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Effect of biomass adaptation to biodegradation of dissolved organic carbon in water

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Abstract In the present study the time of adaptation of fixed biomass for biodegradation of natural organic matter was investigated. The experiments were done in columns that are usually used for rapid determination of biodegradable dissolved organic carbon (BDOC). The biomass was adapted to samples with different concentrations of organic substances before measurements by pumping water to be investigated through the columns for several days. The time of adaptation was dependent on the initial concentration of the organic matter in the water sample. The adaptation time increased from 6 to 24 h with increase of concentration of acetate solution from 2 to 10 mg/l, thus adaptation rate decreased simultaneously from 0.28 to 0.11 min^{-1}. In natural water samples with the initial concentration in the range from 4.61–10.82 mg/l of dissolved organic carbon (DOC) the maximal adaptation time was less than 24 h. During the adaptation period the increase in reproducibility and decrease in the standard deviation was observed. The study showed that adaptation of column to the different concentration of organic matter in water sample is necessary in order to decrease the bias in BDOC measurements when using columns tests.

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Introduction

Biodegradable dissolved organic carbon (BDOC) is the fraction of dissolved organic carbon (DOC) that can be consumed by heterotrophic bacteria within a period of a few days to a few months (Servais et al. 1987). The measurements of BDOC are widely used by water industry for controlling of water treatment and distribution processes. BDOC determination is based on measuring the consumption of DOC by native either benthic or sessile microflora. The approach with biomass attached to the media in the columns is usually used for rapid measurements of BDOC (results in less than 2 h) (McDowell et al. 2006; Ribas et al. 1991; Yavich et al. 2004). For this method the concentration of BDOC is determined as the difference between DOC in inlet and outlet from the columns. However, using this approach the reproducibility of the results is low (viz. unacceptably high error or standard deviation) and often negative BDOC values are obtained. Several causes of this problem may be named including low detection limits of DOC and insufficient adaptation of biomass to the specific organic substances in the samples. The biomass adaptation has been mentioned in earlier studies of degradation of chemicals in wastewater and soil environments (Becker et al. 2006; Brandt et al.

2003, 2004; Kulp et al. 2007; Sanchez et al. 2007; Spain et al. 1980; Spain and Veld 1983; Steiner and Sauer 2001; Swindoll et al. 1988; Wiggins and Alexander 1988) however the importance of this phenomenon for BDOC measurements has not been sufficiently addressed in drinking water systems. The knowledge is important not only to improve reproducibility of BDOC measurements but also to better understand the performances of biological filters for removal of DOC from water sources with variable water quality.

The aim of this study was to determine the time of biomass adaptation to different concentrations of organic compounds in oligotrophic water samples such as drinking water.

The lab scale experiments were made with sodium acetate (NaAc) solution as readily degradable organic substrate to determine effect of concentration to adaptation time. The effect of adaptation was tested with water samples containing high amount of slowly biodegradable natural organic matter.

Materials and methods

Experimental design

The adaptation experiments with biomass of heterotrophic bacteria to measure the time of adaptation to the solution of acetate as easy biodegradable substrate (Khan et al. 2003) and natural sample with various concentrations were done in two glass column system with 2 h empty bed contact time (EBCT) at room temperature (21 ± 2°C). The experimental methodology was based on procedure of BDOC measurements developed by Ribas et al. (1991).

Two glass columns (H = 29 cm, ø = 2.5 cm, Chromaflex, USA) filled with 200 g of glass carrier in the form of balls of 6 mm diameter (Assistant, Germany) with total surface area 3.76 cm^2/g were used. Across the columns, the sample was pumped continuously upwards by a peristaltic pump (Masterflex L/S, Cole-Parmer, USA). A flow rate of 3-5 ml/min was chosen, representing a compromise between the EBCT (1 h/column) and the speed of the assay.

Biomass was cultured by incubation using a special mixture of water which consisted of one-third river water, filtered through 1.2 µm pore diameter membrane filters, and two-thirds of water after biofilters. This mixture was used to support biomass and to recover experimental system for at least 24 h between the different influent samples. To grow the microorganisms, the carriers are stored 16 weeks at 21 ± 2°C in the dark and the water was changed every week.

The BDOC value corresponds to the difference (ΔDOC) between the inlet DOC and the DOC of the outlet of the second column (after 2 h). The reported BDOC values are the mean of three measurements.

Sampling procedure

The adaptation process occurred when not adapted columns with biomass were supplied with acetate solution and concentration of substrate was 2 and 10 mg/l measured as DOC. The samples from the outlet of the second column were taken after 2, 2.5, 3, 4, 4.5, 5, 5.5, 6, 20, 22 and 24 h. The statistical confidence of BDOC measurement procedure using "adapted" column system was confirmed with 10 additional sets and various initial concentrations of acetate solution in the range of 0.86 to 10.1 mg/l (Table 1, $n = 3$ for each concentration).

The same effect was tested in grab water samples with different concentrations of natural organic matter (NOM) taken from river and drinking water as well as treated with ozonation and biofiltration (Tihomirova et al. 2010).

