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Productivity and nutritional quality of *Moina* sp. cultivated in various culture media

Produktivitas dan kualitas *Moina* sp. yang dipelihara dengan berbagai media budidaya

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ABSTRACT

Cultivation of *Moina* sp. is still constrained by its quality, productivity, and sustainability. The alternative solution is the use of cultivation media materials that have high nutritional content and easily available in large quantities to support the quality and productivity of *Moina* sp. and meet the needs of live feed. The objective of the study was to evaluate the effect of various culture medium on the productivity and nutritional quality of *Moina* sp.. Five culture media were tested in laboratory scale, i.e. organic ingredient (BO), *Chlorella* sp. (Ch), *Chlorella* sp. + organic ingredients (ChBO), biofloc (BF) and biofloc + organic ingredients (BFBO). While in mass scale, four culture media were tested, i.e. *Chlorella* sp. (Ch), *Chlorella* sp. + organic ingredients (ChBO), biofloc (BF) and biofloc + organic ingredients (BFBO). The peaks of *Moina* sp. density in different treatments were achieved in different days. ChBO treatments significantly had higher productivity ($P<0.05$). The highest protein content was found in *Moina* sp. cultured with ChBO media, even higher than artemia. *Moina* sp. cultured with *Chlorella* sp. (Ch) showed the highest PUFA (poly unsaturated fatty acids) contents, while the highest MUFA (mono unsaturated fatty acids) contents was obtained from *Moina* sp. cultured with BFBO media lower than artemia. The study results indicates that different culture media produces different productivity and nutrient quality of *Moina* sp. The organic material combination of *Chlorella* sp. + organic material (ChBO) was the best media to improve the productivity and protein quality of *Moina* sp.

Keywords : Biofloc, *Chlorella* sp., *Moina* sp., organic matter, productivity, quality

ABSTRAK

Budidaya *Moina* sp. masih terkendala pada kualitas, produktivitas dan kestabilan dalam ketersediaannya. Untuk itu diperlukan penggunaan bahan media budidaya yang memiliki kandungan nutrisi tinggi dan mudah didapat dalam jumlah banyak untuk mendukung kualitas dan produktivitas *Moina* sp. demi memenuhi kebutuhan pakan hidup. Tujuan penelitian yaitu mengevaluasi pengaruh berbagai media budidaya terhadap produktivitas dan kualitas nutrisi *Moina* sp. Lima media kultur yang diuji dalam penelitian laboratorium yaitu bahan organik (BO), *Chlorella* sp. (Ch), *Chlorella* sp. + bahan organik (ChBO), bioflok (BF), dan bioflok + bahan organik (BFBO). Sedangkan pada penelitian skala massal diuji empat media kultur yaitu *Chlorella* sp. (Ch), *Chlorella* sp. + bahan organik (ChBO), bioflok (BF) dan bioflok + bahan organik (BFBO). Puncak kepadatan *Moina* sp. pada tiap perlakuan dicapai pada hari yang berbeda. Perlakuan ChBO memiliki produktivitas yang lebih tinggi ($P<0,05$). Kandungan protein *Moina* sp. tertinggi ditemukan pada media ChBO dan bahkan lebih tinggi dari pada artemia. *Moina* sp. yang dibudidayakan dengan *Chlorella* sp. (Ch), menunjukkan kandungan PUFA tertinggi, sedangkan kandungan MUFA yang tertinggi terdapat pada *Moina* sp. yang dibudidayakan dengan bahan media BFBO namun masih lebih rendah jika dibandingkan dengan kandungan pada artemia. Hasil penelitian menunjukkan media kultur yang berbeda menghasilkan produktivitas dan kualitas nutrisi moina yang berbeda. Kombinasi bahan organik *Chlorella* + bahan organik (ChBO) merupakan media terbaik dibandingkan dengan perlakuan lainnya untuk meningkatkan produktivitas dan kualitas nutrisi terutama protein *Moina* sp.

Kata kunci : Bioflok, *Chlorella* sp., *Moina* sp., bahan organik, produktivitas, kualitas

INTRODUCTION

Live feed supply is highly required in larvae culture. Both nutrition content and size of the live feed are strongly considered by farmers. Artemia is still the major choice of most farmers due to the decent size and complete nutritional compound (War M *et al*., 2011). Another choice to meet the demand of live feed is needed, especially from freshwater to reduce production cost. *Moina* sp. is one of the live feed that is frequently used in fish and shrimp larvae rearing. Specifically, *Moina* sp. size is similar to artemia, even with more nutritious compound (Mubarak *et al*., 2017; Usman *et al*., 2018). *Moina* sp. can reproduce and grow well in a low level of oxygen and relatively high content of ammonia (Loh *et al*., 2013). *Moina macrocopa* and *Moina micrura* contain both essential and non essential amino acids and 52.4% of protein (Mubarak *et al.*, 2017). *Moina* sp. is a filter feeder that consumes bacteria, organic material, and algae. The nutrition compound in *Moina* sp. can be enhanced using several different media, such as biofloc waste, microalgae, and various organic material, i.e. fish meal, soy meal, and bran meal.

Biofloc is various microorganism aggregate, such as floc-forming bacteria, filament bacteria, and suspension fungi in a biofloc-based aquaculture (Panigrahi *et al*., 2021). Biofloc contains valine, lysin, leusine, phenylalanine, threonine, and a little bit of methionine (Ekasari *et al*., 2014). Therefore, biofloc is a potential alternative feed for *Moina* sp. and expectedly increase its nutritional compound. Castro *et al.* (2017) showed that *Moina* sp. can be cultured using bacteria from biofloc. The survival rate and productivity of *Moina macrocopa* fed using *Chlorella* was relatively high (Morales-Ventura *et al.,* 2012)is to culture algae with fertilizers; however, the demography of zooplankton when fed these algae has not yet been evaluated. We studied the population growth and life table demography of the rotifers Anuraeopsis fissa and Brachionus rubens, and the cladoceran Moina macrocopa. For this, the algae Scenedesmus acutus or Chlorella vulgaris were cultured on defined (Bold's basal. Smirnov (2017) explained that green algae have 14.9–37.2 % linoleate acid (18:3w3).

Various organic material, i.e. fish meal, soy meal, and rice bran meal, can be utilized both directly and non directly by moina. Fish meal incorporates 50.55% of protein with an adequate composition of essential amino acids. Soy meal is commonly used as protein source to substitute fish meal in aquaculture feed industry. Putra (2016) state that *Daphnia* culture using soy meal involved 68.4% of protein compared to the animal manure and coconut dregs meal treatment contained comparably lower protein, i.e. 11.2% and 17.4%, respectively. Rice bran is commonly proceed first before involved in *Moina macrocopa* feed. It was proceed into smaller particle to adjust the mouth size of *Moina macrocopa* (Mubarak *et al*., 2017). All the ingredients mentioned above will be utilized by microorganism as nitrogen and carbon source. It stimulates the growth of microorganism that will be the feed source of *Moina* sp.. According to the natural ingredient, further study about *Moina* sp. reared in productivity and nutrition quality is completely requested. Thus, this study aimed to evaluate nutrition profiles and productivity of *Moina* sp. reared in various rearing media, i.e. *Chlorella* sp., biofloc, and organic material mix (fish meal, soy meal, and rice bran meal) and its combination.

