Engineering review of photoautotrophic, autotrophic, heterotrophic bacterial control of ammonia in zero-exchange production systems

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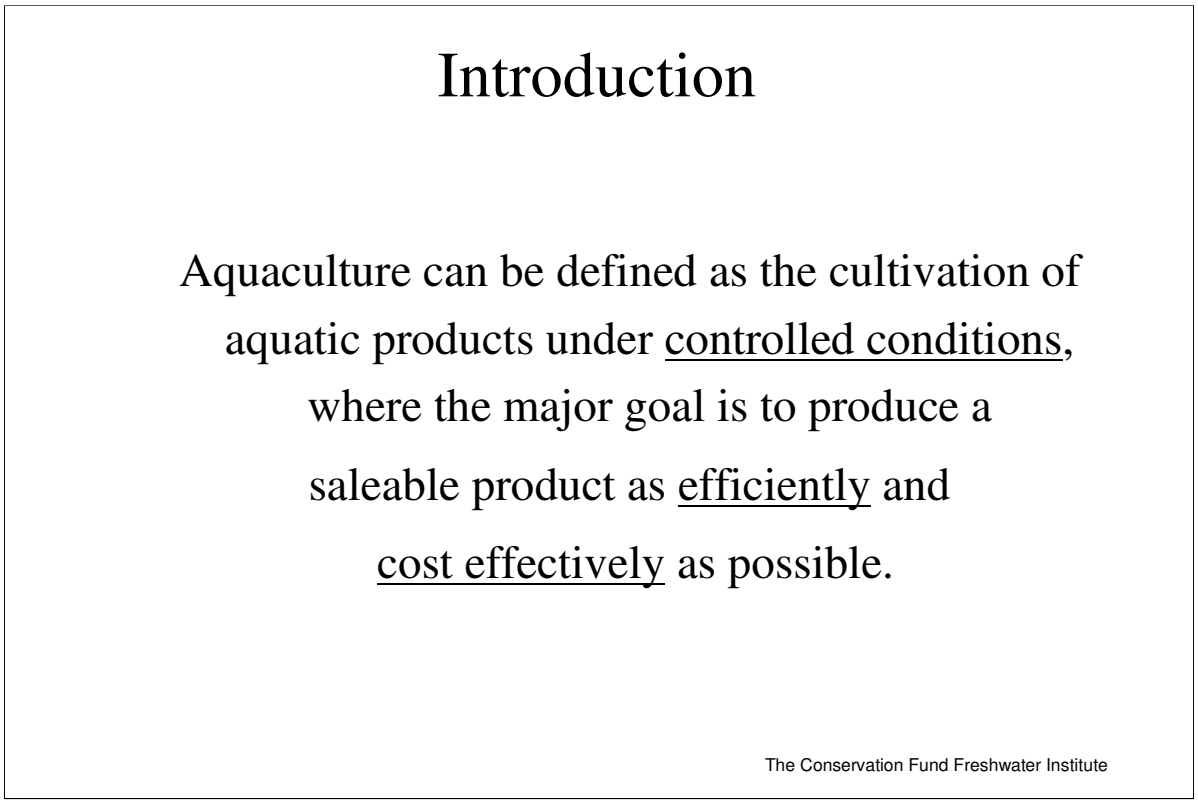
Abstract

In intensive aquaculture systems, ammonia-nitrogen buildup from the metabolism of feed is usually the second limiting factor to increasing 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.



1.0 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), 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.

Introduction

Aquaculture production systems

- extensive pond systems
- intensive pond systems
- intensive recirculating tank and raceway systems

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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, 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/m3. 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/m3 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, 2004).

"New Paradigm"

Zero-exchange Systems "Belize System"

- Shrimp – high health, selectively bred Specific Pathogen Free stock
- Feed – low protein feeds in combination with traditional high protein feeds
- Water management – zero water exchange, recycling water between crops

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Recently, a new production strategy has emerged called intensive zero exchange systems. In these systems, the ammonia build-up is controlled by the manipulation of the carbon/nitrogen ratio in such a way as to promote the growth of heterotrophic bacteria (Avnimelech, 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).

