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# Modelling temperature effects on ammonia-oxidising bacterial biostability in chloraminated systems

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## HIGHLIGHTS

- Exponential curve does not fit the variation of specific growth rate with temperature.
- A model proposed by Ratkowsky et al. (1982) is adopted for sub-optimal temperature.
- A quadratic equation describes the specific growth rate beyond sub-optimal temperature.
- A biostable residual concentration greatly varies with temperature.
- The relationship was validated against full and laboratory scale results between 13-30 °C.

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## ABSTRACT

The biostability concept has been successfully used to predict the onset of nitrification in drinking water distribution systems, but in certain cases deficiencies have been observed in the predictions, indicating that modifications to parameters were needed. At the biostable disinfectant residual concentration (BRC), the rate of ammonia-oxidising bacterial (AOB) growth due to the substrate (free ammonia) and the rate of inactivation due to the disinfectant are balanced. Growth and inactivation rates vary greatly with temperature, but temperature is yet to be considered in the biostability equation. In this paper, two separate novel models are proposed which take into account the temperature effects on the biostability equation. First, a novel model of specific growth rate variability with temperature was shown to be valid for different bacterial species. Then, the biostability model was modified and validated for ammonia-oxidising bacterial activity using data collected from laboratory and full-scale distribution systems. The proposed model has two important uses: while the specific growth rate model and biostability model can be widely adopted for many microbes, the biostability model for AOB also has the potential to aid water utilities in disinfectant residual management throughout yearly temperature variations.

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## 1. Introduction

Microbial chloramine decay, including that due to nitrification, presents a major challenge to water utilities in the management of chloraminated distribution systems. Nitrification, a microbial conversion of ammonia to nitrite, by ammonia-oxidising bacteria (AOB), and then to nitrate, by nitrite-oxidising bacteria (NOB), occurs over a pH range of 6.6 to 9.7 (Odell et al., 1996). Nitrification usually occurs at temperatures above 15 °C, but it can also occur at lower temperatures (Lipponen et al., 2004; Wilczak et al., 1996) in chloraminated distribution systems.

Once nitrification commences, controlling or overcoming it is very difficult even by increasing the chloramine concentration up to 8.0 mg-$Cl_2$ $L^{-1}$ through re-chloramination (Cunliffe, 1991; Skadsen, 1993). Chloramine decays at a much faster rate under nitrification conditions (Sathasivan et al., 2008) which may be due to soluble microbial products produced after the onset of nitrification (Bal Krishna and Sathasivan, 2010; Bal Krishna et al., 2012). It is therefore important to understand the mechanism of nitrification and the point at which the onset of nitrification occurs, so that appropriate measures can be taken in advance. The onset of nitrification has been defined differently by different authors. Pintar et al. (2005) suggested that chloramine concentration was an appropriate indicator to judge the start of this process. Sathasivan et al. (2008) reported that a sudden increase in the concentration of nitrite, a drop in chloramine residual

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concentration or an increase in chloramine decay rate could be used. When batch experiments were conducted on samples collected from Sydney Water systems, these authors found that there was a sharp decrease in chloramine concentration, with increasing nitrite concentration, when the chloramine residual was reduced to 0.5–0.7 mg-$Cl_2$ $L^{-1}$. In this study, the temperature of water samples was maintained at a temperature of about 20° C and, consequently it was not possible to determine the validity of the experiments at different temperatures.

Wolfe et al. (1990) chose *Nitrosomonas europea* as a representative microorganism to study nitrification phenomena. However, the recent use of molecular microbiological techniques demonstrated that *N. europea* was not abundant in chloraminated systems, especially under low ammonia concentration in distribution systems (Regan et al., 2003; Lipponen et al., 2004; Hoefel et al., 2005). In Finnish and US distribution systems, *Nitrosomonas oligotropha* was found to be the most abundant AOB and recent studies also showed the presence of nitrite-oxidising bacterial communities in these systems (Williams et al., 2004). Recently, the presence of ammonia-oxidising archaea (AOA) was reported in drinking water distribution systems (Hoefel et al., 2011), in wastewater treatment plants (Park et al., 2006), in a groundwater treatment plant (Van der Wielen et al., 2009), in soil (Zhalnina et al., 2012) and marine environment (Konneke et al., 2005). Therefore, it is apparent that nitrification is a complex process with no one microorganism being responsible. In this manuscript until it is proven which microorganisms are responsible for the onset, the authors opted to use the terminology AOB to describe the microorganisms responsible.

When nitrification occurs, it is well known that the initial step produces the chloramine consuming substance, nitrite, which is generally used as the indicator of nitrification status (Wolfe et al., 1988). Although other better indicators, such as the NOx-N (sum of nitrite and nitrate) production rate or AOB (or AOA) data, can be used under well-defined conditions, nitrite is still considered the most practical indicator for use in analysis of full-scale systems (Kirmeyer et al., 2004; Fleming et al., 2008).

