

Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia–nitrogen in aquaculture systems

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Abstract

In intensive aquaculture systems, ammonia–nitrogen buildup from the metabolism of feed is usually the second limiting factor to increase production levels after dissolved oxygen. The three nitrogen conversion pathways traditionally used for the removal of ammonia–nitrogen in aquaculture systems are photoautotrophic removal by algae, autotrophic bacterial conversion of ammonia–nitrogen to nitrate–nitrogen, and heterotrophic bacterial conversion of ammonia–nitrogen directly to microbial biomass. Traditionally, pond aquaculture has used photoautotrophic algae based systems to control inorganic nitrogen buildup. Currently, the primary strategy in intensive recirculating production systems for controlling ammonia–nitrogen is using large fixed-cell bioreactors. This option utilizes chemosynthetic autotrophic bacteria, Ammonia Oxidizing Bacteria (AOB) and Nitrite Oxidizing Bacteria (NOB), for the nitrification of ammonia–nitrogen to nitrite–nitrogen and finally to nitrate–nitrogen. In the past several years, zero-exchange management systems have been developed that are based on heterotrophic bacteria and have been promoted for the intensive production of marine shrimp. In this third pathway, heterotrophic bacterial growth is stimulated through the addition of organic carbonaceous substrate. At high carbon to nitrogen (C/N) feed ratios, heterotrophic bacteria will assimilate ammonia–nitrogen directly into cellular protein. This paper reviews these three ammonia removal pathways, develops a set of stoichiometric balanced relationships using half-reaction relationships, and discusses their impact on water quality. In addition, microbial growth fundamentals are used to characterize production of volatile and total suspended solids for autotrophic and heterotrophic systems. © 2006 Elsevier B.V. All rights reserved.

Keywords: Zero-exchange systems; Autotrophic system; Heterotrophic system; C/N ratio

1. Introduction

Aquaculture can be defined as the cultivation of aquatic products under controlled conditions, where the major goal is to produce a saleable product as efficiently

and cost effectively as possible. This usually implies that the system uses the highest stocking density possible, highest quality feeds and active water quality management. In these systems, high levels of ammonia–nitrogen are excretion due to the high protein content of the feed and high production densities, often exceeding 120 kg/m³. Since even low levels of ammonia can be toxic to most cultured animals (Timmons et al., 2002),

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the aquaculturalist needs to provide mechanisms to enhance the removal of ammonia to maintain an acceptable concentration. This also holds true for many other water quality parameters, particularly high concentrations of nitrite, carbon dioxide, and suspended solids or organic loading. The organic carbon loading on the system is particularly important, because it relates to the biochemical oxygen demand (BOD) on the system and whether the water body will require supplemental aeration as this BOD is exerted.

Aquaculture production systems are often classified into three general types: extensive ponds, intensive ponds, and intensive recirculating tank and raceway systems. In both extensive and intensive pond systems, ammonia production is controlled through oxidation to nitrate by a combination of autotrophic processes, driven by nitrifying bacteria and photoautotrophic processes that assimilate ammonia directly into algal biomass (Brune et al., 2004). For example, extensive pond marine shrimp production systems are often very large and with low biomass loading, on the order of 0.5 kg/m^3 . As a result of this low biomass, there is generally no active manipulation of the water quality, other than to provide supplemental aeration during times of high oxygen demand due to algae respiration in early morning hours. Recently to improve economics, marine shrimp biomass loading in ponds has been intensified to as high as 2 to 3 kg/m^3 by providing active mixing of the water column, removal of accumulated sludge, use of high quality formulated feeds, continuous supplemental aeration (McIntosh, 2001) and the development of the partitioned production system (Brune et al., 2004).

Intensive recirculating systems are operated at very low water exchange rates (2% to 10% per day). The inorganic ammonia–nitrogen buildup in these intensive production systems has been traditionally controlled by using large fixed-cell bioreactors that rely on the nitrification of ammonia–nitrogen to nitrate–nitrogen by Ammonia Oxidizing Bacteria (AOB) and Nitrite Oxidizing Bacteria (NOB), autotrophic bacteria. The growth of heterotrophic bacteria and the accumulation of organic carbon are minimized through the rapid removal of solids from the system and by using some level of water exchange (Timmons et al., 2002).

Recently, a new production strategy has emerged called intensive zero exchange systems. In these systems, the ammonia buildup is controlled by the manipulation of the carbon/nitrogen ratio in such a way as to promote the growth of heterotrophic bacteria (Avinimelech, 1999; McIntosh, 1999, 2001). As a result, the ammonia–nitrogen is removed from the system through assimilation into microbial biomass. As a bonus, for some aquaculture

species (marine shrimp and tilapia), this bacterial biomass produced in the intensive zero-exchange systems can be an important source of feed protein, reducing the cost of production and thus improving the overall economics (McIntosh, 1999; Moss, 2002).

In the last few years, research demonstrating low water exchange marine shrimp production systems has been conducted (Ebeling and LaFranchi, 1990; Santos and Ebeling, 1990), and zero-exchange management systems have been developed for large-scale pond production of marine shrimp traditionally photoautotrophic algae based (Hopkins et al., 1996; Avinimelech et al., 1994), and where organic labile carbonaceous substrate is added to the systems to support microbial metabolism (Avinimelech, 1999; McIntosh, 1999). At high organic carbon to nitrogen (C/N) ratios, bacteria will assimilate nitrogen, i.e., ammonia, from the water and produce cell protein. Several attempts have been made to develop the technology for indoor production systems at high densities (Weirich et al., 2002; Otoshi et al., 2003; Davis and Arnold, 1998; Van Wyk, 1999), although it should be noted that in addition to algae and bacterial biomass, each of these incorporated some additional form of fixed-film biofiltration.

In reviewing the literature on zero-exchange systems, there appears to be a limited understanding as to the type of ammonia removal system being employed and whether it is photoautotrophic, autotrophic bacterial or heterotrophic bacterial based, or in reality some mixture of the three. In order to optimize water quality and effectively manage an aquaculture system, it is important to understand what type and the impact on water quality of ammonia removal system. This paper reviews these three ammonia removal pathways, develops a set of stoichiometric balanced relationships using half-reaction relationships, and discusses their impact on water quality. In addition, microbial growth fundamentals are used to characterize production of volatile and total suspended solids for autotrophic and heterotrophic systems.