Due to the environmental impact of these nutrient rich discharges and the need for increased biosecurity, Waddell Mariculture Center, among others, researched the concept of reduced or zero-water exchange culture. Waddell Mariculture Center demonstrated that it was possible to obtain high shrimp yields from ponds using minimal exchange of water with high aeration rates. In the mid-90's, this concept with minor modifications was demonstrated at a commercial farm, BAL in Belize, Central America, hence the reference to the 'Belize zero-exchange system'.

This system was based on the following concepts:

- •Shrimp – high health, selectively bred Specific Pathogen Free stock
- •Feed – low protein feeds in combination with traditional high protein feeds
- •Water management – zero water exchange, recycling water between crops
- •Pond design – square shapes, depth of 1.0 to 1.8 m at center, HDPE liner
- •Pond aeration – 30 to 50 hp/ha, completely mixed
- •Pond management – C/N ratio maintained by feed protein and addition of additional carbon as needed (molasses, sorghum, sugar, cassava or wheat meal)
- •Sludge management – frequent removal from center of pond or by settling between crops in holding ponds.

To prevent the introduction of disease, only disease resistant Specific Pathogen Free (SPR) PL's are stocked in the production pond at densities up to 120 to 200 m2. Recent strains of faster growing shrimp have become available through selective breeding. Feed protein content plays a critical role in maintaining a healthy bacterial population by balancing the carbon to nitrogen ratio. A carbon/nitrogen ratio of 16:1 was found to yield a very health heterotrophic community. This was accomplished by using a grain based feed with a high C/N ratio of 20 to balance the high protein diet used. In addition, molasses (> 40% carbon) was added during initial pond development to stimulate heterotrophic bacterial growth.

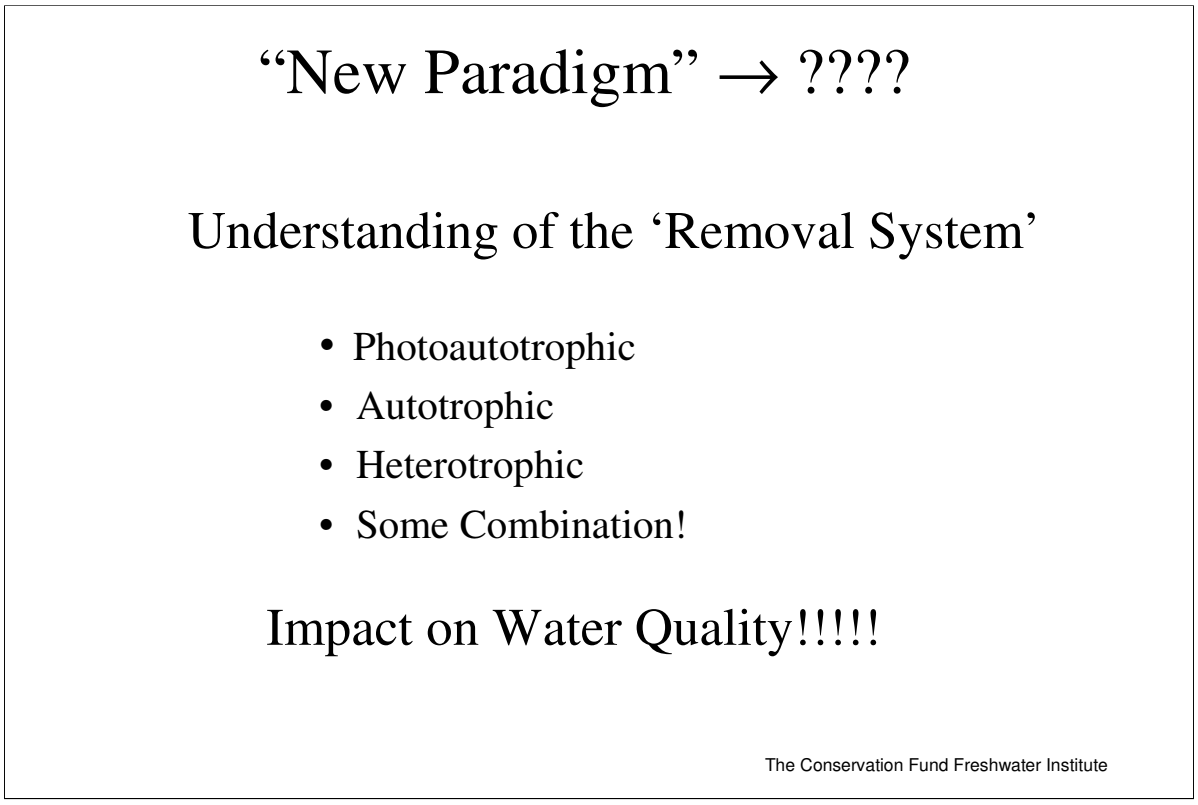
During the production cycle, there was no exchange of water except to make-up water loss to evaporation seepage and solids discharge. At harvest, pond water was routed to a settling basin, where the solids quickly settled out and excess nutrients were removed. After one week, the water was recycled back to the production pond and three days later, shrimp stocked out. This was possible because the recycled water had sufficient nutrients and bacterial population that no extensive pond preparations were needed. Ponds at Belize Aquaculture were square in shape with an average area of 1.6 ha (4 acres) and deeper compared to traditional shrimp ponds (1.8 m). In addition, each production pond was lined with a 40 ml HDPE liner. The liner was critical to allow the high mixing velocities created by the paddlewheel and aspirator aerators. This mixing action maintained floc in suspension and concentrated sludge in the center of the pond. At production levels of 1.8 to 1.9 kg/m2, approximately 1 hp of paddlewheel aeration was required to maintain dissolved oxygen for the production of 500 – 650 kg of shrimp. Ponds at Belize Aquaculture required 50 hp/ha of aeration. The aerators created a circular motion in the pond with water velocities ranging from 23 cm/s at the outside to 5 cm/s at the center.

