OYSTER AND MACROALGAE DENSITY EFFECTS ON THE TREATMENT OF MARINE SHRIMP FARMING EFFLUENT

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Abstract - The evaluation of oysters and macroalgae densities effects in effluent treatment of autotrophic and heterotrophic shrimp culture systems were assessed in laboratory-scale. Native species of oyster (*Crassostrea rhizophorae*) and macroalgae (*Gracilaria birdiae*) were selected due to their local availability and aquaculture potential in northeastern Brazil. Three densities of oyster (0.2, 0.4 and 0.8 oyster.L^{-1}) and macroalgae (2.0, 4.0 and 8.0 g.L^{-1}) were assessed during 48 h to treat effluent water (24 h for each phase). Chemical and physical variables were measured each 8 h during experimental period (0 to 48 h). Variations in the concentration of chlorophyll *a*, pheophytin, total phosphorus, total phosphate, orthophosphate, total ammonia, nitrate, nitrite, total suspended solids, organic suspended solids and inorganic suspended solids showed that the two biological filters reduced significantly the concentration of the different pollutants in the shrimp effluent, however oyster and macroalgae densities definition should be more evaluated.

Palavras-Chave: *Crassostrea rhizophorae*, *Gracilaria birdiae*, *Litopenaeus vannamei*, Water quality, Density

EFEITOS DA DENSIDADE DE OSTRAS E MACROALGAS NO TRATAMENTO DE EFLUENTES DO CULTIVO DE CAMARÃO MARINHO

Resumo - Foram avaliados os efeitos de diferentes densidades de ostra e macroalga no tratamento de efluentes de sistemas autotrófico e heterotrófico de cultivo de camarão. Espécies nativas de ostra (*Crassostrea rhizophorae*) e macroalga (*Gracilaria birdiae*) foram selecionadas devido a disponibilidade local e potencial para a aquicultura no Nordeste do Brasil. Foram avaliadas três densidades de ostra (0,2, 0,4 e 0,8 ostra.L^{-1}) e macroalga (2,0, 4,0 e 8,0 g.L^{-1}) no tratamento do efluente durante 48 h (24 h em cada fase). Variáveis químicas e físicas foram analisadas a cada 8 h durante o experimento (0 a 48 h). Variações nas concentrações de clorofila-*a*, feofitina, fósforo total, fosfato total, ortofosfato, amônia total, nitrato, nitrito, sólidos suspensos totais, sólidos suspensos orgânicos e sólidos suspensos inorgânicos mostraram que os dois organismos filtradores reduziram significativamente a concentração de diferentes poluentes no efluente de cultivo de camarão, entretanto as densidades de ostra e macroalga devem ser mais estudadas.

Keywords: *Crassostrea rhizophorae*, *Gracilaria birdiae*, *Litopenaeus vannamei*, Qualidade de água, Sedimentação, Densidade

INTRODUCTION

Brazilian shrimp aquaculture production had an expressive increment with the culture of the whiteleg shrimp *Litopenaeus vannamei*, which increased from 2,100 t in 1993 up to 64,669 t in 2013 (FAO, 2015). However, that raise, in the majority of the farms, was unplanned and not sustainable, and has caused the culture environment degradation, occurrence of diseases and consequently a decline in production.

Environmental impacts from the expansion of shrimp farms and increasing of culture densities has been widely discussed, mainly the effects of no treated effluent to elevate sediment and nutrient loadings in coastal areas (WANG, 1990; MACINTOSH and PHILLIPS, 1992; ZIEMANN et al., 1992; HOPKINS et al., 1993; BRIGGS and FUNGE-SMITH, 1994; PAEZ-OSUNA et al., 1998; TROTT and ALONGI, 2000). Some authors have warned that the transformation processes of the natural resources and effluent production by shrimp industry could negatively affect itself (CURRIE, 1994; NASCIMENTO, 1998).

Aquatic animal culture produces and releases a large amount of metabolic residues to the environment (BEVERIDGE, 1996). Effluent water often presents higher dissolved nutrient concentrations and suspended particles than affluent water (PHILLIPS et al., 1993; MCINTOSH and FITZSIMMONS, 2003), and its discharge is a waste of energy, that could be used to produce biomass. The culture of oysters and macroalgae is a way to make use of that energy and to reduce the discharges (JONES et al., 2002; MARINHO-SORIANO et al., 2002).