2. Ammonia–nitrogen production

Ammonia is produced as a major end product of the metabolism of protein catabolism and is excreted as unionized ammonia across the gills of aquatic organisms. Ammonia, nitrite, and nitrate are all highly soluble in water. In water, ammonia exists in two forms: un-ionized ammonia, NH_3 , and ionized ammonium, NH_4^+ . The relative concentration of each of these forms is primarily a function of pH, temperature, and salinity (Anthonisen et al., 1976). The sum of the two ($\text{NH}_4^+ + \text{NH}_3$) is usually referred to as total ammonia–nitrogen (TAN) or

simply ammonia. It is common in aquatic chemistry to express inorganic nitrogen compounds in terms of the nitrogen they contain, i.e., $\text{NH}_4^+\text{-N}$ (ionized ammonia–nitrogen), $\text{NH}_3\text{-N}$ (un-ionized ammonia–nitrogen), $\text{NO}_2\text{-N}$ (nitrite–nitrogen), and $\text{NO}_3\text{-N}$ (nitrate–nitrogen). This allows for easier computation of total ammonia–nitrogen ($\text{TAN}=\text{NH}_4^+\text{-N}+\text{NH}_3\text{-N}$) and a mass balances between the various stages of nitrification.

An estimate of ammonia–nitrogen generated per day in an aquaculture production system can be calculated based upon the feeding rate (Timmons et al., 2002):

$$P_{\text{TAN}} = F \cdot \text{PC} \cdot 0.092 \quad (1)$$

where:

P_{TAN}	Production rate of total ammonia nitrogen, (kg/day)
F	Feed rate (kg/day)
PC	Protein concentration in feed (decimal value)

The constant in the ammonia generation equation assumes that protein is 16% nitrogen, 80% nitrogen is assimilated by the organism, 80% assimilated nitrogen is excreted, and 90% of nitrogen excreted as TAN+10% as urea. In addition, the nitrogen in feces and uneaten feed is removed quickly by sedimentation or filtration and the sludge removed from the system.

For heterotrophic bacterial based zero-exchange production systems, this formula needs to be modified to reflect that solids are not removed from the system and there is no traditional fixed-film biofilter. Thus all of the nitrogen excreted, both TAN and urea is available to the bacterial community. In addition for the example used in this paper, research data suggests that 90% of the nitrogen assimilated by marine shrimp is excreted as TAN and urea. Thus for marine shrimp:

$$P_{\text{TAN}} = F \cdot \text{PC} \cdot 0.144 \quad (2)$$

What follows is a review of these three nitrogen conversion pathways — photoautotrophic (algal based), autotrophic and heterotrophic bacteria and their implications on water quality parameters, most importantly ammonia–nitrogen, nitrite–nitrogen, carbon dioxide, and alkalinity.

3. Photoautotrophic (algal based systems)

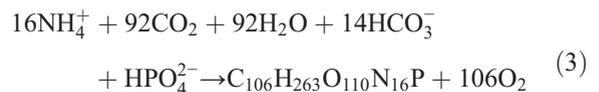
3.1. Background — photoautotrophic systems

Conventional aquaculture ponds rely on the use of algal biosynthesis for the removal of the majority of inorganic nitrogen. The major disadvantage of algal

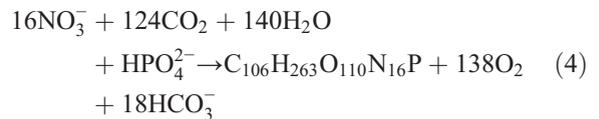
based systems are the wide diurnal variations in dissolved oxygen, pH and ammonia–nitrogen and the long term changes in algal density and frequent ‘die-offs’ (Burford et al., 2003). Unmanaged algal populations in conventional ponds typically can fix 2–3 g carbon/m² day. High rate mixed ponds that are well managed can yield higher rates, 10–12 g carbon/m² day (Brune et al., 2003).

3.2. Stoichiometry — photoautotrophic systems

The biosynthesis of saltwater algae can be described in general by the following stoichiometric relationships (Stumm and Morgan, 1996) for ammonia as the nitrogen source:



Or, for nitrate as the nitrogen source:



where $\text{C}_{106}\text{H}_{263}\text{O}_{110}\text{N}_{16}\text{P}$ represents the stoichiometric formula for seawater algae.

Note that 3.13 g of alkalinity (as CaCO_3) is consumed for every g of ammonia–nitrogen consumed in the first relationship and 4.02 g of alkalinity (as CaCO_3) is produced for every g of nitrate–nitrogen consumed in the second. Using these stoichiometric relationships, for every g of ammonia–nitrogen converted to algal biomass, 18.07 g of carbon dioxide is consumed and for every g of nitrate–nitrogen used 24.4 g of carbon dioxide. Correspondingly, 15.14 and 19.71 g of O_2 are produced respectively per gram of ammonia–nitrogen and per gram of nitrate–nitrogen. Finally, a significant quantity of algal biomass, 15.85 g is generated per gram of either ammonia or nitrate nitrogen. Table 1 summarizes the stoichiometry, including the consumption and production of inorganic and organic carbon.

4. Autotrophic bacteria — nitrification

4.1. Background — autotrophic bacteria

There are two phylogenetically distinct groups of bacteria that collectively perform nitrification. These two groups of bacteria are generally categorized as chemosynthetic autotrophic bacteria because they derive their energy from inorganic compounds as opposed to heterotrophic bacteria that derive energy from organic

Table 1
Stoichiometry for photoautotrophic algal metabolism of 1.0 g NH₄⁺-N

Consumables	Stoichiometry	Consumes (g)	C _{organic} (g)	C _{inorganic} (g)	N (g)
NH ₄ ⁺ -N		1.0	–	–	1.0
Carbon dioxide	18.07 g CO ₂ /g N	18.07	–	4.93	–
Alkalinity	3.13 g Alk/g N	3.13	–	0.75	–
Products	Stoichiometry	Yields (g)	C _{organic} (g)	C _{inorganic} (g)	N (g)
VSS _{Algae}	15.85 g VSS _A /g N	15.85	5.67	–	1.0
O ₂	15.14 g O ₂ /g N	15.14	–	–	–

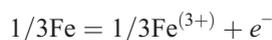
compounds (Hagopian and Riley, 1998). Ammonia Oxidizing Bacteria (AOB) obtain their energy by catabolizing un-ionized ammonia to nitrite and include bacteria of the genera *Nitrosomonas*, *Nitrosococcus*, *Nitrospira*, *Nitrosolobus*, and *Nitrosovibrio*. Nitrite Oxidizing Bacteria (NOB) oxidize nitrite to nitrate, and include bacteria of the genera *Nitrobacter*, *Nitrococcus*, *Nitrospira*, and *Nitrospina*. Nitrifying bacteria are primarily obligate autotrophs, which consume carbon dioxide as their primary carbon source, and obligate aerobes, which require oxygen to grow (Hagopian and Riley, 1998).