MATERIALS AND METHODS

Experimental design

This study was conducted in two steps, i.e. laboratory scale (step 1) and mass scale (step 2). It used experimental method using completed randomized design.

Rearing media composition

The composition of *Moina* sp. rearing media used in the step 1 and 2 was stated in Table 1.

Biofloc media preparation

The culture media for biofloc was sterilized using 30 mg/L of chlorin and neutralized with strong aeration for 72 hours. Biofloc was collected from a tilapia biofloc system. Tilapia was reared in 10.000 L of water volume. With average weight of 100 g, it was reared in 100 fish/m^3 of stocking density. As many of 1 g/L of salt was added into the water for 5 days. After that, as many as 10 g/m^3 of probiotics consisted of *Bacillus subtillis*, *Bacillus megaterium*, and *Bacillus polimixa* was added along with 100 mL/m^3 of molase as a carbon source and 50 g/m^3 of dolomite. Probiotics (3 g/m^3) and molase (30 mL/m^3) were added again every 5 days. Fish were fed 3% of the total biomass using commercial feed with 30% of protein content. Biofloc suspension was produced after 21 days.

***Chlorella* sp. media preparation**

Chlorella sp. culture was started by water sterilizing by adding 30 mg/L of chlorine and neutralizing using strong aeration for 72 hours. *Chlorella* was cultivated in a concrete tank using urea, TSP, fish meal, soy meal, and rice bran meal with each dose, 1000 g/m^3, 250 g/m^3, 100 g/m^3, 100 g/m^3, and 200 g/m^3, respectively. *Chlorella* sp. was initially cultured in laboratory scale, i.e. 1 L, 5 L, 30 L using *Haematococcus Provasolli Medium* (PHM) (Rinanti *et al*., 2013) and furthermore, it was upscaled up to 3000 L using technical fertilizer mentioned before.

***Moina* sp. culture**

Step 1

The laboratory scale culture used 10 units of aquarium sized in 15×15×20 cm^3. The aquarium was washed thoroughly and dried. Mineral water was applied as culture media. The culture was held in a semi indoor room with five treatments of culture media, i.e. organic ingredient (BO), *Chlorella* sp. (Ch), ChBO, biofloc (BF), and BFBO with two replications each for seven days. The initial population of *Moina* sp. was 50 individuals/L with 3 L of total volume. *Moina* sp. inoculant was filtered using mesh sized in 500 µm to get similar size. *Chlorella* sp. media with a minimal abundance 1 × 10^6 cells/mL was used in Ch and ChBO treatment, while the BO, BF, and BFBO treatment media composisition was shown in Table 1. At the end of the culture, biomass calculation was done by manually counting the moina population using a petridisk for further counting using tally counter.

Step 2

On the mass scale study, as many of 12 units of concrete tanks were used. They sized in 1.8×3.8×1 m^3. The water was sterilized using 30 mg/L of chlorine and neutralized by turning strong aeration on for 72 hours in 12 different aeration spots. The sterilized media was poured into the culture tank based on the treatment. Every tools and equipments used in the study were soaked in a chlorine liquid beforehand. To avoid any contamination, each tank was covered using a fabric strainer during the culture.

The study was conducted outdoor with the intial population of moina 173 individuals/L (80 g/tank) and the total volume was 3000 L. *Moina* sp. inoculant was filtered using a mesh sized in 500 µm to harmonize moina size. Furthermore, moina was sterilized by rinsing it with water

Table 1. Rearing media composition of *Moina* sp.

Treatments	Media			
	Ingredients	%	Amount (g/3L)	Amount (g/3000 L)
			Lab. scale (step 1)	Mass scale (step 2)
BO*	Fish meal	30	0,27	-
	Soy meal	35	0,32	-
	Rice bran meal	35	0,32	-
Ch*	*Chlorella* sp	100	4×10^6 cells/mL	4×10^6 cells mL
ChBO*	*Chlorella* sp.	50	2×10^6 cells mL	2×10^6 cells/mL
	Fish meal	15	0.27	270
	Soy meal	17.5	0.32	315
	Rice bran meal	17.5	0.32	315
BF*	Biofloc	100	0.90	900
BFBO*	Biofloc	50	0.45	450
	Fish meal	15	0.14	135
	Soy meal	17.5	0.16	158
	Rice bran meal	17.5	0.16	158

*BO: 30% fish meal, 35% soy meal, 35% rice bran meal; Ch: 100% *Chlorella*; ChBO: 50% *Chlorella* + 15% fish meal, 17.5% soy meal, 17.5% rice bran meal; BF: 100% biofloc; BFBO: 50% biofloc + 15% fish meal, 17.5% soy meal, 17.5% rice bran meal.

*BO: organic ingredient (fish meal, soy meal, rice bran meal); Ch: *Chlorella*; ChBO: *Chlorella* + organic ingredient (fish meal, soy meal, rice bran meal); BF: biofloc; BFBO: biofloc + organic ingredient (fish meal, soy meal, rice bran meal).

and stored for 48 hours before used. The culture was organized for five days (Burgos *et al.*, 2003) using four treatments and three replication. The experimental media was exhibited in Table 1. For Ch and ChBO treatment, a minimal abundance was maintained around 1×10^6 cells/mL. Particularly, the BO treatment was not applied in the mass scale culture because the tested moina in this treatment was realtively undeveloped.

Experimental parameters

Step 1

The analysis of *Moina* sp. consisted of *Moina* sp. biomass/survival rate that conducted in day 7. Water quality parameters were temperature and pH level.

Step 2

Moina sp. and Chlorella sp. analysis

Moina sp. analysis parameters consisted of population dynamics and size distribution of *Moina* sp.. A 1 mL of sub sample of *Moina* sp. was collected and observed to measure the population and size distribution. The observation used sedgewick rafter under the profile projector (Nikon). The result of *Moina* sp. calculation was categorized into 2 sizes, i.e. 300 μm–600 μm (offspring) and 601 μm–1300 μm (broodstock). The abundance of *Chlorella* sp. was observed using microscope and haemacytometer. Total biomass of *Moina* sp. was done in day 5.

Moina sp. nutrition profile analysis

Three different samples from every replication were combined as one sample for amino acids and fatty acids analysis. The nutrition parameters were protein & fat content, water content, crude fiber, and ash.

Water quality analysis and biofloc characteristic

Water quality parameters observed were dissolved oxygen, temperature, pH level, alkalinity, water hardness, ammonia, and nitrate. Meanwhile the observed characteristics for biofloc were total suspended solids (TSS) and volatile suspended solid (VSS).

Data analysis

The step 1 data were analyzed descriptively because the data was relatively not homogeneous, whereas the biomass data was analyzed with completed randomized design and Duncan's test using Microsoft Excel 2010 and SPSS 26. Homogeneity and normality test were organized using Levene's test and Kolmogorov-Smirnov test. The nutrition profiles were analyzed descriptively based on the lab test.

