Quality of Dissolved Organic Matter Driven by Autotrophic and Heterotrophic Microbial Processes in a Large River

Norbert Kamjunke 1,*, Oliver J. Lechtenfeld 2 and Peter Herzsprung 3

1 Department of River Ecology, Helmholtz—Centre for Environmental Research—UFZ, Brückstraße 3a, D-39114 Magdeburg, Germany
2 Department of Analytical Chemistry and ProVIS—Centre for Chemical Microscopy, Helmholtz Centre for Environmental Research—UFZ, Permoserstr. 15, 04318 Leipzig, Germany; oliver.lechtenfeld@ufz.de
3 Department of Lake Research, Helmholtz Centre for Environmental Research—UFZ, Brückstraße 3a, 39114 Magdeburg, Germany; peter.herzsprung@ufz.de
* Correspondence: norbert.kamjunke@ufz.de; Tel.: +49-391-810-9434

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Abstract: Rivers are regarded as important sites for processing of dissolved organic matter (DOM) from terrestrial sources on its way to the ocean. However, little is known about the longitudinal change of DOM molecular composition in large rivers. Here we performed a Lagrangian sampling in the lower part of the Middle Elbe at low discharge conditions to test how DOM composition changes along the river stretch and how this is related to microbial processes. The concentration of dissolved organic carbon and fluorescence indices showed only subtle longitudinal differences. In contrast, ultra-high-resolution mass spectrometry analysis of riverine DOM detected pronounced changes in molecular composition. Also, chlorophyll a concentration, bacterial abundance, and bacterial production all increased downstream. The three microbial parameters were positively related to intensities of CHO and CHNO molecular formulas with high hydrogen/carbon and low oxygen/carbon ratios but negatively to several CHOS surfactants. To disentangle the role of autotrophic and heterotrophic processes, we developed a new approach and compared slopes from linear regression of DOM compound intensities versus chlorophyll a concentration and bacteria abundance. As a result, most of the positive related DOM compounds were produced by bacteria. In conclusion, longitudinal changes of river DOM seemed to be largely driven by microbial processes.

Keywords: Dissolved organic matter (DOM); Fourier transform-ion cyclotron resonance mass spectroscopy (FT-ICR MS); phytoplankton; bacteria; bacterial production

1. Introduction

Rivers receive considerable amounts of terrestrial organic matter and are important links between terrestrial environments and the ocean [1]. Many transfer processes take place on the way to the river mouth. On the one hand, terrestrial dissolved organic matter is degraded although the processing and the net loss of terrestrial dissolved organic matter (DOM) was described to be low in some systems [2,3]. On the other hand, algal photosynthesis contributes to increasing proportions of autochthonous DOM [4,5], and rivers receive molecules from anthropogenic sources such as agriculture, industry, and waste-water treatment [5,6]. Biological processing of DOM in streams and rivers is mainly performed by heterotrophic bacteria whose growth efficiency depends on DOM source [7]. A part of DOM is degraded to smaller molecules or CO₂ while other fractions are buried in
the sediments or exported to the sea [8–10]. The DOM export from rivers depends on river channel connectivity where DOM heterogeneity increases with fluvial complexity [11], and on hydrological conditions where the intensity of microbial processes is predicted to be high at low discharge when the river acts as an “active pipe” [12].

DOM quality and its changes in streams are commonly assessed using fluorescence spectra [5,6,13]. Several indices relating specific excitation and emission wavelengths have been developed, e.g., an increased freshness index [14] indicates more microbially produced DOM, and a decreased humification index [15] indicates less plant-derived DOM. The very high resolution and mass accuracy of Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS) enables the reliable assignment of thousands of CHO, CHNO, CHOS, and CHNOS (C: carbon, H: hydrogen, N: nitrogen, O: oxygen, S: sulphur) molecular formulas in DOM [16]. In recent years, several mass spectrometry-based studies of riverine DOM were performed at single sites of one or more streams [17–19] and also compared the largest world rivers [20,21]. However, data on longitudinal transects of DOM in streams [22,23] and even more so in large rivers are scarce. Thus, we investigated the change of DOM composition in the lower part of a large river by a Lagrangian sampling according to travel time. We tested the hypotheses that: (1) freshness index and low-molecular weight DOM increase along the river course due to planktonic microbial production, and (2) bacterial abundance and activity are increasing as a consequence. Furthermore, we developed a new approach to disentangle the role of autotrophic and heterotrophic processes for DOM composition via comparison of standardized slopes of linear regression.