The major factors affecting the rate of nitrification in suspended growth include: ammonia–nitrogen and nitrite–nitrogen concentration, carbon/nitrogen ratio, dissolved oxygen, pH, temperature and alkalinity. The impact of the carbon/nitrogen ratio will be discussed later in the paper. The effects of dissolved oxygen, pH, temperature, and alkalinity are reviewed by Timmons et al. (2002).

4.2. Stoichiometry — autotrophic bacteria

Classical thermodynamic principles can be applied to biological reactions to describe how they utilize energy through oxidation–reduction processes. The electron equivalent approach is used to account for energy and

electron flow since most of the pertinent reactions are redox reactions. An “electron equivalent (eeq)” of a substance equals the amount of that substance that will deliver 1 mol of electrons for a specified reaction. For example:



Therefore 1/3 mol of Fe is 1 eeq of iron.

Redox reactions consist of compounds losing electrons (electron donors), while others gain electrons (electron acceptors). A series of balanced half-reactions (Table 2) can then be written to describe a biological process, such as nitrification, denitrification, or oxidation of carbohydrates. McCarty (1971, 1975) developed a series of half-reactions for a variety of electron donor and electron acceptors which were used to develop the relationships presented here (see Table 3).

When an electron donor is used for both energy and cell synthesis the split between the electron flow is given by:

$$\begin{aligned} f_s & \text{ fraction of electron donor used for synthesis} \\ f_e & \text{ fraction of electron donor used for energy} \\ \text{and } f_e + f_s & = 1 \end{aligned}$$

Table 2
Oxidation half-reactions (McCarty, 1971, 1975)

Rxn	Reactions for bacterial cell synthesis:	$\Delta G^{\circ}(w)$ kcal/eeq
(1)	$1/20\text{C}_5\text{H}_7\text{O}_2\text{N} + 9/20\text{H}_2\text{O} \rightarrow 1/5\text{CO}_2 + 1/20\text{HCO}_3^- + 1/20\text{NH}_4^+ + \text{H}^+ + e^-$	
	Reactions for electron acceptors:	
(3)	$1/2\text{H}_2\text{O} \rightarrow 1/4\text{O}_2 + \text{H}^+ + e^-$	18.675
	Reaction for electron donors:	
(9)	$1/24\text{C}_6\text{H}_{12}\text{O}_6 + 1/4\text{H}_2\text{O} \rightarrow 1/4\text{CO}_2 + \text{H}^+ + e^-$	–10.0
(19)	$1/8\text{NH}_4^+ + 3/8\text{H}_2\text{O} \rightarrow 1/8\text{NO}_3^- + 5/4\text{H}^+ + e^-$	8.245
(16)	$1/10\text{CH}_3\text{COCOO}^- + 2/5\text{H}_2\text{O} \rightarrow 1/5\text{CO}_2 + 1/10\text{HCO}_3^- + \text{H}^+ + e^-$	–8.545

Where C₅H₇O₂N represents the chemical formula for microbial biomass, and Rxn corresponds to McCarty nomenclature.

Table 3
Stoichiometry for autotrophic bacteria metabolism of 1.0 g NH₄⁺-N

Consumables	Stoichiometry	Consumes (g)	C _{organic} (g)	C _{inorganic} (g)	N (g)
NH ₄ ⁺ -N		1.0	–	–	1.0
Alkalinity	7.05 g Alk/g N	7.05	–	1.69	–
Oxygen	4.18 g O ₂ /g N	4.18	–	–	–
Products	Stoichiometry	Yields (g)	C _{organic} (g)	C _{inorganic} (g)	N (g)
VSS _A	0.20 g VSS _A /g N	0.20	0.106	–	0.025
NO ₃ ⁻ -N	0.976 g NO ₃ ⁻ -N/g N	0.976	–	–	0.976
CO ₂	5.85 g CO ₂ /g N	5.85	–	1.59	–

A free energy balance can then be used to estimate the values for f_c and f_s . Let ΔG_r = free energy released per eq of electron donor converted for energy (respiration). Next combine a donor reaction with an acceptor reaction to balance electrons. For example, Rxn (19)–Rxn (3) from Table 2 can be used, i.e., ammonia–nitrogen being oxidized to NO₃⁻ with O₂ as the acceptor.

Let ΔG_s = ATP energy required to synthesize 1 eq of cells from whatever the carbon and nitrogen sources are. Then let A = eq of electron donor converted to energy per eq of cells synthesized, ignoring endogenous cell decay or maintenance energy.

At steady-state, bacteria must replace ATP at the same rate they are using ATP. Therefore:

$$\varepsilon \cdot A \cdot \Delta G_r + \Delta G_s = 0 \quad (9)$$

where

$$A = \frac{-\Delta G_s}{\varepsilon \cdot \Delta G_r} \quad (10)$$

' A ' is eq of electron donor used for energy per eq of cells synthesized and let ε = efficiency of energy transfer to or from ATP. Rittmann and McCarty (2001) suggest $\varepsilon = 0.6$. McCarty (1972) uses pyruvate as an intermediate in the synthesis process, i.e., organic compounds are first converted to pyruvate then to cell matter through some metabolic pathway.

So the energy process occurs in two steps.

Step 1: ΔG_p = free energy required (or evolved) in conversion of the carbon source to pyruvate (kcal/eq pyruvate). Contribution to the ATP pool will be either:

$$\varepsilon \Delta G_p \text{ (if } \Delta G_p < 0) \text{ or } \Delta G_p / \varepsilon \text{ (if } \Delta G_p > 0)$$

Step 2: Conversion of pyruvate and NH₄⁺ to 1 eq biomass (1/20 C₅H₇O₂N). ΔG_C is the energy required

for this conversion. Through laboratory measurements, $\Delta G_C = 7.5$ kcal.