Mangrove oyster *Crassostrea rhizophorae* and the red macroalgae *Gracilaria* sp. are stocks very exploited at the Brazilian northeast coast (MARINHO-SORIANO et al., 2002; OLIVERA et al., 2006). *C. rhizophorae* (OLIVERA et al., 2006) and *Gracilaria* sp. (MARINHO-SORIANO et al., 2002; MARINHO-SORIANO et al., 2007) have been cultured also in shrimp farm effluent.

Studies demonstrated that macroalgae could efficiently treat effluent water from animal production systems, reducing nitrogen and phosphorus compounds (QIAN et al., 1996; TROELL et al., 1999; NEORI et al., 2000; JONES et al., 2001; NELSON et al., 2001). Oysters, as others filter feeding mollusks, can improve water quality in shrimp ponds, as they effectively remove small-suspended particles from effluents and the organic fraction provides a rich food source (NEWELL and JORDAN, 1983; WANG, 1990; HOPKINS et al., 1993; JONES and PRESTON, 1999; JONES et al., 2001).

In the present study, an integrated treatment system was assessed in laboratory-scale to improve the water effluent quality from *L. vannamei* culture using *C. rhizophorae* and *G. birdiae*.

MATERIAL AND METHODS

Wild mangrove oysters *C. rhizophorae* were collected from estuaries in north coast of Pernambuco State, Brazil. Oysters presented 58.22 g mean total weight, 4.97 g mean dry weight and 8.27 cm mean length. The red macroalgae *Gracilaria birdiae* were collected in an experimental farm at Pau Amarelo beach, Pernambuco, Brazil. These native species were selected due to their local availability and aquaculture potential in northeastern Brazil. The effluent water came from two experimental culture of *Litopenaeus vannamei* in laboratory, an autotrophic and a heterotrophic culture system. Shrimp were reared during four weeks in a 50 L polyethylene tank with closed flow-through system (salinity of 30). In autotrophic system shrimp were fed with commercial food and in heterotrophic system shrimp were fed with commercial food plus probiotic.

This experimental treatment system had two phases: first 24 h with oyster filtration and more 24 h with macroalgae absorption. The effluent from each culture system filled 12 rectangular tanks with 10 L of water. First, three densities of oyster (0.2, 0.4 and 0.8 oyster.L^{-1}) were arranged into the tanks with aeration and no additional food (3 repetitions by density). Then, oysters were removed and three stock densities of macroalgae (2.0, 4.0 and 8.0 g.L^{-1}) were added into the tanks with aeration and photoperiod of 12:12 (3 repetitions by density) (Table 1). Water quality was monitored in the beginning of the experiment and each 8 h.

Table 1. Experimental design.

Treatment	**Oyster (oyster.L-1)**	**Macroalgae (g.L-1)**
Control	0	0
T1	0.2	2.0
T2	0.4	4.0
T3	0.8	8.0

WATER ANALYSIS

Dissolved oxygen, temperature and pH were measured with a Multi Probe System YSI Model 556. Water samples were collected to laboratorial analyzes of total ammonia (KOROLEFF, 1976), nitrite (GOLTERMAN et al., 1978), nitrate (MACKERETH et al., 1978), orthophosphate, total phosphate and total phosphorus (A.P.H.A., 1995), chlorophyll-*a* and pheophytin (NUSCH, 1980), total suspended solids (TSS), organic suspended solids (OSS) and inorganic suspended solids (ISS) (A.P.H.A., 1995).

STATISTICAL ANALYSIS

Differences between treatments were tested using analysis of variance (ANOVA) with time as a repeated measurement and Tukey's test for multiple comparisons of means at a significance level of 0.05.

RESULTS

TEMPERATURE, OXYGEN AND PH

Temperature and dissolved oxygen concentration did not differ among treatments in both effluent sources (autotrophic and heterotrophic culture systems) (Table 3). The pH presented the same pattern in autotrophic and heterotrophic effluent treatment, with a decreasing tendency toward higher densities.

Table 3. Means values of temperature, dissolved oxygen (D.O.) and pH in autotrophic and heterotrophic effluent treatment.

