Standard Harmonized Monitoring Methods for Microplastics in Municipal Wastewater Treatment Plants: A Systematic Review of Current Methodologies and Capabilities

Prepared by

Emma Norman

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Submitted to

Olga Petrov, PhD, Project Course Faculty, Program Head, Environmental Engineering

Fiona Lemon, BSc, MA, TESL, Project Course Faculty, Communications

Advisory Committee

Yao Hung Lan, MASc., P.Eng.

British Columbia Institute of Technology

Burnaby

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Abstract

Microplastics in wastewater have garnered great interest from researchers over the last decade, resulting in numerous publications applying various testing methodologies to determine these contaminant levels in wastewater treatment plants. Given the variability of methods applied throughout these studies, it is difficult to compare the findings as they are subject to different biases. The objectives of this report are to present information on how wastewater microplastics are collected, processed, and analysed and to determine which methods are most feasible for a monitoring protocol. Sixty-seven original research articles on the topic of determining microplastic occurrence in wastewater from January 2018 to September 2021 were identified in a systematic literature search and were each systematically screened, reviewed, and critically assessed. The methods for sampling, processing, and analysing microplastics in wastewater were extracted from each reviewed study and critically evaluated using a decision matrix to determine which methods best met the needs of a proposed microplastic in wastewater monitoring protocol. The results of this study have demonstrated that significant variability exists in the current literature on microplastics in wastewater. A practicable standard methodology is needed to appropriately assess the degree of microplastic contamination in wastewater and make cross-study comparisons. It is recommended that the existing ASTM standards for collection and preparation of microplastics in water matrices for analysis be updated to meet feasibility needs for a monitoring method, and that a standardized method be prepared for the analysis of microplastic in wastewater samples.

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Glossary

American Society for Quality (ASQ)

American Society for Testing and Materials (ASTM)

Collaboration for Environment Evidence Critical Appraisal Tool (CEECAT)

Fourier Transform Infrared (FTIR)

Gas Chromatography Mass Spectrometry (GC-MS)

Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP)

Microplastic (MP)

National Oceanic and Atmospheric Administration (NOAA)

Wastewater Treatment Plant (WWTP)

Water Environment Federation (WEF)

Water Research Foundation (WRF)

List of Symbols and Selected Units

MP – one unit of microplastic particle

MP/L – unit of concentration: microplastic particles per liter of wastewater

µg/L – unit of concentration: micrograms of plastic per liter of wastewater

µm – unit to describe the minimum diameter pore size of a sieve mesh or filter

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1 – Introduction

1.1 Background

Concern over microplastic contamination in the environment has spurred many researchers across the world to characterize regional microplastic discharges from a prevalent point source of pollution: the wastewater treatment plant (WWTP) (for review, see Alvim et al., 2020; Hamidian et al., 2021; P. Kang et al., 2020; Turan et al., 2021). Although plenty of data has been produced from this research, reviewers and authors of original research have cautioned that this data cannot be adequately compared between regions due to a lack of consistency and comparable experimental methods between these studies (Alvim et al., 2020; Hamidian et al., 2021; P. Kang et al., 2020; Long et al., 2019; Magni et al., 2019; Turan et al., 2021). In other words, these studies demonstrate a need for standardized methods for the determination of microplastics in wastewater to understand how different regions and treatment technologies perform and whether these microplastic discharges present a significant risk to the environment.

Accounts of microplastics in the ocean were first reported in the scientific community in the 1970s but were grouped under the general category of marine litter and were not deemed to be an individual pollutant of environmental concern until 2011 by the United Nations Environmental Programme (Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection [GESAMP], 2015). Since then, published research exploring the sources and fates of microplastics in different environmental systems has increased rapidly (GESAMP, 2015) and continues to be a subject of research and development. An official definition of microplastics has not yet been established, but they are generally regarded as being plastic particles smaller than 5mm in diameter and categorized as either primary or secondary microplastics (Nikiema et al., 2020). These microplastics can enter water systems and oceans through surface runoff, atmospheric deposition, or by discharge from wastewater systems and treatment plants. The conventional WWTP has been shown to largely reduce plastic debris in municipal wastewater prior to discharge, but still acts as a significant point source of microplastic pollution to aquatic systems (Alvim et al., 2020; P. Kang et al., 2020). WWTPs may not be capable of removing all microplastic particles in wastewater, and adverse weather conditions can lead to volume overflows which can result in the discharge of untreated wastewater to receiving environments (Nikiema et al., 2020). Once in the ocean, microplastics can spread around the world and decompose into an even greater number of particles (GESAMP, 2015). These particles can be found in samples of air, soil, water, and even food, but the effects of human and animal exposure to microplastics are still being studied (Nikiema et al., 2020).

Microplastics in wastewater may carry physical, chemical, and biological health risks, but the severity of risks are still being studied and regulations on microplastics in wastewater depend on this understanding of risk to establish policy (*Occurrence of Microplastics in Water…Size Does Matter!*, n.d.). Reported physical impacts to aquatic life include starvation and gut blockage (Nikiema et al., 2020). Chemical impacts can occur to both animals and humans due to exposure to toxic monomer constituents of the microplastic polymers. Contaminated surfaces of microplastics also pose a health risk, which could host pathogenic microorganisms or adsorbed inorganic, organic, and metallic chemicals (GESAMP, 2015; Nikiema et al., 2020). However, speakers on the topic of microplastic concerns in water in a 2018 Water Research Foundation webcast expressed that the risks may be overstated (*Occurrence of Microplastics in Water…Size Does Matter!*, n.d.). Specifically, Dr. Allen Burton, a University of Michigan professor for the School for Environment and Sustainability, noted that observed concentrations of microplastics in water samples was far outnumbered by algae food sources to daphnia organisms and therefore did not present significant risk of ingestion. Still, many countries have taken a precautionary stance on this risk to the environment by prohibiting the use of manufactured plastic microbeads in cosmetics and over-the-counter products (Department for Environment, Food & Rural Affairs, n.d.; Health Canada, 2015; Ministry for the Environment, 2021; U.S. Food & Drug Administration, 2020). These products are often rinsed down drains and carried into wastewater systems where they may eventually be discharged to aquatic receiving environments. Microfibers, which enter wastewater systems through washing of synthetic clothing and contribute to 35% of total microplastic releases to the environment (Nikiema et al., 2020, as cited from Boucher and Friot, 2017), have also been targeted for regulation by the government of France, which will require that microfiber filters be installed in all new washing machines by 2025 (US EPA, 2020). These approaches address microplastic pollution from the sources but, to the author's best knowledge, legislation on the monitoring and minimization of microplastic pollution from wastewater discharges has not yet been established anywhere. Shelly Walther, an Environmental Scientist with the Los Angeles County Sanitation District, spoke on the topic of microplastics policy and emphasized a need for policy based on scientific knowledge rather than public perception, which starts with effective and standardized monitoring and testing methods (*Occurrence of Microplastics in Water…Size Does Matter!*, n.d.). Ongoing research will reveal the extent of health risks and impacts that microplastics have on humans and animals, but adequate testing and monitoring on the amount of microplastics entering the environment is necessary for assessing risk and establishing policy.

Microplastics are differentiated from other pollutants by their polymeric composition, and from other plastics by their size, which is typically defined as having an upper limit of 5mm in

diameter (American Society for Testing and Materials [ASTM], 2020a, 2020b; GESAMP, 2015; Masura et al., 2015; Nikiema et al., 2020). They can be classified as primary microplastics, ones manufactured to be less than 5mm in size, or as secondary microplastics, ones that have been shed from larger plastics or degraded down to be less than 5mm in size (Nikiema et al., 2020). The lower size limit of microplastics has not been collectively agreed upon, and different definitions of this limit exist for different organizations. In 2015, the GESAMP defined the lower size limit of microplastics to be 1 nanometer (nm), but in the same year the National Oceanic and Atmospheric Administration (NOAA) recommended using the pore sizes of the nets used to capture microplastics (commonly 330 micrometers [µm]) to establish the lower limit (Masura et al., 2015). The Water Research Foundation (WRF) has even indicated that microplastics are associated to a range of 300-500µm (*Occurrence of Microplastics in Water…Size Does Matter!*, n.d.), and has illustrated the particle sizes that could be captured by a 330µm net (see Figure 1). Later, the ASTM (2020a, 2020b) would differentiate microplastic fibre size definitions from particle size definitions. In their standard practice methods section 3.2.3, a fibre "…no longer than 15 [millimeters (mm)] in length with an aspect ratio of at least 30:1 and <500µm in its smallest dimension" is defined as being a microplastic. Without consistent size definitions of microplastic fibres, one study may be underreporting total microplastic particle counts with respect to another study and vice versa. Without internationally recognized definitions of microplastics and lower size limits, all reported data may be underreporting based on the smallest sample filter pore size or the lowest level of detectability of analytical methods. If researchers and regulators are to properly compare study results of microplastic in wastewater, a standard definition of microplastic size ranges must be developed.

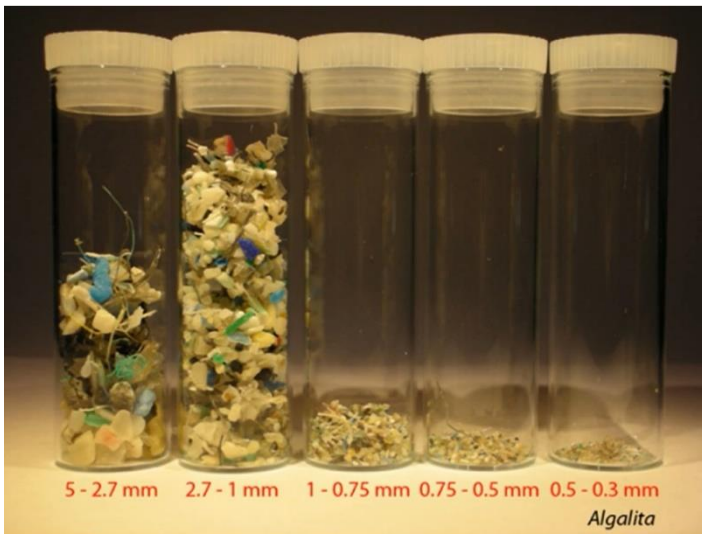

Figure 1. Microplastic particle sizes ranges from a manta trawl net (reproduced from Occurrence of Microplastics in Water...Size Does Matter!, n.d.)

1.2 Research Problem Statement

Until recently, no standard had been published that outlined a procedure for the sampling and preparation of water samples for microplastic analysis that was specific to wastewater treatment plants. Wastewater has been reported to be a challenging media to work with (Cook & Allen, 2020), and therefore careful consideration for how samples are collected, processed, and

analysed is necessary for reliable detection and quantification. In August 2020, the ASTM released two standards: D8332-20 for the sampling of microplastics in water (ASTM, 2020a), and D8333-20 for the preparation of samples for microplastic analysis in water (ASTM, 2020b). To the author's best knowledge, no standard practice for the analysis of microplastics in any environmental water samples has been completed and published by the ASTM at this time. These two standards cite the 2017 works of Dyachenko et al. and Loder et al., who contributed knowledge on methodologies for sampling and processing microplastics in water matrices (P. Kang et al., 2020). Despite the availability of these standards, a large body of research has continued to develop on methods for microplastic testing in wastewater up to the present day that may provide optimizations to the existing standards.

1.3 Research Question and Project Objectives

This study critically reviewed a systematically selected and screened body of research applying established and newly developed methodology for determining microplastic occurrence in wastewater treatment plants. The aim of the research is to make a recommendation for the most efficient, accurate, and representative methods that should be considered for future standard practice revisions and protocols. The discussion and results are presented in four sections: (1) data and reporting units; (2) sampling methods; (3) sample processing methods; and (4) sample analysis methods. The main objective of this research is to recommend a harmonized sampling, sample processing, and sample analysis method for the testing and monitoring of microplastics in wastewater treatment plants. Additionally, a body of recently published research was systematically reviewed and critically appraised, and the methodologies used and results obtained were compiled in a literature review matrix which was used to evaluate the effectiveness of the methods. Finally, these methods were evaluated using a decision matrix, and a recommendation for a harmonized methodology is presented in the ASTM Draft Standard Template for Test Methods (ASTM, n.d.) format.

1.4 Project Scope

The scope of the following project is limited to sampling, sample processing, and sample analysis methods applied to wastewater samples in municipal wastewater treatment plants. Quality assurance and control methods were researched but were not included in the project discussion due to content limitations. The collection, treatment, and analysis of sludge and semi-solid wastewater streams were not included, nor were procedures for testing microplastics in environmental water samples. Additionally, the scope of the literature included in the systematic review is limited to databases freely accessible with a BCIT Student account. The purpose of this project is to evaluate the feasibility of existing and proven methods for finding microplastics in wastewater for a monitoring protocol, and new methods were not proposed in this study.

2 – Methods

2.1 Systematic Literature Review and Search Methods

The available literature on microplastics in wastewater is plentiful, but the applied testing methods are highly variable as illustrated in section 1.1. It was therefore determined that the most appropriate research method for this project would be one that maximized available and focussed studies. The systematic literature review method provided a standardized approach to searching and identifying applicable studies, and was selected as the research method for this project.

The Reporting Standards for Systematic Evidence Syntheses (ROSES) in Environmental Research tool was used to develop the search, screen, review, and writing strategies and methods (Haddaway et al., 2017). The tool was modified to a small degree to meet the scope of the student research project. Modifications included removal of the methods protocol section as no published protocols were used to develop the systematic literature review, removal of reasons for heterogeneity in the review as the selected articles were focussed, and removal of the risks of publication bias as this was evaluated as part of the critical appraisal in the literature matrix instead. This modified tool was used to perform the systematic literature review searches and screening in sections 2.1.1 through 2.1.2.

2.1.1 Systematic Literature Review Search Methods

The systematic literature review search was performed between September 09 and September 13, 2021. Tertiary sources were first searched on September 09, 2021, followed by primary and secondary sources on September 12 and 13, 2021. Tertiary sources were searched only on the BCIT Library database using the keywords *microplastic* AND wastewater** set to a content type of only books. This search identified sources that could provide background on the project topic, but ultimately were not descriptive enough to use as primary resources for the project. Secondary and primary sources were searched in the following order on the bibliographic web databases BCIT Library, Science Direct, MDPI, and Research Gate using the keyword strings: *microplastic AND (wastewater treatment plant) AND (detection OR identification) AND (method OR methodology OR technique)* where possible. The MDPI database search string only contained the keywords *microplastic AND (wastewater treatment plant)* due to limitations of the logical operator. Truncation was applied in compatible search engines, which was limited to the BCIT Library database only, using the keywords *microplastic*, wastewater treatment plant*, method**, and *technique**. Additionally, the WEF and WRF web databases were searched using the term *microplastic* for conference proceedings and webcasts. The purpose of the selected keywords was to focus the search on the contaminant and matrix of concern for research.

The exploratory literature search found that a high volume of research was published between 2012 and the present, and a number of existing microplastic in wastewater methodology review papers had already analysed the studies up to 2018 (Alvim et al., 2020; Hamidian et al., 2021; P. Kang et al., 2020; Turan et al., 2021). Due to research time constraints and the application of 2017 published research in existing ASTM standard practices, it was determined that literature published after 2017 (i.e., January 2018 to September 2021) would provide an adequate basis for studying recent methods and findings that could provide potential optimizations to the ASTM standard practices. Further limitation and expansion operators were applied as they were available in the search engines. Literature type filters were applied to all databases to exclude newspaper articles and editorials, book reviews, dissertations, theses, encyclopedia entries, short communications, perspectives, preprints, posters, presentations, and data. Subject terms or author-specified keyword filters of *microplastics* or *wastewater* were utilized in the BCIT Library, Science Direct, and MDPI databases. Filters through the BCIT Library expanded the search to include non-library literature and limited the search to full-text online and scholarly results only, and all searches were limited to environmental or engineering subjects or disciplines. The purpose of the applied filters was to generate reliable and original research relating to environmental engineering. No search updates were conducted throughout the project due to the short 15-week project writing timespan. The filtered search returns yielded the following hits: BCIT Library (80), Science Direct (233), MDPI (9), Research Gate (21), and WEF (2).

2.1.2 Systematic Literature Review Screening and Source Management Methods

The purpose of the systematic literature search was to focus on articles that were more likely to meet the needs of the project, but the results still included numerous unrelated articles. Systematic screening strategies, outlined in the flowchart in Figure 2, were used to isolate suitable articles for use in the project and eliminate unrelated or unreliable research. The literature results of the database search were screened for title and abstract relevancy to the project. Titles were screened for inclusion of the topic terms *microplastic* and *wastewater* or *wastewater treatment plant*. Articles were excluded from further screening and review if they did not include the term *microplastic(s)* in the title. Titles that included testing methodology terms (*detection, measurement, identification, method, technique, procedure*) were immediately screened into the reviewable literature without abstract screening. Articles that included only the required title topic terms were further screened for their abstract content. The literature abstracts were screened for relevancy to the objectives of this project. The abstract had to include the term *microplastic* and any term relating to a wastewater treatment plant (e.g., *sewage, wastewater treatment facility, etc.*). Furthermore, the abstract was screened for keywords indicating some

experimental testing or methodology (*sampling, collection, processing, preparation, analysis, detection, occurrence, measurement, etc.*). Articles were excluded from further review if they did not indicate inclusion of experimental methodologies in the study, the matrices were not wastewater, the contaminant of interest was not microplastics, or if the methods were developed for specific types of microplastics. The purpose of these screening strategies was to focus the reviewable literature on methods for detecting general microplastic content in wastewater and limit the number of articles to be reviewed in text.

