Effects of treatment on the characterization of organic matter in wastewater: a review on size distribution and structural fractionation

B. Hande Gursoy-Haksevenler and Idil Arslan-Alaton

ABSTRACT

Since it is difficult to analyze the components of organic matter in complex effluent matrices individually, the use of more collective, but at the same time, specific wastewater characterization methods would be more appropriate to evaluate changes in effluent characteristics during wastewater treatment. For this purpose, size distribution and structural (resin) fractionation tools have recently been proposed to categorize wastewater. There are several case studies available in the scientific literature being devoted to the application of these fractionation methods. This paper aimed to review the most relevant studies dealing with the evaluation of changes in wastewater characteristics using size distribution and structural (resin) fractionation tools. According to these studies, sequential filtration-ultrafiltration procedures, as well as XAD resins, are frequently employed for size and structural fractionations, respectively. This review focuses on the most relevant publications including biological treatment processes, as well as chemical treatment methods such as coagulation-flocculation, electrocoagulation, the Fenton's reagent and ozonation. This study aims at providing an insight into the possible treatment mechanisms and details the understanding what structural features of wastewater components enabled or prevented efficient treatment (removal) or targeted pollutants.

Key words | environmental characterization, particle size distribution, structural resin fractionation, treatment, wastewater

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HIGHLIGHTS

- Size distribution and structural fractionation are promising tools to characterize wastewater.
- Understanding removal mechanisms during wastewater treatment.
- Sequential filtration-ultrafiltration procedure for size distribution.
- Resin adsorption with XAD resins for structural fractionation.

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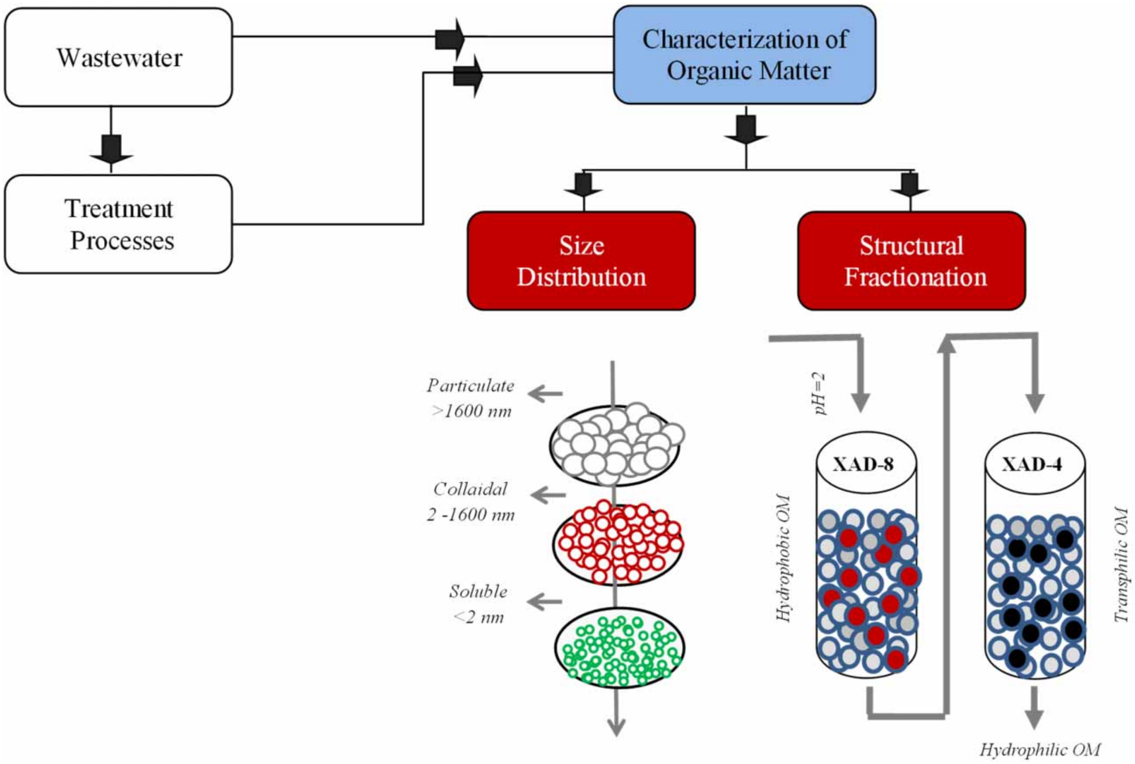
GRAPHICAL ABSTRACT



INTRODUCTION

In many countries around the world, fresh water resources are scarce due to rapid population growth, increasing economic development and extreme weather events caused by climate change. The reuse/reclamation of wastewater is getting more important in order to protect the limited fresh water resources and to protect the receiving environment. Treatment processes have been developed to remove the pollutants in wastewater for elimination of their negative effects on human and environmental health. However, wastewater is of a generally complex nature and may include a wide variety of both organic and inorganic pollutants that affect their treatability. It is known that the organic matter content in wastewater brings about many problems such as treatability, membrane fouling and potential toxic by-products formation during wastewater treatment (Shon *et al.* 2006a). Hence, it is necessary to design, control, and operate a wastewater treatment system for an efficient removal (Tran *et al.* 2015). For wastewater treatment, biological processes (aerobic, anaerobic and fungal treatment), physicochemical methods (flocculation, coagulation, precipitation), advanced oxidation processes (AOPs) (such as Fenton and Photo-Fenton processes), electrochemical treatment processes (involving electrocoagulation) and membrane technologies are well-known treatment alternatives. However, some drawbacks resulting in poor removals due to the characteristics of subjected wastewater have been recorded (Shon *et al.* 2006a, 2006b; Tran *et al.* 2015). For instance, it has been found that the conventional activated sludge is particularly hindered by suspended solids (Tran *et al.* 2015); AOPs are influenced by both concentration of organic matter and its characteristics such as molecular weight (Molnar *et al.* 2013); membrane technologies are negatively affected by fouling, which mostly originates from the hydrophilic and neutral fractions of organic matter (Tang *et al.* 2010).

Since the design, control and operation of a wastewater treatment system mostly depend on the nature of the wastewater, it is essential to characterize the main components of wastewater, especially its organic content for an efficient treatment. There are several methods for organic content characterization of wastewater. For example, it is possible to simply characterize the organic matter content of wastewater by measuring the biochemical oxygen demand (BOD), chemical oxygen demand (COD) and total organic carbon (TOC) parameters. However, within these parameters, BOD has a limited function due to several technical reasons and the fact that TOC does not give any information about the changes in the oxidation state and the rate of organic carbon oxidation (Modin *et al.* 2016). On the other hand, following solely the COD parameter during wastewater treatment is not sufficient for an in-depth evaluation of the treatment and its performance. Neither it is sufficient to identify the type of organic pollutants present in the wastewater, since effluents with similar COD concentrations may have different tendencies for oxidation (Huber *et al.* 2011).

Obviously, it is important to study the characterization of wastewaters in depth in order to explain the impact of different treatment methods on the wastewater's composition/nature (Arimi 2018). Within this scope, the use of sophisticated analytical/instrumental techniques, such as gas chromatography-mass spectrometry (GC-MS) (Rocha *et al.* 2013) and liquid chromatography-mass spectrometry (LC-MS) (Pitarch *et al.* 2007) as well as nuclear magnetic resonance (NMR) (Chen *et al.* 2003) may be other options. However, by using these techniques it is rather difficult to recognize individual components of a wastewater sample due to its complexity (Gursoy-Haksevenler & Arslan-Alaton 2016).

Hence, recently proposed alternative wastewater characterization methods could be more appropriate/feasible in order to examine wastewater characteristics before and after treatment in terms of case-specific but collective parameters. Particle size distribution (PSD) analysis, as an alternative method, appears to be an integral tool for the comprehensive classification of components found in real water and wastewater samples. PSD is based on the distribution of contaminants over specified particle-size ranges between particulate, colloidal and real soluble size fractions. Within recent studies, PSD has been applied to different wastewater types such as slaughterhouse (Sanchis *et al.* 2003), sewage (Wu & He 2010), textile (Dulekgurgen *et al.* 2007) and tannery wastewater (Karahan *et al.* 2008), landfill leachate (Insel *et al.* 2013), pulp and paper mill effluent (Leiviskä *et al.* 2009) as well as several other effluents.

On the other hand, structural (resin) fractionation procedures have also been proposed as a practical tool for classifying the organic matter content of effluents into structurally more specific fractions such as hydrophobics and hydrophilics (Marhaba *et al.* 2003). This approach is based on the serial use of different resin types. However, publications in this area are restricted on structural fractionation applied to freshwater samples (Parsons *et al.* 2004; Kim *et al.* 2006). Within the exception of freshwater, brewery wastewater (Janhom *et al.* 2009) and landfill leachate (Labanowski & Feuillade 2009) were also subjected to resin fractionation.

As is evident in previous related publications, it is difficult to identify all organic compounds present in wastewater. Both PSD analysis and resin fractionation methods allow for the separation of wastewater into pieces according to its size or hydrophilic and hydrophobic affinities. With the implementation of these fractionation methods, besides the clarification of wastewater characterization, it is possible to conclude the questions of how the treatment mechanism proceeds and which part cannot be treated or which part is resistant to treatment. For this reason, PSD analysis and resin fractionation methods seem suitable and applicable for evaluation of the nature of complex wastewater both before and after treatment and understanding wastewater removal mechanisms. To the best of our knowledge, a review devoted to these tools being currently available/that have been specifically developed to examine the effect of treatment methods on the characteristics of organic matter in wastewater is rarely found in the scientific literature. This present review provides a comprehensive overview of scientific work where the effect of treatment processes on organic matter characterization by size and structural fractionation was investigated. Within this concept, the size and structural fractionation studies undertaken with different treatment processes (including biological treatment – aerobic and anaerobic processes as well as chemical treatment – coagulation-flocculation, electrocoagulation, Fenton's reagent and ozonation processes) to evaluate their performance and mechanism are presented. It is aimed to guide future studies related to wastewater treatment and characterization applications.

