New Rapid Methods for Assessing the Production and Removal of Labile Organic Carbon in Water Treatment Using Fluorescence and Oxygen

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New Rapid Methods for Assessing the Production and Removal of Labile Organic Carbon in Water Treatment Using Fluorescence and Oxygen Measurements

Aina McEvoy,* Catherine Paul, Oskar Modin, Amir Saeid Mohammadi, Tomas McKelvey, and Kathleen Murphy

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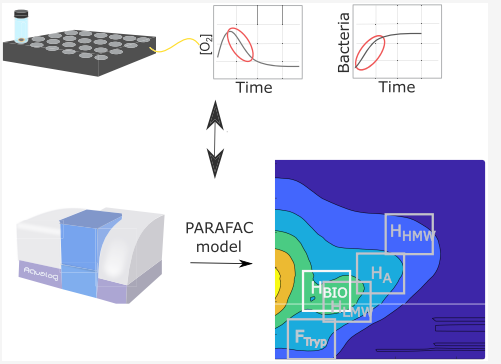
ABSTRACT: Labile organic carbon is a major nutrient, controlling microbial activity in aquatic ecosystems and contributing to the global cycling of carbon. During the production and distribution of drinking water, labile carbon fractions often escape treatment, which threatens water quality and biostability. This study proposes and compares two rapid methods for monitoring the production and removal of labile organic carbon in freshwater. One method measures the consumption of oxygen by bacteria during their initial exponential growth phase and uses this to predict how much labile organic carbon is present. The other method uses an *a priori* model of the fluorescence composition of dissolved organic matter to estimate the relative amount of biodegradable carbon fractions. In water treatment plants and in lab-scale experiments, both methods showed selectivity for biodegradable fractions of natural organic matter and indicated similar changes in scale and direction when water samples were exposed to biodegradation, with higher precision for the fluorescence measurement (coefficient of variation ∼1.5%) compared to the oxygen method (coefficient of variation ∼15%). Software is provided to aid in the implementation of these new methods, enabling their exploration and refinement in future studies.



KEYWORDS: *labile organic carbon, drinking water treatment, PARAFAC, fluorescence spectroscopy, sensor, biostability*

1. INTRODUCTION

Biodegradable dissolved organic carbon (BDOC) refers to the fraction of organic carbon molecules that can be metabolized by bacteria relatively easily in contrast to the typically larger and more abundant carbon molecules that resist biodegradation.[1−4] Microbial processing of BDOC plays an important role in the carbon cycle as well as the cycling of other major and micronutrients affecting ecosystem functioning due to coupled metabolic processes.[5−7] Microbial respiration further leads to degassing of carbon dioxide to the atmosphere and contributes to deoxygenation in water bodies.[8,9]

The traditional purpose of drinking water treatment is to provide safe drinking water to consumers with a negligible risk of microbial contamination. Another critical treatment aim is to reduce the bulk concentration of dissolved carbon molecules.[10,11] Removing bulk DOC improves the esthetic qualities of the water and increases the efficiency of microbial disinfection by reducing chemical demand during chlorination and increasing light penetration during UV treatment.[12,13] BDOC and assimilable organic carbon (AOC), representing the most easily metabolized fraction of BDOC, are major challenges for drinking water treatment due to their tendency to pass through conventional treatment processes and be produced in steps involving chemical oxidation.[14,15] The presence of abundant labile carbon molecules at the conclusion of the water treatment leads to a range of negative consequences. These include undesirable bacterial regrowth within the water distribution network, affecting taste, odor, and coloration.[4,16−18] In extreme cases, high concentrations of BDOC and AOC can lead to biological instability,[19−21] which may favor the spread of pathogens[15,22] or promote biocorrosion in pipe networks.[23,24]

Water treatment plants need to ensure that water is biologically stable, which requires affordable methods for detecting and measuring changes in labile DOC.[25,26] Analytical challenges for quantifying AOC and BDOC include that they cannot be physically isolated from bulk DOC and are present at relatively low concentrations.[2] Previously, Van der Kooij and others developed an assay for estimating AOC concentrations

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based on measuring the maximum growth of bacterial strains *Pseudomonas fluorescens* (strain P-17) and later also *Spirillum* sp. strain NOX in water samples supplemented with N and P. The growth is then compared to their growth when fed with acetate carbon.[27,28] AOC tests can achieve low detection,[28] but are complex and time-consuming to implement, leading to high analytical costs. Method simplifications have been proposed, including replacing time-consuming growth assays with flow cytometry and omitting nutrient supplementation to highlight regrowth potentials under ambient conditions,[29,30] but flow cytometers are expensive and additionally require nucleic acid stains.

Biological oxygen demand (BOD) tests are widely used to assess the biodegradability of wastewater effluents.[31−33] The standard BOD_5 test measures the total amount of oxygen consumed by bacteria while respiring organic and inorganic substances over an incubation period of 5 days.[32,34,35] Although inexpensive and accessible, BOD_5 tests do not achieve the low detection limits needed to measure the biodegradability of carbon in partially or fully treated drinking water.[32,36] In theory, if continuous oxygen measurements are collected during the microbial respiration of BDOC, then the microbial growth potential can be estimated from the oxygen consumption rate occurring when bacteria consume the most labile molecules. Oxygen sensors have been used to estimate microbial respiration rates[37−40] but have, to the authors' knowledge, not yet been explored for the purpose of quantifying labile organic matter in drinking water.

Fluorescence spectroscopy is widely used for detecting the concentration and composition of natural dissolved organic matter (DOM) in water.[41−44] Fluorescence excitation and emission matrices (EEMs) can be exploited by powerful data mining techniques, such as parallel factor analysis (PARAFAC), to retrieve spectral "fingerprints" representing fractions with differing susceptibilities to chemical and biological processes[45−49] or differing natural vs anthropogenic sources.[50,51]

The global application of PARAFAC to DOM fluorescence over the past two decades indicates the likely existence of globally distributed DOM fractions with strongly conserved spectral properties[43,45,47,49] and different susceptibilities to biodegradation.[45,52] For example, the PARAFAC model developed by Moona et al.[52] for Swedish surface waters is highly statistically congruent with an independently derived model of surface and drinking water samples from southern Europe, Asia, Africa, and North America.[41] A rapid method to estimate the relative intensity of labile DOM in new samples is, therefore, to use an appropriate *a priori* PARAFAC model to apportion fluorescence between labile and nonbiodegradable components. This mirrors the approach used by Paradina-Fernández et al.[50] to distinguish between DOM versus pharmaceuticals spiked at low concentrations (3−50 μg/L) into diverse water samples.

