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RESEARCH ARTICLE

Determination of Ammonia Level and Its Protein Conversion in the Water of Biofloc Fish Farming Technology

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ABSTRACT

The biofloc system is a wastewater treatment technology and is used for fish farming by creating the artificial environment. Biofloc Technology (BFT) can enhance the water quality in aquaculture that introduces natural nutrient recycling characteristics with the induction of appropriate Carbon/Nitrogen (C/N) ratio. This study aimed to estimate the effect of BFT implementation on the water quality and production performance of common carp fishes using different concentration of carbon and to attempt a treatment of excess ammonia. The experiment was performed in four tanks (T1, T2, T3 and T4) containing the 10000 liter of water and 5000 shrimps on each tank that has a five weeks' experimental treatment by adding the carbon as (25%, 30%, 35%, 40% and 45% of sugar) according to its feeding diet from sugar in the interval of one week. The ammonia level before and after the treatment with respect to the percentage of carbon from sugar addition on BFT signifies that the 40% of carbon (C:N ratio of 20:1) and 45% of carbon (C:N ratio of 23:1) have a better performance on the ammonia treatment than 25%, 30% and 35% of carbon. The change in the water's color from green to brownish indicates the protein conversion of ammonia, which informs that the water becomes higher in quality after treatment than it was before for the biofloc fish farming system. It also provides an information about the nitrification process that takes place when the water is treated with carbon derived from sugar. The findings of this study have important implications for the improvement of biofloc fish farming systems.

KEYWORDS: Biofloc system, fish farming, aquaculture, ammonia, carbon/nitrogen ratio

INTRODUCTION

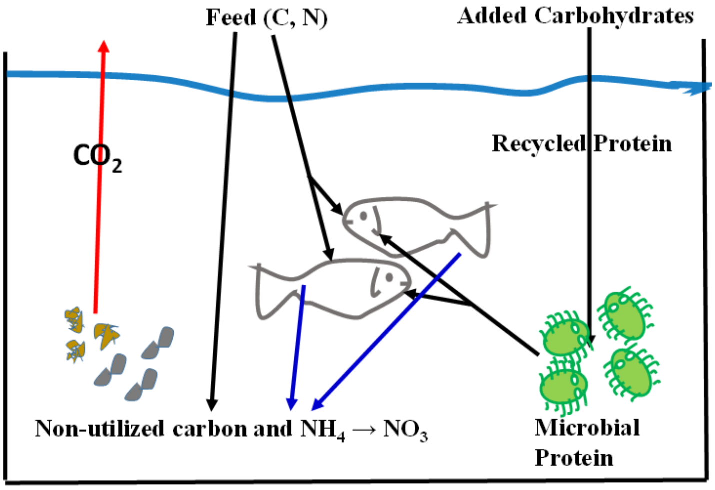
The biofloc fish farming is an innovative and sustainable technology of fish farming that has gained increasing popularity in recent years. In this technique, microorganisms are used to convert excess feed, fish waste, organic waste and other organic matter into protein-rich biomass, which serves as a food source for fishes. This technology is based on the creation of an environment that promotes the growth of these microorganisms in the water. Ammonia (NH_3) is one of the primary waste products in biofloc systems. In this technique, ammonia levels need to be carefully monitored to ensure the health of the fish and the success of this technology. Additionally, understanding the conversion of ammonia into protein is essential for optimizing biofloc systems' productivity (Ahmad et al., 2017). The biofloc system is a wastewater treatment technology that has become more important in aquaculture. Biofloc Technology (BFT) is a method used for fish farming by creating the artificial environment. The technique's principle is to maintain a greater C:N ratio by adding a carbohydrate supply and to improve the water quality by producing high-quality single-cell microbial protein (Emerenciano et al., 2017). Environmental control over the intensive production is in places where the water is scarce or land is expensive. There are strong economic incentives for an aquaculture business to be more efficient with the production inputs, especially the costliest and most limiting the water or land (Hargreaves, 2013). Different components such as ammonia, pH, total dissolved solvent (TDS) etc. are maintained in BFT.

Ammonia is the colorless compound of nitrogen and hydrogen which is characteristically pungent smell. It is toxic for both plants as well as the creatures. In aqueous solution, ammonia has two species: NH_3 and NH_4^+; total ammonia is the sum of $[NH_3] + [NH_4^+]$ and the peak of this ammonia/ammonium ion reaction is around 9.5 parts per million (ppm). The NH_3/NH_4^+ equilibrium both internally in animals and in ambient water depends on temperature, pressure, ionic strength and pH scale; this pH scale is most often of the greatest significance to animals (Ip et al., 2001). Ammonia is present in the aquatic environment due to the decomposition of wastage and can be converted into the microbial protein. In aquaculture, the biofloc system acts like a retention trap for the nutrients in the pond, and reduces maintenance costs because it can be used as a food supplement, improving the food consumption rate for the fish (Azim & Little, 2008).

