An improved method for determining microbially available phosphorus in drinking water

Gang Wen, Qin Deng, Ting-Lin Huang and Jun Ma

ABSTRACT

Microbially available phosphorus (MAP) is the labile phosphorus that is readily assimilated by microorganisms, which is linearly correlated to bacterial re-growth in drinking water in some regions. The conventional MAP bioassay for drinking water was originally developed by Markku based on the growth potential of *Pseudomonas fluorescens* P17 (P17). However, the bioassay bears some demerits, such as time-consuming and labor-intensive enumeration. For convenience, an alternative method based on a similar principle was developed to assess the content of MAP in drinking water, in which natural microbial consortium was used as inoculum instead of pure culture P17, cell number was counted using flow cytometry (FCM), and cultivation at 30°C was adopted. Natural microbial consortium is able to efficiently utilize organic phosphorus and exhibit high sensitivity since more cells are produced per μg P utilized. FCM is a rapid method to count all bacteria growing in drinking water. With incubation temperature increasing up to 30°C, there is a shorter test period (64 h), excellent sensitivity and better utilization efficiency for organic phosphorus. The results show that the developed bioassay is sensitive, time-saving and easily operated.

Key words | bioassay, drinking water, flow cytometry, microbially available phosphorus, natural microbial consortium

INTRODUCTION

Massive microbial re-growth in drinking water distribution systems may cause hygienic and aesthetic problems (Van der Kooij 2000). Limiting the nutrient content is one of the methods of maintaining bio-stability to inhibit microbial re-growth (van der Kooij et al. 1999; Srinivasan & Harrington 2007). In general, assimilable organic carbon (AOC) is considered as the key factor to restrict heterotrophic microbial growth (Liu et al. 2002; Liang & Ma 2009; Hammes et al. 2010a). However, some researchers have pointed out that microbial growth in drinking water is regulated by the level of phosphorus in some regions (Miettinen et al. 1997; Sathasivan & Ohgaki 1999; Lehtola et al. 2002a; Wen et al. 2014). In those drinking waters, even an addition of a very low concentration of phosphate would lead to a massive growth of microbes (Lehtola et al. 1999; Wen et al. 2014).

Microbially available phosphorus (MAP) is the fraction of labile phosphorus that is readily assimilated by microorganisms, resulting in microbial growth. Over several decades, researchers have developed several kinds of chemical extraction methods (Sharpley et al. 1991; Nakajima & Okubo 2003) (i.e. 0.1 M NaOH) to determine the bioavailability of phosphorus in soil or suspended solids. However, these are just indirect methods and unsuitable to monitor bioavailable phosphorus in water. Therefore, bioassays were developed to directly monitor bioavailable phosphorus. Green algae were used to assess bioavailable phosphorus (Sathasivan et al. 1999; Lehtola et al. 2002b; Wen et al. 2014).
phosphorus in waters based on its growth potential (Ekholm & Krogerus 1998; Li & Brett 2012). However, several weeks are required to obtain the result and it is insensitive to a low concentration of phosphorus. Therefore, bacteria were employed in the measurement of bioavailable phosphorus in water, including bacterial growth (Lehtola et al. 1999), dissolved oxygen (DO) consumption by respiration (Nakajima et al. 2006) or CO₂ production by respiration (Nordgren 1992). The period of bioassay using bacteria is a few days and significantly shorter than that using green algae.

In regard to the quantification of bioavailable phosphorus in drinking water, the method originally developed by Markku based on the growth potential of *Pseudomonas fluorescens* P17 (P17) was widely used (Lehtola et al. 1999). Briefly, P17 is inoculated into the pasteurized water samples and incubated at 15 °C until reaching the stationary phase. The maximum cell number at the stationary phase is measured by plating and expressed as colony forming units (CFU) per mL of sample. Thereafter, the maximum cell number is converted to the content of bioavailable phosphorus with a conversion factor obtained from the standard curve (3.73 × 10⁸ CFU μg⁻¹ MAP). However, MAP measurement is not performed routinely in the drinking water treatment and distribution process due to the following shortcomings. Firstly, the inoculum P17 is sometimes difficult to obtain, and even if you get it, the physiology may be not the same at different laboratories. Secondly, underestimating the actual MAP value is probably observed owing to the low phosphate activity of pure culture P17 (Paytan & McLaughlin 2007). In fact, parts of organic phosphorus can be utilized by complex indigenous microbial communities in actual water samples (Bentzen et al. 1992; Dyhrman et al. 2009). Thirdly, the conventional plating is tedious and time-consuming to perform and the existence of viable but non-culturatable bacteria (VBNC) in oligotrophic drinking water leads to an underestimating of the actual total cell number (Hammes & Egli 2010). The emerging flow cytometric enumeration is an alternative method to count all bacteria rapidly and accurately. Lastly, a long incubation time is required to obtain the result as the bioassay is performed at a low temperature (at 15 °C).

