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Determination of carbon in natural freshwater biofilms with total reflection X-ray fluorescence spectrometry ☆

M. Óvári [a,*], C. Streli [b], P. Wobrauschek [b], Gy. Záray [a,c]

[a] Department of Analytical Chemistry, Eötvös University, Budapest, H-1117, Budapest, Pázmány Péter stny. 1/a., Hungary
[b] Atominstitut of the Austrian Universities, TU-Wien, Stadionallee 2, A-1020, Wien, Austria
[c] Cooperative Research Centre of Environmental Chemistry, Eötvös University, Budapest, H-1117, Budapest, Pázmány Péter stny. 1/a., Hungary

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ABSTRACT

There is a growing interest in determination of low *Z* elements, i.e., carbon to phosphorus, in biological samples. Total reflection X-ray fluorescence spectrometry (TXRF) has been already established as suitable trace element analytical method with low sample demand and quite good quantification limits. Recently, the determinable element range was extended towards $Z=6$ (carbon).
Biofilms can be used for biomonioring purposes in the aquatic environment. Besides the trace metals, especially the determination of the carbon content is important for the better understanding of the early stage of biofilm formation. For this, an ATI low *Z* spectrometer equipped with Cr-anode X-ray tube, multilayer monochromator, vacuum chamber, and a Si(Li) detector with ultra thin window was used. Biofilms were grown on two different artificial supports (granite and plexiglass), freeze dried, suspended in high purity water and analyzed. As an internal standard the natural titanium content of the biofilms was used. The accuracy of the method was checked by total carbon measurement using a combusting carbon analyzer.

1. Introduction

Biofilms (periphyton communities) are of particular interest in the aquatic environment, since these communities occur on all immersed surfaces in natural freshwaters [1]. They consist of bacteria, algae, fungi, and their extracellular polymeric substances (EPS) [2]. These latter possess over a very high number of organic function groups and, therefore, are highly capable to bind various trace elements [3]. As biofilms are the first stage of the aquatic food chain, they readily introduce the elements bound into the food chain [4]. Hence, their investigation is an important task for the biomonitoring of the aquatic environment [5]. For the better understanding of the biofilm formation, it is important to determine the main biogenic elements, e.g., carbon.

Total reflection X-ray fluorescence spectrometry (TXRF) was already introduced for trace metal analysis in biofilms for about 10 years [6]. Since that, it has become a recognized analytical method, and is used more and more extensively [7–9]. The major advantage is that, it demands only a low amount of sample and can also be used for the direct analysis of biofilms [6]. The most important drawback of this method was the inability of determination of elements with atomic number $Z<13$. This has changed with the introduction of a new TXRF spectrometer specially designed for light element detection [10]. For the better fluorescence yield, the X-ray source used Cr-anode (5.4 keV). In order to avoid signal losses caused by absorption in the air, the entire beam pathway was set under vacuum. The applicability of the method for the so called low *Z* analysis in biofilms was demonstrated in [11], as well as the internal standardization and self absorption of the fluorescent signal was discussed. Besides Ag–Lα, the use of the naturally present Ti–Kα line as internal standard was described. It has been established that the carbon determination in biofilms grown directly on the quartz supports of the TXRF spectrometer can be performed only during the first week of biofilm cultivation.

The aim of this work was the determination of carbon in natural freshwater biofilms grown for 4 weeks, since only after this period is the biofilm growth completed.

2. Experimental

2.1. Biofilm cultivation

Biofilms were grown at Tiszafüred–Tiszaörvény in the Tisza river, Hungary. Two floating supports containing each 12 slides with area of 10 cm^2 was immersed vertically into the water. The slides in one support were made of roughly polished granite, the others of plexiglass. The biofilm cultivation was performed from May to October for each 4-weeks period.

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* Corresponding author.
E-mail address: ovari@chem.elte.hu (M. Óvári).

