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# DETERMINATION OF BIODEGRADABLE DISSOLVED ORGANIC CARBON IN WATERS: COMPARISON OF SUSPENDED AND ATTACHED BIOMASS METHODS

# BIOLOĢISKI DEGRADĒJAMĀ ORGANISKĀ OGLEKĻA NOTEIKŠANA ŪDENĪ: SUSPENDĒTAS UN ADSORBĒTAS BIOMASAS METOŽU SALĪDZINĀŠANA

**Kristīna Tihomirova,** Department of Water Engineering
and Technology, Riga Technical University
Adress: 16 Azenes iela, LV-1048, Riga, Latvia
PhD student, M.chem.
kristina.tihomirova@uitk.bf.rtu.lv

**Jānis Rubulis,** Department of Water Engineering and Technology,
Riga Technical University
Adress: 16 Azenes iela, LV-1048, Riga, Latvia
Researcher, Dr.sc.ing.
janisrubulis@gmail.com

**Tālis Juhna,** Department of Water Engineering and Technology,
Riga Technical University
Adress: 16 Azenes iela, LV-1048, Riga, Latvia
Asoc. Professor, Dr.sc.ing.
talisj@bf.rtu.lv

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## Introduction

Natural organic matter (NOM) in drinking water can cause various problems including taste, odor, color and formation of disinfection by-products. Biodegradable dissolved organic carbon (BDOC) is fraction of NOM which is used by bacteria leading to regrowth of microbes in distribution systems. Therefore, the control of BDOC has been recognized as an important part of the operation of drinking water treatment plants (WTP) and distribution systems [1]. BDOC determination is based on measuring the consumption of DOC through the ability of a mixed or pure microflora to catabolise organic carbon to carbon dioxide and/or new biomass. Both suspended and attached biomass is being used in the BDOC assay. Although there were several studies aiming at comparing several methods [2, 3, 4 and 5], still it is not clear which methods is most practically applicable by water utilities: rapid, easy to use, sufficiently accurate and reproducible.

The aim of this work was to compare several commonly used BDOC methods to identify the most rapid and easily applicable for control of water quality in drinking water. Both methods which are based on water sample incubation of water sample with suspended biomass and methods based on water sample percolation though porous media with attached

biomass were used. The BDOC concentrations were determined in river water, drinking water and synthetic solution.

## Materials and methods

### Sampling procedure

The concentrations of BDOC were analyzed with several methods in the same samples of drinking water collected from the Daugava WTP and water of river Daugava. The third sample set (synthetic solution) was prepared in laboratory where deionised water was supplemented with the sodium acetate as the carbon source with approx. concentration of 6 mg-C/l.

All the glass bottles, flasks used in these experiments were cleaned thoroughly with a 10% solution of potassium dichromate in concentrated sulfuric acid and rinsed with hot tap water, dried and covered with aluminum septum heated for 6 h at +250ºC in order to avoid organic carbon release [6]. Filtration systems were sterilized for 20 minutes at +121ºC. The filters used must be carefully rinsed, first with 1000 ml of sterile ultra pure water (Elga PureLab Ultra, Veolia Water Ltd., UK) and then with the water sample (200 ml).

### DOC determination

The concentration of dissolved organic carbon (DOC) was determined with a Shimadzu 5000 A TOC analyzer (Shimadzu Corporation, Kyoto, Japan) according to European Standard EN 1484:1997 "Water analysis - Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC)" [7]. Before each measurement all samples were filtered thought a sterile membrane filter (0.45 μm, Sartorius AG, Germany) which had been carefully rinsed first with 100 ml of sterile ultra pure water and then with the sample (10 ml). Each sample was tested in duplicate and then the mean values were calculated (CV≤2%). The blank and control solutions were analyzed with each series of sample in order to verify the accuracy of the results obtained by the method.

