Review of autotrophic and heterotrophic bacterial control of ammonia-nitrogen in zero-exchange production systems: stoichiometry and experimental verification

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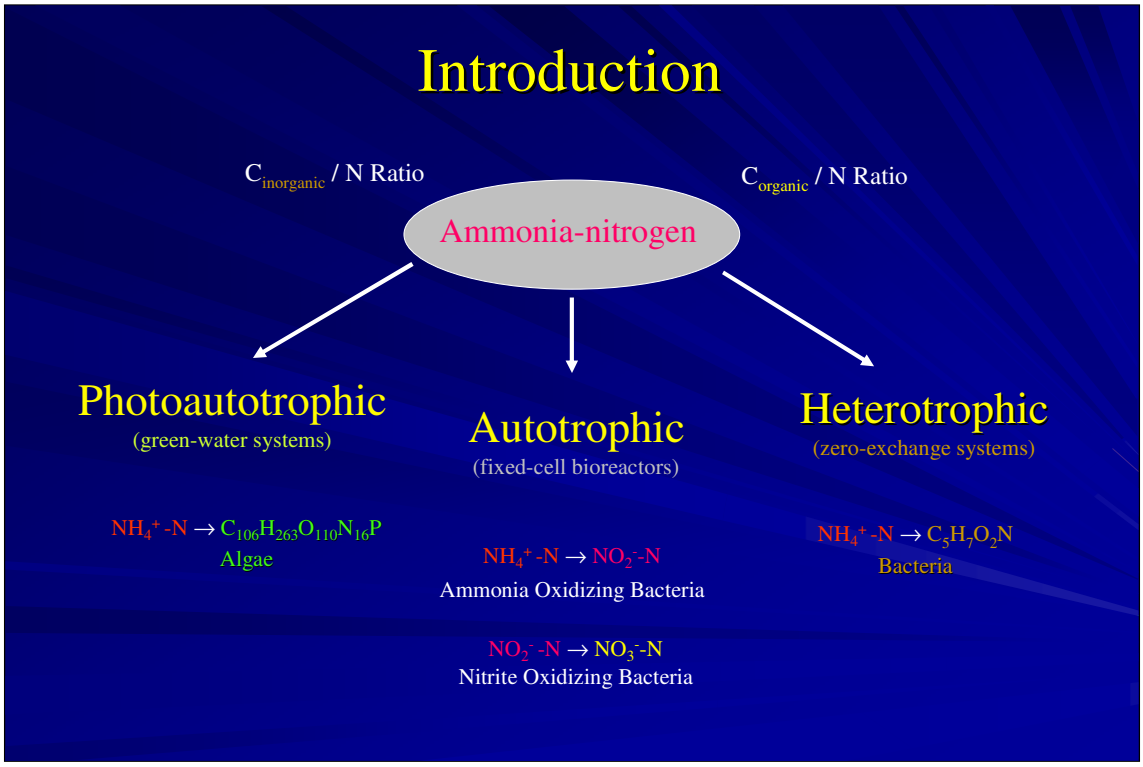
Abstract

In intensive aquaculture systems, ammonia-nitrogen buildup from the metabolism of feed is usually the limiting factor after dissolved oxygen to increasing production levels. Currently, large fixed-cell bioreactors are the primary strategy for controlling inorganic nitrogen in intensive recirculating systems. 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 based on heterotrophic bacteria and promoted for the intensive production of marine shrimp and tilapia. In these systems, heterotrophic bacterial growth is stimulated through the addition of carbonaceous substrate. At high carbon to nitrogen (C/N) feed ratios, heterotrophic bacteria assimilate ammonia-nitrogen directly from the water replacing the need for an external fixed film biofilter. Thus in these types of systems, build-up of suspended solids may become the second limiting factor after dissolved oxygen. This presentation reviews the two nitrogen conversion pathways used for the removal of ammonia-nitrogen in aquaculture systems, autotrophic bacterial conversion of ammonia-nitrogen to nitrate nitrogen, and heterotrophic bacterial conversion of ammonia-nitrogen directly to microbial biomass. The first part reviews in detail the two ammonia removal pathways, presents a set of stoichiometric balanced relationships, and discusses their impact on water quality. In addition, microbial growth energetics are used to characterize production of volatile and total suspended solids for autotrophic and heterotrophic systems. A critical finding of this work was that only a small fraction of the feed's carbon content is readily available to the heterotrophic bacteria. For example, feed containing 35% protein has only 109 g/kg feed of labile carbon. In the second part, the results of a study on the impact C/N ratio on water quality. In this experimental trial, sufficient carbon in the form of sucrose (sugar) was added daily at 0%, 50% and 100% of the feed rate to three proto-type zero-exchange systems. The system was stocked with marine shrimp (*L. vannamei*) at modest density (150 /m^2) and water quality measured daily. Significant differences were seen between the three systems in the key water quality parameters of ammonia-nitrogen, nitrite-nitrogen, nitrate-nitrogen, pH and alkalinity. The control system exhibited water quality characteristics of a mixed autotrophic/heterotrophic system and the two systems receiving supplemental carbon, water quality characteristics of pure heterotrophic systems.



Introduction

The three pathways for the removal of ammonia-nitrogen in traditional aquaculture systems are: photoautotrophic algae, autotrophic bacterial conversion from ammonia-nitrogen to nitrate nitrogen, and heterotrophic bacterial conversion from ammonia-nitrogen directly to microbial biomass. Traditionally, pond aquaculture has used photoautotrophic algae based systems (green-water systems) to control inorganic nitrogen buildup. In intensive recirculating aquaculture production systems large fixed-cell bioreactors are routinely used that rely on the nitrification of ammonia-nitrogen to nitrate-nitrogen by Ammonia Oxidizing Bacteria (AOB) and Nitrite Oxidizing Bacteria (NOB) (Timmons et al., 2002). In intensive recirculating systems, the growth of heterotrophic bacteria and the accumulation of organic carbon are minimized through the rapid removal of solids from the system and water exchange. In contrast, it has been demonstrated that for zero-exchange pond production, the inorganic nitrogen build-up can be controlled by the manipulation of the carbon/nitrogen ratio in such a way to promote the growth of heterotrophic bacteria (Avnimelech, 1999). At high organic carbon to nitrogen (C/N) ratios of feeding, heterotrophic bacteria will take up ammonia-nitrogen directly from the water and produce cellular protein. As an additional benefit, for some aquaculture species (marine shrimp and tilapia), this bacterial biomass can be an important source of feed protein, reducing the cost of production and thus improving the overall economics (McIntosh, 1999; Moss, 2000).

In the last few years, zero-exchange management systems have been developed for large-scale pond production of marine shrimp and tilapia, traditionally photoautotrophic algae based, where carbonaceous substrate is added to the systems

New Paradigm

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.

In reviewing the literature on zero-exchange systems, there was often no clear description of the pathways of ammonia removal employed and whether the removal was fundamentally photoautotrophic, autotrophic or heterotrophic bacterial based, or in reality some mixture of the three. This paper presents a short review of two of these three pathways for the removal of ammonia-nitrogen and the results of a study conducted at The Conservation Fund's Freshwater Institute on the impact C/N ratio on water quality. In these trials, carbon in the form of sucrose (sugar) was added daily at 0%, 50%, and 100% of the shrimp feeding rate to three proto-type zero-exchange systems. The three research systems were shaded by two layers of shade cloth (blocking 90% of the sunlight) and by high concentrations of Total Suspended Solids (TSS) and as a result the role of photoautotrophic bacteria were assumed to be very small. Thus only the autotrophic and heterotrophic bacterial pathways were considered in the analysis.

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:

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

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)

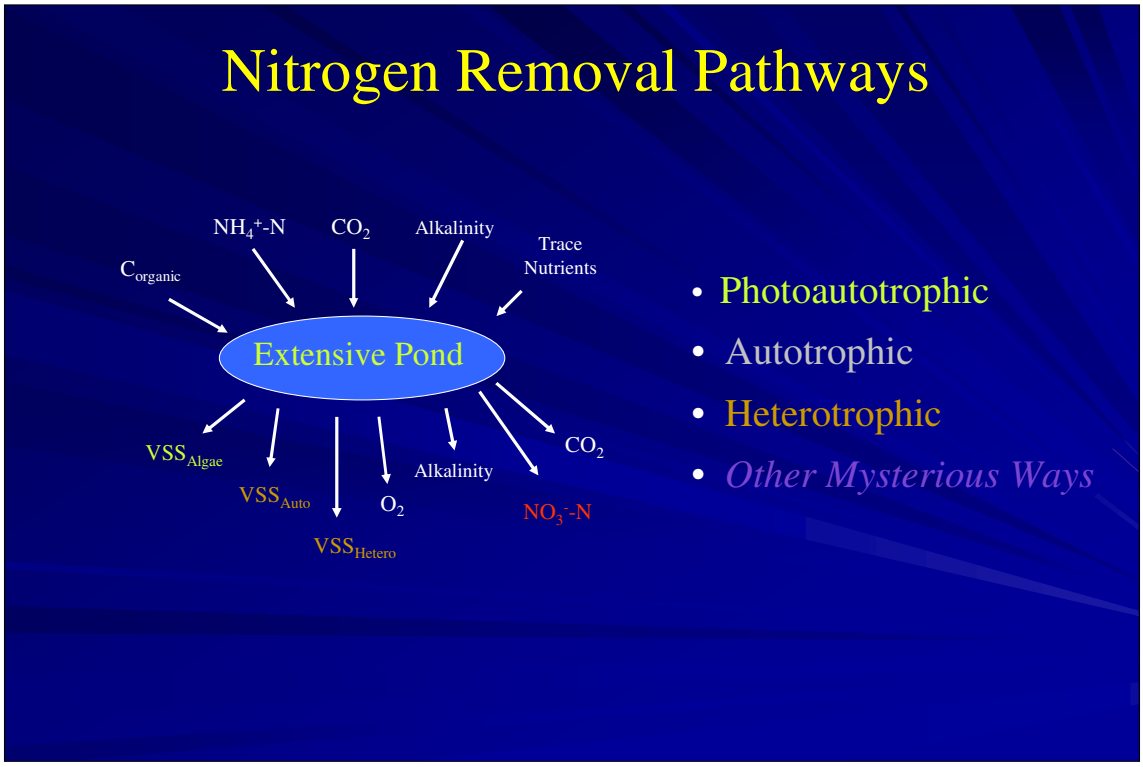
F = Feed rate (kg/day)

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.

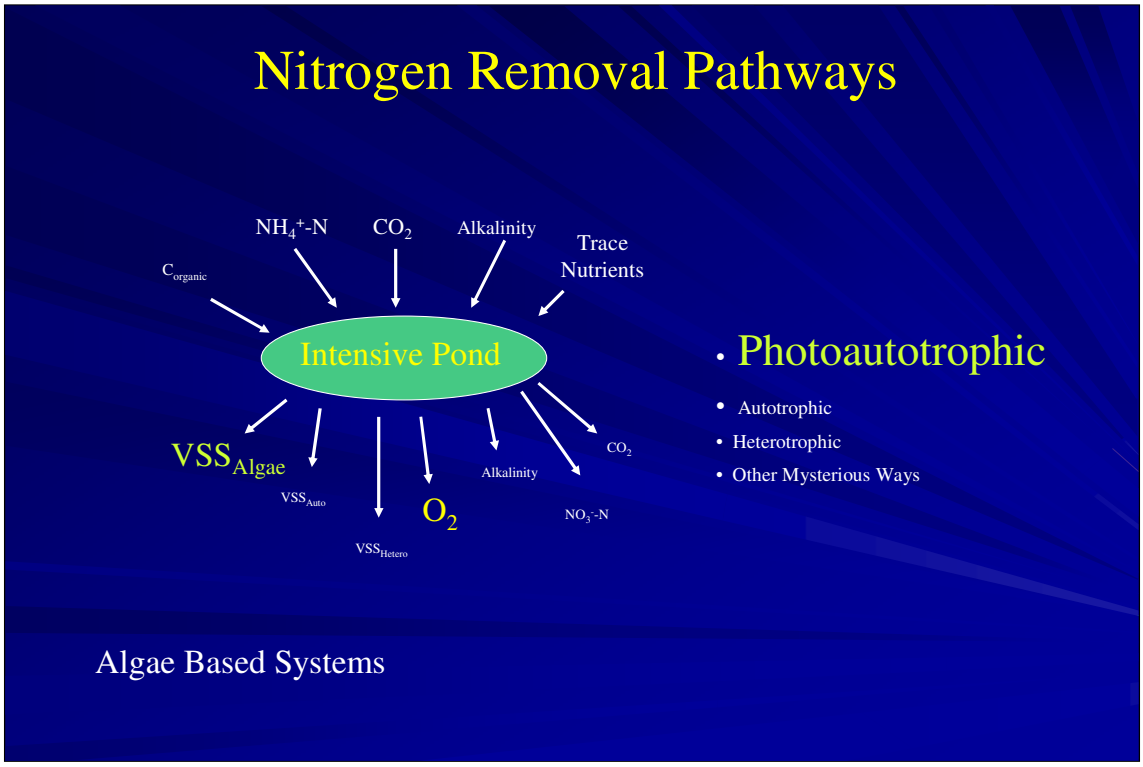
For heterotrophic bacterial based zero-exchange production systems, this formula needs to be modified to reflect that solids are not removed from the system and there is no traditional fixed-film biofilter. Thus all of the nitrogen excreted, both TAN and urea is available to the bacterial community. 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:

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



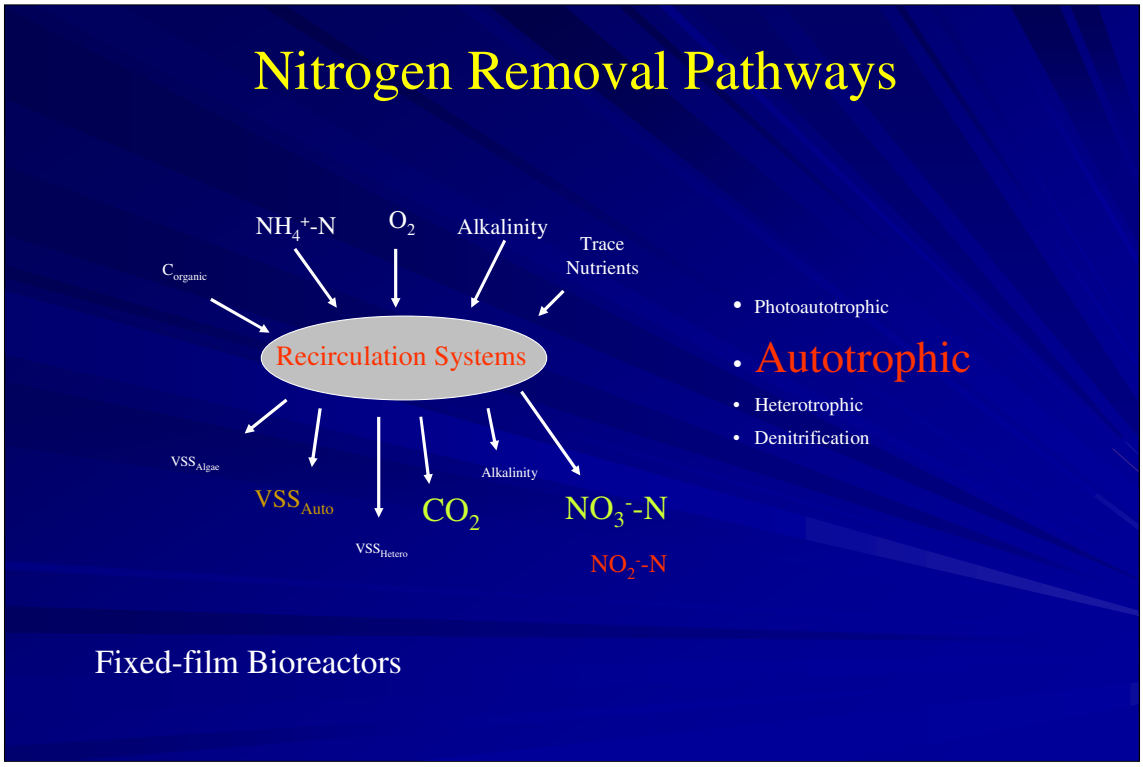
Nitrogen Removal Pathways

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 and combinatorial impact..