The aim of this study was to develop and evaluate two relatively simple and rapid tests for assessing DOM lability by extending two well-established methods using oxygen measurements and fluorescence spectroscopy. The Labile Oxygen Consumption rate (LOCr) method extends the classical BOD test by estimating the microbial regrowth potential from oxygen consumption rates in the early phase of microbial regrowth. The Projection on PARAFAC (PoP) method uses an *a priori* model of natural organic matter fluorescence to estimate the proportion of organic matter that can be metabolized by bacteria. Our approach was to first develop and validate these methods in the laboratory and then apply them to study full-scale treatment processes in drinking water treatment plants.

2. MATERIALS AND METHODS

2.1. Experimental Design and Study Sites. Five drinking water treatment plants (WTPs) located in western Sweden were involved in this study. Two plants (WTP-K, WTP-T) use Göta river as the raw water source. A third (WTP-G) uses a reservoir (Delsjön) that mainly contains water pumped from Göta river. Plant WTP-N receives lake Glottern water while WTP-V receives a combination of groundwater and lake water from Stora Neten. The treatment process chains vary between these plants (Table S1). WTP-K, WTP-G, WTP-N, and WTP-T employ metal coagulants in combination with either sedimentation or membrane filtration. WTP-K has a dedicated biofilter at the beginning of the process chain, consisting of a nonadsorbing expanded clay material with a very high surface area to promote microbial attachment.[52] Two other plants (WTP-T and WTP-N) have traditional slow sand filters located after the coagulation step. Filters containing granular activated carbon are included toward the end of the process trains at WTP-G and WTP-N. All plants implement disinfection using UV irradiation at the end of treatment, and all except WTP-V additionally implement chlorination.

Details of sampling campaigns are compiled in the Supporting Information (Table S2). In summary, eight sites within a drinking water distribution network connected to WTP-V were sampled during four campaigns in the summer of 2021. At all other plants, various stages along the treatment process train were sampled during one or more campaigns in 2022−2023. Samples were collected in baked amber glass bottles (550 °C, 6 h) with Teflon-lined screw caps (acid washed in 10% HCl). Bottles were rinsed twice with the sample and then filled to the top to limit oxygen exposure and bacterial activity. Samples destined for oxygen sensor measurements were pasteurized (if not stated otherwise in the figure captions) and then refrigerated, whereas samples destined for fluorescence measurements were refrigerated directly. Subsequent analyses were initiated within 24 h of sample collection. Delays of this duration are typical of field studies and are not expected to negatively affect the results.[53] During eight sampling campaigns conducted between 2021 and 2023, duplicate samples were sent to a commercial laboratory (Microbial Analytics Sweden AB) for analysis of AOC on the basis of the growth of *P. fluorescens* (P-17). Their methodology followed the AOC protocols established by Van der Kooij and others[27,28] without N and P supplementation.[54]

2.2. Labile Oxygen Consumption Rate Method (LOCr). *Analysis Methodology and Equipment.* The Labile Oxygen Consumption rate (LOCr) method consisted of incubating water samples together with a bacterial inoculum and using oxygen sensors to measure variations in the rate at which microorganisms consumed oxygen during respiration. The amount of labile organic carbon was estimated from a consistent feature in the oxygen consumption profile, whereby a sudden rapid acceleration in oxygen consumption was observed during the first 12−24 h of the experiment. Because in natural samples, this feature was typically very small, it was detected and quantified using a semiautomated data mining

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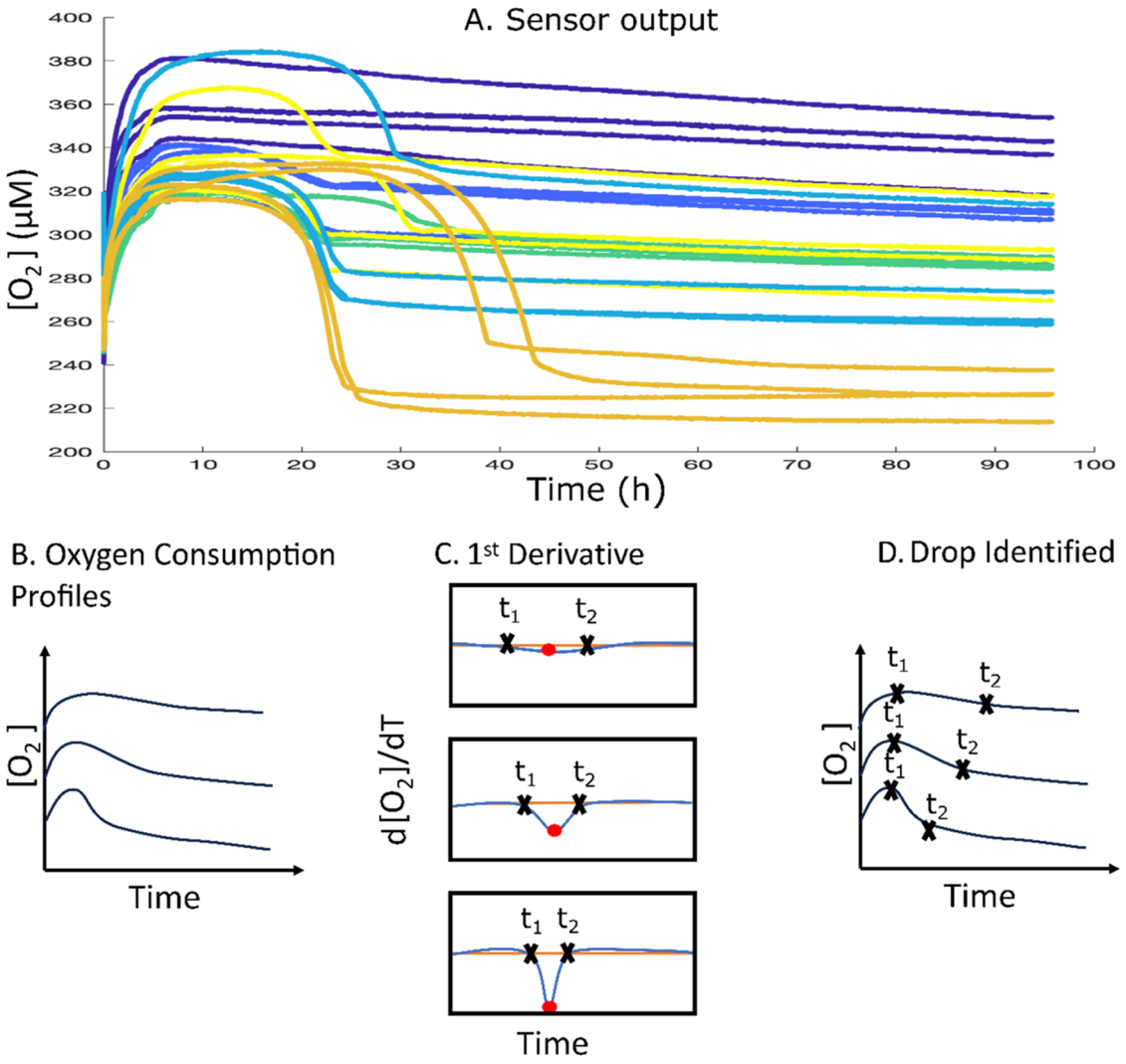

