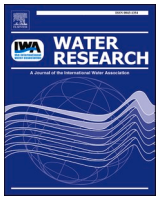
Water Research 271 (2025) 122971

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres



Quantification of high molecular weight organic carbon concentrations with LC-OCD and PHMOC for biological stability investigation of drinking water produced from surface water

R. Schurer [a,b,*], A. Brouwer-Hanzens [c], P.W.J.J. van der Wielen [c], J.H.M. van Lieverloo [d], W.A.M. Hijnen [a]

[a] Evides Water Company, PO Box 4472, Rotterdam 3006 AL, the Netherlands
[b] Department of Environmental Technology, Wageningen University, Bornse Weilanden 9, Wageningen 6708 WG, the Netherlands
[c] KWR Water Research Institute, Groningenhaven 7, 3433 PE Nieuwegein, the Netherlands
[d] Viaeterna, Rijksweg 46, Malden 6581 EN, the Netherlands

ARTICLE INFO

Keywords:
Organic carbon
Biopolymers
Biological stability
Drinking water
LC-OCD

ABSTRACT

The presence of aquatic biopolymeric organic carbon of high (> 10 - 20 kDa) molecular weight (high-MW OC) in drinking water produced from surface water affects its biological stability which may cause regrowth in disinfectant-free distribution. This study compares two analytical methods for determining the concentration of aquatic high-MW OC, namely LC-OCD (liquid chromatography – organic carbon detection) and PHMOC (particulate and colloidal high-molecular weight OC). LC-OCD entails prefiltration of the water sample, chromatographical separation of the relevant biopolymer (BP) OC-fraction, and in-line OC detection. PHMOC is based on the total OC content of the concentrate obtained after 30 kDa crossflow ultrafiltration of the water sample. LC-OCD BP and PHMOC showed a good linear correlation (R^2 0.87) for a suite of treated surface water matrices (except raw water) in the 10 – 200 µg/L concentration range, with PHMOC values being 10% – 30% higher than the corresponding LC-OCD BP value, without a clear impact of other water matrix constituents. The indicative yields and selectivities of both methods for indigenous high-MW OC obtained from the PHMOC concentrate were high (≥ 70% – 88%) but not fully complete, which may explain the observed higher PHMOC values and scatter in the PHMOC – LC-OCD BP correlation. LC-OCD BP and PHMOC displayed similar values and trends across the different seasons and treatment stages, with treated ground water and infiltrated water having the lowest (< 10 µg/L) values. Regrowth (as *Aeromonas*) levels in disinfectant-free distribution networks corresponded with the high-MW OC concentration in the treated drinking water. Overall, the two methods equivalently quantify the concentration of aquatic high-MW OC. Both methods are suitable for use in biological stability studies. The small sample volume renders LC-OCD more practical, whereas the PHMOC method enables further experimentation and characterization of the high-MW OC fraction.

Abbreviations

AIC	Akaike's information criterium (regression analysis parameter) (–)
AOC	assimilable organic carbon (µg/L C)
AOC-P17/NOX	easily assimilable OC (µg/L acetate-C equivalents)
AOC-A3	biopolymeric assimilable OC (µg/L biopolymer mix-C equivalents)
BB	building block LC-OCD fraction (µg/L C)
$BP_{s,\ p,\ c}$	LC-OCD biopolymer content; $_s$ of the water sample; $_p$ of PHMOC ultrafiltration permeate; $_c$ of the PHMOC concentrate
BP	biopolymer LC-OCD fraction (µg/L C)
CBP_{14}	cumulative biomass production during 14 days incubation (ng ATP•d/L)
CF	concentration factor in PHMOC analysis (= V_s/V_c; –)
DOC	dissolved organic carbon (mg/L)
HS	humic substances LC-COD fraction (µg/L C)
HOC	non-chromatographable (hydrophobic) LC-OCD fraction
kDa	kilo Dalton (g/mol)
LC-OCD	liquid chromatography – organic carbon detection

* Corresponding author at: Evides Water Company, PO Box 4472, Rotterdam 3006 AL, the Netherlands.
E-mail address: r.schurer@evides.nl (R. Schurer).

https://doi.org/10.1016/j.watres.2024.122971
Received 12 September 2024; Received in revised form 2 December 2024; Accepted 12 December 2024
Available online 13 December 2024

LMW	low-molecular weight LC-OCD fraction (µg/L C)
MW	molecular weight (g/mol)
OC	organic carbon (µg/L C; mg/L C)
ON	organic nitrogen (µg/L N)
PHMOC	particulate and colloidal high-MW OC (µg/L C)
R_P^2	Pearson coefficient of determination
R_S^2	Spearman-rho coefficient of determination
SEC	size exclusion chromatography
(S)UVA	(specific) UV-absorbance (L/(mg•m); 1/m)
TOC	total organic carbon (mg/L C)
V	volume; $_s$ of the water sample; $_c$ of the PHMOC concentrate (L)

1. Introduction

1.1. Relevance of high-MW OC in surface water treatment

Organic carbon (OC) is commonly present in raw and treated drinking waters as a broad mix of substances of variable molecular weight and chemistry (e.g., Fabris et al. 2008, Leenheer, 2009; Mattilainen et al., 2010). One of the OC fractions encompassed in the total aquatic OC pool comprises substances with a high (~ > 10 – 20 kDa) molecular weight (high-MW OC), which includes biopolymers such as polysaccharides, proteins, fatty acids, etc. of colloidal and particulate size (Stewart et al., 2013). This aquatic high-MW OC fraction comprises typically less than 10% of the totally present OC in raw and treated surface water (Filloux et al., 2012, van der Kooij et al., 2015; Gibert et al., 2015, Park et al., 2016, Hijnen et al., 2018(Tominaga et al., 2022) (Schurer et al., 2023)). Despite this low percentage, the high-MW OC fraction is of particular importance for the production and distribution of drinking water produced from eutrophic surface water sources. Firstly, several case studies observed positive correlations between regrowth as heterotrophic plate counts (HPC), *Aeromonas* and coliform bacteria in disinfectant-free distribution networks, and the presence of high-MW OC residuals in conventionally treated surface water after coagulation – sedimentation – rapid media filtration – main disinfection and/or oxidation – biological activated carbon filtration (BACF) (van der Kooij et al., 2015; Hijnen et al., 2018, 2024; van der Wielen et al., 2023). A maximum guideline value of 47 µg/L was proposed by van der Wielen et al. (2023) to avoid regrowth. Furthermore, high-MW OC compounds act as fouling agents or fouling promotors in membrane water treatment processes as ultrafiltration, nanofiltration andreverse osmosis, thus affecting the membrane permeability (Kennedy et al., 2005; Subhi et al., 2012; Tian et al., 2013; Zheng et al., 2014; Rahman et al., 2014; Kimura et al., 2014, 2018). Hence, the presence of high-MW OC potentially governs the membrane system's design (flux, recovery), operability (chemical cleanings, consumption of energy and chemicals, water loss) and cost (investment and consumables).

1.2. Determination of aquatic high-MW OC concentrations

Several methods are available to assess the concentrations of aquatic OC and its constituent fractions. The TOC and DOC (total organic carbon, dissolved organic carbon) methods are low-cost and commonly used, but only represent the bulk OC quantity without further distinction between its constituent fractions. Spectrophotometric methods (e.g., FTIR, F-EEM, NMR, UVT_{254}, UV-VIS) and solid phase extraction methods provide such distinction based on absorbance or hydrophobicity, but still do not yield information specifically for the high-MW OC fraction of interest (Mattilainen et al., 2011). To overcome these limitations, two methods were developed which specifically target the quantification of the high-MW OC fraction, namely LC-OCD (liquid chromatography – organic carbon detection) and PHMOC (particulate and colloidal high-MW OC) of which the principal characteristics are listed in Table 1. The LC-OCD method involves size-exclusion based fractionation of the water sample's total OC with liquid chromatography followed by in-line detection of the emanating OC, where the OC fraction with the shortest retention time is denoted 'biopolymers' (BP) and represents the high-MW OC fraction (Her et al., 2002a, 2002b; Huber and Frimmel, 1991; Lankes et al., 2009; Huber et al., 2011; Stewart et al., 2013; Brezinski and Gorzcyca, 2019). The PHMOC method involves 30 kDa pore size crossflow ultrafiltration of the water sample followed by TOC determination of the obtained concentrate, which comprises all retained colloidal and particulate high-MW OC as well as other particulate-bound OC (Van der Kooij and Veenendaal, 2013; van der Kooij et al., 2015; van der Wielen et al., 2023). LC-OCD has been widely deployed in research on the OC compositions of raw and treated waters (e.g., Baghoth et al., 2009), whereas PHMOC has been mainly used in biological stability studies in the Netherlands (van der Kooij et al., 2015, 2017; Hijnen et al., 2018; Schurer et al., 2019; van der Wielen et al., 2023). However, despite being applied in water research, several questions remain on the methods' accuracy in detecting the high-MW OC fraction, as described in the next section.