2. Materials and Methods

2.1. Study Area and Sampling

Investigations using the research vessel Albis were performed in the river Elbe in Central Europe which is 1094 km long and drains a catchment of 148,268 km². We applied a Lagrangian approach, i.e., a sampling of nearly the same water body along the way downstream according to its travel time, in the lowland part between Magdeburg-Westerhüsen (river km 318 according to German kilometration) and the weir Geesthacht near Hamburg before the tidal zone (km 585). The cruise was performed between 25 and 28 June 2018 at low discharge of 199 m³·s⁻¹, which is slightly less than the mean low discharge at Magdeburg (231 m³·s⁻¹) in summer, as DOM quality is controlled by instream processes during the late growing season (as opposed to transport control during high flow) [20]. Longitudinal sampling was conducted at 13 sites for chlorophyll a and DOM parameters (three to five points along cross sections, 42 samples in total) and at eight sites for bacterial parameters (left and right bank, 16 samples). Samples were taken with a horizontal sampler from 30 cm water depth.

2.2. Chlorophyll a Concentration, Bacterial Abundance, and Biomass Production

For chlorophyll a analysis, samples were filtered onto glass fiber filters (GF-F, Whatman, Buckinghamshire, UK) immediately after sampling on board of the vessel, filters were frozen, and chlorophyll a was measured spectrophotometrically after ethanolic extraction. Abundance of planktonic bacteria from formalin-fixed samples was estimated after staining with acridine orange and counting using an epifluorescence microscope (Axioskop2, Zeiss, Oberkochen, Germany) according to [24]. Production of planktonic bacteria was measured using the leucine technique [25] as described by [24] after storage of samples at 4 °C until the next day. For measurement of planktonic bacterial production (BP), triplicate 5 mL aliquots and one formalin-treated control (3.7%, final concentration) were spiked with ¹⁴C-leucine (12.2 MBq µmol⁻¹, Sigma, 50 nM final concentration). Samples were incubated in the laboratory at in situ temperature for 1 h in the dark on a shaker. Incorporation was stopped with formalin, and 0.6 mL 50% trichloracetic acid (TCA) was added. Proteins were extracted for 15 min and filtered onto 0.2 µm Nuclepore membranes. Filters were rinsed twice with 1 mL 5% TCA and once with 80% ethanol. After dissolving the filters in 0.5 mL Soluene (PerkinElmer, Waltham, Massachusetts) and adding 2.5 mL Hionic Fluor (PerkinElmer,
Waltham, Massachusetts) to each scintillation vial, radioactivity was measured using a Liquid Scintillation Analyzer (2300 TR, Packard). The external standard ratio method was used for quenching. Carbon production was calculated using the equations of [25].

2.3. Measurement of Dissolved Organic Carbon (DOC) Concentration and Fluorescence

For analysis of DOC, water samples were transferred into acid-rinsed and combusted brown glass bottles, kept at 4 °C for a maximum of 24 h and filtered through glass fiber filters (Whatman GF/F). DOC concentrations were measured by infrared detection of CO2 after high temperature combustion (DIMATOC 2000, Dimatec Analysentechnik GmbH, Essen, Germany). Fluorescence intensity was measured during emission scans (240–600 nm every 3.27 nm; 8 pixel) at set excitation wavelengths in 3 nm increments from 240 nm to 600 nm. A 5 nm band pass for excitation and emission wavelength and 1 s integration time were used. Fluorescence data were corrected (including blank subtraction) using HORIBA internal software. Afterwards, the excitation–emission matrices (EEMs) were Rayleigh-scattering masked and corrected for inner-filter effects [26] using an Aqualog® (HORIBA Jobin Yvon GmbH, Bensheim, Germany) internal recorded Ultraviolet-Visible (UV-Vis) absorbance spectrum recorded in the same quartz cell as the fluorescent sample.

Three fluorescence indices were used to describe the general DOM structure: freshness index, humification index (HIX), and fluorescence index (FI). The freshness index (β/α, where β represents more recently derived DOM and α represents highly decomposed DOM) [14] was computed as the ratio of the emission intensity at 380 nm divided by the highest detected emission intensity between 420 nm and 435 nm, all obtained for excitation at 310 nm. The ratio of β/α is an indicator of autochthonous inputs and indicates relative contributions of recent, microbially produced DOM. The HIX was computed by dividing the peak area under the emission spectra between 435–480 nm with the peak area under the emission spectra between 300–345 nm, both at 255 nm excitation wavelength [15]. The humification index is associated with the degree of condensation of fluorescing molecules and lower hydrogen/carbon (H/C) ratios and supposedly indicates extent of humification. The FI is the emission intensity at 470 nm divided with that of 520 nm at 370 nm excitation [27].