Figure 1. Overview of the Labile Oxygen Consumption rate method: (A) sensor output from 6 × 4 sensor vials, where each color represents a different concentration of acetate degraded by a mixed bacterial community. (B) For example, oxygen-time profiles during incubation at three different concentrations. The rate of change for the largest drop occurring within 48 h is used to predict the abundance of labile organic carbon. (C) The algorithm locates the drop by the sharp negative peak relative to the baseline detected in the first derivative profile and automatically detects its start and end time points. (D) The drop occurs between the time points t_1 and t_2.

algorithm (available via this article: see the Data availability section).

The LOCr methodology is described below, with further details provided in Section S1. Oxygen consumption profiles were measured continuously using SensorVial SV-PSt5−4 mL and SDR SensorDish (PreSens—Precision Sensing GmbH). Our system consisted of 4 mL glass vials affixed with a fluorescent dye patch mounted at the bottom of each vial (sensor vials) to enable the simultaneous monitoring of 48 vials. The system measures dissolved oxygen in each vial via the quenching of fluorescence emitted by the dye patch as detected by the underlying sensor dish. Each sensor dish logged a new oxygen measurement in each vial every 3 min. All sensors were calibrated according to the manufacturer's instructions in April 2021 and April 2022.

At the beginning of each experiment, samples were inoculated with a microbial community by using a newly prepared natural inoculant solution. Early experiments used natural inoculants,[55] which functioned adequately in summer but poorly in winter, so in later tests, the natural inoculant was replaced with a commercial product. Natural inoculant stock solutions were prepared by incubating Göta river water at 35 °C for 1−2 weeks under regular aeration. The commercial inoculum consisted of a proprietary blend of broad-spectrum bacteria developed for Biological Oxygen Demand (BOD_5) tests and marketed as PolySeed capsules (InterLab). To prepare a stock solution, one PolySeed capsule was rehydrated in 500 mL of phosphate buffer solution.

Prior to being inoculated, test water samples were pasteurized to kill the original microbial community. This step was found to improve method sensitivity and reduce the need for an algorithmic data smoothing step. Pasteurization steps are typical in AOC methodologies and previous research found that it does not significantly affect organic matter lability.[13,56] Pasteurization was especially helpful when using a natural microbial inoculant for experiments performed during the winter when microbial activity was relatively suppressed (Section S1 and Figure S1). To prepare the 30 mL of inoculated sample needed for 4 replicate vials, 29 mL (if inoculated with the natural inoculant) or 29.5 mL (if

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inoculated with PolySeed) of sample was dispensed into a 40 mL glass bottle and pasteurized for 30 min at 70 °C, following 15 min warm-up time.[57,58] Samples were then acclimated to 35 °C for approximately 1 h before inoculating with either 1 mL of the natural inoculant or 0.5 mL of Polyseed.

Each batch test consisted of 12 samples × 4 replicates and lasted three to five days. This high number of replicates was chosen to ameliorate a relatively high error rate among individual profiles (~15%) caused by recurring challenges that included lid failures and leakage, bubble entrapment, and unexplained sensor drift. Issues with sensor drift have previously been reported when using optical oxygen sensor systems to measure microbial respiration, including for the SDR system used in this study.[39,40,59] The pasteurized and inoculated sample was carefully shaken to oxygenate the water and then dispensed into four × 4 mL sensor vials. The sensor system containing the sealed vials was then placed in an incubator and maintained at 35 °C for the duration of the experiment.

Acetate was used as a standard carbon source to convert oxygen consumption rates to labile carbon removal as acetate equivalents (Ac-eq).[28,54] To calibrate, a fresh dilution series was prepared as 0 − 500 μg CL^{-1} from a 5000 μg CL^{-1} sodium acetate stock solution, and up to four replicates of each different dilution were measured at the same time as the samples. Because it was time-consuming to perform such a calibration and doing so reduced the number of samples that could be measured in a batch, the calibration step was omitted in many cases, and results are instead reported as oxygen consumption rates (μg L^{-1} h^{-1}). A nutrient broth prepared with NH_4Cl as the nitrogen source and K_2HPO_4 as the phosphorus source in the molar ratio C:N:P of 80:8:1 was added to samples when it was desired to guarantee carbon limitation. Nutrient additions were omitted in many cases to maintain consistency with the method used for AOC analysis at the commercial laboratory. For the results presented, nutrient additions and the type of inoculant used are specified in each figure caption.

LOCr Algorithm. The algorithm workflow is illustrated schematically in Figure 1, while an example of real data is shown in Figure S2. Each curve represents an experimentally obtained oxygen profile measured by a single sensor monitoring a single water sample. Common features in all oxygen consumption profiles include an initial period of increasing values during which equilibrium conditions are established, followed by a short period of maximum oxygen levels, followed by a steep drop in oxygen levels beginning 12−24 h after starting the incubation, followed by a steady decrease in oxygen concentrations over the remainder of the incubation. The steep drop is hypothesized to coincide with the maximum growth rate of bacteria while they consume labile organic molecules. This hypothesis could not be experimentally validated due to the incompatibility of monitoring oxygen concentrations at the same time as cell counts; however, it is supported by the results of the study. The maximum growth rate was used to predict the amount of labile organic carbon substrate available to heterotrophic bacteria.

Data processing begins with plotting the raw and normalized data to visually identify and remove extreme outliers (Section S2 and Figures S3−S5). Thereafter, the baseline is subtracted, and the algorithm calculates the first derivative of each raw oxygen profile to locate rapid changes in oxygen consumption rates relative to background oxygen. A sudden drop in oxygen appears as a reverse "peak" in the derivative plot, and the largest peak after equilibration that fulfills the minimum sensitivity criteria specified by the algorithm is selected. Among replicate sensors, the timing of this peak can vary by up to several hours, which we attribute to random variations in the abundances and activities of the microbial communities in each vial. The consumption of oxygen over the span of the peak is automatically calculated for each individual vial, and then this value is divided by the elapsed time between the beginning and end of each respective peak and defined as the "oxygen consumption rate".