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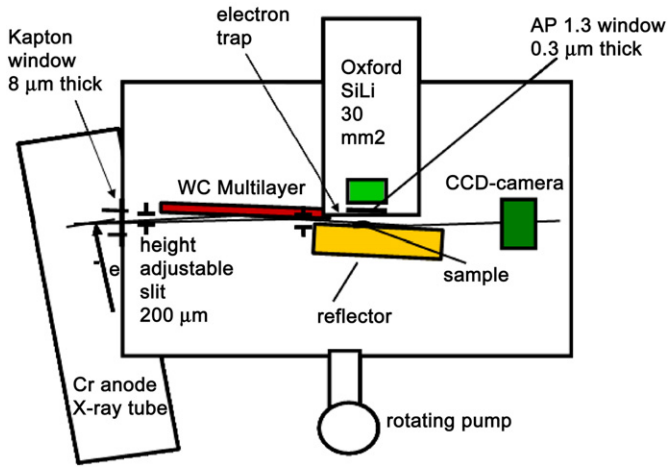

Fig. 1. Scheme of the ATI low *Z* spectrometer.

2.2. Sample preparation

The biofilms were removed carefully from the slides by means of all-ceramic scalpels. In order to avoid contamination from the slides, the bottom layer of the biofilms was discarded. The collected biofilm samples were frozen to −24 °C after being transported into the laboratory. The samples were subsequently freeze-dried and homogeneized in an agate mortar.

Three aliquots of about 50–100 mg of each sample were digested in the same way as described in [5], in order to obtain the Ti mass fraction in the samples. Another three aliquots of about 1–5 mg were suspended in each 1 mL of high purity water (PUR1TE still plus), shaken thoroughly at 6000 rpm for 1 min using a Vortex shaker.

2.3. TXRF spectrometer

For the determination of Ti in the digested sample aliquots, an Extra IIA spectrometer (Atomika, Oberschleißheim, Germany) was used. The operating conditions were as follow: Mo-anode X-ray tube at 50 kV, 38 mA, cut-off filter, Si(Li) detector with 80 mm^2 active area, 1000 s livetime. According to [5], the internal standard was Ga.

The determination of C in the suspended samples was carried out using the ATI low *Z* spectrometer (Atominstitut, Vienna, Austria) described in [11] (Fig. 1). The operating conditions were as follow: Cr-anode X-ray tube at 30 kV, 12 mA, W/C multilayer monochromator set to 5.4 keV, Oxford Si(Li) detector with 30 mm^2 active area and ultra thin window, 200 s livetime. The internal standard was the natural Ti content of the biofilms. Three 2.5-µL aliquots were pipetted onto the Si wafer sample holders from each suspension.

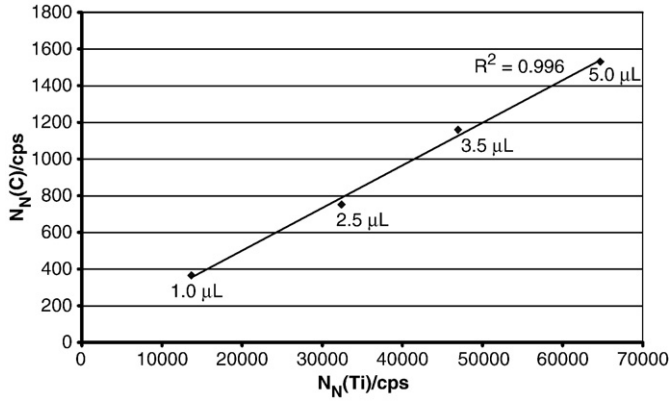

Fig. 2. Results of the linearity test.

Recently, there was a new WOBISTRX spectrometer [13] – installed at the lab of the Eötvös University – used, specially adapted to the requirements of light element detection. This spectrometer is equipped with a Cr tube for excitation and a 10 mm^2 Silicon Drift Detector (KETEK) with an ultra thin window and an electron trap for detection.

2.4. Quality assurance

The accuracy of the measurements was checked by total carbon measurement using a combustive carbon analyzer (N/C 2100, Analytik Jena, Germany). From each sample, three aliquots of 25 mg were weighed into the quartz boats of the equipment, introduced into the furnace and combusted in pure oxygen at 950 °C until no more CO_2 was detected by the infrared detector.

