

# The Impact of Temperature on Nitrification Rate in Fixed Film Biofilters

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**ABSTRACT:** The impact of temperature on nitrification rate was evaluated experimentally in this study. The results show that the impact of temperature on fixed film nitrification rate is less significant than that predicted by the van't Hoff-Arrhenius equation. The effect of temperature on nitrification rate in a fixed film, determined based on bacterial growth rate, was greatly reduced probably due to oxygen limitation. When oxygen is limited, the reduction in saturation concentration of DO that results from increased temperature results in a negative temperature impact on the rate of nitrification.

**KEYWORDS:** Temperature, Nitrification, Fixed film, Biofilm, Biofilter.

## Introduction

Ammonia removal is a major challenge in the design of recirculating aquacultural systems. Ammonia removal is accomplished through biological nitrification in which ammonia is converted into nitrite and then nitrate by nitrifying bacteria called nitrifiers. The oxidation of one gram of total ammonia nitrogen (TAN, including  $\text{NH}_4^+$ -N and  $\text{NH}_3$ -N) during the nitrification process requires approximately 4.57 grams of oxygen (USEPA, 1975). Biological nitrification processes are typically accomplished in one of two configurations: mixed or fixed film bacterial cultures. In a mixed culture under a single limiting-substrate condition, the steady-state kinetics of substrate removal is usually described by the Monod-type expression (Srna and Baggaley, 1975; Rittmann and McCarty, 1980; Drtil *et al.*, 1993):

$$R = \mu \frac{X}{Y_s} \frac{S}{K_s + S} \quad (1)$$

Where

- $R$  = substrate removal rate ( $\text{g m}^{-3} \text{d}^{-1}$ ),  
 $\mu_{max}$  = specific growth rate ( $\text{d}^{-1}$ ),  
 $X$  = bacterial mass concentration ( $\text{g cell m}^{-3}$ ),  
 $Y_S$  = yield of bacterial mass per unit of substrate used ( $\text{g cell g}^{-1}$  substrate),  
 $S$  = limiting substrate concentration ( $\text{g m}^{-3}$ ),  
 $K_S$  = half saturation constant ( $\text{g m}^{-3}$ ).

Developed for suspended culture, equation (1) does not address diffusion related issues encountered in the fixed film nitrification processes. Due to the fact that suspended culture is problematic for the nitrification process because of the low growth rate of nitrifiers and especially at low temperatures, low substrate conditions, a fixed biofilm process is often used in aquacultural applications. A fixed biofilm is a viscoelastic layer of microorganisms attached to a solid surface of the support media. In a fixed film biofilter, a high bacterial retention time can be maintained by immobilization and the growing microorganisms can be retained in a continuous flow reactor (Liu and Capdeville, 1994; Nogueira *et al.*, 1998). Consequently, fixed biofilm systems offer several other advantages in wastewater treatment when compared with suspended culture processes, including handling convenience, increased process stability, little residual sludge, ease of use in small-scale treatment, and the capability to handle shock loads (Fitch *et al.*, 1998; Nogueira *et al.*, 1998).

Since nitrification reactions occur in the biofilm not in the bulk liquid (Moreau *et al.*, 1994), the substrate utilization rate depends on local substrate concentrations within the biofilm. At local reaction sites in a biofilm, reactant concentrations are depressed, and product concentrations are elevated (Boller *et al.*, 1994). It was reported that nitrifier populations deep within the biofilm are maintained by endogenous respiration under oxygen limited conditions, and nitrifying populations on the surface are under ammonia limited conditions (Horn, 1994). Therefore, the kinetics of biofilm reactions is influenced by mass transport (Rasmussen and Lewandowski, 1998). The transfer of nitrogen and oxygen into the biofilm is controlled by a diffusion process in which a concentration gradient has to exist between the bulk liquid and biofilm. Diffusion and transport processes should be considered to better understand the nitrification kinetics of fixed film biofilters.