2.3. Projection on PARAFAC (PoP). An Aqualog spectrophotometer (HORIBA Ltd.) was used to measure fluorescent excitation and emission matrices (EEMs). Measurements were carried out on samples filtered through 0.45 μm polyethersulfone syringe filters in a 1 cm cell with excitation wavelengths ranging from 239 to 500 nm, detecting the emission from 245 to 826 nm. Data corrections for spectral biases and inner filter effects were implemented according to standard methods.[60] Thereafter, the EEMs were fitted with a published PARAFAC model in a procedure referred to here as Projection on PARAFAC (PoP) (for MATLAB code, see the Supporting Information). The traditional approach of developing a new PARAFAC model for each data set was avoided for two reasons. First, PARAFAC models with fewer than five components typically omit one or both of the biodegradable components; however, deriving a model of this complexity requires a relatively large data set.[49] Second, the aim was to develop a simple and rapid method, thus precluding a complex model-building phase.

The drEEM toolbox[60] was used to fit the fluorescence EEM measurements to an *a priori* PARAFAC model to obtain the relative concentrations of FDOM components. The *a priori* model of Moona et al.[52] represents DOM fluorescence as the sum of five PARAFAC components ($F_{\lambda ex/\lambda em}$) with wavelengths ($\lambda$) of maximum excitation (ex) and emission. Four of these components are traditionally described as "humic-like": $F_{340/445}$, $F_{290/420}$, $F_{360/510}$, and $F_{310/400}$ and the fifth is "tryptophan-like" $F_{280/340}$ with only components $F_{290/420}$ and $F_{280/340}$ representing labile DOM. To simplify the discussion in this paper, the four humic-like components have each been renamed: H_A (humic-like aromatic), H_{BIO} (biodegradable humic-like), H_{HMW} (higher molecular weight aromatic), and H_{LMW} (lower molecular weight hydrolyzed). Component $F_{280/340}$ was renamed to F_{Tryp}. Table 1 lists all five components, their peak positions, and characteristics according to recent studies.

Fluorescence Indices. The biological index (BIX) and fluorescence index (FI) are widely used to distinguish between

Table 1. Characteristics of the Five Fluorescent Components of DOM Assumed to be Present in Water Samples in the PoP Method Using the A Priori PARAFAC Model of Moona et al.[52]

components	$\lambda_{Ex}/\lambda_{Em}$ (nm)	characteristics
H_A	340/445	humic-like aromatic[41,61]
H_{BIO}	290/420	humic-like biodegradable[62,63]
H_{HMW}	360/510	humic-like higher molecular weight[41,61]
H_{LMW}	310/400	humic-like lower molecular weight[41]
F_{Tryp}	280/340	protein-like/tryptophan-like[43,64]

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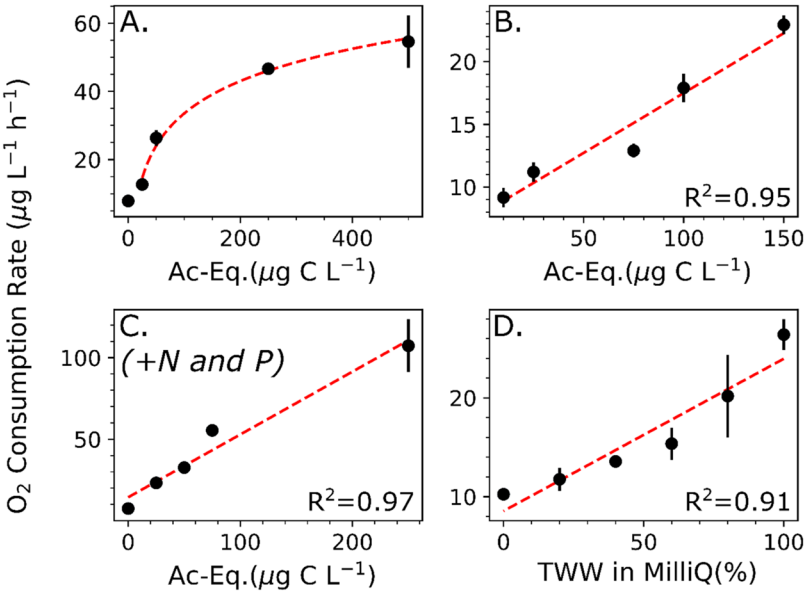

Figure 2. Correlations between the labile O_2 consumption rate and nutrient availability. O_2 consumption rate vs acetate concentrations without the addition of N and P and inoculated with a natural inoculant for (A) 0−500 μg CL^{-1} as Ac-eq, and (B) 0−150 μg CL^{-1} as Ac-eq (C) O_2 consumption rate vs acetate concentrations 0−250 μg CL^{-1} as Ac-eq in the presence of excess N and P inoculated with a natural inoculant. (D) Effect of dilution on the oxygen consumption rate for secondary treated wastewater inoculated with a standardized inoculant PolySeed. Error bars show the standard error of the means.

allochthonous and autochthonous DOM sources in water and so were compared with the new methods for their abilities to indicate changes in DOM lability. BIX measures the ratio of fluorescence intensities at 380 nm over 430 nm at excitation of 310 nm and indicates autotrophic productivity, where values >1 are indicative of recently produced DOM of biological or aquatic bacterial origin.[65] FI is the ratio of fluorescence intensities of 470 and 520 nm at an excitation of 370 nm. High FI (1.8−2.9) is hypothesized to be linked to microbially derived DOM with lower aromaticity and higher lability than terrestrially derived DOM,[66] whereas lower FI (1.3−1.4) indicates that the sample is dominated by relatively recalcitrant terrestrially derived DOM.[66−68]

2.4. Statistical Analysis. Regression analyses were performed in MATLAB using "fitlm" for ordinary least-squares regression and using the built-in "RobustOpts" option to perform robust regressions.[69] Robust regression uses iteratively reweighted least-squares to assign weights to each data point, decreasing the sensitivity to outliers. Error bars are reported as standard errors of the means derived from replicate vials. Pearson correlations were computed using "corrcoef" in MATLAB, and the Pearson correlations coefficients were used to measure the linear correlations between variables.[70] Bland-Altman tests were used to assess whether two different methods (LOCr vs AOC and LOCr vs PoP) each tracked the same underlying phenomenon.[71] In a Bland-Altman plot, the between-method differences are plotted against the average size of the measurements, and nonagreement between test results and biases is indicated by a lack of randomness in the plotted data. Bland-Altman tests are widely recognized to be the most reliable way to assess congruency between alternative methods for quantifying a variable when the true value of the variable is unknown.[72]

3. RESULTS AND DISCUSSION

3.1. Laboratory Validation Phase. *Labile Oxygen Consumption Rate (LOCr) Method.* The first step of validating the LOCr method was to confirm that when samples were diluted with pure (DOC-free) water, concentrations of labile organic carbon decreased in proportion to the amount of water added. Batch tests involving a dilution series of acetate or secondarily treated wastewater diluted into pure water established the relationship between LOCr and carbon substrate concentration (Figure 2). The data points represent the average oxygen consumption within 12−24 h of incubating a pasteurized sample inoculated with either a natural microbial community (Figure 2A−C) or the PolySeed inoculum (Figure 2D).