In the biofloc fish farming technology, the accumulation of ammonia is one of the major challenges. Ammonia can be toxic to fish and its presence in the water can lead to poor fish health and growth. Ammonia is said to be normal up to 0.25 parts per million (ppm) and between 0.50 to 2 ppm, which seems to be a bell of danger, but in more than 2 ppm, no shrimps can exit alive there, which means that shrimps starts to die (Avnimelech et al., 2009). Ammonia is present in the aquatic environment due to the decomposition of wastage especially through the protein containing food supplement and other external waste material. About 78% of nitrogen existing in the aquaculture water body is mostly produced from the artificial food stuff which we use to feed (Huang, 2019). In the biofloc systems, a major factor that controls ammonia concentration is the C:N ratio of feed and other inputs (Dobler et al., 2006). Thus, based on carbon metabolism and nitrogen-immobilizing microbial activities, inorganic nitrogen buildup in ponds is controlled. For nourishment, bacteria and other microbes consume sugar, cellulose, starch and carbohydrates to produce energy and to grow, i.e., to make proteins and new cells (Chavan et al., 2018).

The addition of carbohydrates has the ability to lower the level of inorganic nitrogen, which is a key ingredient in ammonia (Hari et al., 2004; Ghosh & Chattopadhyay, 2005). Figure 1 is the schematic diagram of ammonia as well as its protein conversion through C/N ratio. In addition, C:N ratio can be calculated with respect to the feeding especially protein to the shrimps of the biofloc (Xu & Pan, 2013; Correia et al., 2014).

Figure 1
Schematic Diagram of Ammonia Control



At recent years, a study on this technology was done in maintaining the bacterial growth in biofloc using wheat bran/molasses as the carbon sources (Park et al., 2017; Xu et al., 2016; Lima et al., 2018). Several experiments were also conducted by others for the reduction of ammonia level and making that ammonia level suitable for the growth of shrimps through the carbon components (Luo et al., 2020; Avnimelech, 1999; Abakari et al., 2020). Studies have shown that traditional methods for measuring ammonia levels in the water, such as the Nessler method, may not be accurate in biofloc systems due to the interference from the organic matter and other compounds (Crosby, 1968). The accurate determination of ammonia levels and its protein conversion in the biofloc fish farming water is critical for the success of the technology. However, there is a lack of consensus among the scientific community regarding the best methods for measuring the ammonia levels and protein conversion in the biofloc systems. Therefore, this study deals with the level of ammonia and its control measure in the biofloc fish farming system. It is also shown that the exact level of carbon/nitrogen ratio suitable in the biofloc technology of fish farming by using sugar as the main source of carbon as it contains 40% of carbon per kg (Serra et al., 2015).

In the context of Nepal, most of the people are engaged in the agricultural sector as this technology will be efficient for the people who start the business with a low cost budget, using the scientific treatment of ammonia and the edible organic carbon component. However, this study has been limited to the biofloc fish farming technology. The results may not be applicable to other fish farming technologies. The study has also been limited to a specific geographical location.

MATERIALS AND METHODS

In this study, the ammonia level measurement in the AG biofloc fish farming on 5000 fish (Common Carp) has been conducted in Chitwan district. Sugar was used as the major source of carbon. The biofloc technology can be enhanced by adding sugar, which provides a source of carbon for the microbes and stimulates their growth. Figure 2 shows the method of carbon adding a process in biofloc in which carbon containing a sugar packet was suspended just above the water level of tank so that the sugar was added dropwise through small holes in the biofloc otherwise oxygen deficiency may occur due to the instantaneous mix-up of carbon on the water that may be dangerous to fishes.

Figure 2
Carbon Adding Process from Sugar Packet Suspended Just Above the Water Level of Tank

The four biofloc tanks, designated T1, T2, T3 and T4, each with a water capacity of 10,000 liter, were used for the experiment. Each day, 1000 ml of nutrients from each tank was tested. The initial level of the ammonia before the treatment with sugar and final level of ammonia after the treatment with sugar were noted in all the four tanks using the chemical reagent named API test kit NH_3/NH_4^- (Nunes instruments company, India) shown in Figure 3.