Putting steps 1 and 2 together gives:

$$\Delta G_s = \frac{\Delta G_p}{\varepsilon^m} + \Delta G_C$$

where:

$$m = +1 \text{ if } \Delta G_p > 0$$

$$m = -1 \text{ if } \Delta G_p < 0$$

$$\text{Overall: } A = \frac{-\Delta G_p - \Delta G_C}{\varepsilon \cdot \Delta G_r} \text{ and } f_s = \frac{1}{1+A} \quad f_e = \frac{A}{1+A}$$

If volatile suspended solids (VSS) are used as a measure of bacterial biomass, then some of the VSS produced and then endogenously decayed is non-degradable. According to Rittmann and McCarty (2001), 20% of the endogenously decayed VSS is non-degradable. They showed that:

$$f_s = \frac{1}{1+A} \cdot \left(\frac{(1 + 0.2 \cdot b \cdot \frac{1}{\mu})}{1 + b \cdot \frac{1}{\mu}} \right)$$

where b is the endogenous decay coefficient and μ is the net specific growth rate.

Using the estimated values for f_c and f_s , the half-reactions can then be combined to describe biological processes according to the following relationship (McCarty, 1971, 1975):

$$R = R_d - f_s \cdot R_c - f_e \cdot R_a \quad (11)$$

where:

R	overall balanced reaction
R_d	half-reaction for the electron donor
R_c	half-reaction for the synthesis of microbial biomass
R_a	half-reaction for the electron acceptor

Solving the half-reaction relationships for nitrification of ammonia–nitrogen to nitrate–nitrogen consists of combining the reaction for bacterial cell synthesis Rxn (1) with the reaction for the electron acceptor Rxn (3) and the electron donor Rxn (19), Table 4.

$$\Delta G_r = \text{Rxn (19)} - \text{Rxn (3)} = 8.245 - 18.675 = -10.412 \text{ kcal/eq} \quad (12)$$

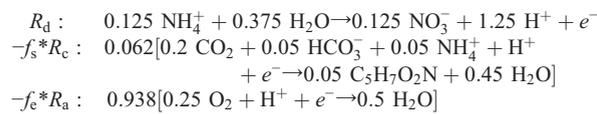
Then solving for A:

$$A = -59.7 / 0.6(-10.43) = 9.540$$

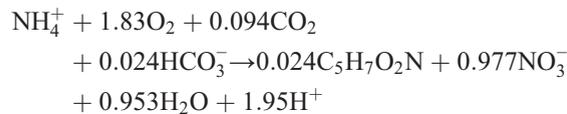
Using the values for endogenous decay coefficient and net specific growth rates for autotrophic bacteria, 0.2 and 15 day⁻¹, yields values for f_s of 0.062 and f_e of 0.938. These reflect the fact that very little energy is used for cell synthesis in the nitrification process, i.e. f_s .

Thus:

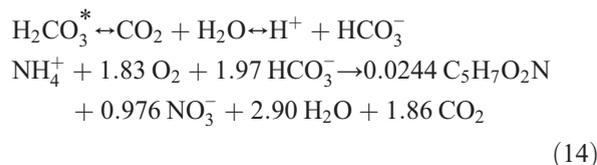
$$R = R_d - 0.062 * R_s - 0.938 * R_a \quad (13)$$



Yields:



Adding the carbonate equilibrium relationship yields:



f_e is a dimensionless cell yield parameter but it can easily be converted to more common yield coefficient with

units of mass of cells produced per mass of substrate (electron donor) used. For example, for autotrophic growth cell mass can be represented as C₅H₇O₂N (MW=113 g) and the electron donor is N₄⁺-N (MW=14 g). From the reactions shown in Table 2 it can be seen that 1/8 mol of N₄⁺-N will yield 1 mol of electrons and 1/20 mol of cell mass can be produced by 1 mol of electrons. So if one prefers to use yield coefficient (Y_a) based on mass f_e can be multiplied by $\frac{\frac{1}{20} \text{ mol C}_5\text{H}_7\text{O}_2\text{N} \cdot 113 \text{ g/mol}}{\frac{1}{8} \text{ mol NH}_4^+-\text{N} \cdot 14 \text{ g/mol}} = 3.23$. Thus if $f_e = 0.062$, $Y_a = 0.2$. This is well within the range of Y_a values reported in Tables 8–11 of Wastewater Engineering (Metcalf and Eddy Inc., 1991).

Using this stoichiometric relationship (Eq. (14)), for every g of ammonia–nitrogen converted to nitrate–nitrogen, 4.18 g of dissolved oxygen, and 7.05 g of alkalinity (1.69 g inorganic carbon) are consumed and 0.20 g of microbial biomass (0.105 g organic carbon) and 5.85 g of CO₂, (1.59 g inorganic carbon) are produced. It should be noted that both the consumption of oxygen and alkalinity is less than that which normally reported, 4.57 g of O₂ and 7.14 g of alkalinity for every g of ammonia–nitrogen converted (Timmons et al., 2002), because in this equation some of the ammonia–nitrogen is converted to biomass. Traditionally, this biomass has not been included in the stoichiometric relationship because it is minor in comparison to the other factors. Table 3 summarizes the stoichiometry for metabolism of 1 g of ammonia–nitrogen by autotrophic bacterial, including the consumption and production of organic and inorganic carbon.