SIZE DISTRIBUTION

Application of characterization methodologies based on size distribution have increased appreciably in recent years. The methods applied within these studies vary depending on the size of the particles analyzed. In traditional methods, gel filtration is mostly used for small size particles (such as molecules and simple compounds) while sieving is used for large particles. However, both of these methods are defined as time consuming, cumbersome and may require high sample volumes (Arimi 2018). Recent methods, including: resonance mass measurement, electrolyte resistance, laser diffraction, particle image analysis, high performance chromatography, size exclusion chromatography and sequential ultra/nanofiltration are recommended as having higher accuracy rate and typically are faster than traditional methods. A significant scientific literature has been devoted to these recent methods. For instance, electrolyte resistance was employed on agricultural wastewater ($0.75 \times 10^3 - 80 \times 10^3$ nm, Chavez *et al.* 2004) and slaughterhouse effluent ($0.01 \times 10^3 - 10 \times 10^3$ nm, Sanchis *et al.* 2003) by applying the Coulter counter method. Laser diffraction was used for pig slurry wastewater ($0.04 \times 10^3 - 2{,}200 \times 10^3$ nm, Marcato *et al.* 2008), tannery effluent ($0.01 \times 10^3 - 100 \times 10^3$ nm, Murugananthan *et al.* 2004) and olive vegetation wastewater ($0.8 - 6.5 \times 10^3$ nm, Stoller 2009) as well as domestic wastewater ($0.02 \times 10^3 - 1.6 \times 10^3$ nm, Hocaoglu & Orhon 2013). Particle image analysis was applied to textile industry wastewater ($5 \times 10^3 - 180 \times 10^3$ nm, Yu *et al.* 2009) and municipal wastewater ($0.2 \times 10^3 - 125 \times 10^3$ nm, Garcia-Mesa *et al.* 2010). Besides the above mentioned methods, PSD based on sequential filtration/ultrafiltration is generally preferred for both characterization of wastewater and the elucidation of removal mechanisms of treatment processes (Tran *et al.* 2015). By using this method it is possible to categorize the organic matter fractions present in wastewater into the groups of (i) particulate (>1,600 nm), (ii) dissolved (<450 nm), (iii) colloidal (2 nm–1,600 nm) and (iv) soluble (<2 nm) (Cheryan 1986). While applying this procedure, a uniform sample is subjected to different pore size membranes sequentially. The permeate obtained from large pore membrane filters is used as the feed in the following (sequentially employed) ultrafiltration steps, where membranes with smaller pore size are employed. The particle mass is obtained from the difference between organic contents of two consecutive membranes. The procedure of size fractionation is schematically displayed in Figure 1. It appears that there is no consensus on unique definition of the size categories (Sophonsiri & Morgenroth 2004). In terms of filtration and ultrafiltration techniques, nominal molecular weight cut-off (such as kDa) and the pore diameter units (such as nm) are frequently used. In order to evaluate the results collectively, the equivalents of size units of ultrafiltration membrane discs, which are 100, 30, 10, 3 and 1 kDa, in the metric system were determined as 13, 8, 5, 3 and 2 nm, respectively (Dulekgurgen *et al.* 2006).

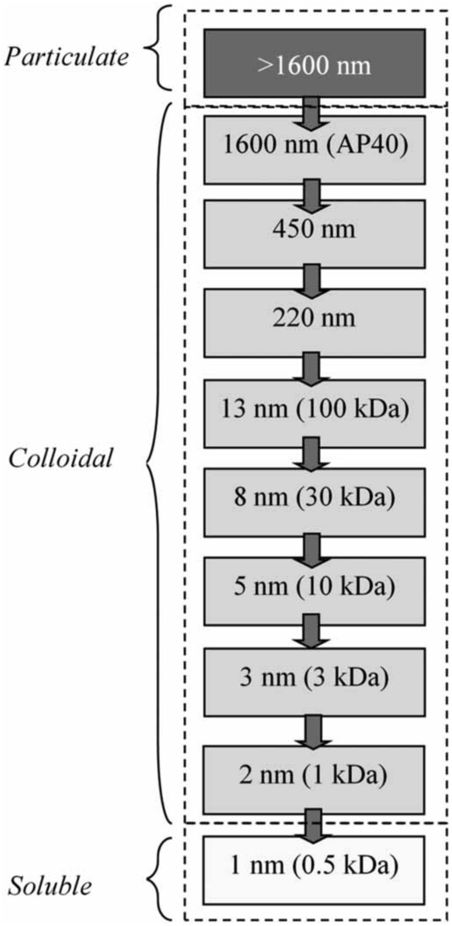


Figure 1 | Schematic of the sequential filtration/ultrafiltration size distribution analysis (Gursoy-Haksevenler & Arslan-Alaton 2015).

Considering previous studies dealing with the sequential ultra/nanofiltration methodology, it is apparent that a variety of wastewater types were subjected to size fractionation including landfill leachate (Wang *et al.* 2006; Xu *et al.* 2006; Berthe *et al.* 2008), pulp and paper mill effluent (Bijan & Mohseni 2004; Leiviskä *et al.* 2009), domestic/urban sewage (Zhang *et al.* 2007; Dogruel 2012; Noyan *et al.* 2017), textile (Dulekgurgen *et al.* 2007; Yaman *et al.* 2016), slaughterhouse (Sanchis *et al.* 2003), agricultural (Chavez *et al.* 2006), tannery wastewaters (Karahan *et al.* 2008), and olive mill wastewater (Gursoy-Haksevenler *et al.* 2014a; Ochando-Pulido *et al.* 2015; Khaligh *et al.* 2017) (Table 1). In some studies, it is seen that the different types of wastewater were compared in terms of their PSD. For example, in a related study conducted by Sophonsiri & Morgenroth (2004) the size distributions of the organic matter in domestic, industrial and agricultural wastewaters were examined. Obtained results indicated that the size distributions of studied wastewaters differed appreciably, such as municipal wastewater was mainly distributed in eight

Table 1 | Size distribution studies reported for different wastewater treatment processes

Treatment process	Wastewater type	Aim and scope of work	Experimental approach	Major findings	Reference
Activated sludge system	Tannery wastewaters	Establishing a scientific relation between biodegradability and PSD	Treatment process: activated sludge system PSD analysis: MF, UF (1,600–2 nm) Investigated parameters: COD, OUR	• 60% of the total COD was distributed at the particulate range while 25% was at the soluble range and 15% was at the colloidal range • Slowly biodegradable COD in particulate form (>1,600 nm) revealed a significant inhibitory effect	Karahan *et al.* (2008)
Activated sludge system	Pulp and paper mill effluent	Evaluation of the biological treatment efficiency on PSD	Treatment Process: Activated sludge system PSD Analysis: MF – 8×10^3, 3×10^3, 450, 220, nm UF – 100, 50, 30, 3 kDa Investigated parameters: COD, COD_{Cr} (COD oxidation with dichromate), BOD_7, TOC, Tot-P, PO_4-P, zeta potential, turbidity, conductivity	• The organic content was mainly distributed under 10 kDa before and after treatment • The highest organic content reduction was in the 6 kDa fraction after treatment • COD_{Cr} removal was 60–70%. COD_{Cr} was mainly in the <30 kDa before and after treatment • BOD_7 removal was >95%. The 3 kDa fractions was increased after treatment	Leiviskä *et al.* (2008)
Activated sludge system	Pulp and paper mill effluent	Evaluation of the biological treatment efficiency on PSD	Treatment process: activated sludge system PSD analysis: MF – 8×10^3, 3×10^3, 450, 220, nm UF – 100, 50, 30, 3 kDa Investigated parameters: COD, BOD_7, TOC, Tot-P, zeta potential, turbidity, conductivity	• By biological treatment the decrease in wood extractives was 89% for resin and fatty acids, 83% for sterols and 60% for b-sitosterol • Lignin both in the influent and effluent was mainly in the <3 kDa • Resin and fatty acids in the influent were 46% as particulate (>45×10^4 nm), 20% as colloidal (45×10^4 nm–3 kDa) and 36% as soluble (<3 kDa) fraction whereas in the effluent they were mainly present in the <3 kDa fraction	Leiviskä *et al.* (2009)

(*continued*)

Table 1 | continued

Treatment process	**Wastewater type**	**Aim and scope of work**	**Experimental approach**	**Major findings**	**Reference**
Anaerobic digestion	Domestic sewage	Investigation of physical characteristics change during anaerobic digestion	Treatment process: anaerobic digestion PSD analysis: by Malvern Zetasizer Investigated parameters: COD, VFA, surface tension and zeta-potential	• The highest biodegradability was in the colloidal (86%) and suspended (78%) fractions while the lowest biodegradability was in the dissolved fraction (62%) • After anaerobic digestion, the volume of particles within the diameters of <4,400 nm and <450 nm were increased • During anaerobic digestion volume of particles was decreased while there was an increase in size distribution • The larger particles ($>10^4$ nm) were more resistant to degradation that resulted in a general distribution shift towards larger sizes	Elmitwalli *et al.* (2001)
Anaerobic digestion	Pig slurry	Evaluation of the removal of metals and macronutrients in different size fractions	Treatment process: anaerobic digestion PSD analysis: by laser diffraction Investigated parameters: major (N, P, K, Ca, Fe, Mg and S) and minor (Al, Cu, Mn and Zn) elements	• There was a general shift towards larger sizes by degradation of small and easily degradable particles with diameters of between 10^3 and 60×10^3 nm • Due to production of large filaments, there was an increase in the ratio of larger particles ($>1.4 \times 10^6$ nm)	Marcato *et al.* (2008)
Chemical-biological flocculation	Domestic wastewater	Evaluation of PSD before and after chemical-biological flocculation (CBF)	Treatment process: CBF PSD analysis: laser diffraction device (0.4×10^3–2×10^6 nm) Investigated parameters: COD, TSS, TP, NH^{4+}-N, heavy metals	• The raw wastewater was mainly distributed in 0.4×10^3–340×10^3 nm (with average size at 29.96×10^3 nm) • Both large and small size particles were removed by CBF • After CBF, the particles were distributed in 0.4×10^3–3.0×10^3 nm (with average size at 819 nm)	Zhang *et al.* (2007)

(*continued*)

