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Performance of feeding *Artemia* with bioflocs derived from two types of fish solid waste

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ABSTRACT

The production of bioflocs with the solid waste from recirculating aquaculture systems (RAS) and feeding *Artemia* results in additional nutrient retention and lowers waste discharged from RAS. The solid waste from the drum-filters of two RAS, which stocked European eel (*Anguilla anguilla*) and Nile tilapia (*Oreochromis niloticus*), was used as substrate to produce bioflocs in suspended growth reactors, referred to as E-flocs and T-flocs, respectively. Mono-diets consisting of 100% E-flocs and 100% T-flocs were added to culture *Artemia*, referred as E-*Artemia* and T-*Artemia*, respectively, in a laboratory scale test. The efficiency of this feeding regime was investigated. A significant difference was observed in terms of crude protein content (35.59 ± 0.2%) for E-flocs, (29.29 ± 0.95)% for T-flocs, (70.01 ± 0.92)% for E-*Artemia* and (65.63 ± 0.89)% for T-*Artemia*. 134 out of the total operational taxonomic units (OTUs) were present in E-flocs and T-flocs from the analysis of high-throughput sequencing data. Most of the shared OTUs belonged to cyanobacteria. C18:1*n*7 of T-flocs was higher than that of E-flocs ($P < 0.05$). C18:2n6 of E-flocs was significantly higher than that of T-flocs ($P < 0.05$). No significant difference was observed in the other fatty acid compositions ($P > 0.05$). The survival rate of E-*Artemia* was (22 ± 0.02) %, significantly higher than that of T-*Artemia* (16% ± 0.02%) ($P < 0.05$). No significant difference was observed between the average body weight of E-*Artemia* (2.38 ± 0.40 mg) and E-*Artemia* (2.91 ± 0.21) ($P > 0.05$). The EPA of *Artemia* fed with E-flocs was (3.00 ± 0.46)%, significantly higher than that of T-*Artemia* (1.57 ± 0.19%) ($P < 0.05$). This study offers a method for reusing the aquaculture waste, which will be helpful to achieve a zero-pollution discharge for aquaculture systems.

1. Introduction

Aquaculture is one of the fastest growing animal food-producing sectors (FAO, 2014). To obtain higher yields, several intensified aquaculture systems were developed through a high stocking density and inputs of artificial food with 25%–45% crude protein (Kutty, 2005). Shrimp or fish naturally utilize a limited amount of the nutrition contained within the food, with up to 75% of the input nitrogen discharged (Hargreaves, 1998; Tovar, Moreno, Manuel-Vez, & GarciaVargas, 2000). If the nitrogen is not treated properly before being discharged, it can be detrimental to the environment. In response to this concern, recirculating aquaculture systems (RAS) were explored as an alternative to pond and cage culture systems (Timmons & Ebeling, 2007). Although RAS provide many advantages over traditional aquaculture systems, 11%–40% of the applied feed was discharged as waste sludge (Davidson & Summerfelt, 2005). As the result of the super-intensive culture in RAS, a considerable amount of sludge is produced that must be treated before it can be disposed (Mirzoyan, Tal, & Gross, 2010; Timmons & Ebeling, 2007). Waste characteristics may vary widely, depending on the fish species (Timmons & Ebeling, 2007). European eel (*Anguilla Anguilla*) and Nile tilapia (*Oreochromis niloticus*) are two of the common species farmed in RAS (Martins et al., 2010; Mota, Martins, Eding, Canário, & Verreth, 2017).

Biofloc technology (BFT) uses aerobic heterotrophic bacteria to convert inorganic nitrogen (NH_4^+-N, NO_2^--N and NO_3^--N) into microbiomass, which is a protein source for the fish or shrimp (De Schryver & Verstraete, 2009). Biofloc is a heterogeneous aggregate of bacteria, bacteria-grazing protozoans, exocellular polymers, and particulate

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organic matter, which is often included in the diets either directly for filter feeding or omnivorous species, such as shrimp and tilapia (Hargreaves, 2013; Xu, Morris, & Samocha, 2016), or in a processed feed for shrimp (Kuhn, Lawrence, Crockett, & Taylor, 2016). However, the processing always requires drying, milling, and storage, which can affect nutritional characteristics (Emerenciano, Gaxiola, & Cuzon, 2013).

Artemia is one of the best sources of live food for cultured fish and shellfish species (Toi, Boeckx, Sorgeloos, Bossier, & Stappen, 2013). *Artemia* can feed on a wide range of diets, such as micro-algae, bacteria, protozoa, and small detritus particles (Fernández, 2001). *Artemia* has been cultured using chicken manure, pig manure, and algae-rich green water as food sources (Sui et al., 2013; Verma, Raghukumar, & Naik, 2011). Previous research has proved that *Artemia* can efficiently feed on the bioflocs produced from aquaculture waste (Luo, Yao, Tan, & Wu, 2017). This makes it possible to avoid the risks of adverse environmental impacts from aquaculture processing. Moreover, feeding *Artemia* with bioflocs may be a promising way to directly deliver probiotics to the digestive tract of the target aquaculture species (Kesarcodi-Watson, Kaspar, Lategan, & Gibson, 2008; Suzer et al., 2008).

Suspended-growth batching reactors (SGBRs) were connected to the drum-filter of the RAS to produce bioflocs with solids waste (Azim, Little, & Bron, 2007; Luo et al., 2017; Schneider, Sereti, Machiels, Eding, & Verreth, 2006). The potential of this technology stems from the additional protein retention harvested and lower overall nutrient discharge from RAS (Schneider et al., 2006). The current experiment was designed to investigate (1) bioflocs production using two types of RAS solid waste and (2) the efficiency of the above two types of bioflocs to feed *Artemia*.