4.3. Autotrophic bacteria — impact on water quality

In the autotrophic nitrification process as opposed to heterotrophic processes, very small amounts of bacterial biomass are produced. And because of the relatively slow maximum growth rate for the nitrifiers in a suspended-growth process, it becomes very easy to ‘wash-

Table 4
Stoichiometry for heterotrophic bacteria metabolism of 1.0 g NH₄⁺-N with carbohydrate as supplemental carbon

Consumables	Stoichiometry	Consumes (g)	C _{organic} (g)	C _{inorganic} (g)	N (g)
NH ₄ ⁺ -N		1.0	–	–	1.0
C ₆ H ₁₂ O ₆	15.17 g Carbs/g N	15.17	6.07	–	–
Alkalinity	3.57 g Alk/g N	3.57	–	0.86	–
Oxygen	4.71 g O ₂ /g N	4.71	–	–	–
Products	Stoichiometry	Yields (g)	C _{organic} (g)	C _{inorganic} (g)	N (g)
VSS _H	8.07 g VSS _H /g N	8.07	4.29	–	1.0
CO ₂	9.65 g CO ₂ /g N	9.65	–	2.63	–

out' the nitrifying bacteria as opposed to a fixed-film system. This is particularly true if there is no sludge recycling that returns the bacteria back into the culture system. Also there is a significant amount of alkalinity consumed (7.05 g (as CaCO₃)/g N) and high levels of carbon dioxide produced (5.85 g CO₂/g TAN). For water with low initial alkalinity this can be a significant problem, requiring the addition of alkalinity, in the form of sodium bicarbonate, lime, sodium hydroxide, to maintain an adequate concentration (100 to 150 mg/L as CaCO₃), especially for systems with limited water exchange. If alkalinity consumption is not compensated for by supplementation, the system pH will drop. Lowering pH will result in an inorganic carbon species shift from bicarbonate to dissolved carbon dioxide, and this increase in dissolved carbon dioxide could affect some aquaculture species. Although CO₂ concentration can be controlled with gas stripping towers, significant energy is required for pumping both the water and air through these systems. The end product of the reaction is nitrate–nitrogen, which is not normally toxic at moderate levels in aquaculture production systems, e.g., several hundred mg/L.

4.4. Autotrophic bacteria — impact of C/N ratio

The ratio of the biodegradable organic carbon to the nitrogen available for nitrification is argued to be one of the critical factors affecting the design and operation of a nitrification system (U.S. EPA, 1993). Heterotrophic bacteria have a maximum growth rate significantly higher than nitrifiers, 5 day⁻¹ compared to 1 day⁻¹ (U.S. EPA, 1993), thus in systems with even relatively modest C/N ratios, the heterotrophs are capable of out performing and significantly inhibiting nitrification. Zhu and Chen (2001) demonstrated the effect of sucrose on the nitrification rate of biofilters under steady-state conditions. They determined that at carbon/nitrogen ratios from 1.0 to 2.0, there was a 70% reduction of total ammonia–nitrogen removal rate as compared to C/N=0. The data suggested that the nitrification rate decreased with an increase in the organic concentration, but the impact became less pronounced when the carbon concentration became sufficiently high.

Additionally in suspended-growth process with high C/N ratios, the increased production of heterotrophic bacteria requires that they be removed from the production system, i.e., using clarifiers. Since the yield of heterotrophic bacteria is greater than the yield of autotrophic nitrifying bacteria there is the potential, when attempting to control the TSS levels in the production system, that the nitrifiers will be washed out of the system.

5. Heterotrophic bacteria

5.1. Background — heterotrophic bacteria

The major factors that affect the rate of nitrification also play a dominant role in heterotrophic bacterial growth. These include: pH, alkalinity, temperature, oxygen, ammonia, and salinity (Timmons et al., 2002).

5.2. Stoichiometry — aerobic heterotrophic bacteria

Classical thermodynamics and the half-reaction relationships used above to describe the nitrification of ammonia–nitrogen to nitrate–nitrogen can also be used to describe the removal of ammonia–nitrogen by incorporation into cell biomass (Table 3). This process can be enhanced by the addition of carbon in the form of glucose, sucrose or any other form of carbohydrate. The same reaction for the bacterial cell synthesis is used; Rxn (1), along with the same reaction for the electron acceptor, Rxn (3), and in this case the electron donor becomes sucrose or any carbohydrate, Rxn (16).

Consider the case where the electron donor is sucrose, the electron acceptor is O₂ and the nitrogen source is NH₄⁺. The energetic calculations yield:

$$\begin{aligned}\Delta G_r &= \text{Rxn (9)} - \text{Rxn (3)} = -10.0 - (18.675) \\ &= -28.675\end{aligned}$$

$$\begin{aligned}\Delta G_p &= \text{Rxn (9)} - \text{Rxn (16)} = -10.0 - (-8.545) \\ &= -1.455 (m = -1)\end{aligned}$$

$$\Delta G_n = 0$$

$$A = \frac{1.455 \cdot 0.6 - 7.5}{0.6 \cdot (-28.675)} = 0.385$$

As previously stated, if volatile suspended solids (VSS) are used as a measure of bacterial biomass, then some of the VSS produced and then endogenously decayed is non-degradable. According to Rittmann and McCarty (2001) 20% of the endogenously decayed VSS is non-degradable. They showed that:

$$f_s = \frac{1}{1 + A} \cdot \left(\frac{(1 + 0.2 \cdot b \cdot \frac{1}{\mu})}{1 + b \cdot \frac{1}{\mu}} \right)$$

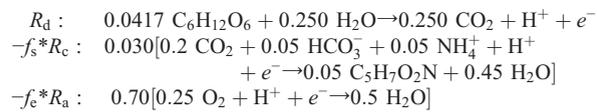
where b is the endogenous decay coefficient and μ is the net specific growth rate.

Using the values for endogenous decay coefficient and net specific growth rates for the heterotrophic

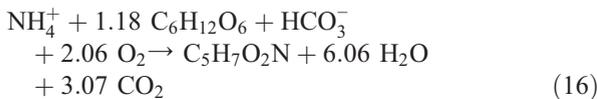
bacteria of interest in zero-exchange systems, 0.2 and 1 day^{-1} , yields values for f_s of 0.70 and f_e of 0.30. In contrast to the nitrification reaction, a majority of the energy is used for cell synthesis, although this number is very dependent on the microbial organism being considered and the substrate being used.

$$R = -R_d - 0.70 * R_s - 0.30 * R_a \quad (15)$$

The following three equations are developed in the same manner as for the autotrophic relationships.