Table 1 | continued

Treatment process	Wastewater type	Aim and scope of work	Experimental approach	Major findings	Reference
Coagulation-flocculation and activated sludge system	Textile wastewater	Evaluation of the effect of chemical and biological treatment on COD fractionation and color profiling by PSD	Treatment process: chemical treatment (coagulation-flocculation), biological treatment (activated sludge system) PSD analysis: MF, UF (1,600–2 nm) Investigated parameters: COD, color	• By chemical treatment, 62% COD was removed; half of this was in particulate and upper colloidal-ranges • By biological treatment, 77% COD was removed; total removal at the particulate ranges and 50% removal at the soluble ranges • Chemical treatment was more effective in removal of color than biological treatment	Dulekgurgen *et al.* (2007)
Mechanical biological pre-treatment	Landfill leachate	Fractionation of the organic matter in leachate (i) recirculated, (ii) before and (iii) after treatment by mechanical biological pre-treatment (MBP)	Treatment process: recirculation and mechanical biological pre-treatment Adsorption: DAX-8, XAD-4 resins PSD analysis: UF -30, 3, 1 kDa Investigated parameters: COD, TOC, BOD_5, SUVA	• For bioreactor leachate, the organic content with <1 kDa was $\approx$70% where high molecular weight was only of 10% • MBP leachates contained more high molecular weight molecules ($\approx$ 20%) than bioreactor leachate (10%) • For MBP leachates, the humification process was more rapid than bioreactor leachate	Berthe *et al.* (2008)
Primary settling and biological treatment	Municipal wastewater (domestic sewage and pretreated tannery effluents)	Evaluation of municipal wastewater's biodegradability by PSD	Treatment process: primary settling and biological treatment PSD: filtration – 450 and 220 nm UF – 100, 50, 30, 3, 1 kDa Investigated parameters: COD, OUR	• Particulate COD was removed in the ratio of 25% (of 27%) by primary settling, • Particulate COD in the ratio of 60% (of 77%) and colloidal COD in ratio of 12% (of 77%) were removed by biological treatment • For soluble size range, there was a slight removal	Dogruel (2012)

(*continued*)

Table 1 | continued

Treatment process	**Wastewater type**	**Aim and scope of work**	**Experimental approach**	**Major findings**	**Reference**
Aerobic and anaerobic biological treatment and coagulation-flocculation	Cotton-dyeing textile wastewaters	Determination of the effect of treatment alternatives on molecular weight distribution	Treatment process: aerobic and anaerobic biological treatment, coagulation–flocculation, MF, UF, RO PSD: filtration – 0.05 μm UF - 0.1 and100 kDa Investigated parameters: COD, BOD, TKN, color, UV_{254}, SUVA	• Aerobic biological treatment alone was unfeasible • Coagulation–flocculation was not appropriate since wastewater was of hydrophilic character • $\approx$50% of the COD and BOD_5 found in the wastewater originated from the bleaching and mixed processes were <1 kDa • 56% of the COD and BOD_5 found in the wastewater came from dyeing and was in the size ranges of 1–100 kDa	Yaman *et al.* (2016)
Coagulation-flocculation	Slaughterhouse wastewater	Evaluation of PSD before and after coagulation-flocculation	Treatment process: coagulation-flocculation with $Al_2(SO_4)_3$ and auxiliary coagulants PSD analysis: MF, UF (0.5×10^3 – 10×10^3 nm) Investigated parameters: Number of particles	• $Al_2(SO_4)_3$ with anionic polyacrylamide provided the highest removal ratio (97%) • The lowest removal rates were in 5.5×10^3 – 8.5×10^3 nm size ranges	Sanchis *et al.* (2003)
Coagulation-flocculation	Agricultural wastewater (treated domestic wastewater)	Comparing the optimum operating conditions by considering PSD	Treatment process: coagulation ($160\ s^{-1}$, 60 s) and flocculation ($20\ s^{-1}$, 15 min) with $Al_2(SO_4)_3$ PSD analysis: Coulter counter (by monitoring the change of electrical resistance zone; 0.5×10^3 – 60×10^3 nm) Investigated parameters: TSS, electrical resistance zone	• Particle size of raw wastewater was 60 to 80×10^3 nm • After coagulation and flocculation >90% TSS was removed • After coagulation and flocculation, the removals were similar in 0.7×10^3 – 1.5×10^3 nm size ranges • During flocculation, removals in 1.5×10^3 – 20×10^3 nm was significantly higher than coagulation • By use of PSD, coagulant dose was three times lower when compared with the use of TSS parameter	Chavez *et al.* (2006)

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Table 1 | continued

Treatment process	Wastewater type	Aim and scope of work	Experimental approach	Major findings	Reference
Coagulation-flocculation	Olive mill wastewater	Enhancing two-stage membrane filtration by applying chemical pre-treatment	Treatment process: coagulation-flocculation with polyaluminium chloride PSD: filtration – 450 and 220 nm UF – 100, 30, 10, 3, 1 kDa Investigated parameters: COD, TOC, TPh, AOA	• More than 95% COD and TPh were removed by coagulation-flocculation • The COD removals were mainly in particulate and also in soluble fractions	Khaligh *et al.* (2017)
Ultrafiltration and Coagulation-flocculation	Polymer industry effluent	Comparing the performance of UF and conventional chemical treatment by PSD	Treatment process: UF and chemical treatment (lime alone and with $FeCl_3$ as coagulants) PSD: filtration – 450 and 220 nm UF – 100, 50, 30, 3, 1 kDa Investigated parameters: COD, OUR	• 70% of the total COD was distributed in the size ranges of 220–450 nm • More than 90% COD was removed by UF and $FeCl_3$ coagulation • 5–7% COD was removed by lime treatment and there was no change in PSD	Dogruel *et al.* (2013)
Fenton's reagent	Olive mill wastewater	Evaluation of the Fenton's reagent efficiency on PSD	Treatment process: Fenton's reagent PSD: filtration – 450 and 220 nm UF – 100, 50, 30, 3, 1 kDa Investigated parameters: COD, TOC, TPh, AOA	• 52% TOC was removed that was mainly in the soluble (30%) and colloidal (21%) size fractions • 46% TPh was removed of which 22% was in the soluble and 13% in the colloidal size fractions	Dogruel *et al.* (2009)

(*continued*)

Table 1 | continued

Treatment process	Wastewater type	Aim and scope of work	Experimental approach	Major findings	Reference
Coagulation-flocculation, electrocoagulation and Fenton's reagent	Olive mill wastewater	Evaluation of the efficiency of chemical treatment processes on PSD	Treatment process: $Ca(OH)_2$ precipitation, $FeCl_3$ coagulation, EC, Fenton's reagent PSD: filtration – 450 and 220 nm UF – 100, 50, 30, 3, 1, 0.5 kDa Investigated parameters: COD, TOC, TPh, acute toxicity	• For raw OMW, TOC was mainly distributed in the particulate size range (43%) whereas TPh was primarily present in the colloidal size range (54%) • $Ca(OH)_2$ precipitation and $FeCl_3$ coagulation resulted in ≈45% COD and TOC removals as well ≈30% TPh removals. By EC, 38% TOC and 27% TPh were removed. Fenton's reagent resulted in poor removals (<10%) • COD, TOC and color reductions were mainly in particulate and colloidal size ranges while TPh removals were in the real soluble size range	Gursoy-Haksevenler *et al.* (2014a)
Precipitation, flocculation-sedimentation, Fenton-like reaction, olive stone filtration, membrane technology	Olive mill wastewater	Evaluation of the treatment alternatives for improving pressure-driven membrane technology	Treatment process: precipitation, flocculation–sedimentation, Fenton-like reaction, olive stone filtration, membrane technology PSD: filtration – 450 nm UF – ranging from 3 to 100 kDa	• More than 90% COD was removed by both precipitation, flocculation–sedimentation, Fenton-like reaction, olive stone filtration • After both treatment alternatives, 75% of particles distributed below 2 μm • 32% of organic pollutants with size below 3 kDa were mainly related with membrane fouling	Ochando-Pulido *et al.* (2015)
Electrocoagulation and Fenton's reagent	Olive mill wastewater	Enhancing the biodegradability by applying chemical pre-treatment	Treatment process: EC and Fenton's reagent PSD: filtration – 450 and 220 nm UF – 100, 50, 30, 3, 1 kDa Investigated parameters: COD, OUR	• 15% of total COD and 11% of soluble COD were removed after Fenton's reagent • 23% of total COD and 20% of soluble COD were removed after EC • Refractory and inhibitory effects were removed by applying chemical pre-treatment	Karahan Ozgun *et al.* (2016)

(*continued*)

Table 1 | continued

Treatment process	Wastewater type	Aim and scope of work	Experimental approach	Major findings	Reference
Electrocoagulation and Fenton's reagent	Domestic sewage	Evaluation the relationship between PSD-COD fractionation and biodegradation	Treatment process: EC and Fenton's reagent PSD: filtration – 450 and 220 nm UF – 100, 50, 30, 3, 1 kDa Investigated parameters: COD, OUR	• Major COD distribution (65%) was >1,600 nm while the second main distribution (10%) was <2 nm • By PSD, all major COD fractions were defined except for the initial soluble and particulate inert COD fractions	Noyan *et al.* (2017)
Ozonation	Pulp and paper effluents	Enhancing biodegradability by ozonation	Treatment process: ozonation PSD analysis: UF (1 kDa) HMW (MW > 1 kDa) and LMW (MW < 1 kDa) Investigated parameters: BOD_5,COD, TC, pH, color, and molecular weight distribution of organics (nominal cut off of 1 kDa)	• The recalcitrant organic matter and color-causing organics were removed (30–60%) by ozonation • The biodegradability of high molecular weight fraction was increased by 50% • There was no improvement in the biodegradability of low molecular weight fraction	Bijan & Mohseni (2004)
Ozonation	Aged raw landfill leachate	Evaluation the effect of O_3 only and O_3/H_2O_2 on PSD	Treatment method: O_3 only and O_3/H_2O_2 PSD analysis: MF, UF (800 nm^{-1} kDa) Investigated parameters: BOD_5, COD, color, metals	• The distribution of metals were in 450 nm–10 kDa; COD and BOD_5 were <10 kDa, as well color was >1 kDa for raw landfill leachate • 29% COD was removed by O_3 only and 42% COD was removed by O_3/H_2O_2 • The H_2O_2 enhanced the reduction of large molecules	Wang *et al.* (2006)

PSD, Particle size distribution.
UF, Ultrafiltration.
NF, Nanofiltration.
RO, Reverse osmosis.

size fractions ranging from 1 nm to 63×10^3 nm, while organic matter in industrial and agricultural wastewaters mainly accumulated in the soluble size fractions (<1 nm) and larger size particles (>1.2×10^3 nm for the industrial and >10×10^3 nm for the agricultural wastewaters) (Sophonsiri & Morgenroth 2004). In the scientific literature, the treatment of surface waters was evaluated by PSD as well as that of wastewater. For example, Nissinen *et al.* (2001) assessed the PSD of raw and drinking waters before and after treatment by conventional processes, adsorption, filtration, ozonation and their combinations. It was observed that the two largest fractions were removed by shifting towards smaller size distribution after applied treatment processes. When the size ranges became smaller, it was recorded that no further removal could be achieved. All humic fractions were removed by the applied treatment processes within the exception of conventional treatment (Nissinen *et al.* 2001). Kitis *et al.* (2002) evaluated the reactivity of dissolved organic matter (DOM) within surface water during formation of disinfection by-products. It was concluded that no correlation existed between disinfection by-product formation and molecular size distribution of DOM (Kitis *et al.* 2002).

