Aquacultural Engineering 72–73 (2016) 58–64

Contents lists available at ScienceDirect

Aquacultural Engineering

journal homepage: www.elsevier.com/locate/aqua-online

# Effects of sludge retention time on water quality and bioflocs yield, nutritional composition, apparent digestibility coefficients treating recirculating aquaculture system effluent in sequencing batch reactor

Wenchang Liu[a], Guozhi Luo[a,b,c], Hongxin Tan[a,b,c,*], Dachuan Sun[a,b]

[a] College of Fisheries and Life Science, Shanghai Ocean University, Shanghai 201306, China
[b] Shanghai Aquacultural Engineering Research Center, Shanghai 201306, China
[c] Shanghai Collaborative Innovation For Aquatic Animal Genetics and Breeding, Shanghai 201306, China

ARTICLE INFO

*Article history:*
Received 5 December 2015
Received in revised form 9 March 2016
Accepted 3 April 2016
Available online 9 April 2016

*Keywords:*
Recirculating aquaculture system
Biofloc technology
Sequencing batch reactor
Sludge retention time
Jellylike bioflocs pellet

ABSTRACT

Bioflocs was produced in sequencing batch reactors (SBRs) that were treating recirculating aquaculture system (RAS) effluent using biofloc technology (BFT). The effects of sludge retention time (SRT = 1 d, 2 d, 3 d, 4 d, and 6 d) on the water quality, bioflocs yield, and nutritional composition were investigated. A type of jellylike bioflocs pellet was designed, and the apparent digestibility coefficients (ADCs) in tilapia (GIFT *Oreochromis niloticus*) were studied. The results showed that SRTs longer than 2 d were good for water re-utilization of the SBRs effluent. The bioflocs yield could be as high as 2.30–2.54 g VSS/g C (SRTs of 1–3 d). The protein values of solid waste (19.23 ± 0.87%) in RAS effluent treated by BFT could be significantly improved, which were 22.58 ± 1.14%, 23.38 ± 1.76%, 24.08 ± 0.94%, 23.92 ± 0.72%, 22.56 ± 1.64% in SRTs of 1–6 d, and that of the bioflocs was the highest in 3 d SRT. The results indicated that the frequently used protein/nitrogen conversion factor of 6.25 causes the protein content to be overestimated in bioflocs. SRT had no significant effects on the essential fatty acid and the polyunsaturated fatty acid contents in bioflocs. Jellylike bioflocs pellets could be successfully fed to tilapia, and the advisable ADCs of dry matter, protein, crude lipid, crude ash, and nitrogen were 57.28–58.64%, 67.04–69.77%, 76.90–80.80%, 53.50–59.82%, and 73.92–77.56%, respectively. In conclusion, a SRT of 3 d was the most advisable one.


## 1. Introduction

Increasing feed prices, wild fish over-exploitation, and environmental pollution have obstructed the development of sustainable aquaculture (Crab et al., 2007). Production of bioflocs from aquaculture systems for use as an alternative aquatic feed material has been attracting worldwide interest (Crab et al., 2007). Bioflocs, microbial community growth taking up dissolved nitrogen with biofloc technology (BFT), contain 20–60% crude protein and 1–5% crude lipid (Azim and Little, 2008; Crab et al., 2010; De Schryver and Verstraete, 2009; Kuhn et al., 2008; Liang et al., 2014; Lu et al., 2012; Luo et al., 2013). Bioflocs are good sources of vitamins and minerals; however, their content of *n*-3 fatty acids is low (Crab et al., 2010; Ju et al., 2008). Several factors affect the nutritional quality of bioflocs. The content of protein is increased when bioflocs grow on glycerol and acetate, and the lipid content is much higher for glucose feeds (Crab et al., 2010). Bioflocs production rates, protein and poly-$\beta$-hydroxybutyrate (PHB) contents increase with higher carbon supplementation levels (De Schryver and Verstraete, 2009; Ruan et al., 2011; Schneider et al., 2006a). *Bacillus subtilis* or another micro-organism inoculation can accelerate the bioflocs development process and improve their nutritional values (Correia et al., 2002; Crab et al., 2010; Lu et al., 2012).

