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Lanthanum-based concentration and microrespirometric detection of microbes in water

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ARTICLE INFO

Article history:
Received 23 July 2009
Received in revised form
3 December 2009
Accepted 23 March 2010
Available online 27 March 2010

Keywords:
Concentration
Lanthanum chloride
Turbidimetric assay
Microrespirometric assay
Concentration efficiency
Recovery rate

ABSTRACT

Rapid concentration and detection of microorganisms, particularly pathogens, are important but remain a challenge. In this research, lanthanum chloride ($LaCl_3$) was used to concentrate *E. coli* in water and the results were compared with those obtained using traditional flocculants, such as ferric sulfate and aluminum sulfate. A turbidimetric assay and a microrespirometric assay were employed to enumerate the bacteria in water samples by monitoring the absorbance of bacteria and the oxygen-based fluorescence intensity, respectively. The microrespirometric method requires less time than the turbidimetric assay. Both assays could linearly enumerate the bacteria at the concentration range from 10^1 to 10^9 cells/mL. Based on the turbidimetric assay, the relative concentration efficiencies of the three flocculants were 75% ($LaCl_3$), 40% ($FeCl_3$) and 33% ($Al_2(SO_4)_3$), while for the microrespirometric assay, the concentration efficiencies were 85% ($LaCl_3$), 34% ($FeCl_3$) and 32% ($Al_2(SO_4)_3$). The microbial recovery rates, defined as the ratio of cell number in the sediment after coagulation/flocculation to that of the controls, were 94% ($LaCl_3$), 69% ($FeCl_3$) and 51% ($Al_2(SO_4)_3$) from the turbidimetric assay. The results demonstrate that compared with traditional flocculants, $LaCl_3$ has higher relative concentration and recovery efficiencies and thus possesses the potential for microbial concentration in water samples.

1. Introduction

The presence of pathogenic microorganisms in drinking water is a great threat to the public health. Rapid detection of pathogens in water is therefore crucial to ensuring safe drinking water supplies. The numbers of microorganisms in water samples are generally very low, and in many cases, even lower than the detection limit of analytical methods. Therefore, most of the detection techniques require pretreatment of water to concentrate the bacteria or viruses before analysis. Filtration is commonly used to concentrate the microbes in water (Brichta-Harhay et al., 2007; Guy et al., 2006; Kamma et al., 2008; Schets et al., 2005; Sobsey et al., 2004). However, the pores of the filter membrane can be easily clogged due to the presence of colloidal materials or organic matter in water (Farrah et al., 1976) whereas smaller size viruses can easily pass through the membrane, resulting in low recovery efficiency (Hijnen et al., 2000; Wohlsen et al., 2006).

Coagulation/flocculation, and in many cases, coupled with filtration is an attractive method for concentration of microbes (Havelaar et al., 1995; Nasser et al., 1995; Rapala et al., 2006). It is a common practice in water treatment

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0043-1354/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.watres.2010.03.029

facilities to remove colloids and microorganisms. Chemicals such as alum and ferric salts remove 40–80% of microorganisms (Meric et al., 2002). However, there is a concern of the changes of cell viability due to significant pH drop after chemical coagulation as a minimal change of microbial physiological properties is often required for downstream detection. The first objective of this research was to evaluate a lanthanum-based method for concentration of microbes in water. Lanthanum chloride ($LaCl_3$) is a less-hydrolyzed flocculant that destabilizes colloids including microbes by strong electrostatic interaction with minimum pH changes (Hu et al., 2002), which may be helpful to retain cell viability.