As mentioned above, the focus of the current study is to understand the effect of wastewater treatment on size and structural fractionation. It appeared that wastewater treatment alternatives preferred in these studies are mostly biological treatment including aerobic and anaerobic processes as well as chemical treatment including coagulation-flocculation, electrocoagulation, Fenton's reagent and ozonation processes. The studies conducted and the results obtained on PSD are summarized below.

Biological treatment

Aerobic process

Recently, there is a tendency towards the studies of PSD to be explored to achieve a better understanding of organic content fractionation and biodegradation (Sophonsiri & Morgenroth 2004; Dogruel *et al.* 2006; Dulekgurgen *et al.* 2006). By using this technique, it is also possible to imply some organic fractions that are responsible for the toxicity or non-biodegradability (Noyan *et al.* 2017). Within these studies, respirometry has been applied as a practical tool for the determination of organic fractions having different biodegradation characteristics in effluents (Ekama *et al.* 1986). For instance, Karahan *et al.* (2008) evaluated the relationship between biodegradability and size distribution of tannery wastewater by monitoring biodegradability-related COD fractions and oxygen uptake rate (OUR; in mg L^{-1} h^{-1}) profiles. In that work, 60% of the total COD was distributed in the particulate size range while 25 and 15% were located in the soluble and colloidal size range, respectively. PSD procedures indicated that the majority of soluble-rapidly-hydrolysable COD was in the soluble range (<2 nm) while the soluble-inert fraction was distributed among the colloidal range (2 nm–1,600 nm) and therefore removed by physical entrapment and adsorption. Slowly biodegradable COD in particulate form (>1,600 nm) revealed a significant inhibitory effect (Karahan *et al.* 2008).

Dogruel (2012) evaluated the biodegradation characteristics of municipal wastewater by employing PSD. Municipal wastewater treatment plant was consisted of an activated sludge process which effluent of domestic sewage and pretreated tannery wastewater were fed. Biological treatment was found to be more effective on the removal of particulate size range (>1,600 nm) with a 60% contribution to the overall COD removal of 77%. On the other hand, by the application of biological treatment, relatively poor removals of 12 and 5% were obtained in the colloidal and soluble size ranges, respectively. PSD analysis indicated that soluble-inert and readily-biodegradable fractions accumulated in the soluble size range (<2 nm), whereas the rapidly-hydrolysable fraction was determined to be distributed in the colloidal range within the exception of the 450–1,600 nm size interval (Dogruel 2012). Similar studies on biodegradability in COD fractions of municipal wastewater also revealed that the readily-hydrolysable organic contents were around 80–90% in the soluble and colloidal fractions. Main COD (45–50%) in the particulate fraction was found to be slowly hydrolysable (Ginestet *et al.* 2002). Levine *et al.* (1985) reported that the size of particles <10^3 nm can be biologically degraded more rapidly than the size of particles >10^3 nm. In studies dealing with biodegradation and its effect on PSD, the general conclusion was that relatively small-sized organic matter could be directly metabolized by microorganisms whereas larger-sized particles could be degraded only after hydrolysis (Ginestet *et al.* 2002; Karahan *et al.* 2008; Garcia-Mesa *et al.* 2010). The above results demonstrate that the performance of a wastewater treatment processes is mainly related to the substrate's size whether it is in soluble form or consists of particulate fractions.

Anaerobic process

Recently, anaerobic digestion processes have improved significantly for wastewater treatment. One of the most important factors affecting the yield in anaerobic digestion is the immobilization of biomass inside the reactor, which is expected to be formed as spherical and mechanically stable particles (Jeison & Chamy 1998). The quality and stability of these particles directly determine the behaviour of the entire treatment process. Settling properties, which is one of the main points in the operation of the anaerobic process, is defined both by the particle's size and density (Arcand *et al.* 1994). There are several studies within various scientific literature dealing with the effects of anaerobic treatments on the size distribution of wastewater. Klinkow *et al.* (1998) aimed to develop a separation procedure for identifying toxic compounds and evaluating the biological degradation of tannery wastewater. The organic content that accumulated within the range of 0.03–0.1 kDa was found to be more significant that displaying similar toxicities for the untreated and the anaerobic/aerobic treated samples. However, the toxicity of the anaerobic treated sample almost doubled that was explained by the formation of non-biodegradable compounds during the anaerobic treatment (Klinkow *et al.* 1998). Barker *et al.* (1999) characterized the soluble residual COD of six different kinds of sample both before and after anaerobic treatment. It was revealed that 89% of the compounds in anaerobic effluents was in the low range of <1 kDa, while the second group (up to 22%) was in the higher molecular weight range (>300 kDa). Although aerobic treatment was shown to be more successful than anaerobic, it was observed that low molecular weight compounds were difficult to decompose aerobically (Barker *et al.* 1999). Elmitwalli *et al.* (2001) investigated the anaerobic digestion of domestic sewage in terms of changes in its size distribution. The highest biodegradability rate was achieved in the colloidal (86%) and suspended (78%) size fractions, while the lowest biodegradability rate was monitored in the dissolved fraction (62%). After anaerobic digestion, the volume of particles within the diameters of <4,400 nm and <450 nm were found to be of a higher volume. During anaerobic digestion the volume of particles decreased while there was an increase in size distribution. Bacterial floc and filaments formation resulted in a slight increase in the volume of larger particles. It was revealed that the most degraded small particles (10^3 nm) contained: amino acids, carbohydrates, polysaccharides, fatty acids, lipids and proteins. It was found that larger particles (>10^4 nm) displayed more resistance to any degradation that resulted in a general distribution shift towards larger sizes. A similar size distribution shift towards larger fractions was obtained by Marcato *et al.* (2008) during anaerobic digestion of pig slurry. Particle size analysis was carried out on raw and digested slurries by applying laser diffraction. During the anaerobic digestion of the slurry, a general shift towards larger sizes was observed by reason of degradation of small and easily degradable particles with diameters of between 10^3 and 60×10^3 nm. A relative increase in the ratio of larger particles (>1.4 mm) was noted due to production of large filaments (Marcato *et al.* 2008).

Chemical treatment

Coagulation-flocculation

In order to improve the removal efficiency of organic compounds and nutrients, the interest in coagulation-flocculation processes increased since the 1970s (Tchobanoglous & Burton 1991). While 80–90% of TSS, 40–70% of BOD, 30–60% of COD, and 80–90% of the bacteria removals were achieved by coagulation-flocculation (Tchobanoglous & Burton 1991), for the removal of DOM, characteristics of organic compounds have significant effect on coagulation-flocculation (Shon *et al.* 2006a).

Shon *et al.* (2006a) investigated the effect of treatment alternatives including coagulation-flocculation, adsorption, and nanofiltration on the organic matter characteristics in a biologically treated sewage effluent. The efficiency of removal was evaluated in terms of TOC, trace harmful chemicals (such as endocrine disrupting chemicals, etc.) reduction, as well size and structural fractionation. Size distributions measured by High Performance Size Exclusion Chromatography displayed that mainly high molecular weight (43.11 kDa) compounds were removed while there was no removal in low molecular weight (0.26 kDa, 0.33 kDa and 0.58 kDa) compounds by $FeCl_3$ flocculation. Mainly low molecular weight compounds were removed by adsorption, while practically all molecular weight compounds could be removed by nanofiltration (Shon *et al.* 2006a).

Dulekgurgen *et al.* (2007) examined the effect of chemical and biological treatments of different textile wastewaters on COD fractionation and color profiling by employing PSD analysis. Before treatment, both different textile wastewaters revealed different COD fingerprints that mainly accumulated in the higher size ranges. After treatment, COD profiles were found similar and mostly remained in the soluble fraction (<2 nm). By coagulation-flocculation with $FeSO_4$ and lime, 62% COD was removed and half of this was obtained at particulate and upper colloidal ranges while almost no removal was obtained in soluble ranges. However, with the use of biological treatment, 77% COD removal was observed as being effective on both size distributions, with total removal in the particulate ranges and 50% removal in the soluble ranges. For color removal, chemical treatment was also effective in color removal originating from the colloidal ranges; however, it was not effective in the soluble size ranges. In contrast, biological treatment was found to be average for all size ranges (Dulekgurgen *et al.* 2007).

Among the examined wastewaters, olive mill wastewater (OMW), which is particularly known for its high-strength and complex organic carbon content, had previously been explored for efficient treatments; however, most of these appeared to be rather inefficient. In most of the studies, membrane units were employed for recovering and concentrating polyphenols from OMW (Paraskeva *et al.* 2007; Coskun *et al.* 2010; Garcia-Castello *et al.* 2010; Cassano *et al.* 2011). In some of these studies, the effect of physicochemical treatment methods on size distribution was examined in more detail (Dogruel *et al.* 2009; Stoller & Bravi 2010). Gursoy-Haksevenler *et al.* (2014a) examined the effect of several physicochemical treatment methods including $Ca(OH)_2$ precipitation and $FeCl_3$ coagulation on PSD of OMW. For untreated OMW, the TOC content was mainly distributed in the particulate size range (43%), whereas total phenols (TPh) were primarily present in the colloidal size range (54%). By $FeCl_3$ coagulation and $Ca(OH)_2$ precipitation ≈57% COD, ≈45% TOC and ≈30% TPh removals were obtained. By $FeCl_3$ coagulation, it was evident that for all parameters (COD, TOC, TP and color) followed, the removal was high in the particulate (>1,600 nm, ≈88%) and colloidal (2 nm–1,600 nm, ≈56%) size fractions while there was no removal observed in the soluble (<2 nm) size ranges. Similarly, by $Ca(OH)_2$ precipitation high removals (94–98%) were obtained in the particulate and colloidal (58–66%) size fractions for all parameters, except TPh (78 and 35% for particulate and colloidal fractions, respectively) (Gursoy-Haksevenler *et al.* 2014a).

