ATP Measurement as a Means for Directly Estimating Active Biomass

ABSTRACT An important step in the treatment of drinking water is the removal of Biodegradable Dissolved Organic Carbon (BDOC). The active microbial biomass established on Granular Activated Carbon (GAC) filters, rapid sand filters and/or slow sand filters used during water treatment is largely responsible for removing BDOC compounds present in water. To evaluate the efficiency of DOC removal and the overall performance of GAC filters, it is necessary to determine the amount of active biomass present on the filter. Because ATP is present in all active microorganisms, monitoring ATP levels can be useful for predicting the active biomass content. Here we describe a method using the BacTiter-Glo™ Assay to measure ATP directly on GAC without prior removal of the biofilm.

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ATP detection of biomass with the BacTiter-Glo™ Assay is a simple and rapid method for estimating active biomass on GAC filters.

INTRODUCTION

ATP is essential for every metabolically active microbial cell; it is the main energy source for cellular functions such as enzymatic reactions (1). Much is known about the ecological and physiological importance of ATP (1–3), and ATP has previously been used to estimate the amount of biomass present in ground water, drinking water and biofilms (4,5). This makes ATP an ideal parameter to quantify the active biomass present in GAC filters used for drinking water treatment (5,6).

GAC filters are normally used to remove organic micropollutants from drinking water through adsorption. GAC filters also serve as an excellent supporting material for biofilm development, and these indigenous microbial communities bring about the continued removal of BDOC, far beyond the point where the adsorption capacity of the filter would be exhausted (6,7). The removal of BDOC is essential for drinking water treatment in order to achieve biologically stable water with minimal regrowth potential (8,9).

To perform accurate kinetic evaluations of BDOC removal during drinking water treatment, and to assess the performance of GAC filter installations, a rapid and sensitive method to detect active biomass on GAC is needed. ATP analysis offers several advantages as an assay for active biomass: it has simple equipment requirements, it can be performed in a short period of time, and it has a sensitive limit of detection. Here we describe the method recently developed by Velten and coworkers (6) to extract and detect ATP directly from biomass on GAC using the BacTiter-Glo™ Microbial Cell Viability Assay[a,b] (Cat.# G8230).

ATP MEASUREMENT DIRECTLY ON GAC

The ATP method described here was developed using a sample from a full-scale GAC filter that had been in use for 20 years. We have also tested the method on new GAC particles during the period of initial colonization in a pilot-scale reactor and on different types/brands of GAC. In all cases, the GAC reactors in question were fed with ozonated surface water (Lake Zürich). While previous methods have removed the biomass from the GAC (e.g., by sonification) prior to measuring the ATP content, the method we developed extracts and measures ATP directly from the biofilm on the GAC particles.

Figure 1 shows a flowchart of the method, and a detailed protocol is provided in Table 1. Briefly, the GAC sample (200 mg GAC) is mixed with phosphate buffer (100 µl), incubated at 30 °C for 3 minutes and then mixed with BacTiter-Glo™ Reagent (300 µl) that is also incubated at 30 °C. The GAC/BacTiter-Glo™ Reagent mixture is further incubated for 1.5 minutes at 30 °C, and then luminescence is recorded with a GloMax® Luminometer exactly 30 seconds later. The luminescence results (relative light units) can be converted to ATP concentrations using a calibration curve, and this can further be converted to a cell concentration using a theoretical or determined ATP-per-cell value (see ATP Calibration Curve with GAC).

PREPARATION OF GAC SAMPLES

GAC samples were collected from the filter bed surface (upper 10 cm) and stored in glass flasks at 4 °C in the dark. Pretreatment consisted of rinsing 5 g wet weight (ww) of GAC three times for 30 seconds in 100 ml of phosphate buffer (3 mg/L KH_2PO_4 and 7 mg/L K_2HPO_4, pH 7).

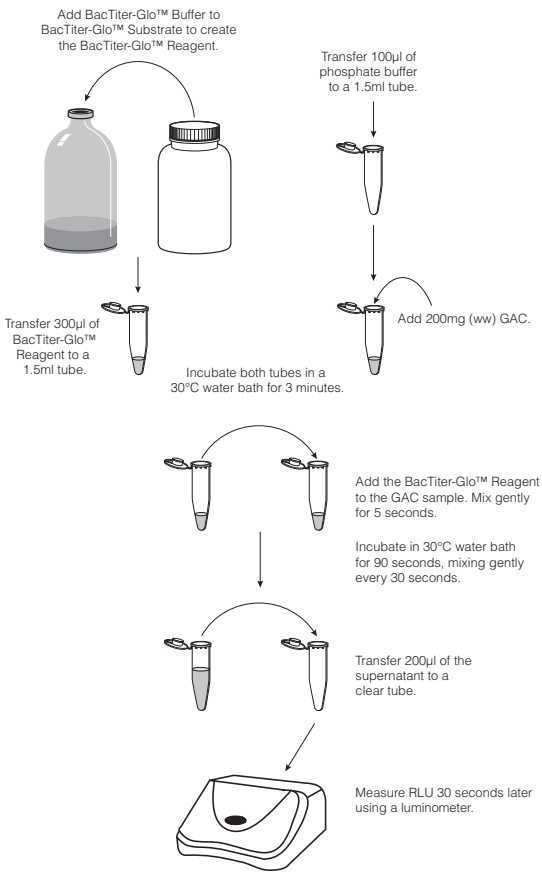

Figure 1. Schematic of ATP detection directly from GAC.

