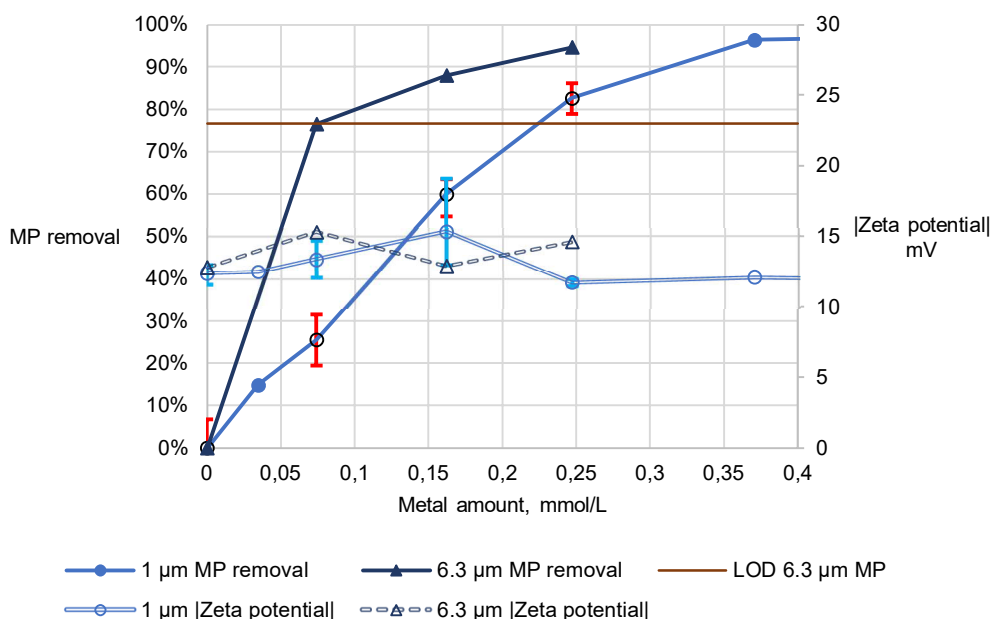


Figure 4 Microplastic (MP) removal and absolute value of zeta potential of 1 µm and 6.3 µm samples after treatment with ferric chloride at pH 7.3. Red lines indicate the highest and lowest values for MP removal of two or three replicate samples. Light blue lines indicate the variation in the absolute value of the zeta potential. The limit of detection (LOD) for 6.3 µm MP is shown with a horizontal line at 76.7%. The zeta potential of all the samples is negative.

4 Conclusions

The typical WW treatment chemicals—ferric chloride, polyaluminum chloride and cationic polyamine—all enhanced the removal of MPs at dosages applicable to tertiary treatment. Based on the literature, MP removal by chemicals has not previously been studied in the municipal wastewater matrix. In addition, the reviewed literature does not report MP removal or MP aggregation with polyamine. Ferric chloride and polyaluminum chloride were observed to be more efficient than polyamine. Ferric chloride and polyaluminum chloride performed similarly to each other. Decreasing the pH did not have a statistically significant impact on the MP removal. However, pH should be considered when optimizing the dosage. Our findings suggest that chemical coagulation plays a key role in the removal of MPs, and the process can be optimized by selecting the right coagulant, dosage, and pH. The suitability of FCM for MP quantification in wastewater effluent was confirmed.

It was observed that the size of the MPs affected their removal at a certain dosage, and this should be considered in optimization. High MP removal above 95% was observed for 1 µm MPs and above 76% for 6.3 µm MPs. For the larger MPs, a smaller coagulant dosage was needed than for the smaller MPs. Finally, MP removal differed from the removal of all particles, and thus MP removal should be included as a criterion when selecting the coagulant.

The study was conducted with clean MP standards, which are noted to be different from MPs found in WW. Thus, further studies should focus on studying MPs that have biofilm on them and that vary in size, shape, and material. It should also be studied if MPs present in WW samples without spiking are possible to quantify with FCM. Concentrating the dilute WW samples might be needed to make use of this method, and that could be studied.

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