

Identification of biofloc microscopic composition as the natural bioremediation in zero water exchange of Pacific white shrimp, *Penaeus vannamei*, culture in closed hatchery system

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Abstract Study on the microscopic composition of biofloc in closed hatchery culture system was carried out to determine the interaction between the aggregation flocs in the bioremediation process for the decomposition and degradation of organic matter loaded in the shrimp culture tanks. The study was done for 105 days of culture period in zero water exchange. All of the organic loaded in the culture tanks identified comes from the shrimp feces, uneaten fed, and the decomposed macro- and microorganisms died in the culture tanks. All of the microscopic organisms in the biofloc were identified using Advance microscopes *Nikon 80i*. From the present study, there were abundances and high varieties of phytoplankton, zooplankton, protozoa, nematodes and algae species identified as aggregates together in the flocs accumulation. All of these microscopic organisms identified implemented the symbiotic process together for food supply, become the algae grazer, act as natural water stabilizer in regulating the nutrients in culture tank and serve as decomposer for dead organic matter in the water environment. Heterotrophic bacteria identified from *Pseudomonas* and *Aeromonas* family consumed the organic matter loaded at the bottom of culture tank and converted items through chemical

process as useful protein food to be consumed back by the shrimp. Overall it can be concluded that the biofloc organisms identified really contributed as natural bioremediation agents in zero water exchange culture system to ensure the water quality in the optimal condition until the end of culture period.

Keywords Biofloc compositions · Organic matter · Bioremediation · Symbiotic process

Introduction

Bioremediation is a process where microorganisms were stimulated with nutrients and other chemicals to enable them to wipe out contaminants in the targeted area and break down the hazardous substances into less toxic or non-toxic substances (Das 2014). Microorganisms are considered as the first living organisms to have evolved and are adaptive with the ecological changes. Nowadays, the use of microorganisms such as bacteria as biodegradation and bioremediation agent has come to attention because of its ability to reduce hazard, success in degrading natural and synthetic substances and accumulating toxic compound (Karigar and Rao 2011). According to Das et al. (2006), microorganisms are responsible for carbon fixation, nitrogen fixation, methane metabolism and sulfur metabolism, thus controlling the biogeochemical cycle. Microorganisms are able to produce diverse metabolic enzymes that can assist for safe removal of contaminants either by direct destruction or converting to safer or less toxic intermediate (Dash and Das 2012). Any microorganisms used as bioremediation has to possess resistant genotype for the particular pollutant and because of it, microorganisms possess certain unique characteristic which make them suitable for bioremediation processes (Stelting et al. 2010).

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Biofloc technology (BFT) is a promising technology which promotes the retention of waste and its conversion to biofloc as natural food for shrimp in the aquaculture system (Panigrahi et al. 2014). Biofloc consists of microorganisms such as heterotrophic bacteria, algae (dinoflagellates and diatoms), fungi, ciliates, flagellates, rotifers, nematodes, metazoans and detritus that conglomerate together and perform symbiotic processes to maintain the water quality, maintain bio-security, support high density of shrimp culture and reduce water exchange in the aquaculture system. In the biofloc technology (BFT) application, protein is utilized as a feed for the shrimp when the heterotrophic microbe in the biofloc converts the nitrogenous waste in the culture tank from the uneaten feed into protein. Development of dense heterotrophic bacterial community rather than algae dominated will overcome the waste generated in the aquaculture system through in situ bioremediation (Panigrahi et al. 2014). By addition of cheap carbohydrate sources such as molasses or tapioca usually in ration around C: N 12–15:1 in the water column, biofloc will convert the toxic nutrients in the water to beneficial food sources for shrimp consumption. Avnimelech (2009) found out that in high stocking density and zero or minimal water exchange the additional carbon source encourage the development of heterotrophic bacteria in the pond or tank. Schneider et al. (2005) discovered that addition of organic nitrogenous waste, ammonium will be converted into bacterial biomass if C:N ratio is balanced at ratio 10–15:1. Usually in BFT, heterotrophic bacteria are more dominant than nitrifying bacteria because of their higher growth rate and microbial biomass yield per substrate, thus making many fold increase of heterotrophic bacteria (Hargreaves 2006). Identification of the microscopic biofloc composition can help in better understanding the application of biofloc. From the identification of each class of organisms' function (phytoplankton as primary producer, zooplankton as the algae grazer, bacteria and protozoa as organic matter decomposer) that occurs in the zero water exchange culture system, the interaction happening between the organisms in the biofloc system can be understood. Because of the potential of biofloc technology for bioremediation in the aquaculture system, present study was conducted to identify the biofloc microscopic composition and to determine the biofloc performance as the natural bioremediation agent for removal of organic matter loader in the culture tank in zero water exchange system.