Table 1
Key properties of the LC-OCD and PHMOC methods.

Method property	LC-OCD	PHMOC
Sample volume	Small (20 mL)	Large (100 L)
Sample pretreatment	Prefiltration with 0.45 µm pore size flat-sheet membranes	None
OC separation	Single liquid chromatography column	Crossflow ultrafiltration
OC separation mechanism	By size exclusion and mass transport (diffusion)	By size exclusion according
OC concentration	Up to several mg/L	Up to several tens of mg/L
OC detection	In-line TOC measurement	TOC analysis of concentrate; correction for CF and sample background DOC
Theoretical OC fraction encompassed in result	All OC < 0.45 µm; for BP fraction > 10 kDa	All OC > 30 kDa
Analytical result (output)	OC, ON and UVA values for high-MW OC and other OC fractions of lower MW	Single value for PHMOC, no information on other OC fractions
High-MW OC concentrate isolate obtained?	No	Yes, can be used for further experimentation

1.3. Knowledge gaps and research objectives

While LC-OCD BP and PHMOC aim to quantify the same high-MW OC fraction, their distinct analytical protocols and principles render it uncertain whether both methods capture exactly the same particle size fraction, and whether they have the same yield and selectivity for the high-MW OC compounds. Consequentially, their analytical results may not be necessarily identical. A preliminary comparison by Schurer et al. (2019, 2022) showed a good correlation (R_S^2 0.77) between both methods' values (paired samples), but this sample set was of limited size and encompassed only a single treated water matrix. Furthermore, evaluation of the LC-OCD's yield and selectivity for high-MW OC has so far mostly been based on model compounds, e.g., alginate and bovine serum albumin (Lankes et al., 2009; Huber et al., 2011; Subhi et al., 2012; Dulaquais et al., 2018; Li et al., 2019; Laforce et al., 2024), for which variable yield values (50% – 110%) were reported. However, yield and selectivity have not yet been evaluated extensively for the indigenous high-MW OC, i.e., as it is present in the water sample. A specific complication here is the nonexistence of a well defined, fixed-composition mix of high-MW OC compounds which could serve as the internal calibration standard (Huber et al., 2011), since the indigenous high-MW OC is a heterogonous mixture of compounds of mostly unknown and variable chemistry (Leenheer, 2009). In addition, no validated standard protocol is available yet for the production of a sufficiently pure isolate of indigenous high-MW OC from the water sample

as is necessary for such calibration. Using a diafiltration protocol for the isolation of indigenous high-MW OC from river water, Tominaga et al. (2022) showed that the LC-OCD chromatographical retention times could differ appreciably from those of model compounds, thus casting doubt on the representativity of model compounds in LC-OCD analysis. With regard to PHMOC, investigation of yield and selectivity is yet scarce and not widely published in scientific literature. Preliminary yield determination showed variable and inconclusive results, whereas the selectivity for high-MW OC was not yet investigated (Hijnen et al., 2015).

Overall, a systematic investigation of the equivalence of both methods for indigenous high-MW OC in raw and treated surface waters is warranted, which the research presented here aims to provide. For this purpose, a suite of raw and treated surface water matrices was sampled in the field and compared on paired LC-OCD BP and PHMOC results. The equivalence of both methods was verified by estimative investigation of their respective yields and selectivities for indigenous high-MW OC using PHMOC concentrate as internal standard for the measurement, which constitutes a new approach compared to previous research which was based on model compounds. The study concludes with an evaluation of the applicability of both methods in investigation of the biological stability of drinking water.

2. Methods and materials

2.1. LC-OCD methodology

LC-OCD analyses were conducted at DOC Labor (Karlsruhe, Germany) according to the regular protocol as schematically presented in Fig. 1a and Table 1 (Huber and Frimmel, 1991; Huber et al., 2011, www.DOC-Labor, Germany). This encompassed successively the injection of 1 mL of water sample from a 20 mL OC-free vial into the phosphate-buffered mobile phase flow, filtration of the mobile phase through a 0.45 μm pore size membrane, fractionation of the DOC by passage over a chromatography column packed with weak cation-exchange resin (Tosoh HW50S), and detection of the OC-fractions after acidification and purging of inorganic carbon in a Gräntzl thin-film UV-destructor with in-line infrared measurement of the generated carbon dioxide amount. The detector's response signal was recorded as the sample's chromatogram where the OC fractions successively appeared. Based on increasing retention time and decreasing apparent MW, the biopolymer (BP), humic substances (HS), building blocks (BB), and low-MW neutral and acidic compounds (LMW) fractions were distinguished as defined by the LC-OCD method (Huber et al., 2011). The OC quantity in each appearing peak was derived with digital deconvolution and integration (Chromcalc software). The calculated difference between the sample's DOC content measured when bypassing the chromatography column and the OC quantity summed for the BP, HS, BB and LMW fractions was denoted as the non-chromatographable hydrophobic (HOC) fraction. The BP fraction was the fraction of main interest for this study as it represented the high-MW OC compounds. The practiced single-column conduct generally yielded sufficient separation of the BP and HS peaks in the chromatogram to enable meaningful quantifications as will be further discussed in Section 3.3. Organic nitrogen (ON; μg N/L) contents and specific UV-absorbances, i.e., per OC mass unit (SUVA, L/mg•m) were detected for the BP and HS fractions with in-line sensoring of the column effluent. Stated limits of detection and quantification were 5 and 11 μg/L for OC and 2 and 3 μg/L for ON (determined with potassium hydrogen phthalate and nitrate, DIN 32645, 10994), whereas high LC-OCD yields were found for various model compounds of high and low MW (Huber et al., 2011, further discussed in Section 4.3). All LC-OCD samples were processed within several days (shipping time) at DOC Labor, Germany.

2.2. PHMOC methodology

The PHMOC method (Van der Kooij and Veenendaal, 2013; Hijnen et al., 2015; Van der Kooij et al., 2015; Van der Wielen et al., 2023) involved the concentration of 100 L of the investigated water sample by recirculation/crossflow-filtration through a 30 kDa pore size hollow-fiber membrane (HF80S, Fresenius Medical Care, Germany) without any further conditioning as shown in Fig. 1b and Table 1. This membrane was used based on its availability with medical-grade quality control at the manufacturer, and its extensive use in biological stability studies which showed a good correlation between the PHMOC concentration and regrowth (Van der Kooij et al., 2015, 2017; Hijnen et al., 2018, 2024; van der Wielen et al., 2023). Verification of the suitability of the 30 kDa pore size was integrated in the current study. The investigated water sample was recirculated (300 L/h) through the membrane fibers while continuously producing permeate (50 L/h). At the end of the concentration run, a small volume of previously collected permeate was poured into the feed container, fed to the membrane module together with air entrained from the emptied feed container, and collected as the PHMOC concentrate. The obtained concentrate volume of 0.3 – 0.5 L (amounts precisely recorded for each run) was equivalent to a concentration factor (CF) value range of 200 – 400.

The collected concentrate was subjected to high-energy sonication to prevent the formation and settling of particulates. The concentrate's total organic carbon content (TOC_c) was determined by acidification of a 24 mL aliquot (vial) to pH 1 – 2 (30% HCl) and subsequent measurement in a calibrated TOC analyzer (Shimadzu V, Japan, by conversion of the OC into carbon dioxide which was measured with an infrared detector) after flushing with the sample, while continuously magnetically stirring the sample vial contents for homogenization. The water sample's dissolved organic carbon (DOC_s) content was measured in a similar way as TOC except that water samples were first filtered through a 0.45 μm membrane (Whatman Spartan 30/0.45 RC, Cytiva, USA, pre-washed with sample, NEN-EN 1484/ISO 8245, 1999). The recovery for TOC and DOC was assumed to be complete. The water sample's PHMOC value was then calculated from $PHMOC = (TOC_c - DOC_s) / CF$ which included the necessary correction for the water sample's background DOC content by the subtraction term DOC_s. All PHMOC samples were analyzed within 24 h at KWR Water Research Institute, the Netherlands, and stored at 4°C in the interim.

2.3. Water sample collection

The high-MW OC quantification as LC-OCD BP and PHMOC was investigated for a variety of water matrices which were representative of various stages of conventional and membrane-based surface water treatment steps and covered seasonal changes in water quality. The water samples were collected at three surface water treatment plants and pilot installations in the Netherlands described in Hijnen et al. (2018) and Schurer et al. (2019, 2023). Sampled matrices encompassed eutrophic raw surface (reservoir) water (the plants' common raw water source), conventionally pretreated water (after coagulation – clari-flocculation or lamella sedimentation – rapid dual media filtration), conventionally-treated water after subsequent biological activated carbon filtration (BACF), distributed drinking water (sampled in the distribution network), permeates of ultrafiltration with 10 kDa, 150 kDa and 0.12 μm pore size membranes fed with BACF filtrate, and the permeate of surface water treated with 1 kDa hollow-fiber nanofiltration and subsequent BACF-filtration (schematically depicted in Fig. S1a). Further LC-OCD BP and PHMOC data for other drinking water types, i.e., produced from (an)aerobic groundwater and dune-infiltrated sources, were taken from literature and own monitoring campaigns.