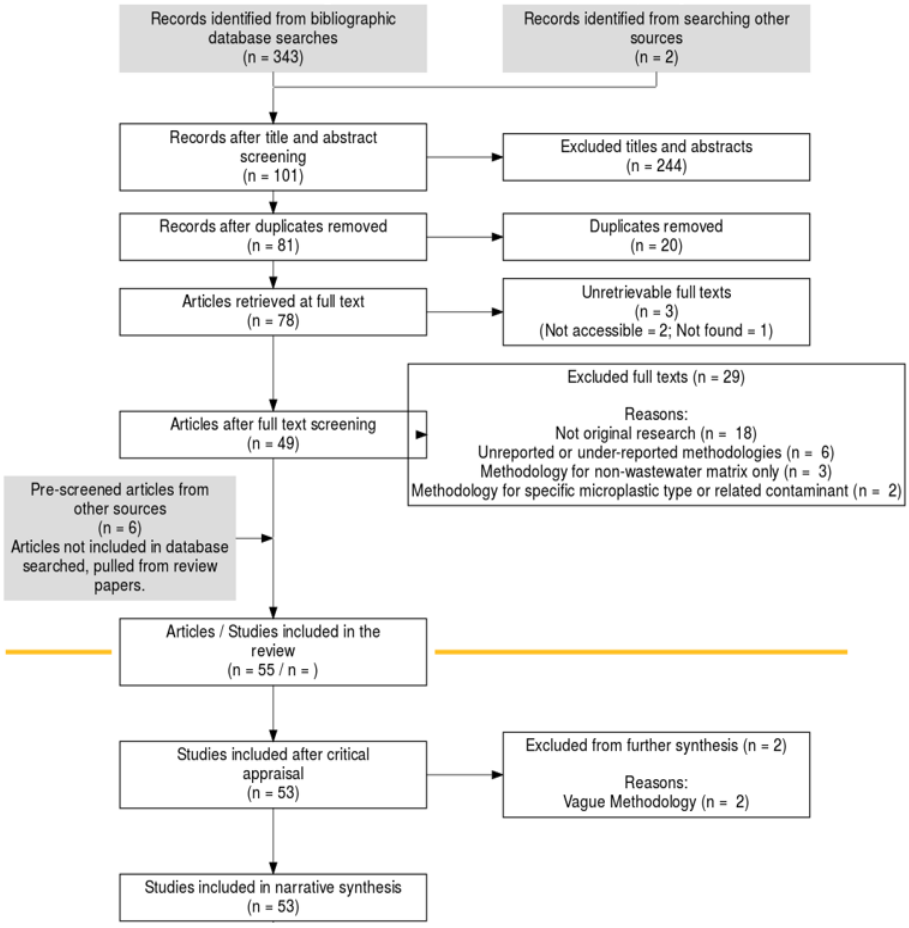

Figure 2. Systematic Literature Search and Review Flow Diagram (adapted from Haddaway, 2020)

Title and abstract screening reduced the search results down to 101 literature sources to be in-text screened. Sources from the BCIT Library and Science Direct databases were saved in ProQuest RefWorks online (*RefWorks*, n.d.). RefWorks lists and literature from MDPI and Research Gate were then exported to Zotero (*Zotero*, n.d.). The database search results were compared for replicates, bringing the final amount to 81 literature sources after de-duplication. The screened sources were compiled of 60 original research, 18 review articles, two conference proceedings, and one book. Three sources were not included in the systematic literature review due to lack of accessibility. Request for access to research was not granted before the project due date for two sources, and one source was removed from the database before it could be downloaded. In total, 78 articles were accepted following abstract and title screening and de-duplication. These articles were downloaded and further screened based on in-text content.

Secondary literature, or review articles, were screened in-text for relevancy and other literature sources. These review articles were assessed for relation to the project topic and must have included sub-headings for the four topics of concern: sampling, sample processing, sample

analysis, and quality assurance/quality control. Four of the 15 review articles were screened for relevancy and included in the reviewable literature. From these review articles, 154 key and fringe references were extracted from the methodology sections and were pre-screened for applicability to this project. These articles were first screened for date of publication, January 2018 to September 2021, then screened for the same title and abstract requirements as for systematically searched articles. After de-duplication, there were seven additional articles to be screened in-text with the systematically searched ones for a total of 67 original research articles. Access to one article was requested but not granted before the project due date, and therefore was not included in the systematic review. Given time limitations, only four of the review articles were included in the project. This was done to allow enough time to review the primary literature, which was the focus of the data extraction and discussion methods in this project.

The primary literature, or original research, were further screened in-text for relevancy and completeness. The in-text screening required that the article contained sub-headings for experimental methods in the methodology section. This included sub-headings for (1) sample location, sampling, collection; (2) sample processing, preparation, digestion, separation; (3) sample analysis, detection, identification, characterization, quantification; and (4) quality assurance and quality control. It was required that at least two of these sub-headings were included and were descriptive enough that the methodology could be reproduced on a step-by-step basis. In-text screening of primary literature was performed in order of notability, starting with sources found in the systematic search and secondary literature, followed by the rest of the literature selected for in-text screening. Articles were excluded from further review if their methods were vague or simply cited as being described in another source not included in the screened literature. Articles that did not contain original research were excluded from further review but included in the secondary literature list. Additionally, articles that were focussed on specific plastic types, other contaminants, or non-wastewater matrices were not included in the reviewable literature given that their methods may not apply to the topic of this project. Based on these restrictions, 13 research articles were eliminated from further review after in-text screening. In total, 56 original research (including conference proceedings) were deemed fit for review following in-text screening and were systematically reviewed for narrative synthesis.

2.1.3 Systematic Literature Review Data Extraction Methods

Primary, secondary, and tertiary literature that was accepted following in-text screening were assessed in a literature review matrix (see Appendix III) using Microsoft Excel 2016. Authors names, publication date, journal title, and APA format reference were used to identify literature in the spreadsheet. Source material relating to the project was briefly summarized for all literature, including research aims or purposes, research methods, key findings and conclusions,

and author discussions on study validity and limitations. A comment on how the source material relates to the project and what significant contributions it makes to the research was included. Secondary and Tertiary sources were additionally reviewed for key and fringe references, which would contribute to the list of reviewable primary source literature as discussed in section 2.1.2.

The primary literature was reviewed more closely for material due to their relation to the project objectives (see section 1.3), requiring significant quantitative and qualitative data extraction. The literature review matrix included a summary of methods for each article, and details of methods were recorded in five tables outlining the equipment, techniques, and procedures used in the methodology. The data was separated into four spreadsheets based on steps in the microplastic determination methodology: Literature Data and Results, Sampling Methods, Sample Processing Methods, and Sample Analysis Methods.

Literature Data and Results was compiled of the WWTP characteristics. This included location, treatment technologies, and quantitative results including microplastic removal efficiencies and microplastic concentrations in influent and effluent. Where necessary, the data units were converted to maintain a single reporting unit through the project. Removal efficiencies were reported in units of percent microplastic removal and concentrations were reported in units of microplastic per liter (MP/L). Where data was available, results were also reported in mass units.

The sampling methods were broken down into multiple categories and recorded for every reviewable article. Any procedural element on sampling methodology was recorded in a table, including the wastewater streams sampled, the sample type taken, the equipment used, the frequency and intervals of sampling, the volumes taken, and the sample storage conditions. For research articles that employed field-sieving of samples, additional details on the number of sieves and mesh sizes were recorded.

Details on how samples were handled, extracted, and prepared after sampling for each reviewed article was included in a sample processing methods table. Processing methods were compiled into three main categories: initial separation and treatment, oxidation and digestion, and final separation and microplastic extraction. Initial separation techniques were given standard terms with a brief description of equipment, conditions, and reagents used. Oxidation and digestion were further categorized based on the three methods observed throughout the reviewed studies: organic matter oxidation, cellulose digestion, and enzymatic digestion. Some of the reviewed research applied a combination of these oxidation and digestion processes, which were tracked as reaction step numbers. Equipment, reagents, and procedures were recorded for all of the oxidation and digestion processes. The final separation and microplastic extraction were recorded similar to the initial separation with the addition of alternate microplastic isolation

techniques. Finally, the estimated preparation time and processing costs were recorded where information was provided.

The sample analysis spreadsheet was separated into three main categories: microplastic pre-selection techniques, microplastic detection and confirmation techniques, and reported parameters. The methods and instruments used, as well as selection criteria if specified, were recorded for each study under the microplastic pre-selection techniques category. Sub-sample sizes, instrument conditions, and microplastic detection techniques were recorded for the microplastic confirmation and detection category. Additionally, reported parameters, detection limits, mass conversions, and any adjustments for microplastic recovery or contamination were recorded under the reported parameters section.

2.1.4 Critical Appraisal Methods

A critical appraisal of reviewable tertiary, secondary, and primary sources was applied in the literature matrix spreadsheet (see Appendix III). A general appraisal was used for tertiary sources in columns J to L, consisting of appraisal of the source reputability, research and method justifications, and validity of results, key findings, and conclusions. Published systematic review checklists were used for the critical appraisal of the secondary and primary sources.

The Checklist for Systematic Reviews and Research Syntheses by the Joanna Briggs Institute (JBI, 2020) was used to define the critical appraisal parameters for secondary sources. The secondary literature was evaluated in columns M to P for review article quality in the presentation of the review question, the applied search strategies, the critical appraisal strategies, conclusions, and potential biases.

The Collaboration for Environment Evidence Critical Appraisal Tool (CEECAT) Version 2.0 (Konno et al., 2021) prototype was determined to be the most relevant tool for primary research that falls under the environmental engineering discipline. This tool, applied in columns L to S, was primarily used to assess for risk of biases in the reviewed literature, including those due to uncontrolled variables, selection criterion, methods used, detection and measurement techniques, missing and unreported or under-reported data, and analytical errors. The primary literature was also critically appraised for how well the research question was presented, justifications for research methods used, and how the evidence supports the key findings and conclusions.

2.2 Statistical Analysis Methods

Most of the extracted data was qualitative and was only analysed through narrative syntheses. Where data needed to be synthesized, basic statistics were applied. The reported results were averaged where multiple results from different sampling instances or different WWTPs using the

same sampling and testing methodologies were reported in a single study. The middle value was reported where results in a single study were expressed as a range. It was assumed that the results of microplastic in wastewater testing would be similar in magnitude across multiple samples or WWTPs in a single study when the same sampling and testing methodology was used. When cross-study comparisons were made, the median values were used. The median is a better representative for the large variations in magnitude of reported results between the reviewed studies (*Discrete Data Sets - Mean, Median and Mode Values*, n.d.) and using it eliminated the need for detecting and removing outliers.

2.3 Evaluation Methods

Following data extraction and critical appraisal, decision matrices were prepared to identify the most preferable sampling, processing, and analysis methods for detecting microplastics in wastewater. This was done using the Decision Matrix Procedure Method 1 outlined by the American Society for Quality (ASQ, n.d.). The rows consisted of methods, techniques, and equipment used in the literature. The columns consisted of a series of quality criteria based on the Canadian Plastics Science Agenda (CPSA) Framework needs for plastics detection, quantification, and characterization methods as well as efficiency criteria. This included method reproducibility, representation, reporting, quality assurance, quality control (Environment and Climate Change Canada, 2019), time, cost, automation (Quevauviller et al., 2006), and environmental impact of the methods. One of three units was assigned to each category to assess how well the methods met the criteria, where a unit of 0 indicated that the criteria was not met, 1 indicated that the method was less than ideal, and 2 indicated that the method was ideal in meeting the criteria. The results of the decision matrix were averaged per row to estimate what the most ideal methods are for use in a harmonized microplastic in wastewater testing methodology.

2.4 Draft Harmonized Method Design

The final deliverable of the project was a draft harmonized method that incorporated the recommendations and results of the literature review, critical appraisal, and decision matrix. This draft was adapted from the ASTM Draft Standard Template for Test Methods (ASTM, n.d.) format, and included the following headings: (2) Scope, (2) Summary of Test Method, (3) Significance and Use, (4) Interferences, (5) Apparatus, (6) Reagents and Materials, (7) Sampling, (8) Conditioning/Processing Procedure, (9) Analytical Procedure, (10) Calculation or Interpretation of Results, and (11) Precision and Bias. The proposed standard method was presented in detail in Appendix II.

3 – Systematic Literature Review Discussion and Results

The systematic literature review research included experimental and review studies, both of which focused their methods and discussion around three methodological stages: sample collection, sample preparation, and sample analysis (Flowchart in Figure 3). Sampling involved methods for capturing and concentrating wastewater samples to be analysed. Sample processing included methods to remove unwanted interferences and extracted microplastics from the samples. Sample analysis involved the methods used to detect, quantify, and characterize microplastics in wastewater. These methods, as well as data and results, were reviewed and critically appraised as part of the following discussion.

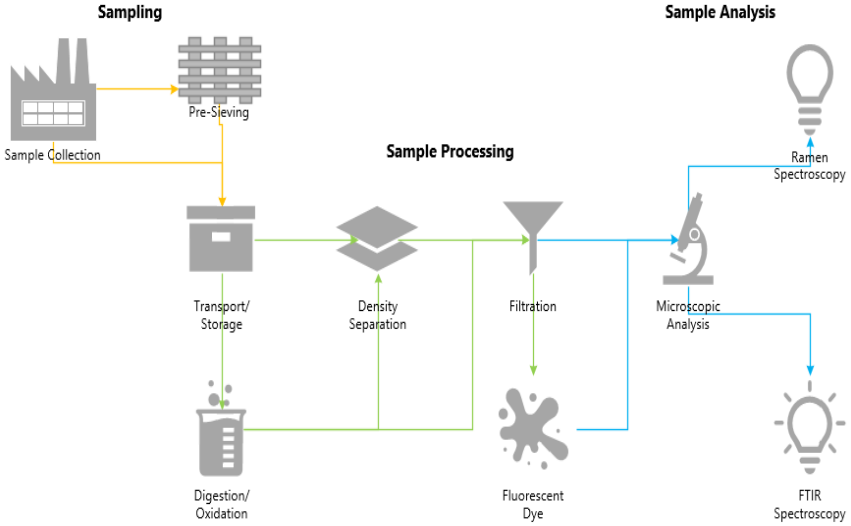

Figure 3. Flow Diagram for Common Microplastic in Wastewater Testing Methods

3.1 Microplastic Removal Efficiencies and Concentration in Wastewater

The research aim of most of the reviewed studies were to characterize the microplastic contamination in a respective region's wastewaters. The microplastics were described by quantitative values, mainly concentration, as well as qualitative values, including type, colour, polymeric composition, and size. It has already been established that the results of these individual research studies cannot adequately be compared due to the use of different methodologies to obtain the data (see 1 – Introduction 1.1 Backgroundfor details). But the data and results can provide insight into the effectiveness and potential biases of the methods used. This section focuses on the connection between the quantitative results between the studies and how they may be influenced by the experimental methods applied in the reviewed literature.

3.1.1 Wastewater Treatment Plant Microplastic Removal Efficiency

The way to describe how well a series of treatment systems remove microplastics from wastewater is to report it in overall removal efficiency (Spellman, 2016, section 1.7.3). The removal efficiency (Equation 1, reproduced from Spellman, 2016, section 1.7.3.1) is a measure of plant performance that determines the overall effectiveness of treatment in removing a pollutant, in this case individual microplastic particles.

$$\%\ Removal = \frac{influent\ concentration\left(\frac{MP}{L}\right) - effluent\ concentration\left(\frac{MP}{L}\right)}{influent\ concentration\left(\frac{MP}{L}\right)} \times 100\% \quad \text{.....(1)}$$

Estimated microplastic particle removal efficiencies of the wastewater treatment plants (WWTPs) in the reviewed literature ranged from 17% to over 99%. There was no significant trend in increased microplastic removal rate for WWTPs employing advanced tertiary wastewater treatment over secondary treatment, but these wastewater quality and testing methodologies were variable and therefore it may not be reasonable to compare their removal efficiencies. Figure 4 shows how studies that reported low concentrations of microplastics tended to also report lower WWTP removal efficiencies than studies with higher microplastic concentrations. A 98.3% removal efficiency of microplastics in a secondary WWTP with an effluent concentration of 0.5MP/L was reported in one study (Gies et al., 2018), but in another study applying tertiary treatment the removal efficiency was only 82.1% with an effluent concentration of 0.05MP/L (Lv et al., 2019). Here, the lower removal efficiency may just be a factor of the low concentration of microplastics rather than a measure of treatment efficacy. It is also affected by the units used to calculate it, where removal efficiency by mass may indicate WWTP performance differently. The study that reported an 82.1% microplastic particle removal efficiency also reported a 99.5% removal efficiency by mass for the same WWTP, indicating excellent removal of particle mass despite lower efficacy in removing total particles. The sampling and testing methodologies applied may also contribute to biases in removal efficiencies. In one study, an unfiltered sample of influent and a pre-filtered sample of effluent were used to assess WWTP microplastic removal rates (Gies et al., 2018). Pre-filtering would eliminate a fraction of particles smaller than the sieve pore size in effluent but not influent, thus contributing to a high bias in the reported WWTP removal efficiency. Therefore, the calculated removal efficiency can only provide a good representation of how well the WWTP removes microplastics in wastewater when consistent measurement methods are used and similar concentrations are observed between wastewater treatment plants. Therefore, it

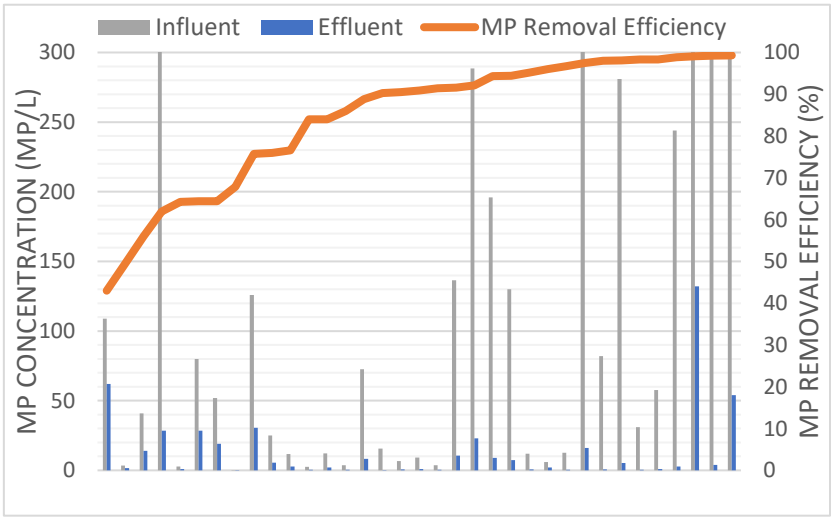

Figure 4. Reported Microplastic in Wastewater Concentrations Less than 300MP/L and Associated WWTP Removal Efficiencies

may be more advantageous to assess treatment performance using a different standard, such as concentrations.