Methodology

Experimental design

Rounded tank with capacity 8 ton (height = 1.2 m and diameter = 3.3 m) was stocked with Pacific White shrimp,

Penaeus vannamei, postlarvae at PL10 with density of 100 PL per cubic meter, m³. Six tanks were used in the experiment for treatments (T1, T2 and T3 and for control tanks, C1, C2 and C3). Molasses as carbohydrate or carbon sources at ratio C:N 10:1 were transferred to the treatment culture tank after being fermented for 24 h to boost the breakdown process by the bacteria or the microorganisms for biofloc formulation. Shrimp were cultured for 105 days until reaching harvested size at PL115. During the culture period, the microorganisms in the culture tank were sampled every week to identify the microorganism's composition in the biofloc aggregation in the closed and zero water exchange system. All water parameters were checked weekly for pH, salinity, DO, TDS, and temperature using YSI multi-probe YSI 556 and nutrients (ammonia, nitrite, nitrate) were analyzed with spectrophotometer by ammonia–salicylate method (Standard Method 8155), nitrite diazotization method (Standard Method 8507) and nitrate–cadmium reduction method (Standard Method 8192) of (DR/2400 Procedure manual 2002).

Sample collection

3 L of water sample from treatment tank was filtered using plankton net 20 µm for microscopic plankton identification. For bacterial identification, sample water was pipetted out using micropipette and serial diluted until 10^{−5} for bacteria analysis. Sample for plankton analysis was left 24 h for the substrate to settle at the bottom and concentrated to 10 ml of water sample and then preserved with 10 % formalin. All water samples were taken back to laboratory for further analysis.

Microbial identification

Bacteria were isolated using trypticase soy agar (TSA) and selective agar thiosulphate–citrate–bile salts–sucrose agar (TCBS) for isolating *Vibrio* sp. The colony-forming unit (CFU) from fifth time serial dilution (10^{−5}) was selected for colony counting. Gram staining also was done to identify Gram-positive and -negative bacteria. Catalase test was done to identify Gram-positive bacilli. API kit (*Bio-merieux*) API20E and API 20NE were used to identify Gram-negative bacteria. Incubation box, tray and lid were prepared for the strip preparation. For the inoculum preparation, an ampule of API NaCl 0.85 % (2 ml) was selected and 1–4 colonies of bacteria were picked up using inoculation loop from the agar plate and then suspension was prepared with the turbidity equivalent to 0.5 McFarland. For the API 20NE strip inoculation, test nitrate reduction (NO₃) and *p*-nitrophenyl-β-D-galactopyranoside hydrolysis (PNPG) were inoculated by distributing the saline suspension into the tubes using Pasteur pipette. API

AUX medium was added to approximately 200 µl of the remaining suspension into the ampule and was homogenized well. Tubes and cupules of test glucose fermentation (GLU) and Phenyl-acetate assimilation test (PAC) were filled with the suspension. Mineral oil was added to the cupules of 3 tests (GLU), arginine hydrolysis (ADH), and urea hydrolysis (URE) until convex menisci formed. The incubation box was closed and incubated at $29 \pm 2^\circ\text{C}$ for 24 h. After the incubation period, the strips were read by referring to the reading table. The reactions for (GLU, ADH, URE, aesculin hydrolysis test (ESC)), gelatine hydrolysis (GEL) and (PNPG) were recorded on result sheet. For NO_3 test, 1 drop of NIT 1 and 1 drop of NIT 2 reagents were added to NO_3 cupule. For tryptophan deaminase test (TRP), 1 drop of JAMES reagent was added and immediate reaction took place. NIT 1, NIT 2 and JAMES reagents were removed using pipette and test NO_3 and TRP were covered with mineral oil. Kit was reincubated at $29 \pm 2^\circ\text{C}$ for 24 h and all tests were read again except for NO_3 , TRP and underlined GLU, which were only read once at 24 h. Identification is obtained with the numerical profile. Database (V6.0) in the API web index was used by entering the seven digit numerical profile in the identification software for species identification.

For API20E strip inoculation, bacterial suspension were distributed into the tubes with pipette for citrate assimilation test (CIT), Voges–Proskauer (VP) test for acetyl methyl carbinol detection, gelatine hydrolysis (GEL) test by filled in both tube and cupules, and for test ADH, lysine decarboxylase test (LDC), Ornithine decarboxylase test (ODC) and production of hydrogen sulfide test (H_2S) and urea hydrolysis (URE) filled with mineral oil in the cupules. The incubation box was closed and incubated at $36 \pm 2^\circ\text{C}$ for 18–24 h. The strip was read by referring the reading table after incubation period. For Tryptophan deaminase test (TDA), 1 drop of TDA reagent was added, for Indole production test (IND) 1 drop of JAMES reagent was added and for VP test, VP 1 and VP 2 reagents were added. Identification is obtained with numerical profile nine digit using the database (v4.1) in the API web index for species identification.