2.4. LC-OCD BP – PHMOC equivalence

The equivalence of LC-OCD BP and PHMOC and the possible impact

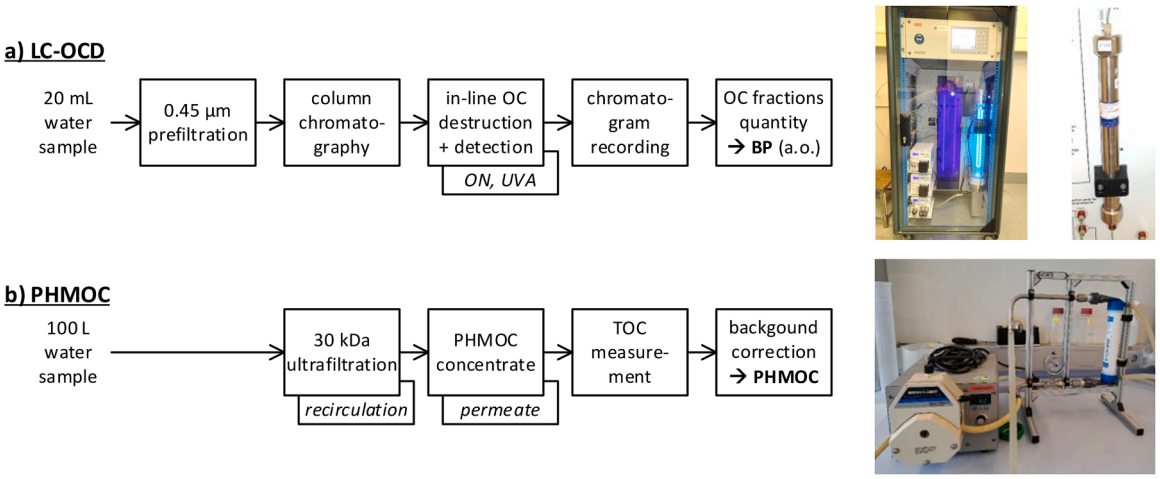


Fig. 1. Schematical depictions and images of the LC-OCD (a) and PHMOC (b) methods.

of water matrix parameters were investigated from a data set of 203 paired values obtained in the field sampling campaign described in Section 2.3 using statistical means further specified in Section 2.7. Investigated water matrix parameters comprised DOC, the LC-OCD OC fractions, calcium, iron, total cell count and ATP as indicators for cellular biomass, and chlorophyll-a as indicator for algae, since these parameters could potentially affect the high-MW OC determination because of their particle size (e.g., being excluded from the analytical result) or because of their possible interaction with the OC compounds (e.g., agglomeration into larger aggregates like humic-calcium complex formation).

2.5. Yields and selectivities of the LC-OCD and PHMOC methods for indigenous high-MW OC

The absence of gross inconsistencies in the quantification of indigenous high-MW OC as LC-OCD BP and PHMOC was checked for selected cases by estimative determination of the method's yields and selectivities using the concentrates produced with the PHMOC ultrafiltration step (Section 2.2). The concentrates were prepared from BACF-filtrate according to the regular PHMOC protocol followed by dilution with milli-q water (< 0.1 mg/L TOC) to TOC levels in the 1 to 5 mg/L range. The assumed predominance of high-MW OC compounds in the (diluted) concentrates was checked by comparing the relative sizeof the BP peak to the other peaks in the LC-OCD chromatograms of the concentrates to that in the chromatograms of the respective BACF-filtrate sample which had been used as source. The LC-OCD yield for high-MW OC was then estimated by comparing the (diluted) PHMOC concentrates' total DOC detected with LC-OCD (i.e., summed for all LC-OCD fractions) to the corresponding concentrates' TOC_c values which had been determined separately as part of the PHMOC protocol (approach akin to the yield determination for model compounds (Lankes et al., 2009; Subhi et al., 2012; Dulaquais et al., 2018; Li et al., 2019; Laforce et al., 2024). The linearity of LC-OCD BP values and the high-MW OC concentration was investigated based on LC-OCD analyses for an addition series prepared by pipetting incrementally increasing volumes of a single PHMOC concentrate (prepared from BACF-filtrate) in a blank water (milli-q water, 0.07 mg/L DOC, 8 µg/L BP, 4 µg/L PHMOC) in OC-free glass Erlenmeyer flasks (600 mL) and mild manual agitation. The impact of the LC-OCD's 0.45 µm prefiltration on the BP result was checked by parallel LC-OCD analyses with and without the 0.45 µm prefiltration for the same sample (raw water, pretreated water, BACF-filtrate, PHMOC concentrate). The LC-OCD selectivity for high-MW OC, i.e., whether the high-MW OC compounds were located exclusively in the BP peak of the chromatograms and not in any other OC fractions' peak, was estimated indirectly from the distribution of ON and SUVA in the concentrate's fractions as measured by LCOCD (Section 2.1), under the assumption that ON was predominantly associated with the protein subfraction of high-MW OC compounds and that UVA was predominantly associated with the LC-OCD HS fraction (Huber et al., 2011; Stewart et al., 2013).

The yield of the PHMOC method for indigenous high-MW OC was estimated from the LC-OCD BP values of the field sample (BP_s) and the permeate which passed the PHMOC ultrafiltration membrane during the concentration step (BP_p; composite of 4 subsamples collected evenly time-distributed during the concentration step) as $(1 - BP_p/BP_s) \times 100\%$, as well as from the PHMOC concentrate (BP_c) as $(BP_c / (CF \times BP_s)) \times 100\%$. Furthermore, the fit of the mass-balance $(BP_c / CF + BP_p) / BP_s \times 100\%$ was checked. The selectivity of the PHMOC method for high-MW OC was estimated from the LC-OCD BP values of (diluted) concentrates as a percentage of the totally present DOC. This included accounting for the contribution of the field sample's residual background OC which was estimated with theoretical calculations assuming complete retention of the BP fraction and full passage of the other (non-BP) OC fractions by the PHMOC ultrafiltration membrane.

2.6. Application of the LC-OCD and PHMOC methods in biological stability studies

The correlation of LC-OCD BP and PHMOC concentrations with biological stability parameters in drinking water, and with regrowth in disinfectant-free distribution networks was evaluated for multiple cases of drinking water prepared from surface water, groundwater and dune-infiltrated water from literature data (Baghoth et al., 2009; Zheng et al., 2015; Hijnen et al., 2015, 2018; van der Kooij et al., 2015, 2017; Schurer et al., 2019, 2023; Ketelaars et al., 2023; van der Wielen et al., 2023) and own additional sampling data. Finally, both methods were appraised on their suitability for application in biological stability studies.

2.7. Statistical procedures

All statistical analyses were conducted with Jamovi® version 2.2.5. Data points were considered to be potential outliers if their value was outside the data set's median value ± three times the p50 – p75 and p50 – p25 interquartile range without a clear explanation at hand. Normality of data was checked with Kolmogoroff-Smirnov, and homogeneity of variances was checked with Levene. The equivalence between LC-OCD BP and PHMOC was investigated with Pearson ordinary least-squares correlation analysis. The impact of water quality parameters on the LC-OCD BP – PHMOC correlation was investigated with multivariate regression analysis by adding the respective water quality parameter as covariate one at a time. The strength of the LC-OCD BP – PHMOC correlation and multivariate regression was assessed as Spearman-rho

coefficient of determination (R^2_S) since the (untransformed and log-transformed) LC-OCD BP and PHMOC data were not not-normally distributed ($p = 0.01$). Correlation and regression significance and reliability were assessed as p-value and 95% confidence interval, normality and homoskedasticity of the residuals, and the (relative) value of Akaike's information criterion (AIC) as further detailed in S2. Similarity between data sets (significance criterion $p > 0.05$) was checked with Student's t-test for normal-distributions and homogeneous variances, and with Wilcoxon signed-rank otherwise.