3.1.2 Wastewater Microplastic Concentration

Microplastic concentration in wastewater influent and effluents was used to assess microplastic contamination of wastewaters in most of the reviewed literature. Where removal efficiency is a measure of treatment performance, the concentration gives information on how much microplastic enters the treatment plant and how much is discharged after treatment. This is an important parameter to report because many of the reviewed studies concluded that although microplastic removal efficiencies of the studied WWTPs were high, significant amounts of microplastics were still being discharged to the receiving environments due to the large volume of wastewater processed on a daily basis (Gies et al., 2018; Liu et al., 2019; Long et al., 2019; Magni et al., 2019).

The most common way to report microplastic concentration in the reviewed studies was by number of microplastic particles per liter of wastewater (MP/L). These units give information on how many microplastic particles are estimated in influent and effluent and give a sense of scale to the contamination. The median influent concentration for 38 researchers reporting this value was 46.5MP/L, varying from as low as 0.28 MP/L (Lv et al., 2019) to as high as 65,953 MP/L (Fortin et al., 2019) (see Table 1 in Appendix I for details). Effluent counts showed similar trends to the influent, for a median concentration of 3 MP/L from 47 articles reporting effluent concentrations. The reported concentrations vary significantly, up to five orders of magnitude, which may be due to considerable differences in water quality between the analysed regions or may be due to biases inherent in the different methodologies used to find these values. Furthermore, mechanical stressors of treatment technologies and testing methods themselves can fragment plastics into multiple smaller particles, leading to higher apparent concentrations.

A major problem with reporting microplastic concentration by number of particles is that it is not a conserved base value, which is to say that a single particle can turn into several particles due to fragmentation as a result of mechanical stressors during wastewater treatment or sample processing (Simon et al., 2018). This can cause unexpectedly high effluent concentrations when compared to influent concentrations which in turn can lead to low biases in calculated removal efficiency. An alternative conserved reporting format is microplastic mass per liter (mg/L or μg/L), which was sparingly measured in the literature with results ranging from less than a microgram per liter to over a milligram per liter (see Table 2). Mass concentrations could either be directly obtained from thermal analysis (Bannick et al., 2019; Rasmussen et al., 2021), or could be estimated based on particle composition and dimensions observed under microscope

and infrared spectroscopy (Lv et al., 2019; Simon et al., 2018, 2019). Although mass values may be less influenced by mechanical stressors than numeric particle values, the current non-destructive methods to find mass are unreliable. As seen in Table 2, Lv et al. (2019) estimated microplastic mass at an order of magnitude higher than Simon et al. (2018) but also reported microplastic particle numbers four orders of magnitude less than what Simon et al. counted. These results are contradictory, which may be due to a calculation or reporting error in one or both studies. There is also potential that the results are not truly comparable due to differences in the methods use to quantify or estimate microplastic mass.

Table 1. Reported Influent and Effluent Microplastic Mass Concentrations in Reviewed Literature

Source	***Publication date***	***Influent (MP/L)***	***Influent (ug/L)***	***Final Effluent (MP/L)***	***Final Effluent (ug/L)***
Lv et. al	2019	0.28	5600	0.09	98*
Simon et al.	2018	7216	250*	54	4.2*
Simon et al.	2019	N/A	N/A	3	0.31
Bannick et al.	2019	N/A	N/A	N/A	45
Rasmussen et al.	2021	533	156.74	4	2.39

**Results are averages*

The results of the microplastic in wastewater studies in the reviewed literature vary in the magnitude of reported concentrations, how concentration is reported, and the calculated removal efficiencies. On the surface, these results could describe how wastewater quality varies across the world or the effectiveness of wastewater treatment in different facilities. But the results are not comparable because they were not all determined in the same way. Different methods used to sample, process, and analyse microplastics in wastewater can impart different types and degrees of biases on the results. Therefore, it is necessary to evaluate how these methods impact results and determine which methods are most appropriate for standardization. Although the results of these studies cannot be directly compared against each other, they can be used as evidence in some of the critical evaluations of the applied testing methods and assist in making conclusions on which methods are suitable for monitoring microplastics in wastewater treatment plants.

3.2 Wastewater Treatment Plant Microplastic Sampling Methods

The first step in determining the microplastic composition in wastewater is sampling. Good sampling techniques are necessary for representative results and must consider wastewater heterogeneity and variability (Quevauviller et al., 2006, section 1.2.1). These are impacted by composition of source wastewater, conditions of significant dilution such as rainfall events, and diurnal or seasonal changes in wastewater quality and flow. The depth that samples are taken can

also contribute to variable results, where microplastics may concentrate at the wastewater surface (Tagg et al., 2020). Five key criteria for a microplastic targeted sampling method were proposed by Bannick et al. (2019): (1) the sample volume must be representative for the source, (2) the sampling technique should be applicable to different water matrices, (3) the sampling methods should be capable of capturing microparticles of varying compositions, sizes, and shapes, (4) the sampling method should support diverse sample processing and analysis methods, and (5) the sampling recovery rates must be expressed. How these criteria and considerations were met, or if they were achieved at all, varied significantly across the reviewed literature, (see flowchart in Figure 5). This section explores the different approaches and methods applied in the reviewed literature and will recommend a suitable sampling strategy for microplastic in wastewater detection.

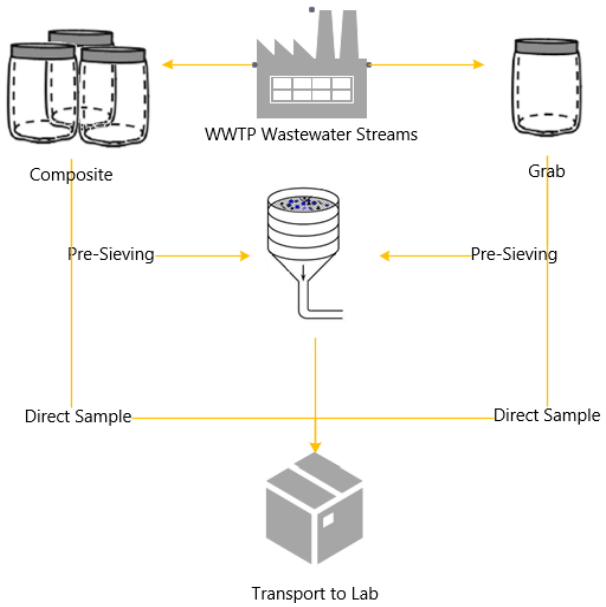

Figure 5. Microplastic in Wastewater Sampling Methods Flowchart (adapted from Bayo et al., 2021, and Long et al., 2019)

3.2.1 Sampling Equipment, Intervals, and Techniques

The methods for sampling in the reviewed research started with the equipment and sample strategies. Researchers had to determine how the samples would be collected and what equipment they needed to perform the sampling. There were two main decisions for researchers in the literature: to take the samples as a grab or composite, and to retain the samples as bulk suspensions in wastewater or to pre-filter them over stacked sieves. There are advantages and disadvantages of each option, and their applicability will depend on what the goals of the research are. Feasibility and representativeness are likely to be primary concerns for monitoring studies, and methods that can best achieve these will be discussed in this section.

The equipment types and materials chosen for sampling microplastics in wastewater varied significantly across the reviewed studies depending on research priorities. Researchers concerned about finding accurate results for smaller volumes of sample may be more concerned about the effects of cross contamination of equipment or the environment into their samples. This could be managed by using non-plastic materials, such as stainless steel buckets or glass jars (Liu et al., 2019; Simon et al., 2018) for manually collecting samples and stainless steel or silicon tubing for conveying pumped samples (ASTM, 2020a; Bannick et al., 2019). Additional strategies may be

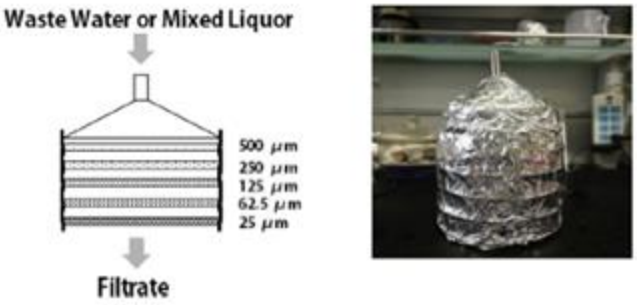

Figure 6. Covered stacked sieve sampling device (reproduced from Lv et al., 2019)

used to minimize exposure of the samples to atmospheric contamination of microplastics, such as using aluminum foil to cover sampling sieves as in Figure 6. Where accurate sample volume measurements were a priority, especially in large volume sampling, researchers could employ a flow meter to find volumes when sampling time is known (ASTM, 2020a; Bannick et al., 2019). Researchers concerned with sample representativeness may select equipment that is optimized for sample volume size or sample particle size. Sample volume size is a priority in the ASTM (2020a) standard procedure, where volumes higher than 1500L are recommended to minimize standard error. To handle such large sample volumes, sieving must be used to concentrate the wastewater solids of interest into a manageable sample vessel. Most of the reviewed studies used sample pre-sieving, but this led to concerns over particle size representation for some researchers. Only solids retained on the sieves were included in the reported results, and therefore the number of particles reported was dependent on the smallest sieve mesh size used. A median sieve mesh size of 55µm was used in 30 of the reviewed studies employing sample pre-sieving, but it has been demonstrated that samples could be sieved down to a particle size of 10-20µm (Bannick et al., 2019; Ben-David et al., 2021). The problem with smaller sieve sizes is that they tend to clog, and therefore their use was dependent on the sample volume collected or the availability of personnel and other sieves to replace clogged ones (ASTM, 2020a; Bannick et al., 2019; Long et al., 2019). The sampling priorities need to be established to determine what equipment is needed for a microplastic in wastewater monitoring protocol, which will also influence the sampling method used.

There are two general methods that can be applied for sampling wastewater: the grab method and the composite method (WEF, 2010, section 5.1.1). The grab method can be performed manually or automatically and involves the collection of a single sample that represents wastewater conditions at one instance in time (Quevauviller et al., 2006, section 1.2.2; WEF, 2010, section 5.1.1). This type of sampling is not considered appropriate for monitoring methods as it does not provide information on how wastewater conditions may change through the day or year (Quevauviller et al., 2006, section 1.2.2.1). The composite method involves the collection of wastewater samples into a single vessel based on time or flow intervals (Quevauviller et al., 2006; WEF, 2010). A composite sample allows the observer to look at an average of wastewater quality over a period of time in a single sample, and is generally automated to collect set

sampling volumes or volume integrated with wastewater flow rates (Quevauviller et al., 2006, section 1.2.2). The choice to collect the samples as grabs or composites is dependent on the research goals, but the drawbacks need to be carefully considered in addition to their advantages.

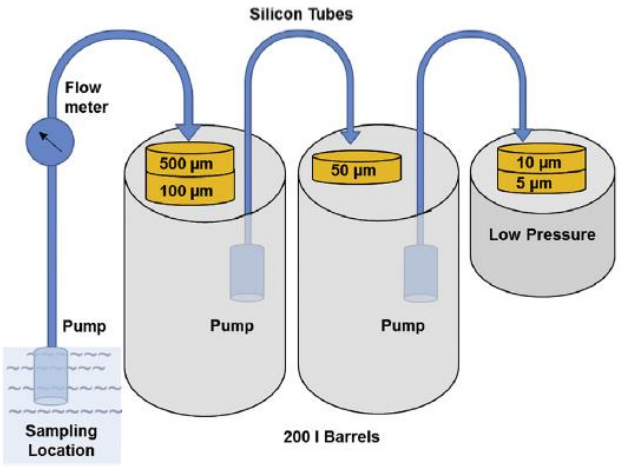

Figure 7. High Volume Automatic Grab Sampling Apparatus (reproduced from Bannick et al., 2019)

Grab samples were taken as the primary method of sampling in most of the reviewed studies. Some studies manually took samples using plastic, glass, or steel collection containers, which offer a quick, easy, and inexpensive method for collecting samples. But this method requires active sampling work by personnel and can pose limitations on sample representativeness. Ideally the sample can be taken from the most homogenous part of the wastewater stream (Quevauviller et al., 2006), but access may be limited when sampling the stream manually. Manual sampling may also limit how much volume is collected due to available personnel time. Researchers in 24 studies reported taking manual grab samples for a median collectable volume of 13L. The largest volume collected manually (81.5L of effluent by Simon et al. (2018)) was still less than 10% of the recommended sampling volume in the ASTM sample collection standard practice (ASTM, 2020a). Automatic sampling methods, such as pumps or sampling faucets, could achieve sampling volumes in excess of 1000L. Bannick et al. (2019) demonstrated an automatic grab sampling method in Figure 7 that could collect up to 1000L of effluent sample in three hours using pre-sieving and vacuum filtration to the smallest sieve mesh sizes. These higher volumes are expected to better represent trace microplastics and overcome standard error. Furthermore, Ben-David et al. (2021) found that larger volume samples collected over longer periods (8-9 hours and 100L) had fewer variable results than small volume samples collected over a shorter period (2-3 hours and 30L) and were comparable in variability to composite samples collected over a 24-hour period. However, both research groups only conducted these sampling tests on effluent streams, which are less variable throughout the day (WEF, 2010) and therefore these sampling methods many not be applicable to higher solids and more variable influent streams. Grab sampling methods can be applied in a way to collect larger and more representative sample volumes, but they may not be the most appropriate method for estimating daily microplastic loads to a wastewater treatment plant, which may be better represented by a composite sampling method.

Composite sampling, unlike grab sampling, provides a method for estimating an average wastewater quality over a period of time using automatic samplers. This method of sampling would improve representativeness of the samples for daily loads, but is limited to the volume it can collect. Composite sampling is recommended over grab sampling for monitoring purposes (Quevauviller et al., 2006), and sampling parameters should be selected according to variability of the matrix: flow based sampling for variable water qualities, as in the influent, and volume based sampling for consistent water qualities, as in the effluents (WEF, 2010, section 5.1.1). The main limitation of composite sampling in the reviewed literature was the total volume that could be collected. The largest volumes collected for studies applying 24-hour flow-based composite sampling was 270L of final effluent (Petroody et al., 2020). The ASTM (2020b) standard practice requires 24-hour composite sampling of high solid or influent streams at a minimum flow rate of 1 GPM, which works out to be 227 L/hr to minimize standard error. The ability to achieve these volumes of collection depend on the composite sampling apparatus and the availability of personnel, and therefore it may not be practical to capture such high volumes. Ben-David et al. (2021) also reported a low coefficient of variability of 24-hour composite samples taken at volumes of 24L or less, so the results may still be representative in smaller volumes. The ASTM (2020a) method would have composite samples directly filtered, but this would require continuous availability of personnel to replace the sieves as they become clogged over a 24-hour period. Smaller composite sampling volumes could be collected into containers and filtered later (Conley et al., 2019), minimizing the need for personnel oversight through the sampling process but potentially increasing standard error. Composite samples offer a solution to representation of wastewater quality throughout the day, but are prone to limitations on volumes that can be collected and therefore may not be applicable for all sampling streams and situations.

Once the sampling method has been established, the sampled volume needs to be managed in a way that is reasonable for processing and analysis. Whether the sample method used is for collecting grabs or composites, there are two main strategies for how the collected volumes are managed: capturing the wastewater suspension directly in a container or collecting the solids on site by pre-sieving the wastewater volume over a series of sieves. Each method for managing sampled wastewater has advantages and disadvantages, but their use ultimately depends on the priorities of the microplastic monitoring study and will be discussed in the next sections.

3.2.2 Direct Sampling

Direct sampling occurred when wastewater was collected as suspended samples in an un-filtered form. This method of sampling is simple and includes all particle size fractions, but may involve limitations in volumes collected and sample integrity during storage. The data recovered from the reviewed literature showed volume limitations of direct sampling, where median collected

volume collected for all streams in 20 studies was 10L with a maximum volume of 60L (Raju et al., 2020). These volumes may be too small to be representative of continuous wastewater quality or meet detection limits for scarce microplastics. However, they allow for researchers to capture and observe very small microplastics that may otherwise be filtered out in a pre-sieved sample. In addition to volume concerns, samples collected directly may be at risk for aging. Ding et al. (2020) found that reference plastic particles incubated in wastewater at 25°C for three days experienced degradation and agglomeration. Sample aging can be minimized by storing samples at reduced temperatures (Quevauviller et al., 2006), but the impacts of this need further research. Direct sampling is useful for determining proportions of microplastics in a larger range of size fractions, but it is limited to the collectable volume and therefore may not be appropriate for quantifying total microplastic loads and releases to and from a wastewater treatment plant.