Plankton microscopic identification

Advance microscope *Nikon 80i* was used for biofloc microscopic identification and for plankton length and size measurements. Qualitative and quantitative analyses of phytoplankton and zooplankton were done by Lackey's method. Compound microscope was used for phytoplankton counting. The cover slip was placed over a drop of water in the slide and whole of cover slip was examined by parallel overlapping strips to count all the organisms in the drop. About 22 strips were examined in each drop. Number of

subsamples to be taken depended on examining 2–3 successive subsamples without addition of an encounter species when compared to the examined subsamples in the same sample (American Public Health Association APHA 1989).

Calculation formula:

$$\text{Density (cells l}^{-1}\text{)} = \frac{C \times A_t}{A_s \times S \times V} \times \frac{\text{volume of concentrated sample (ml)}}{\text{volume of actual water filtered}}$$

where C Number of organisms counted, A_t Area of cover slip ($22\text{ mm} \times 22\text{ mm}$), S Number of strip counted, A_s Area of strip ($22\text{ mm} \times 1\text{ mm}$), V Volume of sample under the cover slip.

Results

There were various types of microscopic organisms identified from biofloculation. All of the microscopic organisms identified come from different classes of phytoplankton algae and also numerous of algae grazer such as rotifer and nematode also the protozoa, *Vorticella* sp. (Fig. 1). From Gram staining, rod-shaped Gram-positive bacteria were also identified, which are *Bacillus* sp., from the positive result of catalase test. From the API kit analysis, species of bacteria identified come from heterotrophic bacteria (*Aeromonas hydrophila*, *Pseudomonas aeruginosa*) and also anaerobic bacteria, *Vibrio* sp. (ex. *V. fluvialis*). For the water parameter results, mean dissolved oxygen, DO, was $6.67 \pm 0.97\text{ mg l}^{-1}$ ($5.9\text{--}9.53\text{ mg l}^{-1}$; $n = 12$), mean temperature $28 \pm 0.30^\circ\text{C}$ ($26\text{--}28^\circ\text{C}$; $n = 12$), mean pH 7.36 ± 0.49 ($6.1\text{--}8.2$; $n = 12$), mean salinity $33.66 \pm 1.45\text{ ppt}$ ($31\text{--}36\text{ ppt}$; $n = 12$) and mean total dissolved solid, TDS 33.52 ± 1.33 ($31.5\text{--}35.5\text{ mg l}^{-1}$; $n = 12$). The bioremediation process was successfully carried out by the microorganism in the biofloc as the nutrients ammonia, NH_3 , drops from 8.0 to 0.3 mg l^{-1} , nitrite drops from 0.8 to 0.5 , and nitrate drops from 15.3 to 5.7 mg l^{-1} during the culture period (Fig. 2). Species *Pseudomonas* sp. and *Aeromonas* sp. were identified to be dominant from the colony-forming unit (CFU) counting. *Vibrio* sp. was also identified as aggregates in the biofloculation (Table 1). The percentage of bacteria identified dominantly come from heterotrophic bacterial species (ex. *Aeromonas hydrophila*) and also from *Vibrio* spp. (*V. alginolyticus* and *V. fluvialis*) (Fig. 3). There were a lot of and varieties of microscopic organism compositions identified from biofloculation in treatment tank 1 (Table 2) treatment tank 2 (Table 3) and treatment tank 3 (Table 4). The density of microorganism was also identified based on the Day of Culture (DOC) of shrimp, density of microorganism in DOC11 shrimp (Fig. 4), DOC17 (Fig. 5), DOC30 (Fig. 6), DOC58 (Fig. 7), DOC65 (Fig. 8), DOC76 (Fig. 9), and for DOC93 (Fig. 10).

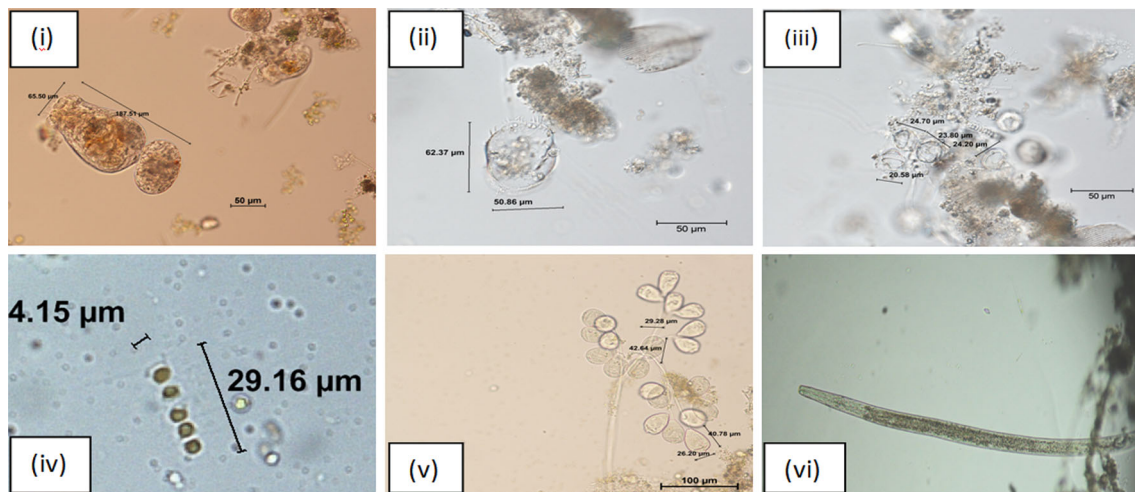


Fig. 1 Microscopic organisms identified in the floc: **a** rotifer; **b** euplotes, the ciliate protozoa; **c** *Alexandrium* sp. of dinoflagellates; **d** *chaetoceros* diatom; **e** *vorticella* the protozoa and **f** nematode. All observed under $\times 400$ magnifications