3. Results

3.1. Equivalence of the LC-OCD BP – PHMOC methods

The paired-sample comparison of the high-MW OC concentrations determined as LC-OCD BP and as PHMOC covered the concentration range of 0 to ~ 400 µg/L. Fig. 2a shows the results for all 203 water samples, whereas Fig. 2b excerpts the 0 – 250 µg/L concentration range encountered in pretreated and treated drinking water. LC-OCD BP and PHMOC values increased both in the order NF permeate < UF permeate < BACF-filtrate ~ distributed water < pretreated water < raw water. The correlation between LC-OCD BP and PHMOC for the complete data set of all water matrices was according to the equation (mean ± 95% confidence margin) PHMOC = – 1 ± 8 + (1.17 ± 0.07) × LC-OCD BP (five data points were excluded as outliers from the statistical analysis, details in S2). Although the coefficient of determination was high (R^2_S 0.92, $p = 0.01$), other reliability criteria (notably the normality of residuals and homoskedasticity) were not met. Omission of the raw water data points from the total set resulted in the reliability criteria being met, and yielded a significant and strong (R^2_S 0.88, p 0.01) linear correlation PHMOC = – 8 ± 6 + (1.23 ± 0.07) × LC-OCD BP thus describing all water samples except raw water (Fig. 2b). Overall, LC-OCD BP and PHMOC yielded values of similar magnitude in the 10 – 200 µg/L high-MW OC concentration range for all water matrices except raw water, with PHMOC having ~ 23% higher average values than LC-OCD BP.

3.2. Water matrix and LC-OCD prefiltration effects

The inclusion of water matrix parameters (compositions of the various matrices presented in Table S1b) as covariate in the multivariate regression analysis did not show any clear unambiguous improvement of the LC-OCD BP – PHMOC correlation (S2). Taking chlorophyll-a as covariate improved correlation for the full sample data set (i.e., including raw water) in AIC (Akaike's Information Criterium) value, but simultaneously reduced the correlation's strength (R^2_S declining to 0.66, $p = 0.01$). Total cell count as covariate improved AIC less but maintained the high R^2_S value. However, the correlation reliability criteria for residuals' normality and homoskedasticity were still not met. For the data set encompassing all matrices but excluding raw water, correlation improved slightly (AIC reduction ≤ 49%, R^2_S remaining high at 0.87) for turbidity, TCC or iron as covariate, whereas DOC, the HS, BB, LMW, HOC LC-OCD fractions, calcium and ATP showed no effect. Overall, no evidence was found for a clear and significant impact of the investigated water matrix parameters on the LC-OCD BP – PHMOC equivalence.

3.3. LC-OCD BP and PHMOC yield and selectivity

Fig. 3 shows typical LC-OCD chromatograms of the field samples (raw water, pretreated water, BACF-filtrate etc.) and of the (diluted) concentrates which were produced by the PHMOC ultrafiltration step of BACF-filtrate samples. The BP peak was generally well distinguishable from the subsequent HS peak, though peak separation up to baseline restoration was not always complete (best visible in Fig. 3 for raw water and PHMOC concentrate). According to the LC-OCD chromatogram peak sizes, the relative BP content of the PHMOC concentrates amounted to 70% ± 7% of the total (sum for all LC-OCD fractions) OC. These levels were clearly increased compared to the 4% ± 2% BP in the BACF-filtrate field samples from which the concentrates had been obtained ($N = 10$, $p < 0.01$). The PHMOC concentrates were thus predominantly composed of BP at contents well above those of the non-concentrated field samples, and were therefore considered to be suitable for further use in the investigation of the yield and selectivity of each method for indigenous high-MW OC.

The LC-OCD's total DOC sum of the BP, HS, BB and LMW fractions amounted to 88 ± 12% of the respective concentrate TOC_c ($N = 10$; Table 2), thus indicative of a high yield for a medium with a high relative and absolute content of indigenous high-MW OC substances. The addition series test showed a strong linear correlation (R^2_S 0.99) between the LC-OCD BP concentration value and the incremental addition of PHMOC concentrate with the regression line intersecting through the origin (Fig. S3). No significant lowering of the BP concentration occurred during sample storage (S4a). LC-OCD BP concentrations with the standard 0.45 µm pre-filtration were slightly lower for the same sample without the prefiltration (5 ± 8%, just significant at p 0.04, N = 12, 70 – 2200 µg/L BP range; Table S4b). Organic nitrogen (ON) was almost exclusively (96 ± 2%) present in the LC-OCD BP fraction of field samples as well as of PHMOC concentrates (Table S4c). Based on these results, no major inconsistencies or errors were evident in the LC-OCD results for indigenous high-MW OC, and the method was thus considered to be useable for further evaluation of the yield and selectivity of the PHMOC method.

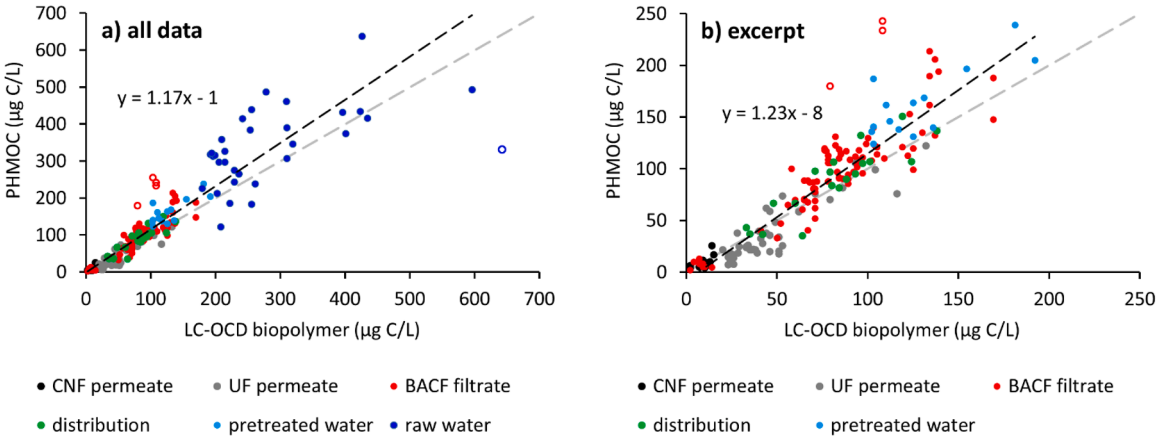


Fig. 2. Correlation between LC-OCD biopolymer (BP) and PHMOC: a) for all (treated) surface water matrices; b) detailed for the 0 – 250 µg/L high-MW OC concentration range and excluding raw water. Striped black lines denote the correlation equation; striped grey lines represent equality; open symbols denote outliers.

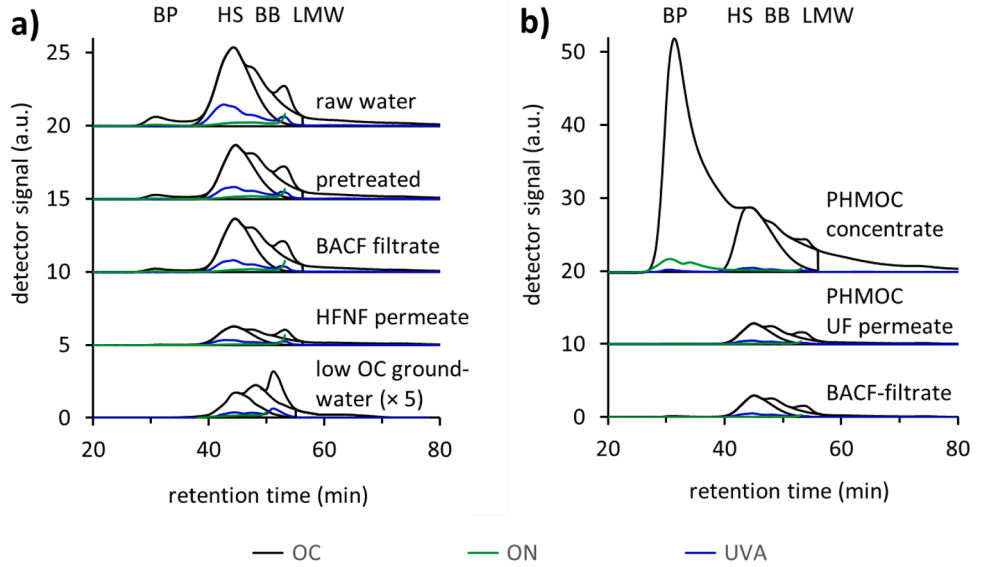


Fig. 3. Typical LC-OCD chromatograms of: a) the investigated water matrices (vertical scale expanded 5× for low OC ground water); b) PHMOC concentrate (undiluted), PHMOC UF permeate, original water sample (BACF-filtrate).

Table 2
LC-OCD and PHMOC yields for indigenous high-MW OC. BP_s: LC-OCD BP content of the original water sample; BP_c: of the PHMOC concentrate; BP_p: of the PHMOC ultrafiltration permeate; CF: PHMOC concentration factor; n.d.: not determined.

