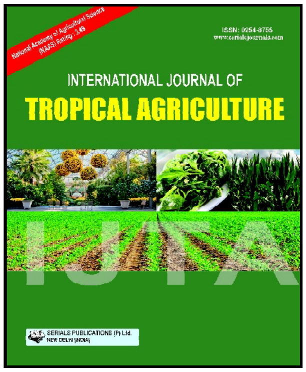

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Microorganisms in maintaining Carbon/Nitrogen (C/N) ratio in Intensive Aquaculture System: A Review

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Abstract: Typically, only 20–25% of fed protein is retained in the fish raised in intensive systems, the remaining being lost to the system as ammonia and organic N in feces and feed residue. One of the major water quality problem in intensive aquaculture system is the accumulation of inorganic nitrogen (NH_4^+ and NO_2^-) in the water. Microbial breakdown of organic matter leads to the production of new bacteria, amounting to 40–60% of the metabolized organic matter. The relationship among the addition of carbohydrates, the reduction of ammonium and the production of microbial protein depends on the microbial conversion coefficient, the C/N ratio in the microbial biomass and the carbon contents of the added materials. The control of inorganic nitrogen accumulation in a pond is the carbon metabolism and nitrogen-immobilizing microbial process. Carbohydrate like starch, sugar and cellulose are used as food by bacteria and other microorganism. Through this they generate energy and grow by producing protein and synthesizing new cells. Under optimum C/N ratio, inorganic nitrogen is immobilized into bacterial cell while organic substrates are metabolized. The conversion of ammonium to microbial protein needs less dissolved oxygen compared to oxygen requirement for nitrification suggesting the preference of heterotrophic community rather than nitrifying bacteria in the intensive aquaculture system. The C/N ratio should be raised from 10.75 to 15.78 to remove TAN from fish pond. Controlling the inorganic nitrogen by manipulating the C/N ratio is a potential control method for intensified aquaculture system.

Key words: C/N ratio, microbial protein, inorganic nitrogen, TAN, Intensive Aquaculture

INTRODUCTION

In intensive systems, the buildup of sludge on the bottom rapidly declines water quality. Thus, regularly sludge removal is necessary before it spoils and becomes a problem to the culture species (McGraw, 2016). One of the major water quality problems in intensive aquaculture system is the accumulation of inorganic nitrogen (NH_4^+ and NO_2^-) in the water (Colt and Armstrong, 1981). A major source of ammonium is protein rich food and aquatic animal (fish and shrimp) excrete ammonium, which may accumulate in pond bottom. Aquatic animals need a high concentration of protein in feed because their energy production pathway depends on catabolism and oxidation of proteins (Hepher, 1988). The manipulation in the C/N ratio may result in a shift from an autotrophic to a heterotrophic system. The heterotrophic bacterial population utilizes the inorganic N to synthesize bacterial protein and new cells (single cell protein) and it may be utilized as a food source by fish, thus lowering the demand for supplemental feed protein. Nitrogen control is induced by feeding bacteria with CH and uptake of nitrogen from the water, by microbial proteins synthesis.

However, the adjustment of C/N ratio in the feed, as a means to control the pond water quality, is presently under active research in many research centers (McGoogan and Galtin, 1998). Frequently the exchange of water in the pond is one of the common solutions for removal of excessive nitrogen. However this is limited as environmental regulations prohibit the release of nutrients rich water into environment. This is due to the degradation of water quality. There is also the danger of introducing pathogen into external water and high expense of pumping huge amount of water. Another method is to enhance nitrification of ammonium and nitrite to inert nitrate. It is done by biofilter, which is a high cost affair and need to treat and digest a large mass of feed residues.

The most economic method for removal of ammonium from water is through its assimilation into microbial proteins by the addition of carbonaceous material to the system. This involve feeding bacteria with carbohydrates and through the subsequent uptake of nitrogen from water, by the synthesis of microbial proteins, the bacteria reduce nitrogen levels. A further important aspect of this process is the potential utilization of microbial protein as a source of feed protein for fish. However, limited study on how much of the aquatic microbial remains neglecting relationships between bacteria with other organisms (Grossart, 2010; Tang *et al.,* 2010).

The most prominent characteristic of intensive aquaculture systems is the water contaminated by dissolved organics and ammonia which reduce the productivity of the system. This paper reviews the role of Microorganisms in maintaining Carbon/ Nitrogen (C/N) ratio in Intensive Aquaculture System with the intention of assisting researchers in selection of new research topics for further innovative research.