Fig. 2 The bioremediation process was successfully carried out by microorganisms in the biofloc (bacteria, algae, plankton) to breakdown the hazardous nutrients in the treatments culture tank into the non-toxic substances and can be consumed back as additional protein feed diet for shrimp consumption

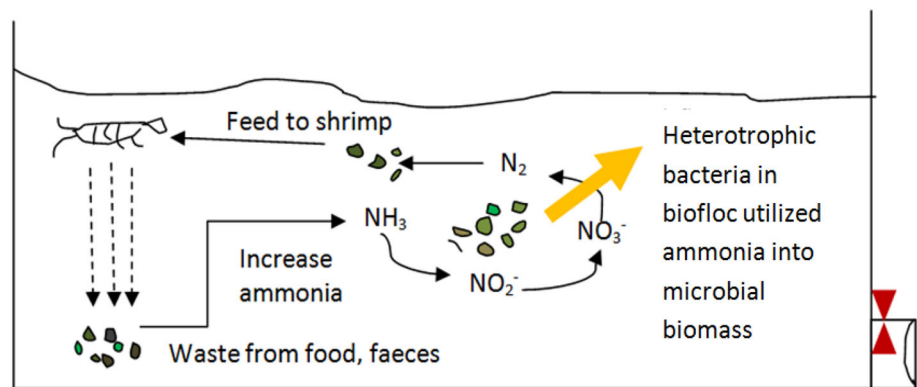


Table 1 Species of Gram-negative bacteria identified using API kit 20E and 20NE *biomerieux*

Tank	Colony	CFU count	Species identify	% id	Id status	Code	API
Tank 1	3 colony	5×10^{-5}	<i>Vibrio fluvialis</i>	98.3	Good id	204650***	20E
		8×10^{-5}	<i>Vibrio alginolyticus</i>	82.6	Good id	7010***	20NE
		23×10^{-5}	<i>Aeromonas hydrophila</i>	98.9	Good id	704612***	20E
Tank 2	2 colony	13×10^{-5}	<i>Aeromonas hydrophila</i>	98.4	Good id	704612***	20E
		34×10^{-5}	<i>Aeromonas salmonicida</i>	99.9	Very good id	1550***	20E
Tank 3	3 colony	38×10^{-5}	<i>Aeromonas salmonicida</i>	99.9	Very good id	1550***	20NE
		16×10^{-5}	<i>Pseudomonas aeruginosa</i>	98.9	Good id	220200***	20E
		3×10^{-5}	<i>Vibrio alginolyticus</i>	86.3	Good id	7434***	20NE

*** Profile coding of the analytical profile index

Discussions

From the identifications done on the biofloc aggregations, the microorganisms can be divided into five overlapping groups which are: floc-forming organisms, saprophytes (organisms that obtain nutrients from dead organic matter), nitrifying bacteria, algae grazers, and pathogenic bacteria

(*Vibrio* spp). All of these types of microorganisms have their own function and interaction between each other in the biofloc system to make the bioremediation process successfully happen. The organisms that forming the floc were identified come from some of algae and bacterial biomasses which they used in activated sludge form the organic matter come from the waste in the tank to secrete sticky EPS

Fig. 3 Percentage of bacterial species identified accumulated together in the biofloc in treatment tank 1 (T1), tank 2 (T2) and tank 3 (T3)