### Method of pure culture inoculation – 1

Before the calculation of BDOC concentration the samples of water (200 ml) were sterilized by filtration through a membrane filter (0.45 μm, Millipore Corporation, USA or 0.45 μm, Sartorius, USA) and supplemented with a inoculums (2 ml) of bacteria *Pseudomonas fluorescens* P17 (MSCL 599). Incubation of the inoculated sample was performed at 21±2ºC in the dark for 28 days with everyday manual shaking. The sub-samples (5 ml) were collected at the beginning of the incubation (just after addition of the bacteria) and at 1, 2, 5, 7, 9, 15, 21 and 28 incubation days for DOC determination [8, 9 and 10]. The BDOC value was calculated as the difference (ΔDOC) between the concentration of DOC in the beginning of the incubation and the highest decreases of DOC concentration.

### Method of mixed bacteria inoculation – 2

The methodology was the same like for method 1 with exception that the concentration of the inoculum (2 ml) was taken from drinking water biofilm sample cultured in the dark and at room temperature (+21±2ºC) on glass carrier beads sized 6 mm (Assistant, Germany) for 3 months (see Method 3).

**The batch test with attached biofilm – 3**

The BDOC concentration is determined by using a fixed biofilm [11, 12 and 13] cultured on sterile, with sample water three times pre-washed glass carrier beads (100 g) sized 6 mm. In the glass bottle filled with mixture of one-third river water filtered trough the membrane filter (1.2 μm Millipore Corporation, USA), and two-thirds of water collected from WTP biofilters. The adaptation of the microorganisms were done on shaker (RPM=150, Multi-Shaker PSU 20, Biosan, Latvia) for 4 weeks at room temperature (21±2ºC) in the dark (weekly water exchange was applied) until concentration of adenosine 5-triphosphate (ATP) measured according to method proposed by van der Kooij and Veenendaal (2001) [14] reached acceptable level [15]. The ATP concentration was determined in all water samples by taking 100 μl of water sample.

The samples for the DOC analysis and BDOC calculation were performed in the same interval (see Method 1).

**The batch test with attached biofilm supplemented with nutrients – 4**

The BDOC concentration is determined similarly to the method 3 with exception that the mixture of the sample (100 ml) water was supplemented with the inorganic nutrients solution [16] of 100 μl. The solution were prepared by dissolving 4.55 g $(NH_4)_2SO_4$, 0.2 g $KH_2PO_4$, 0.1 g $MgSO_4 \times 7H_2O$, 0.1 g $CaCl_2 \times 2H_2O$ and 0.2 g NaCl in sterile ultra pure water (1000 ml) .

**The column system – 5**

The BDOC measurement were performed on fixed biofilm [13, 17 and 18] using two standard chromatography glass columns (H=29 cm, ø=2.5 cm, Chromaflex, USA) connected in series and filled with glass carrier beads (200 g) with total surface area 3.76 cm²/g. The sample was continuously pumped upward to the columns by a peristaltic pump (Masterflex L/S, Cole-Parmer, USA). An optimal flow rate of 3-5 ml/min was used, representing a compromise between the required retention time (1h/column) and rapidness of the assay. Biofilm was cultured by incubation in a mixture of water (see Method 3) [13]. To adapt the microorganisms, the carriers were stored for 16 weeks at roome temperature (21±2ºC) in the dark (weekly water exchange). The BDOC value was calculated as the difference (ΔDOC) between the inlet DOC and the DOC outlet of the second column (after 2h).

**Statistical analysis**

To compare all methods of BDOC determination statically significant assays of the differences (procedure for computing one-way ANOVA) were developed, with paired samples when possible [19].

**Results**

Initial concentration of DOC in drinking water sample measured with several methods was in range from 2.49±1.37 to 4.08±0.16 mg/l. The highest initial concentrations of DOC in drinking water sample showed method (Nr.3) of batch test with attached biofilm which latter was excluded from ANOVA analysis. In all presented BDOC bioassays, except the column system (Nr.5), the changes of DOC concentration must be analyzed within time period of 28 days (Figure 1).