The biostability concept, first developed by Woolschlager et al. (2001) was adopted by Harrington et al. (2002) for the determination of the onset of nitrification and further developed by Fleming et al. (2005) who applied it in a laboratory-scale system. At the biostable disinfectant residual concentration, the rate of bacterial growth and the rate of inactivation due to disinfection are balanced. Fleming et al. (2005) used free ammonia [the sum of ammonia ($NH_3$ – N) and ammomium ($NH_4^+$ – N)] as the substrate for controlling the growth of AOB and dichloramine as the disinfectant, although finally they converted dichloramine to total chlorine. Sathasivan et al. (2008) adopted total chlorine as the disinfectant, since there is reported to be little dichloramine present under distribution system conditions (Valentine, 2007). The resulting equation Eq. (1) for the biostable disinfectant residual concentration (BRC), as proposed by Sathasivan et al. (2008), is:

$$BRC = \frac{\mu_m}{k_d} \cdot \left( \frac{free \quad ammonia\_N}{K_s + free \quad ammonia\_N} \right) \tag{1}$$

where, $\mu_m$ is the maximum specific growth rate of AOB ($day^{-1}$); free ammonia represents the sum of $NH_3$ – N and $NH_4^+$ – N concentrations (mg – N $L^{-1}$); $K_s$ is the half saturation constant for AOB (mg-N $L^{-1}$); $k_d$ is the rate constant for inactivation of AOB by disinfectant (L $day^{-1}$ mg-$Cl_2^{-1}$); and BRC is the biostable disinfectant residual concentration measured as a total chlorine (mg-$Cl_2$ $L^{-1}$).

When the BRC is presented as a function of the free ammonia concentration, the curve produces two regions: a region of nitrification (below the curve) and that of no-nitrification (above the curve) (Fleming et al., 2005). Using such an approach, Fleming et al. (2005) obtained 2 mg-$Cl_2$ $L^{-1}$ and 0.5 mg-N $L^{-1}$ for $\mu_m/k_d$ and $K_s$ values respectively, for a pilot-scale system. Sathasivan et al. (2008) determined a different $K_s$ value (0.18 mg-N $L^{-1}$) for samples from three different sub-systems utilising three different raw water resources in New South Wales, Australia. In a full-scale distribution system, Fleming et al. (2008) reported different values of $\mu_m/k_d$ and $K_s$ to the pilot-scale systems. The reason for the difference was cited as the presence of different microbiological species in different systems (Fleming et al., 2008). However, before assigning the variation in values to microbiological diversity or different physiological status, the effect of temperature also needs to be considered.

The growth rate of nitrifying bacteria depends on various factors including ammonia concentration, temperature, pH, light and dissolved oxygen concentration (Watson et al., 1986). The pH in chloraminated distribution system is usually within a narrow range of 7.5 to 8.5 to maintain the chloramine stability. Light usually does not enter the pipe lines or service reservoirs, except for open service reservoirs. The dissolved oxygen in the distribution systems is rarely below 5 mg $L^{-1}$. However the temperature which is the key factor in controlling growth rate and disinfection rate varies greatly. In the few reported applications of the biostability concept, the impact of temperature was neglected in defining the parameters of biostability curves. An investigation of the impact of temperature variation on bacterial growth and inactivation would assist in understanding the effect of temperature on the onset of nitrification. Therefore, a model that reliably predicts the effects of temperature on these microbes would be very useful in pre-empting the preventive actions.

Traditionally, growth rate is modelled using an exponential function (the Arrhenius equation). However, Ratkowsky et al. (1982) showed that the use of the exponential function was not valid even for the sub-optimal (minimum to optimum) temperature range. The typical temperature range in water distribution systems is 6–35 °C and the optimum temperature for the growth of nitrifying bacteria is 25–30 °C (Wolfe et al., 1990; Skadsen, 1993; Odell et al., 1996). However, the maximum temperature in some distribution systems can be up to 50 °C, 20 °C higher than the optimum temperature, conditions under which the current models have not yet been validated: there is currently no model in the literature that proposes a model to describe maximum specific growth rates around optimum temperature and beyond. Furthermore, it is well known that $k_d$ varies greatly with temperature; such temperature variation is usually modelled using an exponential relationship (Tchobanoglous et al., 2003), implying that the $\mu_m/k_d$ value in the biostability equation would also vary greatly with temperature, as was found in our previous study (Sarker and Sathasivan, 2011). The aim of the current study is to develop and validate a model that describes the maximum specific growth rate, and thus the $\mu_m/k_d$ value, in the region of interest for the temperature, i.e. 6–35 °C, for AOB prevailing in both laboratory-scale and full-scale chloraminated distribution system environments. The model for the first time describes the variation of growth rate with temperature under log growth phase and beyond.

## 2. Model Development

At chemical equilibrium, the temperature effects on the rate constant for chemical reactions can be described clearly by the Arrhenius equation, or an exponential function. Ratkowsky et al. (1982) reported that the effect of temperature on the growth of bacteria cannot be described completely by the Arrhenius equation as the growth of bacteria is a complex process that is composed of a variety of substrates and enzymes. To overcome this problem, Ratkowsky et al. (1982) showed the following relationship for sub-optimal temperatures:

$$\sqrt{\mu_{m,T}} = b(T - \theta_o) \tag{2}$$

where $\mu_{m,T}$ is the maximum specific growth rate ($day^{-1}$) at temperature $T$ (K), $b$ is the regression coefficient and $\theta_o$ is a hypothetical temperature (K) which is an intrinsic property of an organism. This model is

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