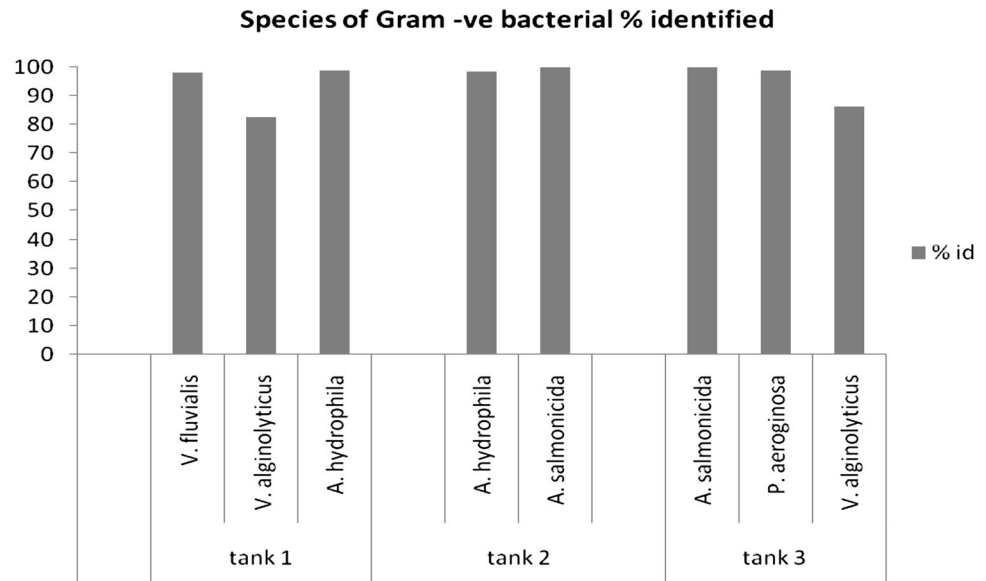


Table 2 Type of microorganisms identified as aggregates in the biofloc in Tank 1

Plankton	Tank	Phylum	Class	Genera
Phytoplankton	Tank 1	Ochrophyta	Bacillariophyceae	<i>Nitzschia</i>
				<i>Navicula</i>
				<i>Licmophora</i>
				<i>Amphora</i>
				<i>Cymbella</i>
				<i>Radiolarian</i>
				<i>Cyclotella</i>
				<i>Leptocylindrus</i>
				<i>Coscinodiscus</i>
				<i>Gomphoperia</i>
Zooplankton	Tank 1	Cynophyta	Cynophyceae	<i>Oscillatoria</i>
				<i>Borodinellopsis</i>
				<i>Chlorella</i>
				<i>Chlamydomonas</i>
				<i>Tetraselmis</i>
				<i>Brachionus</i>
				<i>Euplotes</i>
				<i>Vorticella</i>
				<i>Paramecium</i>
				<i>Euglena</i>
Protozoa	Tank 1	Ciliophora	Ciliatea	<i>Ciliate</i>
				<i>Nematode</i>
Nematode	Tank 1	Nematoda		

(Medina and Neis 2007). These EPS were known to have significant effect on the physiochemical properties of the microbial aggregates including structure, surface charge, flocculation, settling properties, dewatering and absorptive capacity Sheng et al. (2010). The floc itself also as the bioremediation agent is able to stick the detritus from the

wastes together with other organisms such as protozoa and zooplankton during bioflocculation and bacteria in the floc take up ammonia in the water which mostly comes from the metabolic waste of shrimp and convert it into microbial protein. Hargreaves (2013) discovered that bioflocs are accumulation of algae, bacteria, protozoan, and other kinds

Table 3 Type of microorganisms identified as aggregates in the biofloc in Tank 2

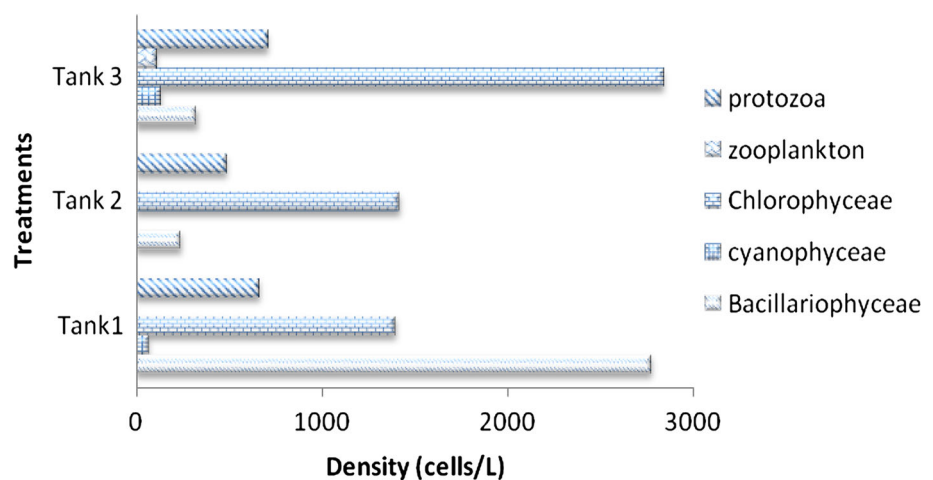
Plankton	Tank	Phylum	Class	Genera
Phytoplankton	Tank 2	Ochrophyta	Bacillariophyceae	<i>Nitzschia</i>
				<i>Leptocylindrus</i>
				<i>Navicula</i>
				<i>Cyclotella</i>
				<i>Melosira</i>
				<i>Licmophora</i>
				<i>Cymbella</i>
				<i>Coscinodiscus</i>
				<i>Oscillatoria</i>
				<i>Gomphosperia</i>
		Cynophyta	Cynophyceae	<i>Gloeocapsa</i>
				<i>Chlorella</i>
				<i>Borodinellopsis</i>
				<i>Scenedesmus</i>
				<i>Tetraselmis</i>
Zooplankton	Tank 2	Dinophyta	Dinophyceae	<i>Protoperdinium</i>
				<i>Alexandrium</i>
				<i>Copepod</i>
Protozoa	Tank 2	Arthropoda	Copepoda	<i>Branchius</i>
		Rotifera	Brachionidae	<i>Radiolaria</i>
		Sarcomastigophora	Radiolaria	<i>Euplotes</i>
		Ciliophora	Euplotidae	<i>Paramecium</i>
		Ciliophora	Parameciidae	<i>Ciliate</i>
		Ciliophora	Ciliatea	<i>Euglena</i>
		Euglenoidea	Euglenaceae	<i>Vorticella</i>
Nematode	Tank 2	Nematoda	Vorticellidae	<i>Nematode</i>