3.2.3 Pre-Sieving Sampling

Pre-sieving of wastewater samples refers to how wastewater is passed through one or more sieves with different pore sizes to separate the solid fraction from the liquid fraction. The solids fraction retained on the sieves is collected for sample analysis, whereas the water fraction is typically discarded. Most of the reviewed studies employed pre-sieving of wastewater samples, and the ASTM (2020a) standard practice mandates use of pre-sieving. Pre-sieving offers an advantage over direct sampling in that larger volumes of sample can be collected, thereby contributing to reduced standard error. Where direct sampling was limited to collection volumes of under 100 L, researchers pre-sieving samples reported collecting up to 2000 L (Kazour et al., 2019). Despite these larger volume captures, the current standard for microplastic in water sampling specifies sampling volumes of 1500-5450 L (ASTM, 2020a). One reason for why these ASTM volumes were not feasible in the reviewed literature is that the sieves are prone to clogging at even small volumes, especially for higher solids influent streams. Samples had to be monitored closely for sieve clogging, which could occur in under 20 minutes of sampling at flows less than 20 L/min for smaller pore sizes sieves (Long et al., 2019). The ASTM (2020a) standard practice requires that sieves be replaced once they are clogged to continue sampling, but this would require continuous monitoring and availability of personnel. The composite samples would be particularly difficult to manage for sieve clogging if personnel aren't available to monitor the system for the full 24-hour cycle. Sampling volumes should be maximized where possible, but volumes greater than 1000 L may not be practicably feasible for monitoring methods due to the need for continuous monitoring of the sieves.

The major drawback of pre-sieving wastewater samples is the inherent elimination of microplastic particles that are smaller than the smallest sieve pore sizes. The ASTM (2020a) standard practice mandates a minimum sieve pore size of 20μm, but the size range of

microplastics has been defined down to 1nm (GESAMP, 2015). The smallest sampling sieve mesh sizes used in the field were 10µm, but in the absence of sieving these microplastics from wastewater could be captured in sizes as small as 0.45 µm by filtering in the laboratory (Bayo et al., 2020a; Ben-David et al., 2021; Conley et al., 2019). This method allows for the capture of microparticles smaller than required by the ASTM standard method, but is limited by workable volumes. One study was able to field filter 1000L of sample through a pore size of 10µm under vacuum (Bannick et al., 2019), which means that the limit of detection can be improved upon the ASTM standard practice. Many researchers used multiple sieves in series to separate size fractions of particles, which may be beneficial in minimizing sieve pore clogging but the number of sieves and sieve pore sizes varied. The number of sieves used to sample may allow for larger sample volumes to be collected. Researchers that collected more than 100 L of wastewater used at least three sieves to pre-filter their samples, but more research would need to be conducted to make a definitive conclusion on any trends relating number of sieves to sample volume size.

Pre-sieved sampling offers the advantage of collection of larger sample sizes, which minimizes standard error and can eliminate the macro-plastic portion from sample processing and analysis. But pre-sieving automatically eliminates smaller microplastic size fractions from the final microplastic estimates in wastewater. Efforts should be made to capture smaller particle sizes while minimizing monitoring time. Additionally, microplastic size fractions should be standardized and defined in the final results to report only the size fractions that were sampled.

3.2.4 Microplastic Sampling Methods Evaluation and Decision Matrix

The possible sampling methods extracted from the reviewed literature were evaluated in a decision matrix in Table 3 of Appendix I. To meet feasibility and quality objectives, the ideal sampling method should be representative, quick, and cost-effective and it should produce high quality and reliable results. Although grab samples are quick and can be done with minimal resources, they represent a snapshot in time which may not be reflective of continuous wastewater conditions. Pre-sieving adds additional resources and time to the sampling method, but greatly increases the volume of sample that can be collected, especially when using sieve mesh sizes greater than or equal to 20µm. High volumes are preferred in samples as they minimize the standard error of results, but too large of volumes may require excessive monitoring to ensure that sieves do not clog and overflow. Additionally, it may only be feasible to sample a medium sized volume for high solids content wastewater such as influent. It has therefore been decided that the most practically feasible and accurate method for sampling microplastics in wastewater is to pre-sieve medium volumes of influent and high volumes of effluent over a minimum 20µm sieve mesh size using 24-hour composite samplers.

3.3 Sample Processing

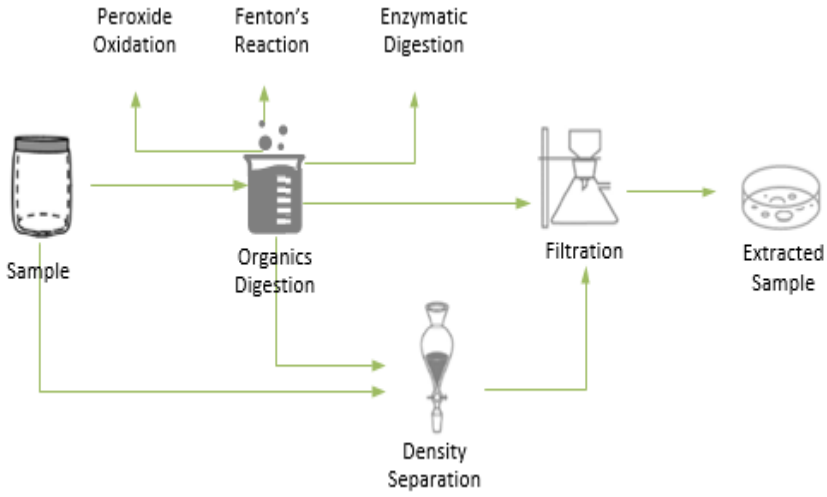

Figure 8. Microplastic in Wastewater Sample Processing Methods Flowchart (adapted from Bayo et al., 2021, and Ben-David et al., 2021)

Samples collected from wastewater treatment plants generally cannot be directly analysed in their original form. Organic and inorganic suspended solids as well as biological and chemical contaminants on microplastic particle surfaces can interfere with or inhibit the spectroscopic analysis and identification of microplastics. To minimize or eliminate the effects of these interferences in analysis, samples are typically first processed to digest interfering solids and extract plastic particles, but the procedures used to do this varied significantly between the reviewed studies (see flowchart in figure 8). There were two main methods applied to isolate microplastic particles from the wastewater samples: organic matter oxidation and/or microplastic particle extraction by density separation. But even for studies that applied the same digestion and isolation techniques, their procedures could be different in the order of processing steps, the materials and equipment used, and the wastewater streams that these methods were applied to. The quality of the results and efficiency of the method is dependent on how these procedures are performed, and therefore the processing methodology needs to be standardized to produce reliable and comparable results. The processing methods need to be carefully selected and performed to minimize potential biases due to contamination and recovery, but also need to be cost-effective and time-sensitive if they are to be applied as monitoring methods. The following sections will introduce and discuss each of the common microplastics in wastewater processing methods and steps encountered in the reviewed studies, and will highlight the advantages and disadvantages of these methods.

3.3.1 Oxidation and Digestion Procedures

Most researchers in the reviewed methods applied at least one procedure to break down organic suspended solids in the wastewater samples that could interfere with the analysis of microplastics. The most common procedure involved oxidation, but it was often supplemented by enzymatic digestions (see **Error! Reference source not found.**. These methods may be effective in decomposing organic material in wastewater, but they need to be gentle on

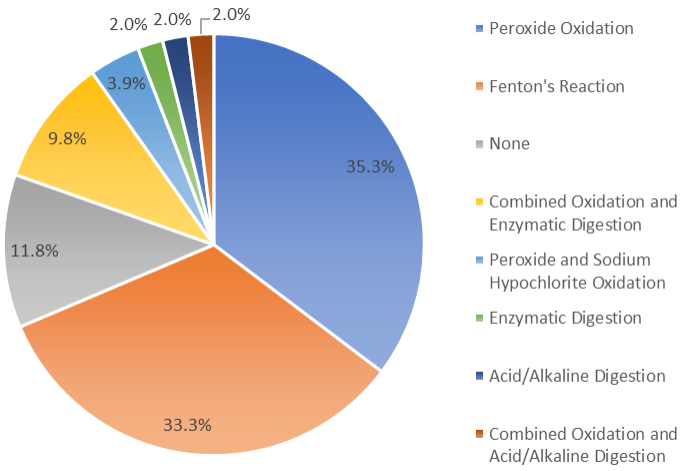

Figure 9. Proportion of Studies Using Different Sample Digestion Techniques

microplastics to give reliable results. Processing time and costs should also be considered when determining how applicable these methods are for monitoring protocols. The current ASTM standard (2020b) specifies a time and materials intensive procedure for extracting microplastics from medium to high suspended solids content waters, including seven steps of digestion and extraction and a number of different reagents. US EPA researchers tested the ASTM method on a sample of influent, and reported that samples could be fully processed in 24 hours. But this method involved four hours of active personnel work to prepare samples for digestion, perform centrifugations and decantation, and to clean up samples and reagents (Cook & Allen, 2020). Therefore, this ASTM standard practice may not be feasible in monitoring applications for high solids wastewater matrices due to time intensive treatment of samples and costs of specialized enzymes and reagents (Al-Azzawi et al., 2020; Raju et al., 2020). This section will discuss alternative processing methods used in the reviewed literature and how applicable they are in microplastic in wastewater monitoring applications with regards to effectiveness and feasibility.

Acid and alkaline digestions have historically been used to manage non-plastic suspended solids in wastewater, but were not as common in the literature published reviewed in this project. The use of acid digestion can target organic solids that may not be effectively removed through peroxide oxidation, such as cotton or semi-synthetic fibers (Conley et al., 2019). However, typical acid and alkaline reagents have also been reported to degrade some plastic compounds, (Al-Azzawi et al., 2020; Raju et al., 2020). One researcher found that 10% potassium hydroxide solution dissolved the plastics polyethylene terephthalate and polylactic acid in wastewater sludge samples (Al-Azzawi et al., 2020), and another found that polyethylene beads were completely destroyed by 1 Molar hydrochloric acid and 10 Molar sodium hydroxide solutions at elevated reaction temperatures (Ding et al., 2020). Naji et al. (2021) were the only researchers to implement alkaline digestion on their samples, but their results showed possible signs of particle decomposition given that 90% of their detected plastic particles were 3-50µm in size despite only sampling down to a particle size of 333µm. Given these findings, acid and alkaline

digestions, even at lower concentration, may be too aggressive for microplastic extraction and therefore should not be considered in a standard method for measuring wastewater microplastics.

Enzymatic digestion is a gentler option for removing organic solids from wastewater samples. Enzymes were used to digest cellulose, proteins, starches, fats, and chitin in wastewater (ASTM, 2020b; Wang et al., 2020), and were usually applied in combination with other enzymes or in addition to peroxide oxidation. The use of enzymes is reported to be very effective in removing organics from wastewater samples (Ding et al., 2020; Raju et al., 2020), but has been criticized for being costly and time-consuming (Al-Azzawi et al., 2020; Raju et al., 2020). Simon et al. (2018) applied cellulase enzyme to samples following an oxidation reaction that required 40 hours of incubation. However, the ASTM (2020b) standard method specifies an enzymatic digestion period of 20 hours for a mixture of protease and lipase, and Ding et al. (2020) even performed their entire sample digestion using only a protein enzyme in the short period of two hours. The use of enzymatic digestion could be a promising method for digesting organic interferences in wastewater samples, but it warrants further investigation into time and cost-effective procedures to determine if it is feasible for a microplastics in monitoring method.

The most common procedure in the reviewed studies for removing interfering solids in wastewater samples was oxidation, where more than 80% of the studies applied oxidation in some form. There were two main methods for performing oxidation: simple peroxide oxidation or iron (II) catalyzed peroxide oxidation, referred to as Fenton's reaction. Simple peroxide oxidation was used in half of the studies performing oxidation procedures, with various volumes of 15-35% concentration hydrogen peroxide using single or iterative digestions spanning 12 to 168 hours (see Table 4 in Appendix I). Alvim et al. (2020) completed their peroxide oxidation reaction within two hours at elevated temperatures on wastewater effluent samples, but it is not clear if this short oxidation period would work for higher solids samples, such as influent. Other researchers enhanced the speed of their oxidation reactions by using Fenton's reagent, where the iron (II) in the reagent catalyzes oxidation reactions when used with hydrogen peroxide at low pH (Simon et al., 2018). Researchers that used Fenton's reagent reported reaction times of less than 24 hours to as little as 30 minutes (see Table 4, Appendix I). Therefore, oxidation reactions can be time-efficient and do not require expensive enzyme reagents, but their impacts on microplastic integrity also need to be considered.

Although widely utilized, these oxidation reactions have been shown to have variable effects on microplastic particles in wastewater. Ding et al. (2020) found that polyethylene beads in a reference sample were destroyed by peroxide oxidation. However, oxidation reactions have generally been reported to remove interfering suspended solids without causing major

degradation to the microplastic particles (Al-Azzawi et al., 2020; Raju et al., 2020). Studies that processed reference spikes along with their samples reported overall recoveries in the 56-100% range (Alvim et al., 2020; Ben-David et al., 2021; Gies et al., 2018; Long et al., 2019; Mayo et al., 2019; Raju et al., 2020; Simon et al., 2018). There was also concern in some studies that the oxidative reactions could lead to interferences in the detection of microplastic particles in the analysis stage. Signs of polypropylene decomposition in the spectral analysis of samples prepared using Fenton's reagent were reported in one study, but the researchers could still successfully identify all reference microplastics (Alvim et al., 2020). Peroxide oxidations may not be aggressive enough to remove biological films from microplastic particles, which can interfere with the identification of plastics in micro-Raman spectroscopy (Ben-David et al., 2021). Additionally, iron precipitates produced in Fenton's reaction could coat microplastic particles and inhibit their detection (Al-Azzawi et al., 2020). Oxidation reactions have been shown to effectively remove suspended solids with adequate treatment without significantly compromising the integrity of plastics. With optimizations to reduce processing time, this makes the oxidation method a feasible option for a microplastics in wastewater monitoring protocol.

3.3.2 Microplastic Extraction and Isolation

To prepare the wastewater samples for microplastic analysis by microscopic and spectroscopic means, the plastic particles need to be separated from other components of the matrix and isolated onto an analytical medium. Typically, this is done by filtering the digested sample solution over a small pore size glass or cellulose fiber filter, but some researchers included an additional extraction stage to separate microplastics from other solids. This separation step is optional and may provide advantages in reducing inorganic interferences from extracted samples undergoing analysis, but it also may produce additional uncertainties and biases in the results.

Table 5. Density Separation Reagents and their Respective Density Values

Reagent/Particle	Density (g/mL)	Source
Microplastics	0.83 – 2.3	Al-Azzawi et al., 2020; Lares et al., 2018
Sodium Chloride (NaCl)	1.08 - 1.2	Bayo et al., 2020a; Jiang et al., 2020; Magni et al., 2019
Calcium Chloride (CaCl2)	1.4	Grbic et al., 2020
Cellulose	1.5	Lares et al., 2018
Sodium Ioidide (NaI)	1.45 - 1.80	Gundogdu et al., 2020; Lv et al., 2019; Mayo et al., 2019; Petroody et al., 2020; Ziajahromi et al., 2021
Zinc Chloride (ZnCl2)	1.5-1.9	Jiang et al., 2020; Rasmussen et al., 2021; Simon et al., 2019; Wang et al., 2020; Wolff et al., (2019, 2021); Yang et al. (2019)
PTFE	2.1 - 2.2	Wolff et al. (2019, as cited from Baur et al., 2013)

Density separation is a method that uses carefully prepared solutions to separate solids based on their physical properties. Microplastics, typically characterized by lower densities, float on the solution surface but denser inorganic particles sink, allowing them to be easily excluded from further analysis. Densities of these reagents vary (see Table 5), where low density NaCl solutions are inexpensive and have a high degree of discrimination for denser particles but may also accidentally exclude microplastics with higher densities, such as polyester (Alvim et al., 2020; Lares et al., 2018). Denser solutions, such as NaI, may not be as prone to losses of denser plastics but they are not as cost effective as NaCl (Alvim et al., 2020), which can be a practical limitation. Other density extraction solutions were used, but often had interferences that could inhibit microplastic analysis. Higher density $ZnCl_2$ solution was reported to be corrosive to sample filters (Jiang et al., 2020), and the canola oil used in Gies et al.'s (2018) oil extraction protocol can appear in infrared spectra and interfere with microplastic identification. Despite its usage in many studies, the benefits of applying density separation to wastewater samples for microplastics analysis is not well justified. It is not clear to what degree non-plastic solids are removed using this method. Organic solids, such as cellulose, have densities similar to that of plastics, and therefore would likely not be effectively density separated from microplastics (Al-Azzawi et al., 2020; Lares et al., 2018). Density separation adds additional time and material to a processing method where it's benefits to microplastic detection are not well defended. It is therefore not recommended to include in a microplastics in wastewater monitoring study, where direct filtration has shown to adequate for the isolate and analysis of microplastic particles.