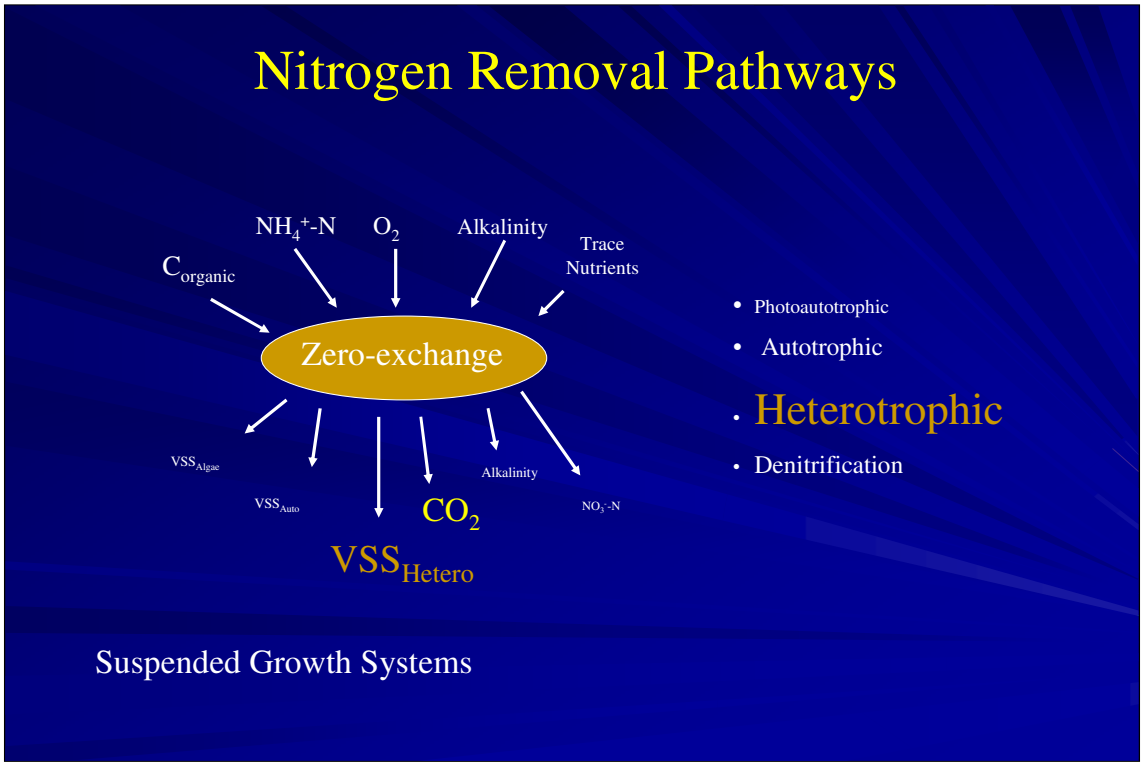
Nitrogen Removal Pathways

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



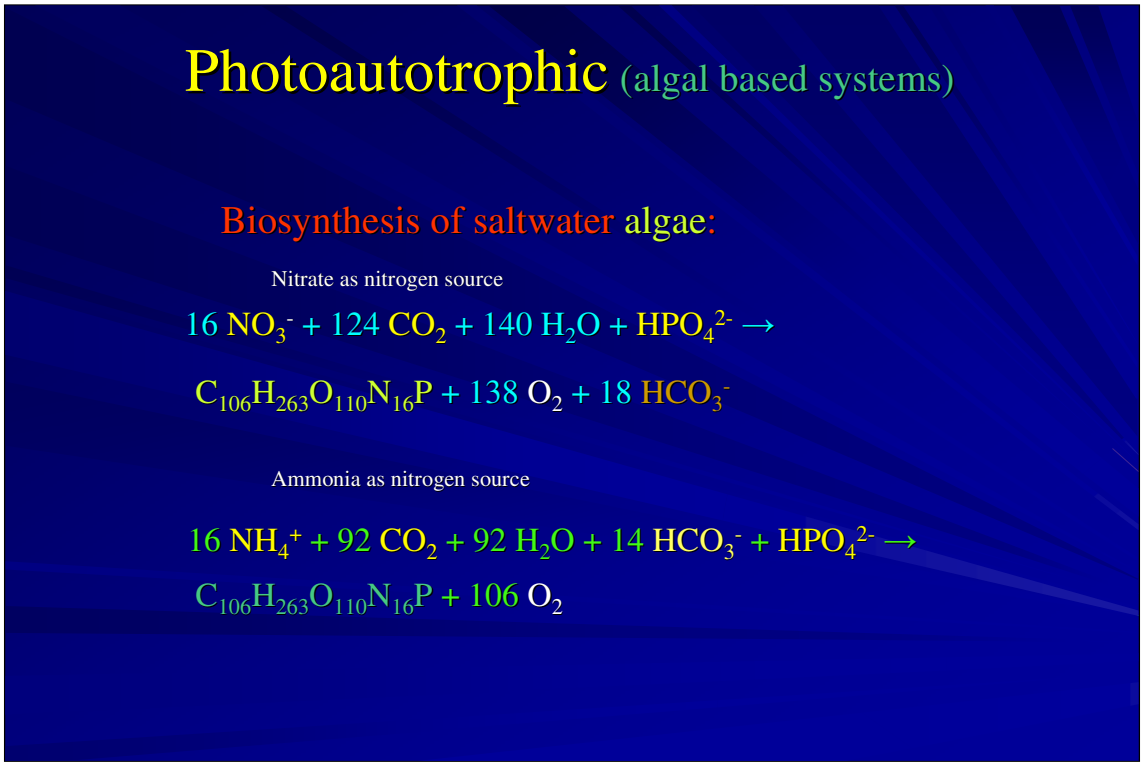
Nitrogen Removal Pathways

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

Heterotrophic systems incorporate the ammonia-nitrogen directly into microbial biomass. Thus no nitrite-nitrogen or nitrate-nitrogen is generated. High C/N ratios are required and feed only systems (35% protein) are only able to incorporate about 1/3 of the ammonia-nitrate into heterotrophic bacteria. Supplemental carbon as carbohydrates or low protein feeds are used to increase C/N ratio to 16, McIntosh, 2002.



Photoautotrophic (algal based systems)

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/m^2–day. High rate mixed ponds that are well managed can yield higher rates, 10 -12 g carbon/m^2 day (Brune, et al., 2003).

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:

$16NH_4^+ + 92\ CO_2 + 92\ H_2O + 14\ HCO_3^- + HPO_4^{2-} \rightarrow C_{106}H_{263}O_{110}N1_6P + 106\ O_2$

Or, for nitrate as the nitrogen source:

$16\ NO_3^- + 124\ CO_2 + 140\ H_2O + HPO_4^{2-} \rightarrow C_{106}H_{263}O_{110}N_{16}P + 138\ O_2 + 18\ HCO_3^-$

where $C_{106}H_{263}O_{110}N_{16}P$ 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. Correspondently, 15.14 g 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.

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	-----	-----	-----

Photoautotrophic (algal based systems)

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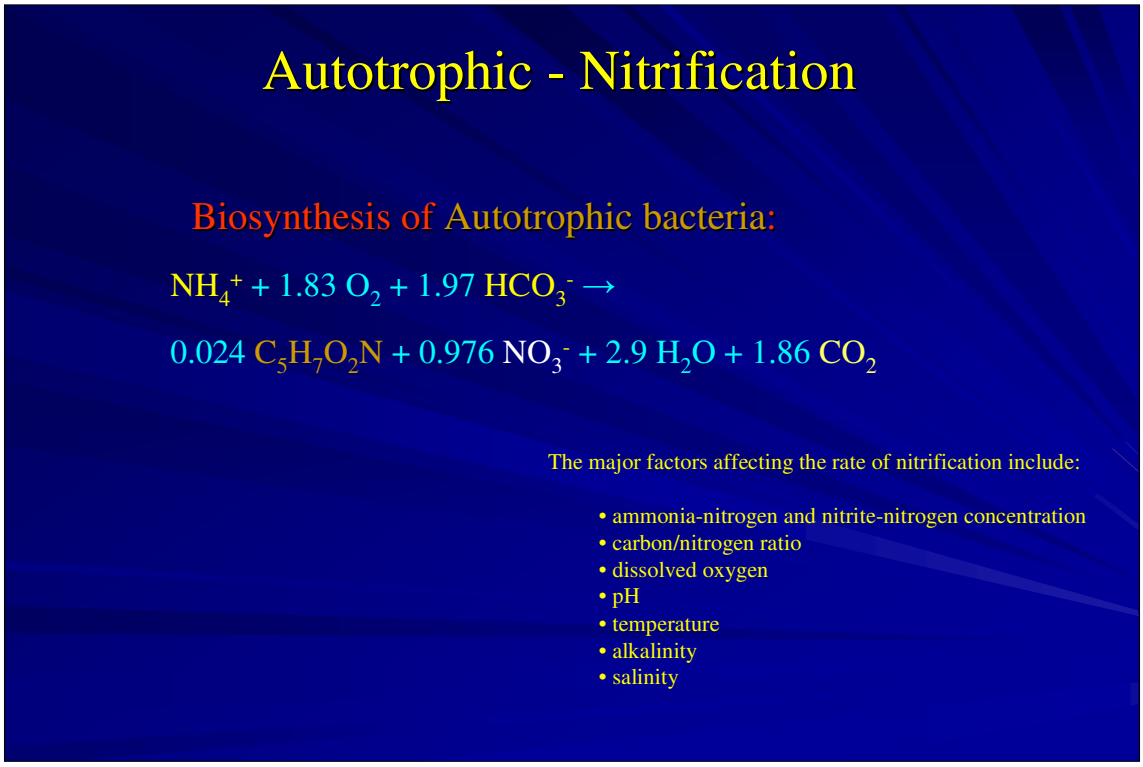


Autotrophic Bacteria - Nitrification

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	-----

Using this stoichiometric relationship, 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 CO_2, (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 O_2 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.

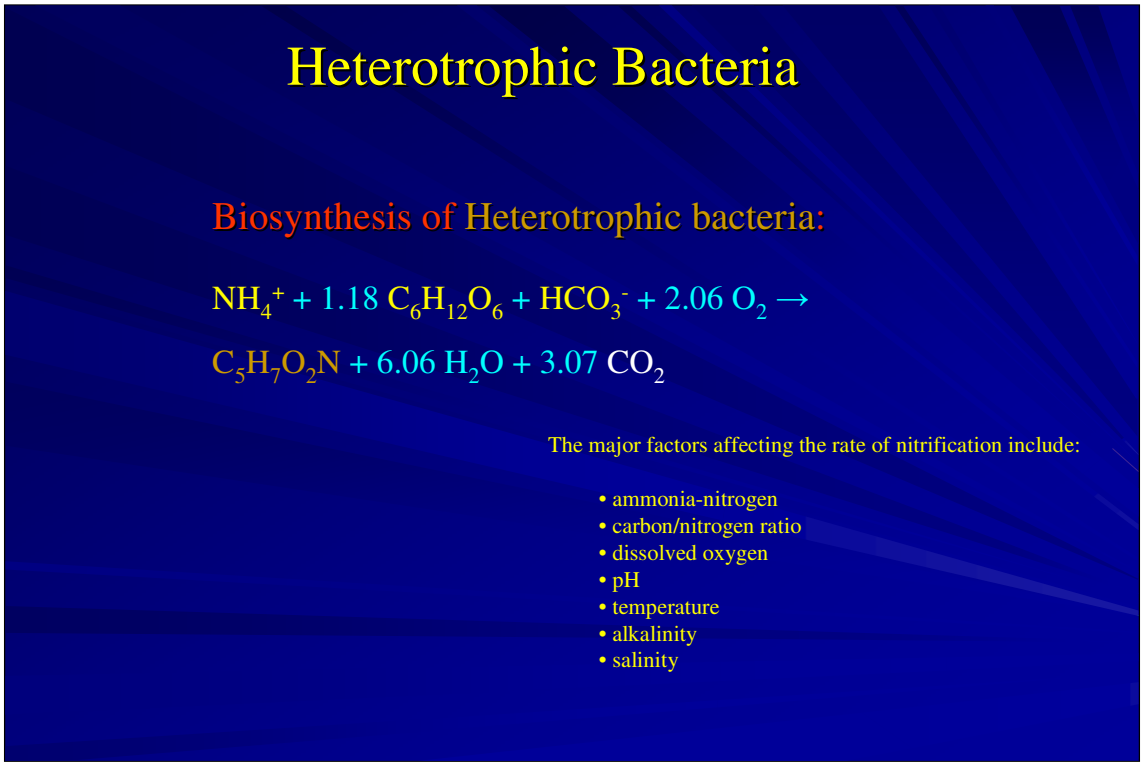
Autotrophic Bacteria – Impact on water quality

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 $CaCO_3$)/g N) and high levels of carbon dioxide produced (5.85 g CO_2 /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 $CaCO_3$), 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 CO_2 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.

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

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 CO_2 (2.63g 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 g versus 0.20 g.