of particulate organic matter such as feces and uneaten feed which are held together in a loose matrix of mucus secreted by bacteria and bound by filamentous algae or held by electrostatic attraction. During siphoning process, it can also be seen that the waste at the bottom of the tank was aggregated in small rounded shape, which means the biofloc microorganisms work in settling down the detritus and acted as the bioremediation agent in neutralizing the pollutant in the bottom of the tank and makes the condition of water optimum. For the saprophytes group or the organisms obtaining nutrients from the dead organic matter, heterotrophic bacteria is in this classification. Heterotrophic bacteria identified from the study are *Aeromonas* spp and *Pseudomonas* spp. Heterotrophic bacteria used the organic compound from the organic matter left in the tank as the sources of energy and food which contrast with the autotrophic organisms such as phytoplankton and algae. Protozoa identified in the biofloc treatment tank such as ciliate, vorticella, euplotes and paramecium also classified as saprophytic protozoa as absorb organic matter through their cell wall for food and takes 40 % of the nutrients for the

production of protozoan biomass (Merriam Co 1913; Lal 2006). All of these saprophytic microorganisms acted as bioremediation agents in neutralizing the nutrients (ammonia, nitrite and nitrate) from the wastes of uneaten feed and shrimp's fecal secretion that produced ammonia product. The denitrifying bacteria, *Pseudomonas* sp., was identified in the biofloculation that worked to convert the nitrates NO_3^- into gaseous nitrogen; N_2 makes the water condition less toxic and maintains the water quality (Schramm et al. 1999). This bacteria also will convert the nitrates in the water to the beneficial protein for shrimp consumption besides getting the food from the pellet given. These are proved through Hargreaves's (2013) study in biofloc system, whereas some of the nitrogen is incorporated into the bacterial cells that become the main component of biofloc, shrimp consumption of this microbial protein will effect for a second time and contribute to shrimp growth. Zooplankton, protozoa and protozoa parasite are classified under the algae grazer category. The organisms identified in the biofloculation were such as nematode, gastrotrich, euplotes protozoa, vorticella

Table 4 Type of microorganisms identified as aggregates in the biofloc in Tank3

Plankton	Tank	Phylum	Class	Genera
Phytoplankton	Tank 3	Ochrophyta	Bacillariophyceae	<i>Nitzschia</i>
				<i>Leptocylindrus</i>
				<i>Cyclotella</i>
				<i>Licmophora</i>
				<i>Navicula</i>
				<i>Coscinodiscus</i>
		Cynophyta	Cynophyceae	<i>Oscillatoria</i>
				<i>Gomphosperia</i>
		Chlorophyta	Chlorophyceae	<i>Gloeocapsa</i>
				<i>Chlorella</i>
		Dinophyta	Dinophyceae	<i>Borodinellopsis</i>
				<i>Protoperdinium</i>
Zooplankton	Tank 3	Arthropoda	Copepoda	<i>Alexandrium</i>
		Rotifera	Brachionidae	<i>Copepod</i>
		Gastrotricha	Chaetonotida	<i>Branchius</i>
		Euglenozoa	Euglenaceae	<i>Gastrotrich</i>
Protozoa	Tank 3	Ciliophora	Vorticellidae	<i>Euglena</i>
		Ciliophora	Euplotidae	<i>Vorticella</i>
		Ciliophora	Parameciidae	<i>Euplotes</i>
		Ciliophora	Ciliata	<i>Paramecium</i>
		Sarcomastigophora	Radiolaria	<i>Ciliate</i>
		Nematoda		<i>Radiolarian</i>
Nematode	Tank 3	Nematoda		<i>Nematode</i>

Fig. 4 Density of microorganisms composition identified from the biofloculation in the water column of treatment culture tanks for Day of culture, DOC11

protozoa, ciliate, rotifer and copepod which in dense composition as higher food from algae and phytoplankton types were available in the biofloc treatment tank (Hargreaves 2013). During culture starting from DOC11 to DOC93 (Figs. 4, 5, 6, 7, 8, 9, 10), algae from three different classes indentified to dominate the biofloc culture treatment are Chlorophyceae (Green algae), Bacillariophyceae (Diatoms), Cyanophyceae (Blue green algae) and occasionally from