Yields the following equation:



This equation predicts that for every g of ammonia–nitrogen converted to microbial biomass, 4.71 g of dissolved oxygen and 3.57 g of alkalinity (0.86 g inorganic carbon) and 15.17 g carbohydrates (6.07 g organic carbon) are consumed. Also 8.07 g of microbial biomass (4.29 g organic carbon) and 9.65 g of CO_2 (2.63 g inorganic carbon) are produced. Note the oxygen demand is slightly higher, the alkalinity requirement about half and the CO_2 production almost 75% greater than the corresponding reaction for nitrification. Most importantly, the increase in microbial biomass production is 40 times greater than the biomass generated from the nitrification process; 8.07 versus 0.20 g. Table 4 summarizes the stoichiometry for the heterotrophic pathways for ammonia–nitrogen conversion.

5.3. Heterotrophic bacteria — impact on water quality

Several aspects are important in the overall heterotrophic bacterial reaction. Paramount is the extremely large amount of bacterial biomass produced by this reaction, compared to the autotrophic reaction. Thus some form of solids management to remove excess TSS is required. A second issue is the modest amount of alkalinity consumed as the carbon source (3.57 g/g TAN) and the resulting high levels of carbon dioxide produced (9.65 g/g TAN). For water with low initial alkalinity, this will generally still require the addition of carbonate, usually in the form of sodium bicarbonate to maintain reasonable alkalinity (100 to 150 mg/L as CaCO_3), es-

pecially for systems with limited water exchange. As a result, zero-exchange production systems that rely on suspended or attached heterotrophic bacteria usually show a modest decrease in alkalinity, large suspended solids production, and high CO_2 levels. Finally, there should be no production of nitrite–nitrogen, or nitrate–nitrogen in a pure heterotrophic system.

6. Conversion of 1 kg of feed at 35% protein

Relating this analysis to the production of marine shrimp in a zero-exchange system, it can be assumed that for every kg of feed at 35% protein, approximately 50.4 g of ammonia–nitrogen will be generated, Eq. (2). Several different nitrogen pathways are available for the system. These are dependent upon the availability of carbon and it forms, either as inorganic carbon as alkalinity or organic carbon from the feed and fecal matter or as supplemental carbohydrates. Thus for a recirculating system where all of the solids containing organic carbon are rapidly removed from the system, the system would be primarily autotrophic, utilizing inorganic carbon from the alkalinity as its carbon source. For a zero-exchange system, the solids remain in the production tank and all of the carbon and nitrogen from the feed and fecal matter are available for heterotrophic bacterial production. In this case, because there is insufficient organic carbon to completely convert the nitrogen to heterotrophic bacterial biomass, some limited autotrophic conversion occurs, which utilizes inorganic carbon from the alkalinity. If however sufficient supplemental organic carbon is added, as for example carbohydrates, then all of the nitrogen is converted to bacterial biomass via heterotrophic bacteria.

For a pure autotrophic nitrification process (Table 5) the mass of microbial biomass generated as VSS can be calculated from the ammonia–nitrogen production rate and the VSS yield, approximately 10.1 g VSS/kg of feed. Since bacterial biomass (VSS) contains 53.1% C and 12.3% N (based on stoichiometry), this translates into 5.35 g of organic carbon and only 1.25 g of nitrogen sequestered in the microbial biomass. It is interesting to note, that only about 6.2% of the carbon available is actually contained in the microbial biomass (5.35 g), and most of the carbon is released as carbon dioxide (295 g). In addition, only 2.5% of the nitrogen is sequestered in the bacterial biomass, again the majority of the nitrogen is converted to nitrate–nitrogen (49.2 g $\text{NO}_3\text{-N}$). The source of the inorganic carbon required by the autotrophic bacteria is from the consumption of 355 g of alkalinity as CaCO_3 . The C/N ratio for optimal conversion by autotrophic systems works out to be 1.69 g

Table 5

Stoichiometry for autotrophic bacteria metabolism of 1.0 kg feed at 35% protein, with no supplemental carbon and 50.4 g NH₄⁺-N ammonia–nitrogen

Consumables	Stoichiometry	Consumes (g)	C _{organic} (g)	C _{inorganic} (g)	N (g)
NH ₄ ⁺ -N		50.4	–	–	50.4
Alkalinity	7.05 g Alk/g N	355.3	–	85.2	–
Oxygen	4.18 g O ₂ /g N	210.7	–	–	–
Products	Stoichiometry	Yields (g)	C _{organic} (g)	C _{inorganic} (g)	N (g)
VSS _A	0.20 g VSS _A /g N	10.1	5.35	–	1.25
NO ₃ ⁻ -N	0.976 g NO ₃ ⁻ -N/g N	0.976	–	–	49.2
CO ₂	5.85 g CO ₂ /g N	294.8	–	80.1	–

inorganic carbon/g nitrogen, compared to the microbial biomass C/N ratio of 4.28 g organic carbon/g nitrogen.

In a pure zero-exchange system (Table 6), all of the solids remain in the production tank and all of the organic carbon and nitrogen from the feed and fecal matter is available for heterotrophic bacterial production. Since the energetics of heterotrophic bacteria is more favorable than those for autotrophic bacteria, it will be assumed that the heterotrophic bacteria will first consume the

available nitrogen using the readily available, labile carbon from the feed and fecal matter. The available organic carbon from feed and fecal matter is difficult to estimate due the wide variation in feed formulations, species assimilation rates, rate of nutrient leaching from the feed particles and numerous other difficulties. Thus as an approximation, we can use literature data to estimate that feeds exert 0.30 to 0.36 kg BOD/kg of feed (Zhu and Chen, 2001; Brune et al., 2003). Using a

Table 6

Stoichiometry for mixed-system of autotrophic and heterotrophic bacteria metabolism of 1.0 kg feed at 35% protein, with no supplemental carbon, and 50.4 g NH₄⁺-N