2. Material and methods

2.1. Overview of experiment strategy

A laboratory-scale integrated RAS-SGBRs-*Artemia* production system was established for the current experiment. The system was comprised of three compartments: (a) two RASs culturing Nile tilapia/European eel; (b) six SGBRs producing bioflocs; and (c) six cylinders rearing *Artemia*. Water and sludge collected in the solid/liquid filter was batch-fed into the SGBRs periodically to produce bioflocs, which were used as food for *Artemia* (Fig. 1).

2.2. RAS, fish waste and SGBRs

The initial solid fish waste was obtained from the solid/liquid separators of two RASs stocked with European eel (*Anguilla Anguilla*) and Nile tilapia (*Oreochromis niloticus*) in Recirculating Aquaculture Engineering and Technology Laboratory, Shanghai, China. The RASs were equipped with eight 1 m^3 tanks, a drumfilter separator, a cold/heat control, a gas/liquid mixing device, two biofilters, an ultraviolet sterilization unit, and two circulating pumps (Fig. 1). The water in the system was recirculated at a rate of 24 times per day. Root-type compressed air blowers were used to aerate the water. The stocking density of eel and tilapia in each RAS was approximately (12 ± 0.5) kg/m^3 and (22.6 ± 1.0) kg/m^3, respectively.

The eels were fed a commercial pellet diet (Tongwei 8912, Sichuan, China) containing 10% moisture, 48% crude protein, 2% crude fibers, 17.0% crude ash, and 1.5% total phosphorus. The tilapias were fed a commercial pellet diet (Tongwei 8912, Sichuan, China) containing 10% moisture, 30% crude protein, 12% crude fibers, 13% crude ash, and 1.5% total phosphorus.

The characteristics of eel waste were as follows: (147.6 ± 2.2) g/L of total suspended solids (TSS), (238.8 ± 11.3) g/L of chemical oxygen demand (COD), and (9.9 ± 0.3) g/L of nitrogen. The characteristics of tilapia waste were as follows: (138.2 ± 1.2) g/L of TSS, (308.6 ± 10.1) g/L of COD, and 5.1 g/L of nitrogen. The nitrogen content of the bioflocs was used instead of the crude protein content because all the organic nitrogen was degraded to ammonium nitrogen first and then assimilated into the bioflocs as micro-biomass (Avnimelech, 1999).

Six SGBRs, with a working volume of 15 L, were used to produce bioflocs in the current study, three for tilapia waste (T-flocs) and three for eel waste (E-flocs). The fish waste from the drum-filters of RASs were completely pulverized and filtered through a 10-μm mesh and the filtrate was added to each reactor in equal amounts as the TSS source at initial concentrations of approximately 5000 mg/L. Each reactor was filled with seawater with salinity (30 ± 1.0) g/L. The temperature of the reactors was maintained at (25 ± 1) °C. The mixture was thoroughly suspended by placing an air stone at the bottom of each reactor. The air blowers

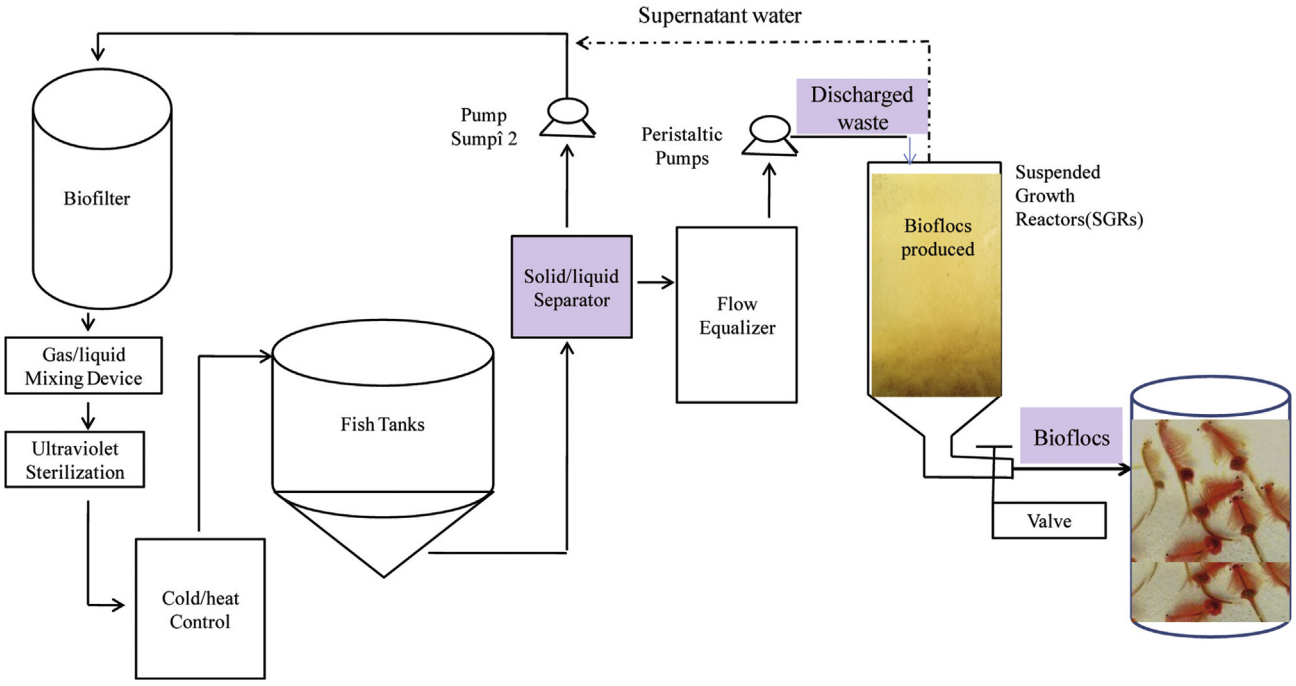

Fig. 1. The technical process diagram of the current experiment.