For coagulation of high molecular weight compounds, the main removal mechanism is known as charge neutralization while for the organic content composed of a lower molecular weight, or non-humic substances, the removal mechanism is estimated to be adsorption on to a metal hydroxide surface (Matilainen *et al.* 2010). From previous studies examining coagulation-flocculation effects on PSD, it can be concluded that the removal was mainly in the particulate and colloidal fractions and the dominant removal mechanism was charge neutralization. The relatively higher reduction of these fractions can be explained by having hydrophobic characteristics and containing more aromatic compounds within these two fractions (Świetlik *et al.* 2004).

Electrocoagulation

The electrocoagulation (EC) process is an advanced alternative to chemical coagulation in water and wastewater treatment to help overcome some disadvantages of the more conventional treatment technologies (Kim *et al.* 2002). For the EC process, aluminum, iron or steel electrodes are generally employed that undergo anodic dissolution during the reaction. After dissolution of these electrodes, metal hydroxide compounds such as $Al(OH)_3$, $Fe(OH)_3$ and $Fe(OH)_3$ are formed. The colloidal/suspended pollution parameters are destabilized and aggregated by these metal hydroxides and followed by absorption, adsorption, flocculation, particle entrapment and also redox reactions (Olmez-Hanci *et al.* 2012). Unlike other treatment processes, the process of EC is a treatment involving precipitation, adsorption, reduction and oxidation, flocculation and flotation (Emamjomeh & Sivakumar 2009). Depending on many variables such as current density, pH value, amount of electrolyte used during the reaction, especially the characterization of the wastewater studied, one or more of the removal mechanisms hosted by the EC process becomes more effective (Hanafi *et al.* 2010).

In our previous work (Gursoy-Haksevenler *et al.* 2014a) OMW was subjected to EC and its effect on PSD was evaluated. After EC, 53% COD, 38% TOC and 47% color removals were achieved, these were mainly in particulate and colloidal size ranges. On the other hand, most TPh removals (25% of 27%) were observed in the real soluble size (<1 nm) range. When the obtained results were compared with $FeCl_3$ coagulation and $Ca(OH)_2$ precipitation to understand which mechanism was dominant in EC, it was concluded that the total COD and TOC removal efficiencies through the entire size spectrum were very similar. After EC application, 53% COD and 38% TOC removals were obtained where 58% COD and 45% TOC removals by $FeCl_3$ coagulation as well as 57% COD and 48% TOC removals were observed by $Ca(OH)_2$ precipitation. This could explain the operated main removal mechanism in EC being coagulation-flocculation. However, differently, a noticeable TPh removal (25%) in real soluble size fraction (<1 nm) was provided after EC, that could be explained by the adsorption of real soluble phenolic compounds by EC (Gursoy-Haksevenler *et al.* 2014a). A related work conducted by Olmez-Hanci *et al.* (2008) examined the effect of the EC process on the PSD of OMW. It was concluded that COD and TOC were removed in the ratio of 20–30%. After EC, the organic content accompanied with ≈90% COD, TOC and antioxidant activity was shifted to the soluble size range (<2 nm) (Olmez-Hanci *et al.* 2008).

Fenton's reagent

Among the AOPs, Fenton's reagent has been determined to be kinetically superior and a more effective treatment of toxic and/or inert wastewater matrices (Vedrenne *et al.* 2012). By Fenton's reagent it is possible to treat industrial wastewater, since it involves both oxidation under acidic pH via Fenton's reagent and ferric hydroxide flocs formed after pH re-adjustment. Hence, the pollutants expected to be removed are not only water-soluble, polar aromatic substances during the oxidation stage, but they are also relatively less soluble, hydrophobic pollutants and colloids during the pH re-adjustment and coagulation stage (Wadley & Waite 2004).

In studies where treatment by Fenton's reagent and its effect on PSD are examined, it is seen that OMW is specifically studied due to the interest in the complex structure of OMW. In a related study investigated by Dogruel *et al.* (2009), the effect of Fenton's reagent on the size distribution of organic carbon in OMW was examined. 52% TOC removal was observed for Fenton's reagent in OMW, which was mainly composed of soluble (30%) and colloidal (21%) size fractions. For TPh removal, 46% reduction was obtained, of which 22% was in the soluble and 13% in the colloidal size fractions (Dogruel *et al.* 2009). In our previous study, OMW treatment by Fenton's reagent and its effect on PSD analysis were also investigated. The obtained removal efficiencies were very poor as only 13% COD, 20% TOC and 7% TPh were recorded as removals. These relatively low removals were mainly obtained in the particulate and colloidal fractions (Gursoy-Haksevenler *et al.* 2014a). These low removals obtained from OMW treatment by Fenton's process can mainly be attributed to the fact that only partial oxidation can be achieved. In particular, oxidation may not be efficient in complex wastewater such as OMW. Even if radicals are formed during the reaction, they react with many components and the efficiency of the chain reactions decreases. Considering that partial oxidation is expected, the results obtained for this complex OMW sample containing high carbon and radical scavenging components were not surprising. Both these studies conducted by Dogruel *et al.* (2009) and Gursoy-Haksevenler *et al.* (2014a) confirmed that treatment efficiencies mainly depend on the removal mechanisms of the selected treatment methods and the characteristics/nature of examined wastewater. Comparing the results of studies conducted by Dogruel *et al.* (2009) and Gursoy-Haksevenler *et al.* (2014a), the differences in distribution of size fractions obtained could be explained by the difference in characteristics of studied OMW effluents. The COD content of the OMW in our previous study (Gursoy-Haksevenler *et al.* 2014a) was very high (155,000 mg/L) and mainly in the particulate form (54%) while the COD of OMW studied by Dogruel *et al.* (2009) was relatively low (40,000 mg/L) and mainly in soluble form (49%). Hence when the organic content is very high and mainly of particulate nature, as in our previous study, it is expected to have different and relatively poor treatment results by employing Fenton's reagent.

Ozonation

Ozone reacts with organic pollutants present in water and wastewater in two different ways as direct molecular reactions and indirect radical chain-type reactions (Gottschalk *et al.* 2009). The ozone reaction is influenced by the water/wastewater characteristics, pH, ozone concentration, presence of initiators for decomposition, promoters and scavengers in the reaction medium (Glaze *et al.* 1987). Ozonation is just one of the effective methods used in the removal of recalcitrant organic fraction in wastewaters such as pulp and paper effluent (Bijan & Mohseni 2004), landfill leachate (Wang *et al.* 2006) and textile wastewater (Arslan-Alaton & Alaton 2007). There are also several other studies dealing with the effect of ozonation on PSD analysis in the literature. For instance, aged raw landfill leachate was subjected to oxidation with ozone only (O_3 only) and ozone combined with H_2O_2 to help us understand inert organic compounds behaviours during treatment (Wang *et al.* 2006). The distribution of COD and BOD_5 were <10 kDa, and that of color was >1 kDa for raw landfill leachate. It was found that 29% COD removal was provided by O_3 only, while 42% COD removal was obtained by O_3/H_2O_2. The addition of H_2O_2 enhanced the oxidation of large molecules (>1 kDa) - including refractory organic molecules (such as humic substances) - did not affect the lower size ranges (Wang *et al.* 2006). In a related study carried out by Bijan & Mohseni (2004), the effect of ozonation on the degradation of recalcitrant compounds in pulp and paper effluents was examined by a molecular weight distribution analysis. The recalcitrant organic matter and color-causing organics were removed (30–60%) by ozonation. Ozonation improved the effluent's biodegradability in the ratio of ≈50%, which was consistent with significant removal of recalcitrant compounds containing high molecular weight organics. No improvement has been achieved for biodegradability of low molecular weight fractions (Bijan & Mohseni 2004). Dogruel *et al.* (2006) applied PSD analysis to evaluate the ozonation impact on COD profiles of textile wastewater before and after its biological treatment. The COD fractions before and after ozonation appeared to be similar in the soluble range (<2 nm) and the major removal effect of ozonation was obtained in the 13–220 nm size interval with 9–15% COD reduction. However, a COD reduction of non-ozonated sample was found to be higher than the ozonated samples. That was explained by solubilization of organic content into smaller compounds as well as polymerization to the higher size range (>220 nm) besides the oxidation mechanism (Dogruel *et al.* 2006). From the previous studies on wastewater treatment by ozonation and its effect on PSD, they revealed that partial oxidation could be achieved due to the nature of the wastewater. The removal mechanism could differ as not only oxidation but also solubilization into smaller species and/or organic radical formation, and/or polymerization to the higher size range (Jekel 1998; Ollis 2001).

As presented above, these studies dealing with the effects of chemical treatment on size fractions can provide a deeper insight into wastewater characteristics and the removal mechanisms of applied treatment processes. However, it should be kept in mind that the main factor for treatment efficiency is wastewater characteristics. Each process can results in different removal efficiencies for each particular type of wastewater.