ATP CALIBRATION CURVE WITH GAC

To convert the result of the BacTiter-Glo™ Assay from Relative Light Units (RLU) to an ATP concentration, a calibration curve must be generated. First, the microbial cells on the GAC are inactivated by suspending 5 g of GAC (ww) in 5 ml of phosphate buffer and incubating for 21 hours at 60 °C. Following the incubation, the GAC is washed gently five times with 15 ml of phosphate buffer per wash. In the meantime, rATP standard (Cat.# E6011) is serially diluted from 1 µM to 0.05 µM. The ATP calibration curve is generated following the protocol we describe in Table 1 by replacing the 100 µl phosphate buffer in Step 1 with 100 µl of the diluted ATP standard solution. A separate tube must be prepared for each dilution. The resulting RLU can be plotted against the added ATP concentration to generate the calibration curve. For proper accuracy, a new calibration curve should be generated for every new batch of BacTiter-Glo™ Reagent and sample.

Table 1. Protocol for ATP Detection from GAC.

Before You Begin

1. Prepare the BacTiter-Glo™ Reagent as described in the *BacTiter-Glo™ Microbial Cell Viability Assay Technical Bulletin* #TB337.
2. Transfer 300 µl of the BacTiter-Glo™ Reagent to a 1 ml microcentrifuge tube.

Protocol

1. Place 100 µl of phosphate buffer (3 mg/L KH_2PO_4, 7 mg/L K_2HPO_4, pH 7; Sigma-Aldrich) in a microcentrifuge tube.
2. Add 200 mg wet weight (ww) GAC to the tube with the phosphate buffer and place it in a 30 °C water bath. Simultaneously place the tube containing the BacTiter-Glo™ Reagent in the water bath. Incubate both tubes for 3 minutes.
3. Transfer the BacTiter-Glo™ Reagent (300 µl) to the tube with the GAC and mix gently for 5 seconds.
4. Incubate the BacTiter-Glo™ Reagent/GAC mixture for 1.5 minutes in the 30 °C water bath. Mix the sample every 30 seconds.
5. Remove the tube from the water bath and transfer 200 µl of the supernatant to a clean tube.
6. Measure the relative light units (RLU) 30 seconds later using a luminometer (GloMax® 20/20 Luminometer).
7. Convert the results to an ATP concentration using a calibration curve constructed using rATP (Cat.# E6011).

DETERMINING ATP CONTENT PER CELL

Cells can vary in their ATP content according to cell size, species, physiological state or stress condition. However, drinking water bacteria typically have stable ATP/cell values in the range of $0.7–2.3 \times 10^{-16}$ g ATP/cell (5,6). For case-specific accuracy, an ATP-per-cell concentration can be determined experimentally. To obtain this, a pretreated GAC sample is shaken manually for 1 minute to remove a fraction of the attached bacteria. The ATP concentration of the suspended bacteria is determined using the BacTiter-Glo™ Assay as described in the *BacTiter-Glo™ Microbial Cell Viability Assay Technical Bulletin* #TB337. Once the total ATP concentration of the sample has been determined, the total concentration of microbial cells in the sample is measured using SYBR® Green I staining followed by flow cytometry as described in Hammes and Egli (8), or alternatively using quantitative epifluorescence microscopy. This method assumes that the detached and GAC-associated cells will exhibit similar properties.

MONITORING BIOMASS DEVELOPMENT

The development of active biomass on a new GAC filter is characterized by two concurrent events: the gradual decrease in the DOC adsorption on the GAC filter as a result of saturation and an increase in ATP on the GAC (Figure 2). We have used this method of direct ATP detection to monitor the startup of a pilot-scale GAC reactor. The ATP concentration peaked after 33 days and then decreased to a stable average by day 53.

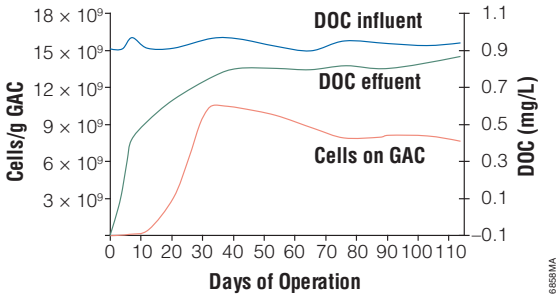

Figure 2. Biofilm formation in a GAC filter pilot-scale reactor over time. The reactor was fed with ozonated lake water. Adapted from Velten and co-workers (2007) (6).

CONCLUSIONS

Detecting ATP directly on GAC offers a simple and rapid method for estimating the amount of active biomass present on the filter material. The method requires simple analytical equipment, can be done in a short time period (~45 minutes per sample) and has a low limit of detection. The method was sensitive enough to determine the biomass of both stable and developing biofilms on GAC filters. In addition to GAC filters, we believe that this method could be applied to similar systems such as rapid sand filters, slow sand filters and soil filters. Finally, the method with some adaptations could work for biofilms on membranes.

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PROTOCOL

- *BacTiter-Glo™ Microbial Cell Viability Assay Technical Bulletin* #TB337
  www.promega.com/tbs/tb337/tb337.html

ORDERING INFORMATION

Product	Size	Cat.#
BacTiter-Glo™ Microbial Cell Viability Assay*	10 ml	G8230
	10 × 10 ml	G8231
	100 ml	G8232
	10 × 100 ml	G8233
rATP, 10 mM*	0.5 ml	P1132
GloMax® 20/20 Luminometer	1 each	E5311

*For Laboratory Use.

(a)U.S. Pat. No. 6,602,677, Australian Pat. No. 754312 other patents and patents pending.

(b)The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.

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Detecting biomass directly on GAC using the BacTiter-Glo™ Assay requires simple analytical equipment, can be done in a short time period and has a low limit of detection.