RESULTS AND DISCUSSION

Results

Step 1

Moina sp. culture in laboratory scale showed that the ChBO treatment had the most population (1.128 ind/L). The BO treatment presented a decresed abundance (Figure 1) from 50 ind/L to 3 ind/L at the end of study. Thus this particular treatment was not involved in the upcoming study (step 2).

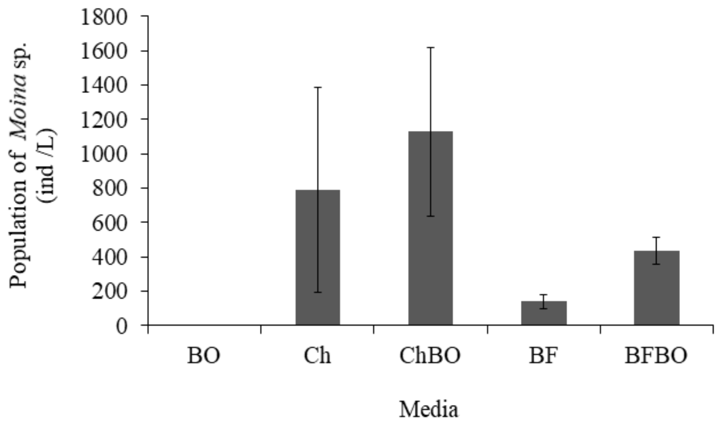


Figure 1. *Moina* sp. abundance culture in laboratorium scale. Note: *BO: 30% fish meal, 35% soy meal, 35% rice bran meal; Ch: 100% *Chlorella*; ChBO: 50% *Chlorella* + 15% fish meal, 17.5% soy meal, 17.5% rice bran meal; BF: 100% biofloc; BFBO: 50% biofloc + 15% fish meal, 17.5% soy meal, 17.5% rice bran meal.*BO: organic ingredient (fish meal, soy meal, rice bran meal); Ch: *Chlorella*; ChBO: *Chlorella* + organic ingredient (fish meal, soy meal, rice bran meal); BF: biofloc; BFBO: biofloc + organic ingredient (fish meal, soy meal, rice bran meal).

Step 2

The *Moina* sp. abundance in the ChBO treatment had the highest population followed by BFBO compared to the other treatment (Table 2). The peak population *Moina* sp. was differ amongst treatment. ChBO reached the peak population in day 5, while Ch had it in day 4. The BF and BFBO treatment have experienced the peak population in day 3 (Figure 2). The biomass of *Moina* sp. in the Ch anf BF treatments was not different significantly amongst treatment, yet lower than the ChBO and BFBO (Figure 3).

Size distribution of Moina sp.

The offspring population of *Moina* sp. in the Ch treatment barely increase after day 2, while the broodstock population approached its peak in day 4. On contrast, the *Moina* sp. offspring in the ChBO treatment started to increase in day 2 and the broodstock population increased to the fullest in day 1 to day 5. In the BF treatment, both the offspring and broodstock, their population increased around the same time, day 1 to day 3. Similarly, the BFBO treatment also described the identical pattern of peak population on both offspring and broodstock of *Moina* sp. (Figure 4).

Nutrition profile of Moina sp.

Generally, *Moina* sp. cultured in ChBO obtained the highest protein and amino acids amongst treatment (Table 3 & 4). The result of fatty acids described that the highest SFA and MUFA were recorded in the BFBO treatment.

Meanwhile, a higher level of PUFA detected in BF and BFBO treatment (Table 5). *Moina* sp. in the Ch treatment had the highest n-3 fatty acid (eicosapentaenoic, linolenic, and docosahexaenoic), while the highest content of n-6 fatty acids (linolenic, linoleic, and arachidonic acid) was shown in BFBO treatment (Table 6 and 7).

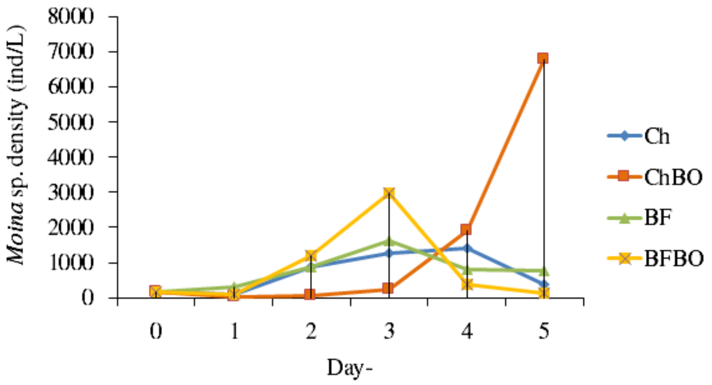


Figure 2. Daily population of *Moina* sp. (ind/L) in a mass scale culture. Note: *Ch: 100% *Chlorella*; ChBO: 50% *Chlorella* + 15% fish meal, 17.5% soy meal, 17.5% rice bran meal; BF: 100% biofloc; BFBO: 50% biofloc + 15% fish meal, 17.5% soy meal, 17.5% rice bran meal. *Ch: *Chlorella*; ChBO: *Chlorella* + organic ingredient (fish meal, soy meal, rice bran meal); BF: biofloc; BFBO: biofloc + organic ingredient (fish meal, soy meal, rice bran meal).

Table 2. Daily population of *Moina* sp. (individuals/mL) cultured in various media.

Parameter	Day-	Treatment			
		Ch*	ChBO*	BF*	BFBO*
Moina sp. population (300–1300 µm)	0	173 ± 0[a]	173 ± 0[a]	173 ± 0[a]	173 ± 0[a]
	1	108 ± 12[a]	28 ± 14[b]	299 ± 64[c]	102 ± 12[a]
	2	875 ± 137[a]	60 ± 13[b]	861 ± 111[a]	1196 ± 75[a]
	3	1283 ± 364[a]	229 ± 13[b]	1623 ± 56[a]	2984 ± 157[c]
	4	1407 ± 513[a]	1922 ± 632[a]	809 ± 198[a]	394 ± 19[b]
	5	391 ± 264[a]	6770 ± 472[b]	776 ± 91[a]	112 ± 25[ac]

*Ch: 100% *Chlorella*; ChBO: 50% *Chlorella* + 15% fish meal, 17.5% soy meal, 17.5% rice bran meal; BF: 100% biofloc; BFBO: 50% biofloc + 15% fish meal, 17.5% soy meal, 17.5% rice bran meal.

*Ch: *Chlorella*; ChBO: *Chlorella* + organic ingredient (fish meal, soy meal, rice bran meal); BF: biofloc; BFBO: biofloc + organic ingredient (fish meal, soy meal, rice bran meal).

*Average value (± SE) in the same row followed by different superscript indicates significant difference (P<0.05).

Total suspended solid (TSS)

The TSS in BF and BFBO treatment were higher than Ch and ChBO treatment. During the culture, TSS in BFBO treatment was likely to increase, in contrast with the Ch treatment. In the meantime, TSS in ChBO treatment increased until day 3 and decreased afterwards and contrast with the TSS in BF. The VSS in ChBO and BFBO fairly elevated compared to the Ch and BF treatment. The VSS in Ch treatment was relatively stable, wheares the BF treatment experienced some increases until day 3.