It was also tested whether the pipetted sample amount has an influence onto the relative signal $N_N(C)/N_N(Ti)$, where $N_N(C)$ and $N_N(Ti)$ are the net signal intensities for the C and Ti, respectively. For this, increasing amounts (1.5, 2.5, 3.5 and 5.0 µl) of the same suspension were pipetted onto the sample supports, dried and analyzed.

The reproducibility of the analysis was also investigated. For this, five aliquots of 2.5 µl volume were analyzed, and the relative standard deviation of the above mentioned relative signal was calculated.

3. Results and discussion

3.1. Quality assurance

The carbon concentration in the biofilms was determined both by TXRF and a combustive carbon analyzer. The recovery was calculated as concentration found by TXRF divided by the concentration found by the carbon analyzer, and was found to be 91 ± 8% for all samples.

3.2. Linearity test

The net signal intensities of carbon and titanium are depicted on Fig. 2. As it can be seen, the linearity is good with $R^2 = 0.996$.

3.3. Reproducibility test

The relative standard deviation of the five consecutive measurements was 9%. It should be emphasized that this deviation originates both from the inhomogeneity of the sample and the deviation of the analytical method and is their sum. This means that the sample is homogeneous enough for this determination.

3.4. Carbon determination in biofilms from the Tisza river

The above described method was used for the regular monitoring of carbon concentration in natural freshwater biofilms grown

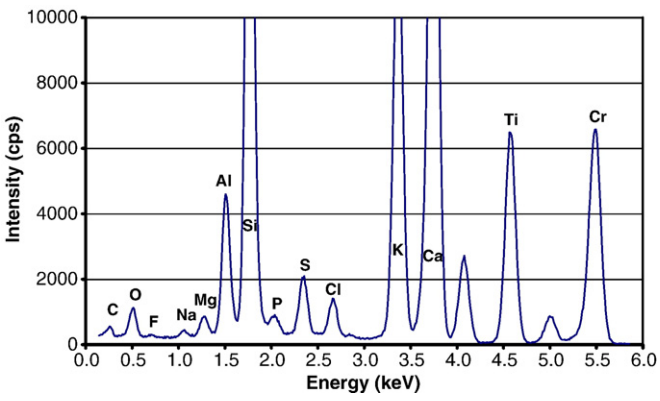

Fig. 3. Spectrum of a natural freshwater biofilm grown on granite substrate, August 2006.

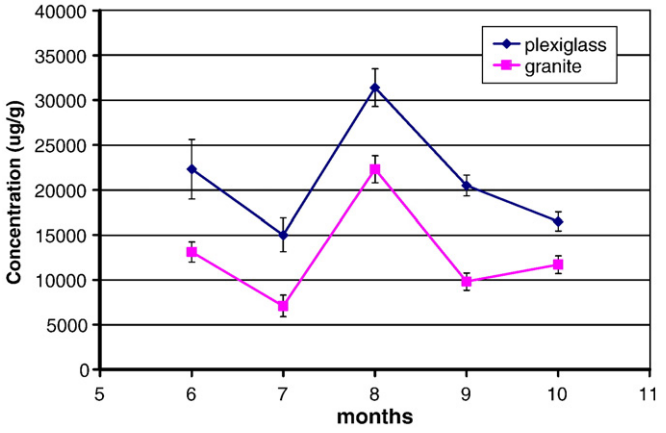

Fig. 4. Carbon concentration distribution in biofilms during the investigation period June–October 2006.

on artificial substrata. A typical spectrum of a biofilm is shown in Fig. 3.

The results of the carbon determination for all cultivation periods are shown in Fig. 4. As it can be seen, there is a difference in the carbon concentration between the two types of supports; biofilms grown on plexiglass have higher concentration. This might be explained by the different biological composition, as different algae and bacteria have different support preferences [12]. There is also a significant seasonal change in the carbon concentration, which also could be explained by the change of biological composition during the 5 months investigation period.

4. Conclusions

It has been demonstrated that the TXRF spectrometry is useful for the determination of carbon in natural biofilm samples. A simple method was described for this determination. The results obtained are in good agreement with those of the independent combustive total carbon analysis. The method is quite robust against self absorption problems, since the sample thickness can be easily kept small with the adequate dilution of the suspension.

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