The second and equally important objective was to validate an oxygen fluorescence based microrespirometric assay for microbial detection. While a wide variety of microbial detection methods are available, a rapid and more accurate means for cell enumeration is preferred. Standard cell culture techniques, although still commonly used today for microorganism enumeration (Abbaszadegan et al., 2007; Nasser et al., 1995; Xagoraraki et al., 2004), are time-consuming and often inaccurate. PCR-based molecular techniques do not require culturing steps and can serve as a standard method to detect various organisms including pathogens (Behets et al., 2007; Hwang et al., 2007; Ko et al., 2005; Schwab et al., 2001; Tanriverdi et al., 2002). However, PCR-based methods require skilled technicians to perform the test and DNA sequence information on a specific microorganism is often unavailable. Flow cytometry may rapidly detect microorganisms stained with fluorescent probes. However, the signal obtained from target cells in environmental samples after fluorescent in situ hybridization is often too low to be detected (Amann et al., 1990; DeLong et al., 1999; MacDonald and Brozel, 2000; Schonhuber et al., 1997). Oxygen fluorescence based microrespirometry provides a new approach to determine aerobic viable cell counts by measuring microbial oxygen consumption and equating O_2 consumption rate to microbial concentration (O'Mahony and Papkovsky, 2006). It has been applied to microbial tests in food samples (O'Mahony et al., 2006, 2009), but its environmental applications have not been well studied. In the microrespirometric assay, dissolved O_2 quenches the phosphorescence of a soluble, oxygen sensitive probe. As microbes in the sample grow and respire in test medium, the decrease of O_2 concentration in the solution results in an increase in phosphorescence signal, which can be recorded in conjunction with the use of high throughput microtiter plate-based assays.

In this study, lanthanum chloride was employed as a flocculant to concentrate microbes in water. The concentrated cells were quantified by a turbidimetric assay and an oxygen fluorescence microrespirometric assay. The results of the concentration efficiencies were compared with those obtained from traditional flocculants (e.g., $FeCl_3$ and $Al_2(SO_4)_3$).

2. Materials and methods

2.1. Bacterial culture

E. coli MG1655 (ATCC 47076) was selected as a model organism because it is a commonly studied K-12 strain with known genomic information (Blattner et al., 1997). The strain was incubated in a BBL medium (BD, Cockeysville, MD) overnight on a shaker (200 rpm) at room temperature. Initial cell numbers were determined using standard agar plate methods. The overnight stock culture was used immediately in subsequent experiments at the required working dilutions in 1× phosphate buffer saline (PBS). A series of cultures were thus prepared at the final concentrations of 10^0, 10^1, 10^2, 10^3, 10^4, 10^5, 10^6, 10^7, 10^8 and 10^9 colonies/mL, respectively.

2.2. Turbidimetric microtiter assay

To enumerate cells using the turbidimetric microtiter assay, aliquots (20 μL) of cell cultures of known concentrations (standards) or samples in 8 replicates were added to the microplate wells followed by the addition of 180 μL BBL medium. The absorbance of cell culture in each microwell was measured at 600 nm every 1 h for about 48 h by a microreader (VICTOR3, PerkinElmer, Shelton, USA).

2.3. Microrespirometric assay

The oxygen based microrespirometric assay was adapted from a previously reported assay with modifications (O'Mahony and Papkovsky, 2006). Oxygen probes (Redlight, Luxcel Biosciences, Ireland) were reconstituted in 1 mL of DI water to give stock solutions of 3 μM, which were stored in the dark at -20 °C for further use. Aliquots (20 μL) of cell cultures or water samples were added to 170 μL BBL medium followed by the addition of 10 μL oxygen probe used at a final working concentration of 150 nM. Each microwell was then sealed with a layer of heavy mineral oil (100 μL/well), which acts as a barrier for ambient oxygen and prevents sample evaporation during the experiments. The time-resolved fluorescence (TR-F) from each water sample was monitored every 15 min over an approximate 48-h period by the microreader using standard sets of filters of 340 nm (excitation) and 642 nm (emission). All samples were prepared in 4 replicates in the microrespirometric assay.

Measured time profiles of absorbance or oxygen based fluorescence signals due to bacterial growth were analyzed to determine the time required to reach a threshold level for each sample. The threshold level was defined as half of the maximum absorbance in the turbidimetric microtiter assay or half of the maximum oxygen probe signal in the microrespirometric assay (O'Mahony and Papkovsky, 2006; Stitt et al., 2002). Calibration curves were established by plotting the time required to reach a threshold level against cell concentration.

2.4. Concentration of microbes in water

An aliquot (1 mL) of overnight *E. coli* culture of known concentration and 3 mL BBL medium were added to 96 mL tap water to prepare a series of bacterial samples. $LaCl_3$, $FeCl_3$ and $Al_2(SO_4)_3$ were employed as flocculants and their stock solutions (0.1 M) were prepared in DI water. Aliquots of these chemicals were added individually at a final cation (La^{3+}, Fe^{3+} or Al^{3+}) concentration of 0.2 mM. To concentrate bacteria in water by coagulation/flocculation, water samples of known cell numbers were rapidly mixed at 200 rpm for 1 min, followed by