	LC-OCD DOC yield on concentrates' TOC_c			PHMOC yield and mass balance for LC-OCD BP						
Concentrate source sample i.d.	PHMOC TOC_c (mg/L)	LC-OCD total DOC (mg/L)	Yield as (LC-OCD DOC/ TOC_c)	BP_s (µg/L)	BP_p (µg/L)	BP_c (µg/L)	Yield as (1 – BP_p/BP_s)	CF (×)	Yield as (BP_c / (CF × BP_s))	Mass balance fit
BACF 1	23	18	77%	64	16	11,431	75%	369	48%	74%
BACF 2	22	22	98%	56	23	12,753	59%	309	74%	115%
BACF 3	16	13	82%	48	6	8,994	88%	211	89%	101%
BACF 4	16	14	88%	60	1	10,594	98%	211	84%	85%
BACF 5	32	29	90%	101	6	21,905	94%	282	77%	83%
BACF 6	32	37	116%	124	2	27,282	98%	282	78%	80%
BACF 7	37.3	35	94%	102	n.d.	29,048	n.d.	346	82%	n.d.
BACF 7+0.12 µm	17.1	13.7	80%	53	n.d.	10,210	n.d.	233	83%	n.d.
BACF 7+150 kDa	9.3	7.4	80%	44	n.d.	4,856	n.d.	246	45%	n.d.
BACF 7+10 kDa	8.9	6.7	75%	41	n.d.	4,169	n.d.	266	38%	n.d.
mean ± stdev			88 ± 12%				85 ± 16%		70 ± 18%	90 ± 15%

The LC-OCD BP concentrations in the PHMOC ultrafiltration permeates were clearly much lower than in the original water sample, resulting in yields based on the LC-OCD BP fraction of 85% ± 16% (based on BP_p and BP_s, N = 6) and 70% ± 18% (based on BP_c and BP_s, N = 10) (Table 2), whereas the BP mass-balance was 90% ± 15% (N = 6). The sum of all LC-OCD fractions in the PHMOC ultrafiltration permeates was 6% ± 10 % lower than that of the respective field sample, which was mainly attributable to the BP retention but possibly also a minor retention of the other OC fractions. The PHMOC concentrates comprised 70% ±7% of BP, 21% ± 6% of HS and building blocks, and 9% ± 3% of low-MW compounds (N = 10, Fig. 4). These values still included the residual background OC of the BACF-filtrate from which the concentrates had been produced. For the conditions applicable for PHMOC concentration of BACF filtrate, theoretical calculations showed that the PHMOC concentrate should theoretically contain approximately 90% BP as further detailed in S5 based on the following assumed conditions: average BP and DOC content of the BACF-filtrate, 100% retention of BP, 100% passage of the other OC-fractions, and average PHMOC CF value. The actually measured BP content of the PHMOC concentrate was thus 20% lower than theoretically expected, suggesting that the selectivity of the PHMOC method for indigenous high-MW OC was incomplete by this amount.

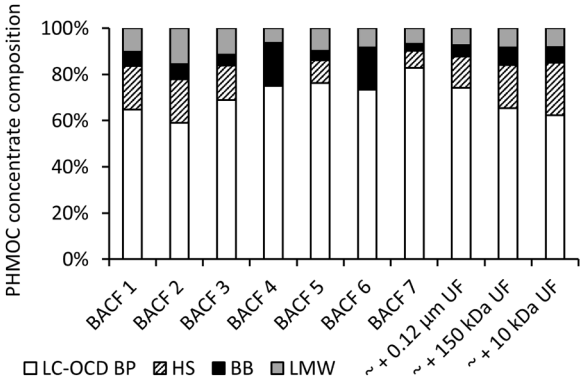


Fig. 4. LC-OCD composition of the PHMOC concentrates.

3.4. Application of LC-OCD and PHMOC methods in water treatment and biological stability studies

LC-OCD BP and PHMOC concentrations displayed similar seasonal variations in raw and treated surface water (S6). The raw water saw high but variable values during the summer periods of long daylight and

increased water temperature due to (extracellular) biopolymer production by algae during blooms. Removal of LC-OCD BP and PHMOC in the pretreatment and BACF filtration steps was higher in summer than in winter. Removal of LC-OCD BP and PHMOC with membrane treatment increased as the membrane pore size reduced. LC-OCD and PHMOC were thus both capable of discerning changes in the high-MW OC content in drinking water treatment conditions.

Fig. 5 shows the average concentrations of high-MW OC as LC-OCD BP and PHMOC, biological stability parameters, and regrowth in disinfectant-free distribution networks for treated surface water, ground water and dune-infiltrated water. Biological stability was represented as biodegradable biopolymers (AOC-A3 bio-assay, Sack et al., 2011), the total microbial growth potential (CBP_{14}, i.e., the cumulative ATP concentration during 14 days in the directly-incubated water sample (van der Kooij and Veenendaal, 2014), and as *Aeromonas* counts which is used in the Netherlands as a technical indicator for regrowth (van der Wielen et al., 2023; Hijnen et al., 2018, 2024). Both LC-OCD BP and PHMOC showed much lower values (< 10 µg/L) for treated groundwater and dune-infiltrated water than for treated surface water. Treated surface water also possessed clearly higher levels of biodegradable biopolymers and total growth potential than treated groundwater and infiltrated water. Moreover, regrowth was substantial in four of the five distribution networks supplied with drinking water produced from surface water, whereas regrowth occurred only in one of the seven studied networks supplied with treated groundwater and dune infiltrated water.

4. Discussion

4.1. Equivalence of high-MW OC quantification by LC-OCD BP and PHMOC

This study demonstrated that two different analytical methods used to quantify high-MW OC in water, LC-OCD BP and PHMOC, are equivalent in the quantification of aquatic high-MW OC in the concentration range of 0 – 200 µg/L with the PHMOC values being on average ~ 20% higher than LC-OCD BP values. Water matrix effects were not evident except for chlorophyll-a in raw water. However, the possible presence of matrix effects may require further evaluation for waters with wider concentration ranges, as was shown by Tominaga et al. (2022) who observed that the presence of calcium impacted the separation of high-MW OC with diafiltration. The values obtained in the current study for indicative yield and selectivity of the methods for indigenous high-MW OC using the PHMOC concentrates were high (average 88% for LC-OCD, 70% – 85% for PHMOC), which supported the validity of the observed equivalence between the two methods. These values must be considered as estimations since the both methods were crosschecked with each other because a complete validation of either method was not available for indigenous high-MW OC. Overall, the step-wise approach applied here revealed no indications of gross inconsistencies in the observed yield and selectivity of either method for indigenous high-MW OC, nor any other major methodological errors, and therefore the adopted approach appeared justified. The details of the investigated aspects of high-MW OC quantification are discussed in the subsequent sections.

4.2. Impact of particle size boundaries and water matrix on LC-OCD BP – PHMOC equivalence

Based on the methods' respective protocols, the LC-OCD BP results nominally represented all OC compounds between 10 kDa and 0.45 µm (which is the pore size of the sample prefiltration prior to the OC-measurement) (Her et al., 2002a; Huber et al., 2011; Hidayah et al., 2016; Brezinski and Gorzcyca, 2019), whereas the PHMOC results represented all OC compounds with a particle size > 30 kDa and all lower-MW compounds associated with particles of > 30 kDa size. The exclusion of the > 0.45 µm particulate-size OC subfraction from the LC-OCD results (due to the sample prefiltration) could theoretically result in LC-OCD BP values lower than PHMOC values. The impact was shown here to be of minor magnitude for treated water, but for raw surface water a potential but variable impact of raw water chlorophyll-a and biomass may exist for high-MW OC quantification. The lacking correspondence between LC-OCD BP and PHMOC for raw water suggests that the two methods are not equivalent for this water matrix. This is possibly explained by the presence of large organic particulates such as algal and bacterial biomass in the raw water, which are included in the PHMOC result, but are excluded from the LC-OCD BP result because of the latter's 0.45 µm pore size prefiltration and the column pore width. The presence of algal matter in the raw water was evidenced from the ~ 5 µg/L chlorophyll-a encountered in the raw water. This equated to ~ 0.3 mg/L TOC when assuming a conversion factor of 0.07 mg TOC per µg chlorophyll-a (Portielje and van der Molen, 1998), which amount was significant compared to the 300 – 600 µg/L PHMOC and LC-OCD BP concentration for this water matrix. Since the size of algal cells is typically > 5 µm, the corresponding amount of high-MW OC would be included in the PHMOC result but excluded from the LC-OCD BP result. Because the presence of algae was highly variable in the investigated raw water (Schurer et al., 2022), the difference in high-MW OC quantification between PHMOC and LC-OCD BP may fluctuate accordingly as observed in this study. Using LC-OCD BP for raw water samples thus gives the high-MW OC concentrations for the size range of the smaller