Therefore, for convenience, an alternative MAP determination method was developed. The present study was to optimize the operational conditions for MAP bioassay, including the inoculum, incubation temperature, the amount of organic carbon spiked and the enumeration method, and compare the new method for MAP determination with the existed methods.

**MATERIALS AND METHODS**

**Preparation of carbon, phosphorus-free equipment**

All glassware and caps were washed with phosphate-free detergent and then prepared as described previously (Hammes & Egli 2005; Wen et al. 2014).

**Preparation of inocula**

*Pseudomonas fluorescens* P17 (ATCC 49642) (P17) was used for comparisons in the bioassay. P17 was inoculated into 0.1 μm-filtered (SLVV 033 RS, Millipore) bottled mineral water (Evian) amended with 1 mg L⁻¹ acetate carbon and 5 μg L⁻¹ PO₄-P (di-sodium hydrogen phosphate), and then cultivated at 30 °C for 6 d to reach the stationary phase.

Mineral water (Evian, France) in a plastic bottle was purchased from a shopping mall as a natural microbial consortium, which is available all over the world in similar water quality (Supplementary Material, Table S3 and Figure S1, available with the online version of this paper). The natural microbial consortiums from mineral water purchased in different places and batches are relatively stable (Supplementary Material, Table S3 and Figures S1 and S2).

**Determination of microbially available phosphorus**

MAP measurement was adapted according to the method developed by Markku (Lehtola et al. 1999). Briefly, an aliquot of 20 mL of 0.22 μm-filtered pasteurized water samples was inoculated with natural microbial consortium (Evian) with the initial concentration of 5 × 10³ cells mL⁻¹. Meanwhile, 1 mg L⁻¹ acetate carbon and 10 μL trace element solution were amended to achieve P-limited microbial growth in the bioassay, and then incubated at 30 °C until reaching the stationary phase. Finally, the total cell count (TCC) was monitored by flow cytometry (FCM) and the concentration of MAP was calculated based on the standard curve.
Growth curves of inocula in the defined waters

Growth curves of different inocula (P17 and natural microbial consortium (Evian)) under varying conditions (different temperatures, phosphorus and acetate carbon spiked) were recorded. An aliquot of 1 mL water sample was taken at regular time intervals, and TCC was monitored by the FCM or adenosine tri-phosphate (ATP) method. The specific growth rate (μ) was based on the cell number increase with the function of incubation time as follows:

\[ \mu = \frac{(\ln(n_t) - \ln(n_0))}{\Delta t} \]

where \( n_t \) and \( n_0 \) are the cell numbers monitored at two subsequent time points and \( \Delta t \) is the time interval.

Enumeration of total cell count by flow cytometry

Enumeration of TCC was conducted by FCM as described previously (Hammes & Egli 2013; Wen et al. 2014). In short, water samples were stained with SYBR Green I and then monitored using a BD Accuri C6. The detection limit was 1,000 cells mL\(^{-1}\) and the instrumental error was below 5% (Hammes et al. 2008).

ATP analysis

Total ATP was monitored using the BacTiter-Glo reagent (Promega Corporation, Madison, WI, USA) and luminometer (Glomax, Turner Biosystems, Sunnyvale, CA) (Hammes et al. 2008b; Vital et al. 2012). ATP was always measured in triplicate and the standard deviation was below 4%. More than 95% of the ATP was intracellular ATP in the experiments.

Heterotrophic plate counts

Heterotrophic plate counts (HPC) were conducted using R2A agar (Reasoner & Geldreich 1985). In short, 100 µL of appropriate decimal dilutions of a sample were placed on the surface of the R2A agar, and were incubated at 30 °C for 48 h. All analyses were performed in triplicate.

RESULTS AND DISCUSSION

Optimization of inocula

It is expected that the composition of the microbial community has a remarkable impact on the utilization of phosphorus (Monbet et al. 2007). Since P17 is a specific pure culture, it is sometimes not easily obtained and the viability differs in different laboratories; on the other hand, P17 is not as efficient as complex microbial consortium at utilizing organic phosphorus (Paytan & McLaughlin 2007). Thus, natural microbial consortium was examined as an inoculum and the differences with P17 were compared.