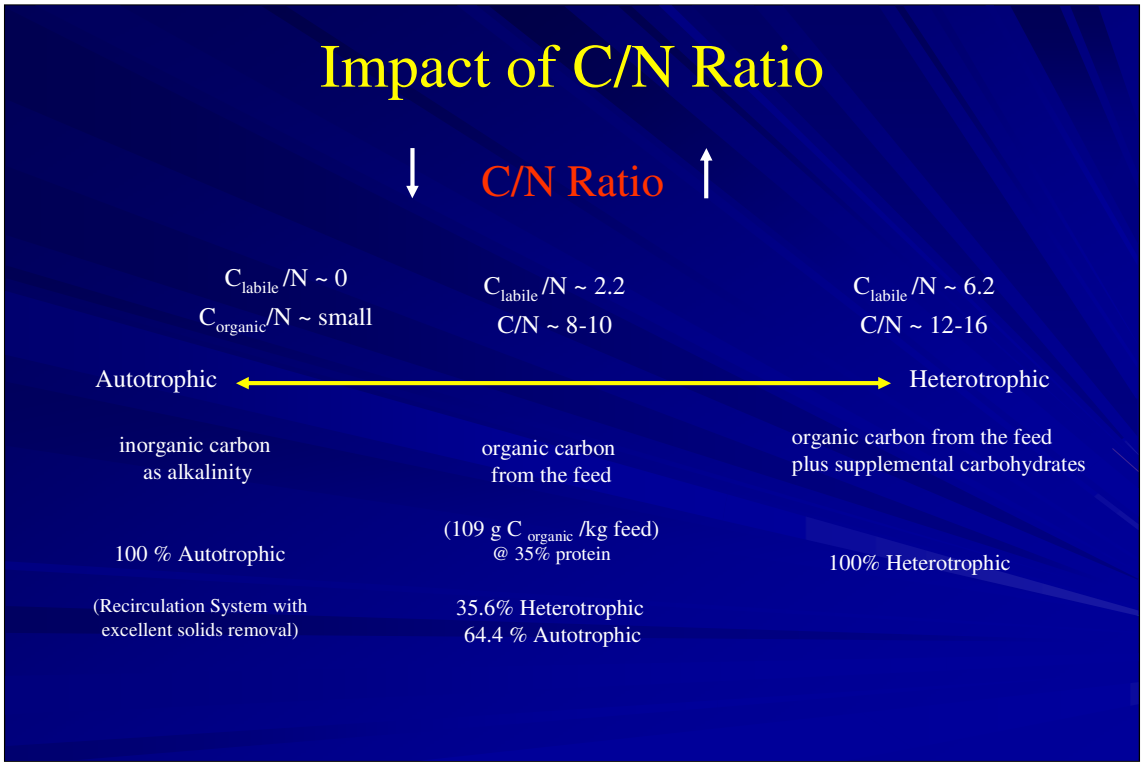
Heterotrophic Bacteria

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	-----

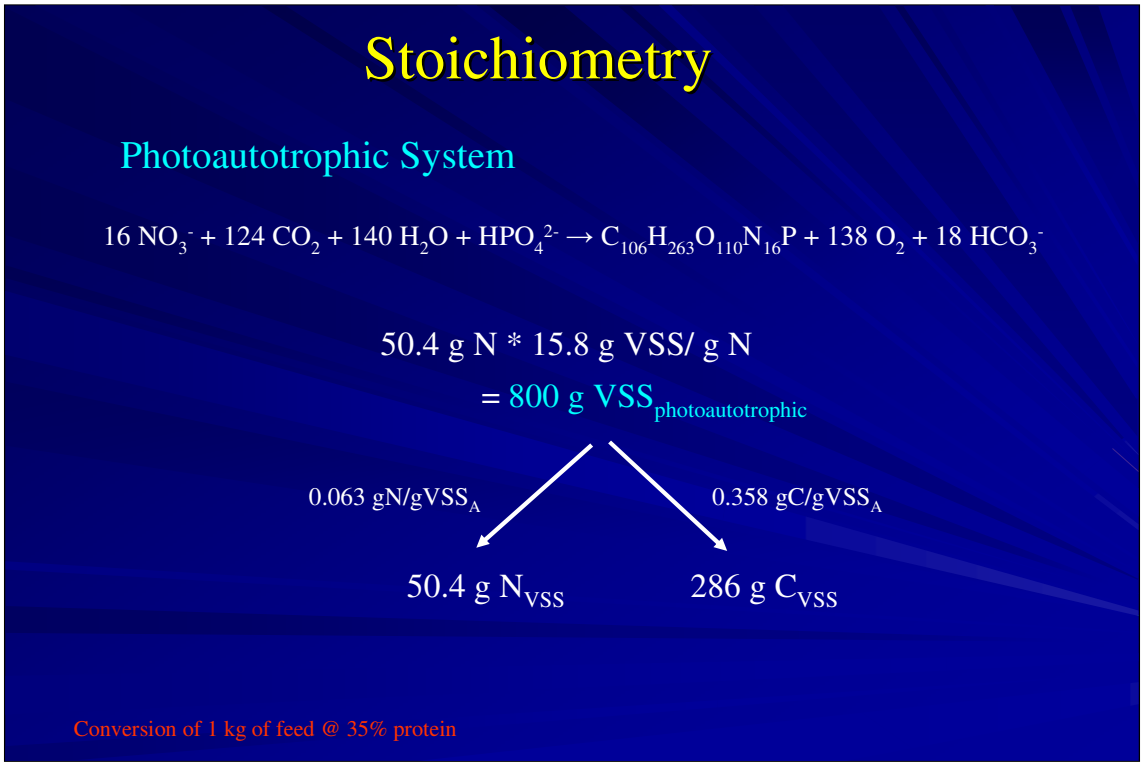
Heterotrophic Bacteria

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 $CaCO_3$), 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 CO_2 levels. Finally, there should be no production of nitrite-nitrogen, or nitrate-nitrogen in a pure heterotrophic system.



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.



Consumption:

911 g CO_2/kg feed

7.06 g P/kg feed

158 g Alk / kg feed

Production:

763 g O_2 / kg feed

Photoautotrophic (Pond intensive system)

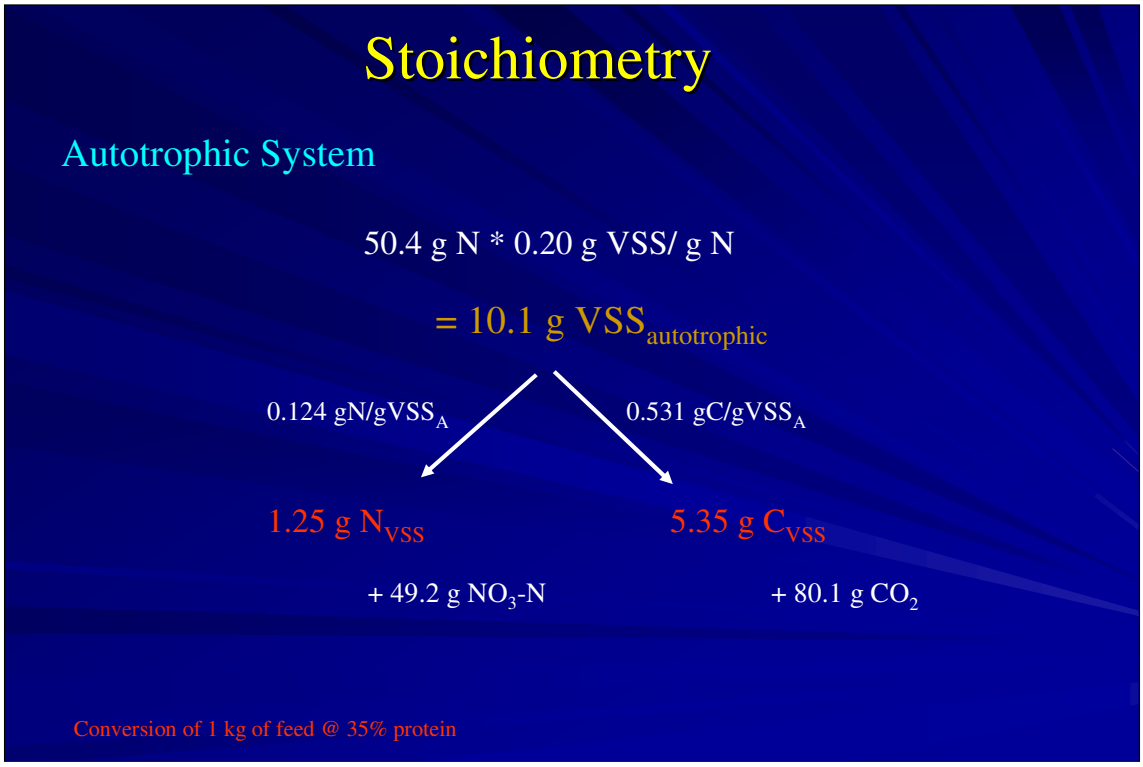
Consumables	Stoichiometry	Consumes (g)	$C_{organic}$ (g)	$C_{inorganic}$ (g)	N (g)
NH_4^+-N		50.4	-----	-----	50.4
Carbon Dioxide	18.07 g CO_2/ g N	911	-----	249	-----
Alkalinity	3.13 g Alk/ g N	158	-----	37.9	-----

Products	Stoichiometry	Yields (g)	$C_{organic}$ (g)	$C_{inorganic}$ (g)	N (g)
VSS_{Algae}	15.85 g VSS_A / g N	800	286	-----	50.4
O_2	15.14 g O_2/ g N	763	-----	-----	-----

Conversion of 1 kg of feed @ 35% protein

Conversion of 1 kg of feed @ 35% protein

For a pure photoautotrophic process (above Table), the mass of algal biomass can be calculated from the ammonia-nitrogen production rate and the VSS yield, approximately 800 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).



Microbial biomass assimilates 1.25 g N and 5.36 g C into bacterial biomass. Only 2.5% of the nitrogen is assimilated into biomass and only 6.2% of the carbon. Most of the nitrogen is converted into nitrate-nitrogen (49.2 g NO_3-N) and the carbon into carbon dioxide (295 g CO_2). In addition, 355 g of alkalinity as $CaCO_3$ is consumed as well as 211 g of oxygen.

Autotrophic (Intensive Recirculation System)

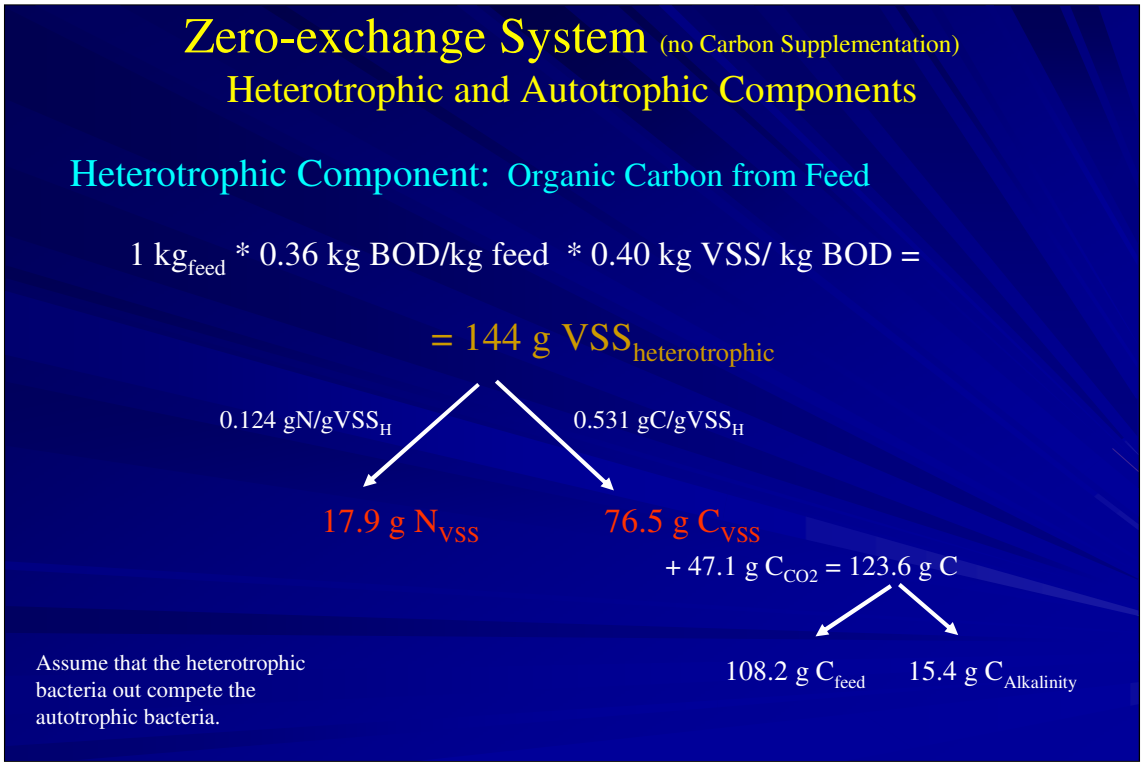
Consumables	Stoichiometry	Consumes (g)	$C_{organic}$ (g)	$C_{inorganic}$ (g)	N (g)
NH_4^+-N		50.4	-----	-----	50.4
Alkalinity	7.05 g Alk/ g N	355	-----	85.6	-----
Oxygen	4.18 g O_2/ g N	211	-----	-----	-----

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	-----

85.6 g C_{Alk} / 50.5 g N $\rightarrow$ C/N ratio of 1.7 and TOC is very, very small

Conversion of 1 kg of feed @ 35% protein

For a pure autotrophic nitrification process (Table above) 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 $CaCO_3$. 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.

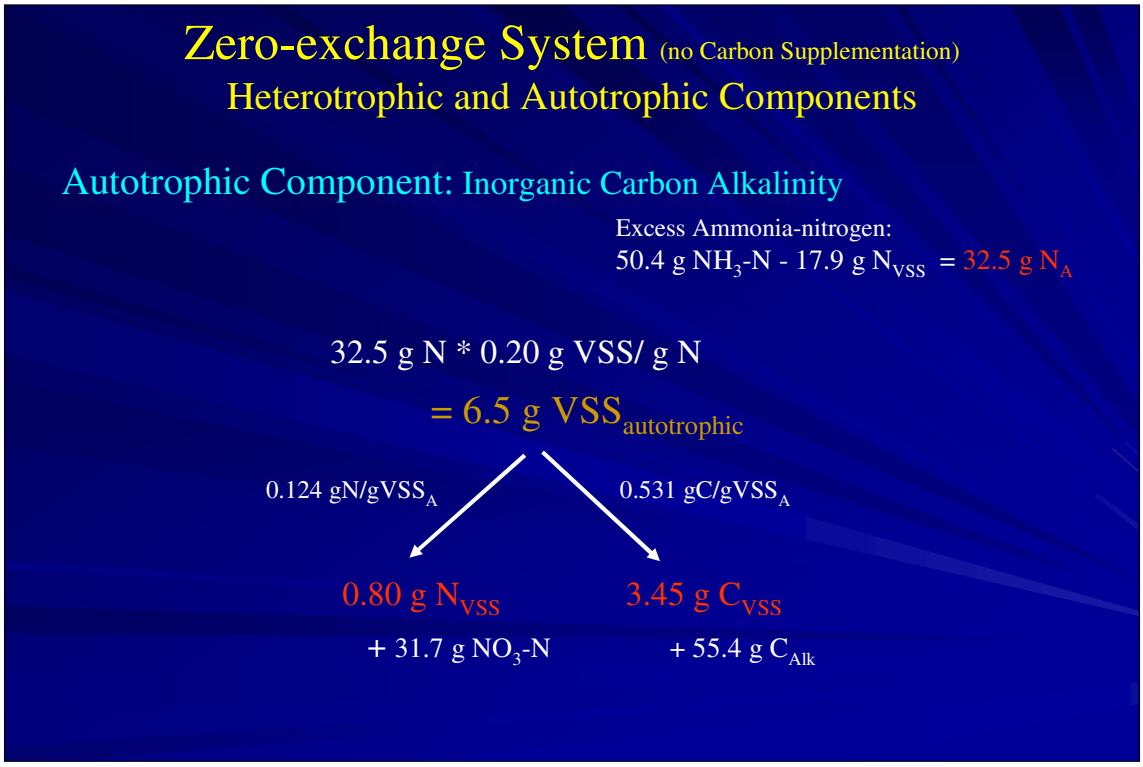


If we examine a simple zero-exchange system with no supplemental organic carbon addition, the solids remain in the production tank and all of the organic carbon from decomposing feed and fecal matter is available to the heterotrophic bacteria (Figure 1). Normally in recirculating systems, uneaten feed and fecal matter containing organic carbon is quickly removed from the production system, to prevent growth and build up of heterotrophic bacteria. In recirculating systems, heterotrophic bacteria are detrimental; in zero-exchange systems heterotrophic bacteria are beneficial. Since the growth rate of heterotrophic bacteria is significantly higher than autotrophic bacteria (Table 1) it is assumed that the heterotrophic bacteria will initially dominate the metabolism of ammonia-nitrogen until the organic carbon source becomes the limiting factor. The remaining ammonia-nitrogen not assimilated by the heterotrophic bacteria will then be assimilated by the autotrophic bacteria using alkalinity as an inorganic carbon source.