These high rates of aeration and mixing are the first major component of a zero-exchange production system. The second is the maintenance of an active heterotrophic bacterial community by controlling the amount of organic loading and the carbon/nitrogen ratio. At sufficiently high stocking densities, there is normally adequate inorganic material (predominately ammonia-nitrogen) to maintain a robust heterotrophic bacterial community. At these high stocking densities, there can be a problem with too high a nitrogen concentration for the available carbon due to the use of high (>30%) protein levels. Belize Aquaculture found that by increasing the carbon/nitrogen ratio in the feed to 16:1 by mixing in a grain based feed (20:1 ratio) the heterotrophic community appeared to be more in balance.

Finally, sludge management is important and consists of primarily removing sludge concentrated in the center of the pond by mixing action. Sludge is very different from the bacterial floc in suspension, consisting of fecal matter and uneaten feed particles. Sludge in the center of the pond is either drained out or pumped out to the drainage canals and eventually to the solids settling pond for treatment and ultimate disposal.

The Belize system, which is a solution to conventional shrimp pond constraints, also suggests the possibility that a recirculating system approach might be used to raise shrimp in an intensive manner. Using an indoor approach would also provide more control over water temperatures and heating costs that might permit a zero-exchange system to produce several crops per year in a moderate climate such as Atlanta GA, where pond systems would be limited by outdoor water temperatures for essentially 6 months of the year. Since maximum shrimp growth rates occur near 86 F (30 C), there are essentially no outdoor sites that have such temperatures year round. Thus, the ability to control water temperatures to optimal temperatures on a year-round basis is a distinct advantage.

Over the past few years, zero-exchange management systems have been developed for large-scale pond production, where carbonaceous substrate is added to the systems to support microbial metabolism (Avnimelech, 1999; McIntosh, 1999). At high carbon to nitrogen (C/N) ratios, heterotrophic bacteria will assimilate ammonia-nitrogen directly from the water and metabolize the ammonia directly into cellular biomass. Numerous researchers have applied this concept to indoor production systems at high densities (Weirich, 2002; Otoshi, 2003; Davis and Arnold, 1998; Van Wyk, 1999), although each includes some form of biofilter in the overall water treatment stream.



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.