CARBON / NITROGEN RATIO (C/N RATIO)

The relationship among the addition of carbohydrates, the reduction of ammonium and the production of microbial protein depends on the microbial conversion coefficient, the C/N ratio in the microbial biomass and the carbon contents of the added materials.

The control of inorganic nitrogen accumulation in a pond is the carbon metabolism and nitrogen-immobilizing microbial process. Carbohydrate like starch, sugar and cellulose are used as food by bacteria and other microorganism. Through this they generate energy and grow by producing protein and synthesizing new cells.

Organic C→Carbon dioxide + energy + carbon assimilated in microbial cells.

Microbial conversion efficiency (E) = Assimilated carbon from feed x 100/Metabolized feed carbon. Range of E is 40 – 60%.

Nitrogen is also required by microorganism since the major component of the new cell material is protein. Thus, microbial utilization of carbohydrate is accompanied by the immobilization of inorganic nitrogen. This process is basic microbial process.

ROLE OF MICROORGANISM IN MAINTAINING C/N RATIO

The microorganisms are composed of bacteria, fungi and plankton that have protein content around 30-40% (Supono *et al.,* 2014). Biofloc Technology is an efficient alternative system based on growth of microorganism in the water since nutrients continuously recycled and reused by adding external carbon source in intensive aquaculture system to stimulate heterotrophic growth and nitrification (Azim and Little, 2008). Thus, maintenance of water quality by microbial, and floc nutrition are the main roles increasing culture feasibility by reducing FCR and feed costs (Emerenciano *et al.,* 2013).

Nitrogen control in the water column is influenced by feeding bacteria with carbon source by the synthesis of microbial proteins. Furthermore, reducing inorganic nitrogen in fish ponds are effective by adding carbonaceous substrate that fish can be fed these produced microbial proteins. Thus, feed protein replacement and feeding costs reduction are approved (Avnimelech, 1999). As it shows in figure 1, bioflocs will be integrately used to feed with a low N content and/or a carbon source addition to maintain floc concentration in the system (Crab *et al.,* 2012).

CALCULATION OF C/N RATIO

Twenty-gram samples of pond bottom (clay soil, from commercial tilapia pond) were shaken for 12 h with 1000 ml tap water enriched with $(NH_4)_2SO_4$, at an initial concentration of about 10 mg/l, and 200 mg/l glucose. Sample were taken periodically and filtered. Ammonium concentrations were determined according to standard method using an auto-analyzer (EPA, 1974).

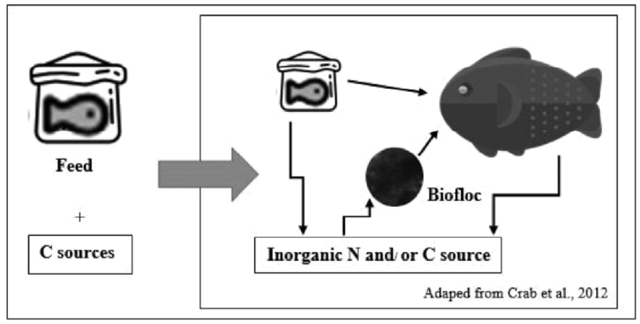

Figure 1: Schematic representation of Biofloc in maintaining C/N Ratio in Aquaculture systems

Tilapias grown in circular 50 m^2 ponds at a density of about 0.5 kg/m^2. Fish will first fed using conventional 30% of protein pellets (C/N=11.1) and then, tested with formulation of low protein diet of 20% protein (C/N=16.7). Finally based on some assumption as mentioned below we get the result concerned with the water quality and feed cost required for the cultured species before and after amendment C/N ratio.

AMOUNT OF CARBOHYDRATE ADDITION (ΔCH) NEEDED TO REDUCE NH_4^+ IN A POND (WITHOUT FISH)

The potential amount of microbial carbon assimilation, according to E (microbial conversion coefficient), is $\Delta C_{min} = \Delta CH \times \% C \times E$.

Where ΔC_{min} = Amount of carbon assimilated by microorganism.

ΔCH = amount of carbohydrate addition

$\%C$ = carbon content of added carbohydrate (roughly 50%)

Again, the amount of Nitrogen needed for production of new cell material i.e. Total NH_4^+ Nitrogen (ΔN) depend on C/N ratio in microbial biomass, which is around 4 (Gaudy and Gaudy 1980).