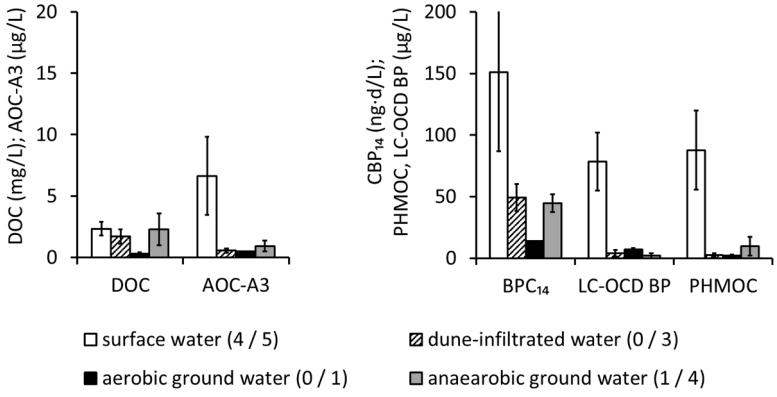


Fig. 5. Comparison of various drinking water types on high-MW OC concentrations as LC-OCD BP and PHMOC, microbial growth potential (CBP_{14}), biodegradable biopolymers (AOC-A3) and regrowth in the distribution network given in parentheses in the legend as number of cases with high *Aeromonas* regrowth versus (/) the total number of studied cases. Bars represent average values, error bars denote standard deviation, results based on paired and unpaired data, data from Baghoth et al. (2009), Zheng et al. (2015), Hijnen et al. (2015, 2018), van der Kooij et al. (2015, 2017), Schurer et al. (2019, 2023), Ketelaars et al. (2023), van der Wielen et al. (2023) and data acquired in the current study, full details provided in S7.

particles which predominate in pretreated and treated drinking water.

Any association of low-MW compounds with inorganic or organic particles or agglomerates of MW > 30 kDa would result in higher PHMOC vis-á-vis LC-OCD BP values. On the other hand, the LC-OCD BP fraction included the OC compounds in the 10 – 30 kDa particle size range unlike the PHMOC result, which could theoretically result in the values of LC-OCD BP being higher than those measured as PHMOC. There is no readily available means to determine the involved OC quantities independently except by conducting PHMOC ultrafiltration with membranes of lower pore size (e.g., 10 kDa). Nevertheless, the relatively low intercept value (– 8 μg/L) and the ratio value of 1.23 being close to unity observed in the LC-OCD BP – PHMOC equivalence equation for the 0 – 200 μg C/L concentration range (Section 3.1) suggest that the majority of the high-MW OC compounds measured with the two methods lie in the same, i.e., largely overlapping, OC particle size range. Still, the size distribution of the OC particles will vary between water samples, and could thus be a partial explanation for the scatter observed in the LC-OCD BP – PHMOC equivalence plot (Fig. 2).

4.3. LC-OCD yield and selectivity

The high yield value of 88% ± 12% and the linear relationship of the LC-OCD BP value with the quantity of the PHMOC concentrates in the addition series indicated that the LC-OCD method was capable of quantifying the indigenous high-MW OC presence appropriately. The largely, but not fully complete BP – HS peak separation (baseline restoration) may introduce a minor inaccuracy (estimated < 10%) in the BP value. The observed yield value was in line with data from Huber et al. (2011) of 85% – 100% for the model compounds ferritin, thyroglobulin, albumin, ovalbumin and myoglobin, and 101% ± 15% for pullulanes, dextranes and sodium polystyrene sulfonates with MW of 5 to 500 kDa. However, also much lower yield values of 50% – 80% have been observed for sodium alginate and bovine serum albumin (Lankes et al., 2009; Subhi et al., 2012; Dulaquais et al., 2018; Li et al., 2019; Laforce et al., 2024), which was mostly attributed to incomplete oxidation in the thin-film reactor and/or possible degradation in the column. Whether this variability in yields would also occur for indigenous high-MW OC was not investigated in the current study but warrants further attention, by running analysis of high-MW OC concentrates as was conducted here.

The near-exclusive presence of ON in the LC-OCD BP fraction indicated that LC-OCD BP was highly selective for high-MW OC compounds of a high ON-content, e.g. proteins and DNA. The TOC, ON and HS-SUVA deployed in this study to assess the yield and selectivity of the LC-OCD method were not capable of detecting high-MW OC substances which have low ON and SUVA values such as polysaccharides and fatty acids, proteoglycans and glycoproteins where carbohydrates and proteins co-exist (Santschi et al., 2020), and therefore no firm conclusions can be drawn specifically for these substances. The BP fraction N:C mass ratio of 0.12 ± 0.05 for the water matrices (Table S1b) investigated here was below the typical value of 0.3 for proteinic matter (Huber et al., 2011), thus indicating that non-proteinic substances comprised about half of the BP fraction. Still, the high yields observed in this study for the concentrates suggested that other non-proteinic compounds were correctly represented in the LC-OCD BP peak, although further confirmation is warranted (Lankes et al., 2009). Furthermore, the LC-OCD's non-chromatographable hydrophobic HOC fraction was potentially also of concern, since the concentration in the field samples (5% ± 4% of the total LC-OCD's DOC) were in the same range as that of the BP and thus not negligible. However, no interference of the HOC fraction was noted, as there was no significant correlation between HOC and PHMOC, BP nor any other LC-OCD fraction, and HOC values were not changed by the LC-OCD's prefiltration, PHMOC ultrafiltration, nor by 10 kDa ultrafiltration of BACF-filtrate (details in S4d). More accurate LC-OCD BP yields could be obtained by running LC-OCD columns in tandem (Kimura et al., 2018; Tominaga et al., 2022).

4.4. PHMOC yield and selectivity

The PHMOC method was a further development of the protocol presented by Heijnen et al. (2009) for the detection of low concentrations of viruses, phages, bacteria and protozoa in large sample volumes with cross-flow ultrafiltration concentration by van der Kooij et al. (2015) to quantify high-molecular and particle-bounded OC or biopolymers in drinking water. Previous attempts to validate the PHMOC method for the quantification of high-MW OC involving repeated PHMOC concentration of a high-MW OC concentrate were inconclusive. Highly variable yield values well below and above 100% were encountered, which may have been caused by possible chemical/physical interactions (e.g., agglomeration) of the various concentrated (in)organic compounds present in the water samples (Hijnen et al., 2015). Also, the selectivity of the PHMOC for high-WM OC compounds was not investigated at that time. The 70% – 86% BP retention observed in the current study during the PHMOC ultrafiltration step indicated a high yield of high-MW OC (measured as LC-OCD BP) for the PHMOC method. The observed values were in the same range as found in similar experiments involving LC-OCD BP determination of concentrates obtained with ultrafiltration (82% for seawater with 1 kDa membrane in Yin et al. (2019); 67% – 85% for lake and river waters with 13 kDa membranes diafiltration in Kimura et al. (2018) and Tominaga et al. (2022)). The slightly incomplete yield could theoretically result in a correspondingly limited underestimation of the high-MW OC quantity as PHMOC. Possible reasons for this are the presence of high-MW OC compounds with a MW of < 30 kDa, and the PHMOC ultrafiltration membrane comprising pores with a higher molecular weight cut-off than the nominal 30 kDa. A more exact value for the PHMOC yield could not be achieved here as the ultrafiltration permeate BP content was near the LC-OCD BP detection limit. The impact of the applied concentration factor and crossflow velocity on the PHMOC yield, e.g., the possible formation of a gel/cake layer on the UF membrane concentrate-side surface which could result in incomplete release of high-MW OC compounds and hence incomplete yield for high-MW OC, was not specifically investigated in the current study. This aspect, as well as the membrane pore size distribution, and the inclusion of a diafiltration step on the yield of the PHMOC method is recommended for further optimization of the high-MW OC separation.

The PHMOC concentrates comprised 70% ± 7% LC-COD BP, and thus 30% of other organic compounds. This presence of non-BP compounds aligned with the chemical characterization of similar PHMOC concentrates showing compositions of 40% – 65% carbohydrates, 10% proteins, and 30% – 40% of other, unidentified OC-compounds (van der Kooij et al., 2015, 2017; Hijnen et al., 2018). Of this 30%, half could be explained by the contribution of the sample's background DOC, but the PHMOC concentrate thus still contained 10% – 20 % less BP than expected, thus suggesting that the PHMOC selectivity for high-MW as LC-OCD BP was high but not 100%. As the PHMOC sample background correction term (Section 2.2) does not compensate for this latter excess, the obtained PHMOC value could theoretically overestimate the high-MW OC content with 10% – 20%. Furthermore, the observed shifts between the respective SUVA-values of the BACF-filtrate and the PHMOC concentrate could theoretically point to a lowering of the PHMOC selectivity for BP of maximally 16% as further detailed in S4e, but this could not be further substantiated with the available data as the involved OC compounds were of unknown chemistry.