3.3.3 Application of Sample Processing to Different Wastewater Streams

Although many of the reviewed studies used similar processing techniques, the application of these methods also varied by order and sample matrix. To the authors best knowledge, no experimental studies have been performed on microplastics in wastewater to determine how order of processing impacts results. It may be more beneficial to perform density separation following oxidation or digestion reactions as organic particles have similar densities to microplastics and would likely be extracted together (Al-Azzawi et al., 2020), but none of the reviewed studies investigated this. On the other hand, variations in how the processing steps are applied to different wastewater streams within a single study can lead to biases in reported results, but it may also be the only way to effectively and efficiently process wastewaters that vary in solids content. This section will discuss how sample processing methods could be modified for different wastewater streams and how that may impact the final reported results.

Most of the methodologies applied in the reviewed research selected a single sampling, processing, and analytical method which was applied to all wastewater streams regardless of suspended solids content. But a handful of studies used different methods for different

wastewater streams, including the ASTM standardized method for sample processing (2020b). Where the ASTM recommends a series of oxidation and digestion steps for samples with medium to high suspended solids (i.e., influent and secondary effluent), they only recommend simple centrifugal separation for samples with low to very low suspended solids (i.e., tertiary effluent). Applying methods based on what is necessary to clean up and detect microplastic particles in wastewater is a good way to maximize efficiency, but it may have limitations on how microplastics in different wastewater streams are compared. Two research studies applied oxidation reactions only to the higher solids streams or sample fractions, opting for simple density separation and/or filtration for the lower solids content samples (Gies et al., 2018; Hidayaturrahman & Lee, 2019). Any effects of the oxidation procedures on microplastics in the higher solids content samples would not be reflected in the lower solids content samples. Similarly, researchers that only performed density separation on some wastewater streams would be subjecting those samples to selection biases, where denser microplastics or microplastics adhered to denser particles would be eliminated from further analysis (see section 3.3.2). Applying different processing methods to different wastewater streams becomes a problem when the researchers intend to use these results to determine WWTP microplastic removal efficiency. For instance, Liu et al. (2019) collected samples from influent and final effluent streams but only applied density separation to the influent stream. Their overall reported WWTP removal efficiency of 64.4% may be biased low if denser microplastic particles were accidentally separated out in the influent, leading to lower than expected influent concentrations. Depending on the goals of the monitoring study, different methods to process higher and lower solids wastewater samples could be beneficial by limiting time and material use to only where it is needed. However, this could cause biases where the differently processed samples are directly compared to each other, and therefore recommendations for how different wastewater streams are processed is directly tied to the goals of the monitoring studies. Where the goal is to only compare influent and effluent discharges independently over time, then different processing methods are highly beneficial. Where the goal is to monitor WWTP performance over time, the same processing methods applied to every measured wastewater stream would be necessary to limit biases in results and therefore the processing method should be consistent between wastewater streams.

3.3.4 Sample Processing Methods Evaluation and Decision Matrix

Desirable qualities in a sample processing method include effective clean-up measures, minimal impact of reagents on plastics integrity, timeliness, and cost-effective parameters. The existing ASTM standard (2020b) for the preparation of microplastic in water samples may utilize effective and proven methods, but it involves multiple digestion reagents and time-intensive

work from laboratory personnel that may limit the feasibility of this method in monitoring applications. Alternative methods for processing wastewater were evaluated in a decision matrix (see Table 6, Appendix I). Enzymatic digestions produced good recovery for microplastics in wastewater, but generally only targeted one type of organic compound at a time which could lead to time and costs accumulation. Oxidation reactions were shown to be effective in removing organics from wastewater samples, and Fenton's reactions in particular achieved rapid reaction times. Density separation could eliminate some inorganic constituents of wastewater, but also risked losses of denser microplastics and added time and material to the method that wasn't well justified. Applying a different processing method based on solids content of the wastewater stream may improve efficiency in the method, but can lead to uncertainties in comparison of results from these different streams. It is therefore recommended that a harmonized microplastics in wastewater sampling method apply Fenton's reaction to remove organic interferences from all sampled wastewater streams followed by direct filtration of the digested samples to isolate the particles on an analytical medium.

3.4 Sample Analysis

The final step in estimating the microplastic content of wastewater is measuring it. Sampling and sample processing are performed to capture a representative portion of the wastewater and to put it into a form that analytical instruments and operators can reliably use to detect microplastic particles. This is what the analysis stage entails – the detection and characterization of microplastics in wastewater. A variety of techniques and analytical instruments have been used to measure and classify microplastics (see Figure 10), each with their individual set of characterization capabilities. The analytical methods selected also determine the type and quality of data that can be retrieved, where commonly reported parameters included microplastic counts, sizes, types or shapes, composition, and colour. Although counts, composition, and colour were

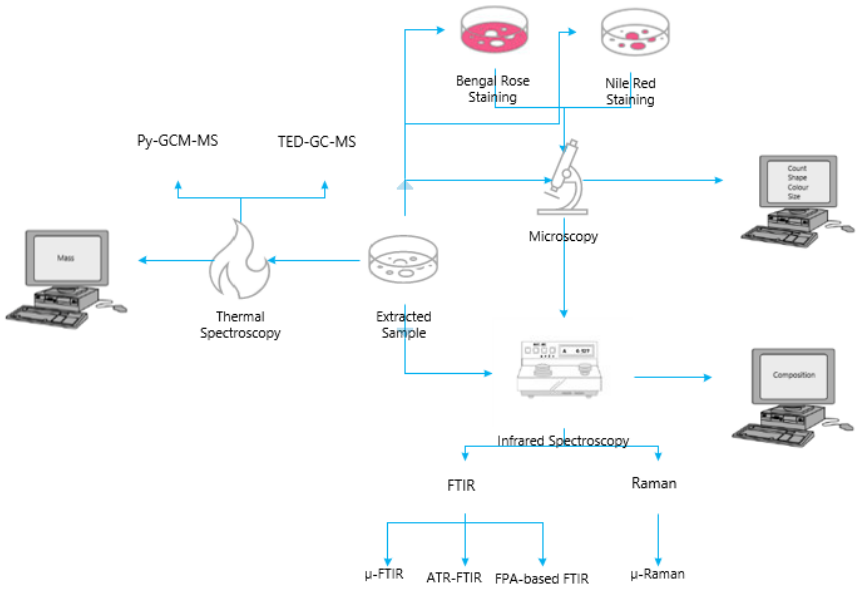

Figure 10. Microplastic in Wastewater Sample Analysis Methods Flowchart (adapted from Bayo et al., 2021)

generally reported in the same way across all studies, sizes and type/shape of microplastics were inconsistently defined and classified and contributed to difficulties in being able to make cross-study comparisons. Another parameter that was only measured and characterized in a few of the reviewed studies is microplastic mass. The benefit of reporting in this unit is that it is a conserved based quantity (Simon et al., 2018), but there were limited methods and applications for finding this value in the reviewed literature and non-destructive methods have been criticized as being unreliable (Magni et al., 2019). As research develops on the fate and ecological impacts of microplastics in the environment, it will become clearer what microplastic features are most important and which analytical methods are best to determine these parameters. For now, all commonly reported characteristics in the reviewed research (count, size, type/shape, colour, and composition) will be considered as key parameters to report and the following section will determine which analytical methods are most suitable for detecting these attributes.

3.4.1 Microscopic and Pre-Selection Methods

Microscope analysis is often performed prior to other analytical methods and may be referred to as a pre-selection method for visually identifying and isolating microplastic particles for further characterization. Researchers typically used stereomicroscopes or dissecting microscopes to observe particles, which was usually performed manually but could be automated in some cases. Microscope analysis allows authors to determine plastic type, colour, and size. It also allows for a preliminary count on suspected microplastic particles, and has been identified as a quick and cheap method for characterizing microplastics (Mayo et al., 2019; Robey, n.d.). One problem with this method is the potential for bias due to human error in identifying particles as plastics (H. Kang et al., 2020). Sample processing does not completely eliminate non-plastic particles from the extracted sample, and therefore careful and reliable analysis needs to be performed in order to correctly identify microplastics, especially if polymer confirmation procedures will not be performed on them. The reliability and accuracy of microscope analysis can be enhanced in two ways: following an established set of criteria for positively identifying microplastics, or using fluorescent staining to distinguish between natural and plastic particles.

Criteria to distinguish plastic particles from natural or non-plastic particles was used in nine of the reviewed studies with varying requirements. Most of these studies adapted criteria from Hidalgo-Ruz et al.'s 2012 article *Microplastics in the marine environment: a review of the methods used for identification and quantification*. This included three criteria for identifying a microplastic: "… (1) the object should not have cellular or organic structures, (2) fibres should be equally thick throughout the entire length, (3) colour of particles should be clear and homogeneous throughout" (Gies et al., 2018, as cited from Hidalgo-Ruz et al., 2012). Additional criteria described by Nor and Obbard in their 2014 article *Microplastics in Singapore's coastal*

mangrove ecosystems was used by Conley et al. (2019) and required that "…3) fibers were not segmented nor appeared as flat, twisted ribbon; and 4) fibers or particles did not fragment when pressed." Furthermore, Ben-David et al. (2021) specified criteria to eliminate particles that exhibited metallic sheens. These criteria provide standard detection procedures to assist the un-trained eye in distinguishing between microplastics and non-plastics, but visual analysis is still subject to biases of individual perception and abilities. These criteria also need to be reviewed for application, as some may not be suitable for microplastics that have been weathered by wastewater processes, such as criteria for shine and tapering (Conley et al., 2019). The use of criteria for visually inspecting microplastic particles is necessary for developing a standardized visual microplastic characterization method. However, a method that could discretely distinguish plastic particles from natural particles would improve confidence in detection of plastics, which can be achieved using dyes.

Fluorescent dyes can be used to enhance the efficiency and accuracy of microscopic analysis methods. Applying dyes to the sample filters make particles easier to distinguish and can even allow for automated detection under microscope. There were two ways to approach this in the reviewed literature: applying dye to stain the non-plastic particles, and applying dye to stain the microplastic particles. In the reviewed literature, Bengal Rose dye was used to stain the natural and non-plastic particles (Ben-David et al., 2021; Petroody et al., 2020; Ziajahromi et al., 2021). It was not clear whether any synthetic polymer types could also be stained by this dye and lead to false negative analysis, but Ben-David et al. (2021) addressed this uncertainty by performing a series of tests on the stained and non-stained particles to confirm suspect particles. Dyed particles were subject to a pressure test, where particles that disintegrated were deemed natural and particles that remained intact were presumed plastic. Additionally, non-stained particles were subjected to the previously discussed criteria to further categorize them as natural or plastic. This method allows for non-plastic particles to be identified and removed from further analysis, but it still requires manual sorting which can be time-consuming and would be better replaced with a staining method that supports automated analysis.

Nile Red dye can be used to stain plastic particles and distinguish them from natural and non-plastic particles using manual and automated means (H. Kang et al., 2020; Mayo et al., 2019; Raju et al., 2020). The primary benefit of staining the plastic particles is that the fluorescence of the stain can be used to apply fully automatized detection methods. This eliminates human error in the usual visual microscopic inspection of particles, and is fast, representative and cost effective. Mayo et al. (2019) reported that 40% of a filter area could be analysed in one hour at an estimated rate of USD $20/hr, and it achieved 100% recovery in analysis of reference spikes. The main problem with using Nile Red dye is that it can also dye natural organic particles, such

as cellulose, which can be misidentified as plastic (Mayo et al., 2019; Raju et al., 2020). One author also noted low recoveries of some polymer types due to incomplete dying of resistant plastic particles or due to plastic dissolution in the dye solvent (H. Kang et al., 2020). Therefore, care needs to be taken to ensure adequate dyeing concentration and time and that the carrier solvent for the dye will not impact the integrity of the plastic particles. Despite being a promising microplastic identification method, Nile Red staining and microscopic detection must be supplemented by polymeric composition detection methods to reliably report microplastic content. Nile Red stain does offer a characterization method for microplastics in wastewater that is affordable, fast, and reliable, and therefore would be a good supplement to microscopic analysis but should be performed along with spectroscopic analysis in order to supplement accuracy of results.

3.4.2 Infrared Spectroscopic and Thermal Detection and Confirmation Methods

Spectroscopic and thermal analytical methods can be used to detect and identify microplastics and their polymeric compositions. The basic operating mechanism for infrared spectroscopy is through the emission of light in the infrared (IR) region of the electromagnetic spectrum and the detection of the light absorbance of the particles being analysed (Robey, 2019). There are two infrared spectroscopic techniques that were applied regularly in the reviewed studies: Fourier Transform Infrared (FTIR) and Raman spectroscopy. These methods operate by similar principles, but FTIR uses an incandescent light source and Raman uses a laser light source (Robey, 2019). Variations of these analytical instruments exist and were applied in many of the reviewed studies to enhance the data signals, including focal plane array based micro FTIR (FPA based μ-FTIR), attenuated total reflectance FTIR (ATR-FTIR), and micro-Raman (μ-Raman) spectroscopy. These spectroscopic methods have the advantage in being non-destructive, meaning that the samples can be retained for further analysis. An alternative analytical detection method is the thermal method, which is destructive but can provide accurate information on microplastic masses. This section will discuss the application of these instruments for detecting microplastics in wastewater.

Infrared spectroscopy is used to accurately identify microplastics in wastewater in most studies. Although these methods are far more reliable in positively identifying microplastics than visual analysis, they often require significant processing times. At an estimated analysis rate of $45/hour USD (Mayo et al., 2019), it can be practically and economically infeasible to assess a full sample. For this reason, many researchers only analysed a small sub-sample of isolated particles, where visual analysis aided in the reduction of particles by pre-screening them for suspected microplastics. Additionally, not all spectroscopic instruments were suitable for identifying all microplastic colours, sizes. It is therefore necessary that the goals of the research

be established before selecting a spectroscopic method of analysis, for which FTIR or Raman spectroscopy are the current mainstream options.

The most common spectroscopic method used to analyse microplastics in the reviewed studies wastewater was FTIR. This instrument can be used to identify particles down to sizes typically captured on a sampling sieve while still processing a representative sub-sample in a reasonable amount of time. Mayo et al. (2019) demonstrated that FTIR could process a 10% portion of extracted particles within two hours at a recovery rate of 92.5%. The smallest particle size that could be detected through this method was 20µm, but this resolution could be improved using FPA-based µ-FTIR, which could resolve down to a particle size of 6.6µm (Simon et al., 2019). The lower size resolution of FPA-based µ-FTIR doesn't necessary significantly increase processing times. It was demonstrated that FPA-based micro-FTIR could be fully automated for microplastic detection, where an analysis of a 2.4% area of sample filter ($12mm^2$ area for a filter diameter of 25mm) only required 10-15 minutes of analysis time (Da Silva et al., 2020). However, the upper limits to detection using FPA-based µ-FTIR meant that larger microplastics may need alternative detection methods. The analysis of larger microplastics could be achieved using ATR-FTIR, which is only appropriate for detecting particles greater than 500µm in size (Wolff et al., 2021) but this requires additional instrumentation that may not be readily available. Aside from detectable particle sizes, FTIR analysis can also experience limitations in positive identification of particles which could lead to low biases in the results. Excessive noise or the presence of interfering signals could impede in microplastic detection (Gies et al., 2018; Mayo et al., 2019; Simon et al., 2018). The thickness and degree of weathering of particles could also lead to their non-detection or misidentification, as can the presence of additives or contaminants within or on the surface of the particle (Bayo et al., 2021; Mayo et al., 2019; Raju et al., 2020). Generally speaking, some form of spectroscopic analysis is required for the identification of polymeric composition of particles and to verify the microplastic particle counts. FTIR is a good candidate for a standard method because it can analyse a representative sub-sample size within a reasonable amount of time, but it's higher detection limits may not meet the needs of a method concerned with small microparticle sizes.

Raman spectroscopy is an alternative spectroscopic detection method to FTIR that was the second most common analytical method in the reviewed studies. This method is advantageous to FTIR in that it can resolve smaller particles and therefore can achieve lower detection limits. Raman spectroscopy can resolve down to particles sizes of 1µm (Fortin et al., 2019) with excellent recovery rates (Mayo et al., 2019). However, the drawback of this method is that the processing times are much longer than FTIR which can lead to added costs and use of non-representative sub-sample portions. Raman spectroscopy can take two hours to process just 1%

of an extracted sample for a detection size of 20µm (Mayo et al., 2019), and 72 hours to process a 33% of a sample for a detection size of 4µm (Wolff et al., 2019). Similar to FTIR, detection of microplastics using Raman spectroscopy can also be limited by signal interferences. High signal to noise ratios can limit the identification of some plastics (Mayo et al., 2019). Additionally, surface contaminants lowered confidence in the positive identification of microplastics, and black fibers that absorb light tended to be difficult to detect (Ben-David et al., 2021). Despite its accuracy and low detection limits, Raman spectroscopy is disadvantaged by it's length processing time which can lead to poor sub-sampling representation and higher analysis costs. Given these limitations, FTIR is preferable over Raman spectroscopy for the characterization of microplastics, but both are still limited as to their ability to characterize particles based on mass.

Microplastic composition was most commonly determined using infrared spectroscopic methods in the reviewed literature, but they can also be found using thermal spectroscopic techniques. These are typically prepared to run on gas chromatography-mass spectrometer (GC-MS) units, using thermal extraction and desorption (TED-GC-MS) (Bannick et al., 2019), and are capable of providing information about microplastic mass in addition to composition. Thermal methods are capable of providing information about microplastic composition as well as mass (Bannick et al., 2019; Funck et al., 2021), a parameter that could not be confidently estimated in infrared spectroscopic methods. The primary benefit of using TED-GC-MS is that the oxidation and separation of non-plastic particles from plastic ones was not necessary, eliminating lengthy sample processing procedures. However, thermal spectroscopic methods do not provide information on particle counts, sizes, types, and colour and are destructive (P. Kang et al., 2020), which means that particles cannot be retained for further analysis. This is a major limitation to widescale application of GC-MS for microplastic in water analysis, and its usage is ultimately dependent on the goals of a research study. Thermal analysis is therefore not recommended for a monitoring protocol, where microscopic and infrared spectroscopy can be used to characterize particles more completely and can be used to make estimates on particle masses.