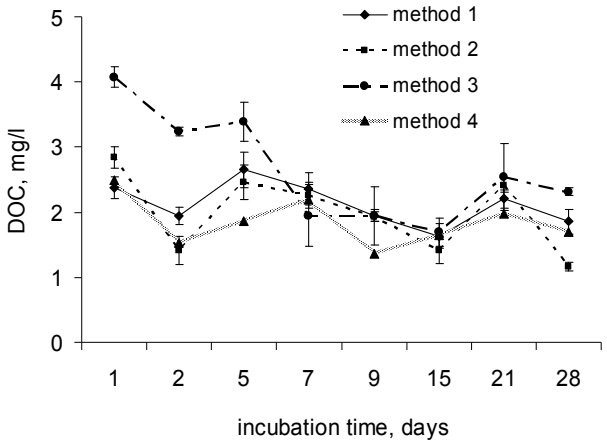


*Fig. 1. Concentration of DOC in sample of drinking water*

Due to bacteria growth processes in the sample the concentration of organic carbon tend to decrease. The highest DOC consumption was reached within 9-15 days when DOC concentration decreased for 31-59%. This decrease of DOC is assumed as BDOC (Table 1) and was calculated in the range from 0.75±0.14 to 2.39±0.27 mg/l.

*Table 1. The concentration of BDOC measured with several methods. Table represents average values (n = 3) with standard deviation*

| Method | River water | | Drinking water | | Synthetic solution (~6 mg-C/l) | |
|---|---|---|---|---|---|---|
| | BDOC, mg/l | BDOC, (%) | BDOC, mg/l | BDOC, (%) | BDOC, mg/l | BDOC, (%) |
| Pure culture inoculation 1 | 2.22±0.29 | 18±2 | 0.75±0.14 | 31±5 | 5.36±0.24 | 92±3 |
| Mixed bacteria inoculation 2 | 2.06±0.25 | 17±2 | 1.43±0.17 | 50±6 | 5.26±0.08 | 93±2 |
| Batch test with attached biofilm 3 | 2.35±0.78 | 20±5 | 2.39±0.27 | 59±5 | 5.43±0.28 | 94±3 |
| Batch test with attached biofilm supplemented with nutrients 4 | 2.18±0.27 | 19±2 | 1.12±0.16 | 45±5 | – | – |
| Column system 5 | 1.71±0.37 | 14±1 | 1.13±0.44 | 48±9 | 5.33±0.09 | 97±2 |

Our experiments showed that procedure of bacteria incubation can be shortened from 28 days to 15 days. The methods with natural bacterial communities (Nr.2 and Nr.3) showed more significant tendencies of DOC consumption in comparison with *P. fluorescens* P17 (Nr.1) grew. The natural culture is more capable to growth in oligotrophic environment. Although microbiological growth in the drinking water samples is phosphorus limiting the sample supplementation with inorganic nutrients (method Nr.4 compared with Nr.3) didn't get expected decrease of DOC concentration.

The ANOVA analysis showed that BDOC concentration in drinking water calculated with method Nr.3 significantly differ from results of other methods and therefore was excluded from ANOVA analysis (Figure 2). Statistical analysis showed that difference between the BDOC results of drinking water calculated for the three samples of each method is not statistically significant: ($F_{3;8}$=8.8746; P<0.05).