Dinophyceae (Dinoflagellates) class. This finding is same as the studies by Galvez (2015) and Schrader et al. (2011) which found out that the most abundant algae in this biofloc study came from cyanobacteria class followed by Chlorophyta, Heterokontophyta, Euglenophyta and Dinophyta. The organisms classified under pathogenic group identified are protozoa that are harmful to the shrimp and the pathogenic bacteria *Vibrio* sp. such as *Vibrio alginolyticus* that

Fig. 5 Density of microorganisms composition identified from biofloculation in the water column of treatment culture tanks for Day of culture, DOC17

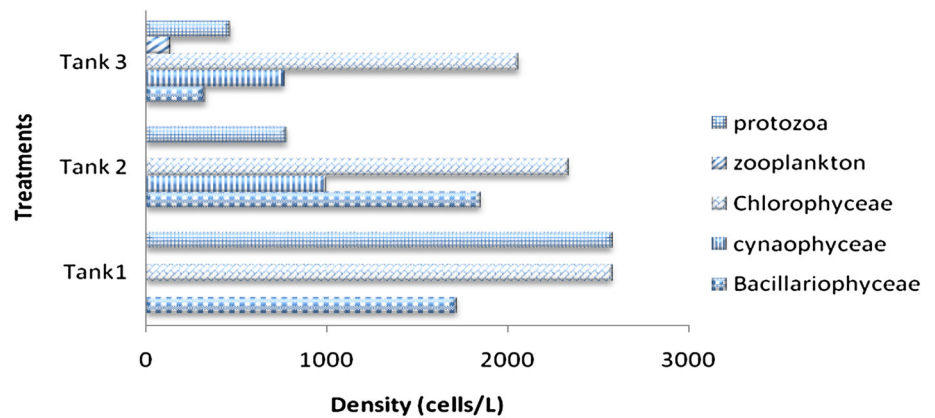


Fig. 6 Density of microorganism composition identified from the biofloculation in the water column of treatment culture tanks for Day of culture, DOC30

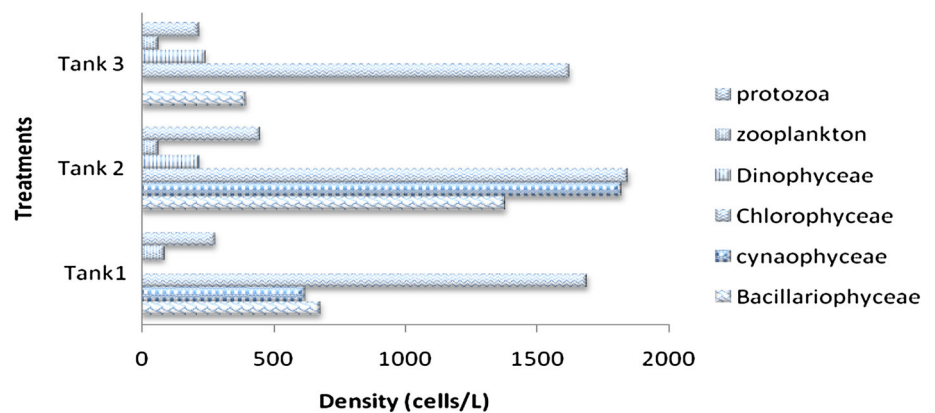
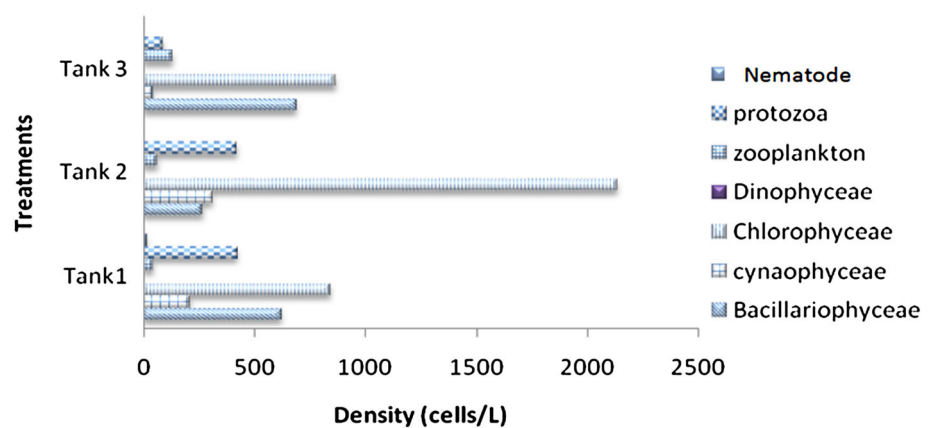