were supplied with a 138-W air pump (ACO-008, SenSen Co., Ltd., Zhejiang, China) operating at a rate of approximately 20 L/min. Dissolved oxygen (DO) was maintained (7.2 ± 1.0) mg/L. The dissolved organic carbon to total ammonia nitrogen ratio (DOC/TAN) in the SGBRs-BFT was maintained at >15 (*w/w*) by adding glucose as the external carbohydrates.

The SGBRs reached a steady state on approximately day 37 (Hargreaves, 2013). From day 38, the bioflocs were harvested from the SGBRs to feed *Artemia*, and the same weight of fresh waste from the RAS was added every day. The crude protein content, and the quantity and quality of the fatty acid (FA) of flocs were measured at every feeding time. Glucose was added to the SGBRs every day to enrich the flocs to maintain DOC/TAN at > 15 (*w/w*) during the feeding period.

2.3. Artemia

The enrichment grade *Artemia* cysts (Shanghai Ocean University, China) were hydrated in tap water for 1 h at 4 °C using a standard protocol, and then the cyst shells were decapsulated (Sorgeloos, Lavens, Léger, Tackaert, & Versichele, 1986). The decapsulated cysts were incubated at a density of 30 g/L in a 160-L conical glass at (27 ± 1) °C, pH (8.50 ± 0.50), salinity (35 ± 1) g/L, and continuous light of 1000 lx for 24 h.

The *Artemia* instar I nauplii were separated from the empty cysts, washed in tap water, and placed in 4-L plastic cylinders containing well-aerated seawater (salinity: 30 ± 1) at (26 ± 1) °C. The cylinders were aerated to supply dissolved oxygen (up to 6 mg/L). All of the cylinders were irradiated with an illumination of 1000 lx on a 12 h/12 h on/off cycle. A stocking density of 3000 nauplii/L was employed in each tank. The two dietary treatments were set up in triplicate. Dietary E-flocs contained only bioflocs produced from eel waste. Dietary T-flocs contained only bioflocs produced from tilapia waste. Crab, Lambert, Defoirdt, Bossier, and Verstraete (2010) suggested that the bioflocs at the concentrations of 0.18 g/L could be eaten efficiently by nauplii. The bioflocs were harvested directly from the SGBRs and fed to *Artemia* in the doses of 0.2–0.5 g/L suspended solids, at intervals determined by the observation of *Artemia* intestines. *Artemia* was cultured over a period of 19 days and checked daily for the ingestion status under a binocular microscope. Uneaten flocs and wastes from *Artemia* were not removed and no water was changed during the experimental period. The individual length, survival rate, biomass production, fatty acid (FA) content, and crude protein content of *Artemia* in each dietary trial were analyzed at the end of the experiment.

2.4. Data collection and sample analysis

2.4.1. Water quality parameters

The water temperature (°C), DO, and pH were measured daily using a YSI-pro plus meter (YSI Incorporated, Yellow Springs, OH, USA). The TSS, volatile suspended solids (VSS), NH_4^+-N, NO_2^--N and NO_3^--N were analyzed according to the Chinese State Environmental Protection Agency (SEPA) standard methods (Chinese SEPA, 2004). The dissolved organic carbon (DOC) was evaluated daily using a total organic carbon analyzer (TOC-V CPH; Shimadzu Seisakusho, Japan).

2.4.2. Artemia performance parameters

Ten *Artemia* from each dietary trial were randomly collected. The individual lengths of the *Artemia*, from the front of the head to the end of the telson, were determined using an electronic dissecting microscope (Nikon SMZ1500, Nikon Instruments Inc., Melville, NY, USA) and converted to the actual length using software *Artemia* 1.0® (courtesy of Marnix Van Domme). After being rinsed thoroughly in deionized water to remove waste, the *Artemia* were then placed on tissue paper to remove excess water and then weighed. All *Artemia* were harvested at the end of the experiment. The survival in each replicate was calculated according to the following equation:

Survival (%) = (final number of *Artemia*/initial number of *Artemia*) × 100.

The biomass production (BP) in wet weight (g/L) in each cylinder was calculated by weighing the total production and the averaged BP for every diet.

2.4.3. Crude protein and fat content of Artemia and bioflocs

Artemia and microbial flocs used to determine crude protein content and crude fat content were freeze-dried at −80 °C before analysis. The crude protein content was determined by measuring nitrogen via the Kjeldahl method (Chinese SEPA, 2004). The protein content was calculated assuming that it contained 16% nitrogen and was expressed as a percentage of the bioflocs' dry matter weight (AOAC, 1999). The volatile suspended solids (VSS) fraction was considered as a measure of the bacteria concentration in the current experiment (Tchobanoglous, Burton, & Stensel, 2003).