Sludge retention time (SRT) may be another important factor affecting the nutritional quality of bioflocs. By adding carbohydrates into the BFT systems, bacterial growth is stimulated and nitrogen uptake occurs through the production of microbial proteins rather than through nitrification. However, nitrification was commonly observed in in-situ BFT aquaculture systems (Azim and Little, 2008; Nootong and Pavasant, 2011) and ex-situ BFT reactors (Liang et al., 2014; Luo et al., 2013). The capability of autotrophic nitrifying bacteria is significantly improved with a long hydraulic retention time (HRT) or SRT (Tay et al., 2002). The age of bioflocs influences the types of organisms that are present, and a young biofloc's age can yield predominately heterotrophic organ-

* Corresponding author at: College of Fisheries and Life Science, Shanghai Ocean University, Huchenghuan Road 999, Shanghai 201306, China.
*E-mail address:* hxtan@shou.edu.cn (H. Tan).

http://dx.doi.org/10.1016/j.aquaeng.2016.04.002
0144-8609/

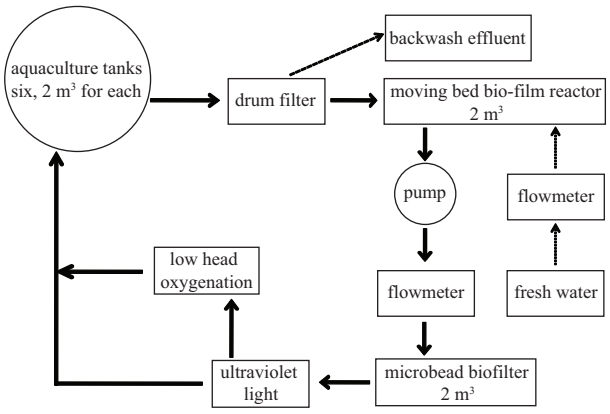


**Fig. 1.** Design schematic of recirclating aquaculture system.

isms (Avnimelech, 2012). The occurrence of nitrification means less nutrient substance is accumulated and stored. In a previous study, the protein and polysaccharide contents decreased when biofloc were cultured too long, and nitrification was observed in the biofloc reactors (Liang et al., 2014). When there is enough carbon in the active sludge reactor, the lower the SRT is (compare 0.5 d SRT, 1 d SRT, and 4 d SRT), the more the cultures use carbon for direct growth rather than storage (Johnson et al., 2010).

Dried bioflocs have been proposed as an ingredient to replace fishmeal or soybean meal in aquafeeds and to improve the growth performance and digestive enzyme activities of aquatic animals (Anand et al., 2014; Kuhn et al., 2010). However, it is unlikely that dried bioflocs can be used in commercial-scale aquafeed manufacturing. One probable problem is that drying bioflocs solids is not commercially efficient (Hargreaves, 2013). In previous studies, wet bioflocs had been directly used as a feed for juvenile *Litopenaeus vannamei* and *Macrobrachium rosenbergii* postlarvae (Crab et al., 2010; Kuhn et al., 2008), but they were only applied to detritivorous species. A type of jellylike bioflocs pellet, which was designed and made from wet bioflocs and an agar solution, was not dried. Additionally, because the temperature was not higher than 40 °C during the manufacture process, vitamins, enzymes, and some 'growth factors' would not have been easily inactivated, and we would have saved energy required for drying.

In the present study, bioflocs were produced in sequencing batch reactors (SBRs) by treating recirculating aquaculture system (RAS) effluent by BFT. We investigated the effects of SRT on bioflocs yield and nutritional composition. Moreover, jellylike bioflocs pellet was designed, and the apparent digestibility coefficients (ADCs) in juvenile genetically improved farmed tilapia (GIFT *Oreochromis niloticus*) were studied.

## 2. Material and methods

### 2.1. Recirculating aquaculture system effluent

A closed RAS was used in the current experiment (Fig. 1), which located in Shanghai Aquacultural Engineering Research Center, Shanghai, China. It consisted of a drum filter (100 μm mesh size), two bio-filters and six culture tanks (2 $m^3$ for each). The water in the RAS was renewed 12 times per day, daily water exchange rate was 5%. *Acrossocheilus fasciatus* was stocked (400 fish/$m^3$) with individual weights of 28–42 g and final fish weights of 30–51 g. The fish were fed a commercial diet (Ri Gao Special Feed Co., Ltd., Zhangzhou city, Fujian province, China) that contained moisture 9.68%, crude protein 30.14%, crude lipid 4.46% and coarse ash 13.06% (data were provided by actual determination). The daily feed rate was 1% of the total body weight.