Thus, $\Delta N = \Delta C_{min} / [C/N]_{min}$. By putting the value of ΔC_{min} from above we get,

$\Delta N = \Delta CH \times \% C \times E / [C/N]_{min}$

{By using approximate value of %C = 0.5, E = 0.4, and $[C/N]_{min} = 4$} We get,

$\Delta N = \Delta CH \times 0.5 \times 0.4/4$ or $\Delta CH = \Delta N / (0.5 \times 0.4 /4)$.

$\Delta CH = \Delta N / 0.05$.

Thus to reduce total ammonium nitrogen (TAN) concentration by 1ppm (1 gm N/cub.m), we need to add 20g/cub.m of carbohydrate (having 50% C) to the system.

The proportion of Nitrogen assimilated by fish from feed and Change in C/N ratio

It has been reported that fish or shrimp could assimilate only 25% of Nitrogen from feed and rest is excreted as ammonium in faces or feed residue.

Hence, ΔN = feed x %N feed x %N excretion. Putting value of ΔN in above in equation of ΔCH we get, ΔCH =feed x %N feed x % N excretion / 0.05. Assume feed has 30% protein with 4.65% N and 50% of feed nitrogen are excreted (%N excretion). ΔCH=feed x 0.0465 x 0.5 / 0.05.

DCH=0.465 x feed.

Hence, to remove TAN the feed (having 30% protein) should be amended by an additional portion of 46.5% carbohydrate as shown in above equation. The corrected protein percentage =30%/ 1.465=20.48%.

Initially, C/N ratio = Carbon content of carbohydrate/Nitrogen content in feed (30% protein) = 0.5 / 0.0465 = 10.75. As Nitrogen percentage in 30 % protein feed is 4.65 % so in 20.48 % (corrected) protein feed will be 3.1744%. Hence New C/N ratio should be=0.5/0.031744=15.78. Thus C/N ratio should be raised from 10.75 to 15.78 to remove TAN from fish pond.

DISCUSSIONS

The addition of carbohydrate**,** essentially changes the 30% protein feed material to 20% protein feed. This leads to significance reduction of inorganic nitrogen accumulation, Provides good water quality by lowering ammonium. Reduction of protein level in the feed. Increased utilization of protein through the utilization of the microbial proteins. A significance reduction of feed expenditure because of the fact that proteins are expensive component of feed. Good growth of fish due to a lower concentration of toxic inorganic compounds.

The addition of carbohydrate to feed may results in accelerated sedimentation of organic matter to the pond bottom. Microbial biomass will not be utilized by fish in greater extent and increase the organic load in pond.

Carbon to Nitrogen (C:N) ratio is fundamental but it is a considerable value in assessing the dietary requirement of animal, which shows as an effective measure of protein percentage in the food. Feeding bacteria with carbohydrates can be used to control nitrogen concentration in water column through the microbial proteins synthesis. The coefficient of microbial conversion, the C:N ratio in the microbial biomass, and the carbon contents of the added material are dependent factors which affect the relationships between the addition of carbohydrates, the reduction of ammonium and the production of microbial proteins. The ammonium removal from the water is done through microbial proteins assimilation by adding carbon source to the system. If properly modified, potentially abolish the problem of accumulation of inorganic nitrogen can also done by adding carbohydrates. The potential utilization of microbial protein as a source of feed protein for fish or shrimp is a further important process of this aspect (Avnimelech, 1999). Azaml et al. (1983) has found that C:N ratio from nitrogen excretions of heterotrophic flagellates and other microzooplankton are similar to their food, so they can utilize as another source of food to complete and transfer energy to higher trophic levels.

Cost-Benefit Analysis for improving water quality by removing Ammonium/ Ammonia

Without Carbohydrate source (Protein content 30%)

Assumption: 0.5 ha ($5000m^2$) Tilapia (*Oreochromis niloticus*) farm. Stocking density = 3 fish/ m^2. Each fish of 200g being harvested. Each fish excrete 120mg NH_4/day. (128 mgNH_3 kg^{-1} for *Oreochromis mossambicus* (Peters) (Jauncey, 1982) have been reported). Feed with (30% protein) contain 4.56% N_2. Out of 30% protein, 25% N_2 is assimilated by fish i.e. 1.1625% N_2

So, we can say that 3.4875% N_2 is wasted as ammonium (NH_4) through faces, which creates chances of ammonia toxicity for the fish.

