MULTI ReUse

“Reverse Isotope Labeling”: A new method to determine the biodegradable dissolved organic carbon (BDOC)

Background

The microbiological stability of water is not only determined by the dissolved organic carbon (DOC) but mostly by the bioavailable portion of the DOC that can be used for bacterial growth. The biodegradable dissolved organic carbon (BDOC) contains the part of the carbon pool that is used for assimilation forming new biomass and the part that is used for dissimilation or metabolism producing CO_2, which leads to a mineralization of the organic carbon. The part of the BDOC that is used for assimilation is called assimilable organic carbon (AOC). Methods for the determination of AOC are based on cultivation and determining growth of microbial cells. However, such methods are hampered by the fact that not all cells can be cultivated and, hence, the bad reproducibility by different laboratories.

The “Reverse Isotope Labeling” (RIL) is a new method to determine BDOC. It was already used to investigate the BDOC degradation in activated carbon filters for drinking water treatment (Dong et al. 2017). The RIL method measures the amount of released CO_2 that forms through the biodegradation of DOC. The big advantage of this determination is that the BDOC is determined as an internationally comparable unit (mg CO_2 L^{-1}), which easily can be converted into mg BDOC L^{-1}.

Another big advantage over the classic BDOC methods is that with RIL the $^{13}C/^{12}C$ isotope ratio of CO_2 is measured rather than the absolute amount of CO_2. Based on the isotope dilution method applied, the exact amount of CO_2 released (mineralisation, resp. BDOC) can be determined by using a mass balance approach. The advantage of the RIL method is that the isotope ratio of CO_2 can be determined very precisely and with simple absorption photometry. Hence, already very small amounts of mineralised organic carbon can be detected.

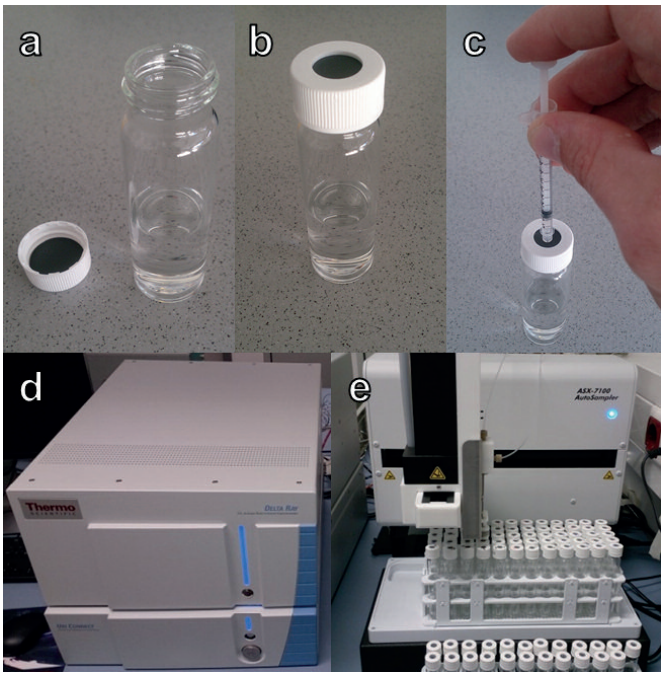

Figure 1: Sample preparation for RIL. a, b, c: addition of ^{13}C-labelled bicarbonate. d, Isotope-Ratio-Infrarot-Spektrometer, Thermo Fisher), and c, autosampler ASX-7100, Teledyne.