Chlorella sp. abundance in Ch and ChBO treatment

Daily *Chlorella* abundance of *Moina* sp. culture in Ch treatment ranged from 6.6×10^5 cells/mL to 1.13×10^6 cells/mL. The highest

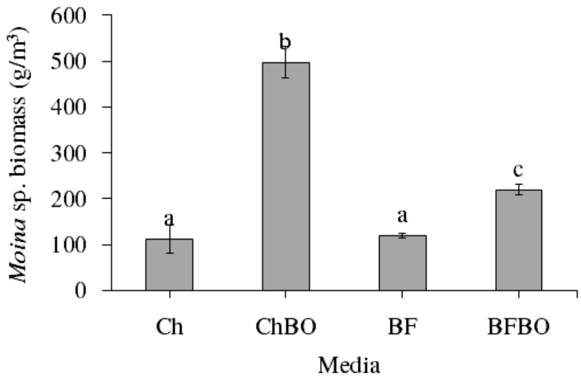


Figure 3. *Moina* sp. biomass culture in various media in mass scale culture. Note: *Ch: 100% *Chlorella*; ChBO: 50% *Chlorella* + 15% fish meal, 17.5% soy meal, 17.5% rice bran meal; BF: 100% biofloc; BFBO: 50% biofloc + 15% fish meal, 17.5% soy meal, 17.5% rice bran meal. *Ch: *Chlorella*; ChBO: *Chlorella* + organic ingredient (fish meal, soy meal, rice bran meal); BF: biofloc; BFBO: biofloc + organic ingredient (fish meal, soy meal, rice bran meal). Different superscript indicates significant difference (P<0.05).

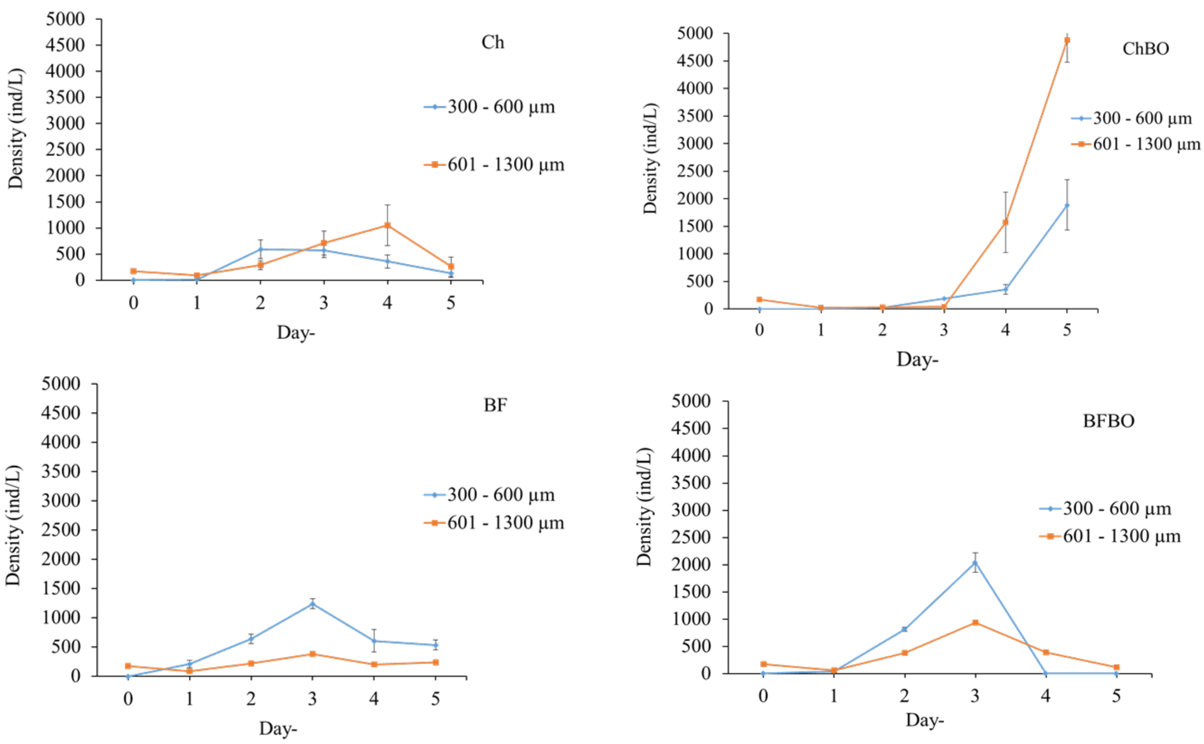


Figure 4. Size distribution of *Moina* sp. cultured in various media in mass scale culture. Note: *Ch: 100% *Chlorella*; ChBO: 50% *Chlorella* + 15% fish meal, 17,5% soy meal, 17.5% rice bran meal; BF: 100% biofloc; BFBO: 50% biofloc + 15% fish meal, 17.5% soy meal, 17.5% rice bran meal. *Ch: *Chlorella*; ChBO: *Chlorella* + organic ingredient (fish meal, soy meal, rice bran meal); BF: biofloc; BFBO: biofloc + organic ingredient (fish meal, soy meal, rice bran meal).

Chlorella sp. abundance in ChBO treatment was gained in day 3 (2.49×10^6 cells/mL). It decresed at day 4 and hit the lowest abundance in day 5 (3.4×10^5 cell/mL) (Figure 6).

Water quality

Ther water quality of *Moina* sp. culture was presented in Table 8 below. The overall temperature during *Moina* sp. culture was 27.5°C–32°C. All treatment experienced a lower dissolved oxygen in the morning from day 1 to day 3 and it elevated in day 4 and 5. The Ch, BF, and BFBO treatments had a fairly stable concentration of dissolved oxygen in the afternoon. On the other hand, the dissolved oxygen in the ChBO treatment diminished in the afternoon from day 1 to day 4. In the morning, the pH level were mostly lower in most treatments, except the BF treatment which had a comparatively stable pH level. The lowest ammonia and nitrate content recorded in the ChBO, while the BFBO resulted the highest ammonia (Figure 7). Water hardness in the Ch and ChBO treatment were relatively lower than the BF and BFBO treatment. As for the alkalinity, the highest level was occurred in the Ch treatment compared to the ChBO, BF, and BFBO.