4.5. High-MW OC and biological stability

The extensive study by van der Wielen et al. (2023) encompassing 34 surface water, groundwater and infiltrated water treatment plants showed significant positive correlations between the presence of high-MW OC and the bacterial indicator parameters for regrowth heterotrophic plate count (HPC) and *Aeromonas*. That study also derived a guidance value for high-MW OC of 47 μg/L as the upper limit for treated

surface water to avoid undesirable regrowth levels. The data used for the current study partially differed from those used in van der Wielen et al. (2023) but resulted in similar findings and thus corroborated the potential importance of high-MW OC for biological stability parameters and regrowth, including the distinction between drinking water produced from surface water and the other water types. Based on average values, higher AOC-A3 concentrations coincided with increased regrowth of *Aeromonas* in treated surface water (Fig. 5, Hijnen et al., 2018), although the correlation between AOC-A3 and high-MW OC (as PHMOC) was weak (van der Wielen at al., 2023). The variability in values of AOC-A3: high-MW OC concentration ratio observed in BACF-filtrate (paired data, Schurer et al., 2022) indicated that AOC-A3 is a small and variable fraction of the total high-MW OC in these samples. No correlation between low-MW AOC (AOC-P17/NOX) and regrowth was found (Hijnen et al., 2018; van der Wielen et al., 2023). Overall, these studies underscore the importance of measuring high-MW OC in drinking water for biological stability studies, with inclusion of other factors such as the presence of iron, biofilm, invertebrates, and the hydraulic configuration of the distribution network (; Wagenvoort et al., 2023; Hijnen et al., 2024).

4.6. Method selection for application in water quality assessment

The results of this study demonstrate that LC-OCD BP and PHMOC both have ample discriminate power to adequately distinguish high-MW OC levels in various water types, water matrices, and biological stability regimes. Their respective values can be used interchangeably (except for raw water) when based on the average of multiple data points and taking the observed relative difference of 20% into account. Hence, in principle either method can be used to quantify aquatic high-MW OC. The small sample volume (20 mL) needed for the LC-OCD method is convenient for water sample collection in the field. Unlike the PHMOC method, LC-OCD yields additional information on the other OC fractions. The PHMOC method is more laborious due to the larger sample volume (100 L) and manual concentration step, but on the other hand yields a high-MW OC isolate which offers the opportunity for further experimentation to determine the organic and inorganic composition of the $>$ 30 kDa particle size fraction (van der Kooij et al., 2015; Hijnen et al., 2015, 2018), to conduct microbial growth potential tests (Schurer et al., 2022), and to perform membrane fouling tests (Jermann et al., 2009; Kimura et al., 2014, 2018; Yin et al., 2019). However, the LC-OCD and PHMOC methods encompass a multitude of manipulable variables (LC-OCD: column type, age, packing and backpressure, eluent type and flowrate, etc., Her et al., 2002b; Mackie et al., 2022; PHMOC: CF, membrane type, crossflow rate, filtration flux, direct filtration/diafiltration; and for both methods: DOC, TOC autosampling and determination). The impact of these variables on the analytical result warrants further study as well as interlaboratory testing (round-robins) with the same batch of (diluted) high-MW OC concentrate to promote reliability and comparability of the results.

5. Conclusions

The quantification of the concentration of aquatic biopolymeric particulate and colloidal organic substances of high molecular weight (high-MW OC) and its relevance for biological stability was investigated in raw and treated surface water comparing two distinct analytical methods, namely the LC-OCD (liquid chromatography – organic carbon detection) biopolymer fraction (LC-OCD BP) and 30 kDa ultrafiltration-concentration based PHMOC (particulate and colloidal high-MW OC), with the following conclusions:

Both methods were equivalent, i.e., yielded similar quantitative results for the concentration of high-MW OC in water samples in the $<$ 200 µg/L concentration range, with PHMOC values being approximately 20% higher than LC-OCD biopolymer values. There were no obvious effects of the water matrix on the equivalence, except for the raw surface water due to exclusion of particulate biomass from the LC-OCD BP result.

- Yield and selectivity of LC-OCD biopolymer and PHMOC for indigenous high-MW OC were high ($\geq$ 70% – 85 %) and no major inconsistencies in the methods' results were observable.
- Biological stability parameters (growth potential, assimilable biopolymers), regrowth in disinfectant-free distribution networks and LC-OCD BP and PHMOC concentration levels were higher for treated (surface) water than for treated groundwater and dune-infiltrated water.
- The LC-OCD method is practical to handle in the field because of the small sample volumes and provides additional information on the whole OC-matrix, whereas the PHMOC method produces a high-MW OC isolate which can be used for further experimentation or characterization of the involved organic compounds.
- Further optimization of both methods for the quantification of indigenous high-MW OC is recommended, by investigating the impact of the methods' operational settings, determination of detection- and quantification limits, and institution of round-robin testing.

CRediT authorship contribution statement

R. Schurer: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **A. Brouwer-Hanzens:** Resources, Methodology. **P.W.J.J. van der Wielen:** Writing – review & editing, Resources, Methodology, Conceptualization. **J.H.M. van Lieverloo:** Software, Methodology, Formal analysis. **W.A.M. Hijnen:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: A. Brouwer-Hanzens and P.W.J.J. van der Wielen: employment at KWR Water Research Institute, the Netherlands.

Acknowledgements

The authors wish to express their gratitude to Dr. S. Huber of DOC Labor, Germany, for proofreading the manuscript and contributing specific expertise on LC-OCD.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2024.122971.

Data availability

Data will be made available on request.

References

Baghoth, S.A., Dignum, M., Grefte, A., Kroesbergen, J., 2009. Characterization of NOM in a drinking water treatment process train with no disinfectant residual. Wat. Sci Technol. 9, 379–386.

Brezinski, K., Gorczyca, B., 2019. An overview of the uses of high performance size exclusion chromatography (HPSEC) in the characterization of natural organic matter (NOM) in potable water, and ion-exchange applications. Chemosphere 217, 122–139.

Dulaquais, G., Breitenstein, A.C.J., Waeles, M., Marsac, R., Riso, R., 2018. Measuring dissolved organic matter in estuarine and marine waters: size-exclusion chromatography with various detection methods. Environ. Chem. 15, 436–449.

Fabris, R., Chow, C.W.K., Drikas, M., Eikebrokk, B., 2008. Comparison of NOM character in selected Australian and Norwegian drinking waters. Water Res. 42, 4188–4196.