Figure 3
Chemical Reagent for Testing Ammonia and Color Chart of Ammonia

At first, the water of biofloc's tank 1 was taken into the test tube to the labeled area and 8 drops of reagent 1 (Nunes instruments company, India) was mixed on it and shaken well. Again, eight drops of reagent 2 (Nunes instruments company, India) was added and shaken very well. Now the solution was hold for five minutes such that the color change of biofloc's water containing ammonia was clearly seen. For the reading of ammonia level in the water, the color change in the water of test tube was compared with the color chart supplied from the Nunes instruments company, India and noted the ammonia level. In this way, the initial level of the ammonia before treatment with using API test kit NH_3/NH_4^- and final level of ammonia after treatment with using API test kit NH_3/NH_4^- were noted in all the four tanks.

The 25% (i.e. around 0.62 kg of sugar is needed to make C:N = 13:1), 30% (i.e. around 0.72 kg of sugar is needed to make C:N =15:1), 35% (i.e. around 0.86 kg of sugar is needed to make C:N =18:1), 40% (i.e. around 0.96 kg of sugar is needed to make C:N

=20:1), 45% (i.e. around 1.1 kg of sugar is needed to make C:N =23:1) of carbon according to its protein diet (Correia et al., 2014; Dobler et al., 2006; Xu & Pan, 2013; Serra et al., 2015) was added in each tank in the interval of one week and ammonia level was noted after two hours of treatment.

The experiments were performed on the interval of one week so that ammonia gets re-increased and helped to check the significant level of carbon. The process of taking data were as follow:

- In first week 25% of carbon according to its protein diet was added (C:N = 13:1)
- In second week 30% of carbon according to its protein diet was added (C:N = 15:1)
- In third week 35% of carbon according to its protein diet was added (C: N = 18: 1)
- In fourth week 40% of carbon according to its protein diet was added (C:N = 20:1)
- In fifth week 45% of carbon according to its protein diet was added (C:N = 23:1)

RESULTS AND DISCUSSION

The study aimed to determine the level of ammonia and protein conversion in the water of a biofloc fish farming technology. The water samples were collected from the biofloc system at different intervals and analyzed them for ammonia concentration and protein conversion rate. Several experimental results were presented by adding sugar as a carbon component according to the feeding component. Table 1 shows the data and comparison between the initial and final level of ammonia before and after the treatment with sugar in each tank T1, T2, T3 and T4 respectively within the time period of five weeks.

Table 1

Ammonia Level Before and After the Treatment of Biofloc Water/Nutrition with Using Reagent

Biofloc tanks	First week - 25% carbon of 1000gm nutrient		Second week - 30% carbon of 1000gm nutrient		Third week - 35% carbon of 1000gm nutrient		Fourth week - 40% carbon of 1000gm nutrient		Fifth week - 45% carbon of 1000gm nutrient	
	Initial ammonia level (ppm)	Final ammonia level (ppm)	Initial ammonia level (ppm)	Final ammonia level (ppm)	Initial ammonia level (ppm)	Final ammonia level (ppm)	Initial ammonia level (ppm)	Final ammonia level (ppm)	Initial ammonia level (ppm)	Final ammonia level (ppm)
T1	4	2	4	2	4	2	2	0.25	4	0.25
T2	2	0.5	1	0	1	0.25	0.5	0	2	0
T3	4	4	2	2	2	1	8	2	8	1
T4	8	8	8	4	4	2	4	0.5	2	0

Figure 4 shows the five-week experimental graph for tank 1 where the concentration carbon factor is plotted on the X-axis and the ammonia dependent variable is plotted on the Y-axis. The black line in Figure 4 represents the ammonia level (on average for seven days) prior to treatment and the red line represents the ammonia level (on average for seven days) following the treatment. In this graph, the level of ammonia changes constantly up to 40% before and after the treatment and the drastic change in the level of ammonia takes place at 45% of carbon.