The influence of temperature over nitrification processes is important for biofilter design and operation. In a suspended culture, biological reaction rates increase with rising temperature until an optimal temperature is reached; above the optimal temperature, enzymatic proteins denature and the rates decrease (Sawyer *et al.*, 1994). The van't Hoff-Arrhenius equation provides a generalized estimate of temperature effects on biological reaction rates (Tchobanoglous and Burton, 1991):

$$\mu = \mu_{20} \theta^{T-20} \quad (2)$$

Where

- $\mu$  = the rate coefficient ( $d^{-1}$ ),  
 $\mu_{20}$  = the value of  $\mu$  at  $20^{\circ}C$  ( $d^{-1}$ ),  
 $\theta$  = temperature coefficient (dimensionless),  
 $T$  = temperature ( $^{\circ}C$ ).

For bacterial growth in a suspended culture, equation (1) will thus become:

$$R = \mu_{20} \theta^{T-20} \frac{X}{Y_s} \frac{S}{K_s + S} \quad (3)$$

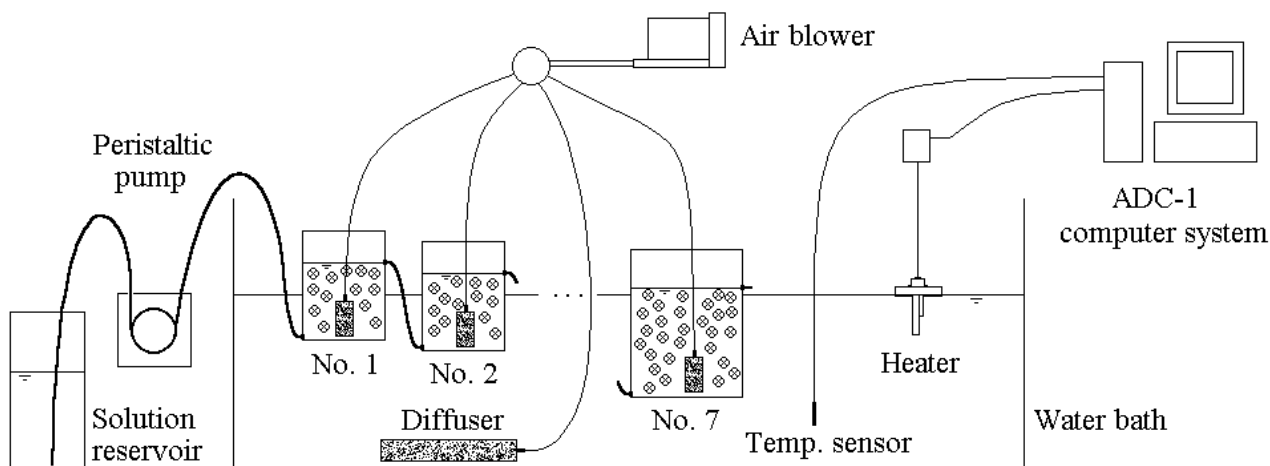
Many researchers have used equation (2) to estimate the impact of temperature change on the nitrification rate of suspended culture processes (Antoniou *et al.*, 1990; Willke and Vorlop, 1996). For fixed film filters, however, it is difficult to address the effects of temperature on nitrification kinetics because the bio-process is also influenced by other temperature dependent phenomena and parameters (Fdz-Polanco *et al.*, 1994), including substrate diffusion and transport, and dissolved oxygen concentration. The impacts of temperature on nitrification rate in fixed film biofilters were less well understood (Okey and Albertson, 1989). Little information is available to qualify the effects of temperature on fixed film nitrification rate (Wheaton *et al.*, 1994).

The objective of this study was to explore the impact of temperature on fixed film nitrification performance through experimental investigations.

## Methodology

A reactor series system (Zhu and Chen, 1999) has been developed for this study (Figure 1). It consisted of seven-stage biofilters connected in series with flexible tubing. A plastic medium, called Purac (Water Management Technologies, Inc., Louisiana, USA), was used as the porous media for nitrifier colonization. Table 1 gives the specifications of the experimental systems. The total biofilm area included the measured media surface area and the submerged inner walls of the reactor vessels. The reactors were positioned at different levels to maintain constant flow by gravity through the reactors in sequence. The water level of each reactor was determined by the outlet position. The inlet of each reactor was located close to the bottom of the vessel and on the side opposite of the outlet so that the water flow shortcut could be minimized.