A nonlinear correlation between LOCr and acetate-C resembling a Monod kinetic curve was observed when there was a wide concentration range of acetate-C (0 to 500 μg CL^{-1}) (Figure 2A). However, correlations were approximately linear both at low acetate concentrations (0−150 μg CL^{-1}) (Figure 2A,B) and at concentrations up to 250 μg CL^{-1} when bacteria were provided with excess N and P (Figure 2C). In diluted wastewater, a nonlinear relationship was observed between the DOM concentration and the oxygen consumption rate (Figure 2D). This may reflect exponential microbial growth, facilitated by the more diverse nutrient substrates available from the pasteurized biomass present in wastewater compared to isolated nutrients (acetate, N, and P).

Labile carbon concentrations expressed as acetate equivalents are typically lower than 150 μg CL^{-1} in treated surface waters.[15,73] Earlier research indicates that although carbon often limits bacterial regrowth in drinking water, N or P can be growth-limiting in specific systems.[74,75] Whether or not to add nutrients can be decided according to the aims of the study; adding N and P ensures carbon limitation but may overestimate the true microbial regrowth potential under ambient nutrient conditions.[54] Figure 2B,C represents linear calibration

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curves that, for a particular batch of samples, can be used to convert LOCr oxygen rate measurements to acetate carbon equivalent units. Since potential changes in the composition and activity of the bacterial inoculant will affect regression slopes, a new calibration curve must be generated for each experimental batch.

Commercial AOC measurements correlated with LOCr measured in samples from a drinking water distribution system (Figure 3). Samples were collected at 6−8 sites during each of the four surveys. The commercial method was according to Van der Kooij et al.[28] but using a single bacterial strain: *P. fluorescens* (P-17). A robust linear regression[69] indicated a weak positive association between the two methods (p-value = 0.08, R^2 = 0.24) with generally increasing AOC and LOCr over the summer during four sequential surveys in June and July. Two outliers were observed in the July 2021 sampling campaign, whereby anomalously high AOC concentrations were not reflected in the LOCr data set (Figure 3A). Excluding these two outliers, a Bland-Altman test showed random error residuals, supporting the conclusion that the two methods track a single underlying phenomenon without systematic biases (Figure 3B). The LOCr method produced a wider range in measurements, with data varying by a factor of around 20 (0.003−0.06 μmolL^{-1}h^{-1}) compared to AOC method data, which varied by a factor of 6 (4−24 μgC^{-1}L^{-1}). This could indicate that the LOCr method has either higher measurement sensitivity than the AOC method or lower measurement precision and/or accuracy. Our AOC measurements at WTP-V represented averages of duplicate subsamples (technical/analytical replicates) for which the median coefficient of variation (CV = stdev/mean) was 19%. CVs for our LOCr measurements were approximately 15% from 2 to 4 replicates remaining after the outlier removal step.

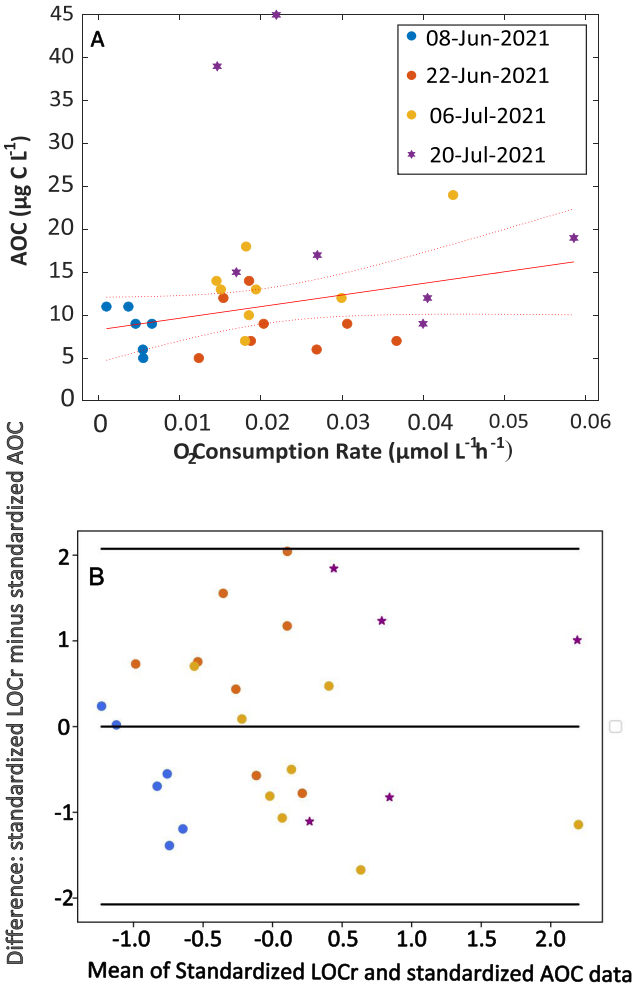

Figure 3. Comparison of commercial assimilable organic carbon (AOC) and LOCr measurements from the WTP-V distribution network. Samples were collected during four campaigns and inoculated with a natural bacterial community without prior pasteurization. The AOC method measured the growth of *P. fluorescens* (P-17) on plate media. (A) Robust linear regression (R^2 = 0.24, p = 0.08) with a 95% confidence interval (dashed line). (B) The Bland-Altman test shows random residuals.