Since, we have 15000 fishes in our pond which will produce NH_4 in the pond is around 1 fish produce 120mg NH_4/day. So, 15000 fishes will produce 1800000 mg NH_4/day. Since the pond volume = $7415m^3$. That is it gives around 0.25 mg NH_4/l/day. Since, LC_{50} value of (NH_4/NH_3) for most of the aquatic organisms ranges between 0.25-2.5 mg NH_4/ l. Based on the above analysis we can draw our conclusion that, the ammonium/ammonia level in our pond is toxic for the cultured fishes which will hamper their growth and finally leads to high fish mortality.

With Carbohydrate source (Protein Content 20%)

Feed with 20% protein contain 3.1744% N_2. Out of which 25% N_2 i.e. 0.80% N_2 is assimilated by fish. So, 2.3744% N_2 will be excreted as NH_4 through faces. As stated above, we have 15000 fishes. 1 fish producing 81.69 mg NH_4/day. So, 15000 fishes will produce 1225350 mg NH_4/day. For total volume of pond water of $7415m^3$. That is it gives around 0.16 mg NH_4/l. So, based on above analysis we can conclude that the ammonium/ammonia level is 0.16 mg NH_4/l/day will not be toxic to the fishes.

Feed Cost Analysis

Without Carbohydrate Source (Protein 30%)

FCR = 2:1 means 2 kg of feed will give 1 kg of fish flesh. In our culture pond average body weight of fish = 200 g. Stocking Density = 15000 nos. Total fish biomass = 3000000g i.e. 3 ton. So, to produce 3 ton of fish we need 6000 kg of feed. Since, Culture Period = 7 month. Total amount of feed required for whole cycle = 6 ton. Suppose, the feed cost Rs. 25/- per kg Total feed cost Rs. 150000/-. Average price of fish Rs. 65/-. Revenue generated by fish Rs.195000/-. Net profit (with respect to feeding) Rs.45000/-.

With Carbohydrate Source (Protein 20%)

FCR = 2:1 means 2 kg of feed will give 1 kg of fish flesh. In our culture pond average body weight of fish = 200 g. Stocking Density = 15000 nos. That is total fish biomass = 3000000g i.e. 3 ton. So, to produce 3 ton of fish we need 6000 kg of feed. Since, Culture Period = 7 month. Total amount of feed required for whole cycle = 6 ton. Suppose, the feed cost=15 Rs/kg. Total feed cost of Rs. 90000/-. Average price of fish Rs.65/-. Revenue generated by fish Rs.195000/-

Net profit (with respect to feeding) Rs. 105,000/-

Further Research need

Additional research in the field concerning management of the microbial protein production, the microbial dynamics in intensive aquaculture systems, the nutritional value and the health effects of bacteria is needed, more specifically the effect on growth and survival of the cultured organisms. Microbiological characterization, possible manipulation of the microbial community and presence of pre/probiotics organisms in the microbial community are challenging fields of interest. There exists scope for further improvement of this management measure by optimizing the quantity of carbohydrate addition at various intensities of culture, and also comparing the potential of other carbohydrate sources. Radio or stable isotope studies are also needed to trace the organic carbon and inorganic nitrogen utilization by the pond microbial flora and also to quantify the contribution of microbial communities to total fish production.Research needed on comparison of several carbon source (the possibility of using byproducts), with combination with several protein level (to find out the optimum protein level). Feasibility of the implementation carbohydrate added diet in different culture systems with different organic material loaded, with different stocking density should be evaluated.

CONCLUSION

This review has drawn some conclusion that how best we can use the microbial protein as a food for fish by incorporating Carbohydrate in feed by maintaining C/N ratio. Carbohydrate source like rice bran, molasses are available in cheaper rate than the proteins feed. By maintaining C/N ratio it will be easier to control nitrogenous waste in the culture system. This will help to improve the water quality of the pond by reducing the feeding level of fish. Controlling the inorganic nitrogen by manipulating the C/N ratio is a potential control method for intensified aquaculture system. This approach is practical and inexpensive. So when this application will encourage the rural farmers to do ecological and economical sustainable intensive fish farming at reducing cost of feed and providing healthy environment to fish. So it is going to be an innovative approach for fish farming. However, further research is needed to make the planning of feed composition more precise way.

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