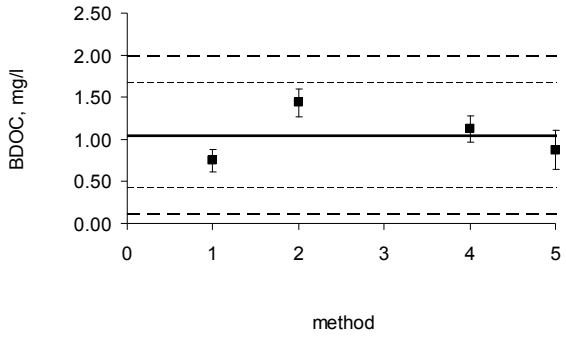


*Fig. 2. BDOC concentration in drinking water samples calculated with four different methods. The data points represents average values (n = 3) of each method, standard errors are indicated by the bars. Black solid line represents average value of all methods, the confidence interval defined by the two standard deviations and three standard deviations of the measured value (2σ and 3σ) are indicated with dotted lines.*

In the river water DOC ranged from 11.52±0.17 to 12.28±0.51 mg/l estimated with five methods. The results of bioassays with attached biofilm (Nr.3 and 4) lasted for 28 days showed tendency of significant decreases of DOC concentration within first 5-7 days and latter increases up to initial concentrations (Figure 3.). The methods with inoculum (Nr.1 and 2) showed highest concentrations of DOC in the sample of river water for day 5. At the last days of incubation these two methods showed lowest concentrations of DOC compared to methods Nr. 3 and Nr.4. The concentrations of BDOC in river water were calculated in the range from 1.71±0.37 to 2.35±0.78 mg/l and these concentrations were 14 to 19% of DOC concentration (Table 1). Similarly to the results for the drinking water samples the highest BDOC concentration showed batch test method with attached biofilm (Nr.3). Although the ANOVA analysis (Figure 4) showed that results of all methods is statistically comparable ($F_{3;11}$=1.6800; P<0.05).

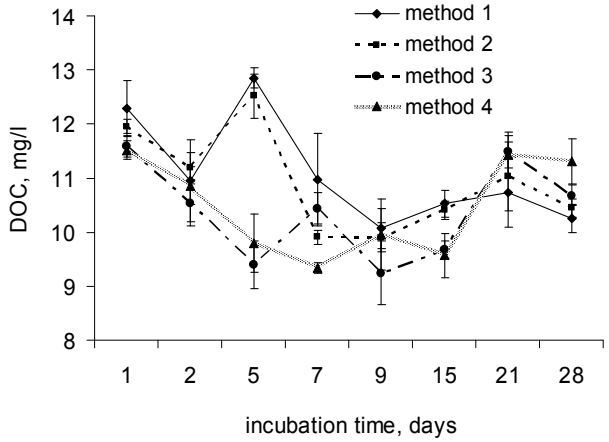


*Fig. 3. Concentration of DOC in sample of river water*

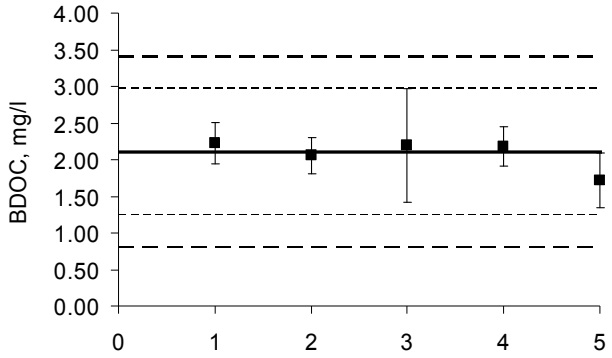


*Fig. 4. BDOC concentration in river water samples calculated with four different methods. Legends: see Fig. 2.*

The acetate is easy convertible substrate for bacterial growth therefore solution of approx. 6 mg-C/l was used as control to compare all methods. The initial concentration of DOC in solution was measured in the range from 5.47±0.04 to 5.81±0.11 mg/l. Within the first 5 days almost all substrate (92-97%) was converted in to the bacterial biomass and used for bacterial growth (Figure 5).