Growth processes

Figure 1 represents the batch growth curve for Evian water and P17 growing with/without inorganic phosphorus (dissodium hydrogen phosphate, Pi) and organic phosphorus (ATP) (Sebastián et al. 2012). It clearly demonstrates that an addition of Pi or ATP significantly promotes bacterial growth. The maximum specific growth rates (\( \mu_{\text{max}} \)) of Evian growing on Pi and ATP are 0.21 and 0.17 h\(^{-1}\), and the \( \mu_{\text{max}} \) of P17 growing on Pi and ATP are 0.17 and 0.14 h\(^{-1}\). There is a slightly higher \( \mu_{\text{max}} \) of Evian compared with P17. However, the required time to reach the stationary
phase of Evian (64 h) is longer than that of P17 (48 h), which is ascribed to a longer adaptation time before extensive growth.

When reaching the stationary phase, the TCC (7 × 10^6 cells mL^-1) by inoculating Evian is remarkably higher than that (1.2 × 10^6 cells mL^-1) of P17 at the same amount of Pi or ATP. The results may be attributed to the fact that there is a higher ratio of low nucleic acid bacteria in Evian, composed of smaller size cells (as shown in Figure 2), and more cells are produced at the same amount of phosphorus. Furthermore, the TCC (reaching the stationary phase) growing on Pi by inoculating Evian is the same as that of ATP, whereas the TCC growing on Pi by inoculating P17 is higher than that of ATP, indicating that there is higher utilization efficiency for organic phosphorus by Evian compared to P17. It demonstrates that the microbial community originating from Evian water appears to contain a strain composition that can take advantage of a rather broad spectrum of phosphorus compounds. P17 also presents the capability to decompose organic phosphorus into inorganic phosphorus due to the significant bacterial growth compared to the control, concurring with the previous study (Lehtola et al. 1999). However, the TCC growing on ATP after reaching the stationary phase is lower compared to that growing on Pi, indicating there is no sufficient phosphatase activity to decompose ATP and utilize it. The excellent phosphatase activity of Evian is attributed to the complex microbial community, which can be seen from the 2D-dot plots of FCM (Figure 2).

### Standard curve

The standard curve was developed by measuring TCC_{max} of bacterial growth at a series of phosphorus concentrations. As shown in the Supplementary Material, Table S1 (available with the online version of this paper), all the four standard curves have perfect regression correlations (R^2 > 0.95). There is no interception since the TCC_{max} of bacterial growth in the control is subtracted. There is a comparable slope for Evian growing on Pi (9.4 × 10^8) and ATP (8.9 × 10^8) as the P source, which further corroborated that there is a stronger phosphatase activity for Evian. However, the slopes of P17 growing on Pi and ATP are 1.8 × 10^8 and 1.3 × 10^8, respectively, which is remarkably lower than that of Evian. The results indicate that there is a better sensitivity for measuring MAP using Evian as inoculum since more cells are produced per µg P utilized. In the previous study (Lehtola et al. 1999), 1 µg of PO_4-P corresponds to 3.73 × 10^8 CFU of P17, which is greater than that of P17 in this study but far lower than the conversion factor of Evian. The different conversion factor reported for P17 should be attributed to the different activity.

Therefore, Evian is proposed as the inoculum for MAP determination for three reasons: Firstly, the complex microbial community is capable of utilizing dissolved organic phosphorus thoroughly. Secondly, there is better sensitivity for measuring MAP since more cells are produced per µg P utilized, and thirdly it is relatively stable (Supplementary Material, Table S3 and Figures S1 and S2) and available world-wide to obtain the inoculum (Evian) for MAP measurement.