Projection on PARAFAC (PoP) Method. Fluorescence measurements behave linearly upon dilution after correcting for artifacts and inner filter effects;[76] therefore, it was not necessary to investigate the effect of dilution on the PoP method. Instead, the first step in validating the PoP method was to confirm that exposing natural samples to biodegradation removed only the H_{BIO} and F_{Tryp} components. The other components are expected to have relatively stable fluorescence intensities or may slightly increase due to the biological production of humic-like DOM.[42,46,52]

The susceptibility of H_{BIO} and F_{Tryp} to biodegradation was confirmed in two experiments (Figure 4). In the first experiment, triplicate vials of river water from WTP-K were pasteurized, inoculated with a natural bacterial inoculant, then incubated for 7 days at 35 °C. Pasteurization itself had a negligible effect on fluorescence composition, reducing H_{BIO} and F_{Tryp} by less than 2% (Figure S1). However, the intensities changed significantly relative to the pasteurized samples following incubation. Components H_{BIO} and F_{Tryp} decreased by 3−6%, whereas H_A, H_{HMW}, and H_{LMW} increased by 18−19% (Figure 4A), indicating that H_{BIO} and F_{Tryp} were slightly removed, whereas the other three components accumulated.

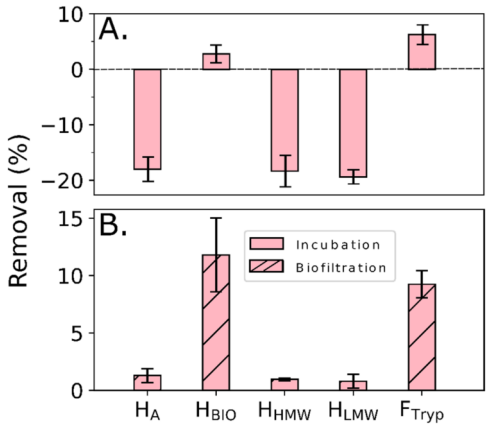

Figure 4. Removal (%) of five fluorescent DOM fractions in water due to biological activity. Percentage of DOM fractions removed from (A) pasteurized vials of water from Göta River inoculated with natural bacterial community and incubated for 7 days at 35 °C. (B) Effluent waters from three parallel rapid sand filters at WTP-K with an average contact time of 80 min at 2−3 °C. Error bars show standard errors of means from independent replicates.

In the second experiment, river water passed through three full-scale biofilters at WTP-K. In this case, the intensities of H_{BIO} and F_{Tryp} decreased by 15−20% across the three filters

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compared to decreases of only 1−2% for the other components (Figure 4B). The much higher levels of biodegradation observed in the treatment plant (15−20%) compared to the laboratory (3−6%) are consistent with the organic matter being exposed to mature microbial biofilms that adapted to the incoming water quality. Also, biofilter granules have a very high surface area and more favorable conditions for microbial attachment and growth compared to glass vials.[77,78]

3.2. Applications at Drinking Water Treatment Plants. The results of the laboratory validation phase suggested that LOCr and PoP are relevant tools for water treatment monitoring, especially if the aim is to measure relative increases or decreases in regrowth-supporting carbon substrates. Therefore, in the application testing phase, these methods were applied to samples from full-scale water treatment plants. During several campaigns at water treatment plants between September 2022 and August 2023, AOC was measured for the same samples that were tested using PoP and LOCr. However, AOC concentrations were below the method detection limits for the commercial laboratory in 44% of these samples, resulting in an insufficient sample size for assessing correlations with AOC (Figure S6).

Instead, correlations between the LOCr and PoP methods were assessed in summer and winter using samples collected before and after the biofilters at WTP-K. At this plant, three biofilters were arranged in parallel, and each received untreated (river) water and was sampled to obtain an average value representing conditions after the biofilters, allowing for both biological (between filters) and technical replicates. According to the PoP method, in summer the biofilters decreased the amount of biodegradable DOM in the incoming water by 20% on average (Figure 5A) compared to 15% in winter (Figure 5B). In comparison, according to the LOCr method, the biofilters decreased biodegradable DOM by 38% in the summer (Figure 5A) compared to 13% in the winter (Figure 5B). A Bland-Altman test showed no systematic biases when comparing the two methods since, relative to the PoP method, the LOCr overestimated the regrowth potential during winter and underestimated the regrowth potential during summer (Figure S7). This seasonal shift in method bias may be related to seasonal changes in the composition of the labile carbon source[79,80] and/or seasonal changes in the function of the biofilter community.[78,81] Higher levels of biodegradation are consistent with warmer temperatures favoring bacterial metabolism as well as there being generally higher BDOC concentrations in surface waters during the summer months.[82,83]

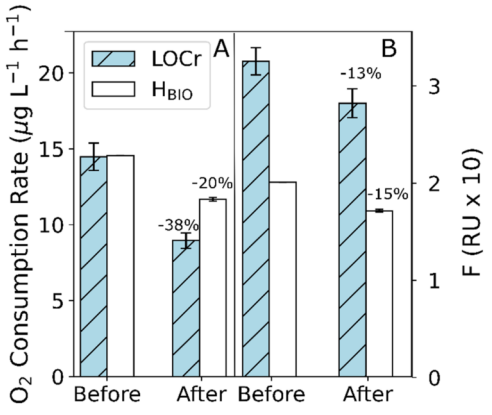

Figure 5. Changes in Labile Oxygen Consumption rate (LOCr) and Projection on PARAFAC component H_{BIO} in water passing through parallel biofilters at treatment plant WTP-K. Sampling was in (A) June 2022 (water temperature 17 °C) and (B) February 2023 (water temperature 2 °C). A natural bacteria inoculant was used for Labile Oxygen Consumption rate measurements, and in (B), signals were enhanced through boosting with 1 μg CL^{-1} Ac. Equation Error bars show standard errors of means from four to three technical replicates (LOCr) and three biological replicates.

It is also instructive to compare the removal of labile DOM fractions by the biofilters with the removal of bulk DOC, which is expected to be lower because it includes recalcitrant DOM. DOC measurements are unavailable for the summer sampling campaign; however, in the winter campaign, DOC concentrations were reduced by <10% from 5.7 ± 0.04 to 5.5 ± 0.01 mg/L ($t(1) = 8.56$, $p = 0.074$) (Table S3). On average, across these and other experiments, the percentage of bulk DOC degraded was approximately half to a third of the measured removals for PoP and half to a quarter of that measured for LOCr. Also, when comparing the new methods with the BIX and FI fluorescence indices used to indicate changes in the age and aromaticity of DOM, BIX and FI were observed to change in the same direction as PoP and LOCr but by a much smaller magnitude, i.e., 3% (summer) and 2% (winter) for BIX and 2% (summer) and 0% (winter) for FI. These results suggest that the new methods are more sensitive predictors of DOM lability than BIX and FI.