2.4. High-Field FTICR Mass Spectrometry of DOM

2.4.1. Solid Phase Extraction (SPE)

Aliquots of 500 mL water were filtered through pre-combusted glass fibre (GF/F; Whatman) and acidified with HCl (Suprapur, Merck, Kenilworth, NJ, USA) to pH 2. Subsamples of 450 mL of the acidified filtrate were passed through 500 mg styrene-divinyl-polymere type (PPL) solid-phase cartridges (Agilent, Waldbronn, Germany) using a SmartPrep® automatic cartridge extractor (Horizon Technology, Salem, MA, USA) to desalt the sample for subsequent direct infusion electrospray ionization mass spectrometry (DI-ESI-MS). The retained DOM was eluted twice with 2 mL methanol (high performance liquid chromatography grade, Baker, Phillipsburg, New Jersey); the extracts (solid phase extractable dissolved organic matter (SPE-DOM) in the following) were diluted with methanol to 5 mL and were stored at −20 °C until analysis [28,29]. The average carbon:sorbens ratio was 209 ± 26 and the average carbon-based recovery (60 ± 11%; n = 42).

2.4.2. FT-ICR MS Measurement

SPE extracts were diluted to 20 ppm, mixed 1:1 (v/v) with ultrapure water and analyzed in random order with an FT-ICR mass spectrometer equipped with a dynamically harmonized analyzer cell (solariX XR, Bruker Daltons Inc., Billerica, MA, USA) and a 12 T refrigerated actively shielded superconducting magnet (Bruker Biospin, Wissembourg, France) instrument (capillary voltage 4.3 kV) was used at an infusion rate of 10 µL min⁻¹. For each spectrum, 256 scans were co-added in the mass range m/z 150–3000 Da with 10 ms ion accumulation time and 4 MW time domain (resolution @ 400 m/z was ca. 500,000). Mass spectra were internally calibrated with a list of peaks (m/z 200–650 Da, n > 147) commonly present in natural organic matter, the mass accuracy after internal calibration was better than 0.11 ppm (n = 42). Peaks were considered if the signal/noise (S/N)
ratio was greater than four. Raw spectra were processed with Compass DataAnalysis 5.0 (Bruker Daltonics Inc., Billerica, MA, USA).

2.4.3. Data Evaluation

Molecular formulas were assigned to peaks in the mass range m/z 150–750 with an error range of ±0.4 ppm according to [30]. Molecular formulas were calculated considering the following elements $^{12}$C$^{0–60}$, $^{13}$C$^{0–1}$, $^{1}$H$^{0–122}$, $^{16}$O$^{0–40}$, $^{14}$N$^{0–4}$, $^{32}$S$^{0–1}$, $^{34}$S$^{0–1}$. Only formulas with $0.3 \leq H/C \leq 2.5$, $0 \leq O/C \leq 1$, $0 \leq N/C \leq 0.5$, $0 \leq DBE \leq 20$ (double bound equivalent, $DBE = 1 + \frac{1}{2} (2C-H + N)$) and $-10 \leq DBE-O \leq +10$ were considered for further data evaluation [31,32]. Isotopologue formulas ($^{13}$C, $^{34}$S) were used for quality control but removed from the final data set as they represent duplicate chemical information. On average 5642 ± 568 molecular formulas were assigned to 15,900 ± 1180 peaks in the considered mass range ($n=42$). Relative peak intensities (RI) were calculated based on the summed intensities of all assigned peaks in each sample [33,34]. Only molecular formulas present in all samples ($n=2462$) were considered for further analysis. These formulas represent on average 82.5 ± 2% of the total intensity of all assigned peaks.

We applied a linear regression between the relative mass peak intensity as dependent variable and (i) the distance of the sampling sites from the source, (ii) the chlorophyll a (Chl a) concentration, (iii) the bacterial abundance, and (iv) the bacterial production as independent variables. Independent variables were standardized (Table S1). The slope values were used at $p < 0.05$. The slope of linear regression can thus be regarded as semi-quantitative measure for the change in compound abundance; a positive/negative slope reflects an increase/decrease of the compound abundance along the flow length, with Chl a concentration, etc. The calculation of linear regression was previously used for comparable data sets and proved useful to disentangle microbial–molecular relationships [34]. The standardization allows the comparison of slopes for different parameters.

Here we used the slope differences for significant regressions, i.e., $|slope$ (peak intensity vs. Chl a concentration)$| - |slope$ (peak intensity vs. bacterial abundance)$|$ indicating the dominant process determining compound abundance changes (for CHO and CHON only positive slopes and for CHOS only negative slopes were evaluated for this calculation).