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**Figure 2 |** 2D dot-plots of (a) FCM of Evian and (b) P17.
Incubation temperature

Incubation temperature is an important factor affecting bacterial growth rate and the time required to reach the stationary phase, thus, the incubation temperatures of 15 and 30 °C were compared with regard to the growth process and standard curve. As shown in Figure 3 (15 °C) and Figure 2(a) (30 °C), it demonstrates that there are remarkable differences with respect to the adaptation time and growth rate at incubation temperatures of 15 and 30 °C. The adaptation times are 100 h and 40 h at incubation temperatures of 15 and 30 °C, respectively, indicating that there is a shorter adaptation time when the incubation temperature is at 30 °C, and correspondingly the test time is greatly shortened. The $\mu_{\text{max}}$ of Evian growing on Pi at 15 and 30 °C are 0.06 and 0.21 h$^{-1}$, and the $\mu_{\text{max}}$ of Evian growing on ATP at 15 and 30 °C are 0.04 and 0.17 h$^{-1}$. There is a higher $\mu_{\text{max}}$ growing at 30 °C compared with that at 15 °C. Therefore, the required time to reach the stationary phase for Evian growing at 30 °C (64 h) is shorter than that growing at 15 °C (166 h).

When reaching the stationary phase, the TCC (7 x 10$^6$ cells mL$^{-1}$) growing at 30 °C is slightly higher than that (5.5 x 10$^6$ cells mL$^{-1}$) growing at 15 °C at the same amount of Pi. Moreover, the TCC growing on ATP by inoculating Evian is lower than that growing on Pi at 15 °C, indicating that there is no sufficient utilization efficiency for organic phosphorus at lower temperature (15 °C), which is ascribed to the lower phosphate activity.

As shown in the Supplementary Material, Table S2 (available with the online version of this paper), the two standard curves also have perfect regression correlations ($R^2 > 0.95$). There is a greater slope for Evian growing on Pi than that growing on ATP at 15 °C, which demonstrates that there is no sufficient phosphatase activity at lower temperature. The results indicate that there is a low sensitivity for determining MAP when the incubation temperature is at 15 °C since fewer cells are produced per µg P utilized and lower utilization efficiency for organic phosphorus is observed.

Based on the above discussions, it is concluded that there is a shorter test time, an excellent sensitivity and better utilization efficiency for organic phosphorus when the incubation temperature is higher (30 °C). Therefore, 30 °C is recommended as the incubation temperature.

The amount of organic carbon spiked

Organic carbon was added to water samples to ensure phosphorus-limited growth in the bioassay. The effect of different amounts of organic carbon (acetate carbon) spiked on the standard curve was tested. As shown in Figure 4, TCC increases linearly with the increase of the amount of phosphorus within a certain concentration range. With the increase of the amount of acetate carbon spiked, the linear range extends proportionally. For example, the linear range is 0–5 µg L$^{-1}$, 0–10 µg L$^{-1}$, and 0–20 µg L$^{-1}$ as the amount of acetate carbon spiked is 0.5 mg L$^{-1}$, 1.0 mg L$^{-1}$, and 2.0 mg L$^{-1}$, respectively. However, TCC increases slowly with continuous increase of the concentration of phosphorus over the critical point. During the linear range, there are significant linear regression correlations ($R^2 > 0.95$) between the TCC and phosphorus concentration, and the slope is comparable irrespective of the amount of acetate carbon spiked, which means that the conversion factor is a constant even if the organic carbon concentration of the water sample is different. According to the result, it means that the amount of acetate carbon spiked is flexible only if the MAP content of a water sample is within the linear range.
Enumeration methods

Generally, the microbial quality of drinking water is measured by HPC, which has been used for more than 100 years and recommended as the standard method in drinking water guidelines. Due to the shortcomings of HPC being time-consuming and the presence of uncultivable bacteria in drinking water, rapid and accurate detection methods including ATP measurement and TCC measurement by FCM are increasingly used. In the present study, the total cell numbers using plating, ATP and FCM were compared.

As shown in Figure 5, the correlations of the three methods are presented. A total of 86 samples were quantified using the FCM and ATP methods, while 51 samples...
were compared with respect to the correlation between TCC and HPC. The correlation coefficient between HPC and TCC \((R^2 = 0.21)\) is considerably weak (Figure 5(a)), which is similar to the previous reports (Siebel et al. 2008; Nescerecka et al. 2014). This is explained by the fact that more than 99% of the bacteria in drinking water are uncultivable on synthetic media under selective laboratory-defined conditions, indicating the limitation for the detection of MAP in drinking water using the HPC method. TCC was measured by FCM and ranged from \(1.2 \times 10^5\) cells mL\(^{-1}\) to \(4.3 \times 10^6\) cells mL\(^{-1}\). ATP concentrations in the samples were from 0.02 to 0.93 nM. In all the samples, there are less than 5% free ATP and more than 95% intact cells. A good correlation \((R^2 = 0.90; n = 86)\) can be observed between the TCC and total ATP concentrations (Figure 5(b)). The average ATP per cell is \(2 \times 10^{-10}\) nmol cell\(^{-1}\), which is comparable to the previous study (Lautenschlager et al. 2010; Hammes et al. 2010b). In summary, TCC by FCM or ATP measurement is time-saving and accurate for MAP measurement.