Consumables	Stoichiometry	Consumes (g)	C _{organic} (g)	C _{inorganic} (g)	N (g)
<i>Heterotrophic bacteria</i>					
NH ₄ ⁺ -N	0.356 * N _T	17.9	–	–	17.9
C ₆ H ₁₂ O ₆ feed	15.17 g Carbs/g N	272	108.9	–	–
Alkalinity	3.57 g Alk/g N	63.9	–	15.4	–
<i>Autotrophic bacteria</i>					
NH ₄ ⁺ -N	0.644 * N _T	32.5	–	–	32.5
Alkalinity	7.05 g Alk/g N	229.1	–	55.4	–
Total consumables	Consumes		C _{organic} (g)	C _{inorganic} (g)	N (g)
NH ₄ ⁺ -N	50.4 g N	–	–	–	50.4
C ₆ H ₁₂ O ₆	272 g Carbs	108.9	–	–	–
Alkalinity	293 g Alk	–	–	70.8	–
Products	Stoichiometry	Yields (g)	C _{organic} (g)	C _{inorganic} (g)	N (g)
<i>Heterotrophic bacteria</i>					
VSS _H	8.07 g VSS _H /g N	144	76.5	–	17.9
CO ₂	9.65 g CO ₂ /g N	173.9	–	47.4	–
<i>Autotrophic bacteria</i>					
VSS _A	0.20 g VSS _A /g N	6.5	3.45	–	0.81
NO ₃ ⁻ -N	0.976 g NO ₃ ⁻ -N/g N	31.7	–	–	31.7
CO ₂	5.85 g CO ₂ /g N	189.5	–	51.7	–
Total products	Yields		C _{organic} (g)	C _{inorganic} (g)	N (g)
VSS	150.5 g VSS	80.0	–	–	18.7
NO ₃ ⁻ -N	31.7 g NO ₃ ⁻ -N	–	–	–	31.7
CO ₂	363.4 g CO ₂	–	–	99.1	–

Table 7

Stoichiometry for heterotrophic bacteria metabolism of 1.0 kg feed at 35% protein, with supplemental carbon as a carbohydrate, and 50.4 g NH₄⁺-N

Consumables	Stoichiometry	Consumes (g)	C _{organic} (g)	C _{inorganic} (g)	N (g)
NH ₄ ⁺ -N		50.4	–	–	50.4
C ₆ H ₁₂ O ₆	15.17 g Carbs/g N	764.6	305.9	–	–
Alkalinity	3.57 g Alk/g N	179.9	–	43.3	–
Oxygen	4.71 g O ₂ /g N	237.4	–	–	–
Products	Stoichiometry	Yields (g)	C _{organic} (g)	C _{inorganic} (g)	N (g)
VSS _H	8.07 g VSS _H /g N	406.7	216	–	50.4
CO ₂	9.65 g CO ₂ /g N	486.4	–	132.6	–

conservative yield fraction of 0.40 kg VSS/kg BOD (Brune et al., 2003), and a BOD content of 0.36 kg/kg feed, suggests that a kg of feed would generate approximately 144 g of heterotrophic VSS. Again since bacterial biomass (VSS) contains 53.1% C and 12.3% N, this translates into 76.5 g of organic carbon and 17.9 g of nitrogen sequestered in the heterotrophic microbial biomass. In addition to the organic carbon from the feed and fecal matter (109.4 g), 15.4 g of inorganic carbon are required; this is obtained from the consumption of 64.0 g of alkalinity as CaCO₃.

Since there is 50.4 g of nitrogen available from the feed, and only 17.9 g of nitrogen is sequestered by the heterotrophic bacteria, there remains 32.5 g of nitrogen to be assimilated by the autotrophic bacteria. Again using 0.20 g VSS/g of nitrogen, yields a production of 6.5 g of autotrophic bacteria VSS. Since bacterial biomass (VSS) contains 53.1% C and 12.3% N, this translates into 3.45 g of organic carbon and only 0.81 g of nitrogen sequestered in the autotrophic microbial biomass. Thus, only a small fraction of the nitrogen is sequestered by the autotrophic bacteria, most of the

nitrogen is contained in the nitrate–nitrogen (31.7 g) and most of the carbon is released as carbon dioxide (51.7 g). The source of the inorganic carbon (55.4 g) required by the autotrophic bacteria is the consumption of 288.3 g of alkalinity as CaCO₃. Thus two forms of carbon are consumed during this pathway, 108.9 g of organic carbon and 15.4 g of inorganic carbon. The resulting C/N ratio based on the organic carbon is 2.16. Although the exact percentage is dependent upon the protein content of the feed, in this case 35.6% of the nitrogen is removed by the heterotrophic pathway and 64.4% by the autotrophic pathway. Note that only 4.3% of the VSS are from autotrophic bacteria, demonstrating how quickly heterotrophic bacteria will dominate a system with adequate organic carbon. And also how easy it is to ‘wash-out’ autotrophic bacteria during harvesting of excess bacterial biomass, since the autotrophic bacteria growth rate is significantly slower than the heterotrophic bacteria.

Finally consider a zero-exchange system where carbon is added to make up the difference between the available organic carbon from feed (108.9 g) and the

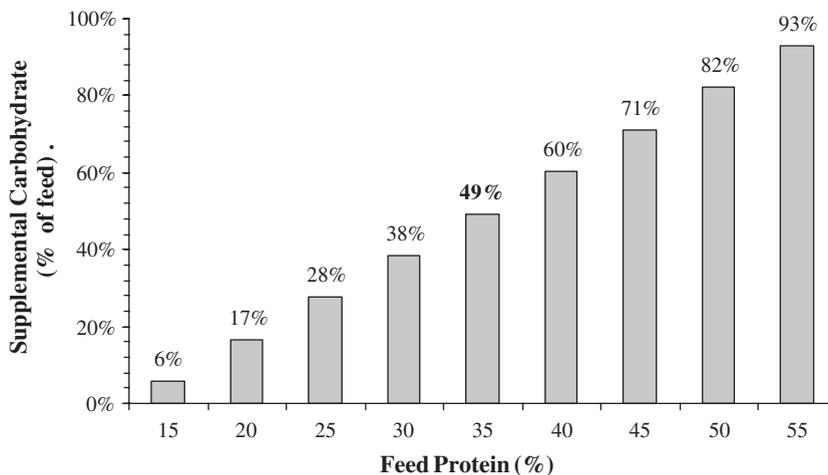


Fig. 1. Supplemental carbohydrate as percentage of feed rate for heterotrophic metabolism of ammonia–nitrogen to microbial biomass.