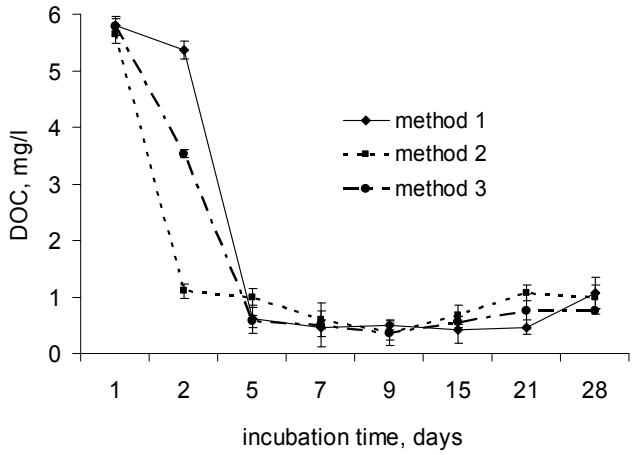


*Fig. 5. Concentration of DOC in sample of sodium acetate solution.*

The ANOVA analysis showed that the difference in mean of the tree samples is not statistically significant for sodium acetate solution ($F_{3;8}$=2.7868; P<0.05) and average values of all methods, each of them are shown in Figure 6.

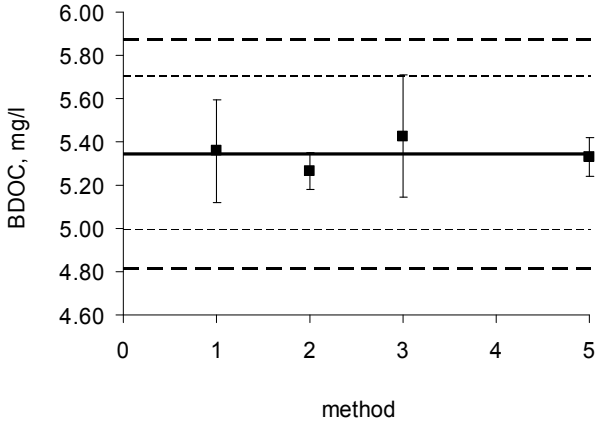


*Fig. 6. BDOC concentration in synthetic water samples calculated with four different methods. Legends: see Figure 2.*

## Discussion

BDOC bioassay is based on measurement of changes in DOC concentrations. BDOC content represents the fraction of DOC which is assimilated and mineralized by heterotrophic flora. Our results showed that all methods (inoculum, attached biofilm and column system) generated similar results of BDOC concentrations for river and drinking water and acetate solution (see Figure 2, 4 and 6, respectively). The water sample was incubated either up to 28 days with suspended bacteria and with attached bacteria to glass beads [10] and 2 hours - with attached bacteria in two column system [13]. For methods with incubation period there was observed fluctuation of concentration of DOC (Figure 1 and 3). This can be explained with detachment of bacteria from glass beads duet to shaking and/or bacteria lysis due to death, the same hypothesis is mentioned by Volk [10] which used sand as support material of attached bacteria. The fluctuation of DOC within incubation time was more expressive for river water which contains different type of carbon source – easy and slowly biodegradable. A steady decrease of DOC was observed with easily utilized acetate solution (Figure 5).

The concentration of BDOC beyond 28 days was considered as slowly biodegradable organic matter [10]. In our study, the period of BDOC determination was significantly shorter than proposed in previous studies [3, 4, 5 and 10] and ranged from 5 to 15 days, for methods were BDOC analyzed by using suspended biomass inoculum and fixed biomass inoculum in the batch test.

The concentration of BDOC measured with all methods represents a 14-59% of fraction of total DOC analyzed in drinking, river and synthetic sample. This variation can possibly be attributed for different fractions of organic matter, such as easy degradable like acetate and slowly degradable in the form of complex or macromolecular substrate (humics).

The most important factor and the drawback of two column system method is the acquirement of mature biofilm on support material for stable conditions for continuous BDOC monitoring [13]. In this study, to adapt the microorganisms in two column systems, the carriers were stored for 16 weeks, but the period of BDOC determination ranged from 2 h to 4 h, until DOC determination becomes stable.

