

# Aerobic Heterotroph and Autotrophic Nitrifier Competition in Trickling Filters

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May 17, 2018

## Introduction and Background

Trickling filters are used to treat biological oxygen demand (BOD) and nutrient rich wastewater. Trickling filters can be used in combined secondary treatment applications for BOD removal and nitrification in the same reactor, or as tertiary treatment reactors for nitrification. In combined secondary treatment applications, the main bacterium of interest are aerobic heterotrophs which degrade BOD and autotrophic nitrifiers which are responsible for nitrification. Aerobic heterotrophs consume influent carbon as an energy and carbon source while autotrophic nitrifiers consume  $CO_2$  as a carbon source and inorganic nitrogen as an energy source.

Aerobic heterotrophs and autotrophic nitrifiers do not directly compete for energy sources, but they do compete for space, oxygen, and other nutrients that enter the biofilm from the influent waste. Heterotrophs can outcompete nitrifiers due to higher growth rates. Carbon to nitrogen (C/N) ratio, dissolved oxygen (DO) concentrations, and temperature are relevant factors for modeling microbial competition. BOD at high enough concentrations can inhibit nitrification. Within a single trickling filter, there can be different zones of kinetic and metabolic activity due to changes in influent ammonium concentration decreasing over reactor depth, making theoretical modeling of such systems very complex (Okey & Albertson, 1989).

Research on heterotroph and nitrifier competition has mostly employed empirical models to determine appropriate parameters for trickling filter operation. Maximum BOD influent loadings in the form of C/N ratios have been recommended based on field experiments, many of which were prepared and published with support from the U.S. EPA (Scheible et al., 1991). Maximum C/N ratios must acknowledge that nitrifiers rely on heterotrophs in to form biogrowth that nitrifiers adhere to, but too high ratios allow heterotrophs to overgrow (U.S. EPA, 2000). Table 1, adapted from Metcalf & Eddy, highlights typical loadings for single-stage reactors (Metcalf & Eddy, 1991). Table 2, adapted from Rittmann & McCarty, shows additional guidelines for biofilm reactor treatments based on surface loadings where nitrogen loading is calculated from TKN concentration.

Table 1: Typical Loading Rates for Single-Stage Nitrification, adapted from Metcalf & Eddy

Trickling Filter Media	% Nitrification	Loading Rate lb BOD/1,000 $ft^3$ /d (g BOD/ $m^3$ /d)
Rock	75-95	10-3 (160-48)
Plastic	75-85	181-12 (288-192)
Tower Trickling Filter	85-95	12-6 (192-96)

Table 2: Nitrogen and BOD loadings used successfully in various nitrifying biofilm processes, adapted from Rittmann & McCarty

Process Type	N Loading, kg N/1,000 $m^2$ -d	BOD Loading, kg BOD/1,000 $m^3$ -d
Trickling filter	0.5-0.8	< 4.4
Rotating biological contactors	0.2-0.6	< 6
Biolite granular filters	<0.7	< 6
Fluidized beds	0.5	not given
Circulating beds	<0.1	not given

# Science Questions and Objectives

Design of trickling filters relies heavily on empirical models and observations. Using empirical models can lead to transferability issues, in that models derived for a specific reactor geometry, packing material, and influent may not apply to other treatment scenarios. Inappropriate use of empirical models may lead to inefficient or unoptimized reactors and treatment schemes for combined BOD removal and nitrification trickling filters.

Using first-order fundamental models to understand trickling filter treatment would provide a more robust analysis of trickling filters, specifically competition between heterotrophs and nitrifiers. It is understood that heterotrophs can outcompete nitrifiers because of the higher growth yield for heterotrophs, and because organic carbon is a stronger electron donor than inorganic nitrogen.

A model for metabolic interactions that focuses on competition for oxygen would give more insight into trickling filter operation. If oxygen use is understood and the heterotroph/nitrifier competition is understood from a metabolic perspective, work could be done from a kinetic perspective to ultimately lead to better reactor design and operation choices to make trickling filters more efficient.

In a design context, it is important to know wastewater influent characteristics to understand BOD loading, because that could impact reactor design decisions. In cases with high BOD loading, two-stage treatment would allow for first BOD removal and second nitrification. However, multiple reactors may be more costly. Recent research into heterotroph/nitrifier robustness under long- and short-term exposure to different C/N ratios provide more insight into the microbial competition and the impact that controlled changes in influent could have on treatment (Knutsen, 2017). Understanding the balance between BOD removal and nitrification needs is important to trickling filter innovation. Theoretical models for oxygen and space constraints could inform better design decisions for trickling filters.