Figure 4

Graphical Representation of Five Week's Ammonia Level in Tank 1

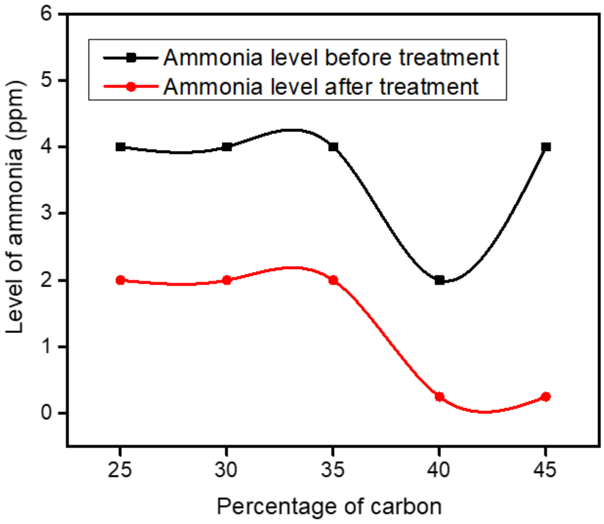


Figure 5 is the graph for tank 2 with five weeks' experiments on different concentration levels of carbon that belongs to the percentage of carbon added along the X-axis and the level of ammonia (average of seven days) along the Y-axis. In this figure, the level of ammonia denoted by black line and red line before and after the treatment respectively concludes that changed at the ammonia level. It is seen that the change becomes maximum at 45% concentration of carbon after the treatment.

Figure 5

Graphical Representation of Ammonia Level in Tank 2 for Five Weeks

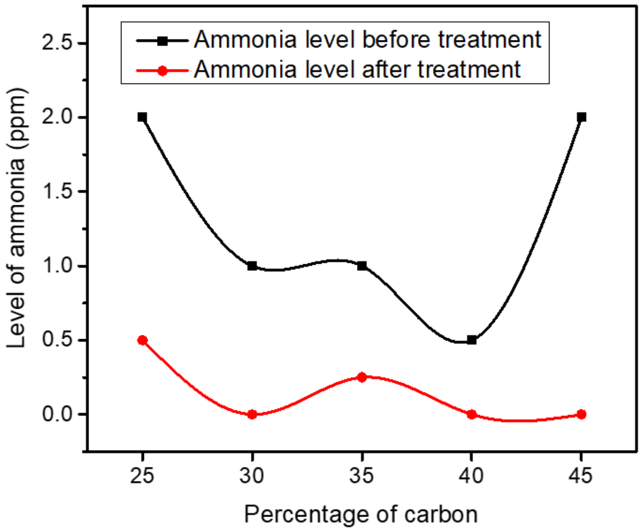


Figure 6 is the graph for five weeks' experiment on different concentration levels of carbon to the tank 3. Figure 6 contains the X-axis having percentage of carbon added and Y-axis with the ammonia level before the treatment and after the treatment. In this figure, there is no change in ammonia level from 25% to 30%, which is because of the absence of bacteria present in the tank 3. Since the tank 3 had been cleaned in the first and second week regularly so there was the absence of the microbial containing water. From the third week (i.e. after 30% concentration of carbon), one bucket of microbial containing water has been poured into the tank 3 from the tank 2 containing the biofloc water. Here, the microbial bacteria go on multiplication. Due to this, the ammonia level is changed, which can be seen in Figure 6. It concludes that bacteria must be there because bacteria help in nitrification and through the nitrification, the ammonia level is changed into the protein. This gradual changing in the ammonia level takes place at 35%, 40% and 45% of carbon.

Figure 6

Graphical Representation of Five Weeks' Ammonia Level in the Tank 3

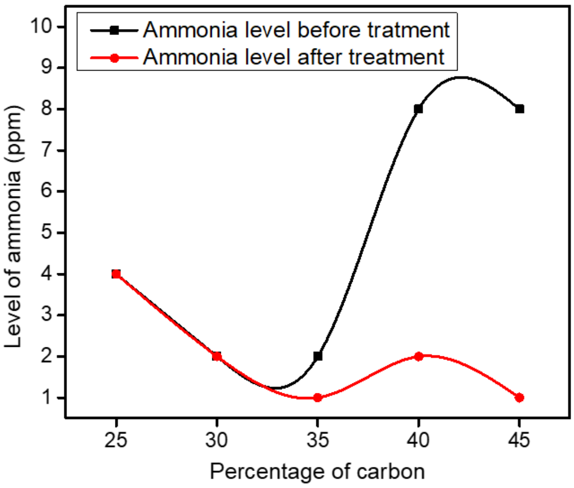
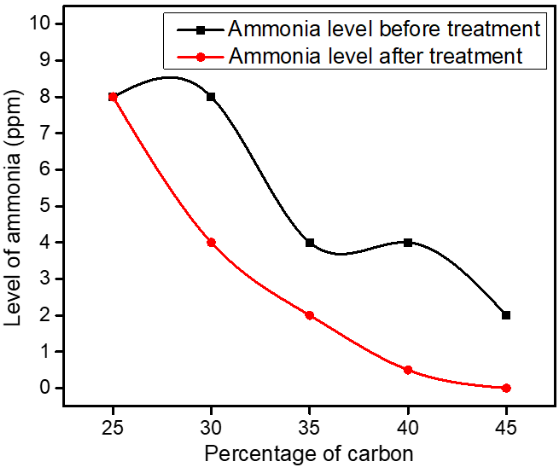