For this analysis, marine shrimp are being grown and for every kg of feed at 35% protein approximately 50.4 g of ammonia-nitrogen will be generated (Timmons et al., 2002; Brune, 2003). This was estimated based on chemical composition of protein being 0.16 g nitrogen per g protein and 90% of the nitrogen being excreted by the shrimp or:

1 kgfeed * [0.35 g protein/g feed * 0.16 g nitrogen/g protein * 0.90 excreted] = 50.4 g NH_3-N

One of the difficulties in this analysis was determining the fraction of the organic carbon that was available to the heterotrophic bacteria. It is straight forward to measure the carbon content of feed (approximately 40 to 50%), but only a small fraction of this organic carbon not metabolized by the shrimp is available to the bacteria. Thus using engineering data, an estimate was made based on the carbon sequestered by the Volatile Suspended Solids (VSS) generated and their known carbon content. It has been shown that aquaculture feeds express approximately 0.30 to 0.36 kg BOD per kg of feed (Zhu and Chen, 2001, Brune, 2003). Using a yield fraction of 0.40 kg VSSH per kg BOD and a BOD content of 0.36 kg per kg feed, suggests that a kg of feed should generate approximately 144 g of VSS_H. Since bacterial biomass (VSS) contains 53.1% C and 12.3% N based on stoichiometry (Ebeling et al., 2006), this heterotrophic microbial biomass would assimilate approximately 17.9 g nitrogen and 76.5 g of organic carbon. In addition from previous research trials, the long-term ratio of VSS to TSS for an autotrophic/heterotrophic system was found to average about 0.72. Thus, about 200 g of heterotrophic bacterial TSS_H is produced for every kg of feed fed into a system



Note that only 36% of the nitrogen is incorporated into by the heterotrophic bacteria, the remaining nitrogen (32.5 g N) is thus available to the autotrophic bacterial population. Using a yield fraction of 0.20 g VSS_A/g N (Table 1), produces 6.5 g VSS_A. Using the same C/N ratios as above yields 0.80 g of nitrogen and 3.45 g of carbon assimilated by the autotrophic microbial biomass. Thus only 0.80 g of nitrogen is incorporated into the autotrophic bacteria, and that the remaining is excreted as nitrate-nitrogen. Using the same ratio of TSS to VSS as above, only 9.0 g of TSS_A for every kg of feed is produced by the autotrophic bacteria. Combining the two forms of TSS, yields a total of 209 g TSS produced per kg feed. It is interesting to note that only about 1.6% of the nitrogen available is actually contained in the autotrophic microbial biomass, and about 36% in the heterotrophic microbial biomass. In addition, the mass of heterotrophic bacteria is more than twenty times the mass of the autotrophic bacteria produced.

Zero-exchange System (no Carbon Supplementation)
Heterotrophic and Autotrophic Components

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	-----

Consumables for a Zero exchange system with no carbon supplementation

In a pure zero-exchange system (Table above), 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 $CaCO_3$.

Zero-exchange System (no Carbon Supplementation)
Heterotrophic and Autotrophic Components

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	-----

460 g C_{feed} / 50.5 g N $\rightarrow$ C/N ratio of 9

Products for a Zero exchange system with no carbon supplementation

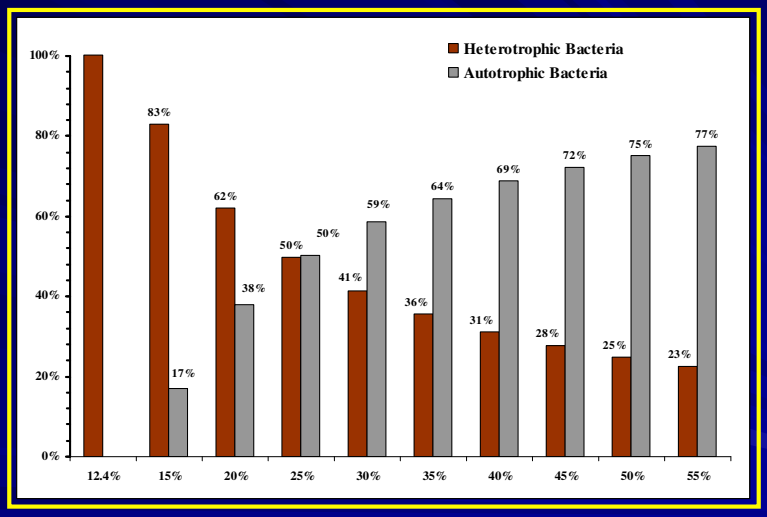
Since there is 50.4 g of nitrogen available from the feed, and only 17.9 g of nitrogen is sequestered by the heterotrophic bacteria, there remains 32.5 g of nitrogen to be assimilated by the autotrophic bacteria. Again using 0.20 g VSS per g of nitrogen, yields a production of 6.5 g of autotrophic bacteria 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.

It is somewhat more difficult to follow the carbon (inorganic and organic) consumption, since the carbon source can be either organic carbon from the feed (heterotrophic) or inorganic carbon from alkalinity (autotrophic).

Using the stoichiometric relationships developed in Ebeling et al., 2006, the total carbon consumed by the process is 124 g C, divided between organic carbon (109 g C_{feed}) metabolized directly by the heterotrophic bacteria and the depletion of alkalinity inorganic carbon (15.4 g $C_{alkalinity}$). All of the inorganic carbon consumed by the autotrophic bacteria (55.8 g Calkalinity) comes from alkalinity. Thus a total of 180 g of C per kg of feed is consumed by this pathway, divided between organic carbon (109 g C_{feed}) and alkalinity carbon (70.8g $C_{alkalinity}$) or 293 g of alkalinity as $CaCO_3$. Thus if feed contains on average approximately 46% carbon, only 25% of that organic carbon is available to the heterotrophic bacteria as labile carbon. In addition 220 g of oxygen are consumed and 363 g of carbon dioxide are produced.

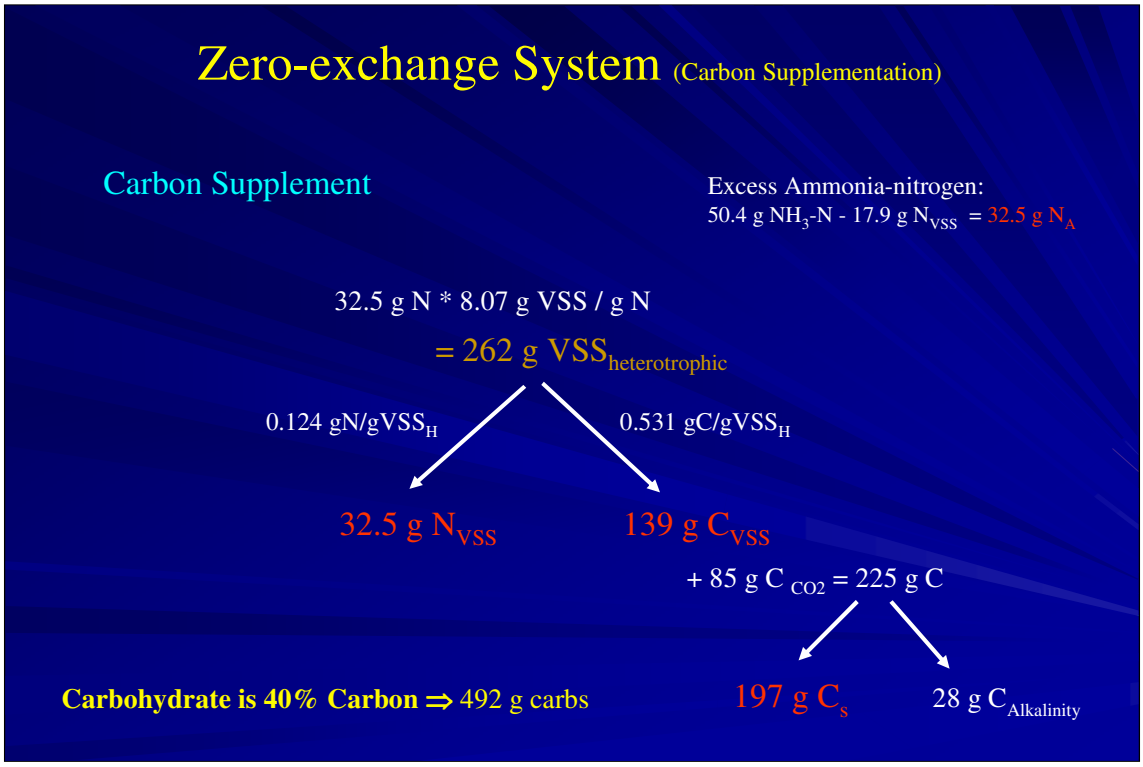
Zero-exchange System (no Carbon Supplementation)

Heterotrophic and Autotrophic Components



Percent removal of ammonia-nitrogen by Heterotrophic or Autotrophic Process as a function of % Protein

The percent protein content of feed determines the ratio of autotrophic versus heterotrophic removal of ammonia-nitrogen. This is because of the direct relationship between protein content and quantity of ammonia-nitrogen that is generated and that only a fixed quantity of labile carbon is available from the feed. The above graph shows how as the protein content of the feed increases, the percent removal of ammonia-nitrogen by the autotrophic pathway increases from complete removal by heterotrophic bacteria at 12.4 % protein content to 75% removal of ammonia-nitrogen by the autotrophic pathway at 50% protein content.



Heterotrophic bacteria – carbon supplementation

Consider next, a zero-exchange system where organic carbon is added to make up the difference between what is available from the feed and the total demand by the heterotrophic bacteria for complete conversion of all available nitrogen (Figure 2). From the above analysis, 32.5 g of nitrogen needs to be consumed by the additional heterotrophic bacteria from the supplemental organic carbon source. From Table 1, 8.07 g VSS_H per g of N are produced, thus an additional 262 g VSS_H are generated by the supplemental carbon. This additional VSS_H requires 225 g of carbon, divided between organic carbon (197 g CS) metabolized by the heterotrophic bacteria and the depletion of inorganic carbon (28 g $C_{alkalinity}$). Thus the total VSS_H generated is 406 g per kg feed. The research described in this paper found a ratio TSS to VSS of 81%, which then suggests a total TSS_H production of 500 g for every kg of feed. Thus a total of 349 g of C per kg of feed is consumed by this pathway, with the heterotrophic bacteria metabolizing organic carbon from the feed (109 g C_{feed}) and the supplemental organic carbon (197 g C_S). In this case sucrose ($C_{12}H_{22}O_{11}$) at 42% carbon was used requiring 470 g sucrose per kg feed. In addition, inorganic carbon as alkalinity was depleted (43.3 g $C_{alkalinity}$) or 180 g of alkalinity as $CaCO_3$. In addition 220 g of oxygen are consumed and 486 g of carbon dioxide are produced. In addition 237 g of oxygen (50.4 g NH_3-N x 4.71 g oxygen per g of nitrogen produced) are consumed and 486 g of carbon dioxide are produced.

Zero-exchange System (Carbon Supplementation)

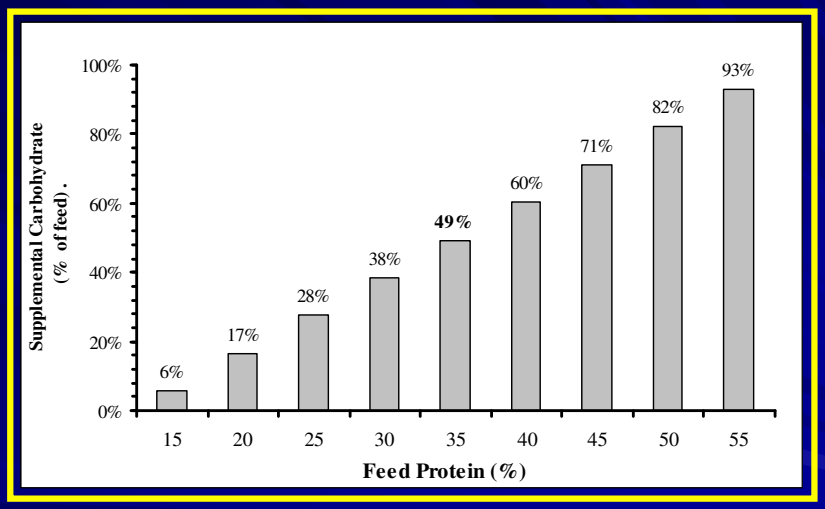
Consumables	Stoichiometry	Consumes (g)	$C_{organic}$ (g)	$C_{inorganic}$ (g)	N (g)
NH_4^+-N		50.4	-----	-----	50.4
$C_6H_{12}O_6$	15.17 g Carbs/ g N	765	306	-----	-----
Alkalinity	3.57 g Alk/ g N	180	-----	43.3	-----
Oxygen	4.71 g O_2/ g N	237	-----	-----	-----

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	-----

(460 g C_{feed} + 197 g C_{carb}) / 50.5 g N $\rightarrow$ C/N ratio of 13

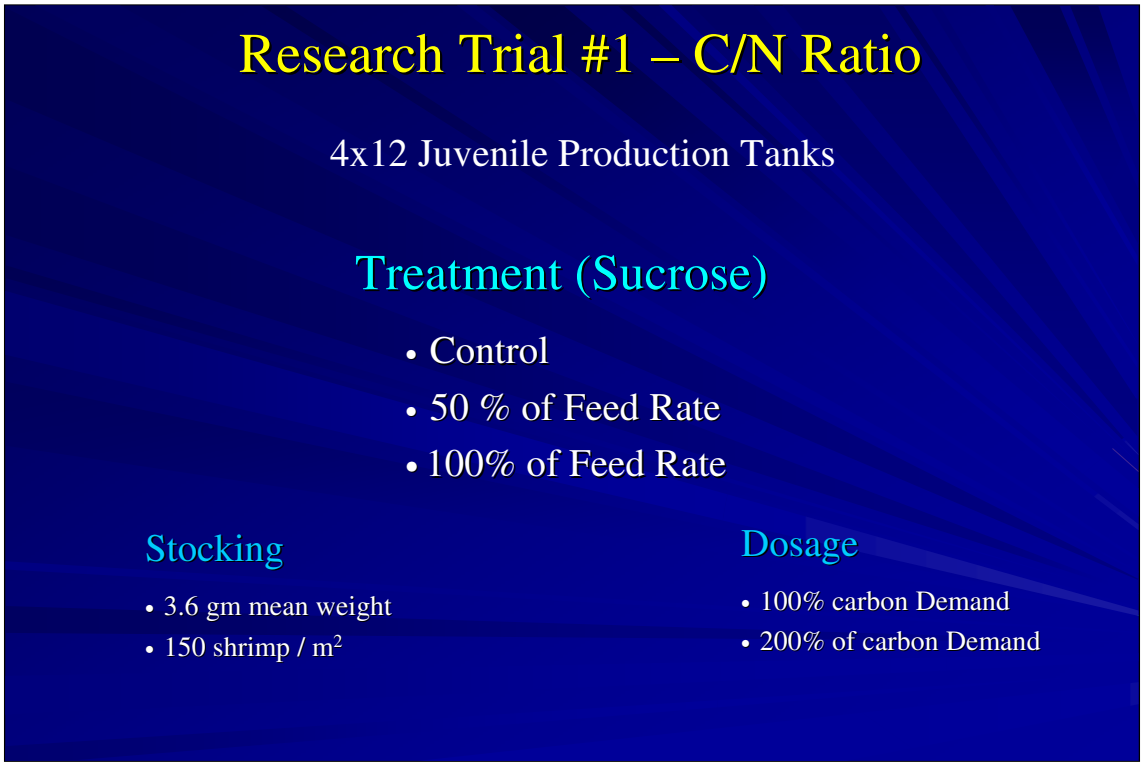
Thus the total VSS_H generated is 407 g per kg feed. The research described in this paper found a ratio TSS to VSS of 81%, which then suggests a total TSS_H production of 500 g for every kg of feed. Thus a total of 349 g of C per kg of feed is consumed by this pathway, with the heterotrophic bacteria metabolizing organic carbon from the feed (109 g C_{feed}) and the supplemental organic carbon (197 g C_S). In this case sucrose ($C_{12}H_{22}O_{11}$) at 42% carbon was used requiring 470 g sucrose per kg feed. In addition, inorganic carbon as alkalinity was depleted (43.3 g $C_{alkalinity}$) or 180 g of alkalinity as $CaCO_3$. In addition 220 g of oxygen are consumed and 486 g of carbon dioxide are produced. In addition 237 g of oxygen (50.4 g NH_3-N x 4.71 g oxygen per g of nitrogen produced) are consumed and 486 g of carbon dioxide are produced.