## Mathematical Modeling/Theoretical Considerations

Rittmann and McCarty report a successful load for trickling filter processes to be less than  $4.4 \frac{kgBOD_L}{1,000m^2d}$  and a range for recommended BOD loading from 2 to  $6 \frac{kgBOD_L}{1,000m^2d}$  for different nitrifying biofilm processes. Modeling consumption and competition for oxygen in a biofilm from theoretical metabolic models could provide important insight into what the precise limit of trickling filter treatment capacity is.

## 1 Metabolic Model

A simple metabolic framework was developed for total BOD for heterotrophs and nitrifiers in a trickling filter biofilm. For conservative estimates, temperature was assumed to be  $10^\circ C$  and the minimum solids retention time was determined from the ammonium oxidizers which grow more slowly than heterotrophs. Using representative wastewater organics, half-reactions for energy metabolism and cell synthesis were used to write overall reaction equations to determine BOD. Then, net yield was calculated to compare growth rates. This first-order approach is a simplification of biofilm processes and can be used as the basis for future work to consider kinetic models and biofilm space requirements.

### Definition of variables

$\Delta G^{\circ'} (\frac{KJ}{e^{-eq}})$ : Gibbs Standard Free Energy Equation at pH = 7.0

$R_e$ : Energy reaction

$R_s$ : Synthesis reaction

$R$ : Total reduction-oxidation reactions for energy and cell synthesis

$f_e^o$ : Theoretical fraction of electrons allocated to energy metabolism

$f_s^o$ : Theoretical fraction of electrons allocated to cell synthesis

$f_e$ : Actual fraction of electrons allocated to energy metabolism

$f_s$ : Actual fraction of electrons allocated to cell synthesis

$A$ : Number of electron equivalents of electron donor converted to energy per electron of cells synthesized

$\Delta G_p (\frac{KJ}{e^{-eq}})$ : Free energy consumed (or evolved in conversion of a carbon-source to pyruvate

$\Delta G_{pc} (\frac{KJ}{e^{-eq}})$ : Free energy required to convert pyruvate to cell carbon

$\Delta G_r (\frac{KJ}{e^{-eq}})$ : Energy released per electron equivalent of electron donor substrate used in catabolism

$\epsilon$ : Efficiency of energy transfer to or from an energy carrier (ATP)

$n$ : Indicates that an amount of energy greater than  $\Delta G_p$  is needed to yield an exergonic energy transfer due to inefficiencies

$f_d$ : Fraction of cell mass that is biodegradable

$b$  ( $\frac{1}{day}$ ): decay rate

$\theta_x^{min}$  (day): Minimum solids retention time to avoid washout

$\theta_x^{design}$  (day): Design solids retention time to ensure treatment

$SF$ : Safety factor applied to minimums

*correction*: Correction factor applied to synthesis calculations to account for cell decay

$Y$  ( $\frac{biomass_{mass}}{e^{-donor_{mass}}}$ ): Microbial yield of a reaction

## 2 Aerobic Heterotrophs:

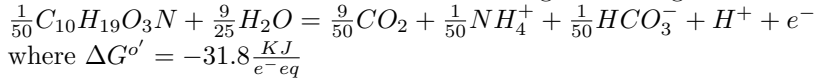
Electron donor:  $C_{10}H_{19}O_3N$

Electron acceptor:  $O_2$

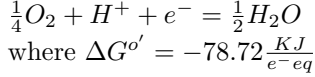
Nitrogen source:  $NH_4^+$

### 2.1 Energy equation:

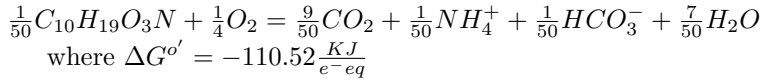
Half-reaction for domestic wastewater with biodegradable organics as an electron donor:



Half-reaction for reduction of oxygen:

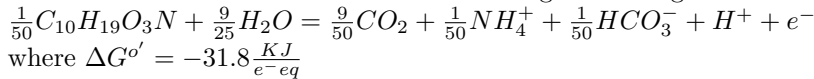


#### 2.1.1 Total energy equation, $R_e$ :

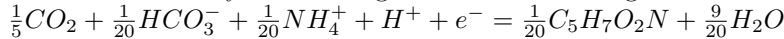


### 2.2 Cell synthesis equation:

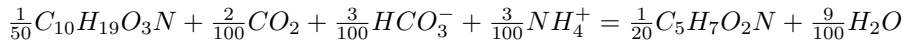
Half-reaction for domestic wastewater with biodegradable organics as an electron donor:



Half-reaction for cell synthesis using ammonium as nitrogen source:



#### 2.2.1 Total synthesis equation, $R_s$ :



### 2.3 Calculation of $f_e$ and $f_s$

$$A = \frac{-(\frac{\Delta G_p}{\epsilon n} + \frac{\Delta G_{pc}}{\epsilon})}{\epsilon \Delta G_r}$$

$$\Delta G_r = \Delta G_{R_e} = -110.52 \frac{KJ}{e^{-eq}}$$

$$\Delta G_p = \Delta G_{pyruvate} - \Delta G_{C-source} = (35.09 - 31.8) \frac{KJ}{e^{-eq}} = 3.29 \frac{KJ}{e^{-eq}}$$

$$\Delta G_p > 0 \text{ therefore, } n = 1$$

$$\Delta G_{pc} = 18.8 \frac{KJ}{e^{-eq}}$$

$$\epsilon = 0.6$$

$$A_{heterotroph} = 0.555$$

$$f_s^o = \frac{1}{1 + A} = 0.643$$

$$f_e^o = 1 - f_s^o = 0.357$$

Correction factor to account for aerobic heterotroph cell decay =  $\frac{1+(1-f_d)(b\theta_x)}{1+b\theta_x}$

Values for heterotrophs were found on page 309 of Rittmann and McCarty and are shown below.

$$f_d = 0.8$$

$$b = 0.15 \frac{1}{\text{day}}$$

$\theta_x^{min} = 2.8$  days, chosen from  $\theta_x^{min}$  for ammonium oxidizers at  $10^\circ C$ , as found on page 472 from Rittmann and McCarty.

$$SF = 2$$

$$\theta_x^{design} = 5.6 \text{ days}$$

$$correction = 0.635$$

$$f_s = correction * f_s^o = 0.408$$

$$f_e = 1 - f_s = 0.591$$

## 2.4 Total reaction equation, R

Only taking into account  $O_2$ ,  $C_{10}H_{19}O_3N$ ,  $NH_4^+$ , and  $C_5H_7O_2N$ :

$$f_s R_s : 0.00816 C_{10}H_{19}O_3N + 0.01224 NH_4^+ = 0.0204 C_5H_7O_2N$$

$$f_e R_e : 0.01182 C_{10}H_{19}O_3N + 0.14775 O_2 = 0.01182 NH_4^+$$

$$R : 0.01998 C_{10}H_{19}O_3N + 0.14775 O_2 + 0.00042 NH_4^+ = 0.0204 C_5H_7O_2N$$

From this equation for heterotrophic metabolism and cell synthesis,  $0.14775 \text{ mol } O_2$  is required for 1  $e^-eq$ .

## 2.5 Expected yield

$$Y = \frac{biomass_{mass}}{e^-donor_{mass}}$$

$$0.0204 C_5H_7O_2N * \frac{113g C_5H_7O_2N}{mol} = 2.3052g C_5H_7O_2N \text{ required per } e^-eq$$

$$0.01998 mol C_{10}H_{19}O_3N * \frac{201g C_{10}H_{19}O_3N}{mol} = 4g C_{10}H_{19}O_3N \text{ required per } e^-eq$$

$$Y = \frac{2.3052g C_5H_7O_2N}{4g C_{10}H_{19}O_3N} = 0.5762 \frac{g C_5H_7O_2N}{g C_{10}H_{19}O_3N}$$

## 3 Aerobic nitrifiers:

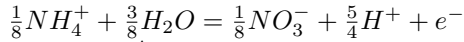
Electron donor:  $NH_4^+$

Electron acceptor:  $O_2$

Nitrogen source:  $NH_4^+$

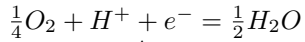
### 3.1 Energy equation:

Half-reaction for reduction of ammonium:



$$\text{where } \Delta G^{o'} = 35.11 \frac{KJ}{e^-eq}$$

Half-reaction for reduction of oxygen:



$$\text{where } \Delta G^{o'} = -78.72 \frac{KJ}{e^-eq}$$

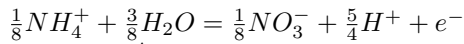
#### 3.1.1 Total energy equation, $R_e$ :



$$\text{where } \Delta G^{o'} = -43.61 \frac{KJ}{e^-eq}$$

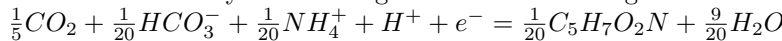
### 3.2 Cell synthesis equation:

Half-reaction for reduction of ammonium:

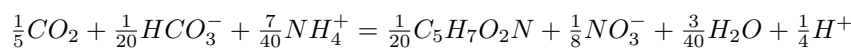


$$\text{where } \Delta G^{o'} = 35.11 \frac{KJ}{e^-eq}$$

Half-reaction for cell synthesis using ammonium as nitrogen source:



### 3.2.1 Total synthesis equation, $R_s$ :



### 3.3 Calculation of $f_e$ and $f_s$

$$A = \frac{-(\frac{\Delta G_p}{\epsilon^n} + \frac{\Delta G_{pc}}{\epsilon})}{\epsilon \Delta G_r}$$

$$\Delta G_r = \Delta G_{Re} = -43.61 \frac{KJ}{e^-eq}$$

$$\Delta G_p = \Delta G_{pyruvate} - \Delta G_{energy-source} = (35.09 + 35.11) \frac{KJ}{e^-eq} = 70.2 \frac{KJ}{e^-eq}$$

$$\Delta G_p > 0 \text{ therefore, } n = 1$$

$$\Delta G_{pc} = 18.8 \frac{KJ}{e^-eq}$$

$$\epsilon = 0.6$$

$$A_{heterotroph} = 5.67$$

$$f_s^o = \frac{1}{1+A} = 0.15$$

$$f_e^o = 1 - f_s^o = 0.85$$

$$\text{Correction factor to account for aerobic nitrifier cell decay} = \frac{1+(1-f_d)(b\theta_x)}{1+b\theta_x}$$

Values for heterotrophs were found on page 492 of Rittmann and McCarty and are shown below.

$$f_d = 0.8$$

$$b = 0.06 \frac{1}{day}$$

$\theta_x^{min} = 2.8$  days, chosen from  $\theta_x^{min}$  for ammonium oxidizers at  $10^\circ C$ , as found on page 472 from Rittmann and McCarty.

$$SF = 2$$

$$\theta_x^{design} = 5.6 \text{ days}$$

$$correction = 0.799$$

$$f_s = correction * f_s^o = 0.12$$

$$f_e = 1 - f_s = 0.88$$

### 3.4 Total reaction equation, $R$

Only taking into account  $O_2$ ,  $NH_4^+$ ,  $NO_3^-$  and  $C_5H_7O_2N$ :

$$f_s R_s : 0.021NH_4^+ = 0.006C_5H_7O_2N + 0.015NO_3^-$$

$$f_e R_e : 0.11NH_4^+ + 0.22O_2 = 0.11NO_3^-$$

$$R : 0.131NH_4^+ + 0.22O_2 = 0.006C_5H_7O_2N + 0.125NO_3^-$$

From this equation for autotrophic nitrifier metabolism and cell synthesis,  $0.22 \text{ mol } O_2$  is required for  $1 \text{ e}^-eq$ .

### 3.5 Expected yield

$$Y = \frac{biomass_{mass}}{e^-donor_{mass}}$$

$$0.006C_5H_7O_2N * \frac{113gC_5H_7O_2N}{mol} = 0.678gC_5H_7O_2N \text{ required per } e^-eq$$

$$0.131molNH_4^+ * \frac{18gNH_4^+}{mol} = 2.36gNH_4^+ \text{ required per } e^-eq$$

$$Y = \frac{0.678gC_5H_7O_2N}{2.36gNH_4^+} = 0.28 \frac{gC_5H_7O_2N}{gNH_4^+}$$

## 4 Metabolic Model Analysis

From the simple metabolic framework shown above, it is clear that heterotrophs can outcompete nitrifiers in ways that greatly impact the functioning of a trickling filter. The analysis supports current standards that limit C/N ratios to control heterotroph populations. Key parameters that show differences between the heterotrophs and nitrifiers are summarized in Table 3.