2.4.4. Fatty acid extraction and analysis

Crude fat was extracted from the sampled *Artemia* as described by Folch, Lees, and Sloane Stanley (1957). The samples, coupled with 50 mL of nonadecanoic acid, were used as substitute then added with standard working solution. The samples were lapped by pellet pestle (Sigma-Aldrich, USA), homogenized with chloroform/methanol (2/1) to a final 20 times volume and stored at 4 °C overnight for fat extraction. After centrifugation at 2000 r/min for 10 min, the supernatant was transferred to 50 mL storage bottles and thoroughly homogenized with 3 mL of saturated saline using the pellet pestle (Sigma-Aldrich, USA) on ice for at least 2 h. he upper liquid phase layer was then removed and the lower layer organic phase layer mixed with 1 mL of the chloroform: methanol: water (*v*: *v*: *v* ¼ 3:48:47) solution. After discarding the upper layer, the extracts were evaporated under a moderate nitrogen flow until dry. The residuum was mixed with 2 mL of boron trifluoride methanol solution (14%, *w/w*) in every test tube followed by water bath at 100 °C for 20 min, before immediate transfer into cold water. After that, 2 mL of benzene and 2 mL of methanol saturated normal saline were added into each tube and mixed with a violent shake in a water bath at 100 °C for 20 min, and then the supernatant was transferred into 50 mL stripping tubes and eluted using 2 mL n-hexane followed by an addition 4 mL of n-hexane. The last procedure was repeated once. The resulting extracts were evaporated until dry under a nitrogen stream and transferred into 2 mL glass tube. Each stripping tube was washed with n-hexane three times, with the washing liquid transferred into glass tubes. All extracts were evaporated until dry under a nitrogen stream to 1 mL in a water bath at 40 °C, transferred into sample vials, sealed with parafilm, and stored in darkness at −20 °C until the GC-MS analysis.

A 7890A gas chromatograph coupled with a 5975C mass spectrometer (Agilent Inc., USA) was used. The FA methyl ester was separated using a DB-5 ms capillary column (30 m × 0.25 mm × 0.25 μm, Agilent Inc., USA). The identification and quantification of the fatty acids were performed according to the method of Toi et al. (2013). The FAs were identified by comparing the peak area of each peak with the peaks of the known standards (Guinot et al., 2013), 0.2 g fresh tissue +1.0 mL ($1NH_2SO_4$) + 100 μg internal standard (19:0 methylester,10 μL from 10 mg/mL) in a screw-capped tube.

2.4.5. Bacteria community analysis

On day 38, the bioflocs were taken out of SGBRs for the bacterial community analysis. In addition, *Artemia* adults were kept in ethanol (70%) and washed three times with sterile ultrapure water at the end of the experiment. They were dissected with sterile tools under a stereoscope microscope to isolate the guts, which were homogenized with a pipette by blowing up and down until complete homogenization of the soft *Artemia* tissues was achieved. Then, the *Artemia* adults were incubated with lysozyme (Sigma) for 45 min. The genomic DNA was extracted using the Qubit 2.0 DNA and the polymerase chain reaction products were determined by pyrosequencing using Miseq Illumina by

Majorbiotech Co., Ltd. (Shanghai, China) according to the standard protocols and software (data collection software, Illumina). The raw sequencing data obtained from this study were deposited to the NCBI Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra/) with the project accession number of SRR5366148 - SRR5366151.

The sequences were clustered into OTUs by setting a 0.03 distance limit (equivalent to 97% similarity) using the UCLUST v1.1.579. The Shannon index, Chao 1 index, and abundance-based coverage estimator (ACE) indices were calculated to compare the microbial diversity and richness between these flocs samples. Sequences were phylogenetically assigned to taxonomic classifications using an RDP classifier Bayesian Algorithm (http://rdp.cme.msu.edu/). The sequences were allocated down to the phylum, class, and genus levels. The relative abundance of a given phylogenetic group was set as the number of sequences affiliated with that group divided by the total number of sequences per sample.

2.5. Data analysis

Statistical analysis of the experimental data was conducted using SPSS 16.0 software (SPSS, Inc., Chicago, IL, USA). The data of crude protein content, fatty acid composition of biofloc and *Artemia*, survival rates of *Artemia* were checked for homogeneity of variance and normal distribution by Levene's *F* test and *P*-P plot. The data failed to meet these assumptions and were logarithmically transformed to satisfy normal distribution and to homogenize variance. An ANOVA followed by Tukey post hoc test at 0.05 probability level was employed for the parameters of two types of flocs and each feed regime.

3. Results

3.1. Nutritional quality of biofloc

There were no observed differences in the VSS concentrations of E-flocs and T-flocs ($P > 0.05$) (Table 1). The crude protein content of T-flocs (35.59 ± 0.23%) was significantly higher than that of E-flocs (29.29% ± 0.95%) ($P < 0.05$). The biofloc was further analyzed to see whether it contained essential fatty acids on day 37 (Table 1). There were no significant differences among the other compositions between E-flocs and T-flocs ($P > 0.05$), except for the C18:1*n*7 and C18:2*n*6.

3.2. Diversity of bacteria community in bioflocs

The Illumina MiSeq sequencing was applied to evaluate the differences in the bacteria community composition of E-flocs and T-flocs. A 97% (cut off = 0.3) was used to cluster OTUs in the downstream analyses.

Table 1
Concentrations of total suspended solids (TSS), volatile suspended solids (VSS) and crude protein content, Fatty acid composition (>1%) of T-flocs and E-flocs.