Zero-exchange System (Carbon Supplementation)



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

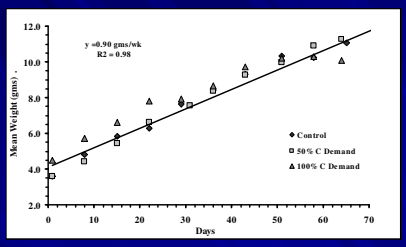
The above analysis is for a feed with a protein content of 35%. Additional calculations for other feed protein content are straight forward with additional organic carbon supplementation at high protein level, i.e. high ammonia-nitrogen production. The above figure shows this relationship for feed protein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



The two pathways for nitrogen removal are very different in terms of substrate utilization, bacterial biomass generated and by-products produced. The difficulty in practical application is that both may be present to some degree depending upon the availability of inorganic and organic carbon. The ability to control the carbon to nitrogen ratio by feed formulation, solids removal, or addition of organic carbon allows the aquaculture producer to manage what type of system is used. To examine this potential, a study was conducted were sufficient organic carbon in the form of the carbohydrate (sucrose) was added daily at 0%, 50% and 100% of the shrimp feed rate to three proto-type zero-exchange systems. These systems had been operated for several months as marine shrimp juvenile production systems and all had well matured bacterial 'soups'. The three systems were stocked with 675 *L. vannamei* marine shrimp at a density of 150/m2 with an initial average weight of 3.60 gms.

Research System

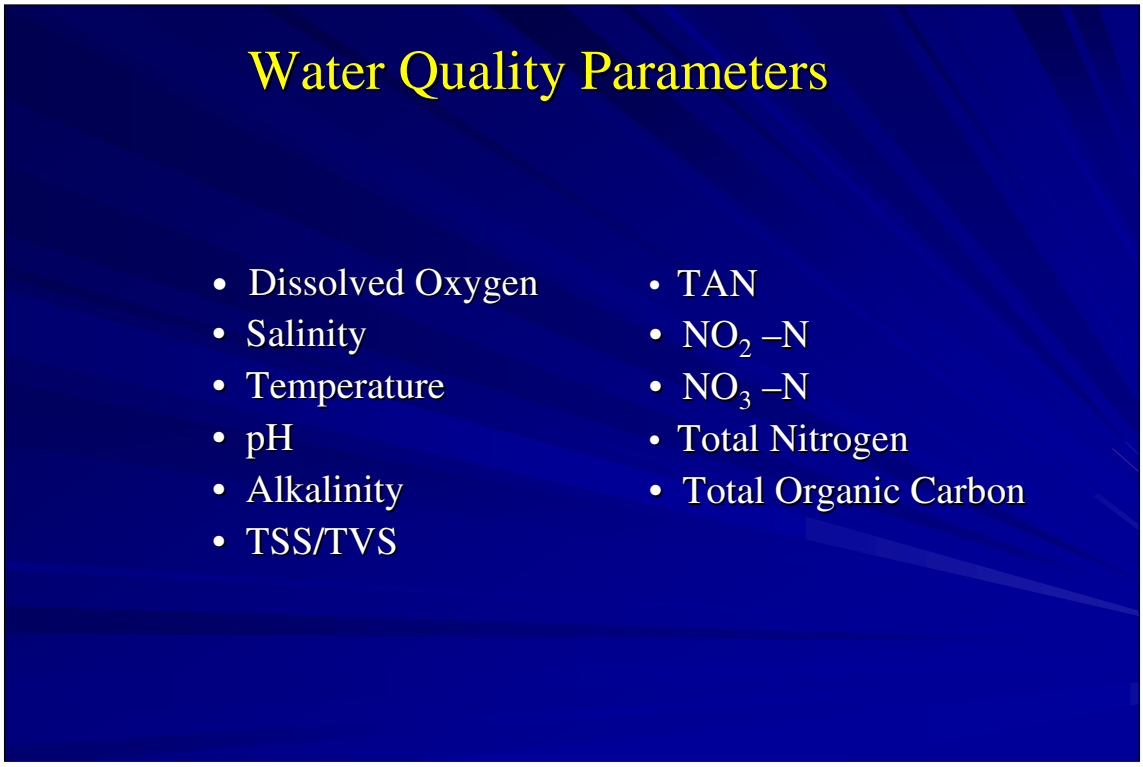
4x12 System with sludge settling tank, automatic feeders, bacterial floc



Weekly Growth – about 0.9 gm/wk

The juvenile production system (Figure above) consisted of rectangular fiberglass tanks, measuring 1.22 m x 3.66 m x 0.76 m (4 ft x 12 ft and 30 in). Water depth was maintained at 61 cm (24 in) with an outside standpipe. Outside stand pipes, 5 cm (2 in) in diameter were used to manage water removal and control water depth and a 7.6 cm (3 in) PVC drain line pipe was used to remove water or to harvest shrimp in bulk. In addition, a ¼" PVC mesh screen was placed at the discharge from the tanks. Tanks were initially covered with ¼" PVC mesh tops, but shade cloth was later added to help reduce stress on the juvenile shrimp and limit growth of photoautotrophic algae.

Two titanium, 1.8 kW, 240 VAC bayonet style heaters were mounted in each tank to maintain system temperature at approximately 30 ºC. These were controlled by a Ranco temperature control with temperature LED display and a DPDT power relay. In addition the control systems were designed to monitor low and high water levels and turn off the heaters if the water level dropped, at which time, an audible alarm alerted staff. The control systems worked extremely well, maintaining temperature with 1º C of set point. Aeration in the tanks was provided by four 5 x 30 cm (2x12 in) air stones and two 3.66 m (12 ft) lengths of aeration hose on each side of the tank. The aeration hose provided good mixing by creating two counter rotating cells along the long axis of the tank. Additional air stones were used when needed to maintain dissolved oxygen levels. Two Sweeny automatic feeders hung above the tanks and could be activated as often as every half hour. Fresh water was added as needed to make up for evaporation and minor losses. A simple clarifier (Foreground of figure on left) was used to harvest suspended solids from the tank when the TSS approached approximately 450 mg/L. Figure on the right shows the sampled weekly average weight of a sample of approximately 50 to 100 animals. Over the first four weeks of growout, survival average 90% in the three tanks with an average feed conversion ratio (FCR) of 1.8. During this phase of research, the shrimp were seen primarily as 'food processors' for conversion of the feed to either small particles or fecal matter.



Dissolved oxygen, temperature, and salinity were measured daily between the hours of 8 to 9 am. At the same time, grab samples were taken and filtered through an 8 - 12 µm filter paper (Hach, 506-59 Filter Paper) with the filtrate then used determine dissolved constituent concentrations, total ammonia-nitrogen (TAN), nitrite-nitrogen, nitrate-nitrogen, pH, and alkalinity. In addition, daily samples were also analyzed for total suspended solids (TSS) and volatile suspended solids (VSS). Weekly samples were analyzed for total organic carbon and total nitrogen. Standard methods were routinely used and where appropriate, primary standards were analyzed along with the samples for quality assurance.

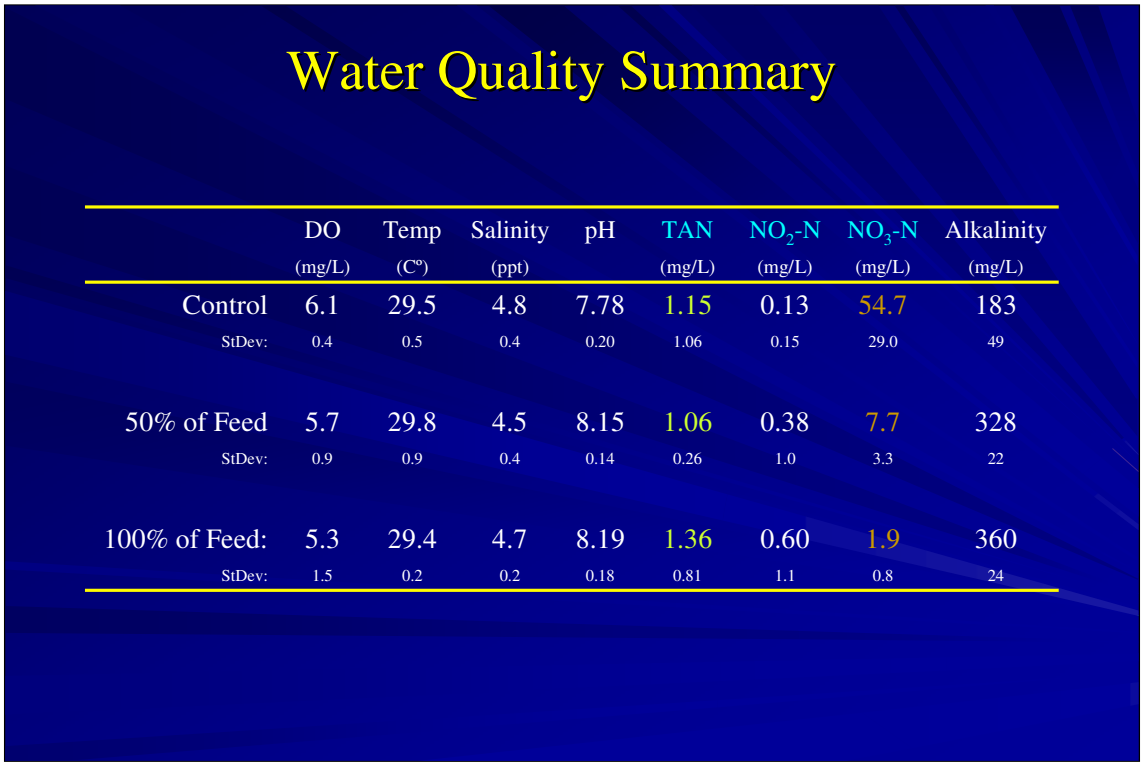


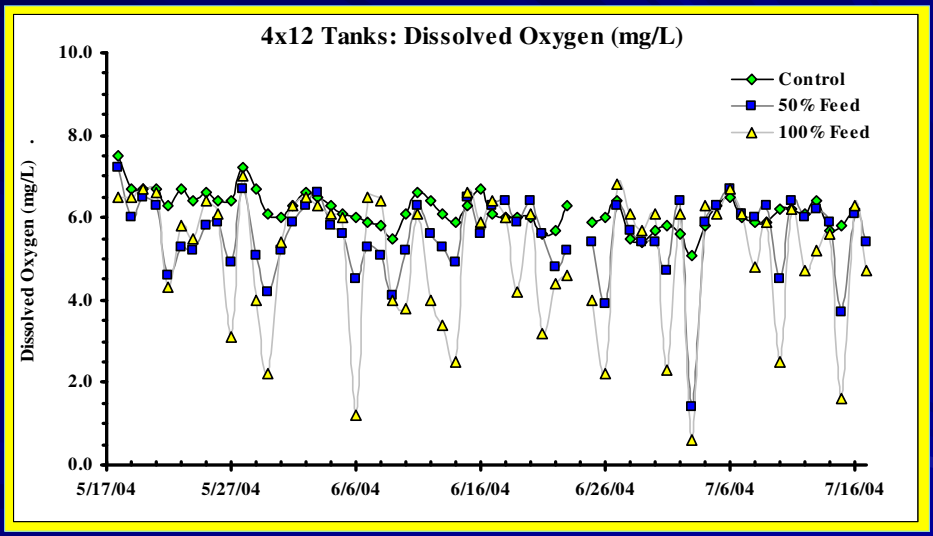
Table 3 presents the water quality data for the three treatments over the research period. Overall water quality in all three tanks was excellent. Note the substantial difference in nitrate-nitrogen and alkalinity between the control tank and the two tanks receiving supplemental carbon.

Water quality

As can be seen in the following figures, very different water quality is exhibited for the three treatments. Since the control tank received no supplemental organic carbon, it should exhibit water quality that is a combination of a heterotrophic and autotrophic system. For example, the above Table shows a significant lower mean pH for the control versus the two other treatments, which would be expected in an autotrophic system. The impact of the autotrophic bacteria is especially apparent in Figs. 8 and 9, with the increase of nitrate-nitrogen and the rapid decline in alkalinity. The alkalinity became so low in fact, that sodium bicarbonate was added on day 58 to increase it above the minimum recommended level of 150 mg/L (Timmons et al., 2002). In all three systems, TAN increased slowly over the research trial, but was never higher than 1.5 mg/L –N. For the control, nitrite-nitrogen was typically less than 0.10 mg/L, although it reached a maximum of 0.25 mg/L near the end of the trial.

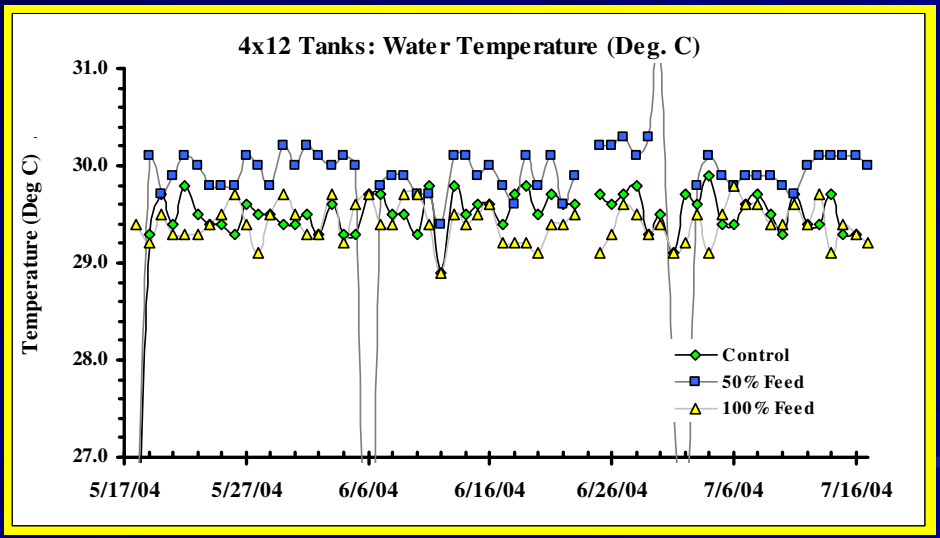
Both the treatments (50% and 100% of feed as carbohydrate) exhibited similar pH values, decreased slightly during the initial start-up phase, then increased and finally remained constant throughout the trial at about a pH of 8.3. The direct conversion of ammonia-nitrogen to bacterial biomass in these systems is nicely demonstrated in Fig. 8, where the nitrate-nitrogen concentrations are either very low or at barely detectable limits. The absence of autotrophic bacteria implies that no nitrite-nitrogen or nitrate-nitrogen is produced. The higher than expected nitrite-nitrogen concentrations in the 50% of feed as sucrose (Fig. 7) might be explained by a limited population of autotrophic bacteria that are inhibited by the high carbon/nitrogen ratios in the system from completing the conversion of TAN to nitrate. Near the end of the growout, the concentration of nitrite-nitrogen was significantly reduced, although it needs to be noted at no time was the concentration high enough to have any significant impact on the marine shrimp juveniles. The fact that the alkalinity (Fig. 9) increased and then remained constant during the growout trial is unexplained. Theoretically, alkalinity should be consumed by the heterotrophic bacteria, although at a much lower rate than for an autotrophic system. One explanation might be the recovery of alkalinity during some limited denitrification that may have occurred. Denitrification might be occurring in the interior of the large floc particles, where oxygen would be limited and anoxic conditions would prevail, which would potentially cause denitrification. This is an area that needs further research.

