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The effect of different carbon sources on the nutritional composition, microbial community and structure of bioflocs

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ABSTRACT

The objective of this study was to document how different carbon sources affect the quality of biofloc (BF). The experiment consisted of three types of biofloc systems in which biofloc was produced by daily (25 days in all) supplementation with three different carbon sources, glucose (Glu), starch (Sta) and glycerol (Gly), in each 45-L tank; the C/N ratio was 15. The highest protein content was obtained in the BF (Glu), with a value of 41.2 ± 0.8% dry weight (DW). The BF (Sta) and BF (Gly) had lower values of 31.5 ± 0.6% and 35.5 ± 1.2% dry weight (DW). A higher lipid content was observed in the BF (Sta). The essential amino acid and nonessential amino acid contents were similar in BF (Glu) and BF (Gly), but both were higher than those of BF (Sta). The Essential Amino Acid Index (EAAI) for BF (Glu), BF (Sta) and BF (Gly) was 0.99, 0.93 and 0.98, respectively; which indicated that the biofloc produced in this experiment can be considered a good-quality protein source for shrimp. High-throughput sequencing of different bioflocs revealed that three types of bioflocs were dominant in *Proteobacteria* and *Bacteroidetes*. In addition, *Cyanobacteria* were the dominant biofloc in BF (Sta). Microscopic examination revealed that BF (Sta) was more closely related to the formation of the floc and contained more algae. Overall, this study demonstrated that bioflocs grown on different carbon sources have different qualities and suggested that the choice of carbon source used for growing bioflocs is of prime importance.

Statement of relevance: The present study indicated that different carbon sources could affect nutritional composition of biofloc, morphostructure of biofloc and the microbial community of biofloc. This result may provide a theoretical basis for the biofloc technology used in aquaculture.

1. Introduction

In recent years, biofloc technology (BFT) was introduced into aquaculture. It is an emerging environmentally friendly aquaculture production system. This technology was developed to create economic and environmental benefits via reduced water use, effluent discharges, artificial feed supply and improved biosecurity (Wasielesky et al., 2006; Avnimelech, 2007; Mishra et al., 2008). Biofloc (BF) is the core of BFT. It can provide nutrients such as "native protein" (Emerenciano et al., 2011), lipids (Wasielesky et al., 2006), amino acids (Ju et al., 2008) and fatty acids (Izquierdo et al., 2006; Ekasari et al., 2010) in the form of diverse microorganisms. Another advantage of BF has is that it is available as a food source all day and can reduce artificial feed inputs and costs (Browdy et al., 2001; Avnimelech, 2007; Samocha et al., 2007). Some studies have indicated that the different types of carbon sources can affect the composition of the biofloc. The structure and stability of a biofloc are determined by the selection of an organic carbon source to some extent (Hollender et al., 2002; Oehmen et al., 2004). The cost of an organic carbon source also determines the use of the biological flocculation (Wilén et al., 2000). Crab et al. (2010) conducted a small–scale laboratory experiment and found that different carbon sources led to differences in the protein, lipid, carbohydrate, and fatty acid compositions of the bioflocs. Literature describing the difference in quality of BFs as a result of different carbon sources remains scarce, especially concerning the relationship between amino acid content and carbon source.

A BF is mainly composed of different microorganisms, which play a key role as producers and consumers of dissolved oxygen, as nutrient recyclers, and as a food source for other organisms from higher trophic levels in aquaculture (McIntosh et al., 2000; Ray et al., 2010,; Martínez-Córdova et al., 2016). Therefore, analyzing the microbial community of biofloc can help to understand the nutritional differences of different bioflocs. Different carbon sources can affect the composition of the microbial community.

Knowledge of the community structure of a biofloc and its nutritional value, especially its amino acid (AA) composition, will help in the development of cost-effective shrimp feed formulations (Ju et al., 2008). Biofloc can be used as an additional feed source in aquaculture. Yet, its effect is dependent on the carbon source used (Crab et al., 2010). The

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goal of the present study was to assess the nutritional value of biofloc grown on different organic carbon sources.

2. Materials and methods

2.1. Experimental design and conditions

The experiment was conducted at the laboratory of life science (South China Normal University, China). The biofloc was produced in outdoor plastic tanks, containing 42 L of dechlorinated tap water and 3 L of fresh pond water to inoculate the tank with natural microorganisms such as bacteria and algae. No water exchange was performed, but evaporation losses were compensated with dechlorinated fresh water. The salinity was maintained at approximately 5 ppt. Three carbon sources were investigated: glucose, starch and glycerol. Each carbon source has three replicates and was assigned randomly. Feed pellets containing 42% protein (Haid Feed Co., Ltd. Guangdong, China) were used. The carbon source and feed were added once a day at an amount corresponding to a C/N ratio of 15. The daily quantity of carbon added was calculated according to Avnimelech (1999). The preweighed carbon source and feed were mixed in a beaker with tank water and then uniformly dispersed into the tanks. Each tank was inoculated with yeast only once at the beginning of the experiment. After inoculation, the final concentration of yeast in each tank was 1.0×10^5 cells·mL^{-1}. The tank water was aerated and agitated continuously using air stones connected to an air pump. The diameter of the aeration tube was 6 mm, and the diameter of the air stone was 40 mm. The experiment was carried out for a period of 25 days.

