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

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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 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 BF 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).
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 used 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 pre-weighed 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 $1.0 \times 10^9$ 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 from the three treatments was BF (Sta) (Gly). The BF (Gly) treatment had the lowest concentrations of NH$_4$–N (0.13 ± 0.01 mg/L) and 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 significantly different at P < 0.05. When significant differences were found, Duncan’s multiple range test was used to identify differences between the experimental groups.

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 biofloc. 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 according to the following formula: carbohydrate (% DW) = 100

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, 4000g). Total DNA was extracted from the samples using a water DNA extraction kit (E.Z.N.A.$^\text{TM}$, Omega) according to 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 BF (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 significantly different at P < 0.05.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BF (Glu)</th>
<th>BF (Sta)</th>
<th>BF (Gly)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>23.3 ± 1.9$^a$</td>
<td>23.5 ± 1.6$^a$</td>
<td>23.8 ± 1.3$^a$</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>7.5 ± 0.2$^{b}$</td>
<td>7.9 ± 0.1$^{b}$</td>
<td>7.1 ± 0.3$^{b}$</td>
</tr>
<tr>
<td>pH</td>
<td>7.6 ± 0.2$^{b}$</td>
<td>8.0 ± 0.3$^{b}$</td>
<td>7.7 ± 0.2$^{b}$</td>
</tr>
<tr>
<td>NH$_4$–N (mg/L)</td>
<td>0.13 ± 0.01$^{b}$</td>
<td>0.20 ± 0.03$^{b}$</td>
<td>0.12 ± 0.03$^{b}$</td>
</tr>
<tr>
<td>NO$_2$–N (mg/L)</td>
<td>0.04 ± 0.02$^{b}$</td>
<td>0.13 ± 0.02$^{b}$</td>
<td>0.02 ± 0.01$^{b}$</td>
</tr>
<tr>
<td>NO$_3$–N (mg/L)</td>
<td>0.14 ± 0.01$^{b}$</td>
<td>0.09 ± 0.01$^{b}$</td>
<td>0.10 ± 0.02$^{b}$</td>
</tr>
</tbody>
</table>

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