The series reactors were inoculated with sludge from a nitrification biofilter. A synthetic substrate solution containing ammonia chloride, sodium bicarbonate and other necessary nutrients (Table 2) was continuously fed into the first reactor by a metering pump. Continuous feeding was maintained until a steady-state culture, as evidenced by a stable TAN concentration for each reactor at a fixed TAN feeding rate, was established. This required about five weeks of operation. Sampling then began. The major advantage of this experimental system was the easy maintenance of a stable, but different substrate concentration in each biofilter, allowing the evaluation of nitrification kinetics in response to substrate concentration changes.



**Figure 1.** Schematic of the series reactor experimental system

**Table 1.** Specifications of the Experimental Reactor Series System

Reactor number	1	2	3	4	5	6	7
Water volume (L)	3	3	3	3	3	12	15
Total biofilm area (m <sup>2</sup> )	0.36	0.36	0.36	0.36	0.36	2.65	3.18
Residence time (h)							
8 and 14°C: flow rate 94 L d <sup>-1</sup>	0.77	0.77	0.77	0.77	0.77	3.06	3.83
20°C: flow rate 109 L d <sup>-1</sup>	0.66	0.66	0.66	0.66	0.66	2.64	3.30
27°C: flow rate 117 L d <sup>-1</sup>	0.62	0.62	0.62	0.62	0.62	2.46	3.08

**Table 2.** Composition of substrate nutrients (modified from Liu and Capdeville, 1994). The solution was further diluted for different experimental conditions.

Ingredient	Composition
NH <sub>4</sub> Cl	1377 g
NaHCO <sub>3</sub>	3500 g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	36 g
Na <sub>2</sub> HPO <sub>4</sub>	159 g
KH <sub>2</sub> PO <sub>4</sub>	153 g
FeCl <sub>3</sub> ·6H <sub>2</sub> O	5 g
Water	75 L

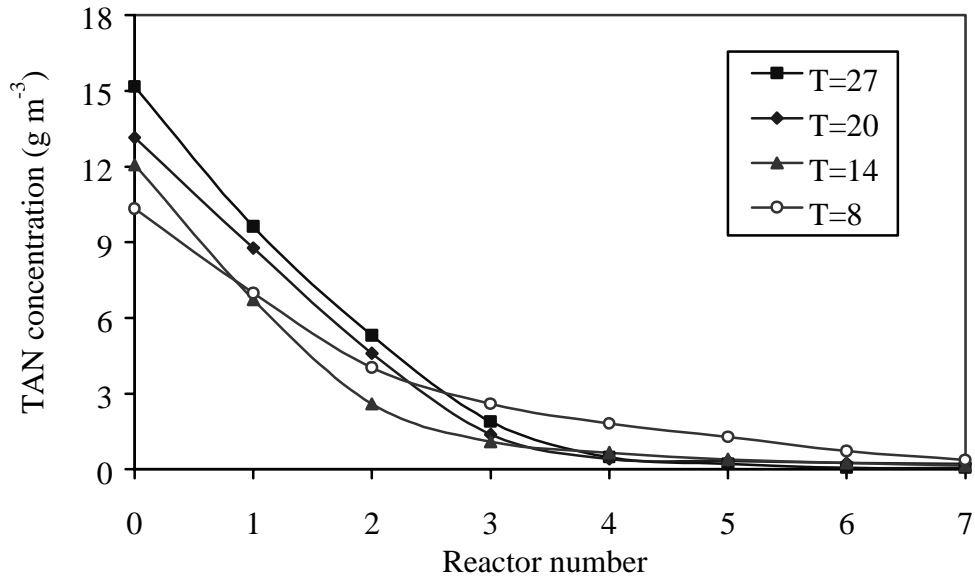
The experiment began with the treatment using the highest temperature of 27°C, followed by 20, 14 and 8°C. For the two high temperature treatments (20 and 27°C), the reactor vessels were placed in a water bath where water temperature was controlled by a computer interfaced with an ADC-1 data acquisition and control board (Remote Measurement Systems, Inc., Washington, USA). An air diffuser was placed in the water bath for mixing to keep the temperature homogeneous. For the lower temperatures of 8 and 14°C, the reactor series system was placed in an environmental control chamber. In order for nitrifying bacteria to acclimate to different conditions, temperature was gradually decreased between two treatments over a week, and then maintained at least three weeks before sampling started.

