

## **EFFECT OF C/N RATIO ON WATER QUALITY USING DISTILLERY SPENTWASH AS A CARBON SOURCE IN INDOOR AEROBIC MICROBIAL FLOC TANKS**

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**Abstract:** Application of Aerobic Microbial Floc (AMF) technology allows minimal or zero-water exchange practice improve sustainability, biosecurity and production in fish and shrimp culture. The driving force of AMF systems is the development of floc, which is responsible for water quality control, waste assimilation, and nutrient recycling, which contribute to improved performance of cultured animals. The results of the present study showed that the C/N ratio had a significant influence on the development of Aerobic Microbial Floc and affects the inorganic nitrogen. Distillery spent wash obtained as effluent from sugar industries showed heterotrophic bacterial floc at the C:N ratio of 20-25:1. Thus distillery spentwash can be used as a potential carbon source in the C:N ratio of 20-25:1 for the maintenance of good and stable water quality to improve the animal performance in zero water exchange system.

**Keywords:** Aerobic Microbial Floc, C/N ratio, Distillery spent wash, water quality.

### **Introduction**

Spent wash is of purely plant origin and contains large quantities of soluble organic matter and plant nutrients. As the Spent wash generated in the distilleries have high potential to produce methane, it is subjected to anaerobic digestion for methane recovery. The efficacy of aquaculture as a tool for the treatment of wastes has been demonstrated at Madras, where the effluent after undergoing the biomethanation process, is fed into fish ponds. With production rates of 50 tonnes per hectare per year, about 6 hectares of land area has been shown to be adequate for treating 100m<sup>3</sup> of effluents (Rangaswami, 1987). This has opened avenues for utilizing a variety of agro-based industrial effluents for aquaculture practices, largely based on microbiological processes, in terms of oxidation or nutrient removal through algae and other macrophytes.

### **Materials and Methods**

The experiment was conducted in 75L capacity experimental tanks throughout the 15 days of period. Ten tanks of dimensions 60×30×46 cm and volume of 50 L each were used and the experimental set up housed in a greenhouse. All treatments had one replicates and allocation

for each treatment was completely randomized. Aeration was provided in all tanks to uniform mixing. C: N ratio was maintained at different level viz: Non-AMF, Treatment 1- 10:1, Treatment 2- 15:1, Treatment 3 - 20:1 ,Treatment 4: - 25:1. The levels of feed and C:N ratio were calculated by dividing total input carbon with total input nitrogen as followed in shrimp culture systems (Avnimelech, 1999). The main carbon source was from the feed and spentwash, while the main nitrogen source was feed. Furthermore, the nitrogen content of feed was determined by the assumption that 30 % protein feed containing 4.65% nitrogen (Avnimelech, 1999). The nitrogen content of feed with more than 30% protein, therefore, was calculated by conversion of that assumption while the carbon content of feed and spent wash used in each study is 38.5% and 29.71%, respectively. The amount of spent-wash required per 1gram of feed in each treatment, therefore, is 0.00116g, 0.00174g, 0.00232g, 0.0029g for C:N ratio 10:1, C:N ratio 15:1 C:N ratio 20:1 C:N ratio 25:1 respectively.

### **Water Quality Parameters**

During the experimental period, water quality parameters such as Temperature, Dissolved oxygen, pH, ammonia, were recorded three times a day (morning, afternoon, evening) in the glass tanks. Modified/Winkler's titration method APHA (2012) was adopted to estimate the dissolved oxygen. pH and Total Ammonia-N. The AMF volume was the measure of aerobic microbial biofloc in treatment tank and which was determined by adopting the method followed by Avnimelech and Kochba (2009). C: N ratio of the AMF treatment tank was monitored regularly and adjusted by adding spent wash at the rate of 20 times the ammonia-N content of culture water.