Ammonia Production

In general:

$$P_{TAN} = F * PC * 0.092$$

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

For marine shrimp:

$$P_{TAN} = F * PC * 0.144$$

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2.0 Ammonia-nitrogen Production

Ammonia is produced as a major end product of the metabolism of protein catabolism and is excreted as un-ionized 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 ($NH_4+ + NH_3$) 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., $NH_4+–N$ (ionized ammonia-nitrogen), $NH_3–N$ (un-ionized ammonia-nitrogen), $NO_2-–N$ (nitrite-nitrogen), and $NO_3-–N$ (nitrate-nitrogen). This allows for easier computation of total ammonia-nitrogen (TAN = $NH_4+–N + NH_3–N$) 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):

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

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:

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

Photoautotrophic (algal based systems)

Biosynthesis of saltwater algae:

Nitrate as nitrogen source

$$16\ NO_3^- + 124\ CO_2 + 140\ H_2O + HPO_4^{2-} \rightarrow$$

$$C_{106}H_{263}O_{110}N_{16}P + 138\ O_2 + 18\ HCO_3^-$$

Ammonia as nitrogen source

$$16\ NH_4^+ + 92\ CO_2 + 92\ H_2O + 14\ HCO_3^- + HPO_4^{2-} \rightarrow$$

$$C_{106}H_{263}O_{110}N_{16}P + 106\ O_2$$

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3.0 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 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:

16NH4+ + 92 CO2 + 92 H2O + 14 HCO3- + HPO42- → C106H263O110N16P + 106 O2 (3)

Or, for nitrate as the nitrogen source:

16 NO3- + 124 CO2 + 140 H2O + HPO42- → C106H263O110N16P + 138 O2 + 18 HCO3- (4)

where C106H263O110N16P represents the stoichiometric formula for seawater algae.

Note that 3.13 g of alkalinity (as CaCO3) is consumed for every g of ammonia-nitrogen consumed in the first relationship and 4.02 g of alkalinity (as CaCO3) 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. Correspondently, 15.14 g and 19.71 g of O2 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.

Photoautotrophic (algal based systems)

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
Oxygen	15.14 g O_2/ g N	15.14	-----	-----	-----

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3.0 Photoautotrophic (algal based systems)

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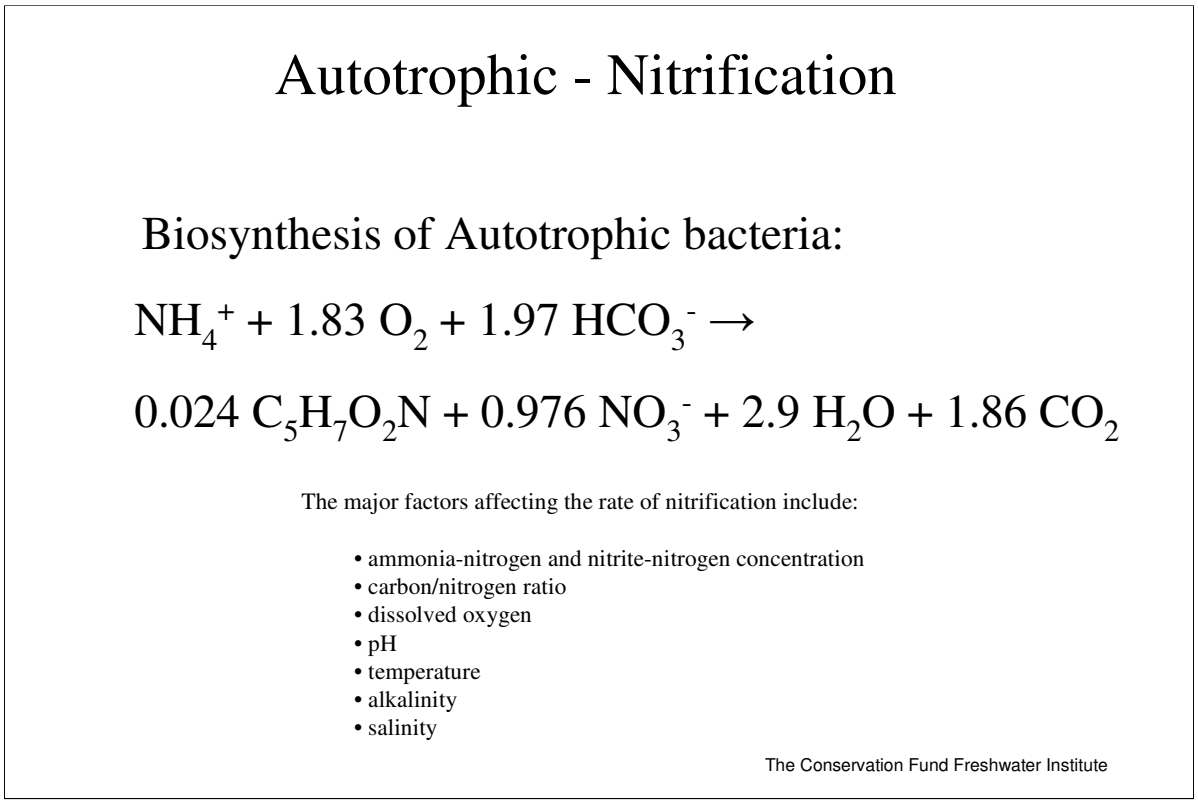

4.0 Autotrophic Bacteria - Nitrification

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 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, Nitrosospira, 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).

