Aquaculture 482 (2018) 103–110

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

The effect of different carbon sources on water quality, microbial community and structure of biofloc systems

Min Deng[a], Jieyu Chen[a], Jingwei Gou[a], Jie Hou[a,b,c], Dapeng Li[a,b,c], Xugang He[a,b,c,*]

[a] College of Fisheries, Huazhong Agricultural University, Wuhan 430070, PR China
[b] Freshwater Aquaculture Collaborative Innovation Center of Hubei Province, Wuhan 430070, PR China
[c] Hubei Provincial Engineering Laboratory for Pond Aquaculture, Wuhan 430070, PR China

ARTICLE INFO

Keywords:
Ammonia
Biofloc
Illumina-MiSeq
Microbial diversity
Ammonia-oxidizing bacteria

ABSTRACT

The main objective of this work was to investigate how different carbon sources affect microbial diversity of biofloc. The experiment consisted of the control and three types of biofloc systems using different carbon sources, tapioca starch (TS), plant cellulose (PC) and the combination of tapioca starch and plant cellulose (TS + PC), and conducted for 42 days in twelve 300 L Fiber Reinforce Plastic (FRP) circular tanks. *Pelteobagrus vachelli* ($n = 72$) of mean weight (38.1 ± 5.9 g) were randomly distributed into twelve tanks. Community characterization of total bacteria and ammonia-oxidizing bacteria in all treatments were respectively investigated by Illumina MiSeq analysis using PCR-amplified 16S rRNA gene fragments (primer 27F-338R for total bacteria and primer CTO189F-CTO654R for ammonia-oxidizing bacteria). The ammonia-nitrogen concentration in control (3.6 ± 4.6 mg L^{-1}) was significantly higher than that in all biofloc systems (2.4 ± 2.9 mg L^{-1}, 1.8 ± 2.4 mg L^{-1} and 2.2 ± 2.5 mg L^{-1} for TS, PC and TS + PC, respectively) ($P < 0.05$). Weight gain ratio in TS was significantly higher than the control ($P < 0.05$), while there is no significant difference between the control and treatment groups added plant cellulose (PC and TS + PC) ($P > 0.05$). The Illumina MiSeq sequencing results showed that biofloc systems incorporating plant cellulose treatment groups had a higher total bacterial diversity and greater microbial richness than those with no plant cellulose treatment groups (Control and TS) ($P < 0.05$). There was significant increase of Betaproteobacteria in the control and TS treatments, whereas Alphaproteobacteria was higher in treatment groups added plant cellulose ($P < 0.05$). Illumina sequencing analysis of ammonia-oxidizing bacteria V3 region also detected a higher community diversity in biofloc systems (Shannon index, > 1.21; Simpson index, < 0.3949) compared to control (Shannon index, 0.84; Simpson index, 0.6149). We conclude that organic carbon sources in biofloc system can decrease ammonia-nitrogen concentration and improve the community diversities of overall and ammonia-oxidizing bacteria.

1. Introduction

Much interest has been shown for biofloc technology (BFT) in shrimp and tilapia aquaculture (Azim and Little, 2008; Asaduzzaman et al., 2008; Wasielesky et al., 2013). BFT is a technique of enhancing water quality through the addition of extra carbon to the aquaculture system, through an external carbon source of elevated carbon content of the feed (Crab et al., 2012). In such system, a conglomerate of microbes, algae and protozoa develops in the water column, along with detritus and dead organic particles (Avnimelech, 2012). BFT involves stimulating the process of inorganic nitrogen assimilation by heterotrophic bacteria. These bacteria utilize ammonium, in addition to the organic nitrogenous wastes, to synthesize new cells by consumption of carbohydrates (Hargreaves, 2006). Carbon and nitrogen are well balanced in BFT system. This promotes nitrogen uptake by growth of heterotrophic bacterial decreases the ammonium concentration more rapidly than nitrification by nitrifying bacteria. Today, the research priority for BFT is to measure and describe the complex microbial biofloc community and to develop methods that will establish diverse and stable microbial communities (Cardona et al., 2016). A more diverse microbial community structure can facilitate decomposition of chemical pollutants and improve water quality (Ibekwe et al., 2007). Many studies have implied that the different types of carbon sources can affect the composition of the biofloc (Hollender et al., 2002; Oehmen et al., 2004). High-throughput sequencing is a culture-independent method which can enable the high-resolution characterization of microbial communities. Today, through the use of Illumina MiSeq sequencing technique, for the first time, authors can characterize the composition of the