LOCr results and DOM composition, including the relative concentrations of labile organic carbon, changed in response to various treatment processes in WTPs (Figure 6; sampling points are depicted in Figure S8). DOC typically decreases throughout drinking water treatment plants, especially during coagulation and adsorption onto GAC.[14,41,79] However, organic matter can leach from cells grown within biological treatment processes,[52,79] and small biodegradable molecules are produced when oxidation processes (e.g., chlorination, ozonation) break down larger molecules.[84,85]

Changes in the ratio of the biodegradable H_{BIO} component relative to components H_{HMW} and H_{LMW} in this study indicated which treatment steps selectively removed labile aromatic fractions versus higher or lower molecular weight DOM fractions. When the ratio decreased across a treatment step, it indicated that H_{BIO} was selectively removed. Conversely, when the ratio increased relative to the previous step, it indicated that H_{BIO} was removed less effectively than H_{HMW} and/or H_{LMW}. The ratios of H_{BIO}/H_{HMW} and H_{BIO}/H_{LMW} were observed to decrease following treatment steps T3 (after sand filters/UV disinfection/chlorination in WTP-T) and N2 (after flocculation/flotation/sand filters in WTP-N). This is consistent with results from WTP-K (Figure 5) and with the expectation that sand filters promote the removal of biodegradable organic carbon.[86,87] In contrast, increasing ratios were observed in connection with filtration through activated carbon media (N3 and G3), which is also consistent with expectations because hydrophobic high molecular weight organic matter fractions typically have a higher sorption affinity to activated carbon.[88−91]

In treatment plants, WTP-T and WTP-G, which both receive water from Göta river, LOC, and PoP measurements were correlated with $R^2 = 0.43$ (Figure 7 and Table S4) in three sampling campaigns in spring, winter, and summer. Apart from measurement error, potential sources of error include varying inoculant compositions and seasonal changes in

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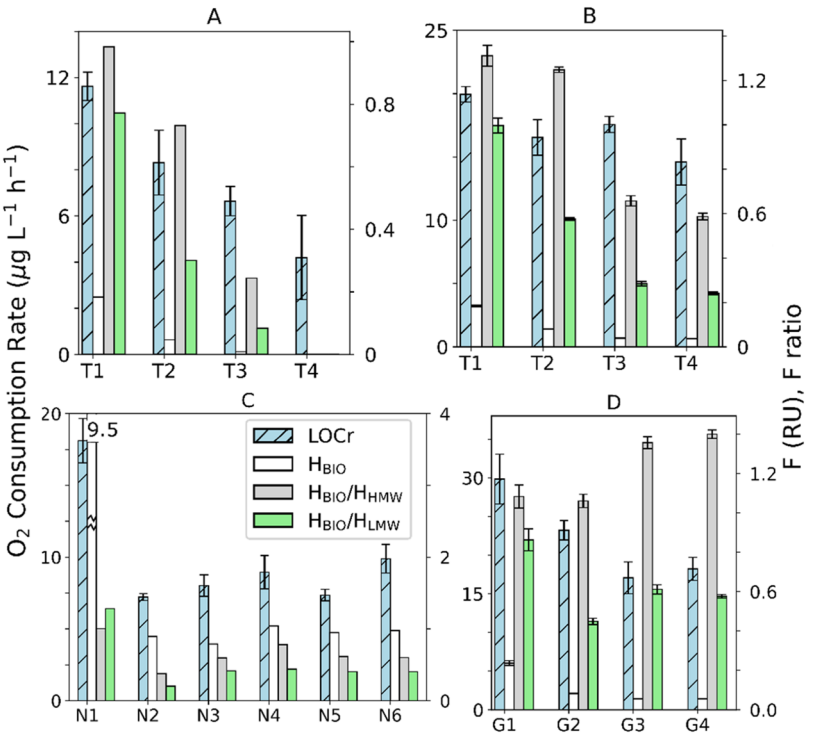

Figure 6. Changes in LOCr and PoP components and ratios in drinking water treatment plants. (A, B) WTP-T in (A) winter and (B) spring (January and May 2023, respectively): raw water (T1), after flocculation and sedimentation (T2), after rapid and slow sand filters, UV disinfection, and chlorination in outgoing water (T3) and in the distribution network (T4). (C) WTP-N water treatment plant in September 2022: Raw water (N1), after flocculation, after flotation and rapid sand filter (N2), after aged granular activated carbon filter (N3), after UV (N4), in outgoing water after chlorination (N5) and in the distribution network (N6). (D) WTP-G in August 2023: raw water (G1), after flocculation and sedimentation (G2), after carbon filter (G3), and after ultrafiltration and chlorination in outgoing water (G4). (A, C) were inoculated with a natural bacteria community, and (B, D) using PolySeed. Error bars show standard errors of means with propagated standard errors shown for ratios.

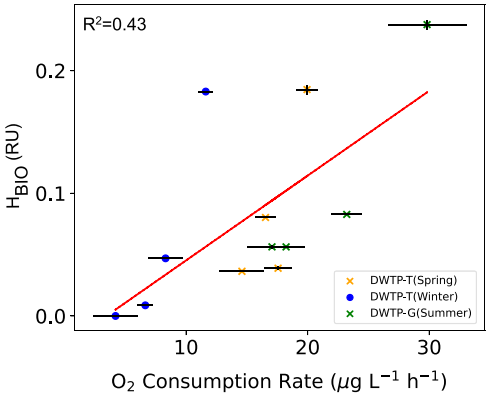

Figure 7. Correlation between H_{BIO} and Labile Oxygen Consumption rate (LOCr) measurements in seasonal sampling campaigns at two WTPs treating water from the same river. For the Labile Oxygen Consumption rate method, a mixed natural inoculant was used in WTP-T (winter), whereas a PolySeed bacterial community was used in WTP-G (Summer) and WTP-T (Spring).

organic matter composition.[79,80] This could cause the measurements to diverge because the PoP method only detects fluorescent molecules, so is insensitive to changes in colorless DOM.

3.3. Practical Considerations. The SDR SensorDish system has previously been used for assessing microbial respiration rates in aquatic samples based on the linear or nonlinear fitting of oxygen consumption curves collected during 3-day or 28-day incubations.[37,38,59] The LOCr method developed here instead detects the period and rate when respiration is greatest and uses it to estimate the microbial regrowth potential under ambient nutrient conditions or biodegradable carbon concentrations after adding excess N and P. Once the sensors have finished collecting oxygen profiles, data interpretation is largely automated and takes only a few minutes. The results can be expressed as oxygen consumption rates or can be converted to acetate equivalent units by running an acetate dilution series at the same time as running a batch of samples (Figure 2), although doing so increases measurement time and complexity.

Several methodological developments were important for improving the precision of LOCr measurements, and further improvements may be achievable with additional refinements of the protocols and algorithms. Using PolySeed as the inoculant greatly improved method sensitivity relative to using a natural inoculant, especially for experiments performed in winter (Supporting Information Section S1). Also, the negative peak in the derivative profiles was more prominent in samples that had been pasteurized prior to inoculation (Figure S1).

