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Macronutrients of Brown and Green Water Types of BFT Systems May Behave Differently During Recirculation in Saline Aquaponics

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HIGHLIGHTS

- Brown and green waters of BFT systems had different nutrient compositions.
- Reduction of 28–72% (nitrate and potassium) after recirculation of brown water.
- Green water recirculation increased nitrate and nitrite concentrations.

Abstract: Biofloc technology (BFT) systems are sustainable for aquaculture due to the growth of microorganisms in the culture medium that can uptake toxic nitrogen compounds, which generates “in situ” microbial protein that can be eaten, decreasing feed costs. BFT brown (formed with no direct natural light and bacterial dominance) and green (exposed to direct natural light, algal system) waters can be potentially used for aquaponics of commercial halophytes. This study evaluated changes in physical-chemical parameters and macronutrient concentrations of brown and green saline waters of BFT systems, with marine shrimp (*Litopenaeus vannamei*) breeding stock, subjected to a one-week recirculation cycle in saline aquaponics with halophytes (*Salicornia neei*, *Apium graveolens* and *Paspalum vaginatum*). For 30 days in the summer of 2019, clarified brown and green waters of BFT systems of *L. vannamei* were used for aquaponic cultivation of these three halophytes. Water samples were collected before and after one week of water recirculation in the aquaponic systems. Halophytes grow well with both water types. The brown water had a higher phosphate concentration and its nitrate content reduced 3-fold in magnitude after one week of recirculation. The green water had higher average concentrations of nitrate, potassium, calcium, magnesium and sulfate compared to the brown water, and its nitrate and nitrite contents significantly increased after one week of recirculation. The remediation of dissolved macronutrients using aquaponics can be ineffective for green water types, mainly due to the death and decay of photoautotrophic microorganisms during the water recirculation process inside aquaponic structures.

Keywords: biofloc technology; flocponics; halophytes; *Litopenaeus vannamei*; marine aquaponics.

INTRODUCTION

Green technologies have been strongly supported for aquaculture production, for example, a biofloc technology (BFT) system. This sustainable approach is based on the growth of microorganisms in a culture medium, where aggregates (flocs) of algae, bacteria, protozoans, and particulate organic matter (e.g., feces and uneaten feed) uptake toxic nitrogen compounds (i.e., ammonia nitrogen), which generates "in situ" microbial protein that can be eaten, decreasing feed costs [1]. A biofloc system is based on the minimal renewal of water, allowing the conversion of waste into bioflocs, since constant aeration and mixing of the water column and the addition of an organic carbon source are provided [2]. Biofloc composition varies and is affected by many factors, for instance, carbon application and light limitation can benefit growth of heterotrophic microorganisms but restrict photoautotrophic microorganisms [3].

Bioflocs are basically categorized as brown water, where heterotrophic bacteria are dominant and there is no direct exposure to natural light or light restriction (e.g., structures covered with a shading screen), and green water, where there is a greater abundance of algae and photoautotrophic flagellates (composed of a diverse microbial community), generating large bioflocs under direct exposure to light [2,4]. In greenhouses, a predictable sequence of changes occurs over time in sunlight-exposed biofloc systems as the feeding rate increases, which leads to the transition from green to brown water [2,5]. Xu and coauthors [6] showed that the color of suspended bioflocs differentiates from green to brown with an increase in the C/N ratio, and tanks with higher C/N ratios predominately had a heterotrophic biofloc. A mixed type of biofloc dominated by both microalgae and autotrophic bacteria seems to be more beneficial to shrimp performance in high density BFT systems than those dominated by heterotrophic bacteria [4,6].

Overall, in a biofloc system the amounts of suspended solids, dissolved nitrate and phosphorus increase overtime [1], and nitrate building up results from decomposing organic matter but mainly ammonia oxidation by nitrifying bacteria and much of the phosphorus is derived from excretion and decomposition of uneaten feed [6,7]. An option for controlling water quality is aquaponics (i.e., hydroponics with recirculating aquaculture water) [7,8]. Cultivating vascular plants in aquaponic systems used for marine animal production is growing in the food sector, which is also called maraponics, haloponics and saline aquaponics [9]. Halophytes (highly salt tolerant plants) are promising candidates for saline aquaponics [9–11], and waters in BFT systems can be a nutritional source for these plants [7,8,12,13]. Brazilian halophytes, for example, *Salicornia neei*, *Apium graveolens* and *Paspalum vaginatum*, have high quality biomass [14,15] and great potential for saline aquaponic cultivation [8,11,16].