Discussion

The abundance and biomass of *Moina* sp. were influenced by the type of media. Microorganism in *Cladocera* group, included *Moina* sp., consume decomposition material of organic waste, algae, and bacteria (Smirnov, 2017). In the step 1 study, it inferred that, except the BO treatment, the other treatments were able to induce *Moina* sp. abundance. A low abundance of *Moina* sp. in the BO treatment was presumably caused by the size of the particle that the organic matter had which not suitable for *Moina* sp. It led to the lack of nutrition intake. Nandini *et al.* (2019) stated that a hunger *Moina macrocopa* barely survive more than a day. The population will reduce drastically in the next day and experience a total mortality in day 4. According to the size distribution in the mass scale culture, *Moina* sp. growth and

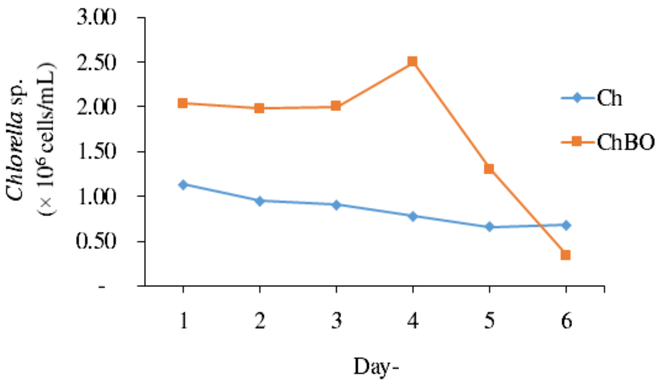


Figure 6. *Chlorella* sp. abundance as a additional feed for Moina sp. cultured in the culture media added with *Chlorella* sp. Note: Ch: *Chlorella*; ChBO: *Chlorella* + organic ingredient (fish meal, soy meal, rice bran meal).

Table 8. Ther water quality of *Moina* sp. culture

Parameter	Treatments			
	Ch*	ChBO*	BF*	BFBO*
Temperature (°C)	28.2–32	27.5–32.1	27.9–33	28.2–32.6
Nitrate (mg/L)	0,028–0.071	0.001–0.009	0.012–0.072	0.034–0.075
Hardness (mg $CaCO_3$/L)	66–86	73–100	86–164	67–157
Alkalinity (mg/L)	96–121	33–59	45–75	44–96
VSS** (g/L)	0.011–0.012	0.009–0.027	0.012–0.014	0.012–0.021
TSS** (g/L)	0.334–0.761	0.236–0.396	0.229–0.572	0.381–0.822

*Ch: 100% *Chlorella*; ChBO: 50% *Chlorella* + 15% fish meal, 17.5% soy meal, 17.5% rice bran meal; BF: 100% biofloc; BFBO: 50% biofloc + 15% fish meal, 17.5% soy meal, 17.5% rice bran meal. Ch: *Chlorella*; ChBO: *Chlorella* + organic ingredient (fish meal, soy meal, rice bran meal); BF: biofloc; BFBO: biofloc + organic ingredient (fish meal, soy meal, rice bran meal).

**VSS: volatile suspended solid, TSS: total suspended solid development are mostly affected by the amount, type, size, and the nutritional compound of the feed, especially amino acids and fatty acids (Nwachi, 2013; Neri *et al.*, 2020; Yuslan *et al.*, 2021).

In the first day after inoculation, *Moina* sp. population decreased, except for the BF treatment. It was reasonably caused by the adaptation to the new environment. Started from day 2 and day 3, all treatments except the ChBO, showed a significant increase 8–9 times greater than the initial abundance. We can infer that *Moina* sp. was well-adapted, grow, and reproduce well. In day 3, the *Moina* sp. abundance in BF and BFBO treatment reached the maximum abundance which indicated that biofloc, especially the smaller particle, was edible by *Moina* sp. According to the observation, the particle size of biofloc ranged from 300–500 μm. Biofloc particle changes overtime. Along with the culture period, the particle size of biofloc will get bigger and undoubtly will affect the eating habit of *Moina* sp. (Milligan *et al.*,

Table 3. The proximate result of *Moina* sp. cultured in various media in mass scale culture.

Parameter (% wet weight)	Treatment			
	Ch*	ChBO*	BF*	BFBO*
Protein	4.67	5.08	4.62	4.98
Water content	92.18	92.29	92.64	91.67
Ash	0.23	0.47	0.23	0.73
Fat	1.20	0.69	0.57	0.79
Crude fiber	0.83	0.50	0.51	0.98

*Ch: 100% *Chlorella*; ChBO: 50% *Chlorella* + 15% fish meal, 17.5% soy meal, 17.5% rice bran meal; BF: 100% biofloc; BFBO: 50% biofloc + 15% fish meal, 17.5% soy meal, 17.5% rice bran meal. *Ch: *Chlorella*; ChBO: *Chlorella* + organic ingredient (fish meal, soy meal, rice bran meal); BF: biofloc; BFBO: biofloc + organic ingredient (fish meal, soy meal, rice bran meal).

Table 4. Amino acids content (% wet weight) of *Moina* sp. cultured in various media in mass scale culture.

Parameter	Treatments (media)			
	Ch*	ChBO*	BF*	BFBO*
Essential amino acids (% wet weight)				
Phenylalanine	0.19	0.23	0.19	0.19
Isoleucine	0.14	0.19	0.20	0.17
Valine	0.19	0.25	0.25	0.21
Arginine	0.10	0.29	0.26	0.20
Lysine	0.19	0.28	0.27	0.22
Leusine	0.23	0.33	0.30	0.28
Threonine	0.17	0.25	0.24	0.21
Histidine	0.11	0.13	0.11	0.11
Non-essential amino acids (% wet weight)				
Serine	0.13	0.19	0.22	0.12
Glutamate acid	0.33	0.49	0.44	0.46
Alanine	0.21	0.29	0.25	0.27
Glycine	0.21	0.25	0.25	0.22
Aspartate acid	0.19	0.34	0.29	0.30
Thyroxine	0.09	0.21	0.16	0.17
Proline	0.16	0.21	0.20	0.19

*Ch: 100% *Chlorella*; ChBO: 50% *Chlorella* + 15% fish meal, 17.5% soy meal, 17.5% rice bran meal; BF: 100% biofloc; BFBO: 50% biofloc + 15% fish meal, 17.5% soy meal, 17.5% rice bran meal. *Ch: *Chlorella*; ChBO: *Chlorella* + organic ingredient (fish meal, soy meal, rice bran meal); BF: biofloc; BFBO: biofloc + organic ingredient (fish meal, soy meal, rice bran meal).

1998). Castro *et al.* (2017) explained that bacteria administration as the main feed for moina could not produce the similar moina abundance with the one fed with microalgae. *Moina* sp. abundance in BFBO treatment was 84% greater than the BF treatment. The combination of fish meal and soy meal as nitrogen source and rice bran meal as the carbohydrate sourcein the BFBO treatment could be the nutrition source for moina and the bacteria that later will be consumed by moina. The organic matter holds essential role in term of nutrition sources to regenerate the bacteria in *Cladocera* culture that used bacteria as feed source (Sambode *et al*., 2013).