Variable	Control	T1	T2	T3
Autotrophic:				
Temperature (ºC)	27.29±1.46 ^{a}	27.16±1.43 ^{a}	27.37±1.46 ^{a}	27.42±1.73 ^{a}
D.O. (mg.L^{-1})	5.15±0.30 ^{a}	5.09±0.32 ^{a}	5.04±0.29 ^{a}	4.94±0.35 ^{a}
pH	7.92±0.02 ^{a}	7.87±0.05 ^{ab}	7.85±0.07 ^{bc}	7.81±0.12 ^{c}
Heterotrophic:				
Temperature (ºC)	26.81±1.85 ^{a}	26.73 ±1.81 ^{a}	26.95±1.86 ^{a}	26.77 ±1.92 ^{a}
D.O. (mg.L^{-1})	5.38±0.37 ^{a}	5.32±0.37 ^{a}	5.29±0.36 ^{a}	5.29±0.39 ^{a}
pH	8.17±0.02 ^{a}	8.14±0.02 ^{b}	8.13±0.02 ^{b}	8.12±0.03 ^{b}

NITROGEN COMPOUNDS

In heterotrophic effluent, nitrogen compounds levels did not differ among treatments after oyster and macroalgae filtration phases. However, differences were found when the period of treatment was considered (0 to 48 h). Mean nitrate concentration presented differences among times, decreasing from 25.676 to 21.787 mg.L-1 in 24 h and to 15.628 mg.L-1 in 48 h, a reduction of approximately 39% (Figure 3a). Nitrite concentration showed differences between time 0 h and the other sample times, with a reduction from 0.107 to 0.082 mg.L-1 (Figure 3b). Total ammonia concentration had a similar statistical pattern observed for nitrite, reaching undetected levels after 24 h (Figure 3c).

In autotrophic effluent, nitrate and total ammonia concentration did not present differences among Control, T1 and T2 treatments in relation to densities and over the time (Figures 3d and 3f). Nevertheless, the treatment with highest densities (T3) differed to the others after macroalgae phase (48 h), where total ammonia increased from 0.052 mg.L-1 in time 24 h to 0.218 mg.L-1, just as nitrate concentration raised from 3.045 to 5.376 mg.L-1. During the experiment, nitrite concentration in the Control did not vary, but in T3 it was higher than in other treatments and increased significantly over the time (Figure 3e). Nitrite level in T1 and T2 differed to Control only after macroalgae filtration phase (48 h).