Autotrophic - Nitrification

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	-----

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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) is consumed and 0.20 g of microbial biomass (0.105 g organic carbon) and 5.85 gm of CO2, (1.59 g inorganic carbon) is 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-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.

Heterotrophic Bacteria

Biosynthesis of Heterotrophic bacteria:

$$NH_4^+ + 1.18\ C_6H_{12}O_6 + HCO_3^- + 2.06\ O_2 \rightarrow$$
$$C_5H_7O_2N + 6.06\ H_2O + 3.07\ CO_2$$

The major factors affecting the rate of nitrification include:

- ammonia-nitrogen
- carbon/nitrogen ratio
- dissolved oxygen
- pH
- temperature
- alkalinity
- salinity

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5.0 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).

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 CO2 (2.63g inorganic carbon) are produced. Note the oxygen demand is slightly higher, the alkalinity requirement about half and the CO2 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 g versus 0.20 g. Table 4 summarizes the stoichiometry for the heterotrophic pathways for ammonia-nitrogen conversion.

Heterotrophic Bacteria

Consumables	Stoichiometry	Consumes (g)	$C_{organic}$ (g)	$C_{inorganic}$ (g)	N (g)
NH_4^+-N		1.0	-----	-----	1.0
$C_6H_{12}O_6$	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	Yield (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	-----

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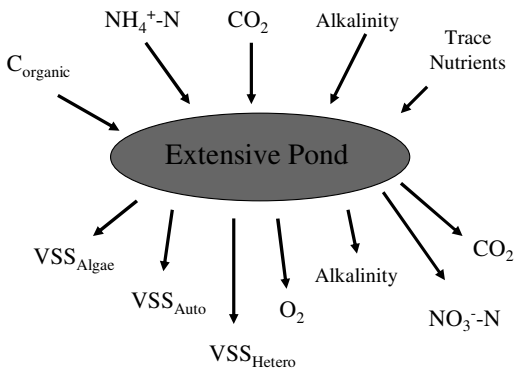
5.0 Heterotrophic Bacteria

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 CaCO3), especially 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 CO2 levels. Finally, there should be no production of nitrite-nitrogen, or nitrate-nitrogen in a pure heterotrophic system.

Nitrogen Removal Pathways



- Photoautotrophic
- Autotrophic
- Heterotrophic
- Other Mysterious Ways

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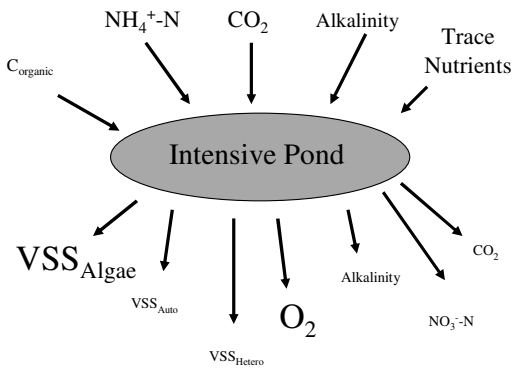
Nitrogen Removal Pathways

Extensive ponds are stocked at very low biomass densities and multiple removal systems are used to remove nitrogen. These would include photoautotrophic, autotrophic and heterotrophic systems as well as numerous other pathways based on other organisms, soil-water interactions, etc. Very difficult to model and track pathways due to low concentrations.