Despite several advantages, practical challenges were encountered when the LOCr method was implemented using the SDR sensor system. A strong advantage of the SDR system is the possibility to run many samples simultaneously, but this is traded off against a relatively high error rate for individual sensors. Due to low sample volumes, even small temperature fluctuations affect oxygen measurements, although this type of error is easily detected because all sensors are similarly affected (Figure S4A). However, if there are leaks or if air bubbles are trapped when sealing a sensor

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vial, the oxygen profile will diverge strongly from other replicates, invalidating the results for the affected replicate (Figure S4B,C). Usually, an outlier sensor recorded a much steeper decline in oxygen levels than its replicate sensors, and in many cases, the reason why this occurred was unclear (Figure 4D). Insuring against sensor drift and other errors necessitates a relatively high degree of replication, which constrains the number of independent samples that can be feasibly analyzed in the same analytical batch. Sensitivity and reproducibility in microbial respiration measurements are improved by using larger incubation vials.[40,59] However, it was not possible to increase the vial sizes in this study due to the geometry of the SDR detector plates.

The PoP method was comparatively simple and robust due to employing sensitive and stable instrumentation and a well-established data modeling procedure. A PoP method limitation is that it is impossible to reliably calibrate fluorescence intensities against a standard curve to obtain concentrations of biodegradable DOC because the specific molecular structures responsible for the H_{BIO} component are unknown,[92] and many labile molecules, including acetate and glucose, lack aromatic rings and do not fluoresce. Using a customized PARAFAC model developed for a specific instrument and/or water source is likely to improve method accuracy relative to using a general PARAFAC model, as was done in this study, since any lack of fit by the model to the specific chemical composition of the water will increase prediction error.[60]

The tryptophan-like fluorescence peak F_{Tryp} has been used as a proxy of both DOM lability and microbial contamination in many previous studies.[52,93−95] In the treatment plants, F_{Tryp} correlated with H_{BIO} (R = 0.87, Figure S10) but no more strongly than H_{BIO} correlated with other PARAFAC components (R = 0.94−0.98 Figure S9). The relatively poor correlation between F_{Tryp} and H_{BIO}, although both are much more labile than other fluorescent DOM (Figure 4), probably reflects two confounding factors that each reduce the suitability of F_{Tryp} as a proxy of DOM lability. First, it is difficult to precisely measure F_{Tryp} due to the easy adsorption of proteins onto glass cuvette surfaces, leading to higher inter-replicate variability than is typical for other fluorescent components.[93,96,97] Second, F_{Tryp} is correlated to the production of proteinaceous extracellular polymeric substances (EPS) that are excreted by microbes and released during cell lysis.[98,99] These EPS can be subsequently used as a growth substrate and biodegraded by other microorganisms.[100,101]

4. CONCLUSIONS

The European Union's recent drinking water directive[25] requires that drinking water treatment plants deliver clean and safe drinking water showing "no abnormal changes" in regrowth potential for treated water monitored over time. Microbial regrowth potential is therefore an important water quality parameter for WTPs and can be linked to labile organic carbon concentrations whenever carbon is the limiting nutrient. All existing tools for quantifying labile organic carbon provide partly overlapping windows into DOC biodegradability rather than a complete picture, and all methods have practical limitations or high costs. This means there is value in exploring additional methods, especially if they are relatively simple and inexpensive.

The tools evaluated in this paper (PoP and LOCr) offer a novel window into DOC lability with potential applications in water quality monitoring. Both are simpler and cheaper than measuring AOC and, hence, are more suited to repeated measurements if the aim is to detect abnormal water quality changes. In this study, they were capable of detecting changes in the biodegradability of DOM across drinking water treatment processes and in an unchlorinated drinking water distribution system, suggesting that there may be further potential applications for studying DOM biodegradability in surface waters (rivers and lakes) and wastewaters. However, they are probably most suited to comparative studies that mainly require knowing relative concentrations; for example, when assessing the removal or production of labile organic carbon across water treatment steps, following BDOC dynamics at a specific location over time, or detecting "abnormal" changes in regrowth potential for treated drinking water.

■ ASSOCIATED CONTENT

Data Availability Statement

The algorithms for implementing the LOCr method are provided as three MATLAB files (*.m) and three test data sets (*.xlsx). These files are available at https://github.com/ainamcevoy as *LOCr_toolbox_for_oxygensensors*.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestwater.5c00153.

> Treatment steps at collaborating DWTPs; additional methodological details, including procedures for removing outliers in the LOCr method; removal percentages for DOC, H_{BIO}, and LOCr are given as tables; and code for implementing the PoP method in MATLAB using a pre-existing PARAFAC model is provided (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Aina McEvoy − *Department of Architecture and Civil Engineering, Chalmers University of Technology, Gothenburg SE-412 96, Sweden;* orcid.org/0009-0003-8277-0614; Email: ainam@chalmers.se

Authors

Catherine Paul − *Department of Building and Environmental Technology, Lund University, Lund SE-221 00, Sweden;* orcid.org/0000-0003-0323-8359

Oskar Modin − *Department of Architecture and Civil Engineering, Chalmers University of Technology, Gothenburg SE-412 96, Sweden*

Amir Saeid Mohammadi − *Department of Architecture and Civil Engineering, Chalmers University of Technology, Gothenburg SE-412 96, Sweden;* orcid.org/0000-0002-4600-773X

Tomas McKelvey − *Department of Electrical Engineering, Chalmers University of Technology, Gothenburg SE-412 96, Sweden;* orcid.org/0000-0003-2982-5535

Kathleen Murphy − *Department of Architecture and Civil Engineering, Chalmers University of Technology, Gothenburg SE-412 96, Sweden; Department of Building and Environmental Technology, Lund University, Lund SE-221 00, Sweden;* orcid.org/0000-0001-5715-3604

Complete contact information is available at: https://pubs.acs.org/10.1021/acsestwater.5c00153

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Author Contributions

CRediT: **Aina McEvoy** data curation, formal analysis, investigation, methodology, project administration, validation, visualization, writing - original draft, writing - review & editing; **Catherine J Paul** supervision, writing - review & editing; **Oskar Modin** supervision, writing - review & editing; **Amir Saeid Mohammadi** supervision, writing - review & editing; **Tomas McKelvey** software, writing - review & editing; **Kathleen R. Murphy** conceptualization, formal analysis, funding acquisition, methodology, project administration, resources, software, supervision, validation, writing - original draft, writing - review & editing.

Notes

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