In the scientific literature case studies, the efficiency of different treatment processes was comparatively evaluated for size fractionation. For example, OMW was one of the wastewaters preferred to study comparatively different treatment processes due to its complex structure. Gursoy-Haksevenler *et al.* (2014a) examined the effect of several physicochemical treatment methods including $Ca(OH)_2$ precipitation, $FeCl_3$ coagulation, EC and Fenton process on size distribution of OMW. For untreated OMW, the TOC content was mainly distributed in the particulate size range (43%), whereas TPh were primarily present in the colloidal size range (54%). Among the chemical treatment alternatives examined, coagulation and chemical precipitation appeared to be the most efficient processes in terms of COD (≈57%), TOC (≈45%) and TPh (≈30%) removal from OMW, while the Fenton's reagent resulted in poor removal efficiencies (<10% for each examined parameter). TOC reduction was mainly obtained in the particulate size fraction (>1,600 nm, 35% of 45% removals) while the major TPh reduction was observed in the colloidal size fraction (1,600–1 nm, 19% of 30% removals). It could be concluded that 'phase-transfer' (concentration in one phase) was the dominant removal mechanism among the selected treatment options. By the Fenton's reagent, which is a 'destructive' (oxidative) treatment method, only partial oxidation could be achieved. This was due to the high organic content (initial COD = 155,000 mg/L) and particulate concentration (Gursoy-Haksevenler *et al.* 2014a). Arslan-Alaton *et al.* (2009) evaluated the efficiency of lime precipitation–coagulation, Fenton's reagent, and EC on PSD of OMW. It was concluded that organic carbon content of raw OMW mainly originated from the soluble fractions. Moreover polyphenols are thought to constitute a significant part of the soluble fractions that known as inert for biological treatment. None of the chemical treatment alternatives examined were found to be sufficient for effective treatment of OMW (COD removals were found as 25% by coagulation, 23% by EC and 17% by Fenton's reagent). Among these alternatives, EC that accommodates the mechanisms of phase-transfer (like adsorption, coagulation, flotation, and particle entrapment) was found to be more effective than Fenton's reagent. By EC it was possible to remove organic content mainly in particulate and colloidal fractions while it was only possible to decrease the organic content in the soluble ranges by Fenton's oxidation. On the other hand, Fenton's reagent was more effective than EC in removal of polyphenols that mainly accumulated in the soluble size range (Arslan-Alaton *et al.* 2009). In another work conducted by Olmez-Hanci *et al.* (2008), the effect of the Fenton's reagent and EC on the size distribution of OMW was also compared. Results indicated that EC was more efficient in the removal of particulate and colloidal TOC fractions while the Fenton's reagent was more effective in soluble TOC fractions removal, as expected from their removal mechanisms (Olmez-Hanci *et al.* 2008).

Size fractionation studies published for different wastewater treatment processes are presented in Table 1 (after the year 2000).

STRUCTURAL FRACTIONATION

Structural (resin) fractionation procedures have also been proposed as a practical tool for classifying the organic matter content of effluents into structurally more specific fractions (Leenheer 1981). This approach is based on the use of different resin types to fractionate the organic matter into different structural portions and basically into hydrophobics (HPOs) and hydrophilics (HPIs) as follows; hydrophobic neutral (HPON), hydrophobic acid (HPOA), hydrophobic base (HPOB), hydrophilic neutral (HPIN), hydrophilic acid (HPIA) and hydrophilic base (HPIB) fractions. According to the previous studies, the hydrophobic fraction is mainly composed of humic substances, with mainly carboxylic and phenolic functional groups such as humic acids and fulvic acids. Hydrophilic fraction comprises acids such as amino acids, bases such as primary and secondary protein groups and neutrals such as polysaccharide groups (Cho 1998; Thurman 2012; Zheng *et al.* 2014). Fractions having an intermediate polarity ranging between HPOs and HPIs are called Transphilic (TPI) and contain lower aliphatic and aromatic carbon contents than the hydrophobic fraction, such as sugar acid, sulfonic acid. Obviously, XAD-8 and XAD-4 resins have been most widely used for characterization based on the hydrophilic and hydrophobic properties of the effluent components (Marhaba *et al.* 2003; Chen *et al.* 2011; Labanowski & Feuillade 2011; Lin & Wang 2011). While applying this procedure, DOM isolated by XAD-8 fractionated into HPOs while DOM isolated by XAD-4 fractionated into TPIs. Effluents from XAD-8 followed by XAD-4 are fractionated into HPIs (Leenheer 1981; Imai *et al.* 2002; Marhaba *et al.* 2003) (see Figure 2).

The structural fractionation studies are rather limited and mostly focused on surface and drinking water samples in order to help identify toxic by-product formation in the disinfection processes (Ma *et al.* 2001; Kitis *et al.* 2002; Marhaba *et al.* 2003; Parsons *et al.* 2004; Kim *et al.* 2006; Iriarte-Velasco *et al.* 2007; Chen *et al.* 2011; Lin & Wang 2011). However, in several studies, resin fractionation has also been applied to municipal wastewater (Gong *et al.* 2008), landfill leachate (Labanowski & Feuillade 2009) and OMW (Ferri *et al.* 2011; Gursoy-Haksevenler & Arslan-Alaton 2014b). These wastewater types can be defined as refractory, hence the structural fractionation studies were mostly aimed at highlighting the inert organic components that prevent treatment. The reported treatment alternatives subjecting the effect of treatment on the structural fractionation mainly focused on biological treatment including aerobic and anaerobic processes and physicochemical treatment including coagulation, electrocoagulation, Fenton's

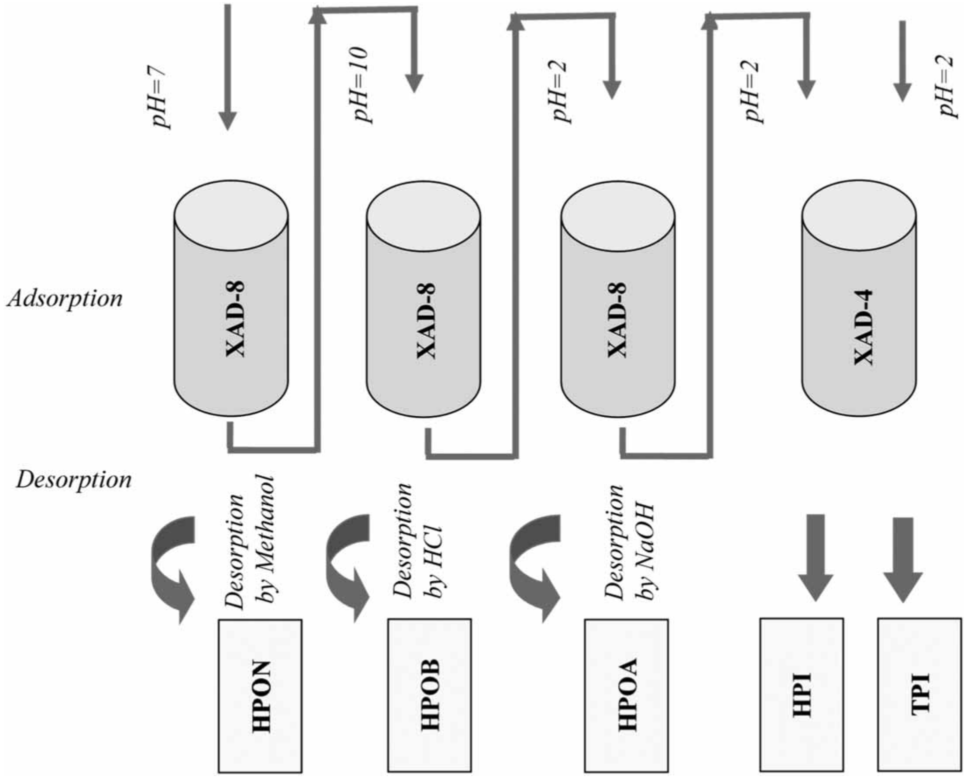

Figure 2 | Schematic of the structural fractionation by applying XAD-8 and XAD-4 resins (Gursoy-Haksevenler & Arslan-Alaton 2015).

reagent and ozonation. The most frequently applied treatment alternatives to evaluate the effect on structure are presented below.

Biological treatment

Aerobic process

For achieving an effective biodegradation of wastewater, it is required to have a deeper evaluation of biodegradation characteristics and to define any potential inhibitory effects on the degradation process (Dignac *et al.* 2000). Structural fractionation procedure would provide valuable information for optimization of treatment by answering the questions of which structural fraction is more essential within the overall organic content and how the biodegradation characteristics of the organic content vary during treatment. Among the studied wastewaters in the literature, landfill leachate was a strong one with complex characteristics. Biological treatment by activated sludge process was applied on landfill leachate and the obtained results from different studies were similarly poor with less than 20% COD removal (Imai *et al.* 1995; Aziz *et al.* 2011; Cui *et al.* 2016). Cui *et al.* (2016) reported that the acidic fractions composed of HPOA and transphilic acid were the major components in the untreated leachate (corresponding to more than 59% of DOM). After applying an activated sludge process, the ratio of these fractions raised from 59% to 77%. Moreover, the hydrophilic fraction (10%) did not alter during biodegradation and was identified as refractory. It was concluded that the main reason for the refractory characteristic of the leachate was the majority of HPOA, transphilic acid and HPI fractions (69%). It was revealed that the bacteria could preferably consume the neutral fractions, and then acidic and hydrophilic fractions (Cui *et al.* 2016). Nguyen *et al.* (2012) examined the biological treatment effect by applying membrane bioreactor-granular activated carbon for a synthetic wastewater including pharmaceutically active compounds, pesticides, hormones and industrial chemicals. It was confirmed that the biological treatment was effective in the removal of HPOs and biodegradable organic materials, but was in fact insufficient for the removal of HPIs and persistent compounds (Nguyen *et al.* 2012). A previous study by Namour & Muèller (1998) investigated the organic matter fractionation from sewage treatment plants (by activated sludge system) both before and after biodegradation and this brought light to the refractory compounds. Biodegradation resulted in a 40% increase in the HPOs fraction, which was equivalent to the humic substances and inert to biodegradation. Differently than other works, it was observed that hydrophilic compounds had no characteristics refractory to biodegradation (Namour & Muèller 1998). These dissimilar results obtained by biodegradation could be explained by the different wastewater matrices examined (such as landfill leachate, sewage effluent, synthetic wastewater with trace elements). However, ultimately, it is possible to reveal the distribution of organic matter in the effluent during biodegradation and indicate the inert fraction by employing the structural fractionation procedure.