Technology

The degradation of substrates to CO_2 is often evaluated by adding a ^{13}C-labelled carbon source and measuring the evolution of the product $^{13}CO_2$ in the background of a $H^{12}CO_3^-$-buffer to make use of the extremely sensitive analytical techniques to measure stable isotope-labelled CO_2. However, natural substrates such as DOC or BDOC cannot be labelled with stable isotopes. Furthermore, synthesized DOC would not represent the chemical composition of natural DOC of water. We therefore simply "reverse" the system by following the BDOC degradation in a ^{13}C-bicarbonate buffer. The natural DOC is than degraded to $^{12}CO_2$ and changes the carbon stable isotope ratio $^{13}C/^{12}C$ of the carbonate buffer. This change can be measured very precisely and allows calculating the developed $^{12}CO_2$ in the sample, using a mass balance approach.

Similar approaches for the determination of dissolved inorganic carbon, short DIC (Freije-Carrelo et al. 2018), as well as photochemical mineralisation of DOC (Powers et al. 2017) showed the successful application of isotope dilution methods. Using of a new infrared spectrometer (Delta Ray, Thermo Fisher, Bremen, Germany) the $^{13}C/^{12}C$ isotope ratio of CO_2 can be measured very precisely over a large range of $^{13}C/^{12}C$ isotope ratios. This in return allows for sensitive determination of the CO_2 production.

The method

Water samples are put into screw cap vials closed with a septum. Then, ^{13}C-labelled bicarbonate ($H^{13}CO_3^-$) is added (see figure 1 a–c). To measure the DIC, liquid samples (0.5 ml) are taken and put into 12 ml vials amended with 85 % phosphoric acid (50 µl). All CO_2 evaporates into the gas phase and is channelled over a gas flow into the infrared spectrometer (figure 1 d and e) where it is measured. The CO_2 concentration in the sample is then calculated based on the isotope ratio. The difference CO_2 concentrations between day 0 (original state) and day 7 (substrate metabolism after 7 days) give the amount of BDOC degraded. The method is displayed schematically in figure 2.

RIL for determination of BDOC in wastewater treatment

The BDOC in the effluent of a wastewater treatment plant was determined with the RIL method before and after an additional purification step by a combination of flocculation and ultrafiltration. Figure 3 shows the BDOC determined after incubating the different waters for 7 days. The positive BDOC was reduced by ca. 60 % through flocculation and ultrafiltration indicating the positive effect for the stabilisation of the produced water.

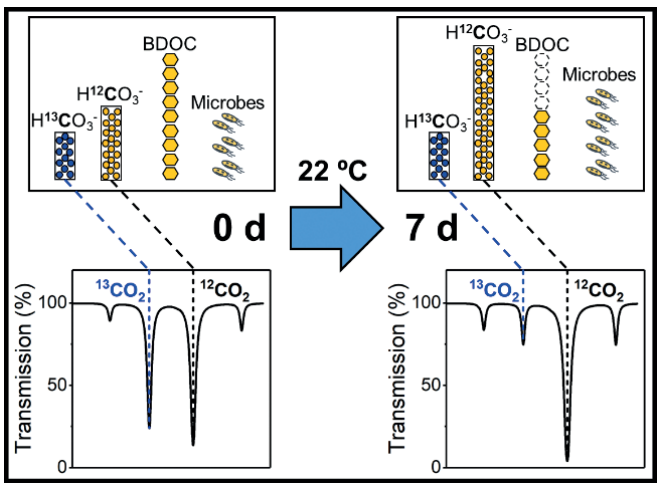

Figure 2: Schematic view of quantifying microbial respiration with RIL. At day zero, ^{13}C-labelled bicarbonate is added to a water sample containing the indigenous microbial community and the organic substrate (BDOC) (a). The stable isotope ratio of the bicarbonate buffer is measured as the starting value (c). At day 7, the BDOC is degraded and released $H^{12}CO_3^-$(b), which changes the isotope ratio of the bicarbonate buffer (d). The difference of the stable isotope ratio together with the DIC concentration allows to calculated the amount of CO_2 released.