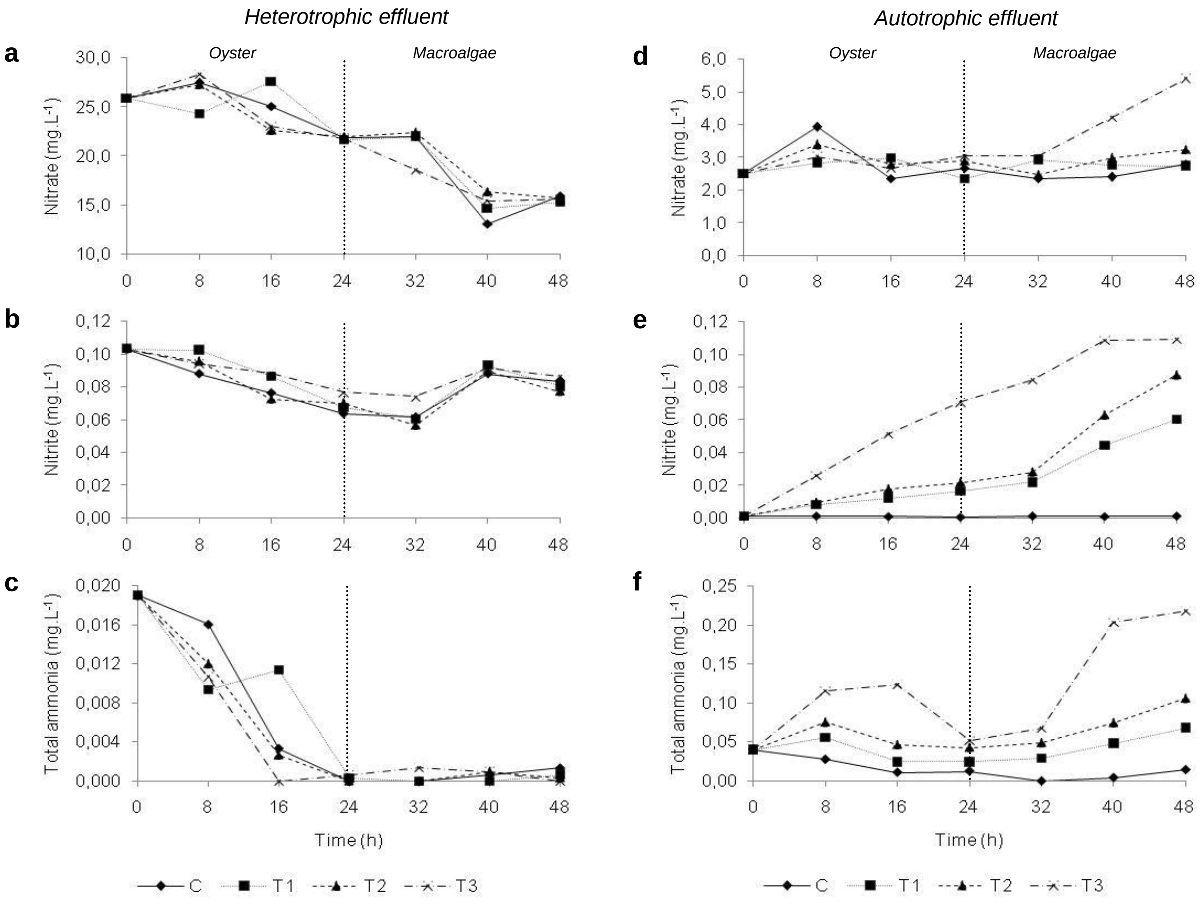

Figure 3. Concentration of nitrate (a, d), nitrite (b, e) and total ammonia (c, f) during integrated treatment of effluent from autotrophic and heterotrophic shrimp culture system (C - control; T1 – treatment 1; T2 – treatment 2; T3 – treatment 3).

PHOSPHORUS COMPOUNDS

In heterotrophic effluent, phosphorus compounds presented no differences among control and the three combinations of oyster and macroalgae densities (Figures 4a and 4b). Nevertheless, orthophosphate concentration presented differences over the time, unlike total phosphorus concentration. Orthophosphate concentration was significantly lower in the end of the trial, reaching 1.929 mg.L-1 with a reduction of 8.6% (Figure 4b).

In autotrophic effluent, concentrations of orthophosphate in the treatments increased after oyster (24 h) and macroalgae (48 h) phases (Figure 4c). Significant differences among the treatments were only observed after the last phase, when Control presented the lowest concentration (1.739 mg.L-1) and T1 (1.913 mg.L-1) was similar to T2 (2.016 mg.L-1), which was similar to T3 (2.141 mg.L-1). Total phosphorus concentrations had a reduction after oyster phase, but increased during macroalgae phase (Figure 4d). Total phosphorus levels did not present differences among treatments.

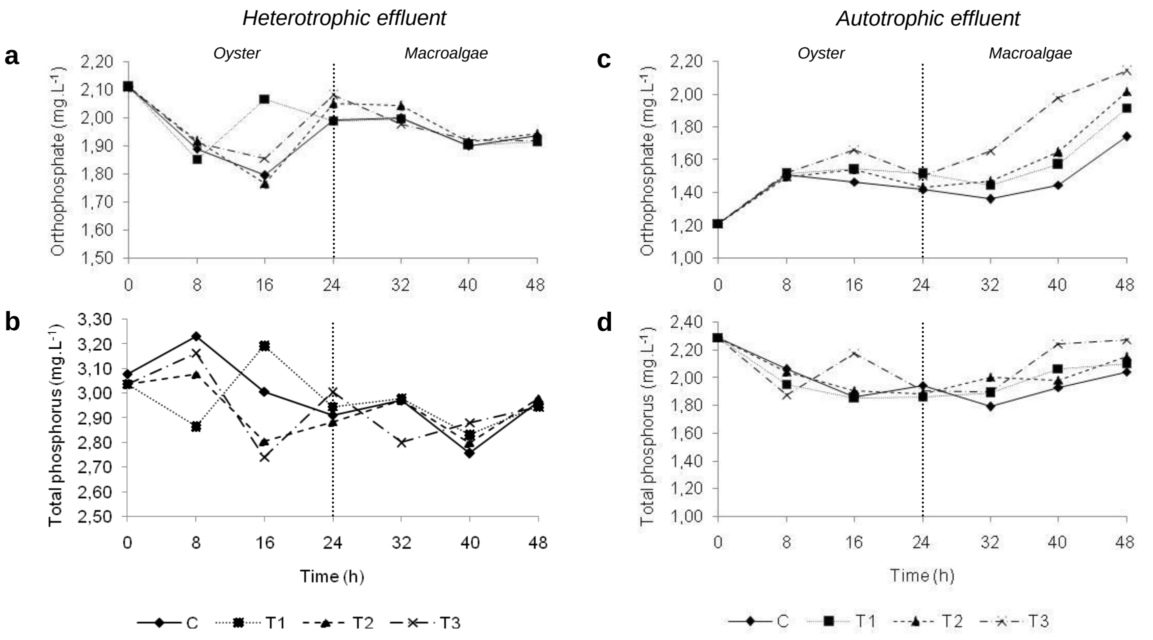

Figure 4. Concentration of orthophosphate (a, c) and total phosphorus (b, d) during integrated treatment of effluent from autotrophic and heterotrophic shrimp culture system.