2.2. Assessment of water quality parameters

During the 25-day experimental period, the water temperature, dissolved oxygen (DO) and pH were measured on site every day using a YSI556 meter (YSI Incorporated 1725, Yellow Springs, OH, USA). Water samples (100 mL) were collected weekly at approximately 08:00 h and filtered through 0.45-μm GF/C filter paper under vacuum pressure. Ammonia nitrogen (NH_4–N), nitrite (NO_2–N) and nitrate (NO_3–N) concentrations in the filtrate were analyzed according to standard methods (APHA, 1998).

2.3. Proximate analysis of different bioflocs

Proximate analysis was carried out on the different types of bioflocs. Concentrated biofloc samples were collected from each tank by passing tank water through a 10-μm mesh nylon bag (Xu and Pan, 2012) after 25 days. The samples were dried in an oven at 105 °C until they reached a constant weight and then preserved in a refrigerator (−20 °C) until proximate composition analysis. The crude protein content was calculated based on the assumption that protein contains 16% nitrogen (AOAC, 1999). For ash content, a known amount of dry sample was burnt in a muffle furnace at 550 °C for 4 h before the ash was cooled and weighed. The lipid content was determined with Soxhlet apparatus. Protein, lipid and ash contents were expressed as a percentage of the dry weight (% DW) of the bioflocs. The total carbohydrate amount was calculated according to the following formula: carbohydrate (% DW) = 100 − (crude protein (% DW) + lipid (% DW) + ash (% DW)) (Manush et al., 2005). The gross energy content of the diets was calculated using kJ·g^{-1} DW values of 23.0, 38.1 and 17.2 for protein, lipids and carbohydrates, respectively (Tacon, 1990). The amino acid composition of the biofloc was measured by a professional laboratory using high-performance liquid chromatography (HPLC).

A selection of amino acids that are considered essential for aquatic animals in general was chosen as described by Tacon (1987) and Babarro et al. (2011). The essential amino acid ratio (E/A) for each essential amino acid (EAA) was expressed as a percentage of each amino acid of the total amount of essential amino acids measured (Arai, 1981). The essential amino acid index (*EAAI*) was calculated according to Peñaflorida (1989) using the formula:

$$EAAI = \sqrt[9]{aa1/AA1 \times aa2/AA2 \times \cdots \times aa9/AA9}$$

with *aa* being the essential amino acid ratio (E/A) in the biofloc, *AA* being the E/A ratio in the animal body, and 1, 2, 3, …, 9 being each of the essential amino acids.

2.4. Bacterial metagenome sequencing and bioinformatics analysis

When the experiment was finished, water samples were taken from 50 mL of each biofloc tank, and biomass was collected by centrifugation (10 min, 4000*g*). Total DNA was extracted from the samples using a water DNA extraction kit (E.Z.N.A.^{R}, Omega) according to the manufacturer's protocol. MisSeq metagenome sequencing and bioinformatics was conducted by the Shenzhen HENGCHUANG Gene Technology Co Ltd. using a sequencing machine (Illumina HiSeq2500 sequencing, United States Illumina company completed). Community structure analysis examines the relative distribution of species at the phylum level.

2.5. Biofloc volume and morphostructure

Floc volume was determined by sampling 1000 mL of water into a series of Imhoff cones (1000–0010, Nalgene) at 10:00 am every 3 days. The volume of the floc plug accumulating on the bottom of the cone was determined 15 min after sampling. Then, the floc plug was collected from the turn-knob at the bottom tip of the cone, and the floc morphostructure was observed with a biologic microscope (BX51, Olympus) and a fluorescence microscope (Leica, DMI3000B).

2.6. Statistical analysis

Data obtained from the experiment were analyzed using SPSS 17.0 software (SPSS, Chicago, USA) for Windows. One-way ANOVA was performed on the experimental parameters. Differences were considered significant at $P<0.05$. When significant differences were found, Duncan's multiple range test was used to identify differences between the experimental groups.

3. Results

3.1. Water quality

The physical and chemical water quality parameters monitored throughout the experiment are presented in Table 1. There was no significant difference in temperature and pH observed between different BFs ($P > 0.05$). However, DO, NH_4-N、NO_2-N and NO_3-N had significant differences ($P < 0.05$). The relationship between the resulting DO concentrations from the three treatments was BF (Sta) > BF (Glu) > BF (Gly). The BF (Gly) treatment had the lowest concentrations of NH_4-N (0.12 ± 0.03 mg/L), NO_2-N (0.02 ± 0.01 mg/L) and NO_3-N (0.10 ± 0.02 mg/L). The concentrations found in the BF (Glu) treatment were

Table. 1
The water quality parameters in three kinds of bioflocs treatment during the experiment.

Parameter	BF (Glu)	BF (Sta)	BF (Gly)
Temperature (°C)	23.3 ± 1.9^a	23.5 ± 1.6^a	23.8 ± 1.3^a
DO (mg/L)	7.5 ± 0.2^{ab}	7.9 ± 0.1^b	7.1 ± 0.3^a
pH	7.6 ± 0.2^a	8.0 ± 0.3^a	7.7 ± 0.2^a
NH_4—N (mg/L)	0.13 ± 0.01^a	0.20 ± 0.03^b	0.12 ± 0.03^a
NO_2—N (mg/L)	0.04 ± 0.02^a	0.13 ± 0.02^b	0.02 ± 0.01^a
NO_3—N (mg/L)	0.14 ± 0.01^a	0.69 ± 0.01^b	0.10 ± 0.02^a

Each value represents mean ± S.D. Values in the same row with different superscript letters are significantly different at $P < 0.05$.