Water Quality Parameters - Dissolved Oxygen



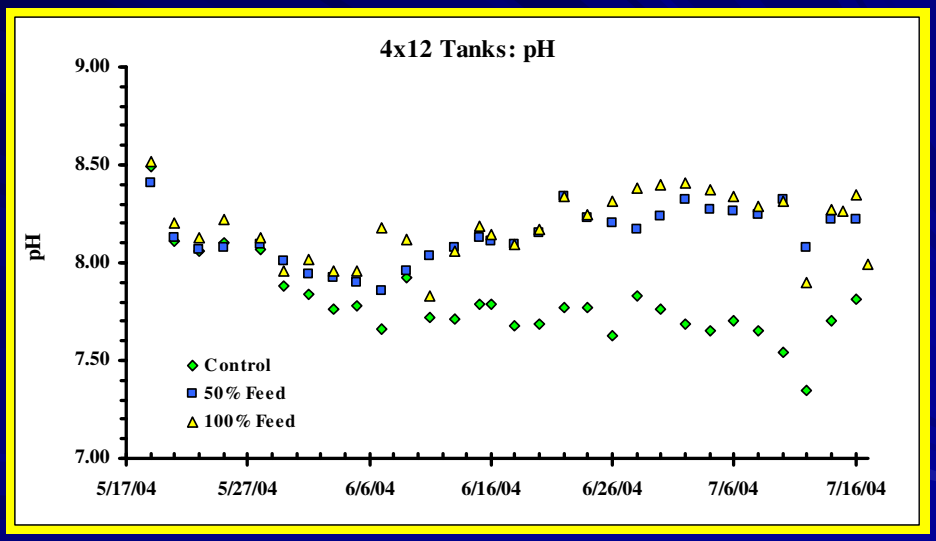
The dissolved oxygen for the control remained around 6 mg/L consistently over the research period. Both treatments exhibited low DO values as the heterotrophic bacteria increased over time and immediately before harvesting of excess microbial biofloc. At no times did the shrimp appear to be stressed by the low values.

Water Quality Parameters - Temperature



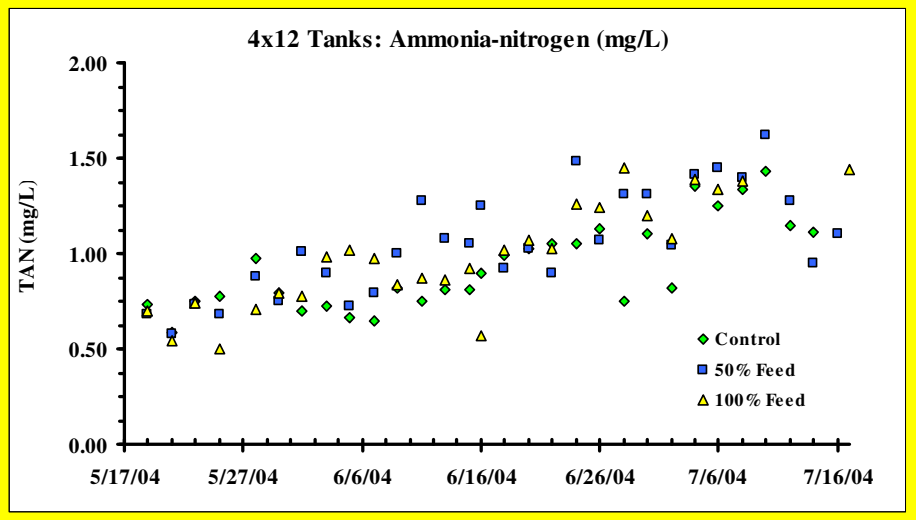
All three tanks maintained a temperature between 29 and 30 Deg C, except on two occasions due to equipment problems in the 50% feed tank. Both problems was traced back to electrical problems, a blown fuse in one case and a short circuit in a wiring junction box.

Water Quality Parameters - pH



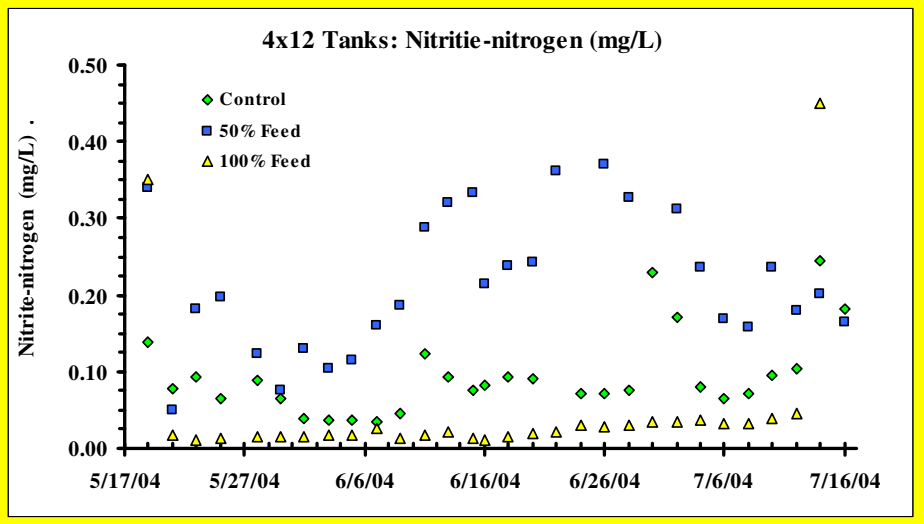
All three tanks started out initially at a pH of about 8.5. After two weeks, they all began to diverge from one another, with the control tank falling as would be expect with an autotrophic process and the two heterotrophic tanks stabilizing at about 8.3.

Water Quality Parameters - TAN



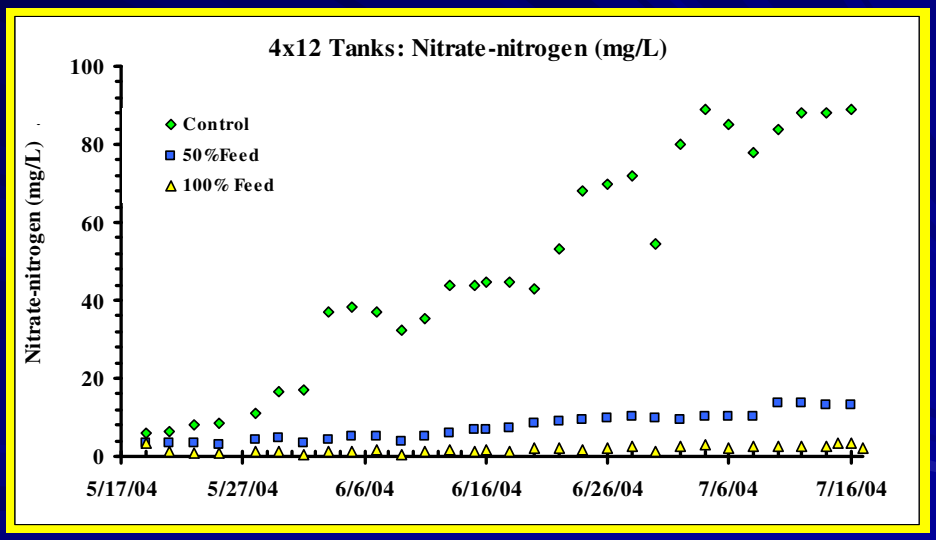
Ammonia-nitrogen concentration remained relative constant and consistent between the three tanks, with only a slight increase over the research period.

Water Quality Parameters – NO_2- N



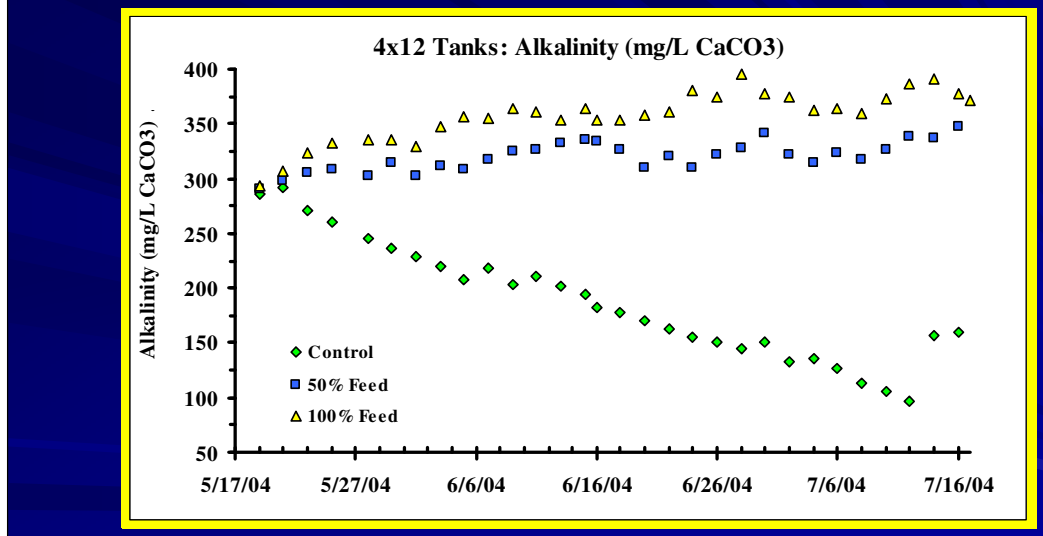
There is a significant difference in the nitrite-nitrogen concentrations between the three treatments, although all values are far below levels that would impact production by several orders of magnitude. The 100% of feed addition points out nicely that with a pure heterotrophic system, there is potentially no nitrite-nitrogen production.

Water Quality Parameters – NO_3- N



The graph of nitrate-nitrogen shows exactly what one would expect from an autotrophic/heterotrophic system, increasing nitrate-nitrogen over time and the very low values in a pure heterotrophic system.

Water Quality Parameters – Alkalinity



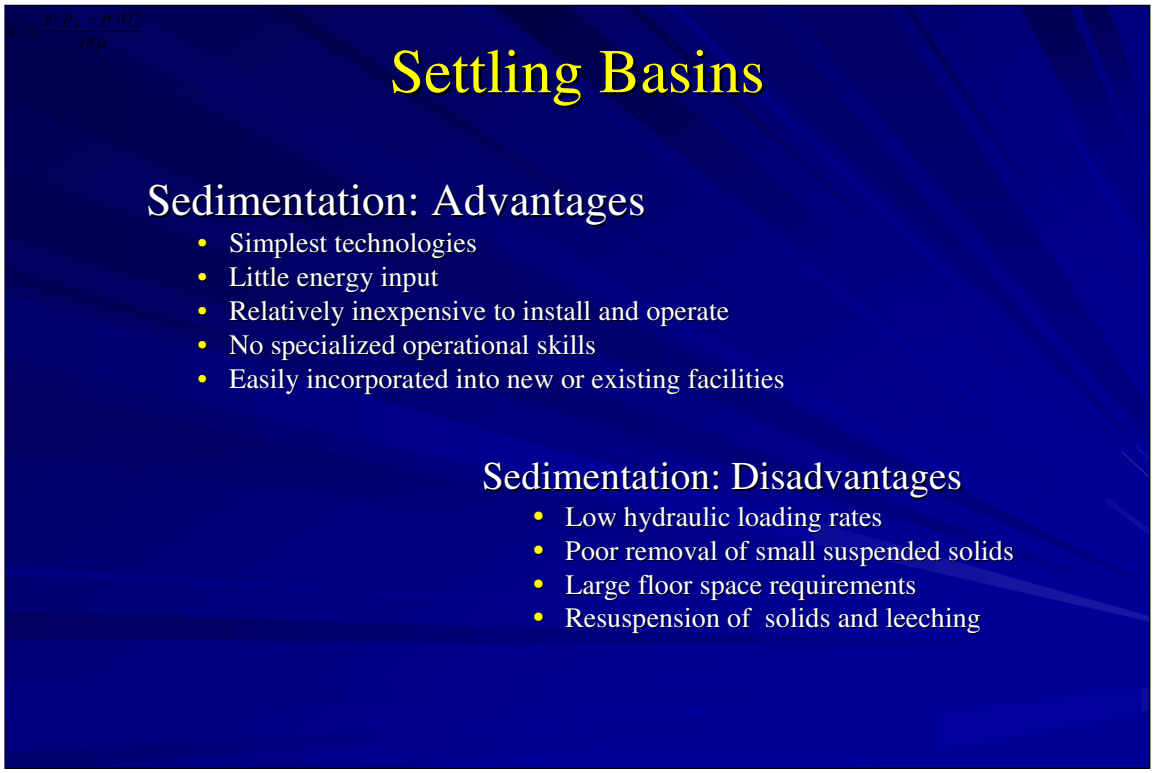
The impact of the autotrophic bacteria is especially apparent in the above figure, with the rapid decline in alkalinity. The alkalinity became so low in fact, that sodium bicarbonate was added on day 58 to increase it above the minimum recommended level of 150 mg/L (Timmons et al., 2002). The fact that the alkalinity increased and then remained constant during the growout trial is unexplained. Theoretically, alkalinity should be consumed by the heterotrophic bacteria, although at a much lower rate than for an autotrophic system. One explanation might be the recovery of alkalinity during some limited denitrification that may have occurred. Denitrification might be occurring in the interior of the large floc particles, where oxygen would be limited and anoxic conditions would prevail, which would potentially cause denitrification. This is an area that needs further research.

Solids Management

Solids Management:

- Settling cones

A simple settling cone was designed and tried with several experimental juvenile production tanks managed at three levels of C/N ratios.



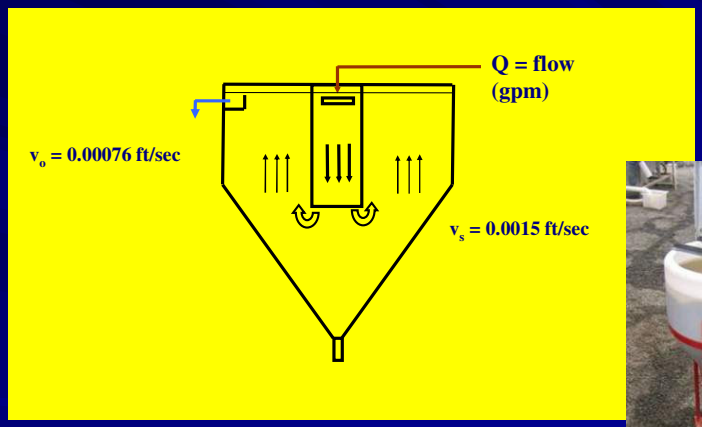
Settling basins are very effective if properly configured and operated. Sedimentation, i.e., gravity separation, is one of the simplest of technologies available to control particulate solids in process water and wastewater. Sedimentation basins require little energy input, are relatively inexpensive to install and operate, require no specialized operational skills, and can be easily incorporated into both new and existing facilities.

The disadvantages of sedimentation are low hydraulic loading rates and poor removal efficiency of small suspended solids (<100 μm). Also, they require additional floor space for their incorporation in comparison to microscreen filters. Innovative uses of vertical space over the settling bed or placing the settling bed in less expensive space can reduce this cost considerably.

Another potentially serious disadvantage is that settled manure remains in the system until the settling basin is cleaned. This condition is one of the major concerns in their use. Dissolution of nutrients and the resuspension of solids that have settled and collected on the bottom of settling basins can markedly reduce the expected performance of these clarifiers (Cripps and Kelly, 1996). Henderson and Bromage (1988) estimated that settling ponds could capture an estimated 97% of their solids loading if resuspension of settled solids was not a factor. They suggest that settling basins are not effective in removing TSS when inlet concentrations are <10 mg/L or attaining effluent concentrations of <6 mg/L. Eliminating resuspension of TSS is difficult at best in most settling basins. Thus, settling basins will generally require further TSS treatment to meet the stringent removal criteria necessary to achieve mandated levels of TSS.