### **Maintenance of biofloc**

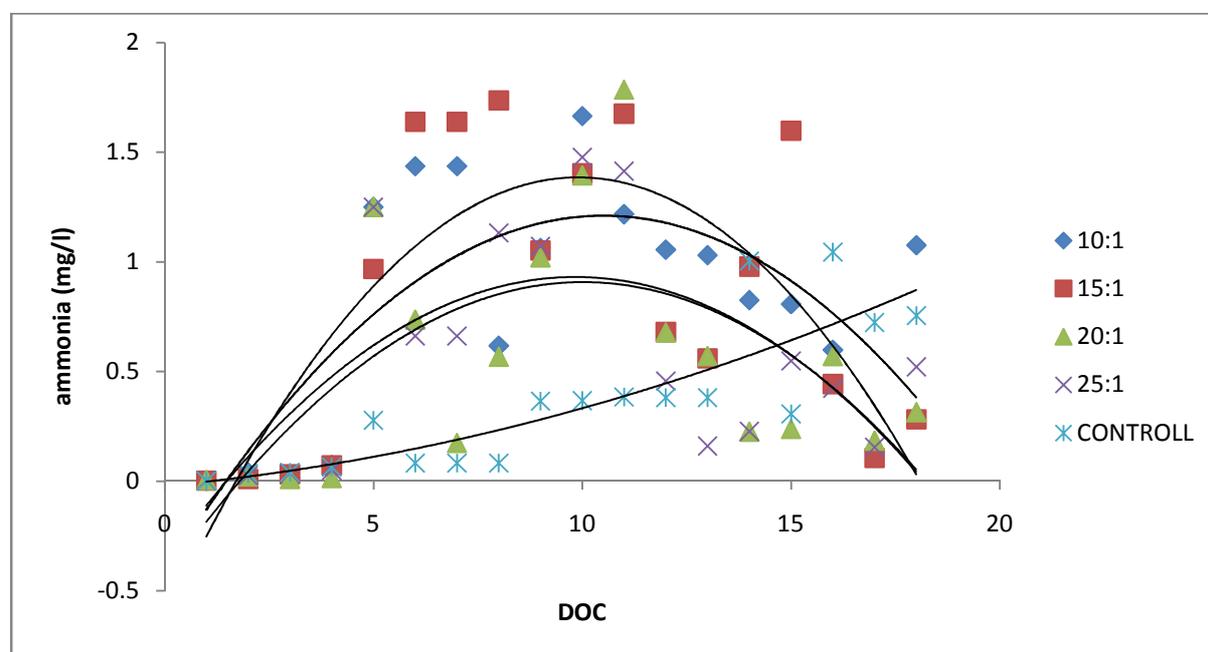
Combination of spent wash and sugar, jaggery, corn flour and wheat flour were formulated and added at 10 day intervals. For the first ten days spent wash and jaggery mixture were applied. Followed by spent wash with sugar mixture. For the next ten days spent wash with wheat flour and corn flour were added for developing the biofloc. All the above mentioned mixture combination were fermented using by yeast before adding into the system.

## **Results and Discussion**

### **Water Quality Parameters on Biofloc**

Levels of C: N ratio maintained in the water significantly affected the concentrations of ammonia, dissolved oxygen, pH in the experiment (Table 1). Generally, concentrations of ammonia, dissolved oxygen, pH decreased in response to increasing levels of C: N ratio. The

results indicated that the C: N ratio of 20:1 and C:N ratio 25:1 were effective for biofloc development and control of dissolved ammonia in the water.



**Fig.1. Ammonia concentration as a function of C: N ratio and DOC**

**Table 3. Water quality parameters affected by varying C:N ratio**

Treatments C:N ratio	TEMPERATURE (°C)	Dissolved Oxygen (ppm)	pH	AMMONIA (ppm)
<b>10:1</b>	28.94±0.43	4.39±0.63	8.29±0.29	0.83±0.53 <sup>a</sup>
<b>15:1</b>	28.94±0.43	4.49±0.64	8.26±0.30	0.80±0.64 <sup>a</sup>
<b>20:1</b>	28.94±0.43	4.52±0.59	8.19±0.24	0.54±0.51 <sup>b</sup>
<b>25:1</b>	28.94±0.43	4.61±0.70	8.19±0.30	0.51±0.49 <sup>b</sup>
<b>Control</b>	28.94±0.43	4.65±0.64	8.29±0.37	0.35±0.32 <sup>c</sup>

Data are presented as mean ± SD of duplicate tanks in each treatment. Values in the same column with different superscript indicates statistical significance at  $p < 0.05$  as indicated by one way ANOVA followed by Tukeys' multiple comparison test.

Control of bacterial community over autotrophic microorganisms can be achieved using a high carbon to nitrogen ratio (C: N, 15-20:1) to control nitrogenous waste metabolites accumulating in the water (Avnimelech et al. 1994; Taw 2010) and this has been proved

clearly from the present study. Chamberlain et al. (2001) recommended the use of balanced mixtures of carbonaceous and nitrogenous materials with a C: N ratio of approximately 20:1 to stimulate bacterial decomposition of organic wastes. In the present study, control of total ammonia concentrations in the system was achieved at a C: N ratio of 20:1(Fig.1), as reported by Chamberlain et al. (2001). Generally, concentrations of ammonia in the experiment decreased in response to increasing levels of C: N ratio and it was probably due to increase in the proliferation of heterotrophic bacteria when the levels of C: N ratio increased. Ootshi (2011) reported in biofloc production systems, ammonia-oxidizing bacteria process ammonia to nitrite, which provides substrate for nitrite oxidizing bacteria that process nitrite to relatively non-toxic nitrate. Ammonia uptake by heterotrophic bacteria occurs rapidly after addition of external carbon source (Mousavi *et al.*, 2014). It can be concluded that a more stable and reliable control over ammonia can be achieved through the heterotrophic pathway than algal uptake or nitrification, as suggested by Hargreaves (2013).

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