Table 3: Relevant parameters for comparison of heterotrophs and nitrifiers

Parameter	Heterotrophs	Nitrifiers
Energy $\Delta G^o' \left( \frac{KJ}{e^{-eq}} \right)$	-110.52	-43.61
$f_s$ (fraction)	0.41	0.12
$O_2$ Requirements $\left( \frac{mol O_2}{e^{-eq}} \right)$	0.15	0.22
$Y \left( \frac{biomass_{mass}}{e^{-donor_{mass}}} \right)$	0.58	0.28

Theoretical parameters were used from the literature to determine the values in Table 3. As shown, the more negative  $\Delta G$  for the heterotroph means that oxygen is more likely to be used for heterotrophic metabolism than the nitrifier metabolism. The greater yield for heterotrophs is a function of a higher fraction of the electrons being used on cell synthesis. This explains the ability for heterotrophs to outcompete nitrifiers by outgrowing them. Heterotrophs typically grow near the biofilm surface while nitrifiers grow near the biofilm base because heterotrophs are able to survive on the high shear surface by growing and replacing themselves fast enough. Theoretical metabolic analysis dictates oxygen levels in the biofilm that would be needed to serve both heterotrophs and nitrifiers, but does not take into account the observed spatial “layering” of the bacteria.

Within the biofilm, heterotrophs and nitrifiers compete for oxygen. Metabolic models are limited by simply demonstrating the stoichiometric need for  $O_2$  and the respective growth rates. However, in practice, trickling filters designed to complete nitrification should be BOD limited to ensure that heterotrophs do not take over. Kinetic models could be used to model growth rates with  $O_2$  as the limiting substrate. Direct metabolism models can be employed for both bacteria, giving:

$$\mu_{heterotroph} = \hat{\mu} \frac{BOD}{K_{BOD} + BOD} \frac{O_2}{K_{O_2} + O_2} - b$$

$$\mu_{nitrifier} = \hat{\mu} \frac{NH_4^+}{K_{NH_4^+} + NH_4^+} \frac{O_2}{K_{O_2} + O_2} - b$$

A dual substrate model could be used to determine the appropriate  $K$  values for  $K_{BOD}$ ,  $K_{NH_4^+}$ , and  $K_{O_2}$ . In Rittmann and McCarty,  $K_{O_2}$  values for nitrifiers are given as  $0.5 \frac{mg O_2}{L}$  while  $K_{O_2}$  for heterotrophs is  $0.4 \frac{mg O_2}{L}$ ; heterotrophs have higher affinity. Different values for  $K_{NH_4^+}$  and  $K_{BOD}$  would show important insight into competition between the two bacteria. Because growth rate is related to cell growth by  $\mu_{synthesis} = \frac{1}{X_a} \frac{dX_a}{dt}$ , changes in biomass density could indicate heterotroph or nitrifier response to different C/N loading ratios. Model work should develop kinetic analyses that work with metabolic models to confirm existing empirical standards.

## Work Plan and Expected Results

Competition between heterotrophs and nitrifiers centers around competition for oxygen and space in a trickling filter biofilm. Proposed research work focuses on two components: 1) to develop a joint metabolic and kinetic model to show the fate of oxygen and cell growth and, 2) to test treatment efficiency at different BOD loadings and determining suitable parameters to update the metabolic and kinetic model.

Because heterotrophs grow faster in higher BOD loadings, important physical and spatial parameters may impact their growth and retention in the trickling filter biofilm. Experimental design should include running trickling filters at steady-state with different BOD loadings. Initially, comparison of experimental with theoretical results should use literature parameter values. However, varying BOD loading should lead to research into optimal design decisions that could lead to natural balance between heterotrophs

and nitrifiers. For example, heterotrophs are more likely to slough off if they grow too much, so decay and sloughing parameters may change dynamically with changing BOD loads. Biofilm composition testing should be done to determine population distribution over time, using tools such as denaturing gradient gel electrophoresis (DGGE). Varying recycle ratios may also impact heterotroph/nitrifier competition because very high recycle ratios may increase velocity high enough to slough off. This would be related to metabolic growth parameters but also support-media characteristics and physical properties of the biofilm adhesion to the support.

In industrialized contexts, where trickling filters are mechanized and monitored, dynamic understanding of fluctuating BOD loads offer an opportunity for trickling filter innovation. Using metabolic and kinetic models to determine oxygen and space needs for nitrifiers to succeed could lead to operational changes that make trickling filters more efficient. Opportunities could include increasing ventilation to the trickling filter to decrease oxygen competition, and to change recycle ratio and bulk fluid velocities to control heterotrophic growth at biofilm surfaces.

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