	E-flocs (%)	T-flocs (%)
TSS (mg/L)	5149.33 ± 341.90[a]	5266.00 ± 373.77[a]
VSS (mg/L)	1492.00 ± 34.95[a]	1591.83 ± 57.36[a]
Crude protein content (%)	35.59 ± 0.23[a]	29.29 ± 0.95[b]
Fatty acid composition (> 1%)		
C14:0	1.54 ± 0.78[a]	1.65 ± 0.22[a]
C16:0	23.58 ± 1.92[a]	22.94 ± 3.06[a]
C16:1	1.29 ± 0.53[a]	2.25 ± 0.51[a]
C18:0	5.80 ± 1.56[a]	4.91 ± 0.36[a]
C18:1n9c	43.84 ± 3.10[a]	42.09 ± 1.74[a]
C18:1n7	2.02 ± 1.70[a]	4.75 ± 0.22[b]
C18:2n6	23.28 ± 2.00[a]	20.21 ± 0.19[b]
C18:3n3	1.72 ± 0.35[a]	1.28 ± 0.33[a]

Values are mean ± standard deviation (n = 3). Different superscripts in the same row denote significant differences among values. E-flocs: flocs produced with solids waste from recirculating aquaculture system stocked European eel (*Anguilla anguilla*). T-flocs: flocs produced with solids waste from recirculating aquaculture system stocked Nile tilapia (*Oreochromis niloticus*).

Following sub-sampling, a total of 39853 sequences with an average length of 430 bp for E-flocs and a total of 30239 sequences with an average length of 433 bp for T-flocs were acquired. These results indicated that there were no significant differences in the species diversity (Shannon index), the species richness indices of ACE and Chao1 between E-flocs and T-flocs (Table 2). The total classified OTUs in the bacterial communities were 162 and 179 for E-flocs and T-flocs, respectively. 134 out of the total OTUs were present in E-flocs and T-flocs. Most of the shared OTUs belonged to cyanobacteria.

At the phyla level, the dominant bacteria communities were similar, and the abundance showed a little difference. The top three phylum in terms of average abundance of E-flocs were cyanobacteria (60.62%), Proteobacteria (20.00%), and Bacteroidetes (9.48%). The top three phylum in terms of average abundance of T-flocs were cyanobacteria (49.79%), Proteobacteria (26.58%), and Bacteroidetes (11.32%).

There were 11 out of 135 genera with relative abundances higher than 1% for E-flocs. The top three genera, in terms of average relative abundance of E-flocs, were Parvibaculum (60.61%), Propionibacteriaceae (6.69%), and Bradyrhizobium (3.93%). There were 13 out of 128 genera with relative abundances higher than 1% for T-flocs. The top three genera, in terms of average abundance of T-flocs, were Parvibaculum (49.79%), Dietzia (8.42%), and Bradyrhizobium (6.14%). Parvibaculum was the most dominant genera in both E-flocs and T-flocs (Fig. 2).

3.3. Artemia performance

The survival rate (22 ± 0.02%) of *Artemia* fed E-flocs (E-*Artemia*) was significantly higher than that (16 ± 0.02%) of fed T-flocs (T-*Artemia*) ($P < 0.05$). No significant differences in individual length, wet weight, and biomass produced were observed between the two groups ($P > 0.05$) (Table 3).

3.4. Crude protein content and fatty acid composition of Artemia

There was a significant difference in the crude protein content between E-*Artemia* and T-*Artemia* ($P < 0.05$), (70.01 ± 0.92)% and (65.63 ± 0.89)%, respectively ($P < 0.05$) (Table 4).

At the end of the culture period, the fatty acid analysis of *Artemia* suggested that C18:1*n*9c of T-*Artemia* was significantly higher than that of E-*Artemia* ($P < 0.05$). The other fatty acids of E-*Artemia* were slightly or significantly higher than those of T-*Artemia* ($P < 0.05$). It is worthy to highlight that C20:5*n*3 (Eicosapntemacnioc Acid, EPA) was (3.00 ± 0.46) % in E-*Artemia*, which was higher than that of T-*Artemia* ((1.57 ± 0.19)%) ($P < 0.05$).

3.5. Bacteria community in the gut of Artemia

The top three in terms of phyla of the bacteria community in the gut of E-*Artemia* were cyanobacteria (43.21%), Proteobacteria (43.45%), and Firmicutes (16.13%). 10 out of 175 genera had relative abundances higher than 1% for the gut of E-*Artemia*. The top three phyla of the bacteria community in the gut of T-*Artemia* were Proteobacteria

Table 2
Comparison of diversity estimators of the bacteria communities of E-flocs and T-flocs.

Sample	OTU	Ace	Chao	Shannon	Simpson	Coverage (%)
E-flocs	179[a]	207[a]	210[a]	2.15[a]	0.38[a]	99.84[a]
T-flocs	162[a]	190[a]	197[a]	2.37[a]	0.26[a]	99.85[a]

Values are mean ± standard deviation (n = 3). Different superscripts in the same row denote significant differences among values. E-flocs: flocs produced with solids waste from recirculating aquaculture system stocked European eel (*Anguilla anguilla*). T-flocs: flocs produced with solids waste from recirculating aquaculture system stocked Nile tilapia (*Oreochromis niloticus*).