Several repetition of experiments with each sample ($n = 3$) were performed to check reproducibility of adaptation period.

Glassware and reagents

All the glassware used in these experiments were cleaned thoroughly with 10% solution of potassium dichromate in concentrated sulfuric acid, rinsed with hot tap water, rinsed with distilled water, dried and covered with aluminum septum and heated for 6 h at +250°C in order to avoid organic carbon release (van der Kooij et al. 1982). Filtration systems for DOC measurements were sterilized for 20 min at 121°C. The 0.45 µm pore size membrane filters (Millipore Corporation, USA) and sterile membrane filters (Sartorius AG, Germany) used were carefully rinsed, first with sterile ultra pure water (Elga PureLab Ultra, Veolia Water Ltd., UK) and then with the water sample.

Table 1 The accuracy of the BDOC determination relative to average DOC measurements ($n = 3$) of acetate solution by "adapted" columns system

Solution	DOC_{in} (mg/l)	DOC_{in} repeatability		DOC_{out} (mg/l)	DOC_{out} repeatability		Accuracy (%)
		sd	sd (%)		sd	sd (%)	
1	10.065	0.096	1.0	0.652	0.398	61	94
2	9.489	0.145	1.5	0.524	0.329	63	94
3	7.551	0.11	1.5	0.501	0.104	21	93
4	7.193	0.105	1.5	0.459	0.213	46	94
5	6.456	0.152	2.4	0.487	0.045	9	92
6	5.318	0.139	2.6	0.492	0.132	27	91
7	3.701	0.09	2.4	0.344	0.246	72	91
8	3.058	0.181	5.9	0.399	0.217	54	87
9	1.951	0.076	3.9	0.224	0.059	26	89
10	0.861	0.152	17.7	0.253	0.171	68	71

DOC_{in} and DOC_{out}—dissolved organic carbon concentration in inlet and outlet water, mg/l; repeatability of measurements calculated as standard deviation (mg/l) and relative standard deviation (%); accuracy (%) = BDOC/DOC × 100%

Acetate standard solution (NaAc), γ (DOC) = 1 g/l, was made in a 1000 ml volumetric flask where 5.6648 g of sodium acetate trihidrate (CH_3CO-$ONa \times 3H_2O$, ≥99.5%, Fluka, Germany) was dissolved and made up to 1000 ml volume with water. The solution is stable at 4°C for about 6 months.

DOC determination

The DOC measurements were performed with a TOC-5000A Analyzer (Shimadzu Corporation, Kyoto, Japan) according to European Standard (EN 1484:1997). The samples were filtered through a 0.45 μm pore size membranes. The blank and control solution were analyzed with each series of DOC sample in order to verify the accuracy of the results obtained by the method. Every DOC sample was tested in duplicate and the mean value calculated (CV ≤ 2%).

Results and discussion

The time of biomass adaptation was measured with set of columns used for BDOC measurements and adaptation rate was calculated. The columns prior experiments were inoculated with native biomass over the period of half a year.

The results from experiments with NaAc solution showed that "not adapted" biomass reduced 2.022 ± 0.089 mg/l acetate to less than 0.348 ± 0.126 mg/l within 6 h in the column system (Fig. 1.). The same experimental system was used to check the adaptation capability of bacteria for higher acetate concentration − 10.059 ± 0.106 mg/l (Fig. 1.). Within 24 h the columns were adapted and substrate degraded to less than 0.652 ± 0.398 mg/l. These both tests with "not adapted" systems confirmed that bacteria can switch their catabolic properties within 1 day and metabolize 71–94% of concentration of easily degradable substrate sample (Fig. 1). The adaptation rate was obtained by fitting the exponential function to data and expressed as first order kinetic constant (min^{-1}). Figure 1 shows that adaptation rate for lower concentration of sodium acetate (0.28 min^{-1}) is more than twice higher comparing with the higher concentration (0.11 min^{-1}) which agrees with previously published results (Wiggins and Alexander 1988).

The statistical confidence of this effect was confirmed with 10 additional sets and the average results (Table 1) showed that in the adapted columns (adaptation time 24 h) 89.6 ± 6.9% from the initial concentration (DOCin) consisting of easily degradable substance (acetate) was utilized within 2 h (EBCT in experimental system). Thus the assimilation capabilities of bacteria in column system