Each reactor was provided with an air diffuser connected to an air blower, so that the dissolved oxygen concentrations in the reactors were always maintained above 6 gm<sup>-3</sup>. The air diffuser also served as an excellent mixer, ensuring each reactor behaved as a CSTR (continuous stirred-tank reactor). The pH value in the reactors was kept in the range of 7.0-8.6, which is the optimal range for nitrifier growth (Wheaton *et al.*, 1994).

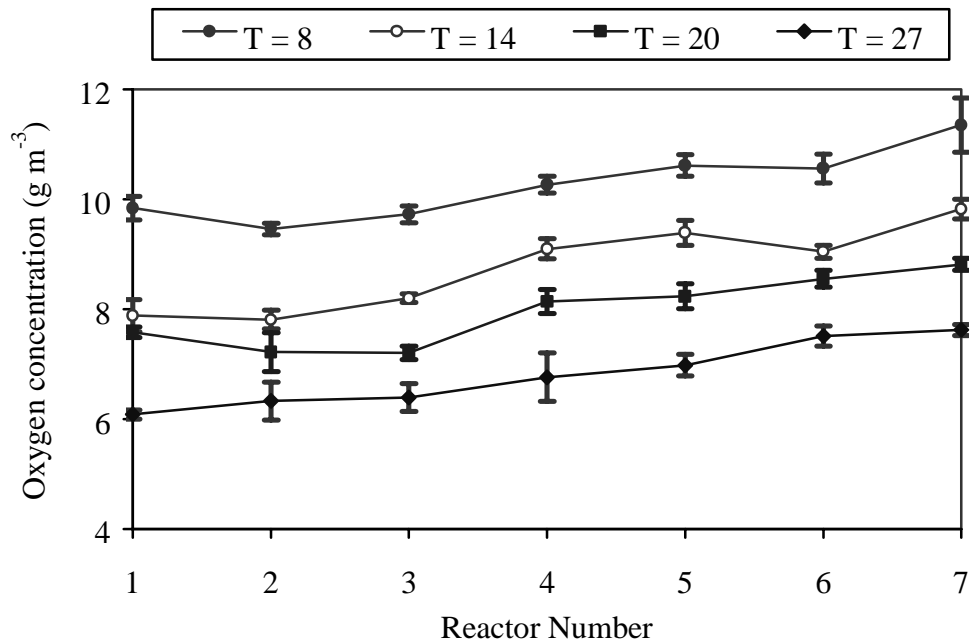
Daily samples were collected from each reactor for the analysis of TAN, nitrite nitrogen and nitrate nitrogen. Other parameters such as temperature, pH, and dissolved oxygen concentration were monitored before sampling. All sample analyses were performed in the Water Quality Laboratory at Washington State University, a laboratory certified by the Washington Department of Ecology for the analysis of wastewater related parameters. TAN concentration was determined using Kjeltac 2300 Analyzer Unit (Foss Tecator AB, Sweden). Nitrite and nitrate nitrogen analyses were performed using the standard methods of 4500-NO<sub>2</sub>-B and 4500-NO<sub>3</sub>-E, respectively (APHA, 1995).

## Results and Discussion

**Experimental Results.** Each reactor in the series was capable of maintaining a different TAN concentration at steady state (Figure 2). TAN was rapidly oxidized in reactors 1 and 2, with a decrease in reaction rate for the following reactors. This indicated that TAN oxidization was reduced due to the low TAN concentration. The slope of the TAN reduction curve at 8°C was significantly less than that at the higher temperatures. This means that the nitrification rate at 8°C was much lower than that at other temperatures. With TAN reduction, nitrite nitrogen (NO<sub>2</sub>-N) was accumulated in the first three reactors and dropped thereafter, which is consistent with the report of Gujer and Boller (1986). NO<sub>2</sub>-N concentration reached its highest value in reactor 3 with the mean values (n = 3) of 4.6, 6.3, 9.3, and 13.5 gm<sup>-3</sup> for 8, 14, 20, and 27°C, respectively. The DO concentrations in the series system varied with temperature and reactor position (Figure 3). For a given temperature, the DO level in the first three reactors was lower than that in the others because most of the oxygen was consumed due to the high nitrification rates in these reactors. For the same reactor, a higher temperature resulted in a lower DO concentration because the DO saturation level dropped as temperature increased. For the four temperatures, the first three reactors were at a DO saturation level of 76-84%.