Nitrogen Removal Pathways



- Photoautotrophic
- Autotrophic
- Heterotrophic
- Other Mysterious Ways

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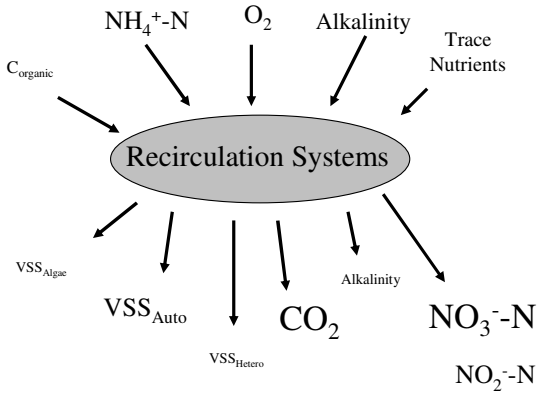
Nitrogen Removal Pathways

Intensive ponds are usually managed to promote algae production through the addition of trace nutrients, flushing of water or cropping of algal biomass.

Nitrogen Removal Pathways



- Photoautotrophic
- Autotrophic
- Heterotrophic
- Denitrification

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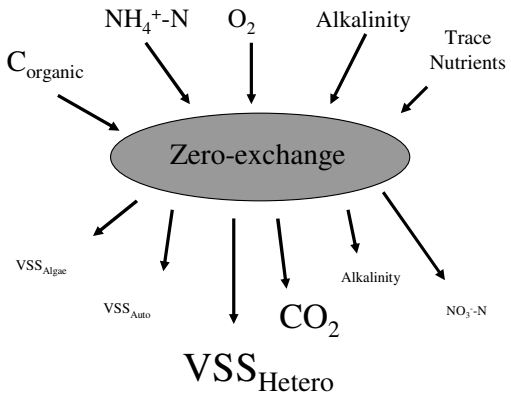
Nitrogen Removal Pathways

Recirculation systems are managed to remove as much of the organic carbon (uneaten feed, fecal matter, etc) as quickly as possible. Autotrophic process are further encouraged using fixed-film bioreactors. Very little microbial biomass is generated, but large amounts of nitrate-nitrogen and carbon dioxide.

Nitrogen Removal Pathways



- Photoautotrophic
- Autotrophic
- Heterotrophic
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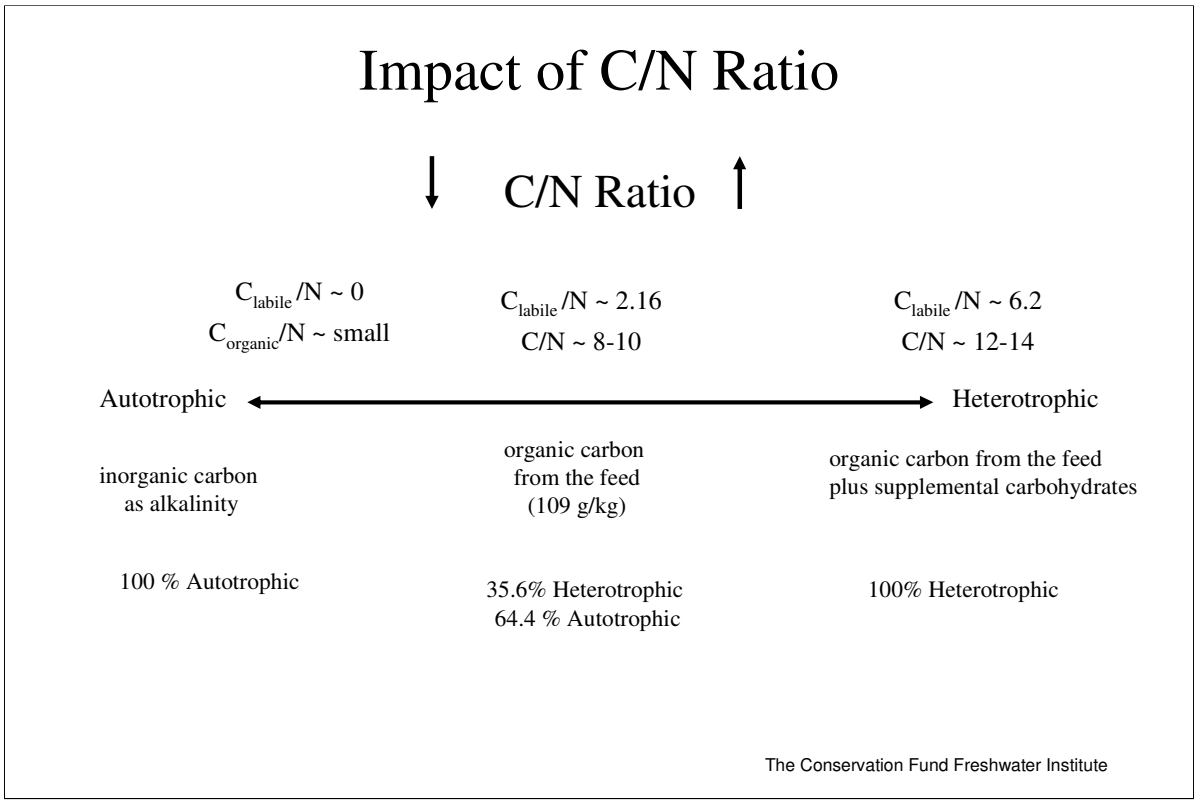
Nitrogen Removal Pathways