Anaerobic process

Generally, anaerobic treatment processes have only been subjected to high strength industrial wastes (Lim *et al.* 2020; Show *et al.* 2020) or municipal sludges' digestion (Baccot *et al.* 2017; Chow *et al.* 2020) Based on the literature examined, there are only a few studies regarding the characterization of the wastewater after anaerobic treatment. In one of these studies, biological treatment steps of pectin production wastewater was investigated to find out the correlation of wastewater's chemical characteristics and its toxic effects (Reginatto *et al.* 2009). Biological wastewater treatment steps were composed of denitrification, anaerobic treatment, activated sludge, clarifier and sand filter. Unfortunately, the fractionation results of anaerobically treated samples were not given in the paper. However, it was reported that after anaerobic treatment, there was an increase in the toxicity that was explained by the desorption of substances present in anaerobic sludge while the final effluent indicated a lower biodegradability and toxicity towards *V. fisheria* and *Scenedesmus subspicatus*. XAD resin fractionation displayed that that the toxicity was related with the organic content. Molecular weights were distributed through 14.3, 25.0, 24.4 and 29.6 kDa after treatment. The pyrolysis-GC-MS demonstrated that the polyaromatic compounds increased (23%) during biological treatment steps. Within the first step, COD and BOD were reduced and the effluent's chemical structure changed, such as increasing polyaromatic compounds that correlated with the toxicity in the effluent. Zhang *et al.* (2012) studied the biodegradability of leachate by applying XAD-8 resin. The leachate was received from different landfill reactors composed of aerobic, semi-aerobic, and anaerobic degradations. A relationship was examined between the COD of the final solution after XAD-8 resin adsorption (COD_{XAD}) and the difference in the ($COD-BOD_5$). It was concluded that leachate was expected to be stable when the COD_{XAD}/COD ratio was higher than 0.432. According to the trends of volatile solid and biologically degradable matter contents, the waste degradation in anaerobic condition was inhibited after day 295; that was associated with accumulation of long chains of fatty acids during this time.

Chemical treatment

Coagulation-flocculation

The coagulation process is known to be more effective in removing the hydrophobic fractions than the hydrophilics (Fearing *et al.* 2004; Haberkamp *et al.* 2007; Matilainen *et al.* 2010). Since the hydrophobic organic content mainly contains a negative charge in high levels that relate to the existence of ionized groups (like phenolic and carboxylic groups), the hydrophobic organic fraction has greater affinity to metal hydroxide surfaces used as a coagulant (Bose & Reckhow 2007; Bond *et al.* 2010). In the scientific literature there are numerous studies confirming the higher removal of HPOs by coagulation. Labanowski & Feuillade (2011) worked on landfill leachate treatment by coagulation and EC. After both processes, 45% COD and 38% TOC were removed and the remaining organic matter concentration was recorded as the same with different compositions. The reduction was mainly recorded in the hydrophobic and aromatic character of organic content. By coagulation and EC, >50% of humic-like acids were decreased. The removal for both of the processes were humic-like acids > HPOs > TPIs > HPIs. After EC, a higher percentage of organic content was transformed into smaller hydrophilic structures which were more inert. The removal mechanisms of these processes on landfill leachate was explained as having the ability to easily absorb the hydrophobic compounds at the flocs' surface and their more easy neutralization. It was determined that as the hydrophobic character of the molecules decreased, their reactivity declined, as obtained for HPIs (Labanowski & Feuillade 2011). A study conducted by Shon *et al.* (2006a) investigated the effect of treatment on biologically treated effluent's structural distribution by applying $FeCl_3$ coagulation, adsorption, ion exchange and photocatalysis. $FeCl_3$ coagulation resulted in 60% removal in HPI, 39% removal in TPI and 22% removal in HPO fractions. Generally known, coagulation was mainly successful in removing HPOs. The obtained higher removal in HPIs was explained in association with ionic effects of organic content in effluent and the high dose of $FeCl_3$ used in the process (by the mechanism of sweep flocculation) (Shon *et al.* 2006a). A related study done by Imai *et al.* (2002) characterized any dissolved organic matter in effluents received from different wastewater treatment plants practicing coagulation, ultrafiltration, activated carbon adsorption and ozonation. It could be established that the organic content was transformed into a relatively small size and a more hydrophilic structure after each treatment process. Aquatic humic substances (3–28%) and HPIA (32–74%) were found to be dominant in all examined effluents, corresponding to ≈55% of organic content. Aquatic humic substances, HPON, and HPIA fractions varied due to the kind of wastewater and the treatment processes applied.

Electrocoagulation

The effect of the EC process known as a multiple-step mechanism, including precipitation, adsorption, sweep flocculation, particle entrapment, and redox reactions, relies on the effluent's characteristics. Considering that coagulation is a more effective process at removing HPO organic compounds, EC, defined as advanced coagulation, is expected to also be as effective in HPI organic compounds removal as well as HPOs. Gursoy-Haksevenler & Arslan-Alaton (2014b) confirmed this by investigating the structural fractionation of OMW's organic content after applying EC and $FeCl_3$ coagulation (after pre-treatment with thermal acid cracking). Removals of 66% for COD, 54% for TOC and 72% for TPh were provided by EC, while removals of 62% for COD, 48% for TOC and 47% for TPh were achieved by $FeCl_3$ coagulation. It was evident that $FeCl_3$ coagulation was effective in the removal of HPOs while EC was also efficient in the reduction of HPIs. A shift was observed from mainly HPOs to more HPIN fractions after EC, and the hydrophilic content increased from 25% to 40% for the TOC parameter. It was concluded that the destructive mechanism of EC may convert organics into more polar and hydrophilic compounds. On the other hand, the TPh parameter mainly present in the hydrophobic fractions (91%), particularly in the HPON fraction (56%) in raw OMW, did not alter considerably after treatment (Gursoy-Haksevenler & Arslan-Alaton 2014b). In another work, the effect of coagulation and EC processes on landfill leachate considering the change in structure of the organic content was examined by Labanowski *et al.* (2010). The composition was modified, whereas its concentration remained almost the same. After EC, the organic structure was changed into smaller hydrophilic structures that were less biodegradable (Labanowski *et al.* 2010).

Fenton's reagent

During the oxidation of pollutants with Fenton's reagent, more oxygenated and hence more polar hydrophilic substances are estimated to form. Fang *et al.* (2016) examined the distribution of the polarity of the organic matter in papermaking wastewater after coagulation and Fenton's reagent. It was found that lignin, which was present in the colloidal form and relatively HPO as well as aromatic, was the major resistant organic matter in the effluent. The coagulation process resulted in 60% removal of the HPO fraction that was associated with the mechanisms of charge neutralization and sweep flocculation. PAC, when used as the coagulant agent, provided the positive charge while lignin provided the negative charge and formed metal complexes. Unlike coagulation, the Fenton's reagent non-selectively degraded organic compounds upon generation of $HO^{\bullet}$, and the carbon content remaining after oxidation (73% DOC removal) was distributed in 42% HPI and 56% HPO fractions (Fang *et al.* 2016). In another work, OMW was subjected to Fenton's reagent (after thermal acid cracking) and its effect on the structure was investigated (Gursoy-Haksevenler & Arslan-Alaton 2014b). Fenton's reagent resulted in 63% COD, 50% TOC and 61% TPh removals. A shift from hydrophobic to hydrophilic fraction was obtained and COD and TOC contents became 40 and 37% hydrophilic after treatment while they were 19 and 26% before treatment, respectively (Gursoy-Haksevenler & Arslan-Alaton 2014b). Wang *et al.* (2018) examined pulp mill wastewater degradation by a heterogeneous Fenton process. The aromatic structures presented in raw effluent were determined as resistant to biodegradation. The hydrophobic compounds were found to be the major (≈60%) fraction of the organic content of raw effluent, while the hydrophilic fraction constituted a proportion of 38%. By GC-MS analysis, it was confirmed that HPO fractions, which were rich in aromatic carbon including lignin derivatives and had a high molecular weight, were resistant to biological treatment. After heterogeneous Fenton treatment, the structural fractionation was not changed significantly (the HPO fraction decreased from 59 to 56%). Further oxidation of HPO and HPI fractions resulted in higher COD reduction (75%). The organic content in raw wastewater was mainly (70%) defined in high molecular weight (>10 kDa) that was not biodegradable. After employing the heterogeneous Fenton process, the molecule fractions with a molecular weight of 10–30 kDa and >30 kDa were mainly transformed into 0–1 kDa (consisting of 88% of the total).

Ozonation

Ozonation directly affects both the composition and characterization of organic matter within the wastewater. During treatment, the polarity and hydrophilic natures are altered, which may be significant when the effluent is discharged to the environment or re-used. Gong *et al.* (2008) investigated structural changes in the structure of biologically pre-treated municipal wastewater when subjected to ozonation and UV-C-assisted ozonation. After ozonation alone and with UV-C irradiation, 36% and 90% DOC reductions were obtained, respectively. The poor efficiency in DOC removal found for ozonation alone was explained by its oxidative capacity and selectivity for hydrophilic aromatic organics. However, in the presence of UV-C irradiation, the ozone chemistry and hence the reaction mechanism changed into an advanced oxidation process. Therefore, organic matter removal increased in all structural fractions. The oxidation of dissolved organics resulted in the decreasing order of HPOs > TPIs > HPIs (Gong *et al.* 2008). Jin *et al.* (2016) studied the reactivity of municipal wastewater's organic content after ozonation. Their findings were that after ozonation, the HPOA fraction converted to the HPON fraction, and then the HPON fraction transformed into the HPI fraction, respectively. It was revealed that ozone preferably reacted with protein-like HPO fraction having a size fraction of <100 kDa. During ozonation, the aromaticity of HPON reduced dramatically at the lowest ozone dose, and it was also recorded that the aliphatics and ketones increased. It was concluded that original HPON comprised more electron-enriched aromatics when compared with original HPOA, hence ozone had a tendency to react with HPON before HPOA (Jin *et al.* 2016).