Water must have a minimum residence time recirculating in an aquaponic system to allow plant growth and nutrient uptake [17,18]. During the recirculation period, water is in contact with plant roots, any other available substrate, biofilms and other microorganisms (e.g., from the biofloc), leading to the uptake of nutrients by plants and other processes, such as mineralization of organic compounds, adsorption of elements/compounds on walls and particles, and biochemical reactions involving microbes. Additionally, the ion content in the water can affect growth and survival of marine shrimp during BFT system cultivation [19]. The different characteristics of brown and green water may result in a prevalence of one of the processes cited above, affecting water quality and nutrient removal efficiency by the aquaponic system. However, there is a lack of information about how different water types in BFT systems behave during recirculation when using aquaponics with halophytes. This study evaluated changes in the physical-chemical parameters and macronutrient concentrations of brown and green saline waters of BFT systems with a *Litopenaeus vannamei* breeding stock subjected to a one-week recirculation cycle in saline aquaponics with halophytes (*S. neei*, *A. graveolens* and *P. vaginatum*).

MATERIAL AND METHODS

Brown and green water types from BFT systems of *L. vannamei* breeding stock were used in decoupled aquaponics with nutrient film technique (NFT) benches. Each water type came from a different tank (40 m^3 each) of adult shrimp (average of 50 g; brown water= 555 breeders per tank and green water= 520 breeders per tank). The shrimp were fed daily with 300 g of commercial extruded feed per tank (Poti Evolution 35 Guabi®, 1.6 mm, containing 35% crude protein, 1530 g of calcium and a minimum of 15 g of phosphorus, 10 mg of manganese, 75 mg of zinc and 34 mg of copper per kg). The tanks were placed in an unheated greenhouse. A shading screen over the brown water tank was the only distinction in maintenance practices for these tanks. The brown water tank was covered with a black 50 percent shade cloth. During the four-week experiment, between January and February 2019, the automatic meteorological station of the Instituto Nacional de Meteorologia-INMET (32°04'43" S; 52°10'03" W) recorded mean values (± standard error) for air temperature (24.6 ± 0.5 °C) and daily solar radiation (19.2 ± 1.3 MJ m^{-2} day^{-1}). The light incidence on the surface of the brown and green water tanks was 19% and 76% of the daily solar radiation, respectively (measured with a Protomatic light meter mod. 8060 Grand).

The experiment was carried out at Estação Marinha de Aquacultura, at the Universidade Federal do Rio Grande-FURG (Rio Grande, RS, Brazil), using three NFT benches (replicates) per treatment with a mixture of 153 halophytic plants. The biometry of all plants of the three species was quantified in the beginning and at the end of the experimental trial. The differences between the initial and final values for height of each plant were used to calculate the absolute vertical growth rate. At the end of cultivation, all plants were harvested and shoots were weighed on a precision scale to determine the fresh weight. The initial mean values (± error standard) for shoot height of *S. neei* (n= 66), *P. vaginatum* (n= 66) and *A. graveolens* (n= 21) were 9.40 ± 0.63 cm, 12.17 ± 0.83 cm and 9.34 ± 1.05 cm, respectively. Throughout the experiment, every week a different water type (brown or green) was recirculated in the NFT benches. Thus, for each water type was obtained six (6) measurements of water recirculation (3 benches X 2 weeks per water type), including physical-chemical parameters and macronutrient concentrations. Both water types of the BFT systems were clarified (500 L conical cylinder tank) and filtered (BP-420-50 Pentair® filter bags; 50 microns) before being stored in a reservoir (3,000 L). The stored water was then pumped to 500 L reservoirs (filled to a useful volume of 450 L) below the NFT benches (six 10 cm X 5 cm X 3 m PVC pipes). Water recirculated from each reservoir to its bench of the hydroponic unit at a rate of 13.1 L^{-1} min^{-1} every other 15 minutes (393 L^{-1} h^{-1}) and it had an overall average of total suspended solids (TSS) of 104.93 ± 0.58 mg L^{-1}. Every week, before the water exchange, the storage reservoir, hydroponic unit reservoirs and pipes, clarifier tank and filter bags were cleaned with sodium hypochlorite.

Water physical-chemical parameters and macronutrient concentrations

Physical-chemical parameters and macronutrient concentrations of the water were monitored in the aquaponic systems twice a week, before and after one-week of water recirculation. The following parameters were measured *in situ*: pH and electrical conductivity with a FEP20 Mettler Toledo® pH meter and HI9835 Hanna® conductivity meter, respectively. Total ammonia nitrogen was measured according to UNESCO [20], nitrite was measured using the methodology in Bendschneider and Robinson [21], and nitrate and phosphate were measured according to Aminot and Chaussepied [22]. Water analyses of potassium, calcium, magnesium and sulfate followed the methodology of the EPA [23]. All the sample bottles were cleaned with 10% chloridric acid and rinsed with distilled water three times.