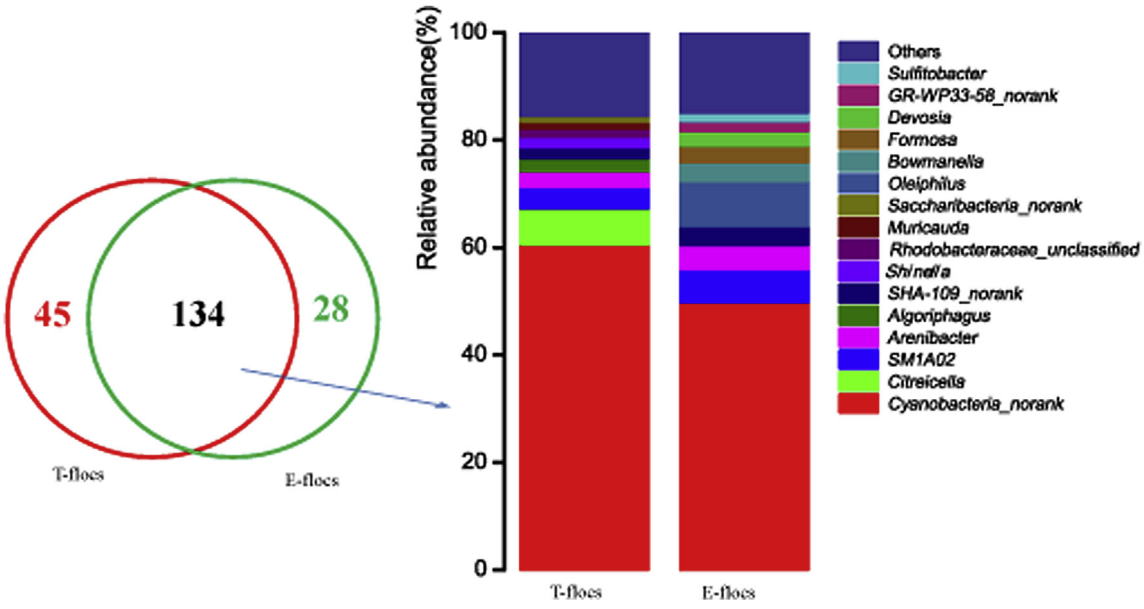

Fig. 2. Overlap of the two bacterial communities and phylogenetic classification at the genus level of the bacteria communities of T-flocs and E-flocs.

Table 3
Survival rate (%), individual length (mm), individual weight (mg) and biomass production (g/L) of E-*Artemia* and T-*Artemia*.

Groups	Survival rate (%)	Individual length (mm)	Individual weight (mg)	Biomass production (g/L)
E-*Artemia*	22 ± 0.02[a]	5.63 ± 1.02[a]	2.38 ± 0.40[a]	1.57 ± 0.12[a]
T-*Artemia*	16 ± 0.02[b]	5.90 ± 1.92[a]	2.91 ± 0.21[a]	1.40 ± 0.08[a]

Table 4
Fatty acid composition and crude protein content of E-*Artemia* and T-*Artemia*.

Fatty acids (%)	E-*Artemia*	T-*Artemia*
C14:0	0.80 ± 0.07[a]	0.97 ± 0.13[a]
C15:0	0.67 ± 0.11[a]	0.74 ± 0.13[b]
C16:0	10.53 ± 1.63[a]	12.13 ± 0.75[b]
C16:1	4.77 ± 0.78[a]	4.27 ± 0.43[a]
C17:0	1.81 ± 0.04[a]	0.91 ± 0.12[b]
C18:0	5.94 ± 0.88[a]	5.37 ± 0.76[a]
C18:1n9t	–	1.72 ± 0.19[b]
C18:1n9c	23.07 ± 2.79[a]	35.39 ± 1.20[b]
C18:1n7	11.21 ± 3.43[a]	7.56 ± 1.78[b]
C18:2n6c	19.36 ± 4.45[a]	16.43 ± 1.61[a]
C20:0	4.28 ± 2.12[a]	2.26 ± 0.51[b]
C18:3n6	1.80 ± 0.25	–
C18:3n3	1.47 ± 0.33[a]	0.87 ± 0.12[b]
C20:4n6	3.54 ± 2.49[a]	1.11 ± 0.35[b]
C20:5n3(EPA)	3.00 ± 0.46[a]	1.57 ± 0.19[b]
Crude protein content (%)	70.01 ± 0.92[a]	65.63 ± 0.89[a]

Values are mean ± standard deviation (n = 3). Different superscripts in the same row denote significant differences among values. E-*Artemia*: *Artemia* fed on flocs produced with solids waste from recirculating aquaculture system stocked European eel (*Anguilla anguilla*). T-*Artemia*: *Artemia* fed on flocs produced with solids waste from recirculating aquaculture system stocked Nile tilapia (*Oreochromis niloticus*). "-" means non-detected.

(34.94%), cyanobacteria (31.07%) and Firmicutes (26.56%) (Fig. 3). 11 out of 183 genera had relative abundances higher than 1% for the gut of T-*Artemia*. The bacteria genera in the T-flocs, E-flocs, T-*Artemia* gut, and E-*Artemia* gut are shown in Table 5 in order to understand the bacteria communities among the two bioflocs and the two types of *Artemia*. Parvibaculum was the most dominant genus in T-flocs and E-flocs. At the genera level, similarity is relatively high between the bacteria community of E-flocs and T-flocs. The difference is more obvious between the bacteria community in the guts of T-*Artemia* and E-*Artemia,* and the genera in the T-*Artemia* gut and E-*Artemia* gut were significantly different from those in the bioflocs.