* Corresponding author at: College of Fisheries, Huazhong Agricultural University, Wuhan 430070, PR China.
E-mail address: xgh@mail.hzau.edu.cn (X. He).

http://dx.doi.org/10.1016/j.aquaculture.2017.09.030
Received 7 March 2017; Received in revised form 17 September 2017; Accepted 18 September 2017
Available online 20 September 2017
0044-8486/

bacterial communities in situ. For instance, Cardona et al. (2016) provided the first information describing the complex microbial community in biofloc system using sugar cane molasses as organic carbon source. Wei et al. (2016) demonstrated that bioflocs grown on different carbon sources (glucose, starch and glycerol) have different nutritional composition and microbial community. Our previous study revealed that the abundance of some genera was significantly different between ponds with grass carp fed Sudan grass and ponds with grass carp fed commercial feed. Some potential pathogens were found to be significantly decreased, while some probiotics were significantly increased in ponds with grass carp fed Sudan grass (Qin et al., 2016). Literature describing the different microbial communities of biofloc systems as a result of different carbon sources, especially for plant cellulose, remains scarce.

Different from heterotrophs which gain their energy primarily form organic carbon sources, ammonia-oxidizing bacteria (AOB) are autotrophic organisms which require inorganic carbon for growth. However, many researches implied organic carbon source also affect the community composition of AOB. Many studies indicated that there was a significant increase of nitrate in biofloc system (Irshad et al., 2016; Zhao et al., 2012). Zhang et al. (2010) illustrated rotating annular bioreactors with high total organic carbon level produced more nitrite nitrogen, which was consistent with the AOB counts. Jones and Hood (1980) observed that *Nitrosomonas* spp. increased ammonium oxidation by 150% when grown in the presence of heterotrophs (*Nocardia atlantica* and *Pseudomonas* sp.). Racz et al. (2010) also provides evidence that the organic carbon source affects the make-up of the heterotroph community as well as AOB in mixed cultures. They hypothesize that in a mixed culture of heterotrophs and AOB, the more complex the organic carbon source will lead to a more diverse heterotrophic and, in turn, the more diverse the AOB community. Therefore, it is possible that there is some symbiotic relationship between heterotrophs and AOB although both of these feed different carbon sources. However, to the best of our knowledge, no reports have addressed the effects of plant cellulose on biofloc system.

Today, BFT is widely used in culture pond. The intensive microbial community present in BFT can serve as a pond water quality treatment system and the microbial protein can be used as a nutrient. However, very little information is available on the bacterial communities present in the biofloc system. To the best of our knowledge, no author has study the effect of plant cellulose on biofloc system. The goal of the present study was to assess the effect of different organic carbon on microbial community diversity and structure of biofloc. Knowledge of the community structure (include nitrifying bacteria) of a biofloc will help to understand the nitrogen reducing effect in biofloc system. Our research also provides references for BFT application and development in culture pond.