3.4.3 Alternative and Upcoming Analytical Methods

The use of microscope and FTIR and Raman spectroscopy dominate in recent research on microplastics in wastewater, but other methods for the microplastics quantification and characterization have been proposed in the research community. There are two methods in particular that warrant discussion and further research, including newly developed infrared spectroscopy technology and correlations to other monitored wastewater parameters. The research for these methods is limited for the detection of microplastics in wastewater, but they may be good approaches for future studies into suitable microplastic monitoring methods.

Laser direct infrared imaging (LDIR) is a newly proposed method for detecting microplastic particles that is similar to the other infrared technologies (Robey, 2019, n.d.). This technology is optimized to generate more concentrated microplastic signals in less time than FTIR and Raman spectroscopy. Where FTIR took 11 hours to detect a set of particles, LDIR was able to detect the same particles in approximately two hours. LDIR detection limits are also comparable to that of FTIR and Raman spectroscopy, which is 20μm used automated methods and can be as low as 1.5μm when paired with ATR. Due to its similarity to other infrared methods, established microplastic in wastewater processing methods are expected to be appropriate for this analysis. However, LDIR was not applied in any of the reviewed studies and applications to studying microplastics in wastewater have only been presented by the manufacturer. The description of the LDIR analytical technique sounds promising, but experimental results on wastewater studies will be needed to understand the limitations and challenges of this instrument before it can be recommended for standardized microplastic monitoring methodologies.

The correlation of microplastic particle occurrence to total suspended solids could also provide estimates on microplastic concentration without ongoing sampling and analysis routines. Two research groups found a linear relationship of microplastic content to suspended solids in wastewater (Long et al., 2019; Ziajahromi et al., 2021). Long et al. (2019) suggested that, given a measured ratio of microplastics to suspended solids content, microplastics could be estimated by simply applying a correlation factor to suspended solids measurements, which are easier and faster to obtain than microplastics concentrations. This could be a sufficient technique for monitoring microplastic concentrations in wastewater streams, but it would not be able to fully characterize the microplastics. Additionally, accurate microplastic testing methodologies would still be needed to find the relationship between microplastics and suspended solids through different times of the day and year (Ziajahromi et al., 2021). These correlation studies could be beneficial by providing information on microplastic particles that may be too small to practicably sample and analyse regularly (< 20μm). Further studies are required to determine how well microplastics and suspended solids content correlate in different wastewater treatment plants and through different time scales, but it may be a good way to conservatively estimate microplastics in wastewater in the future.

3.4.4 Microplastic Characterization and Detection Limits

Microplastics in wastewater are characterized to better understand their sources and their occurrence and fate in wastewater treatment plants. In the reviewed literature they have been characterized on the basis of quantities, concentrations, sizes, types or shapes, polymeric compositions, colour, and sometimes mass. Different analytical techniques are needed to capture all of these characteristics, but generally visual microscopic analysis and infrared spectroscopic

methods can fulfill these data needs. Microplastic sizes, types/shapes, and colour can be determined through microscopic analysis as well as an estimate of quantity, and infrared spectroscopic methods can be used to find polymeric compositions and verify the quantity of microplastics. Thermal methods are needed to accurately quantify microplastic mass, but it can also be conservatively estimated using conversion formulas. This section will discuss the characterization parameters and how they apply to monitoring of microplastics in wastewater.

Microplastic colour was reported in several studies as it could indicate the source and potential environmental risk of the identified particles, and was easily obtained through visual analysis. Particle colour could be used to make corrections to sample results based on colour of contaminant microparticles detected in control blank standards (Gies et al., 2018). The colour of the particles can also indicate whether they pose a risk to aquatic species in receiving waters, where blue, grey, and white particles could resemble plankton and be mistaken as food sources (Bayo et al., 2021). However, it is not clear from the reviewed research if fully automated microscopic methods can accurately detect colour, especially if fluorescent stains are used. Ultimately, the significance of colour is not well justified without evidence of increased harm to aquatic species, and it would not be recommended to alter methods to measure colour unless it is found to have significant contribution to ecological fate.

Particle type or shape can indicate the source of plastic and is typically classified into multiple categories. Microplastics were frequently differentiated as fibers and other particles, where other particles could be further categorized as pellets, beads, fragments, films, foams, granules, glitter, and many other shapes (Bayo et al., 2021; Gies et al., 2018; Raju et al., 2020). It is recommended that microplastics be categorized by five standardized types that describe the source of the plastic (Miller et al., 2021; *Zooming in on the Five Types of Microplastics*, 2016). This includes:

- Fibers, which describe particles released from textiles and cigarettes
- Spheres/pellets, which describe microbeads and nurdles
- Foams, which describe Styrofoam and other similar texture plastics
- Films, which describe thin sheets such as from bags or wrapping products
- Fragments, which describe any irregular shaped particle or secondary plastic

Microplastic types were measured by visual or infrared-microscopic methods, and were important for researchers to understand the source of plastics and which ones were effectively removed in wastewater treatment. For instance, microfibers were frequently reported as making up the majority of microplastics detected in wastewater, suggesting that clothes washing was a significant source of microplastics to WWTPs (Ben-David et al., 2021; Petroody et al., 2020; Raju et al., 2020). Particle type is an important characteristic for determining fate of

microplastics in wastewater treatment plants and therefore should be included as a standardized set of classifications.

Quantities of microplastics were almost always reported in the reviewed literature as this value describes the degree of contamination in wastewater and is the basis for estimating WWTP removal efficiency. Microplastics could be quantified manually or automatically using microscopic techniques. Generally, particles identified as suspected plastics in visual analysis would need to be positively verified using spectroscopic analysis before reporting a total number of microplastics detected. Therefore, quantification of microplastics requires spectroscopic measurements at minimum to validate the results. However, given that spectroscopic methods have been reported to be slow (see section 3.4.2), many researchers found it beneficial to only characterize a sub-sample of suspected microplastics and apply the findings to the whole sample. A randomized sub-sample of the visually pre-selected microparticles should be performed in a way that minimizes biases due to location on the filter or human preferences (an example is in Figure 11). Long et al. (2019) developed such a method, where sample filters were analysed based on 20 randomly set circles and then further subdivided by selecting only 24-48 particles from these circles using two personnel to minimize preferential bias. Mathematical operators were then used to estimate the number of microplastic particles on the filter from verified plastic counts in the sub-samples. Quantities and concentrations of microplastics in wastewater can be found using only visual analysis, but to mitigate bias due to human or automated detection error, the polymeric composition of a whole or partial sample must be found to validate the results.

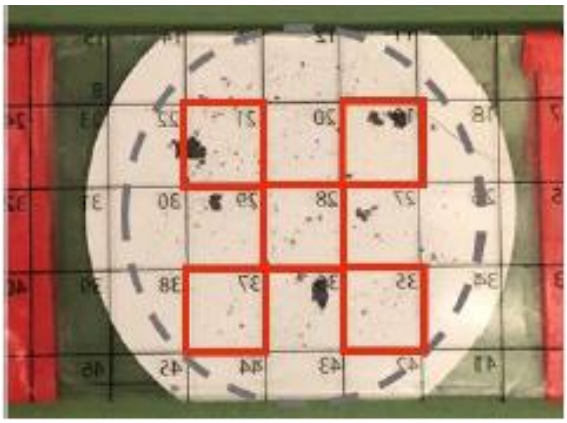

Figure 11. Subsampling area for Nile Red Microscopic Analysis (reproduced from Mayo et al., 2019)

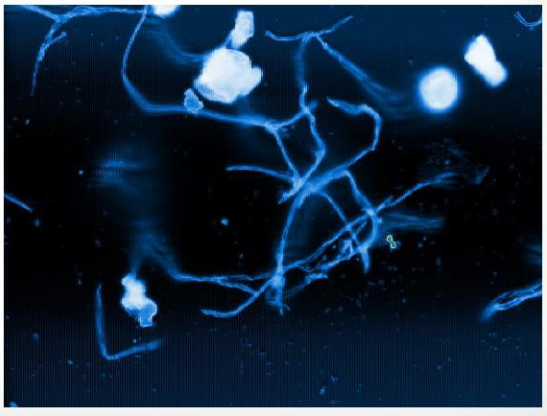

Figure 12. Visual Representation of cellulose fibers and plastic fibers (reproduced from Cook and Allen, 2020)

Polymeric composition of microplastic particles can only be accurately estimated using spectroscopic means and requires the use of infrared or thermal analysis to determine. This parameter is used to further characterize the microplastic contamination sources and is necessary

for providing accurate numerical estimates of microplastics in wastewater. In one study, researchers noted that toilet paper fibers could be misidentified as plastic fibers in visual analysis due to their similarity in appearance (see Figure 12), and could only be confirmed non-plastic through polymer identification methods (Cook & Allen, 2020). In the reviewed literature, the median ratio of particles positively identified as plastic from a sub-sample of suspected microplastic particles was 76.3%, thus indicating the need for spectral confirmation. Polymeric compositions are typically determined using analytical software and spectral library databases, but there are limitations to spectral identification as discussed in section 3.4.2. It would be necessary for spectral analysis software and libraries to include weathered and impure plastics to improve detection accuracy for wastewater matrices. Polymeric composition can only be measured through spectroscopic methods, and because it acts as a necessary verification technique for finding microplastic quantities it should be included as a characterization parameter in a standardized method.

Microplastic sizes are important for understanding the fate of plastics through a WWTP and can be classified as to their precise sizes or by size fractions. Precise size can be estimated using microscopic or imaging techniques, whereas size fractions, which describe particles in a range of sizes, can be sampled and individually analysed by microscopic, infrared, or thermal methods. A number of researchers were interested in what microplastic size fractions were most prevalent in wastewater samples, where many studies concluded that the smallest size fraction, ranging from 0.45-355µm, contained the most number of microplastic particles (Ben-David et al., 2021; Fortin et al., 2019; Petroody et al., 2020; Raju et al., 2020). There were some exceptions where particles were mostly detected in middle size ranges (i.e. 0.5 – 2 mm), but this could happen in studies that employed manual particle sorting prior to spectroscopic analysis where human ability to detect smaller microparticles could be limited (Rasmussen et al., 2021). These findings are significant because they mean that the microplastic estimates for many studies may be biased low due to their lowest detectable size fraction (Ben-David et al., 2021; Fortin et al., 2019).

The smallest detectable particle may be limited by the pore size of the sieve or filter used to collect and process samples, or it could be restricted by the resolutions of the analytical methods used. The relationship between reported microplastic in wastewater concentrations and particle sizes captured and analysed in the reviewed literature was evaluated in Figure 13. The analytical detection limits were assumed to be the smallest particle size reported unless otherwise stated in the literature. There were no observable trends for the lowest detection limit as relating to the analytical method, but a concrete conclusion cannot be made on this relationship when only a few authors reported their analytical detection levels. However, it did appear that higher concentrations of influent microplastics coincided with smaller particle capture sizes. Therefore,

there may be a relationship between the reported microplastic concentration and the particle size collected, where studies that could detect smaller particles tended to report higher concentrations of microplastics. The results of cross-study wastewater microplastic concentrations cannot be appropriately compared because different researchers studied different size fractions, meaning their results are not equivalently measured. It is therefore important that a standardized method includes common size fraction measurements and a minimum particle size detection limit. Where very small detection limits may be too difficult to achieve for every study, standard microplastic size fractions can assist in comparability of results by providing data on common subsets of particle sizes. Ideally the detection limit is as small as possible, but it is dependent on filtration and instrumental capabilities. Ultimately, a standardized method should include microplastic size measurements and fractions to facilitate cross-study comparisons of results and to establish detection limits to illustrate the smallest filterable and detectable particle size.

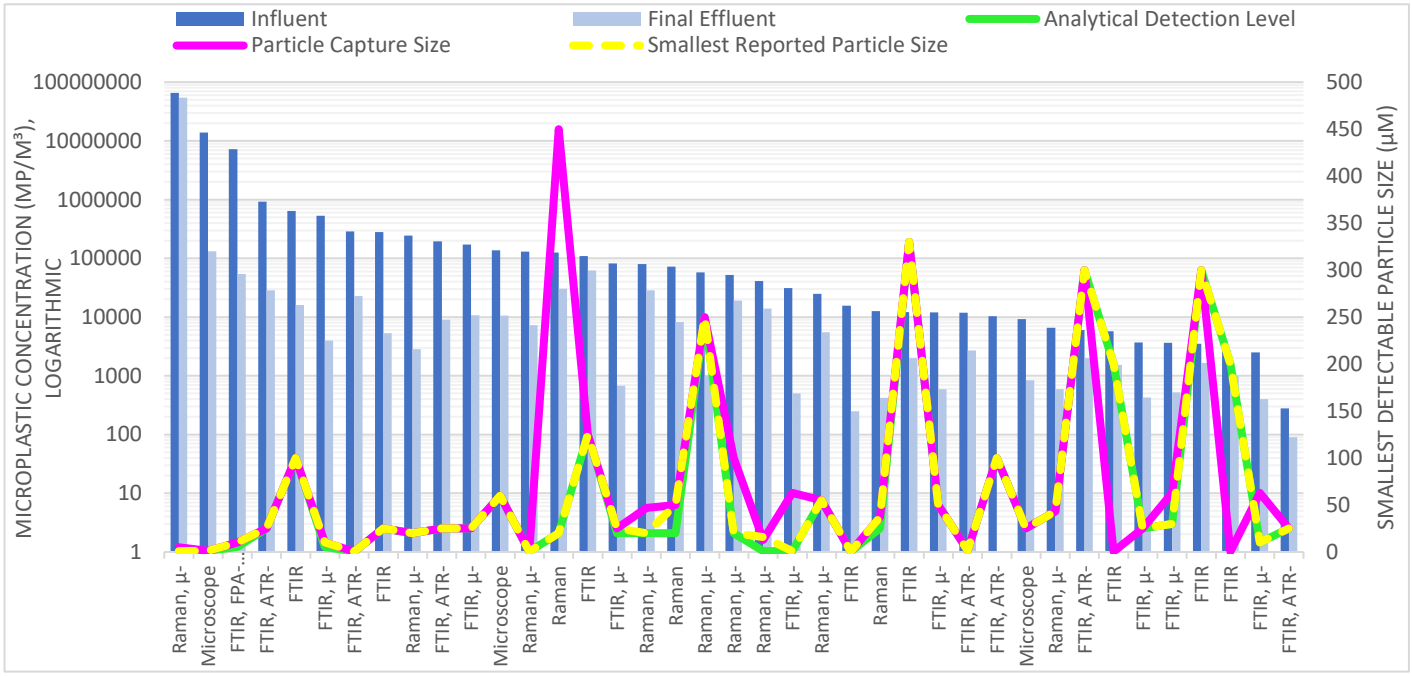

Figure 13. Reported Microplastic in Wastewater Concentrations as Relating to Particle Capture Sizes and Analytical Detection Limits

The primary data output for most of the reviewed studies was the microplastic concentration in wastewater, which describes the degree of contamination entering and exiting the WWTP. This was typically reported in microplastic units per liter (MP/L) based on quantification results, but a small number of researchers also reported their results in mass units per liter (mg/L or ug/L). As discussed in section 3.1.2, microplastic mass is an appealing characteristic for evaluating WWTP efficiency because it is base conserved. However it can only be accurately determined using

destructive thermal analysis or by finding weight on a balance, for which only particles larger than 500µm can reasonably be weighed (Rasmussen et al., 2021). Alternatively, microplastic mass can be estimated from microscopic and infrared spectroscopic analysis using polymeric composition and particle dimensions. Simon et al. (2018) estimated microplastic mass from polymer density and size under the assumption that all particles were oval or cylindrical in shape and that particle thickness was equal to the ratio of the particle dimensions. However, the authors did not validate their method with known mass values and therefore it cannot be known how accurate it is. Understanding the mass of particles entering and leaving WWTPs would be valuable information, but further researcher should be conducted in estimating mass from non-destructive analytical methods before using it in a standardized method.

3.4.5 Analytical Methods Evaluation and Decision Matrix

A suitable analytical method for standardized microplastic in wastewater monitoring should be able to reliably characterize microplastic particles down to the smallest sampled particle size in a timely and cost-effective manner. Currently there is no ASTM standard practice for the analysis of microplastics in wastewater, but the quality and feasibility of various analytical methods are well tracked in the reviewed literature and they were evaluated in a decision matrix (Table 7, Appendix I). Microscopic analysis is simple and cheap, but it is prone to human error in detection and identification of plastic particles. The use of fluorescence microscopy on Nile Red stained samples allows for automation and rapid detection of plastic particles, but cannot fully characterize microplastics as to their polymeric composition. Infrared spectroscopy methods can determine polymeric composition of microplastics, but are time-consuming and costly. Raman spectroscopy can detect very small microplastics, but cannot analyse much of the sample in a reasonable amount of time. FTIR can analyse a representative portion of sample within a couple of hours, but cannot resolve down to the detection levels that Raman spectroscopy can. Based on this evaluation, it was determined that the most appropriate analytical method for characterizing microplastics in wastewater is one that uses Nile Red staining and automated microscopic analysis to detect and characterize microplastic particles as to their size and shape, followed by FTIR analysis to identify the polymeric composition of the suspected microplastic particles.