Figure 7 is the graph regarding the tank 4 where dependent variable is ammonia, which is plotted on the Y-axis, adding the percentage of carbon, which is plotted on the X-axis. The figure contains the five weeks' experiment, having a black line indicating the ammonia level before treatment and red line indicating the ammonia level after treatment. In this figure, there is no change at the ammonia level in the first week, i.e., at 25% carbon concentration. The reason behind this is that the tank 4 had been already cleaned and due to this there were no bacteria present in the tank 4. Therefore, to make a difference at the ammonia level from the second week, one bucket of biofluc water from the next tank 1 has been put into the tank 4, which initially did not have any bacteria. Here, it shows that a gradual change at the ammonia level, which are seen after treatment in 30%, 35%, 40% and 45 %.

Figure 7

Graphical Representation of Ammonia Level in the Tank 4 for Five Weeks



From the experimental data and analysis shown in the figures above, the best results to change of ammonia level by adding the carbon concentration component are only possible if the biofloc water in the cleaned tank containing the cleaned water is added such that it makes a difference of ammonia level in between the untreated (original cleaned water) and the treated water of the biofloc system.

Table 2

Difference between Ammonia Level Before and After the Treatment

Biofloc tanks	Difference in ammonia level in first week - 25% carbon of 1000gm nutrient	Difference in ammonia level in second week - 30% carbon of 1000gm nutrient	Difference in ammonia level in third week - 35% carbon of 1000gm nutrient	Difference in ammonia level in fourth week - 40% carbon of 1000gm nutrient	Difference in ammonia level in fifth week - 45% carbon of 1000gm nutrient
T1	2	2	2	1.75	3.75
T2	1.5	1	0.75	0.5	2
T3	0	0	1	6	7
T4	0	4	2	3.5	2

From the careful observation of Table 2, the test on the addition of 25%, 30% and 35% carbon according to the daily nutrient diet are not effective for making the qualitative water for the shrimps in the biofloc fish farming technology. However, an addition of 40% and 45% of carbon has a great significance for the ammonia treatment and protein conversion. In the left hand side of Figure 3, there is a tube containing a solution in which it is observed that the color of water to brownish from green color indicating the protein conversion of ammonia. This informs that the qualitative water is found after the treatment than before the treatment. This finding in this paper agrees well with the finding of Ogello et al. (2021) in which it reduces the feed cost by a large amount and ensures a higher profitability. Therefore, it is concluded that nitrification occurs on the treatment of water with the help of carbon from sugar (Luo et al., 2020).

CONCLUSION

BFT is seen as the novel blue revolution since nutrients can be continually recycled and reused in the culture medium. It is an aquaculture method that is beneficial to the environment and relies on in-situ microbe production. The research conducted an analysis on the water samples of a biofloc fish farming system to determine the concentration of ammonia and protein conversion rate. The impact of BFT deployment on water quality and Common Carp fish production performance utilizing various carbon concentrations has great significance and tries to treat excessive ammonia. By incorporating natural nutrient recycling properties and the proper Carbon/Nitrogen (C/N) ratio, BFT can improve the water quality in aquaculture systems. The ammonia level before and after the treatment with respect to the percentage of carbon from sugar addition on BFT, the 40% of carbon (C:N ratio of 20:1) and 45% of carbon (C:N ratio of 23:1) have better performance on the ammonia treatment than 25%, 30% and 35% of carbon. The color of water to brownish from green color informs the protein conversion of ammonia and it indicates that the qualitative water is found after the treatment than before the treatment. It gives the information about the nitrification occurring on the treatment of water by the help of carbon from sugar. The results revealed that there is a gradual decrease in ammonia concentration in the system and becomes lowered at fourth and fifth week. So, the protein conversion rate increases gradually after treatment in comparison to before treatment. The study recommends regular monitoring of ammonia levels and protein conversion rates to maintain the optimal health and growth of the fish in biofloc systems. The findings of this study have important implications for the improvement of biofloc fish farming systems. In fish culture systems, it improves feed conversion, growth performance, survival rate and efficient generation of feed. It also lowers the expense of regular feed and the usage of protein-rich feed.

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