3. Results

3.1. Longitudinal Changes

The concentration of chlorophyll a was 29 µg·L$^{-1}$ in Magdeburg (km 318) and increased to maximum values of 234 µg·L$^{-1}$ at km 536 (Figure 1). Bacterial abundance increased from 1.7 to 3.6 × 10$^9$ L$^{-1}$ and bacterial production from 20 µg C L$^{-1}$·d$^{-1}$ to 160 µg C L$^{-1}$·d$^{-1}$. The concentration of DOC increased slightly along the river stretch starting from 5 mg L$^{-1}$ in Magdeburg and reaching 6 mg L$^{-1}$ in Geestacht (km 585). The fluorescence index FI decreased within a narrow range from 1.39 to 1.32. The freshness index $\beta/\alpha$ did not show a consistent longitudinal pattern whereas the humification index HIX decreased slightly from 5 to 4.5. Relative intensities of SPE-DOM molecular formulas detected by FT-ICR MS either declined or increased along the river stretch (see Figure 1 for two examples) or did not change significantly. The slopes of all SPE-DOM molecular formula intensities against river kilometers showed a longitudinal increase of many CHO and CHON formulas but a longitudinal decrease of several CHOS compounds (Figure S1).
Figure 1. Lateral averages of averages of chlorophyll a, bacterial abundance bacterial production, concentration of dissolved organic carbon (DOC), fluorescence index (FI), freshness index (β/α), humification index (HIX), and two examples for molecular formulas with decreasing and increasing relative intensities along the river Elbe between river kilometers 318–585.

3.2. Relation to Microbial Processes

When intensities of FT-ICR MS components were correlated (by linear regression) to standardized chlorophyll a concentration, we detected positive slopes for CHO formulas with H/C ratios between 0.9–1.6 and O/C ratios between 0.1–0.6 and a molecular mass up to 500 Da (Figure 2). Few formulas with negative slopes were observed with H/C ratios >1.6 and <0.9. Formulas of the CHNO compound class showed positive slopes in the same range of O/C and H/C ratios as CHO compounds but with lower molecular weight (<400 Da). Almost no negative slopes were calculated for CHNO formulas. In contrast, negative slopes were found for CHOS formulas along two characteristic lines in the van Krevelen space. The regression of peak intensities with bacterial abundance resulted in a similar pattern (Figure 3), where strongly positive slopes for the CHO class extending up to 600 Da and almost no negative slopes. The slopes of the CHNO class were slightly less positive than those of the CHO class. In contrast to the results for chlorophyll a, CHNO formulas with positive slopes from the regression to bacterial abundance covered a larger molecular diversity (0.7 < H/C < 1.6) as compared to the CHO formulas (1.1 < H/C < 1.6). An extended mass range for the CHNO formulas with positive slopes after bacterial abundance regression (up to 500 Da) was found as compared to the chlorophyll a regressions. CHOS formulas related negatively to bacteria showed similar H/C, O/C, and mass ranges as compared to CHOS formulas related to Chl a. The number of molecular formulas with significant regression to bacterial production was slightly lower than those related to bacterial abundance but were located in similar regions in the van Krevelen space (Figure S2).
Figure 2. Slopes of regressions between intensities of solid phase extractable dissolved organic matter (SPE-DOM) molecular formulas and standardized chlorophyll a concentration for CHO (a); CHNO (b); and CHOS components (c). See also the data base in the Supplementary Materials.
Figure 3. Slopes of regressions between intensities of solid phase extractable dissolved organic matter (SPE-DOM) molecular formulas and standardized bacterial abundance for CHO (a); CHNO (b); and CHOS components (c). See also the data base in the Supplementary Materials.

The difference between slopes of SPE-DOM molecular formulas versus chlorophyll and those versus bacterial numbers showed were negative for the CHO compound class (i.e., higher slopes versus bacterial numbers; Figure 4). Slope differences were less pronounced for CHON formulas. Slope differences for CHOS formulas were slightly positive (chlorophyll-dominated) for the C₆H₄(CH₂)ₙO₅S and negative (bacteria-dominated) for the C₈H₆(CH₂)ₘO₅S. A similar pattern as for bacterial numbers was found for bacterial production (Figure S3).
Figure 4. Differences of slopes between solid phase extractable dissolved organic matter (SPE-DOM) molecular formulas versus chlorophyll a concentration (Figure 2) minus those versus bacterial abundance (Figure 3) for CHO (a); CHNO (b); and CHOS components (c).