Recirculation systems are managed to remove as much of the organic carbon (uneaten feed, fecal matter, etc) as quickly as possible. Autotrophic process are further encouraged using fixed-film bioreactors. Very little microbial biomass is generated, but large amounts of nitrate-nitrogen and carbon dioxide.



6.0 Conversion of 1 kg of feed @ 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 form, 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.

Photoautotrophic (Pond intensive system)

Consumables	Stoichiometry	Consumes (g)	$C_{organic}$ (g)	$C_{inorganic}$ (g)	N (g)
NH_4^+-N		50.4	-----	-----	50.4
Carbon Dioxide	18.07 g CO_2/ g N	911	-----	249	-----
Alkalinity	3.13 g Alk/ g N	158	-----	37.9	-----

Products	Stoichiometry	Yields (g)	$C_{organic}$ (g)	$C_{inorganic}$ (g)	N (g)
VSS_{Algae}	15.85 g VSS_A / g N	799	287	-----	50.4
O_2	15.14 g O_2/ g N	763	-----	-----	-----

Conversion of 1 kg of feed @ 35% protein

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6.0 Conversion of 1 kg of feed @ 35% protein

For a pure photoautotrophic process (Table 5), the mass of algal biomass can be calculated from the ammonia-nitrogen production rate and the VSS yield, approximately 799.8 g VSS per kilogram of feed. Since algal biomass (VSSalgal) contains 35.8% C and 6.31% N (based on stoichiometry), the algal biomass sequesters 286.4 g organic carbon and 50.4 g N. Note the large amount of organic carbon in the algae that originates from the carbon dioxide (248.5 g C) and alkalinity (37.9 g C).

Autotrophic (Intensive Recirculation System)

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	-----	85.2	-----
Oxygen	4.18 g O_2/ g N	211	-----	-----	-----

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	295	-----	80.1	-----

Conversion of 1 kg of feed @ 35% protein

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6.0 Conversion of 1 kg of feed @ 35% protein

For a pure autotrophic nitrification process (Table 6) 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 per kilogram 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 NO3-N). The source of the inorganic carbon required by the autotrophic bacteria is from the consumption of 355 g of alkalinity as CaCO3. The C/N ratio for optimal conversion by autotrophic systems works out to be 1.69 g inorganic carbon/g nitrogen, compared to the microbial biomass C/N ratio of 4.28 g organic carbon/ g nitrogen.

Autotrophic / Heterotrophic (Zero-exchange System)

Heterotrophic Bacteria	Stoichiometry	Consumes (g)	$C_{organic}$ (g)	$C_{inorganic}$ (g)	N (g)
NH_4^+-N	0.356 * N_T	17.9	-----	-----	17.9
$C_6H_{12}O_{6\ feed}$	15.17 g Carbs/ g N	272	108.9	-----	-----
Alkalinity	3.57 g Alk/ g N	63.9	-----	15.4	-----

Autotrophic Bacteria	Stoichiometry	Consumes (g)	$C_{organic}$ (g)	$C_{inorganic}$ (g)	N (g)
NH_4^+-N	0.644 * N_T	32.5	-----	-----	32.5
Alkalinity	7.05 g Alk/ g N	229.1	-----	55.4	-----

Total Consumed	Consumes	$C_{organic}$ (g)	$C_{inorganic}$ (g)	N (g)
NH_4^+-N	50.4 g N	-----	-----	50.4
$C_6H_{12}O_6$	272 g Carbs	108.9	-----	-----
Alkalinity	293 g Alk	-----	70.8	-----

Conversion of 1 kg of feed @ 35% protein

The Conservation Fund Freshwater Institute

6.0 Conversion of 1 kg of feed @ 35% protein

In a pure zero-exchange system (Table 7), 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 per kg of feed (Zhu and Chen, 2001, Brune, 2003). Using a conservative yield fraction of 0.40 kg VSS per kg BOD (Brune, 2003), and a BOD content of 0.36 kg per 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.