Table 8

Fraction of nitrifying bacteria and percent of ammonia–nitrogen removed as a function of the BOD₅/TKN ratio (Metcalf and Eddy, 1991) and C/N ratio for aquaculture feeds

BOD ₅ /TKN	Modified BOD ₅ /TKN	g VSS _(A) /g VSS	% Removed by autotrophs	Aqua C/N ratio	g VSS _(A) /g VSS	% Removed by autotrophs
0.50	0.19	0.35	96	0.50	0.151	92
1.00	0.38	0.21	91	0.75	0.126	88
2.00	0.75	0.12	85	1.00	0.105	84
3.00	1.13	0.083	79	1.50	0.072	75
4.00	1.50	0.064	73	2.00	0.050	67
5.00	1.88	0.05	68	2.50	0.035	59
6.00	2.25	0.043	64	3.00	0.024	51
7.00	2.63	0.037	61	3.50	0.017	42
8.00	3.00	0.033	58	4.00	0.012	34
9.00	3.38	0.029	55	5.00	0.006	18
10.00	3.75	0.025	51	6.00	0.003	1

requirements of the heterotrophs (Table 7). From the stoichiometry, 15.17 g of carbohydrates are required per g of N, or 764.9 g of carbohydrates containing 305.9 g of organic carbon. As was shown above, feed provides only 108.9 g of organic carbon, so the remaining 197 g must be made up for with a supplemental carbon source. The carbon available from a generic carbohydrate (C₆H₁₂O₆) is 0.40 g C per g carbohydrate. Thus to add the additional 197 g of carbon, would require approximately 492 g of carbohydrate, or 49% by weight of the feed. Overall, a total of 305.9 g of organic carbon would be required to convert heterotrophically 50.4 g of N. This yields a C/N ratio of 6.07.

The above analysis is for a feed with a protein content of 35%. Additional calculations for other feed protein content are straightforward with additional organic carbon supplementation at high protein level, i.e. high ammonia–nitrogen production. Fig. 1 shows this rela-

tionship for feed protein contents from 15% to 55% and as a percent of feed the required to provide the necessary supplemental carbohydrate required for complete heterotrophic metabolism of the ammonia nitrogen produced from the feed being fed to the shrimp.

7. Impact of C/N ratio

For wastewater treatment suspended growth systems, it has been determined that the fraction of nitrifying bacteria found in the mixed liquor is reasonably well related to the BOD₅ to TKN ratio (Metcalf and Eddy Inc., 1991). Total Kjeldahl Nitrogen (TKN) is determined by digestion of the sample in boiling sulfuric acid, where the organic nitrogen is converted to ammonia and subsequent measurement of ammonia–nitrogen by Nesslerization method. Thus TKN is the sum of the organic and ammonia–nitrogen, or from a wastewater treatment

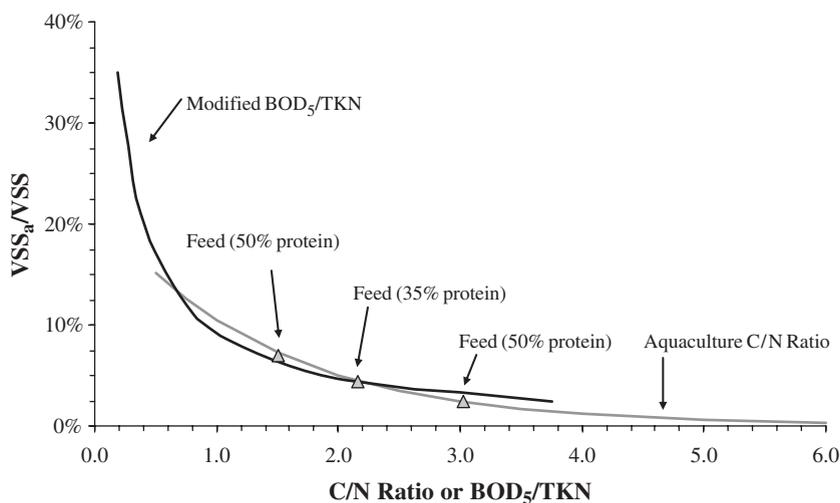


Fig. 2. Fraction of nitrifying bacteria as a function of the BOD₅/TKN ratio (Metcalf and Eddy Inc., 1991) and C/N ratio for aquaculture feeds.

viewpoint, an indicator of oxygen requirement to convert the available nitrogen to nitrate–nitrogen. Table 8 shows the fraction of nitrifiers to heterotrophs as a function of the BOD₅/TKN ratio (Metcalf and Eddy Inc., 1991). It can be seen from this table that as the BOD₅/TKN ratio increases the fraction of nitrifying bacterial falls off quickly. Since BOD₅ is approximately a measure of the oxygen consumed by the organic carbon, using a simple mass balance suggests that the ratio of carbon to oxygen consumption should be 12 g mol C to 32 g mol O₂ or 0.375 g carbon/g oxygen. The modified BOD₅/TKN column reflects this adjustment to an approximate C/N ratio. In a zero-exchange system without carbon supplementation 1 kg of aquaculture feed contains approximately 109 g of labile carbon and removes approximately 18 g of ammonia–nitrogen via heterotrophic bacteria. The remaining ammonia–nitrogen is removed via autotrophic bacteria using alkalinity as the inorganic carbon source. Based on the previously developed stoichiometry, VSS production for both the heterotrophic and autotrophic bacteria can be estimated. Table 8 presents C/N ratios for aquaculture feeds and demonstrates the same rapid fall off in the fraction of nitrifiers to heterotrophs as the C/N ratio increases. Fig. 2 shows the excellent correspondence between aquaculture C/N ratio or modified BOD₅/TKN ratio and the fraction of nitrifying VSS for wastewater treatment suspended growth systems.

Zhu and Chen (2001) demonstrated the effect of sucrose carbon on the nitrification rate of biofilters under steady-state conditions using an experimental system. They determined at carbon/nitrogen ratios from 1.0 to 2.0, which are where most RAS systems operate, there was a 70% reduction of total ammonia–nitrogen removal rate as compared to C/N ratio of zero. Table 8 supports this observation, with a reduction from 67% at a C/N ratio of 2.0 to 84% at a ratio of 1.0.

8. Conclusions

The three pathways for nitrogen removal are all very different in terms of substrate utilization, bacterial biomass generated and by-products. The difficulty in the complex world of production aquaculture is that all three may be present to some degree and compete for the same substrate. The ability to control the carbon to nitrogen ratio by feed formulation, solids removal, or addition of organic carbon does allow the aquaculture producer to manage what type of system is used. In the end though, it is the cultured species that determine which system is most appropriate based on their water quality needs.

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