Fig. 7 Density of microorganism composition identified from the biofloculation in the water column of treatment culture tanks for Day of culture, DOC58



can give infection to the shrimp as suggested by Wei and Wendy (2012). The *Vibrio* sp. was also identified in the biofloc aggregation but in less CFU number. These were not lethal to the shrimps as it is being controlled by the biofloc itself through higher diversity of phytoplankton and algae and also can compete with dominant number of hetero-

trophic bacteria. Refer to study done by Emerenciano et al. (2013) they discovered that the natural probiotic in the biofloc could internally or externally against the *Vibrio* sp. and ectoparasite from giving harmful to the shrimp. Competing with the dominant heterotrophic bacteria and nitrifying bacteria for the essential nutrients such as nitrogen

Fig. 8 Density of microorganism composition identified from the biofloculation in the water column of treatment culture tanks for Day of culture, DOC65

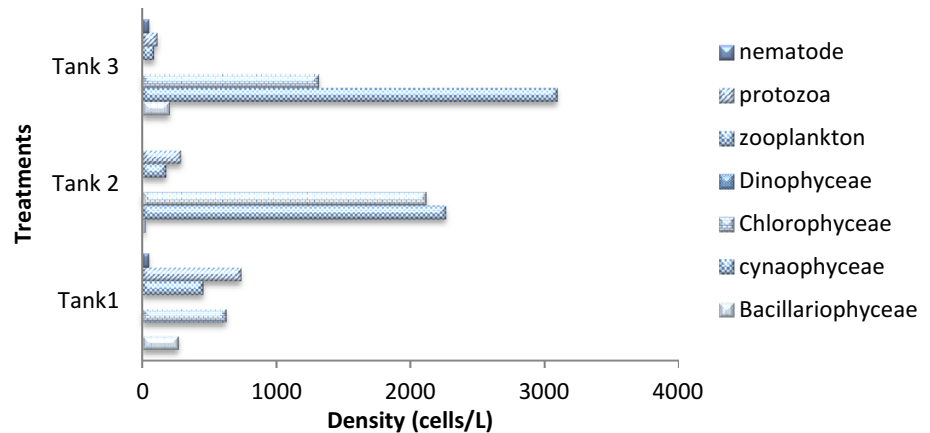


Fig. 9 Density of microorganism composition identified from the biofloculation in the water column of treatment culture tanks for Day of culture, DOC76

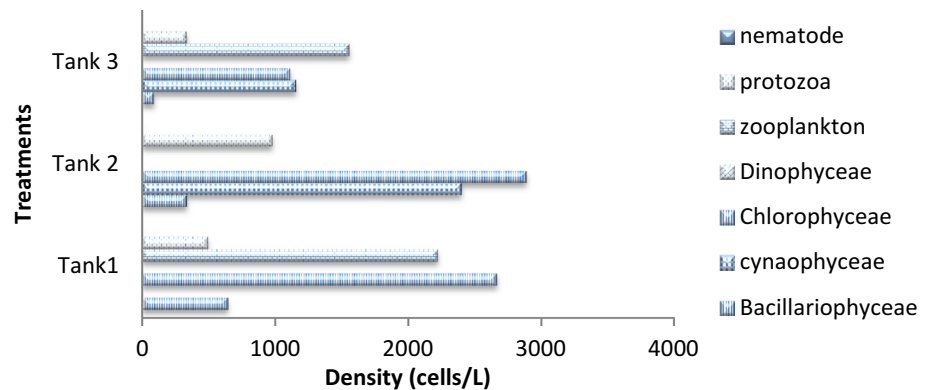
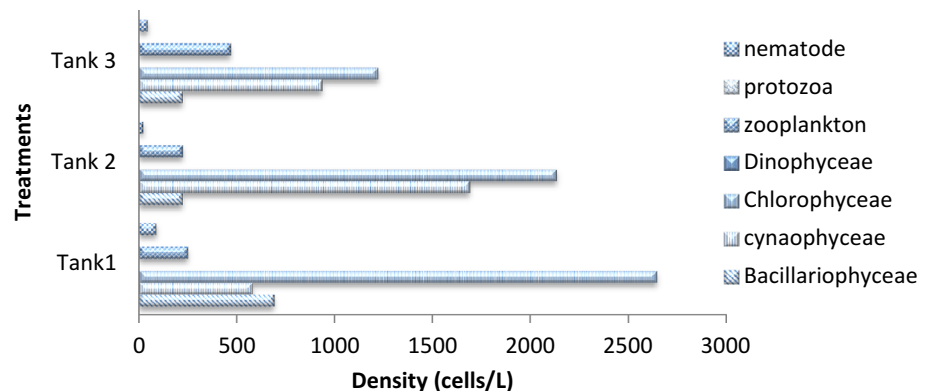


Fig. 10 Density of microorganism composition identified from the biofloculation in the water column of treatment culture tanks for Day of culture, DOC93



also will limit the *Vibrio* sp. group from uncontrolled growth (Emerenciano et al. 2013).

Conclusion

Biofloculation is a promising technology towards friendly aquaculture environment. In fact, it can supply additional diet for shrimp's consumption from the biofloculant of a variety microorganisms identified in the floc; biofloc also

were recognized to be efficient and successful as a bioremediation and biodegradation agent for maintaining the water quality in the close aquaculture system with the zero water exchange.

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