Autotrophic / Heterotrophic (Zero-exchange System)

Heterotrophic Bacteria	Stoichiometry	Yields (g)	$C_{organic}$ (g)	$C_{inorganic}$ (g)	N (g)
VSS_H	8.07 g VSS_H / g N	144	76.5	-----	17.9
CO_2	9.65 g CO_2/ g N	174	-----	47.4	-----

Autotrophic Bacteria	Stoichiometry	Yields (g)	$C_{organic}$ (g)	$C_{inorganic}$ (g)	N (g)
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	-----	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	-----

Conversion of 1 kg of feed @ 35% protein

The Conservation Fund Freshwater Institute

6.0 Conversion of 1 kg of feed @ 35% protein

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 per 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.

Heterotrophic (Zero-exchange System with C supplementation)

Consumables	Stoichiometry	Consumes (g)	$C_{organic}$ (g)	$C_{inorganic}$ (g)	N (g)
NH_4^+-N		50.4	-----	-----	50.4
$C_6H_{12}O_6$	15.17 g Carbs/ g N	765	306	-----	-----
Alkalinity	3.57 g Alk/ g N	180	-----	43.3	-----
Oxygen	4.71 g O_2/ g N	237	-----	-----	-----

Products	Stoichiometry	Yield (g)	$C_{organic}$ (g)	$C_{inorganic}$ (g)	N (g)
VSS_H	8.07 g VSS_H / g N	407	216	-----	50.4
CO_2	9.65 g CO_2/ g N	487	-----	133	-----

Conversion of 1 kg of feed @ 35% protein

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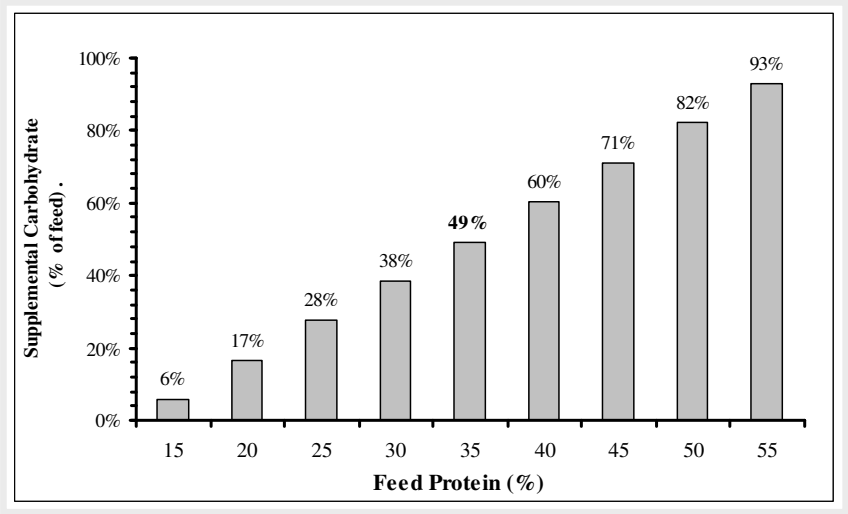
6.0 Conversion of 1 kg of feed @ 35% protein

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 requirements of the heterotrophs (Table 8). 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.

Heterotrophic (Zero-exchange System with C supplementation)



Supplemental Carbohydrate as percentage of feed rate
for heterotrophic metabolism of ammonia-nitrogen to microbial biomass

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6.0 Conversion of 1 kg of feed @ 35% protein

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

Conclusions

Further work is needed to characterize the impact on production system performance at various C/N ratios.

Alternative forms of Carbon need to be evaluated for effectiveness and economics.

Fundamental research is needed on carbon assimilation and conversion efficiency for heterotrophic bacteria.

Development of optimal strains of bacteria for zero-exchange systems.

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Questions?

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