CHLOROPHYLL-*A* AND PHEOPHYTIN

The concentrations of chlorophyll-*a* and pheophytin were not significant different among treatments and over the time in heterotrophic effluent (Figure 5a and 5b), as observed for chlorophyll-*a* in autotrophic effluent for T2 and T3 (Figure 5c). However, chlorophyll-*a* concentration decreased significantly in the first 16 h from 0.011 to 0.001 mg.L-1 for Control and T1 in autotrophic effluent. These treatments also showed a reduction in pheophytin concentration of approximately 91% in 16 h (Control) and 78% in 24 h (Control) (Figure 4d).

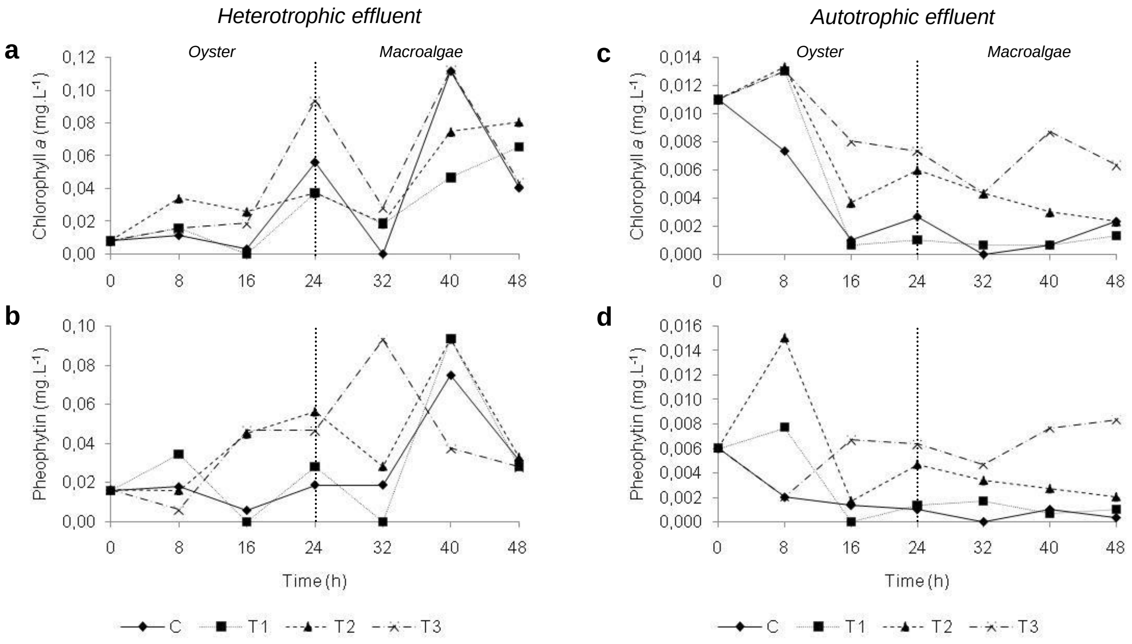

Figure 5. Concentration of chlorophyll-*a* (a, c) and pheophytin (b, e) during integrated treatment of effluent from autotrophic and heterotrophic shrimp culture system.

DISCUSSION

In the present study was verified a reduction of approximately 76% for chlorophyll *a* and 91% for pheophytin in autotrophic during first 16 h in the Control treatment, indicating that this treatment operated as a settlement tank. Under laboratory scale, the sedimentation of *Marsupenaeus japonicus* pond effluent effectively reduced the concentration of chlorophyll *a* (72%) (JONES et al., 2001). The present experiment also confirmed previous findings that around 60% of the chlorophyll *a* was removed by the settlement under still-water (non-flow) condition and the remained phytoplankton was removed by the oyster filtration (JONES and PRESTON, 1999). Nevertheless, Jones et al. (2002) argued that flow-through systems could improve the removal of chlorophyll *a* by oysters as the phytoplankton remains in suspension.