4. Discussion

The objective of the current experiment was to compare the efficiency of feeding two types of bioflocs to *Artemia*. The crude protein content (DW%) was within the range of the 23%–50% stated in the related reports (Avnimelech, 2012; Azim et al., 2007; Khanjani, Sajjadi, Alizadeh, & Sourinejad, 2017). The crude protein content in E-flocs was higher than that of T-flocs. This result might be because the nitrogen content in European eel waste was higher than that of tilapia waste in the current experiment.

The crude protein contents of *Artemia* (70.01% ± 0.92% for E-*Artemia* and 65.63% ± 0.89% T-*Artemia*) were higher than those (40%–60%) reported in related studies (Anh, Van, Van, & Sorgeloos, 2009; Sorgeloos, 1989). This was perhaps because the crude protein content of the bioflocs was higher than the other food offered to *Artemia*, e.g., *Chlorella* (approximately 14%) (Luo et al., 2017). In our previous research, the crude protein contents of the *Artemia* that were only fed flocs and *Artemia* fed flocs mixed with *Chlorella* were significantly higher than that of the *Artemia* fed only *Chlorella* (Luo et al., 2017). Docosahexaenoic acid (DHA), which is one of the essential fatty acids, was not identified (Table 5). The low levels of DHA usually found in *Artemia* raise concerns by researchers (Cutts, Sawanboonchun, Mazorra de Quero, & Bell, 2006). The previous study suggested that the DHA level in *Artemia* was particularly hard to increase through the using of any enrichment process (Haché & Plante, 2011).

In addition to the essential nutrients in bioflocs, such as crude protein and fatty acid, the presence of bacteria in bioflocs is believed to play a prominent role in the value of the bioflocs as a food source for aquatic animals. Bacterial enzymes in bioflocs were detected in many research

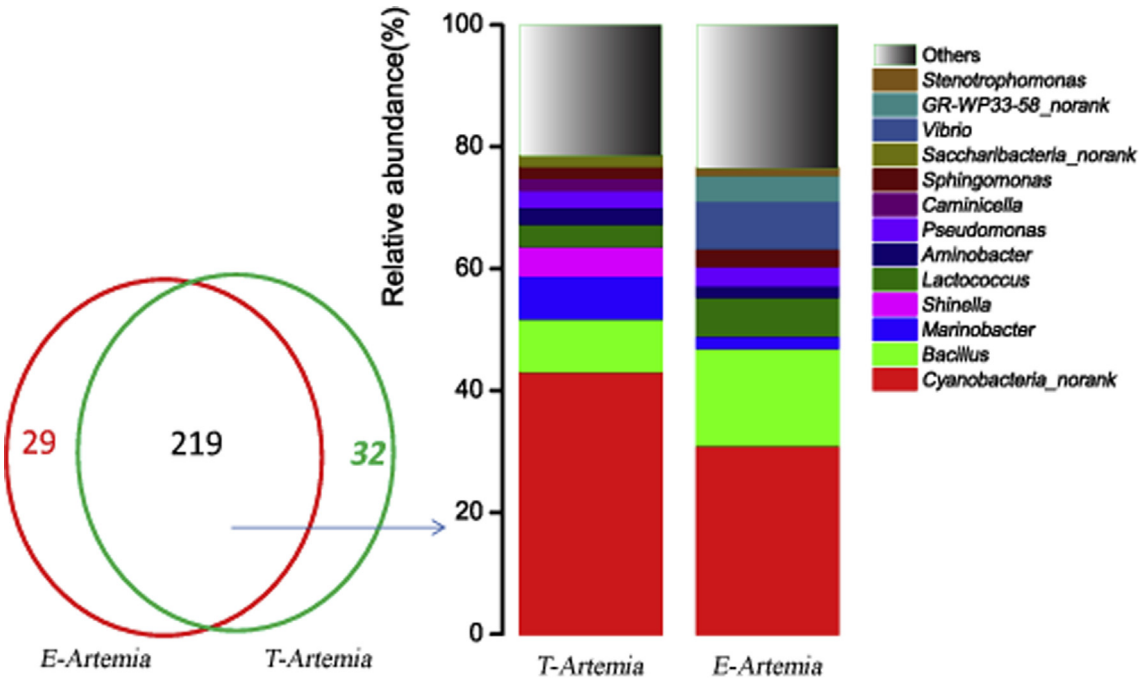

Fig. 3. Overlap of the two bacterial communities and phylogenetic classification at the genus level of the gut bacteria communities of E-*Artemia* and T-*Artemia*.

Table 5
Bacteria genera (abundance top 10) in T-flocs, E-flocs, and gut of T-*Artemia* gut and E-*Artemia*.

T-flocs	Abundance (%)	E-flocs	Abundance (%)
Parvibaculum	60.61	Parvibaculum	49.79
Propionibacteriaceae norank	6.69	Dietzia	8.42
Bradyrhizobium	3.93	Bradyrhizobium	6.14
Sporosalibacterium	2.96	Sporosalibacterium	4.55
Thermomonas	2.45	Brevibacillus	3.51
Brevibacillus	2.05	Salimesophilobacter	3.45
Bdellovibrio	1.92	mle1-27	3.14
Carnobacterium	1.60	OPB35 soil group	2.70
Flavobacteriaceae	1.18	Microbulbifer	1.88
Brachybacterium	1.05	Arcobacter	1.53
T-*Arteima*	Abundance (%)	E-*Arteima*	Abundance (%)
Cyanobacteria	43.21	Parvibaculum	31.05
Bacillus	8.61	Sneathiella	15.94
Marinobacter	7.09	Aequorivita	7.83
Shinella	4.87	Lachnospiraceae	6.40
Lactococcus	3.64	Microbulbifer	4.14
Aminobacter	2.81	Chryseobacterium	3.17
Pseudomonas	2.74	Azospira	2.89
Caminicella	1.97	Holosporaceae	2.01
Sphingomonas	1.86	Terrisporobacter	1.92
Saccharibacteria	1.73	Armatimonadetes	1.13