By improving the carbon source solubility, such as rice bran meal, nitrogen waste can be converted into microbe biomass using heterothropic bacteria (Dauda *et al.*, 2017; Ekasari *et al.*, 2014). The Ch treatment exhibited that the highest moina abundance was obtained in day 4, followed by a drastic decreasing in the next day. It might be caused by low concentration of *Chlorella* sp. suspension which had been consumed by *Moina* sp. In the ChBO treatment, the availability of feed (organic ingredient and *Chlorella* sp.) was sufficient, even higher along the day. The existence of organic ingredient supported no only moina growth, but also *Chlorella* sp. as nutrition source. Sarma *et al*. (2001) explained that *M. macrocopa* fed with *Chlorella vulgaris* demonstrated a higher abundance from day to day, along with the increase of *Chlorella* abundance. Armin *et al.* (2014) even supported that *Chlorella* has an important role in boosting fecundity of *Moina micrura.*

The highest protein content was observed in ChBO treatment which was even higher than most artemia (Firmansyah *et al*., 2013), followed by the BFBO treatment (Table 3 & 4). The protein content in Ch and BF treatments were similar and both were lower than ChBO and BFBO treatment. Those results confirmed that the combination of several media positively affected the quality and productivity of *Moina* sp. The organic ingredient in ChBO treatment worked as nutrient source for *Chlorella* sp. Meanhile, the addition of organic ingredient to the media in BFBO treatment induced microorganism growth in a floc aggregate that was beneficial for *Moina* sp. Generally, the essential and non essential amino acids in ChBO treatment was greater compared to the others. It could be explained by the highest protein content which ChBO initially had.

Algae and bacteria can accumulate more than 20% of fat in their cells (Yousuf *et al*., 2010). The fat content in ChBO was inferior (0.2008%) compared to the Ch treatment (0.2226%). It seemingly due to the addition of organic ingredient in ChBO treatment. It is supported by Qi *et al*. (2016) who stated that fat accumulation in *Chlorella* sp. is frequently found in a nitrogen-lacked environment. Cheah *et al*. (2018) explained that fat accumulation in bacteria is not related to the biomass growth. Despite of for fat accumulation, the nutrient-rich energy will be used firstly for biomass growth. Moreover, Qi *et al*. (2016) and Chen *et al*. (2015) mentioned that a high level of fat was not in accordance with biomass production. Nitrogen existence from fish meal and soy meal were positively involved in the bacteria biomass growth, but not in fat accumulation. *Bacillus cereus* grows rapidly by duplicating nutrition and organic matters to produce greater biomass (Karim *et al.*, 2019). Thus, it was assumed that the BFBO treatment that rich in nutritious compounds will produce more bacteria biomass compared to the BF treatment. However, Zhang *et al*. (2014) described that *Bacillus* sp. can accumulate 39.8% of fat in 48 hours so that the BFBO, where in the process of biofloc making used *Bacillus* sp., possibly carried higher fat content than the BF treatment. The fat content in *Moina* sp. in BF and BFBO treatment was influenced by floc-forming bacteria. It can be inferred from the fat content in BFBO (0.8350 %) which is higher than the BF treatment (0.2350 %).

Based on the fatty acids tests, it was known that linoleate acid (C18:2) and linolenate acid (C18:3) were discovered in the tested *Moina* sp. in all media treatment (Table 5). Arachidonic acid (AA, C20:4n-6) was also detected in all treatments, except the ChBO treatment. The highest level of arachidonic acid was found in the BF treatment, while the lowest level was in the BFBO treatment. Docosahexaenoic acid (DHA, C22:6n-3) content was only discovered in the BF treatment, whereas the eicosapentaenoic acid (EPA, C20:5n-3) was disclosed both in the Ch and BF treatment (Table 6 & 7). EPA and DHA were not noticed in *Moina* sp. cultured in ChBO treatment. It was supported by Smirnov (2017) who wrote that 20:4 w6 (AA); 20:5 w3 (EPA) dan 22:6 w3 (DHA) are not commonly discovered in green algae. Otles *et al*. (2001) also stated that *Chlorella* sp. majorly carries PUFA (36–43 %), especially linoleate and linolenate acid (21.55% in *C. pyrenoidosa*). In contrast, DHA was only detected in an extremely small percentage. The highest MUFA content in tested *Moina* sp.

was noticed in the BFBO treatment, most of if was oleic acid. Meanwhile, the Ch and ChBO treatment carried the PUFA higher compared to the BF and BFBO treatment. Generally, the fatty acid content in all four treatment were still inferior compared to artemia (Francis *et al*., 2019). It was presumably generated by *Chlorella* sp. domination in both Ch and ChBO, the BF and BFBO treatments were dominated by bacteria.

The SFA content in BF and BFBO treatment was superior compared to the Ch and ChBO because of the high content of palmitic acid (C16:0) in the biofloc. It is in accordance with a former study by Anand *et al.* (2014) who stated that palmitic acid, oleic acid, and linoleic acid are the dominated fatty acids in biofloc. The BF and BFBO treatment contained a higher HUFA (highly unsaturated fatty acid) content than Ch

Table 5. Fatty acid content of *Moina* sp. cultured in various media in mass scale culture.

Fatty acids (% fat)	Treatment (media)			
	Ch*	ChBO*	BF*	BFBO*
∑SFA**	0.0663	0.0620	0.0856	0.4595
∑MUFA**	0.0330	0.0195	0.0567	0.2771
∑PUFA**	0.1233	0.1193	0.1099	0.0976
C 18:2 (linoleic acid)	0.0412	0.0695	0.0350	0.0801
C 18:3 (linolenic acid)	0.0624	0.0483	0.0273	0.0096
C 20:4n-6 (AA**)	0.0086	0	0.0308	0.0073
C 20:5n-3 (EPA**)	0.0099	0	0.0133	0
C 22:6n-3 (DHA**)	0	0	0.0028	0

*Ch: 100% *Chlorella*; ChBO: 50% *Chlorella* + 15% fish meal, 17,5% soy meal, 17.5% rice bran meal; BF: 100% biofloc; BFBO: 50% biofloc + 15% fish meal, 17.5% soy meal, 17.5% rice bran meal. *Ch: *Chlorella*; ChBO: *Chlorella* + organic ingredient (fish meal, soy meal, rice bran meal); BF: biofloc; BFBO: biofloc + organic ingredient (fish meal, soy meal, rice bran meal).

**SFA: Saturated fatty acid, MUFA: Mono unsaturated fatty Acid, PUFA: Poly unsaturated fatty Acid, AA: Arachidonic acid, EPA: Eicosapentaenoic acid, DHA: Docosahexaenoic acid.

Table 6. The fatty acids of *Moina* sp. cultured in various media in mass scale culture.

Fatty acid (% fat)	Treatment (media)			
	Ch*	ChBO*	BF*	BFBO*
C 8:0	-	-	-	-
C 10:0	0.0043	-	-	-
C 12:0	-	-	-	0.0037
C 14:0	0.0054	-	0.0041	0.0136
C 15:0	0.0051	0.0031	0.0057	-
C 16:0	0.0374	0.0335	0.0480	0.3795
C 16:1	0.0040	-	0.0089	0.0050
C 17:0	0.0028	0.0034	0.0060	0.0025
C 17:1	0.0151	0.0047	0.0040	-
C 18:0	0.0091	0.0160	0.0189	0.0449
C 18:1 w9c	0.0139	0.0148	0.0438	0.2721
C 18:2 w6	0.0412	0.0695	0.0350	0.0801
C 18:3 w3	0.0598	0.0483	0.0224	0.0087
C 18:3 w6	0.0026	-	0.0049	-

*Ch: 100% *Chlorella*; ChBO: 50% *Chlorella* + 15% fish meal, 17.5% soy meal, 17.5% rice bran meal; BF: 100% biofloc; BFBO: 50% biofloc + 15% fish meal, 17.5% soy meal, 17.5% rice bran meal. *Ch: *Chlorella*; ChBO: *Chlorella* + organic ingredient (fish meal, soy meal, rice bran meal); BF: biofloc; BFBO: biofloc + organic ingredient (fish meal, soy meal, rice bran meal).