Statistics

One-way repeated measures analyses of variance (ANOVA) were used to contrast physical-chemical parameters and concentrations of macronutrients of the BFT system waters, where the repeated (within-subject) factor was water recirculation in aquaponics (values before and after recirculating in the system for a week) and the fixed (between-subject) factor was water type (brown and green water). Nitrite, calcium and magnesium were transformed by $\log_{10}(x)$ to meet the requirements of normality (Shapiro-Wilk test) and homoscedasticity (Levene test) described in Zar [24]. Significant differences among averages were found using the Tukey HSD post-hoc test. All the values were reported as mean ± standard error and a 5% significance level was considered for all statistical analyses.

RESULTS

Halophytes grow well with both water types. Average monthly growth (difference between initial and final plant size) and final biomass were 19.10 ± 0.40 cm and 43.15 ± 3.18 g of fresh biomass (FW) for *S. neei*, 19.40 ± 1.02 cm and 8.15 ± 0.66 g FW for *P. vaginatum*, and 14.95 ± 1.40 cm and 1.90 ± 0.26 g FW for *A. graveolens*.

The brown and green waters of the BFT systems showed no statistical difference between their pH values (global average= 7.82 ± 0.04), electrical conductivity (20.66 ± 1.57 mS cm^{-1}; ≈ 13.9 ± 1.1 g NaCl L^{-1}), total ammonia nitrogen (0.06 ± 0.01 mg L^{-1}) and nitrite (0.20 ± 0.02 mg L^{-1}) contents (Table 1). Values for phosphate concentration were 51.3% significantly higher in brown water compared to green water (Table 1). Average concentrations of nitrate (Table 1), potassium, calcium, magnesium and sulfate (Table 2) were 46.4% to 89.6% higher in green water than in brown water.

Table 1. Mean ± standard error of water quality parameters (Total Ammonia Nitrogen-TAN, Nitrite-NO_2, Nitrate-NO_3 and Phosphate-PO_4= mg L^{-1}) from saline aquaponics with two distinct water types of BFT system. One-way repeated measures Analyses of Variance (ANOVA) were performed contrasting water type (brown water and green water) and water recirculation (before and after one-week recirculation; n= 6). Different lowercase letters (within a column) represent significant differences between the averages (p < 0.05), according to the Tukey's HSD test.

Water type	Water recirculation	TAN			NO₂			NO₃			PO₄		
Brown water	Before	0.07	± 0.02		0.18	± 0.03	ab	125.00	± 7.64	b	3.33	± 0.16	bc
	After	0.07	± 0.01		0.18	± 0.02	ab	35.00	± 1.73	a	3.63	± 0.32	c
Green water	Before	0.09	± 0.02		0.15	± 0.01	a	39.33	± 1.54	a	2.18	± 0.28	a
	After	0.01	± 0.01		0.28	± 0.06	b	151.67	± 4.77	c	2.42	± 0.35	ab
Factors		**F**	**Sig.**		**F**	**Sig.**		**F**	**Sig.**		**F**	**Sig.**	
Water type		1.52	ns		0.83	ns		13.51	**		43.28	***	
Water recirculation		3.52	ns		5.03	*		5.59	*		0.34	ns	
Interaction		2.46	ns		4.43	ns		458.76	***		0.01	ns	

∗p < 0.05; ∗∗p < 0.01; ∗∗∗p < 0.001; ns: non-significant (p > 0.05), according to Tukey HSD test.

Nitrate and potassium concentrations decreased after one-week of recirculation for the brown water in the aquaponic systems (Table 1, 2). Additionally, nitrite and nitrate concentrations significantly increased in green water after recirculation (Table 1).

Table 2. Mean ± standard error of macronutrients (Potassium-K, Calcium-Ca, Magnesium-Mg and Sulfate-SO_4=mgL^{-1}) in the water from saline aquaponics with two distinct water types of BFT system. One-way repeated measures Analyses of Variance (ANOVA) were performed contrasting water type (brown water and green water) and water recirculation (before and after one-week recirculation; n= 6). Different lowercase letters (within a column) represent significant differences between the averages (p < 0.05), according to the Tukey's HSD test.

Water type	Water recirculation	K			Ca			Mg			SO₄		
Brown water	Before	250.67	± 20.17	b	162.33	± 5.78	a	474.67	± 13.64	a	1093.67	± 66.06	ab
	After	180.67	± 9.67	a	163.33	± 5.84	a	485.67	± 13.25	a	1060.67	± 131.87	a
Green water	Before	322.67	± 2.60	c	312.00	± 1.53	b	895.33	± 4.06	b	1584.00	± 157.98	bc
	After	309.00	± 13.65	bc	305.33	± 10.27	b	880.33	± 29.49	b	1624.67	± 202.91	c
Factors		**F**	**Sig.**		**F**	**Sig.**		**F**	**Sig.**		**F**	**Sig.**	
Water type		35.16	**		344.44	***		408.21	***		20.90	*	
Water recirculation		28.56	**		0.10	ns		0.01	ns		0.00	ns	
Interaction		12.95	*		0.31	ns		0.94	ns		0.04	ns	

∗p < 0.05; ∗∗p < 0.01; ∗∗∗p < 0.001; ns: non-significant (p > 0.05), according to Tukey HSD test.