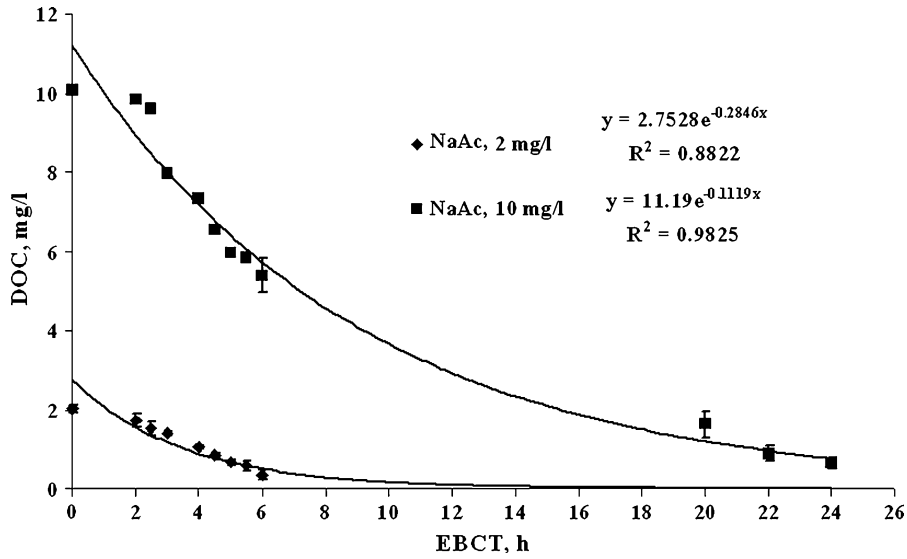

Fig. 1 The period of adaptation and the rate of biodegradation of easily degradable substrate (sodium acetate solution) applying BDOC determination method in not adapted column system with fixed heterotrophic bacteria ($n = 3$). *NaAc* sodium acetate, R^2 regression coefficient

provided repeatable values and the only limitation was the sensitivity of BDOC method itself.

The effect of adaptation of biomass in the same glass column system was performed also with natural samples containing four different initial concentrations of DOC (Fig. 2). Natural samples were passed through two types of column systems: (1) not adapted and (2) adapted, where the adaptation time was 24 h using the same sample. For all the natural samples the utilization capabilities of adapted columns were 1.4–3.5 times higher than those of the not adapted columns (viz. detection error of BDOC results ranged from 47 to 82% in not adapted columns and was much lower −8 to 12% in adapted column system).

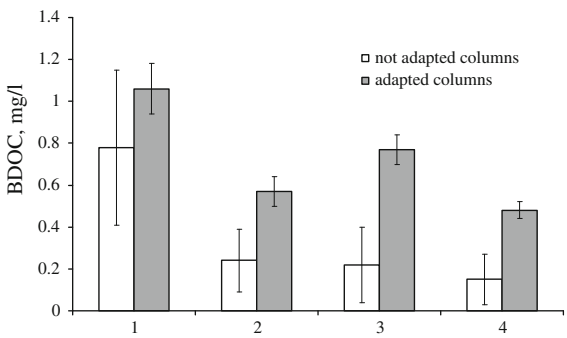

Fig. 2 The concentration of BDOC ($n = 3$) in the natural water samples containing different initial DOC concentrations. Legends: 1—river water (DOC 10.82 ± 0.65 mg/l), 2—ozonated water (6.75 ± 0.07 mg/l), 3—water after biofilters (5.97 ± 0.03 mg/l), 4—drinking water (4.61 ± 0.14 mg/l)

NOM in drinking water can cause various problems including taste, odor, color and formation of disinfection by-products. 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 and distribution systems (Volk et al. 2002). The most practically applicable, rapid and easy to use BDOC determination methods are with attached biomass in the column system (McDowell et al. 2006; Ribas et al. 1991; Yavich et al. 2004), while can be characterized as with low reproducibility and inaccuracy of results.

Microorganisms are able to transform or mineralize most of the organic substances present in natural waters (Brandt et al. 2003, 2004). However the rate of biodegradation is highly variable and dependent of many parameters including the chemical structure and concentration of the organic substance, temperature, salinity, pH, concentration of oxygen, inorganic nutrients, and concentration of microorganisms (Becker et al. 2006; Brandt et al. 2003, 2004; Kulp et al. 2007; Sanchez et al. 2007; Spain et al. 1980; Spain and Veld 1983; Steiner and Sauer 2001; Swindoll et al. 1988; Wiggins and Alexander 1988). When a change in some of environmental factors occurs the biological systems are not able to react quickly (Somova et al. 2005). Therefore it is necessary to know the time needed to adapt biomass to an effective DOC removal in systems used for BDOC

determination after change of type of the substrate. This study showed that before each BDOC measurement in water samples the sample should be passed though columns to adapt the biomass to the new substrate. The adaptation period can range from 6 to 24 h depending on the concentration and type of DOC. During the adaptation period an increased reproducibility and decreased error of measurements or standard deviation were observed. Therefore, these conclusions are relevant because the accuracy in determination of BDOC is essential for the assessment of DOC changes in both a water treatment plant and in the water distribution network.

Conclusions

The obtained results showed:

- Not adapted bacteria were adapted to the acetate and degraded acetate from 2 mg/l to less than 0.348 and 10 mg/l to 0.644 mg/l in a column system within 6 and 24 h, respectively. The adaptation rate was more than twice higher for lower concentration of initial substrate.
- To correctly perform the measurement of BDOC first of all the experimental column system should be passed though with water sample to adapt the biomass to new substrate.
- From the initial concentration 0.861–10.065 mg/l 71–94% of acetate was utilized in adapted columns within 2 h.
- During the adaptation period an increased reproducibility and decreased error of measurements or standard deviation were observed.
- The utilization capability of all the natural samples within a 24 h period in the adapted columns was 1.4–3.5 times higher than in not adapted columns.

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