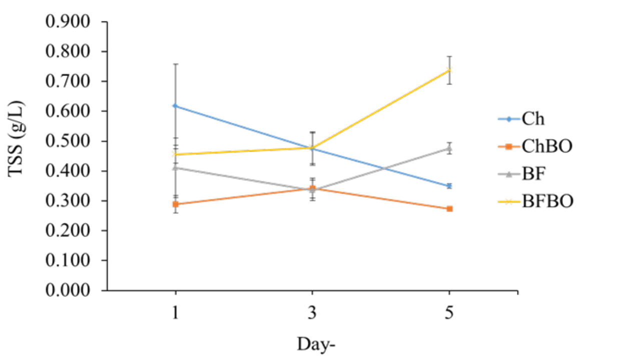
and ChBO treatment. It was majorly caused by the AA, EPA, and behenic caid (C22:0) content that was higher in the BF and BFBO treatment. It was in line with Otles *et al*. (2001) who stated that arachidonic acid was not found in *Chlorella pyrenoidosa* and Thoi (2014) mentioned that behenic and arachidonic acid were uncovered in artemia fed with bacteria.

The TSS and VSS were strongly related to the *Moina* sp., *Chlorella* sp., and organic matters in culture media (Figure 5). The TSS concentration in Ch treatment was relatively low in day 1 and it might be related to the low population of *Moina* sp. and the *Chlorella* sp. which was suspended at the bottom. The TSS in ChBO treatment increased until day 3 and decreased in day 5 along with the dimishing of *Chlorella* sp. abundance although moina population keep improving. It was caused by the organic waste that greatly utilized by both *Chlorella* sp. and *Moina* sp.. The TSS in BF treatment declined at day 3 and returned to increase at day 5. It was related to the biolfoc utilization along with the peak population of *Moina* sp. On the contrary, the BFBO treatment experienced a high level of TSS due to the floc agregate and organic matters addition that induced phytoplankton, floc, and moina growth. The ChBO and BFBO treatment had a greater VSS level compared to the Ch and BF treatment due to the peak population of moina and phytoplankton, also the organic metters and floc agregate. A relatively stable VSS level was noticed in Ch treatment along with and the balanced *Chlorella* sp. abundance and the increase population of moina. Similarly, the BF treatment had a pretty stable VSS level due to the floc existence and moina abundance. *Chlorella* sp. abundance in the Ch treatment did not significantly change because

Table 7 (continued). The fatty acids of *Moina* sp. cultured in various media in mass scale culture.

Fatty acid (% fat)	Treatment (media)			
	Ch*	ChBO*	BF*	BFBO*
C 20:0	-	-	-	0.0029
C 20:4 w6	0.0086	-	0.0308	0.0073
C 20:5 w3	0.0099	-	0.0133	-
C 22:0	-	0.0023	-	0.0047
C 22:6 w3	-	-	0.0028	-
Total n-3	0.0696	0.0483	0.0385	0.0087
Total n-6	0.0524	0.0695	0.0706	0.0874
Total n-9	0.0139	0.0148	0.0438	0.2721

*Ch: 100% *Chlorella*; ChBO: 50% *Chlorella* + 15% fish meal, 17.5% soy meal, 17.5% rice bran meal; BF: 100% biofloc; BFBO: 50% biofloc + 15% fish meal, 17.5% soy meal, 17.5% rice bran meal. *Ch: *Chlorella*; ChBO: *Chlorella* + organic ingredient (fish meal, soy meal, rice bran meal); BF: biofloc; BFBO: biofloc + organic ingredient (fish meal, soy meal, rice bran meal).



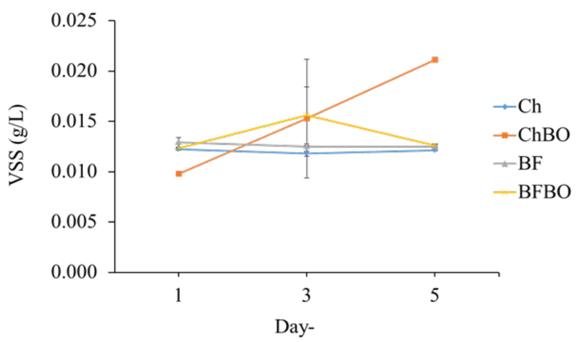


Figure 5. TSS and VSS of *Moina* sp. cultured in various media in mass scale culture. Ch: 100% *Chlorella*; ChBO: 50% *Chlorella* + 15% fish meal, 17.5% soy meal, 17.5% rice bran meal; BF: 100% biofloc; BFBO: 50% biofloc + 15% fish meal, 17.5% soy meal, 17.5% rice bran meal. Ch: *Chlorella*; ChBO: *Chlorella* + organic ingredient (fish meal, soy meal, rice bran meal); BF: biofloc; BFBO: biofloc + organic ingredient (fish meal, soy meal, rice bran meal).

the supply was greatly maintained as moina feed. On the other hand, *Chlorella* sp. abundance in ChBO dropped off in day 4 along with the elevating moina abundance and declined nutrient from organic matters.

The water pH level during the culture ranged from 6–8 in the morning and increased up to 8–9 in the afternoon, particularly the Ch, ChBO, and BFBO (Figure 7). It was indeed related to the *Chlorella* sp. abundance and organic matter. The photosynthesis conducted by *Chlorella* sp. can possibly influence pH level. This particular mechanism was also occurred in BFBO treatment because the floc itself contained phytoplankton that utilise nutrients from organic matters. Unlike the others, the BF treatment showed a stable pH level ranged from 6–7. It was presumably due to the low photosynthesis activity by phytoplankton in the biofloc because no nutrient was added to the system. Nevertheless, the pH fluctuation was in a tolerable range.

Moina sp. productivity was mostly influenced by type of feed and its supply during the culture. Besides aeration, dissolved oxygen was also related to the abundance of *Moina* sp. and *Chlorella* sp. It can be inferred from the declining of dissloved oxygen abundance in BF and BFBO treatment due to the peak of moina abundance in day 3. Meanwhile, the dissolved oxygen in Ch and ChBO treatment was directly influenced by the *Chlorella* sp. existence, photosynthesis and *Moina* sp. abundance. The ammonia concentration in Ch, ChBO, and BF treatment were relatively stable compared to the BFBO treatment. In the BFBO treatment, decomposition was occurred and unevitably released the NH_3 that only partly utilized by the bacteria in the biofloc during the study. It could be stated that C/N ratio was imbalance. However, ther organic decomposition in ChBO was assumed to be used for *Chlorella* sp. growth.