4 – Recommended Harmonized Standard Sampling and Testing Methodology for the Determination of Microplastics in Wastewater

The microplastic in wastewater testing methods in the reviewed literature were analysed and critically appraised for their ability to generate reliable, representative, and reproducible results. Based on the results of a decision matrix for desirable sampling, sample processing, sample analysis, and quality assurance/quality control methods, the most optimal procedures were recommended for each of these categories to apply towards a proposed harmonized microplastic in wastewater testing method. It was determined that the existing ASTM standard methods for sample collection and processing (ASTM 2020a, 2020b) may not be feasible in a wastewater monitoring application due to very large sample collection volumes, personnel time commitments, and costly reagents. Given that the ASTM methods were developed for a variety of water matrices that may not experience the same sample collection and processing difficulties that high organic solids wastewaters do, it is recommended that a method for microplastic in wastewater testing be prepared specifically for wastewater matrices.

The proposed harmonized standard testing method for microplastics in wastewater (see Appendix II) was developed using successful applications of sampling, sample processing, and sample analysis procedures as described in the reviewed literature. A 24-hour medium to large volume composite sample as described by Petroody et al. (2020) was recommended for influent and effluent sampling, as sieved through multiple sized meshes down to a minimum mesh size of 20µm as described in the existing ASTM (2020a) standard sampling method. The Fenton's reagent catalyzed oxidation reaction as used by Lv et al. (2019) was recommended for sample processing, followed by a Nile Red staining procedure described by Mayo et al. (2019) to prepare the sample for automated microscopic analysis of particle quantity, shape, and size. A standard and randomized sub-sampling procedure described by Long et al. (2019) was recommended to analyse a representative sample portion by FTIR spectroscopy (Mayo et al., 2019) to gather information on polymeric composition of particles. Finally, a set of quality control samples including field and procedural blanks described by Blair et al., 2019, and a reference standard described by Mayo et al. (2019), was recommended to be collected and analysed with each iteration of the testing procedure. The success of these testing methods has been demonstrated in the reviewed literature, and they are recommended towards a standardized testing method for microplastics in wastewater given their time and cost effectiveness and practical feasibility.

5 – Conclusion and Recommendations

The results and methods applied in the systematically reviewed literature confirm what has been expressed in previous review studies and research – that a standardized method for microplastic in wastewater sampling, processing, and analysis is needed to properly compare results across studies. Based on the concerns and limitations discovered in the reviewed research, the existing ASTM standard test methods may not be practical or feasible in microplastic in wastewater monitoring applications. Very high sampling volumes proved too difficult to collect for most researchers, where presence of high amounts of organic solids led to rapid clogging of sampling sieves. Additionally, the application of multiple oxidation and digestion procedures for sample processing was time and materials intensive, and enzyme reagents in particular were expensive to obtain and often required lengthy digestion periods. There is no ASTM standard practice for the analysis of microplastics in wastewater, but it was clear from the reviewed literature that different analytical techniques were subject to different biases and that there was no standard classification system for particle types and size fractions. Given this assessment of standardized and non-standardized methods, there is a need for a standardized methods for sampling, processing, and analysis of microplastics in wastewater as well as standard reporting parameters.

Although microplastic in wastewater methods were variable across the reviewed literature, the different applications of these testing methods provided information on what was effective, practicable, and feasible. From the results of this systematic review of research from 2018 to the present, a harmonized method that meets the needs of a microplastic in wastewater monitoring protocol was prepared. It is recommended that an inter-laboratory study on microplastics in WWTPs in different regions be conducted using this harmonized method to determine how suitable the methods are in application. Additionally, as more research is published on the ecological impacts of microplastics it may be necessary to modify the procedure to ensure efficiency and detectability of higher risk microplastic particles.

In conclusion, testing methods that do not require excessive resources but still provide reliable, representative, and high-quality results should be applied in wastewater microplastic monitoring. The harmonized method prepared in this project is expected to achieve those needs using Fenton’s Reaction to prepare pre-sieved composite samples for analysis using Nile Red stain with fluorescent microscopy and FTIR spectroscopy. It is ultimately recommended that the existing ASTM microplastic in water standard practices be modified to provide more feasible wastewater sampling and sample processing methods in addition to the inclusion of standardized analysis methods and reporting parameters.

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Appendix I - Tables

Table 1. Reported Influent and Effluent Microplastic Particle Count Concentrations in Reviewed Literature

Source	***Publication Date***	***Location***	***Influent (MP/L)***	***Final Effluent (MP/L)***
Alvim et al.	2020	Valencia, Spain	N/A	0.44
Bayo et al.	2020a	Cartegena, Spain	15.7	0.25
Bayo et al.	2020b	Murcia, Spain (average*)	5.71	1.54
Bayo et al.	2021	Murcia, Spain	2.74	0.98
Ben-David et al.	2021	Karmiel, Israel	130	7.3
Blair et al.	2019	Scotland (average)	6	2
Cao et al.	2020	Hong Kong, China	10.36	N/A
Conley et al.	2019	South Carolina, USA (average)	136.5	10.65
Ding et.al.	2020	Beijing, China	109	62
Edo et al.	2020	Madrid, Spain	N/A	10.7
Fortin et al.	2019	Virginia, USA	65,953	54,750
Franco et al.	2021	Cadiz, Spain	645	16
Gies et al.	2018	Vancouver, Canada	31.1	0.5
Grbic et al.	2020	Toronto, Canada (average)	N/A	13.3
Gundogdu et al.	2018	Turkey (average)	24.9995	5.555
Hidayaturrahman and Lee	2019	South Korea (average)	13,813	132
Hongsparith et al.	2020	Bangkok, Thailand (average)	12.2	2
Jiang et al.	2020	Harbin, China	126	30.6
Kazour et al.	2019	Le Havre, France	244	2.84
Lares et al.	2018	Mikkeli, Finland	57.6	1.0
Liu et al.	2019	Wuhan City, China	79.9	28.4
Long et al.	2019	Xiamen, China - (average)	6.55	0.59
Lv et. al	2019	Eastern China (average)	0.28	0.09
Magni et al.	2019	Northern Italy	2.5	0.4
Mayo et al.	2019	Kansas - average (average)	281	5.3
Naji et al.	2021	Iran (average)	N/A	2.019
Petroody et al.	2020	Sari, Iran	12.667	0.423
Pittura et.al.	2021	Central Italy	3.64	0.52
Ragoobur et al.	2021	Mauritius (average)	N/A	237
Raju et al.	2020	NSW, Australia	11.8	2.7
Sierra et al.	2020	Montevideo, Uruguay	N/A	6.75
Simon et al.	2018	Denmark (average)	7216	54
Simon et al.	2019	Denmark	N/A	3
Tagg et al.	2020	East Midlands, United Kingdom	N/A	1.5
Takdastan et al.	2021	Iran	9.2	0.84
Tang et al.	2020	Wuhan City, China (average)	51.9	19.1
Vardar et al.	2021	Istanbul, Turkey	72.6	8.2
Wang et al.	2020	Changzhou, China (average)	41	14
Wolff et al.	2018	Germany	N/A	4.6
Wolff et al.	2021	Germany (average)	N/A	0.011
Xu, X et al.	2019	Changzhou, China (average)	196	9.04
Xu, X et al.	2021	Changzhou, China (average)	928.9	28.48
Xu, Z et al.	2020	China	725	600
Yang et al.	2019	Beijing, China	12.03	0.59
Yang et al.	2021	Xi'an China	288.5	22.9
Yano et al.	2021	South Korea	3.52	1.65
Zhang et al.	2021	Guilin City, China (average)	3.71	0.43
Ziajahromi et al.	2021	Australia (average)	82	0.68
Median			**46.45**	**3**

*Results have been averaged across multiple WWTPs or for a range of reported values.

Table 3. Microplastic in Wastewater Sampling Methods Decision Matrix

Decision Matrix			Criteria							Total
			Sampling							
Method			representation	reproducibility	feasibility	quality assurance and quality control	time	cost	automation	
Sampling	Type	Grab	0	1	2	2	1	2	1	1.29
		Composite	2	2	2	2	1	1	2	1.71
	Pre-Sieving	None	1	0	0	2	2	2	2	1.29
		lower sieve size <20um	2	2	1	1	1	1	1	1.29
		lower sieve size ≥20um	1	2	2	1	2	1	1	1.43
	Volume	Low (1L - 10L)	0	0	1	1	2	2	2	1.14
		Medium (10-100L)	1	1	1	1	1	1	2	1.14
		High (100-1000L)	2	2	1	1	1	1	2	1.43
		Very High (1000-10,000L)	2	2	0	2	0	1	2	1.29

Table 4. Oxidation Digestion Procedures and the Concentration, Temperature, and Time Parameters Applied in the Reviewed Literature

Source	Digestion Procedure	Peroxide Concentration (%)	Reaction Temperature (ºC)	Reaction Time (hours)
Ben-David et al. (2021)	Fenton's Reaction	30	70	N/A
Franco et al. (2020)		30	75	0.5
Franco et al. (2021)		30	75	0.5
Gundogdu et al. (2018)		30	75	N/A
Hongsparith et al. (2020)		30	75	N/A
Jiang et al. (2020)		30	75	1
H. Kang et al. (2020)		35	24-25	72
Liu et al. (2019)		30	N/A	12
Long et al. (2019)		30	60-70	N/A
Lv et al. (2019)		30	60	3
Mayo et al. (2019)		30	70	N/A
Nguyen et al. (2021)		30	50	0.5
Raju et al. (2020)		30	Room Temp.	24
Sierra et al. (2020)		30	N/A	0.5
Yang et al. (2019)		30	60	0.5
Yano et al. (2021)		30	65	N/A
Zhang et al. (2021)		30	N/A	N/A
Alvim et al. (2020)	Peroxide Oxidation	35	60	2
Blair et al. (2019)		30	N/A	72
Cao et al. (2020)		30	60	24
Edo et al. (2020)		33	50	24
Fortin et al. (2019)		30	50	72
Gies et al. (2018)		30	N/A	168
Hidayaturrhman and Lee (2019)		30	N/A	N/A
Magni et al. (2019)		15	N/A	72
Petroody et al. (2020)		30	N/A	N/A
Pittura et al. (2021)		15	50	<24
Ragoobur et al. (2021)		30	Room Temp.	>168
Tagg et al. (2020)		30	N/A	168
Takdastan et al. (2021)		30	80	N/A
Tang et al. (2020)		30	N/A	24
Xu et al. (2019)		30	60	48
Xu et al. (2021)		30	60	48
Yang et al. (2021)		30	65	12
Ziajahromi et al. (2021)		30	60	24

Table 6. Sample Processing Methods Decision Matrix

Decision Matrix			Criteria							Total
			Processing							
Method			sample recovery	reproducibility	feasibility	quality assurance and quality control	time	cost	environmental impact	
Processing	Sample Digestion	acid/alkaline	0	1	1	1	2	2	1	1.14
		peroxide oxidation	1	1	1	1	1	2	1	1.14
		Fenton's reaction	1	1	2	1	2	1	1	1.29
		enzymatic digestion	2	1	1	2	1	0	2	1.29
		oxidation and enzymatic digestion	1	1	1	2	2	0	1	1.14
	Extraction	None	2	2	1	1	1	2	2	1.57
		Low density separation (i.e. NaCl)	1	1	1	2	1	2	2	1.43
		High Density separation (i.e. NaI)	2	1	1	2	1	1	1	1.29
		Filtration only	1	2	2	2	2	1	1	1.57
	Application	By wastewater stream	2	0	1	0	2	2	N/A	1.17
		Same for all samples	1	2	1	1	1	1	N/A	1.17

Table 7. Sample Analysis and Reporting Methods Decision Matrix

Decision Matrix			Criteria							Total
			Analysis							
Method			reliability	representativeness	detection limit	quality assurance and quality control	time	cost	automation	
Analysis	Quantification Methods	Microscopy	0	2	1	1	1	2	1	1.14
		Nile Red Assisted Microscopy	1	2	1	1	2	2	2	1.57
		Bengal Rose Assisted Microscopy	1	2	1	1	0	2	0	1.00
		Infrared Spectroscopy	1	1	1	2	1	1	1	1.14
	Characterization Methods	Microscopy	0	2	1	1	2	2	0	1.14
		FTIR	2	1	1	2	1	1	1	1.29
		Raman Spectroscopy	2	0	2	2	0	1	2	1.29
		Thermal Spectroscopy	1	0	1	2	1	1	2	1.14
	Reportable Parameters	Colour	2	2	N/A	0	1	2	0	1.17
		Size	1	2	N/A	2	2	2	2	1.83
		Count	1	2	N/A	2	1	2	2	1.67
		Composition	1	2	N/A	2	1	1	2	1.50
		Shape/Type	1	1	N/A	2	1	2	1	1.33
		Mass	1	2	N/A	1	1	1	1	1.17

Appendix II – Proposed Standard Test Method for the Sampling, Processing, Analysis, and Reporting of Microplastics in Wastewater

The following content is a proposed standard microplastic in wastewater sampling, processing, and analysis that was prepared from results of the decision matrix and the most suitable methods as reviewed in the literature using the ASTM Draft Standard Template (ASTM, n.d.). It has been prepared entirely for the purpose of demonstration in this project and will not be officially submitted as a draft standard method to the ASTM organization.

1 - Scope

1.1 This test method is being proposed for standardization of the sampling, processing, analysis, and reporting of microplastics in wastewater influent and effluent streams. The methods proposed have been established in previous literature on the topic of characterizing microplastics in wastewater. This is not an official standard method but is a proposed method to fulfill the project requirements of the British Columbia Institute of Technology EENG 8460 Capstone Project.

1.2 This test method uses only SI units.

1.3 The particle size of microplastics is defined as being less than 5µm in size, but given practical limitations of collecting very small microplastics this microplastic size definition for this standard method will be 20µm to 5000µm.

2 - Summary of Test Method

2.1 This test method is recommended for use on wastewater influent and effluent streams. It involves high volume, 24-hour composite sampling, and pre-sieving of collected wastewater. The solids collected on sample sieves are to be processed under Fenton's Reagent catalyzed oxidation reactions and filtered over small pore size paper or glass fiber filters under vacuum. The processed samples on filters are to be stained with Nile Red dye for automated analysis under microscope, then a sub-fraction of the sample is to be characterized under FTIR. Finally, sample parameters are to be reported in terms of quantity, size, type, and composition.

2.2 This test method is meant to quantify microplastic particles between 20-5000µm in size of their smallest dimension.

3 - Significance and Use

3.1 Wastewater treatment plants have been identified as a primary contributor of microplastics to aquatic environments. The high solids content of wastewater streams can make the collection and analysis of microplastics in samples difficult. Large volumes must be collected to produce representative results, and pre-sieving must be employed to concentrate the sample into a solids only fraction. Small microplastics must be captured to the greatest extent possible, and a maximum sieve mesh size of 20µm should be used to maximize the particle capture sizes. Solid samples must be processed to eliminate interfering organic solids in a timely and efficient manner. Samples should be processed using an oxidation procedure catalyzed by Fenton's reagent, followed by isolation of the un-digested particles on a small pore size filter through vacuum filtration. Extracted samples should be characterized by their quantity, size, type, and composition in the most representative and most efficient ways possible. Nile Red staining should be performed on extracted samples for automated quantification and type and size detection of particles through microscopy, then a 10% sub-sample should be prepared for analysis of polymeric composition and verification of plastic composition.

3.2 Effective quality assurance and quality control techniques should be applied throughout the sampling, processing, and analysis of samples. Equipment and samples should be handled to minimize contamination and sample losses, and control standard must be used to monitor for sample contamination and recovery.

4 - Interferences

4.1 Residual organic and inorganic particles can interfere with microplastic detection and analysis, and therefore effective oxidation reaction is necessary.

4.2 Some natural organic particles may be stained with Nile-Red dye and be mis-identified in microscopic analysis, therefore FTIR spectroscopic analysis of particle composition is necessary to verify the particle as plastic or not.

4.3 Some weathered plastic particles may not be effectively signal matched in FTIR analysis and therefore up-to-date spectral libraries with signals for weathered and contaminated plastics should be used.

5 - Apparatus and Equipment

5.1 Sampling

- Automatic flow-based composite sampler
- Flowmeter

- 6 stacked sieves of the following mesh sizes: 5000µm, 1000µm, 500µm, 100µm, 50µm, 20µm

5.2 Sample Processing

- Beaker
- Watch Glass
- Filtration Apparatus

5.3 Sample Analysis

- Microscope with automated fluorescence detection
- FTIR apparatus with detection down to 20µm particle size and signal matching software and spectral libraries

6 - Reagents and Materials

6.1 Sampling

- Purified water

6.2 Sample Processing

- Purified water
- 30% Hydrogen Peroxide
- Fe (II) catalyst
- ≤ 20µm pore size PTFE filter
- ≤ 20µm glass fiber filter
- 1µg/mL Nile Red dye

6.3 Analysis

- Glass slides for microscopic analysis

7 - Sampling

7.1 Samples are to be collected from wastewater influent and effluent streams using automatic flow-based composite samplers connected to a flow meter capable of recording total volume collected. Influent samples should be taken at a volume of 30L, and effluent samples should be

taken at a volume of 270L (Petroody et al., 2020). Samples should be pre-sieved over a stack of 6 sieves in order of largest mesh size (5000µm) to smallest mesh size (20µm) (ASTM, 2020a).

7.2 Solids on the 20µm to 1000µm mesh size sieves are to be retained for analysis (Ding et al., 2020), but the 5000µm fraction may be discarded. Samples should be transferred to a cool dark container for transport to the laboratory for further processing.