Settling Basins

Design to minimize turbulence:



All continuous flow settling basins are conceptually divided into four zones according to function, see above. The inlet zone serves to uniformly distribute the suspension over the entire cross-section of the basin. Sedimentation occurs in the settling zone and, upon removal from the water column, the solids accumulate in the sludge zone. The clarified liquid is generally collected over the entire cross-section of the basin at the outlet zone and is discharged. Under ideal conditions (no mixing or turbulence), required retention time is the time required for a particle that starts at the top of the inlet zone and settles to the floor of the basin at or before the junction of the outlet zone. The key parameter for the design of settling basins is the volumetric flow of water per unit surface area of the basin or overflow rate (V_o).

Any particle with a settling velocity (V_s) greater than the overflow rate (V_o) will settle out of suspension. Other particles, for which $V_s < V_o$, will be removed in the ratio V_s/V_o, depending upon their vertical position in the tank at the inlet.

Autotrophic/Heterotrophic Model

Control
Tank "A"

Day	Initial (mg/L)	Final (mg/L)	TSS Removed (gms)	% Removed
24	466	210	666	55%
43	487	260	590	47%
56	462	263	517	43%
		Mean:	591	

Tank “A” received no additional carbon as sucrose and required culling of excess solids every two weeks. The settling cone was operated for only 6 to 8 hours to remove about 50% of the Total Suspended Solids.

Heterotrophic Model (50% feed)

50% of Feed
Tank "C"

Day	Initial (mg/L)	Final (mg/L)	TSS Removed (gms)	% Removed
11	379	218	419	42%
22	441	134	798	70%
34	449	206	632	54%
42	441	242	517	45%
52	482	218	686	55%
60	485	313	447	35%
		Mean:	583	

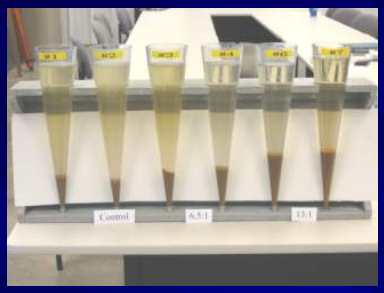
Tank “B” received sufficient carbon to completely convert the ammonia-nitrogen from the feed into microbial biomass. Total suspended solids were harvested about every 10 to 11 days with good removal efficiency.

Heterotrophic Model (100% feed)

100% of Feed
Tank "B"

Day	Initial (mg/L)	Final (mg/L)	TSS Removed (gms)	% Removed
7	381	165	562	57%
13	448	220	591	51%
20	604	192	1071	68%
25	569	304	689	47%
28	490	219	705	55%
32	445	208	616	53%
35	422	187	611	56%
40	507	185	837	64%
45	520	264	666	49%
49	490	254	614	48%
54	463	350	294	24%
58	673	587	224	13%
62	579	338	627	42%
		Mean:	623	

TSS Production Model



Solids Production Model:

- autotrophic/heterotrophic
- heterotrophic

A simple model to predict VSS and TSS concentrations in the three systems was written using an EXCEL spread sheet. The three systems were modeled as a mixed autotrophic/heterotrophic system (control) and as a pure heterotrophic system (50% and 100% of feed as sucrose). As was previously shown, the amount of sucrose required to fulfill the carbon requirement to consume all of the ammonia-nitrogen produced by the feed is approximately 470 g sucrose / kg feed, or 47% of the feed as sucrose. Thus the system supplemented with 50% of feed as sucrose should be a pure heterotrophic system and the system supplemented with 100% of feed as sucrose should be overdosed and the effect on TSS is unknown.

Autotrophic/Heterotrophic Model

- allocated the daily feed organic carbon to heterotrophic bacterial production,
- calculated VSS_H, [VSS_H = feed g/m^3 day * 0.36 g BOD/g feed * 0.40 g VSS_H / g BOD]
- calculated amount of ammonia-nitrogen assimilated in the VSS_H, [$TAN_H = 0.123 * VSS_H$]
- subtracted TAN_H from the daily TAN_{feed} produced,

  [TAN_{feed} = feed g/m^3 day * (0.35 * 0.16 * 0.9)]
- allocated excess ammonia-nitrogen to autotrophic bacterial consumption,

  [$TAN_A = TAN_{feed} - TAN_H$]
- determined VSS_A [$VSS_A = TAN_A$ * 0.20 g VSS_A/g N]
- calculated Total VSS and TSS.

In the case of the control, the model:

- allocated the daily feed organic carbon to heterotrophic bacterial production,
- calculated VSS_H, [VSS_H = feed g/m3 day * 0.36 g BOD/g feed * 0.40 g VSS_H / g BOD]
- calculated amount of ammonia-nitrogen assimilated in the VSS_H, [TAN_H = 0.123 * VSS_H]
- subtracted TAN_H from the daily TAN_{feed} produced,

  [TAN_{feed} = feed g/m^3 day * (0.35 * 0.16 * 0.9)]
- allocated excess ammonia-nitrogen to autotrophic bacterial consumption,

  [$TAN_A = TAN_{feed} - TAN_H$]
- determined VSS_A [VSS_A = TAN_A * 0.20 g VSS_A/g N]
- calculated Total VSS and TSS.

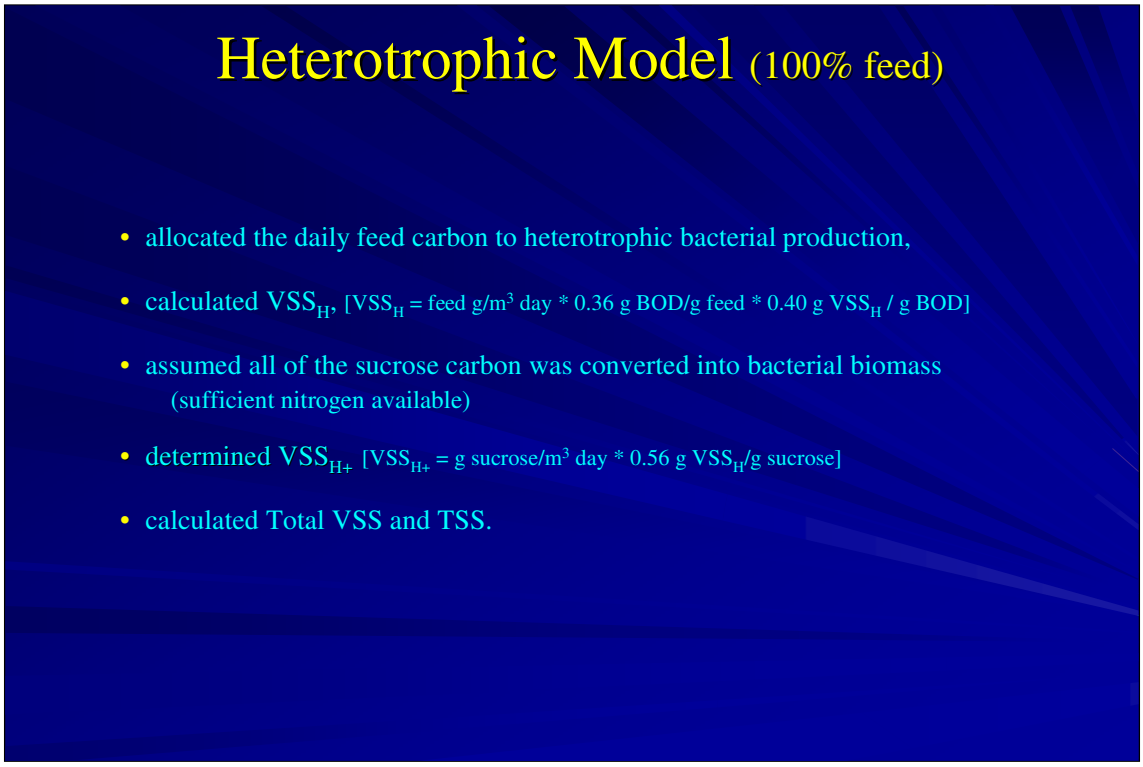
Heterotrophic Model (50% feed)

- allocated the daily feed carbon to heterotrophic bacterial production,
- calculated VSS_H, [VSS_H = feed g/m^3 day * 0.36 g BOD/g feed * 0.40 g VSS_H / g BOD]
- calculated amount of ammonia-nitrogen sequestered in the VSS_H, [$TAN_H = 0.123 * VSS_H$]
- subtracted from the daily TAN_{feed} produced, [TAN_{feed} = feed g/m^3 day * (0.35 * 0.16 * 0.9)]
- allocated excess ammonia-nitrogen to additional heterotrophic bacterial production,

  [$TAN_{H+} = TAN_{feed} - TAN_H$]
- determined VSS_{H+} [$VSS_{H+} = 8.07$ g VSS_H/g N * g N]
- calculated Total VSS and TSS.

In the case of 50% of feed as sucrose, the model:

- allocated the daily feed carbon to heterotrophic bacterial production,
- calculated VSS_H, [VSS_H = feed g/m^3 day * 0.36 g BOD/g feed * 0.40 g VSS_H / g BOD]
- calculated amount of ammonia-nitrogen sequestered in the VSS_H, [$TAN_H = 0.123 * VSS_H$]
- subtracted from the daily TAN_{feed} produced, [TAN_{feed} = feed g/m^3 day * (0.35 * 0.16 * 0.9)]
- allocated excess ammonia-nitrogen to additional heterotrophic bacterial production, [$TAN_{H+} = TAN_{feed} - TAN_H$]
- determined VSS_{H+} [$VSS_{H+} = 8.07$ g VSS_H/g N * g N]
- calculated Total VSS and TSS.



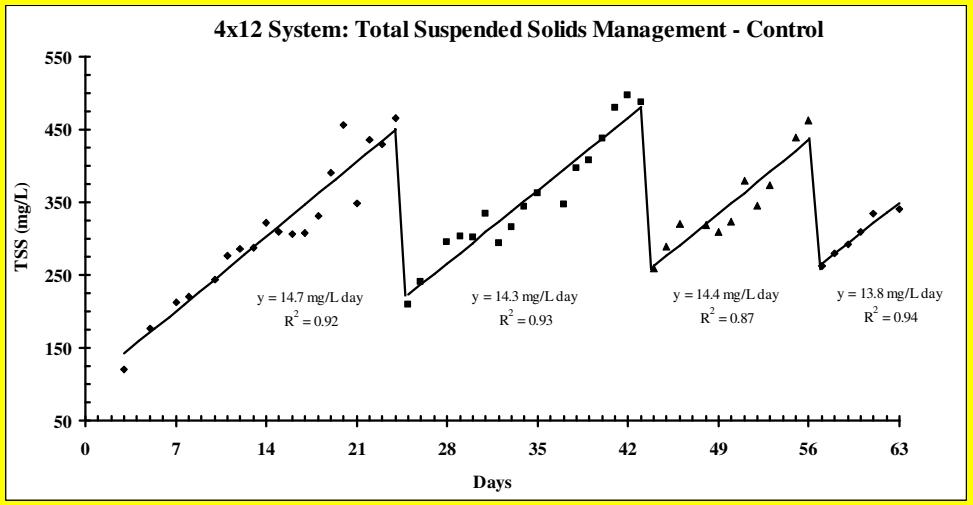
Finally, in the case of 100% feed as sucrose, it was observed that significant quantities of TSS were produced far in excess of the available nitrogen. Thus the assumption was made that somehow there was sufficient nitrogen to react with all of the available carbon from the sucrose.

In the case of 100% of feed as sucrose, the model:

- allocated the daily feed carbon to heterotrophic bacterial production,
- calculated VSS_H, [VSS_H = feed g/m^3 day * 0.36 g BOD/g feed * 0.40 g VSSH / g BOD]
- assumed all of the sucrose carbon was converted into bacterial biomass
- [VSS_{H+} = g sucrose/m^3 day * 0.56 g VSS_H/g sucrose]
- calculated Total VSS and TSS.

In each case, the TSS values were estimated based on the long term average of the measured ratio of TSS to VSS determined during the course of this research period for the heterotrophic system.

Autotrophic/Heterotrophic Model

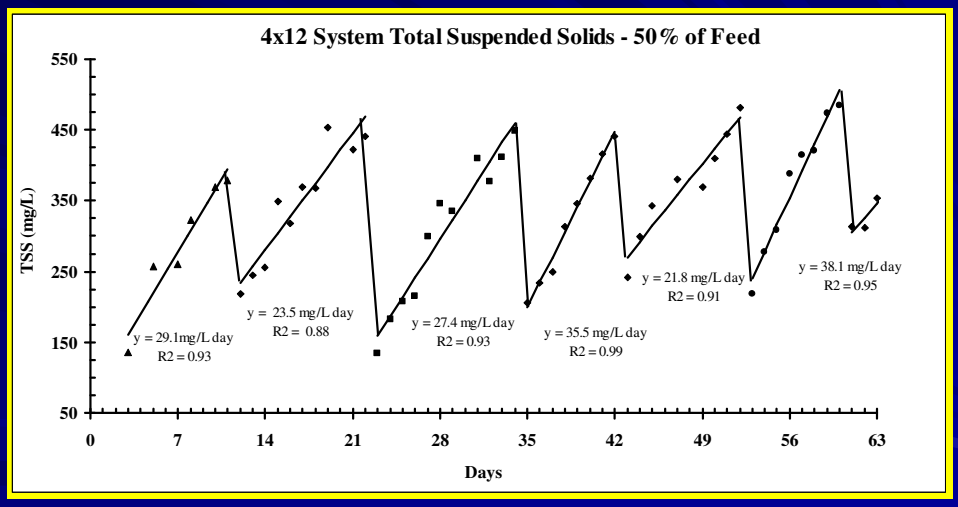


Control: Autotrophic and Heterotrophic Bacteria – No carbon Supplementation

For this analysis it will be assumed that for every kg of feed at 35% protein, approximately 50 g of ammonia-nitrogen will be generated per kg of feed, if we include the urea. If we look at a simple zero-exchange system with no supplemental carbon addition, the solids remain in the production tank and all of the carbon is available for either autotrophic or heterotrophic bacterial production. Since the growth rate of heterotrophic bacteria is much higher than autotrophic bacteria, it can be assumed that the available carbon will first be used by the heterotrophic bacteria, and any additional carbon and nitrogen then assimilated by the autotrophic bacterial. It has been determined that aquaculture feeds express approximately 0.30 to 0.36 kg BOD per kg of feed (Zhu and Chen, 2001, Brune, 2003). Using a yield fraction of 0.40 kg VSS per kg BOD and a BOD content of 0.36 kg per kg feed, suggests that a kg of feed would generates approximately 144 g of VSS. This heterotrophic microbial biomass sequesters approximately 76.3 g of carbon and 17.7 g N, since bacterial biomass (VSS) contains 53.1% C and 12.3% N based on stoichiometry. From this set of research trials, the long-term average ratio of TSS to VSS for heterotrophic bacteria was found to be about 0.72. Thus, about 200 g of heterotrophic bacterial TSS is produced for every kg of feed fed into a system. Note that less than half of the nitrogen is sequestered by the heterotrophic bacteria. The remaining nitrogen is available to the autotrophic bacterial population and using a yield fraction of 0.31 g VSS/g N, produces 10.0 g VSS. The autotrophic microbial biomass sequesters approximately 5.3 g of carbon and 1.24 g of nitrogen. This quantity of nitrogen may seem small, but is due to the poor conversion efficiency of carbon and nitrogen by autotrophic bacteria to microbial biomass and the larger fraction of NO_3 –N produced. Using the same ratio of TSS to VSS, yields a TSS production of 13.9 g for every kg of feed or a total of 214 g per kg feed. It is interesting to note, that only about 2.5% of the nitrogen available is actually contained in the autotrophic microbial biomass, and about 35.4% in the heterotrophic microbial biomass. The same is true of the carbon (20% retained in microbial biomass) with most of the carbon released as carbon dioxide. Normally in recirculating systems, the remaining carbon that is not used by the autotrophic bacteria is quickly removed from the production system, to prevent build up of heterotrophic bacteria. In recirculating systems heterotrophic bacteria are ‘bad’, in zero-exchange systems heterotrophic bacteria are ‘good’.