**Table 1**
The characteristics of recirculating aquaculture system effluent.

| Parameters | | | N = |
|---|---|---|---|
| TAN | mg/L | 2.13 ± 0.95 (1.23–4.42) | 17 |
| $NO_2^-$-N | mg/L | 0.36 ± 0.29 (0.10–1.17) | 17 |
| $NO_3^-$-N | mg/L | 23.38 ± 4.81 (16.28–31.22) | 17 |
| TN | mg/L | 48.35 ± 8.12 (34.43–63.48) | 25 |
| DOC | mg/L | 15.53 ± 5.70 (7.00–28.22) | 25 |
| TOC | mg/L | 511.06 ± 106.78 (338.33–762.52) | 25 |
| TP | mg/L | 25.08 ± 4.87 (18.88–33.12) | 10 |
| SRP | mg/L | 4.25 ± 1.48 (1.62–6.34) | 17 |
| pH | | 6.26 ± 0.23 (5.65–6.58) | 17 |
| Ash | mg/L | 615.10 ± 135.61 (271.10–865.05) | 25 |
| TS | mg/L | 1522.60 ± 231.37 (1071.02–1915.01) | 25 |
| TSS | mg/L | 1046.78 ± 182.03 (783.51–1430.06) | 25 |
| VSS | mg/L | 865.49 ± 162.90 (629.93–1212.99) | 25 |
| COD | mg/L | 1214.27 ± 200.56 (915.21–1580.82) | 13 |
| TOC/TN [a] | | 10.58 ± 2.07 (7.50–14.99) | 25 |
| TOC/TN [b] | | 18.68 ± 2.94 (14.78–23.45) | 25 |

*Note*: Values represent averages ± standard deviation (minimum – maximum). N, number of samples; TAN, total ammonia nitrogen; $NO_2^-$-N, nitrite; $NO_3^-$-N, nitrate; TN, total nitrogen; DOC, dissolved organic carbon; TOC, total organic carbon; TP, total phosphorus; SRP, soluble reactive phosphorous; TS, total solids; TSS, total suspended solids; VSS, volatile suspended solids; COD, total chemical oxygen demand; TOC/TN [a], TOC/TN of RAS effluent; TOC/TN [b], TOC/TN of RAS effluent after sodium acetate was dosed into it.

Two hours before the RAS effluent was used for bioflocs production, a 100-L polyethylene tank was connected to the drum filter to receive the drum filter backwash effluent. The collected effluent was re-suspended by stirring before analyzing the waste composition (Table 1) and being used as a substrate. Before the RAS effluent was fed into SBRs, 1.25 g of sodium acetate (30% organic carbon, analytical reagent, China National Medicines Corporation Ltd., Shanghai, China) was added per liter, thus improving the carbon to nitrogen ratio (C/N) to higher than 15 according to BFT (Avnimelech, 2012).

### 2.2. Biofloc reactor operation and experimental design

Fifteen sequencing batch reactors were installed in the present study. The top internal diameter, bottom internal diameter, and height of the polyethylene reactors were 20.5 cm, 9.0 cm, and 38.0 cm, respectively, and the liquid-filled height of the reactors was 30.0 cm (equivalent to a 4.0-L working volume). The reactors were intensively mixed using 15 air stones placed at the bottom of each reactor, which were intensively powered by three 135-W air pumps (ACO-008, SenSen Co., Ltd., Zhejiang, China) operating at a total rate of 250 L/min. The reactors were maintained at 27–30 °C, and the dissolved oxygen levels (DO, 6.7–7.6 mg/L during the reaction phase) and pH (8.0–8.9) were monitored using a Multi 3430 meter (WTW, Germany).

Two liters of mature bioflocs was initially inoculated into each reactor. The bioflocs had been cultured from the above-mentioned RAS effluent using our previous method (Lu et al., 2012). The characteristics of the bioflocs were as follows: ash = 2.70 g/L, total solid (TS) = 6.17 g/L, total suspended solids (TSS) = 3.05 g/L, and volatile suspended solids (VSS) = 2.63 g/L.

Five different SRTs (1 d, 2 d, 3 d, 4 d, and 6 d) were maintained, and each SRT group consisted of three replicates. The HRT of all SBRs was 24 h. The operation of the SBRs was based on 12-h batch cycles consisting of feeding 2 L of sodium acetate containing RAS effluent (with anaerobic condition) as the first phase (10 min); a reaction phase (660 min); a bioflocs withdrawal phase (10 min) in which 2 L (1 d SRT), 1 L (2 d SRT), 0.667 L (3 d SRT), 0.5 L (4 d SRT), or 0.333 L (6 d SRT) of mixed bioflocs liquor was withdrawn; a settling phase (30 min); and an effluent withdrawal phase (10 min) in which 0 L (1 d SRT), 1 L (2 d SRT), 1.333 L (3 d SRT), 1.5 L (4 d SRT), or 1.667 L (6 d SRT) of reactor supernatant was withdrawn and discharged into