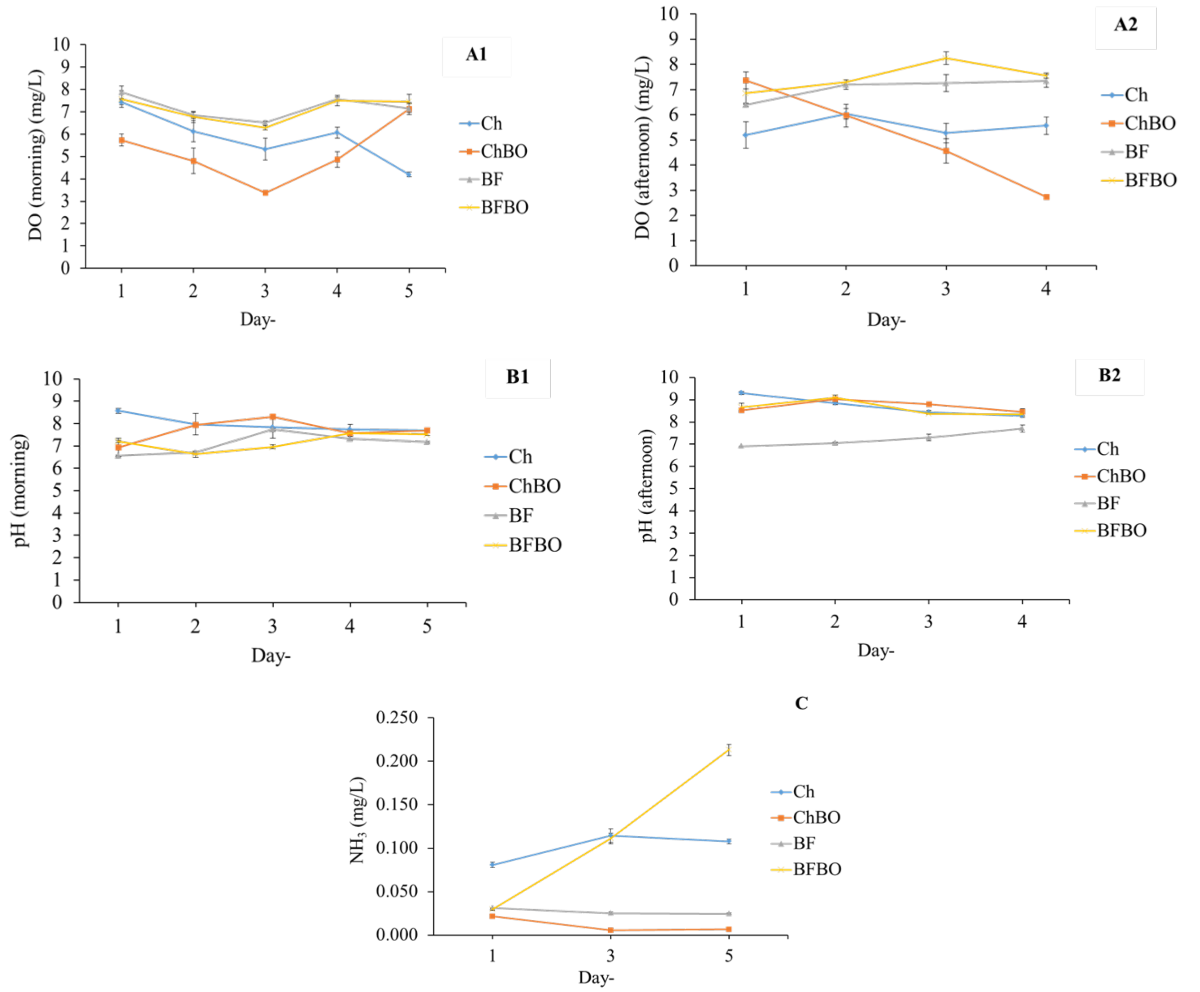


Figure 7. Dissolved oxygen (A1 and A2), pH level, (B1 and B2), and NH_3 concentration (C) in *Moina* sp. culture in various culture media. Note: Ch: 100% *Chlorella*; ChBO: 50% *Chlorella* + 15% fish meal, 17.5% soy meal, 17.5% rice bran meal; BF: 100% biofloc; BFBO: 50% biofloc + 15% fish meal, 17.5% soy meal, 17.5% rice bran meal. Ch: *Chlorella*; ChBO: *Chlorella* + organic ingredient (fish meal, soy meal, rice bran meal); BF: biofloc; BFBO: biofloc + organic ingredient (fish meal, soy meal, rice bran meal).

Based on the result, it can be implied that the nutrient in the culture media, feed size, feed amount available in the culture media, and environmental factors greatly influenced moina growth. Fish meal contained 60.4 % of protein and 24.2 g/100 g of dry weight of amino acids (Radhakhrisnan *et al.*, 2017). The soy meal contained 39.1 % of amino acids, while rice bran meal as carbohydrates source, contained vitamin B, linoleic acid (6.35–6.85%), and α-linoleic acid (0.20–0.27%) (Mubarak *et al*., 2017; Aparecida *et al*., 2012). *Chlorella* sp. that commonly used in zooplankton culture (Malla & Banik, 2015, Morales-Ventura *et al*., 2012), contained PUFA 38.46 % (Otles & Pire, 2001) and 30.2 g/100 g dry weight of total amino acids (Radhakhrisnan *et al.*, 2017). Those components would be strongly related to *Moina* sp. biomass and harvest period. A mass scale *Moina* sp. culture in BF and BFBO treatment obtained a comparably low biomass and the shortest harvest period (3 days). The optimal harvest period was shown by the Ch treatment (4 days), yet the biomass was the lowest. The most productive or the highest *Moina* sp. biomass was appeared in the ChBO treatment and it had the longest harvest period (5 days). By knowing the harvest period, hopefully, the greatest biomass would be achieved within the various media treatment.

Based on the results, it was known that the highest concentration of amino acids and fatty acids in the tested *Moina* sp. were the ChBO and BFBO treatment, respectively. Therefore, with such a promising nutrition profile and productivity, moina was applicable to substitute artemia. *Moina* sp. has a lot of advantages in terms of live feed in aquaculture. It is easier to culture *Moina* sp. in a limited space, coexisted with the aquaculture site. In addition, *Moina* sp. is easily cultured in a mass scale due to the parthenogenesis reproduction mechanism as long as the nutrition and environment are condusive. The administration of various culture media could be applied in *Moina* sp. mass culture with abundant biomass and accurate harvest period to meet the demand of live feed for fish larvae culture. Likewise, referring to the operational cost comparison amongst *Moina* sp. and artemia, it was admitted that *Moina* sp. culture reduced the live feed cost up to 38% compared to artemia.

CONCLUSION

Productivity and nutrition profile of *Moina* sp. was determined by the culture media. *Moina* sp. reared in *Chlorella* sp. presented the highest PUFA content, while the greatest MUFA was recorded in the BFBO treatment. The combination of *Chlorella* sp. and organic ingredient in the culture media in mass culture produced the most excellent productivity and protein content.

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