The average values in samples is not significantly different ($P<0.05$) for all methods (see Figure 2, 4 and 6). The two columns method gives results within 2 h and shows similar tendencies for BDOC measurement with methods with incubation period of 28 days. Therefore two column system method can be recommended for future studies and useful for rapid determination of biodegradable fraction of natural organic matters to set treatment efficiency.

## Conclusions

The results are expressed as the mean of the three samples of experiments. The DOC is higher in the river water, but the ratio of BDOC/DOC (%) is higher in drinking water. Incubation period was 28 days for each method. Methods using fixed and free bacteria showed similar results. The minimum DOC value for methods Nr.1 and Nr.2 was checked at the 5 days of incubation beginning. After 15 days DOC value was increased. The period of BDOC determination depends of type of sample. Column method is faster (about 2 hours) and therefore preferable. We recommend fixed biomass inoculum in the two columns system method for future studies and it can be used in the measurement of biodegradability of different samples of water treatment.

**Acknowledgement**

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***Tihomirova K., Rubulis J. un Juhna T. Bioloģiski degradējamā organiskā oglekļa noteikšana ūdenī: suspendētas un adsorbētas biomasas metožu salīdzināšana.***
*Rakstā salīdzinātas trīs metodes bioloģiski degradējama organiskā oglekļa (BDOC) noteikšanai. Mēs novērtējām metožu atkārtojamību un reproducējamību. Rezultātu salīdzināšanai un datu statistiskai apstrādei, pārbaudīti trīs veidu ūdeņi (dzeramais ūdens, upes ūdens un sintētiskais paraugs) ar trīs dažādām metodēm un divām šo metožu modifikācijām, izmatojot baktēriju suspensiju, fiksēto biomasu pudeļu testā un fiksēto biomasu divu kolonu sistēmā. Pētījuma rezultāti parāda, ka BDOC noteikšanas metožu precizitāte ir līdzīga (4-26%), atšķirībā no tā, ka BDOC varië (no 14% līdz 19% no izšķīduša organiskā oglekļa (DOC) upes ūdenim un no 45% līdz 59% no DOC dzeramajam ūdenim). Turpmākajiem pētījumiem rekomendējam divu kolonu metodi.*

***Tihomirova K., Rubulis J. and Juhna T. Determination of biodegradable dissolved organic carbon in waters: comparison of suspended and attached biomass methods.***
*This article compared the three methods for measuring biodegradable dissolved organic carbon (BDOC) in water. Here, we evaluate the comparability and reproducibility of methods. In order to allow a comparison between the results and to perform statistical analysis of the data, three types of water (drinking water, river water and synthetic sample) were analyzed by using suspended biomass inoculum, fixed biomass inoculum in the batch test and fixed biomass inoculum in the two columns system. Our results showed that the precision of various BDOC methods was similar (4-26%) across a broad range of BDOC (from 14% to 19% of total dissolved organic carbon (DOC) in the river water and from 45% to 59% of total DOC in the drinking water). We recommend two columns method for future studies.*

***Тихомирова К., Рубулис Я. и Юхна Т. Определения биологически разрушаемого органического углерода: сравнивнение методов суспендированной и адсорбированной биомассы.***
*В этой статье мы сравниваем три метода определения биологически разрушаемого органического углерода (BDOC). Мы оцениваем воспроизводимость методов, возможность их сравнения. Чтобы произвести сравнение результатов и статистическую обработку данных, тестированны образцы трёх видов (питьевая вода, речная вода и синтетический образец) тремя разными методами, используя суспензию бактерий, фиксированную биомассу в методе с бутылками и фиксированную биомассу в системе двух колонн. Наши результаты показывают, что точность методов похожа (4-26%), в отличие от результатов BDOC, которые варьируют (от 14% до 19% от количества растворимого органического углерода (DOC) в речной воде и от 45% до 59% от DOC в питьевой воде). Мы рекомендуем метод системы из двух колонн для дальнейших изучений.*