4. Discussion

4.1. Longitudinal Changes

The concentration of chlorophyll a increased along the investigated stretch of the river Elbe of 270 km length. After an eight-fold rise, it reached high maximum values of 230 µg·L\(^{-1}\). This was observed before in the river Elbe [35] and in other rivers [36]. Long travel times at base flow and high light availability on end of June enabled high growth rates of phytoplankton. This pattern was not reflected by DOC concentration and fluorescence of DOM. DOC concentration increased only slightly, FI even decreased, and \(\beta/\alpha\) did not show a longitudinal regression. This was in contrast to findings of longitudinal increases of FI and BIX in other rivers [37,38]. Only the slightly decreasing values of HIX in the river Elbe were in line with reports of declining HIX [37,38]. Obviously, fresh organic carbon potentially produced by growing phytoplankton was immediately taken up by heterotrophic planktonic bacteria. Their production increased by factor eight while their abundance increased by factor two, i.e., the specific bacterial production increased by factor four, probably by higher substrate supply due to algal photosynthesis.

4.2. Molecular Formula Components and Their Relation to Microbial Processes

In contrast to fluorescence indices and bulk DOC concentration, results of single FT-ICR MS derived molecular formulas showed significant and specific pattern of DOM changes along the river transect. Many CHO and CHNO formulas with high H/C and low O/C ratios increased along the
river stretch and with increasing chlorophyll concentration. A relative enrichment of aliphatic compounds by instream processes was detected also in other rivers [39,40] and may be attributed to microbial production of fresh DOM [32,41,42]. In contrast, we did not detect a substantial decline or removal of oxygen-rich, unsaturated compounds in our study, as observed in a smaller stream [23] and in other rivers [39,40]. This is in accordance with the observation from HIX values. The HIX is positively related to the abundance of oxygen-rich and relatively unsaturated compounds [43]. Strongly decreasing intensities of such compounds would result in decreasing HIX values which was not the case in this study.

Obviously, terrestrial DOM was already processed in the lower part of the river Elbe confirming the conservative behavior of aromatic DOM [44]. Instead, we detected a decline of specific CHOS formula abundances. These compounds are potentially metabolites of surfactants as described by [45] for effluent organic matter and previously found in anthropogenically influenced surface waters [23,46]. For example, C6H4(CH2)nO5S and C8H6(CH2)mO5S series were also detected after the inflow of a waste-water treatment plant (WWTP) effluent to a small river, and decreased subsequently [23]. In the river Elbe, these components were evidently further degraded along the river stretch.

Our new approach enables to us disentangle the role of autotrophic and heterotrophic processes for DOM composition along a river. The absolute value of slopes was higher for bacteria (responsible for heterotrophic processes) than for Chl a (autotrophic processes) for nearly all significant related SPE-DOM formulas. Using slope differences, we conclude that predominantly bacterial activity (compared to algal production) was responsible for the observed increase of relatively aliphatic molecules. This indicates that evidently the major part of algae derived DOM was rapidly processed by bacteria. Observed increases in molecular formula intensities point to a pool of relatively stable microbial metabolites, which do not undergo fast mineralization and are transported to the coastal ocean, a mechanism that may play a role in long term stabilization of organic carbon in marine systems [42].

5. Conclusions

We detected a longitudinal change of DOM molecular composition in a large river concurrent with increasing chlorophyll concentration. While DOC concentration and DOM fluorescence did not vary considerably, aliphatic CHO and CHON compounds measured by FT-ICR MS increased while CHOS surfactants decreased in their abundances. Therefore our first hypothesis was partly confirmed. Chlorophyll concentration, bacterial abundance and bacterial production were correlated to specific SPE-DOM molecular formulas, confirming the second hypothesis. Our difference approach enabled the separation between few dominant autotrophically and a majority of dominant heterotrophically produced DOM compounds. Further experiments are needed to show the clear effect of photosynthetic production in approaches with and without light and the effect of bacterial degradation in approaches with and without high bacterial biomass. Nevertheless, our study indicates that longitudinal changes of DOM composition in rivers are due to microbial processes.

Supplementary Materials: The following are available online at www.mdpi.com/2073-4441/12/6/1577/s1, Figure S1: Slopes of regressions between intensities of solid phase extractable dissolved organic (SPE-DOM) molecular formulas and standardized river kilometer, Figure S2: Slopes of regressions between intensities of solid phase extractable dissolved organic (SPE-DOM) molecular formulas and standardized bacterial production, Figure S3: Differences of slopes between solid phase extractable dissolved organic (SPE-DOM) molecular formulas versus chlorophyll a concentration (Figure 2) minus those versus bacterial production (Figure S2).

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