2. Materials and methods

2.1. Experimental design

The experiment was conducted for 42 days (June to July 2015) in 12 FRP circular tanks (300 L) with three treatments and one control viz., Control (no extra organic carbon addition), TS (tapioca starch as carbon source), PC (plant cellulose as carbon source), TS + PC (the combination of tapioca starch and plant cellulose as carbon source). Tapioca (*Manihot esculenta* Crantz) starch (Total carbon content: 58.2%) was locally purchased and plant cellulose (Total carbon content: 47.2%) was the stools collected from grass carp (*Ctenopharyngodon idellus*) rearing pond 8 h after feed *Sorghum sudanense* and dried at 106 °C to constant weight in the oven. All tanks were filled with water (initial pH 7.7 ± 0.2, ammonia nitrogen (NH_4-N): 0.2 ± 0.1 mg L^{-1}, nitrite nitrogen (NO_2-N): 0.2 ± 0.1 mg L^{-1}, nitrate nitrogen (NO_3-N): 4.2 ± 0.9 mg L^{-1}, mean volume of 190 L, mean depth of 0.4 m) collected from rearing ponds located in Wuhan, China (30°28′32″N–114°21′41″E). A 35-kW blower was attached to each of the six tanks to deliver continuously diffusing air from the bottom of the tanks through an airstone to maintain a suspension of organic solids and biomass and provide sufficient oxygen for rearing fishes. The daily quantity of carbon added was calculated according to Avnimelech (1999). The total carbon in different treatments was measured by a Multi N/C 3100 TOC analyzer (Analytik Jena AG, Jena, Germany) and the C/N ratio was schematically calculated according to Cardona et al. (2016). Carbon sources with a rate of 0.6 g per gram of feed were added twice a day between 08:00 to 09:00 and 18:00 to 19:00 to maintain a high C/N ratio (8.8 ± 0.3 for the control, 13.3 ± 0.3 for TS, 14.3 ± 0.4 for PC and 13.8 ± 0.3 for TS + PC). *Pelteobagrus vachelli* were obtained in June 2015 from a hatchery at Huazhong Agriculture University. The fishes were acclimatized in the FRP tanks (300 L × 3) for 14 days and were fed with a commercially produced floating pelleted feed (Xinhong Feed Co., Ltd. Hunan, China) having a protein content of 40% approximately. Also during acclimatization period, any fish showing apparent signs of disease or malnutrition were separated out. *Pelteobagrus vachelli* (n = 72) of mean weight (38.1 ± 5.9 g) were randomly distributed into 12 tanks to form four experimental groups in triplicate following a completely randomized design. All the experimental fishes were fed twice a day between 08:00 to 09:00 and 18:00 to 19:00. The commercially produced floating pelleted feed was formulated with fishmeal, defatted soybean meal, α-starch, wheat flour, yeast powder, forage calcium hydrophosphate, common salt, amino acid mix, vitamin and mineral mix. The proximate composition of the experimental feed is crude protein (40%), crude fat (4.5%), crude fiber (5.0%), crude ash (12.0%), calcium (0.7%–2.3%), total phosphorus (0.75%), common salt (0.4%–1.0%), lysine (1.8%), methionine (0.5%) and moisture (10%).

2.2. Assessment of water quality parameters

Water quality parameters including Dissolved oxygen (DO), pH and temperature were measured in-situ at 18:00 on daily basis using a multi-parameter YSI Professional Plus handheld water quality sonde (Yellow Spring, OH, USA). Water samples were collected at 18:00 on a weekly basis from each tank during the experiment using a horizontal water sampler. Analytical measurements of chemical oxygen demand (COD), ammonium nitrogen (NH_4-N), nitrite nitrogen (NO_2-N) and nitrate nitrogen (NO_3-N) in the water were performed weekly according to the standard methods (Chinese NEPA, 2012) using a Nanodrop® ND-2000C UV/vis spectrophotometer (Thermo Scientific). Concentrations of dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) were determined by a Multi N/C 3100 TOC analyzer (Analytik Jena AG, Jena, Germany).

2.3. Assessment of growth parameters

Fishes were temporarily cultured for 24 h without feeding at the end of the experiment. Fishes (n = 18) of different treatment groups were all weighed and assessed for the growth parameters including weight gain (%), feed conversion ratio (FCR), feed efficiency ratio (FER), and specific growth rate (%) (SGR) as previous report (Asaduzzaman et al., 2008).

2.4. Biofloc parameters and morphostructure

The biofloc volume (BFV), represented by the volume occupied by the flocs in 1 L of tank water after 30 min sedimentation, was determined using an Imhoff cone (1000-0010, Nalgene). Total suspended solids (TSS) were analyzed following the standard methods for water and wastewater analysis (Chinese NEPA, 2012). The sludge volume index (SVI) is defined as the volume in mL occupied by 1 g of TSS after settling. The floc 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).