**Figure 2.** Mean concentrations of total ammonia nitrogen (TAN) of the series reactors at different temperatures (where T is temperature (°C), and reactor 0 means the stocking solution).



**Figure 3.** Dissolved oxygen concentration of the series reactors at different temperature (T, °C). Data points and error bars indicate mean values and standard deviation, respectively.

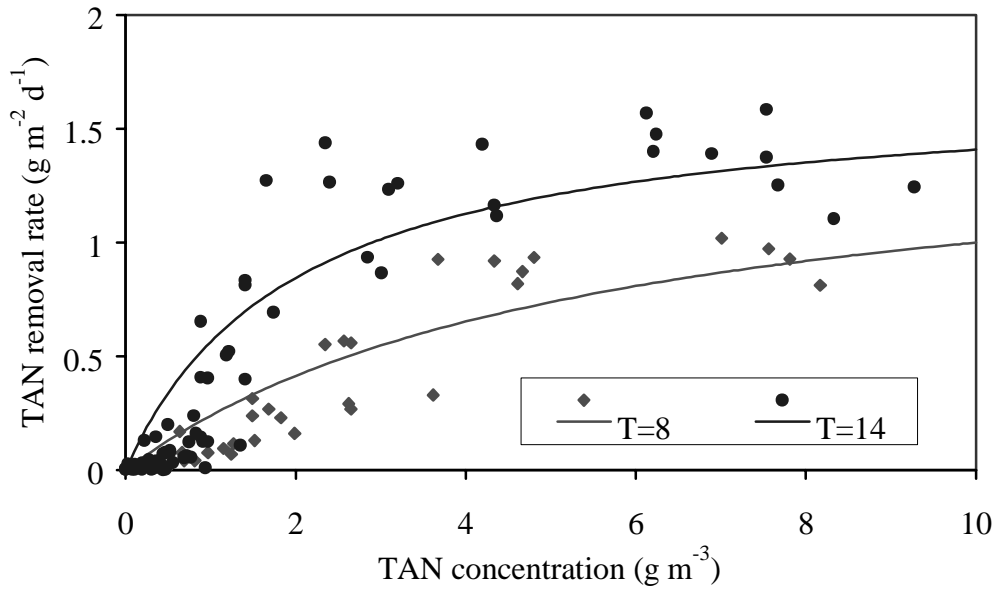
From TAN concentration, the nitrification rate of each reactor can be determined based on the mass balance principle. Since measurements were performed at steady state, TAN removal rate was calculated as follows:

$$J_{Si} = Q(S_{i-1} - S_i) / A_i \quad (4)$$

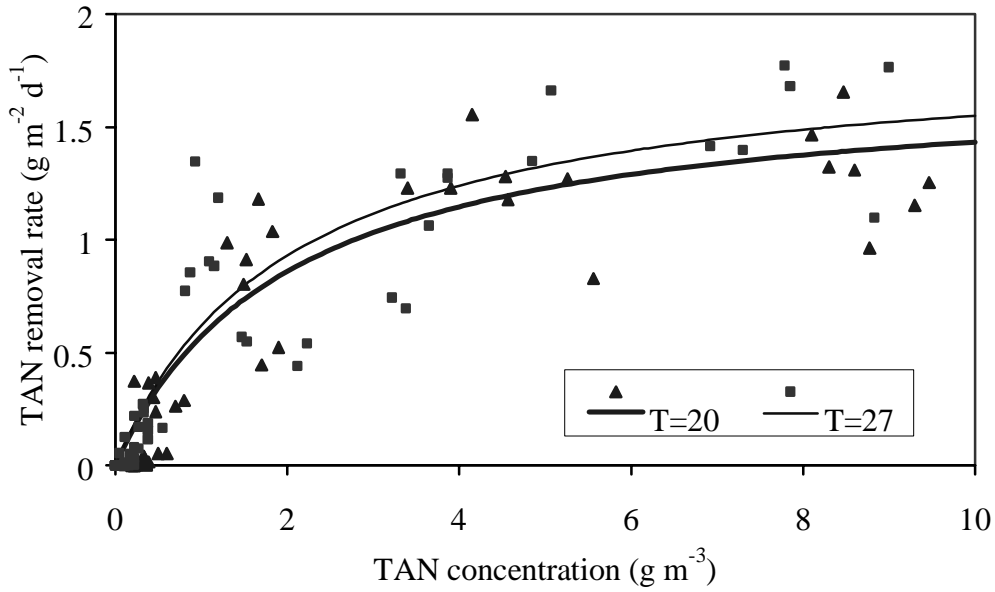
Where

- $J_{Si}$  = TAN removal rate of reactor  $i$  ( $\text{g m}^{-2} \text{d}^{-1}$ ),
- $Q$  = flow rate through the series ( $\text{m}^3 \text{d}^{-1}$ ),
- $S_i$  = TAN concentration of reactor  $i$  ( $\text{g m}^{-3}$ ),
- $S_0$  = TAN concentration of reservoir solution ( $\text{g m}^{-3}$ ),
- $A_i$  = biofilm area in reactor  $i$  ( $\text{m}^2$ ).

For each TAN concentration tested, a related TAN removal rate was obtained. As a result, the relationship between TAN concentration and removal rate was demonstrated as in Figures 4 and 5. The difference in nitrification rate was not significant among the three temperatures of 14, 20 and 27°C. The lowest temperature (8°C) had a pronounced impact on the nitrification rate (Figure 4).



**Figure 4.** Relationship between total ammonia nitrogen (TAN) removal rate and concentration at 8 and 14°C.



**Figure 5.** Relationship between total ammonia nitrogen (TAN) removal rate and concentration at 20 and 27°C.

The Monod equation was used to express the empirical relationship between TAN concentration in bulk water ( $S_w$ ) and nitrification rate of a fixed biofilm (Tchobanoglous and Burton, 1991).

$$J_s = J_{s_{\max}} \frac{S_w}{K_{s_w} + S_w} \quad (5)$$

Where

- $J_s$  = TAN removal rate ( $\text{g m}^{-2} \text{d}^{-1}$ ),
- $J_{s_{\max}}$  = the maximum value of  $J_s$  ( $\text{g m}^{-2} \text{d}^{-1}$ ),
- $S_w$  = TAN concentration of bulk water ( $\text{g m}^{-3}$ )
- $K_{s_w}$  = half saturation TAN concentration ( $\text{g m}^{-3}$ ).

The maximum TAN removal rate and the half saturation concentration were estimated using the Least Square Method with the experimental data (Table 3). Statistical results showed that Equation (5) was applicable to the fixed film nitrification process, but the half saturation value of bulk water TAN concentration for fixed film was different from those reported for suspended culture (Knowles *et al.*, 1965; WPCF, 1983). In this study, the half saturation concentration remained at a similar value of 14, 20 and 27°C, but reached a higher value at 8°C (Table 3 and Figure 4). In a suspended culture processes, the half saturation concentration increases as temperature rises (Knowles *et al.*, 1965).

**Table 3.** Estimated values of the maximum TAN removal rate ( $R_{\max}$ ) and TAN half saturation constant ( $K_{ws}$ ) at four temperature levels.

Temperature (°C)	$K_{ws}$ (g m <sup>-3</sup> )	$R_{\max}$ (g m <sup>-2</sup> d <sup>-1</sup> )	Statistical result		
			$R^2$	n	P
27	2	1.86	0.90	42	<0.01
20	2	1.72	0.79	49	<0.01
14	2	1.69	0.62	56	<0.01
8	5.5	1.55	0.66	35	<0.01

### Summary

The results of this study demonstrated that the temperature impacts on the nitrification rate of fixed film were not as significant as predicted by the van't Hoff-Arrhenius equation, and suggest that the fact that the saturated DO concentration of bulk water decreases as temperature increases, results in a negative impact of temperature on nitrification rate due to oxygen limitation.

### Acknowledgments

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