Values are mean ± standard deviation (n = 3). Different superscripts in the same column denote significant differences among values. E-flocs: flocs produced with solids waste from recirculating aquaculture system stocked European eel (*Anguilla anguilla*). T-flocs: flocs produced with solids waste from recirculating aquaculture system stocked Nile tilapia (*Oreochromis niloticus*). E-*Artemia*: *Artemia* fed on flocs produced with solids waste from recirculating aquaculture system stocked European eel (*Anguilla anguilla*). T-*Artemia*: *Artemia* fed on flocs produced with solids waste from recirculating aquaculture system stocked Nile tilapia (*Oreochromis niloticus*).

studies (Luo et al., 2014; Xu & Pan, 2012). This was the main reason that the current experiment directly fed *Artemia* fresh bioflocs, to protect the biological activities of the bioflocs. High bacteria diversity in bioflocs was reported in the current experiment and the related research (Crab, Kochva, & Verstraete, 2009; Wei, Liao, & Wang, 2016).

Two types of bioflocs were dominated by cyanobacteria, Proteobacteria, and Bacteroidetes, consistent with the results of the related research (Ferreira et al., 2015; Wei et al., 2016). cyanobacteria can grow well under heterotrophic conditions and is characterized by long filamentous flattened cells (Baeza, Lopez, & Ehas, 2017). The Proteobacteria detected is symbiotic in aquaculture (Sakami, Fujioka, & Shimoda, 2008). Proteobacteria removes organic matter, especially in wastewater treatment (Miura et al., 2007), and bioflocs aquaculture systems (Wei et al., 2016). Similar to our results, it was reported that *Bacteroidetes* were dominant in both biofilm and granules in a sequencing batch biofilter reactor (De Sanctis, Di Iaconi, Lopez, & Rossetti, 2010).

The survival rate (16%–22%) was slightly lower than the previous research (21.90%–46.90%) of Toi et al. (2013). The biomass production (1.40–1.57 g/L) in our experiment was within the range of 1.00–5.40 g/L in the previous research (Toi et al., 2013). This result may be due to the sizes of flocs (0.01–1.00 mm, not presented here) in the current experiment being too large for *Artemia*, especially younger individuals, decreasing the feeding efficiency. *Artemia* is a filter feeder of small food particles ranging 1–50 μm in size (D'Agostino, 1980). Biofloc size may not only relate to the uptake potential of the biofloc by *Artemia*, but also the digestibility of the flocs as well as the nutritional value of the flocs (De Schryver, Crab, Defoirdt, Boon, & Verstraete, 2008). The result of Ekasari et al. (2014) showed that the biofloc class of >100 μm contained the highest levels of proteins (27.8%) and lipids (7.5%), whereas the biofloc of <48 μm seemed to be richest in essential amino acids. The biomass production can be improved by breaking the produced bioflocs into different particle sizes according to the size of *Artemia*. Perhaps it would be better if the bioflocs were crushed or autoclaved to make them more accessible for the nauplii to feed on. However, autoclaving or crushing might cause changes in the structure of the bioflocs, destroying extracellular polymers and cell wall material, which will destroy the healthy probiotic. Consequently, if the bioflocs were not fed on time, they would perish and pollute the water. Our experiment lasted for 19 days. We fed the live bioflocs, without crushing or autoclaving the bioflocs before feeding. This feeding strategy might have decreased the availability of bioflocs to *Artemia*. More effective feeding techniques must be developed

in the future. In addition, a full analysis would need to also assess the economic costs and benefits of this way of utilizing the aquaculture waste stream.

5. Conclusion

A significant difference was observed in terms of crude protein content (35.59% ± 0.2%) for E-flocs, 29.29% ± 0.95% for T-flocs, 70.01% ± 0.92% for E-*Artemia* and 65.63% ± 0.89% for T-*Artemia*. The survival rate of E-*Artemia* was 22% ± 0.02%, significantly higher than that of T-*Artemia* (16% ± 0.02%). No significant difference was observed between the average body weight of E-*Artemia* (2.38 ± 0.40 mg) and E-*Artemia* (2.91 ± 0.21 mg). The EPA of *Artemia* fed with E-flocs was 3.00 ± 0.46%, significantly higher than that of T-*Artemia* (1.57% ± 0.19%). At the genera level, similarity is relatively high between the bacteria community of E-flocs and T-flocs. The difference is more obvious between the bacteria community in the guts of T-*Artemia* and E-*Artemia*and the genera in the T-*Artemia* gut and E-*Artemia* gut were significantly different from those in the bioflocs. This result suggests that the nutritional composition of the flocs produced with different fish waste were different. The flocs could efficiently be fed to *Artemia*. The current study supplied a method to deal with aquaculture waste.

Values are mean ± standard deviation (n = 3). Different superscripts in the same column denote significant differences among values. E-*Artemia*: *Artemia* fed on flocs produced with solids waste from recirculating aquaculture system stocked European eel (*Anguilla anguilla*). T-*Artemia*: *Artemia* fed on flocs produced with solids waste from recirculating aquaculture system stocked Nile tilapia (*Oreochromis niloticus*).

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