Detection range and detection limit

As discussed above in the section on the amount of organic carbon spiked, there is a positive linear correlation between the stationary phase TCC and the content of MAP, and the detection range extends with the increase of acetate carbon spiked. Generally, MAP in drinking water is less than \(10 \mu\text{g L}^{-1}\) (Lehtola et al. 2002b; Polanska et al. 2005; Wen et al. 2014); in addition, there is a certain amount of AOC in drinking water, which would extend the detection range. Therefore, the detection range of \(0–10 \mu\text{g L}^{-1}\) with \(1 \mu\text{g L}^{-1}\) acetate carbon spiked is suitable for MAP measurement in most water samples. Of course, the detection range can extend proportionally with the increase in the amount of acetate carbon spiked.

In the present method, MAP was calculated by dividing the conversion factor \((9 \times 10^8\) cells \(\mu\text{g}^{-1}\) MAP) using the stationary phase TCC (the control was subtracted). Because of the differences of incubation conditions and experimental operations between different laboratories, the conversion factor might not be a fixed value. It is better to determine the value of the conversion factor once new stock solution or inoculum is prepared. Although no additional phosphorus was added to the control experiment, a number of cells can grow probably due to the residual phosphorus from the inoculum or the added chemical compounds. Therefore, it is essential to conduct the control experiment without the addition of phosphorus to exclude the influence of background matrices. In theory, the detection limit of MAP is dependent on the precision of FCM. The detection limit of FCM is \(1,000\) cells mL\(^{-1}\) (Hammes et al. 2008), therefore, the detection limit of the present MAP method is \(1.1 \times 10^{-3}\) \(\mu\text{g L}^{-1}\).

Comparisons of different methods for MAP quantification

Bioavailable phosphorus is an important parameter for indicating the utilization efficiency for phosphorus by microbes in water or soil. In order to measure bioavailable phosphorus, several methods have been developed, as shown in Table 1. All methods have their shortcomings, such as lacking sensitivity, being time-consuming, or being tedious to perform. In this section, comparisons of different methods for the quantification of MAP are made in terms of detection principle, operational methods, detection spectrum and detection range (Table 1).

In those methods, the principle is quite different. Green algae such as Selenastrum capricornutum have been widely used to measure bioavailable phosphorus in wastewater by way of quantifying the algal growth potential (Ekholm & Krogerus 1998; Li & Brett 2012). However, several weeks are required to obtained a result, therefore bacteria were applied for the measurement of bioavailable phosphorus in water, including bacterial growth of Pseudomonas fluorescens P17, DO consumption or CO\(_2\) production by respiration (Nordgren 1992; Lehtola et al. 1999; Nakajima et al. 2006). Recently, Dollard & Billard (2003) constructed a phosphate sensor plasmid for the assessment of phosphate bioavailability in wastewater by the molecular biological method, but this method bears high fluctuations due to the instability of plasmid and possible variations in plasmid copy number in the cells. Therefore, it is concluded that it is more feasible to measure MAP in water using bacterial growth due to its stability and being time-saving.

In terms of methods for measuring bioavailable phosphorus using bacteria, the different detection methods
Table 1 | Comparisons of different methods for the quantification of bioavailable phosphorus