DISCUSSION

The brown and green water types of the BFT systems had a similar pH and electrical conductivity but distinct dissolved nutrient concentrations (Table 1, 2). Both waters exhibited an active nitrification process, with high concentrations of nitrate, and either this compound or the physical-chemical conditions cited above remained within the range that is the best growth condition for *L. vannamei* [25,26] and nutritionally advantageous for the studied halophytes [11].

In our aquaponic systems, the concentration of nitrate in the brown and green water types was affected differently by water recirculation. Highly nitrified brown water had a nitrate concentration that decreased 70% after one week of recirculation, whereas the green water showed a massive increase of 112 mg NO_3 L^{-1} for the same time period. Pinheiro and coauthors [12] showed a fast decrease in nitrate after a week of BFT water recirculation in a coupled aquaponic system (i.e., *L. vannamei* tank and *S. neei* NFT bench connected and managed concomitantly), which is similar to that observed in our brown water trials. This was associated with denitrification within the clarifiers and their reduction of TSS. Since in our experiment water clarification was held before recirculation in the decoupled aquaponic systems, nitrate reduction of recirculated brown water might be associated with the biofilm covering pipes and tank surfaces, as well as with uptake by halophytes, which have been reported as nitrate loving plants [7,11,16].

The contrasting increase of nitrate content in the recirculated green water suggests that light limitation may be an issue for photoautotrophic microorganisms abundant in this water type, leading to their death [2,3]. The water reservoir of each aquaponic system was covered, and incident light was also severely reduced by sealed circulation pipes. Dead photoautotrophic microorganisms can quickly decay, and organic matter can be mineralized by heterotrophic microorganisms, which would explain the strong nitrate and potassium incorporation into the recirculating water. Additionally, light restriction inside aquaponic structures might reduce nitrate assimilation by microalgae [27].

Phosphate concentrations were intermediate between those found in *L. vannamei* grow-out tanks in intensive biofloc systems (1.4-5.5 mg L^{-1}) [7,28]. However, higher concentrations of phosphate were observed in the brown water type. Similarly, for other BFT systems, Xu and coauthors [6] and Cavalcante and coauthors [29] reported greater amounts of dissolved phosphate in brown waters than in green waters. High phosphate content in BFT brown waters might be related to a small amount of microalgae and/or other autotrophic organisms that assimilate this compound [28]. Independently of the water type, BFT water recirculation through the halophyte aquaponic units resulted in no changes in dissolved phosphate concentrations. The growth of halophytes, and consequently phosphorus incorporation into their biomass, was not large enough to reduce the phosphate concentration after one week of recirculation. However, an increment in phosphorus concentration is common (not observed in our recirculation experiment) along the cultivation process in super-intensive BFT systems, where no relevant water exchange occurs [28]. Thus, the lack of changes in phosphate concentrations after one week of recirculation seems to point out that the aquaponic units were able to counteract the ongoing phosphorus mineralization processes in the BFT system waters.

The green water type showed the highest mean concentrations of potassium, calcium, magnesium and sulfate (Table 2), with values (except for sulfate) that were low compared to other waters of saline BFT systems with *L. vannamei* (K= 400-798 mg L^{-1}, Ca= 600-1034 mg L^{-1}, Mg= 1300-1448 mg L^{-1} and SO_4= 1100-1200 mg L^{-1}) [19,30]. These low values can be explained by the intermediate water salinity of the breeding stock shrimp tanks (mean= 20.66 mS cm^{-1} ≈ 14 g NaCl L^{-1}) and these ions being major components of seawater [19]. Regarding water recirculation, there was a reduction in the potassium concentration (significantly for brown water) on a weekly basis (Table 2). Potassium requires particular attention for clean water aquaponics, since it is not usually added to aquatic animal feed formulas [31], which leads to an insufficient amount for plant growth and reproduction of some crops [32]. In aquaponics with waters from a saline biofloc system, the concentration of potassium might not be an issue for plants because it tends to increase along with the production cycle and, according to Zacarias and coauthors [33], potassium concentration is direct correlated with the addition of cane molasses (main source of carbon for biofloc formation). Furthermore, a decrease in the potassium concentration over one week of brown water recirculation might also be related to uptake by heterotrophic bacteria, which dominate the brown water of biofloc systems [33].

CONCLUSION

Clarified brown and green waters of saline BFT systems are suitable for halophyte growth. However, the remediation of dissolved macronutrients using aquaponics can be ineffective for green water types, mainly due to the death and decay of photoautotrophic microorganisms during the water recirculation process inside aquaponic structures.

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