Phosphorus in natural waters is usually found in the form of phosphates (PO4-3), which can be in inorganic (including orthophosphates) or organic forms (organically-bound phosphates). Organic phosphate is formed primarily by biological processes and its increase in effluents is mainly due to the excretion and food leftovers decomposing in tanks. In the present study in autotrophic effluent, filtration by oyster and macroalgae did not interfered on total phosphorus and the reduction in the first 24 h can be associated to settlement of organic particles, and the increase after this time could be explained by decomposition of organic matter settled. Orthophosphate increased since the beginning of the experiment probably due mineralization of organic matter. In heterotrophic effluent treatment, orthophosphate concentration was reduced sharply in first 16 h probably due bacterial uptake, since aerobic heterotrophic bacteria convert nitrogen and phosphorus into bacterial biomass (EBELING et al., 2006; SCHNEIDER et al., 2007), and slightly raised until 48 h probably due organic matter contribution by oysters and macroalgae.

Nitrogen is often considered a limiting factor in marine ecosystems (DAY et al., 1989) and its discharge from aquaculture in large amounts can create unhealthy eutrophication in natural coastal waters (HOPKINS et al., 1995a,b; WU, 1995; COSTA-PIERCE, 1996). Although this problem was intensified in our integrated treatment system by the excretion of the oysters, the macroalgae biofiltration decreased significantly the concentration of total ammonia at the end of the heterotrophic treatment. Macroalgae can assimilate high quantities of dissolved organic and inorganic nutrients, usually with ammonia preference (D’ELIA and DeBOER, 1978; RYTHER et al., 1981; VERGARA et al., 1993; SCHUENHOFF et al., 2003). Several *Gracilaria* species quickly assimilate ammonia from aquaculture effluent, including *Gracilaria edulis* (JONES et al., 1996; JONES et al., 2001), *Gracilaria parvispora* (GLENN et al., 1999) and *Gracilaria conferta* (NEORI et al., 1998). In the present study was observed two tendencies, where in the heterotrophic effluent the values of ammonia remained low near undetected levels, on the other hand in the autotrophic effluent the values of ammonia increased in all the densities evaluated.

It has been suggested that *Gracilaria* respond more rapidly to ammonia than nitrate (HANISAK, 1983; GLENN et al., 1999; JONES et al., 2001), consistent with our finding that ammonia rather than nitrate was significantly reduced during macroalgal absorption compared to the control treatment. Nitrifying bacteria in the aerobic sediment layers and free-living forms in the water column are probably related with the NO2-/NO3- increase in the biological treatment in autotrophic effluent, in addition, increases since 32 h until 48 h can be associated to oyster feces. Additionally, oysters could also stimulate nitrification by enhancing the movement of N to the aerobic superficial sediments and nitrifying bacteria in their digestive tract (BOUCHER and BOUCHER-RODONI, 1988). In heterotrophic effluent treatment total ammonia concentration was sharply decreased and nitrate was reduced slower not due oyster or macroalgae filtration, but due heterotrophic bacteria uptake. It seems that nitrate was more effectively reduced when level of total ammonia reached near zero, which can be explained by the preference of this second nitrogen source for bacteria (VRIENS et al., 1989; RITTMANN and McCARTY, 2001; SCHNEIDER et al., 2006).

The densities of oysters and macroalgae evaluated in this study, seems to be very low to interfere in the effluent treatment. Therefore, oyster densities should be higher for *C. rhizophorae,* since the same densities of Sydney rock oyster *Saccostrea commercialis* were used to treat *Penaeus japonicus* culture effluent with significant results in improving water quality (JONES and PRESTON, 1999). JONES at al. (2002) detected that a low density of 0.2 oyster.L^{-1} (10 g.L^{-1}) presented best results in relation to survival in effluent treatment system. Macroalgal absorption inefficiency seems to be related to the no occurrence of photosynthesis process during last 24 h.

CONCLUSIONS

The results of this study showed the viability to improve the water quality from *L. vannamei* effluent by biological integrated treatment system using native species of oyster (*C. rhizophorae*) and macroalgae (*Gracilaria birdiae*). Furthermore, this species of oysters and macroalgae can provide an additional source of income for shrimp farmers. Unfortunately, appropriated densities of oyster and macroalgae to effluent treatment could not be evaluated in this study, which should be repeated with higher densities. Nevertheless, dramatic differences between autotrophic and heterotrophic effluent were recorded during integrated treatment system and different strategies should be developed for each one. The impact that autotrophic or heterotrophic effluents have to the environment is significant if they were not properly treated respecting their specificities

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