The above figure shows the daily measured TSS and the model predication for the control case of no carbon supplementation. As can be seen from the figure, there is an excellent agreement between the measured and the modeled TSS production over most of the growout period. For this case, autotrophic bacteria convert a substantial fraction of the ammonia-nitrogen to nitrate-nitrogen and Figure 4.5 shows the measured (1.63 mg N/L day) and the modeled production for nitrate-nitrogen (1.43 mg N/L day). Again, there is good agreement over most of the growout period, although the model under predicts the rate of nitrate-nitrogen production by about 12%., suggesting a higher than predicated ammonia-nitrogen production. The fall off at the end is probably due to other water quality problems experienced, but is beyond the normal growout period of from 45 to 60 days for either juvenile production or final growout to market size animals.

Heterotrophic Model (50% feed)

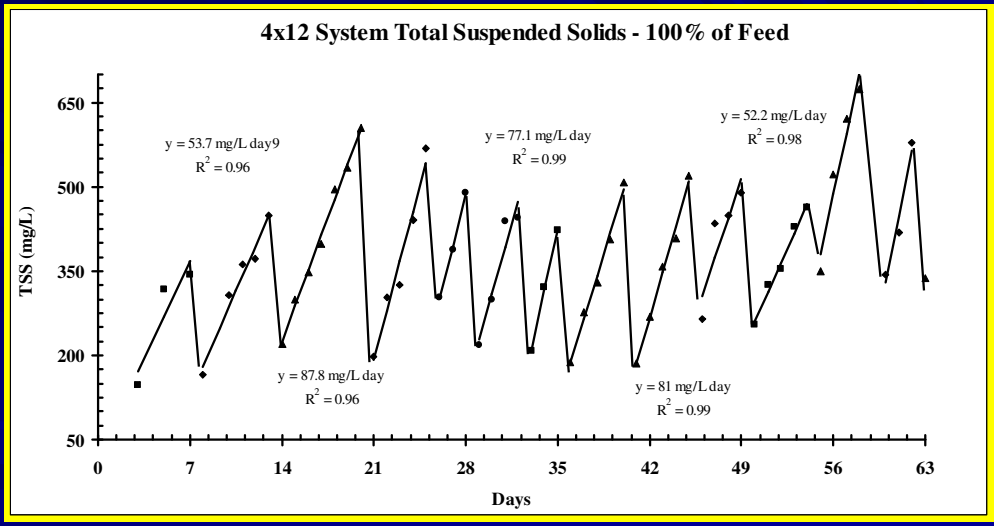


50% of feed as sucrose (carbon)

Consider next, a zero-exchange system where carbon is added to make up the difference between that being available from feed and the total demand from the heterotrophic bacteria for complete conversion of available nitrogen. From above, 32.3 g of nitrogen needs to be consumed by the additional heterotrophic bacteria and from Table 4.3 the stoichiometric conversion rate is 14.51 g sucrose/g N. Thus the sucrose requirement will be 469 g, or approximately 47% of the feed as sucrose., very close to the ratio used in this research trial. Using a yield factor of 0.56 g VSS/g sucrose, produces an additional 262 g VSS per kg of feed, or a total of 424 g of VSS per gram of feed. Using the long term average ratio of TSS to VSS of 81% determined from this research trial, yields a total TSS production of 524 g for every kg of feed.

The above figure shows the daily measured TSS and the model predication for the case where sucrose was added at 50% of the feed rate. Based on the stoichiometry, this should be about equal to the required supplemental carbon to completely assimilate the ammonia-nitrogen production from the feed. The graph shows a relatively close agreement between the predicated and the measured values for TSS. In addition, the model reflects very nicely the change in TSS production rate around day 60, when sucrose was inadvertently not added to the system. Since all of the ammonia-nitrogen should be assimilated by the heterotrophic bacteria, there should be minimal nitrate-nitrogen production. Daily measured values though indicate a rate of production of 0.19 mg N/L day, again suggesting a higher than projected ammonia-nitrogen production rate from the feed. Since the yield of biomass from nitrifying bacteria is so low, the effect of the autotrophic bacteria on the total TSS is small.

Heterotrophic Model (100% feed)

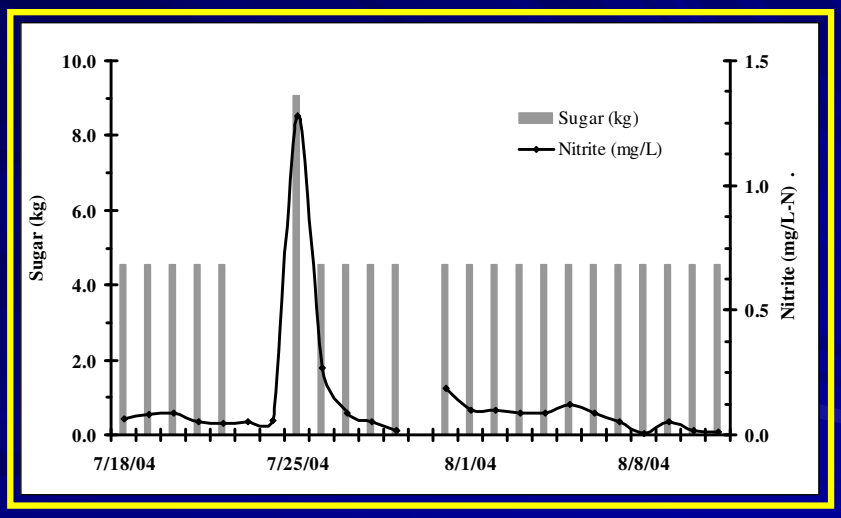


100% of feed as sucrose (carbon)

Finally, consider a zero-exchange system where excess carbon is added beyond what is stoichiometrically required to consume all of the nitrogen from the feed. In this case, there should be the same TSS production as the previous one, since the optimal demand for sucrose is approximately 47%. Yet the research trial showed an excessive amount of TSS production and no excess total organic carbon buildup, which suggests that sufficient nitrogen was made available to convert all of the available carbon into microbial biomass. Thus the VSS production of the sucrose would amount to 560 g per kg of feed, based on a yield factor of 0.56 g VSS/g sucrose or a total VSS production of 704 g per kg of feed. Using the ratio of TSS to VSS from research trials of 86%, yields a total TSS production of 819 g for every kg of feed.

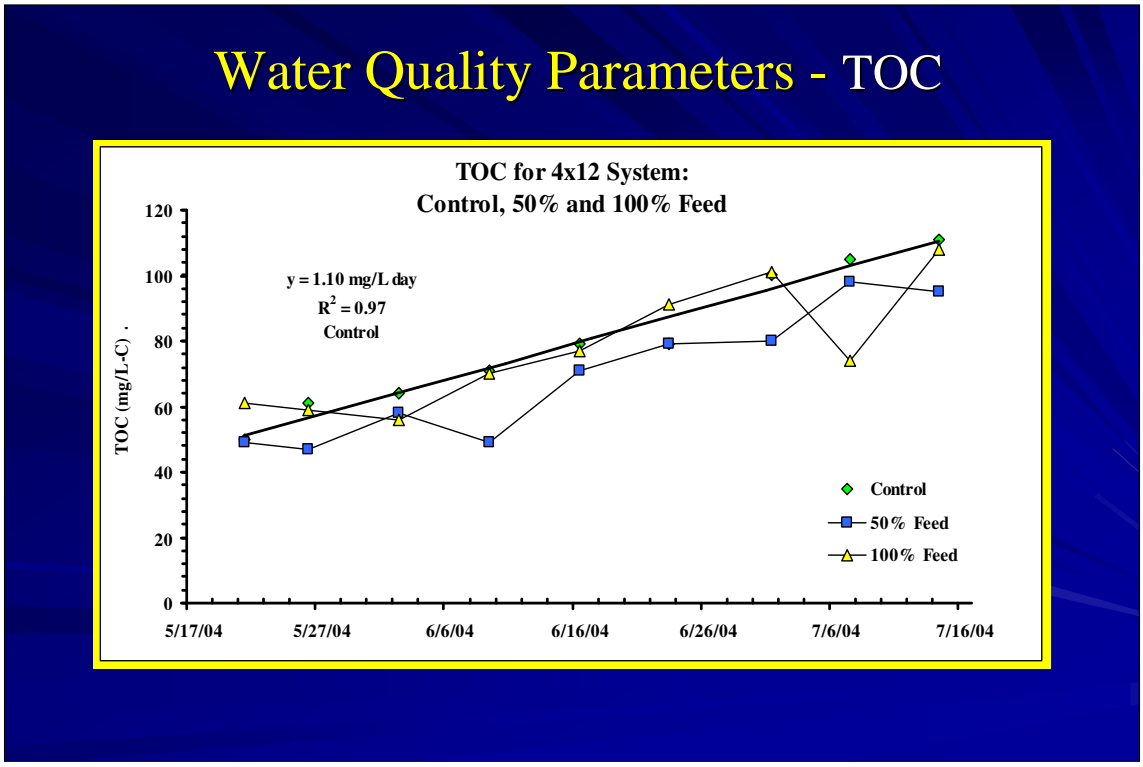
Finally, the daily measured TSS and the model predication for the case where sucrose was added at 100% of the feed rate is shown in the above figure. Suspended solids production is so high that solids needed to be harvested almost every other day. As such, the model does a fairly good job of predicting TSS concentrations over the growout period. Again, in this case, it is assumed that a source of nitrogen was available to completely assimilate all of the carbon available from the feed source and the sucrose. The exact source of the nitrogen is not readily apparent, but it must be there, based on these experimental results. Again, since all of the ammonia-nitrogen should be assimilated by the heterotrophic bacteria, there should be minimal nitrate-nitrogen production. Daily measured values were almost at the detectable limit for the nitrate-nitrogen test.

Water Quality Management – C/N Ratio



Impact of carbon supplementation (or lack of) on nitrite-nitrogen

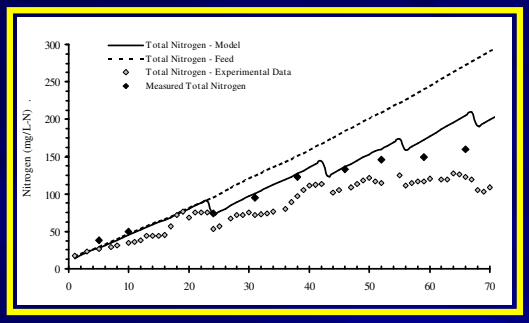
The above figure shows one problem with heterotrophic bacterial based systems that rely on supplemental carbon. Due to miscommunication, over several days, carbon as sugar was not added to the production tank. As can be seen from the graph, nitrite-nitrogen concentrations spiked within several days, but subsided immediately upon resumption of carbon supplementation.



Dissolved Organic Carbon and Total Nitrogen

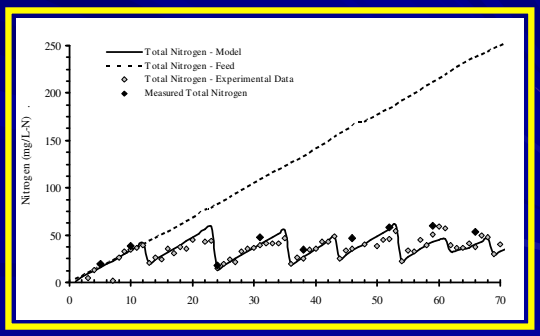
The above Figure shows the dissolved organic carbon concentration (DOC) in the three treatments over the ten week research trial. As can be seen, there appears to be no major difference in the DOC and that there is a consistent increase in the DOC over the growout period. This is probably the result of the gradual buildup in all the systems of humic substances, the 'tea' color seen in intensive recirculation systems that accumulates when ozone or UV is not used to remove it. Humic substances correspond to the non-biodegradable part of the dissolved organic carbon and are not available as a carbon source to the bacteria. Humic substances are hydrophobic dissolved organic matter produced by the auto-oxidation of polyunsaturated fatty acids released by fish feces, uneaten feed and the lysis of dead bacteria.

Water Quality Parameters - TN



Mass balance on nitrogen for the autotrophic/heterotrophic system without carbon supplementation and with periodic harvesting of excess bacterial biomass

Impact of carbon supplementation at 50% of the feed as sucrose on the system with excess bacterial biomass and nitrogen being periodically removed from the system



The above figure on the left shows the results of a mass balance on nitrogen for the autotrophic/heterotrophic system without carbon supplementation and with periodic harvesting of excess bacterial biomass. Total Nitrogen – Model was calculated using the VSS concentrations predicted by the previous presented model and assuming it contains 12.4% nitrogen based on the stoichiometry of bacterial biomass. Total Nitrogen – Experimental Data represents the sum of the nitrogen contained in the experimentally measured VSS plus experimentally measured concentrations of TAN, NO_2-N, and NO_3-N. The Measured Total Nitrogen is the sum of the nitrogen contained in the experimentally measured VSS plus the experimentally measured Total Nitrogen. Finally, the Total Nitrogen-Feed is the estimated nitrogen content of the feed (35% protein), 0.0504k g N/ kg feed.

This figure shows the stair step nature of total nitrogen as bacterial biomass is removed from the system. The experimentally measured value for Total Nitrogen falls below the model for several possible reasons including the difficulty in measuring nitrate-nitrogen accurately with the analysis methods employed and the impact of denitrification, especially noticeable near the end of the research period. The use of Total Nitrogen appears to due a better job of estimating the nitrogen and also shows a falling off near the end of the research period, most likely due to denitrification. Notice that over the growout period, almost all the nitrogen remains in the system.

The figure on the right shows the impact of carbon supplementation at 50% of the feed as sucrose on the system with excess bacterial biomass and nitrogen being periodically removed from the system. Since this is a pure heterotrophic system, there is no nitrate-nitrogen created. Thus the systems total nitrogen remains at very low levels, fluctuating within a very narrow range. The system supplemented at 100% of feed as sucrose showed similar characteristics, except for a greater rate of increase in nitrogen per harvesting cycle and more numerous culling of biomass.

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.

The pathways for nitrogen removal are very different in terms of substrate utilization, bacterial biomass generated and by-products generated. Using simple stoichiometry for autotrophic and heterotrophic bacteria, it is possible to characterize and model the two pathways for nitrogen removal. The difficulty in the real world is that each bacterial pathway may be present to some degree and the bacterial communities associated with each will compete for the same substrate, possibly resulting in dominance by one group over another. The ability to control the carbon to nitrogen ratio by feed formulation, solids removal, or addition of organic carbon allows the aquaculture producer to manage what type of system is created.

Conclusions

"New Paradigm"

- Zero-exchange systems
- Mixed-cell raceways
- SPF, low salinity growout

"Engineering Sustainability"

Organic Carbon + Nitrogen → Bacterial Biomass

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Opinions, conclusions, and recommendations are of the authors
and do not necessarily reflect the view of the USDA.

All experimental protocols involving live animals were in compliance
with Animal Welfare Act (9CFR) and have been
approved by the Freshwater Institute Animal Care and Use Committee.

Questions?