As mentioned above, by size and structural fractionation procedures, it is possible to characterize the wastewater in more detail to evaluate the structure-treatability relationship of compounds found in wastewater and to reveal the structural changes during treatment. By coupling these procedures with instrumental analysis such as GC-MS, LC-MS and Fourier transform infrared spectroscopy (FTIR), this may provide a deeper analysis to identify the recalcitrant components of wastewater. However, due to the complexity of wastewater samples, application of these techniques is restricted. In the scientific literature, related studies are quite limited and most of them have not yet established the relationship between the inert fraction and the size or structure. These procedures have only been included in the studies as a tool (Wang *et al.* 2017; Vimala *et al.* 2020). In one of our previous studies (Gursoy-Haksevenler & Arslan-Alaton 2015), we aimed to determine the inert size and structural fractions in OMW before and after treatment with thermal acid cracking. Our results indicated that the 'inert' OMW fractions were found to be distributed in the

Table 2 | Structural fractionation studies reported for different wastewater treatment processes

Treatment processes	Wastewater type	Aim and scope of work	Experimental approach	Major findings	Reference
Activated sludge system	Landfill leachate	Evaluation of the change of organic matter fractions after biological treatment	Treatment process: sequencing batch reactor (SBR) activated sludge process Resins used: XAD-8 and XAD-4 Investigated parameters: COD, Three-dimensional excitation-emission matrix	• By SBR, less than 14% COD was removed • Bacteria preferably consumed the neutral > acidic > hydrophilic fractions • After SBR, HPOA increased (from 43 to 60%) while HPON (from 16 to 7%) and transphilic neutral (from 15 to 5%) decreased	Cui *et al.* (2016)
Anaerobic process	Landfill leachate	Investigation of the biodegradability of leachate by applying XAD-8 resin	Treatment process: aerobic, semi-aerobic, and anaerobic batch reactors Resins used: XAD-8 Investigated parameters: BOD_5, COD, COD_{XAD} (COD of the final solution after XAD-8 resin adsorption), volatile solids and biologically degradable matter contents	• Leachate was stable when the COD_{XAD}/COD ratio was higher than 0.432 • By the formula of 1-2.084COD_{XAD}/COD, the degradability of leachate was determined • The waste degradation in anaerobic condition was inhibited after day 295	Zhang *et al.* (2012)
Coagulation, ultrafiltration, activated carbon adsorption and ozonation	Effluents from different wastewater treatment plants	Determination of the effect of treatment alternatives on structural fractionation	Treatment: coagulation, ultrafiltration, activated carbon adsorption and ozonation Resins used: XAD-8, AG-MP 50 and AG-MP 1 PSD: gel-filtration chromatography Investigated parameters: TOC and UV absorbance	• Organic compounds transformed into much smaller pore sizes and hydrophilic structures after each treatment process • HPIA (32–74%) and aquatic humic substances (AHS, 3–28%) were the main components in all effluents (corresponded to ≈55% of DOC) • AHS, HPON, and HPIA fractions varied due to the kind of wastewater and the treatment processes applied	Imai *et al.* (2002)

(*continued*)

Table 2 | continued

Treatment processes	Wastewater type	Aim and scope of work	Experimental approach	Major findings	Reference
Coagulation, adsorption, ion exchange and photocatalysis	Biologically treated sewage effluent	Characterization of organic fractions to identify ultrafiltration membrane fouling	Treatment: $FeCl_3$ coagulation, PAC adsorption, ion exchange and photocatalysis Resins used: XAD-8 and XAD-4 Investigated parameters: DOC	• Coagulation resulted in 60% removal in HPI, 39% removal in TPI and 22% removal in HPO fractions • PAC adsorption resulted in ≈70% removal in each fraction • Ion exchange resulted in 69% removal in HPI, 32% removal in TPI and 57% removal in HPO fractions • Photocatalysis resulted in 27% removal in HPI, 32% removal in TPI and 51% removal in HPO fractions	Shon *et al.* (2006a)
Thermal acid cracking	Olive mill wastewater	Determining the inert size and structural fractions before and after thermal acid cracking	Treatment: thermal acid cracking Resins used: XAD-8, XAD-4, AG-MP-50 and Duolite-A-7 PSD: filtration – 450 and 220 nm UF – 100, 50, 30, 3, 1, 0.5 kDa Investigated parameters: TOC, TPh, acute toxicity, GC-MS and FTIR	• The inert fractions were distributed in the soluble (<2 nm) and colloidal (especially in the 5–8 nm) size fractions • The inert fractions mainly originated from the HPON components • The HPON fraction was toxic/ inhibitory • GC-MS and FTIR analyses confirmed that 80% of the polyphenolic organic matter was relatively high molecular weight compounds such as lignin-like structures • 20% was relatively low molecular weight compounds such as catechol and hydroxytyrosol	Gursoy-Haksevenler & Arslan-Alaton (2015)

(*continued*)

Table 2 | continued

Treatment processes	**Wastewater type**	**Aim and scope of work**	**Experimental approach**	**Major findings**	**Reference**
Thermal acid cracking, coagulation, electrocoagulation and Fenton's reagent	Olive mill wastewater	Evaluation of the effect of chemical treatment alternatives on structural fractionation	Treatment: thermal acid cracking (pre-treatment), $FeCl_3$ coagulation, EC and Fenton's reagent Resins used: XAD-8, XAD-4, AG-MP-50 and Duolite-A-7 Investigated parameters: COD, TOC, TPh, acute toxicity	• HPO structures were the main organic components (75–95%) of the untreated OMW • Before treatment, the toxicity (100%) and TPh (56%) were distributed in the HPON fraction • HPON fraction was defined as chemically inert • Coagulation was effective in the HPOs removal while EC and the Fenton's reagent were also efficient in the HPIs removal	Gursoy-Haksevenler & Arslan-Alaton (2014b)
Coagulation and electrocoagulation	Landfill leachate	Evaluation of the effect of coagulation and EC on structural fractionation	Treatment: coagulation with ($Al_2(SO_4)_3.14H_2O$, and EC Resins used: XAD-8 and XAD-4 PSD: UF-0.5, 3, 30 kDa Investigated parameters: TOC, COD, UV_{254}, anion and cation concentrations	• After both processes, the remaining organic matter concentration was the same with different compositions • After EC, a higher percentage of organic content transformed into smaller hydrophilic structures, which were more inert • After both processes, mainly HPOs were removed (30% removal of the biodegradable DOC) • The removal for both processes were humic-like acids > HPOs> TPIs > HPIs	Labanowski *et al.* (2010)
Coagulation and Fenton's reagent	Papermaking wastewater	Evaluation of the change of organic matter after coagulation and Fenton's reagent	Treatment process: coagulation and Fenton's reagent Resins used: XAD-8 and XAD-4 Investigated parameters: DOC	• Lignin (in the colloidal form and relatively HPOs) was the major resistant organic matter • The coagulation process resulted in 60% of the HPOs fraction removal • After Fenton's reagent 73% DOC was removed and the remained distributed in 42% HPIs and 56% HPOs	Fang *et al.* (2016)

(*continued*)

Table 2 | continued

Treatment processes	Wastewater type	Aim and scope of work	Experimental approach	Major findings	Reference
Heterogeneous Fenton process	Pulp mill wastewater	Evaluation the change of organic matter after heterogeneous Fenton process	Treatment process: Heterogeneous Fenton process Resins used: XAD-8 and XAD-4 PSD: filtration - 450 nm UF-30, 10, 3 and 1 kDa Investigated parameters: COD, GC-MS, EEM fluorescence spectra	• After treatment, the highest COD removal was 75% • HPO was the largest fraction of DOC (60%) in raw wastewater • The HPO fraction decreased from 59 to 56% after treatment • In raw wastewater 70% of the organic content was >10 kDa • After treatment, the molecules with 10–30 kDa, and >30 kDa were mainly transformed into 0–1 kDa	Wang *et al.* (2018)
Chloramination	Wastewater effluent	Evaluation the cytotoxicity and genotoxicity of organic matter before and after chloramination	Treatment process: chloramination Resins used: XAD-8 and XAD-4 Investigated parameters: TOC, TN, biological assays (Chinese hamster ovary cell assay, CHO cell chronic cytotoxicity assay and CHO cell single cell gel electrophoresis assay)	• The toxicity of chloraminated effluent was significantly higher than the toxicity of raw effluent • The hydrophobic fraction isolated by XAD-8 resin was more toxic (99%) than the fraction isolated by XAD-4 (79%)	Le Roux *et al.* (2017)
Ozonation	Biologically treated municipal wastewater	Investigation the change of organic structure before and after Ozone and UV/Ozone	Treatment: ozone and UV/ozone Resins used: XAD-8 and XAD-4 Investigated parameters: TOC, UV absorbance	• After ozonation alone and with UV-C irradiation, 36 and 90% DOC were removed • In the presence of UV, organic matter removal increased in all structural fractions • The oxidation of dissolved organics resulted in HPOs > TPIs > HPIs	Gong *et al.* (2008)

BDOC, Biodegradable dissolved organic carbon.
AOC, Assimilable organic carbon.
HAAFP, Haloacetic acid formation potential.
THMFP, Trihalomethane formation potential.

soluble (<2 nm) and colloidal (especially in the 5–8 nm size interval) size fractions. These mainly originated from the HPON components present in the wastewater. The use of these tools also enabled us to identify the origin of acute toxicity in the OMW causing photoluminescence inhibition in marine photobacteria. The soluble-colloidal size fractions and particularly the HPON fraction were found to be toxic/inhibitory. For revealing, the inert fraction of OMW, GC-MS and FTIR analyses were carried out. Numerous organic compounds could be determined via employing these analyses. Relatively low molecular weight compounds were identified by GC-MS analysis, whereas FTIR results displayed a significant loss of aliphatic structures together with a rise in aromatic structures after treatment. It was confirmed that almost 80% of the polyphenolic organic matter is constituted of relatively high molecular weight compounds such as lignin-like structures where the rest (20%) was constituted of relatively low molecular weight compounds such as catechol and hydroxytyrosol (Gursoy-Haksevenler & Arslan-Alaton 2015).

Structural fractionation studies reported for different wastewater treatment processes after 2000 are summarized in Table 2.

CONCLUSIONS

It is of major importance to evaluate the nature and structural properties of wastewaters more deeply to improve the treatment performance of the applied processes. Size and structural fractionation analysis offer advantages of providing comprehensive information about wastewater characteristics and understanding the nature and removal mechanism of effluents. It is apparent that sequential filtration-ultrafiltration and resin fractionation procedures are mostly used in the scientific literature as promising tools to characterize and evaluate wastewater treatability. In this paper, sequential filtration-ultrafiltration and resin fractionation procedures were reviewed that are employed during wastewater treatment in order to more deeply investigate structural changes and removal mechanisms being observed during the applied treatment process. Case studies indicated that by employing the above-mentioned tools, it was also possible to characterize relatively ‘difficult’ wastewaters like effluents from olive oil or the textile industry. In this way, the biochemically inert components present in these wastewaters could be identified that also enabled determination of the origin of its high toxicity/poor biodegradability.

Since the efficiency of wastewater treatment process is mainly influenced by the structural- and size-based properties of the effluent, these characteristics should be carefully assessed before the selection of the most appropriate/suitable treatment system. Conclusively, the above-mentioned sequential filtration-ultrafiltration and resin fractionation procedures can assist scientists and engineers to relate wastewater characteristics and treatment performance. Moreover, these fractionation tools may also serve as major design and operation criterion during scale-up of wastewater treatment units.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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