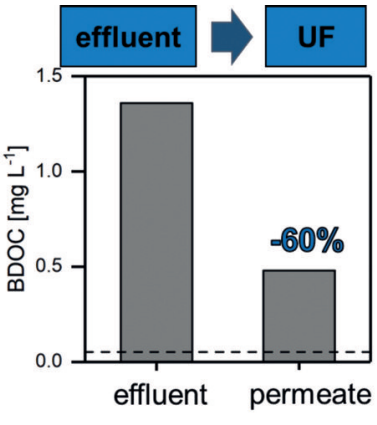

Figure 3: Determination of BDOC in wastewater treatment plant effluent and after flocculation and ultrafiltration. The ultrafiltration removed over 60 % of the BDOC. The measurement precision and detection limit was below 50 µg/l in these samples (dashed line).

Long-term measurements of water with RIL for almost 100 days showed that the microbial mineralisation of the DOC persists for long time with almost constant activity. Figure 4 shows the chronological sequence of the BDOC for raw water and after flocculation and ultrafiltration.

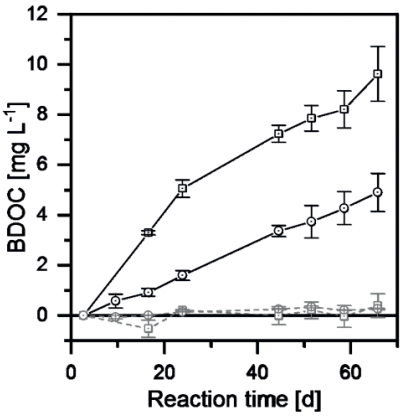

Figure 4: Long-term analysis of BDOC in effluent of the wastewater treatment plant (black squares) and after ultrafiltration (black circles). Grey symbols represent sterile filtered controls (0.2 µm) of effluent (grey square) and after ultrafiltration (grey circles). The data depict that BDOC degradation is more or less constant over months.

Potential of the method

The RIL method offers an internationally comparable parameter for BDOC analysis. Hence, the biological stability of waters can be better classified for different applications. For example, it is possible to examine the BDOC of service waters of different quality, independent from hygienic conditions, particle, and nutrient load. This is especially interesting for the further treatment of local wastewaters, because they can differ strongly in their physical, chemical, and biological properties. Since RIL makes use of the indigenous microbial community with the original water samples it is a cultivation and nutrient independent procedure reflecting the natural conditions in the water as good as possible.

The RIL procedure is also applicable for oxygen free waters in the presence of other electron acceptors (oxidizing agents) such as nitrate and sulphate. In principle, anoxic waters (treated local wastewater, anoxic service water or ground water) can be analysed for BDOC. Because of its high sensitivity, the RIL procedure is an alternative to other standards, e. g. ISO 7827 and ISO 14593. The measuring precision for the DIC is about 30 µg L^{-1}. Right now, the sensitivity of the RIL procedure is improved in order to determine the BDOC in drinking and ground water.

Conclusion

The new RIL method provides a sensitive and easy to handle method for the determination of the BDOC and delivers comparable results with precise specifications of concentrations. The RIL procedure measures the degradation of carbon sources, i. e. DOC in the water and therefore indicates the BDOC. Hence, it measures the biological stability of the treated water and indicates when the BDOC reaches levels where measures for risk minimization need to be taken.

Literature

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Abbreviations

AOC	Assimilable organic carbon
BDOC	Biodegradeable dissolved organic carbon
DIC	Dissolved inorganic carbon
DOC	Dissolved organic carbon
UF	Ultrafiltration
RIL	Reverse Isotope Labeling

Imprint

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Short description of the MULTI-ReUse project

Treated wastewater is an important part of the water cycle. It usually is fed into rivers, something that is acceptable from an environmental point of view but for the use in agriculture or industry the water often is unsuitable. MULTI-ReUse closes this gap by developing and implementing of new procedures for the reuse of service water. The aim of MULTI-ReUse therefore is the development, demonstration and evaluation of a modular water treatment system, in order to offer service water in different qualities and quantities for the different purposes and to competitive prices.

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