<table>
<thead>
<tr>
<th>Method</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>Our method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Principle</td>
<td>Bacterial growth</td>
<td>Algal growth</td>
<td>DO consumption</td>
<td>CO₂ production</td>
<td>Bacterial growth</td>
<td>Bioluminescence production</td>
<td>Bacterial growth</td>
</tr>
<tr>
<td>Target</td>
<td>Drinking water</td>
<td>Wastewater</td>
<td>Lakes and estuaries</td>
<td>Soil</td>
<td>Groundwater</td>
<td>Wastewater samples</td>
<td>Drinking water</td>
</tr>
<tr>
<td>Inoculum</td>
<td>P17</td>
<td><em>Selenastrum capricornutum</em></td>
<td>Indigenous bacteria</td>
<td>Indigenous bacteria</td>
<td>Nitrifying bacteria</td>
<td><em>E. coli/pPHO-lux</em></td>
<td>Evian</td>
</tr>
<tr>
<td>Sterilization</td>
<td>60 °C, 45 min</td>
<td>121 °C, 30 min</td>
<td>N.A.</td>
<td>N.A.</td>
<td>60 °C, 35 min</td>
<td>0.45 μm filtration</td>
<td>70 °C, 60 min</td>
</tr>
<tr>
<td>Inoculation</td>
<td>1,000 CFU mL⁻¹</td>
<td>10,000 cells mL⁻¹</td>
<td>N.A.</td>
<td>N.A.</td>
<td>37 ± 20 cells mL⁻¹</td>
<td>N.A.</td>
<td>1,000 cells mL⁻¹</td>
</tr>
<tr>
<td>Temperature</td>
<td>15 °C</td>
<td>24 ± 2 °C</td>
<td>25 °C</td>
<td>22 °C</td>
<td>N.A.</td>
<td>26 °C</td>
<td>30 °C</td>
</tr>
<tr>
<td>Time</td>
<td>4–6 d</td>
<td>14 d</td>
<td>36 h</td>
<td>30 h</td>
<td>N.A.</td>
<td>140 min</td>
<td>72 h</td>
</tr>
<tr>
<td>Cell count</td>
<td>Plating</td>
<td>Particle size analyzer</td>
<td>DO consumption</td>
<td>CO₂ production</td>
<td>qPCR</td>
<td>Luminometer</td>
<td>FCM</td>
</tr>
<tr>
<td>Substrate</td>
<td>Na₂HPO₄</td>
<td>K₂HPO₄</td>
<td>N.A.</td>
<td>Na₂HPO₄</td>
<td>Na₂HPO₄·12H₂O</td>
<td>K₂HPO₄</td>
<td>Na₂HPO₄</td>
</tr>
<tr>
<td>Carbon spiked</td>
<td>1 mg acetate-C L⁻¹</td>
<td>N.A.</td>
<td>1% glucose</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>1 mg acetate-C L⁻¹</td>
</tr>
<tr>
<td>Detection range</td>
<td>0.05–10 μg L⁻¹</td>
<td>0–50 μg L⁻¹</td>
<td>N.A.</td>
<td>10–300 μg g⁻¹</td>
<td>0–100 μg L⁻¹</td>
<td>&lt;60 μg L⁻¹</td>
<td>0–10 μg L⁻¹</td>
</tr>
</tbody>
</table>

N.A.: not available; FCM: flow cytometry.
including bacterial growth, DO consumption and CO₂ production were introduced. In comparison to the quantification of bacterial biomass, the quantification of DO consumption or CO₂ production is more prone to be affected by the environment due to the ubiquitous presence of oxygen and CO₂ in air. In regard to DO consumption, the relationship between DO consumption and biomass production does not seem to be proportional since the growth-induced reduction in DO concentration of the sample will eventually result in the suppression of further growth once it approaches anaerobic conditions. Similar to DO consumption, CO₂ production will not be proportional to biomass production once DO approaches anaerobic conditions. Therefore, direct measurement of bacterial biomass is of obvious advantage.

With respect to the quantification methods, as discussed above in the section on enumeration methods, TCC by FCM or ATP measurement is suitable for MAP measurement if the indigenous bacterium was adopted as the inoculum due to the presence of VBNC bacteria in actual waters. Of course, it is also feasible but tedious to quantify bacterial biomass using plating if pure culture (such as P17) is adopted as the test inoculum. However, indigenous bacterium has the capability to utilize a wider spectrum of phosphorus, and a better sensitivity was observed (see the section above on optimization of inoculums).

The above discussions clearly demonstrate that the adapted MAP method in the present study is more sensitive, time-saving and easily operated. We have applied this method to the actual waters (Supplementary Material, Figure S3, available with the online version of this paper).

CONCLUSIONS

A rapid and accurate MAP quantification method based on bacterial growth potential was developed. Natural microbial consortium (Evian) was used as the inoculum instead of pure culture P17; high temperature (30 °C) cultivation was adopted to shorten the test period; flow cytometric enumeration is recommended to measure all bacteria growing in drinking water. In summary, the developed method is sensitive, time-saving and easily operated.

Having this rapid MAP quantification method at hand, it is possible to routinely measure the level of MAP during drinking water treatment and estimate the biostability of drinking waters accurately.

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REFERENCES


Hammes, F., Goldschmidt, F., Vital, M., Wang, Y. & Egli, T. 2009 Measurement and interpretation of microbial adenosine tri-


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