8 - Sample Processing

8.1 Samples and QC on sieves should be transferred to a beaker with 100mL of water, then oxidized in 5-20mL of 30% hydrogen peroxide in the presence of Fe (II) catalyst for three hours at room temperature (Lv et al., 2019).

8.2 Digested samples should first be filtered over a PTFE filter with pore size of 5µm, then covered in Nile Red solution and allowed to stain in the dark for 60 minutes at a temperature of 60°C (Mayo et al., 2019). Finally, samples should be transferred to a second 0.2µm glass fiber filter using purified water.

9 - Analytical Procedure

9.1 The sample filter area should be sub-sectioned so as to analyse only a representative fraction of the filter surface. The filter surface should sub-sectioned into 20 randomly selected circles of the same diameter making up at least 40% of the filter area for microscopy analysis (Long et al., 2019; Mayo et al., 2019). Furthermore, at least 10% of the total estimated microplastic particles in microscopic analysis should be randomly selected for FTIR analysis by randomly selecting identified particles from the 20 circles using two laboratory analysts to minimize selection bias (Long et al., 2019).

9.2 The sub-selected areas of the filter should be analysed under fluorescent microscopy using a Texas Red filter and analytical software to automatically identify and characterize microplastic particles by quantity, shape, and size (Mayo et al., 2019).

9.3 The second sub-selection of particles for FTIR analysis should be transferred to a Barium Fluoride substrate and analysed under FTIR using analytical software and spectral libraries to identify polymeric composition of the selected particles (Mayo et al., 2019). FTIR scans, resolution, and wave range should be optimized for detecting microplastics down to a particle size of 20µm.

10 - Calculation or Interpretation of Results

10.1 Microplastic loading and discharge results should be reported in units of microplastic items per liter (MP/L) for influent and effluent.

10.2 Verified plastic particle counts from FTIR analysis should be applied to the total counts found in microscopy analysis, and the total counts of the sub-sampled filter area found under microscopy should be applied to the whole sample filter to produce total results. Equations 2-4 (reproduced from Long et al., 2019) can be used to apply the findings to the whole sample:

$$N = a + b \quad (2)$$

$$b = c \times \varphi \quad (3)$$

$$c = \left(\sum_{i=1}^{20} n_i\right) \times \left[\pi D^2 / \left(20 \times \pi d^2\right)\right] \quad (4)$$

where, N—the total number of MPs.

a—the number of MPs identified by micro-Raman spectroscopy.
b—the number of MPs estimated by statistical analysis.
c—the number of remaining suspected MPs on the filter paper.
φ—the proportion of confirmed MPs by micro-Raman spectroscopy.
n_i—the number of remaining suspected MPs in each circle.
D—the effective diameter of the filter paper, in this study, $D = 70$ mm.
d—the diameter of the circle, $d = 8$ mm.

10.3 Removal efficiency can be quantified using the findings from section 12.2 by applying them to the Equation 5 (reproduced from Franco et al., 2021)

$$RE = \frac{MP\ concentration\ influent - MP\ concentration\ effluent}{MP\ concentration\ influent} \times 100\% \quad \ldots\ldots(5)$$

10.4 Characterization of microplastic particles should be reported in terms of particle sizes, polymeric composition, and plastic type. Plastic type should be defined by one the following five categorizations: fibers, spheres/pellets, foams, films, and fragments (Miller et al., 2021; Zooming in on the Five Types of Microplastics, 2016)

11 - Precision and Bias

11.1 Results of blank control and reference spike studies should be reported in terms of contamination and reference spike recovery, respectively.

11.2 Results of quality control studies should not be used to correct the sample results due to uncertainties in the variability of contamination and recovery factors (Miller et al., 2021; Simon

et al., 2018). Blank control standard results can be used to qualify the sample results as a level of uncertainty (Miller et al., 2021)

Appendix III – Literature Review Matrix

Bibliographic Information				Abstracting: record relevant characteristics and findings						
Source number / short name	Publication date	Reference list entry (using the style required by your program)	Journal	Research question/aim and rationale	Research design and main methods	Summary of Methods	Key finding(s) and/or claims relating to project	Main conclusion (if any / if different than key finding) relating to project	Keywords	Summarize author comments on validity and generalizability, including significant limitations, threats or weaknesses
Gies et. al.	2018	Gies, E. A., LeNoble, J. L., Noël, M., Etemadifar, A., Bishay, F., Hall, E. R., et al. (2018). Retention of microplastics in a major secondary wastewater treatment plant in Vancouver, Canada. Marine Pollution Bulletin, 133, 553-561. doi:https://doi.org/10.1016/j.marpolbul.2018.06.006	Marine Pollution Bulletin	To characterize microplastics in Vancouver influent, effluent, and sludge (p 554)	To perform sampling of influents, effluents, and sludge followed by MP extraction from samples and analysis by FT-IR to present results in number of MP found in the samples (p 554)	Sampling: Bulk influent sample and 1-mesh sieved effluent samples; Processing done by separating settled solids from liquid sample - MPs in solids extracted via peroxide oxidation and filtration, and MPs in liquid extracted via oil extraction protocol; Analysis: visual pre-selection using microscopy and MP confirmation using FTIR - final results adjusted for recovery and contamination; QA/QC: control spikes of all samples during processing, background blank during processing, procedural blank from sample collection to analysis - corrections to sample results made based on recoveries and contamination of these QC	Methods used had limited losses of MP (81-100% recovery); small bulk influent sample volumes mean that microplastics can be detected to much smaller sizes than pre-sieved samples (1um, based on filter pore size in this study); larger volume samples are still required due to needing to meet the detection limit, and cannot follow the faster OEP protocol	Bulk sampling is superior to sieved sampling due to no loss of MP smaller than smallest sieve mesh size; analyzing settled solids separate from liquids can take advantage of cheaper extraction/digestion methods and reduce exposure to harmful chemicals.	microplastics, plastic, FT-IR, wastewater, ocean, pollution	Two main limitations discussed in this paper: limitation of detection limit for low-solids effluent and limitation of correction factors applied based on QA/QC results. Limitation of detection limit means that effluents must be sampled at higher volumes, meaning they cannot be reasonably extracted using the OEP and must be pre-sieved, opening up issues with particle losses (smaller than sieve pore size) and contamination during sieving. Limitations of correction factors can cause biases in final results - samples correct for contamination in blanks could lead to low biases in reported results, and corrections due to control particle recovery could lead to inaccurate results
Magni et. al.	2019	Magni, S., Binelli, A., Pittura, L., Avio, C. G., Della Torre, C., Parenti, C. C., et al. (2019). The fate of microplastics in an Italian wastewater treatment plant. Science of the Total Environment, 652, 602-610. doi:https://doi.org/10.1016/j.scitotenv.2018.10.269	Science of the Total Environment	To characterize microplastics throughout different steps and wastes in an Italian WWTP (p 603)	Method research design separated into 5 sections: sampling, MP separation, MP characterization, contamination control, and statistical analysis.	Sampling: all streams filtered over 3 sieves ranging from 5mm to 63um in mesh size; Processing: density separation first performed using NaCl solution then filtered over 8um membrane filter and digested in peroxide to remove organics for 3 days; Analysis: visual pre-selection using stereomicroscope followed by confirmation of all pre-selected particles using ATR-FTIR; QA/QC: procedural blanks used throughout entire processing to show some contamination of fibres for an average of 10% the fibre count found in samples.	Despite retention of many MPs in WWTP, a large number are still discharged in effluent and removed in sludge (p.607); differences in MP concentration in different WWTPs across the globe may be attributed to a lack of standardized detection methods used across all the research studies	The main conclusion relating to the methods is that significant MP is still released to the environment despite good retention by the WWTP (pp 608-609)	microplastics, wastewater treatment plants, effluents, sewage sludge, treatment efficiency	The author does not give much discussion on method limitations or validity. They do comment that their contamination control was successful by finding background fibres in an amount of 10% of the average fibre count in samples. There is no discussion on potential biases in the sampling or processing methods, but the authors acknowledge a potential low bias in visual pre-selection of MPs due to human limitations for finding very small particles (10-30um)
Lv (et. al.)	2019	Lv, X., Dong, Q., Zuo, Z., Liu, Y., Huang, X., & Wu, W. (2019). Microplastics in a municipal wastewater treatment plant: Fate, dynamic distribution, removal efficiencies, and control strategies. Journal of Cleaner Production, 225, 579-586. doi:https://doi.org/10.1016/j.jclepro.2019.03.321	Journal of Cleaner Production	To perform research needed for establishing strategies to manage microplastics in WWTPs	Experimental design - methods include sampling, sample processing, and sample characterization	Sampling: samples taken and directly sieved over a series of sieves in a contained vessel; Processing: samples first oxidised with peroxide solution then microplastics separated using density separation; analysis: MP particles pre-selected by visual means and stereoisotope, then confirmed using ATR-FTIR to find concentration of MP in number MP/L and in mg/L; QA/QC: negative control was used in the processing phase to assess for potential contamination	The processing method in this method would appear to have excellent contamination control given a negative return on the procedural blanks for microplastics (p 581); mechanical stirring may cause higher MP counts after treatment due to effect of breaking existing MP particles down into several smaller ones (p 584); MP removal efficiency measured by mass is not consistent with removal efficiency measured by MP number - should report both (p 585)	The main conclusions relate to MP characteristics in this particular WWTP and what removal and management methods are most effective, which do not relate to the research goals of my project	microplastics, removal, wastewater treatment plant, oxidation ditch, membrane bioreactor	The only limitation the authors address are the validity of the results with respect to how they represent average wastewater conditions - the authors acknowledge that the one-time sample does not represent wastewater conditions throughout the day or year but justify the results as still being within the expected magnitude of average conditions. (p 585)
Simon et. al.	2018	Simon, M., van Alst, N., & Vollertsen, J. (2018). Quantification of microplastic mass and removal rates at wastewater treatment plants applying focal plane array (FPA)-based Fourier transform infrared (FT-IR) imaging. *Water Research (Oxford), 142*, 1-9.	Water Research	To develop a method for reporting microplastic contamination in WWTPs in units of mass and particles, and to characterize microplastic occurrence in Danish WWTPs (p 2)	experimental design - methods include sampling, sample processing, sample analysis, and sample mass estimates	Sampling: influent samples taken as 24hr composites and effluent samples collected on sieves; Processing: surfactants utilized to separate particles in solution prior to further processing, then enzyme digestion and peroxide oxidation were utilized to decompose organic components; analysis: FPA-based FTIR used to quantify total MP and to make mass estimates; QA/QC: negative controls and spike controls were used to estimate contamination and recovery, respectively, and carried through the entire processing phase.	Final results of this study were much higher than peer studies, which could be due to smaller MP size inclusion and/or processing and detection methods used in this study that may contribute to better resolution or may break MPs into multiple pieces (p 4); Masses can only be roughly estimated using FPA-based FTIR given the assumption and uncertainties surrounding measurements (pp 5, 8)	Reporting microplastics in mass units is favourable over particle count because the value is conserved throughout the process and therefore is expected to be more comparable to other research and methods using alternative sampling, processing, and analysis techniques (even if FTIR isn't the best method for determining mass) (p 8)	microplastic mass quantification, wastewater treatment plants, FT-IR spectroscopy	The author goes into great detail on validity of results and limitations, weaknesses, and advantages of the methods applied throughout the whole article. The author specifies all assumptions and uncertainties relating to the MP mass estimates (pp 3,5). The author also breaks down the QC results to explain potential reasons for and concerns with contamination and extraction recoveries, as well as much higher final results when compared to peer studies (pp 3-4). These relate to limitations of detection limits (contamination), choosing to not use a correction error for extraction recovery (due to limited diversity of reference particles), and procedural reasons for why this studies results are so much higher than others (including mechanical stressors leading to MP fragmentation and increased numbers, use of higher resolution detection equipment, and inclusion of smaller MP particles than in other studies)
Hidayaturrahman and Lee	2019	Hidayaturrahman, H., & Lee, T. (2019). A study on characteristics of microplastic in wastewater of south Korea: Identification, quantification, and fate of microplastics during treatment process. Marine Pollution Bulletin, 146, 696-702. doi:https://doi.org/10.1016/j.marpolbul.2019.06.071	Marine Pollution Bulletin	To determine the MP removal effectiveness of different degrees of WWTP treatment and treatment technologies (p. 696 - abstract)	experimental design - methods include sampling, sample processing, and sample analysis	Sampling - researchers took 5 samples of wastewater from each treatment stage using grab methods and stored at lowered temperatures; Processing: researchers directly filtered samples over 1.2um pore size filters, then further treated the higher organic solids samples (influent, primary and secondary effluents) with peroxide oxidation on the filter; Analysis: samples were visually detected using a microscope and analysis software. (p 697)	The key findings in this article relate to MP characterization for different WWTP's and treatment types, which to not relate to my project. The researchers find that most MP are removed by the time secondary effluent is discharged, and found that microbeads make up the highest proportion of MP in wastewater. Authors also found that coagulant could significantly reduce MP from secondary effluent, but is inhibited when too much coagulant is added. (p 701)	Tertiary WWTPs with coagulation significantly reduce MP in wastewater (>98%), but sheer volume of wastewater passing through the plant means that large amounts of MP are still discharged as pollutants to the water environment (p 701)	microplastics, wastewater treatment plant, coagulation, ozone, membrane disc-filter, rapid sand filtration	The author does not discuss validity of results with respect to the methodology used to find the results in this article.
Liu et. al.	2019	Liu, X., Yuan, W., Di, M., Li, Z., & Wang, J. (2019). Transfer and fate of microplastics during the conventional activated sludge process in one wastewater treatment plant of China. Chemical Engineering Journal, 362, 176-182. doi:https://doi.org/10.1016/j.cej.2019.01.033	Chemical Engineering Journal	To characterize behaviour of microplastics through the activated sludge process of a WWTP in China	experimental design - main methods include sampling, sample processing, and MP detection and characterization	Sampling - researchers took 4 samples from each treatment stage using grab and pre-sieving to only process solids retained on the sieve; Processing - processing varied slightly depending on solids content of wastewater, where lower solid wastewater (secondary and disinfected secondary effluents) were oxidized then filtered, and higher solids wastewater (influent and primary effluent) were oxidized with Fenton's reagent to catalyze the reaction and then separated by liquid and solid layer - both layers were filtered, but the solid layer was first treated to density separation using saturated NaCl solution. (p 177)	No key findings regarding the experimental methods used in this study - all findings relate to treatment processes. These include the finding that most MP transfers out of wastewater into wastewater sludge, and that secondary treatment is effective in removing 64.4% of MP particles (pp 181-182)	Wastewater secondary treatment removes significant MP from wastewater prior to discharge but still releases enough MP to have a potential negative impact on aquatic ecosystems (pp 181-182)	microplastics, wastewater, activated sludge, transparent, ellipse	The authors do not make any comments on the validity of results with respect to the methods applied or any limitations of the methods used. No apparent control standards were used to track for biases.
Long et. al.	2019	Long, Z., Pan, Z., Wang, W., Ren, J., Yu, X., Lin, L., et al. (2019). Microplastic abundance, characteristics, and removal in wastewater treatment plants in a coastal city of China. Water Research, 155, 255-265. doi:https://doi.org/10.1016/j.watres.2019.02.028	Water Research	To characterize microplastics and removal efficiencies in Xiamen WWTPs and to introduce a new sampling method for finding MP in wastewater (p 256)	experimental design - sampling, sample processing, sample analysis, quality assurance and quality control	Sampling was performed using pumps to take grab samples of wastewater collected over stacked sieves until sieves clogged - total volumes were recorded using cameras to capture start and end times for water flow. Sample processing was conducted the same way on all samples - drying, series of wet peroxidations over Fenton's reagent catalyst, density separation, then filtration separation. Analysis was performed by using visual pre-selection followed by random selection of subset of suspected MP particles on micro-Raman spectroscopy to verify composition of suspect MPs. Quality assurance was performed by minimizing background contamination or cross contamination of equipment and working environment, and quality control was checked using reference spikes carried through all methods and by using background blank controls to check for background contamination during sampling. (pp 256-258)	Results of this study and other research applying other methods to quantify MP in wastewater cannot be adequately compared due to a lack of standard methodology applied across these studies (p 260). There is a correlation between total suspended solids and MP count, therefore opening up an easier and quicker method for estimating MP in wastewater without direct measurements (p 260). There is also a correlation of MP count to manufacturing activity served by the WWTP, where higher MP counts were found where there were higher amounts of plastics related manufacturing (p 261). There is also a relation between operating loads and MP in effluent, where full operating loads tend to have more MP in effluents (p 261)	MP discharges to the environment are dependent on the MP characteristics themselves as well as operating loads of the WWTPs, where overloaded operating loads have lower MP removal efficiencies (p 264)	wastewater treatment plant, microplastic, abundance, characteristics, removal	The authors approach their methodology and results with a good discussion on results validity and limitations of the method. One limitation is the need to subsample visually identified particles for further confirmation due to practical infeasibility of confirming all particles (p 257). The authors evaluate their results with the results of other studies, but concedes that results are not truly comparable due to the application of different methodology across these studies (p 260). Authors acknowledge that issues with the processing of samples at one site may have contributed to inaccurate results in which the processing method may not be appropriate for that matrix and MP concentration may be better estimated by a different method (p 260). The author also discussed results of the spike and blank control tests, which gave favourable results and therefore the authors could conclude that their processing methods and sampling methods did not contribute to significant bias in results (p 258)

Please refer to the attached spreadsheet, *Literature Review Matrix and Collected Data – 2021-12-18*, for the complete matrix.