Filloux, E., Labanowski, J., Croué, J.P., 2012. Understanding the fouling of UF/MF hollow fibres of biologically treated wastewaters using advanced EfOM characterization and statistical tools. Biores. Technol. 118, 460–468.
Gibert, O., Lefèvre, B., Teuler, A., Bernata, X., Tobella, J., 2015. Distribution of dissolved organic matter fractions along several stages of a drinking water treatment plant. J. Water Process Eng. 6, 64–71.
Heijnen, L., Brouwer-Hanzens, A.J., Veenendaal, H.R., 2009. Concentration method using hemoflow ultrafiltration; the protocol. Techneau Project Deliverable 3.2.11.
Her, N., Amy, G., Foss, D., Cho, J., Yoon, Y., Kosenka, P., 2002a. Optimizing of method for detecting and characterizing NOM by HPLC size exclusion chromatography with UV and online DOC detection. Environ. Sci. Technol. 36, 1069–1076.
Her, N., Amy, G., Foss, D., Cho, J., 2002b. Variations of molecular weight estimation by HP-size exclusion chromatography (HPSEC) with UVA versus online DOC-detection. Environ. Sci. Technol. 36, 3393–3399.
Hidayah, E.N., Chou, Y.C., Yeh, H.H., 2016. Using HPSEC to identify NOM fraction removal and the correlation with disinfection by-product precursors. Water Sci. Technol. Water Supply 16 (2), 305–313.
Hijnen, W.A.M., Bereschenko, L., van Veen, A., van der Wielen, P.W.J.J., 2015. Validation and standardization of the hemoflow determination of particle-bound high-molecular weight organic carbon in water (in Dutch). KWR, the Netherlands, BTO 2015.057.
Hijnen, W.A.M., Schurer, R., Bahlman, J.A., Ketelaars, H.A.M., Italiaander, R., van der Wal, A., van der Wielen, P.W.J.J., 2018. Slowly biodegradable organic compounds impact the biostability of non-chlorinated drinking water produced from surface water. Water Res. 129, 240–251.
Hijnen, W.A.M., Brouwer-Hanzens, A., Schurer, R., Wagenvoort, A.J., van Lieverloo, J.H. M., van der Wielen, P.W.J.J., 2024. Influence of biopolymers, iron, biofouling and Asellus aquaticus on Aeromonas regrowth in three non-chlorinated drinking water distribution systems. J. Water Process Eng. 61, 105293.
Huber, S.A., Frimmel, F.H., 1991. Flow injection analysis of organic and inorganic carbon in the low-ppb range. Anal. Chem. 63, 2122–2130.
Huber, S.A., Balz, A., Abert, M., Pronk, W., 2011. Characterisation of aquatic humic and non-humic matter with size-exclusion chromatography e organic carbon detection - organic nitrogen detection (LC-OCD-OND). Water Res. 45, 879–885.
Jermann, D., Pronk, W., Boller, M., Schäfer, A.I., 2009. The role of NOM fouling for the retention of extradiol and ibuprofen during ultrafiltration. J. Membr. Sci. 329, 75–84.
Kennedy, M.D., Chun, H.K., Quintanilla Yangali, V.A., Heijman, B.G.J., Schippers, J.C., 2005. Natural organic matter (NOM) fouling of ultrafiltration membranes: fractionation of NOM in surface water and characterisation by LC-OCD. Desalination 178, 73–83.
Ketelaars, H.A.M., Wagenvoort, A.J., Peters, M.C.M.F., Wunderer, J., Hijnen, W.A.M., 2023. Taxonomic diversity and biomass of the invertebrate fauna of nine drinking water treatment plants and their non-chlorinated distribution systems. Water Res. 242, 120269.
Kimura, K., Tanaka, K., Watanabe, Y., 2014. Microfiltration of different surface waters with/without coagulation: clear correlations between membrane fouling and hydrophilic biopolymers. Water Res. 49, 434–443.
Kimura, K., Shikato, K., Oki, Y., Kume, K., Huber, S.A., 2018. Surface water biopolymer fractionation for fouling mitigation in low-pressure membranes. J. Membr. Sci. 554, 83–89.
Laforce, E., Dejaeger, K., Vanoppen, M., Cornelissen, E., De Clercq, J., Vermeir, P., 2024. Thorough validation of optimized size exclusion chromatography-total organic carbon analysis for natural organic matter in fresh waters. MDPI Mol. 29, 2075.
Lankes, U., Müller, M.B., Weber, M., Frimmel, F.H., 2009. Reconsidering the quantitative analysis of organic carbon concentrations in size exclusion chromatography. Water Res. 43, 915–924.
Leenheer, J.A., 2009. Systematic approaches to comprehensive analysis of natural organic matter. Ann. Envron. Sci. 3, 1–130.
Li, X., Rao, N.R.H., Linge, K.L., Joll, C.A., Khan, S., Henderson, R.K., 2019. An evaluation of measurement techniques for algal-derived organic nitrogen. Water Res. 165, 114998.
Mackie, A., Park, Y., Gagnon, G.A., 2022. Calibration of high performance size exclusion chromatography (HPSEC) for molecular weight estimation of aquatic humic substances. J. Environ. Chem. Eng. 10, 108913.
Matilainen, A., Vepsäläinen, M., Sillanpää, M., 2010. Natural organic matter removal by coagulation during drinking water treatment: a review. Adv. Colloid Interfaces 159, 189–197.
Matilainen, A., Gjessing, E.T., Lahtinen, T., Hed, L., Bhatnagar, A., Sillanpää, M., 2011. An overview of the methods used in the characterisation of natural organic matter (NOM) in relation to drinking water treatment. Chemosphere 83 (11), 1431–1442.
NEN-EN 1484/ISO 8245, 1999. Water Analysis–Guidelines for the Determination of Total Organic Carbon (TOC) and Dissolved Organic Carbon (DOC).
Park, J.W., Kim, H.C., Meyer, A.S., Kim, S., Maeng, S.K., 2016. Influences of NOM composition and bacteriological characteristics on biological stability in a full-scale drinking water treatment plant. Chemosphere 160, 189–198.
Portielje, R., van der Molen, D.T., 1998. Relations between eutrophication variables and system characteristics of Dutch lakes and ponds (In Dutch). RIZA, Lelystad, the Netherlands, subreport II of the fourth investigation on eutrophication, 98.007.
Rahman, I., Ndiongue, S., Jin, X., Van Dyke, M.I., Anderson, W.B., Huck, P.M., 2014. Fouling of low-pressure membranes during drinking water treatment: effect of NOM components and biofiltration pretreatment. Water Supply 14 (3), 453–460.
Sack, E.L.W., van der Wielen, P.W.J.J., van der Kooij, D., 2011. Flavobacterium johnsoniae as a Model Organism for Characterizing Biopolymer Utilization in Oligotrophic Freshwater Environments. Appl. Environ. Microbiol. 77, 6931–6938.
Santschi, P.H., Xu, C., Schwehr, K.A., Lin, P., Sun, L., Chin, W-C., Kamalanathan, M., Bacosa, H.P., Quigg, A., 2020. Can the protein/carbohydrate (P/C) ratio of exopolymeric substances (EPS) be used as a proxy for their 'stickiness' and aggregation propensity? Mar. Chem. 218, 103734.
Schurer, R., Schippers, J.C., Kennedy, M.D., Cornelissen, E.R., Salinas-Rodriguez, S.G., Hijnen, W.A.M., van der Wal, A., 2019. Enhancing biological stability of disinfectant-free drinking water by reducing high molecular weight organic compounds with ultrafiltration posttreatment. Water Res. 164, 114927.
Schurer, R., Hijnen, W.A.M., van der Wal, A., 2022. The significance of the biomass subfraction of high-MWorganic carbon for the microbial growth and maintenance potential of disinfectant-free drinking water produced from surface water. Water Res. 209, 117898.
Schurer, R., de Ridder, D.J., Schippers, J.C., Hijnen, W.A.M., Vredenbregt, L., van der Wal, A., 2023. Advanced drinking water production by 1 kDa hollow fiber nanofiltration – Biological activated carbon filtration (HFNF – BACF) enhances biological stability and reduces micropollutant levels compared with conventional surface water treatment. Chemosphere 321, 138049.
Stewart, T.J., Traber, J., Kroll, A., Behra, R., Sigg, L., 2013. Characterization of extracellular polymeric substances (EPS) from periphyton using liquid chromatography-organic carbon detection–organic nitrogen detection (LC-OCD-OND). Environ. Sci. Pollut. Res. 20, 3214–3223.
Subhi, N., Leslie, G., Chen, V., Le-Clech, P., 2012. Organic fouling of ultrafiltration membrane: detailed characterization by liquid chromatography with organic carbon detector (LC-OCD). Sep. Sci. Technol. 48 (2), 199–207.
Tian, J.Y., Ernst, M., Cui, F., Jekel, M., 2013. Correlations of relevant membrane foulants with UF membrane fouling in different waters. Water Res. 47 (3), 1218–1228.
Tominaga, K., Nagai, R., Hafuka, A., Yu, W., Kimura, K., 2022. Isolation of LC-OCD-quantified biopolymers from surface water: significant differences between real biopolymers and model biopolymers. J. Membr. Sci. 658, 120714.
Van der Kooij, D., Veenendaal, H.R., 2013. Determination of the biomass concentration in drinking water by means of the hemoflow (in Dutch). In: BTO, 2013. KWR Watercycle Research Institute, Nieuwegein, NL.
Van der Kooij, D., Veenendaal, H.R., 2014. Regrowth problems and biological stability assessment in the Netherlands. In: van der Kooij, D., van der Wielen, P.W.J.J. (Eds.), Microbial Growth in Drinking-Water Supplies. Problems, Causes, Control and Research Needs. IWA Publishing, London, UK, pp. 291–337.
Van der Kooij, D., Martijn, B., Schaap, P.G., Hoogenboezem, W., Veenendaal, H.R., van der Wielen, P.W.J.J., 2015. Improved biostability assessment of drinking water with a suite of test methods at a water supply treating eutrophic lake water. Water Res. 87, 347–355.
Van der Kooij, D., Veenendaal, H.R., van der Mark, E.J., Dignum, M., 2017. Assessment of the microbial growth potential of slow sand filtrate with the biomass production potential test in comparison with the assimilable organic carbon method. Water Res. 125, 270–279.
Van der Wielen, P.W.J.J., Brouwer-Hanzens, A., Italiaander, R., Hijnen, W.A.M., 2023. Initiating guidance values for novel biological stability parameters in drinking water to control regrowth in the distribution system. Sci. Total Environ. 871, 161930.
Wagenvoort, A., van Asperen, R., Sandrini, G., Hijnen, W.A.M., 2023. Changes in water source cause shifts in invertebrate biomass, composition, and regrowth in a non-chlorinated drinking water distribution system. MDPI Water 15, 732.
Yin, W., Li, X., Suwarno, S.R., Cornelissen, E.R., Chong, T.H., 2019. Fouling behavior of isolated dissolved organic fractions from seawater in reverse osmosis (RO) desalination process. Water Res. 159, 385–396.
Zheng, X., Khan, M.T., Croué, J.P., 2014. Contribution of effluent organic matter (EfOM) to ultrafiltration (UF) membrane fouling: isolation, characterization, and fouling effect of EfOM fractions. Water Res. 65, 414–424.
Zheng, J., Koreman, E., Martijn, B., Galjaard, G., 2015. IJssel Lake NOM characteristics and control through a process comprising ion exchange, ceramic microfiltration, UV/peroxide AOC and carbon filtration. In: Proceedings of the NOM6 IWA. Malmö.