Effects of biofloc promotion on water quality, growth, biomass yield and heterotrophic community in *Litopenaeus vannamei* (Boone, 1931) experimental intensive culture

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Abstract

Six 1.2 m³ tanks were stocked with an initial biomass of 500 g m⁻³ of *Litopenaeus vannamei* juveniles (individual weight: 1.0±0.3 g), to evaluate the effect of biofloc promotion on water quality and on shrimp growth and production, and to identify the dominant taxa in the heterotrophic communities present in experimental closed cultures. Feeding was *ad libitum* twice daily with 35% protein shrimp feed. Three tanks were managed as biofloc technology (BFT) systems, adding daily an amount of commercial feed equal to 50% of the shrimp feed supplied. The remaining three received only shrimp feed and served as controls. Experiment lasted 21 days. The mean concentrations of P·PO₄³⁻ and inorganic dissolved N species (TAN, N-NO₂⁻, N-NO₃⁻) were significantly lower (P<0.05) in BFT than in the control. The individual final weight, increase in biomass, food, and protein conversion rates were significantly better in BFT than in the control (P<0.05). The mean N content of the shrimp biomass gained in the BFT cultures was equivalent to 45.7% of the protein-N added as feed, and was significantly higher than the 34.7% recycled into shrimp biomass in the control cultures. Bacterial concentrations were not significantly different. Vibriocaceae dominated in both systems; in both some isolates were potential pathogens, and diversity was higher in the control than in the BFT treatment. The advantages of BFT technology are confirmed by the significantly lower TAN and N-NO₃⁻ concentrations, as well as by the better shrimp performance in terms of growth, biomass yield, and food and protein conversion efficiency.

Introduction

Land-based aquaculture generates high volumes of nutrient-rich water. These are discharged to the natural environment and may cause eutrophication of the receiving water bodies, which is a widespread source of concern. This requires a reassessment of the current practices used in aquaculture, in order to maintain high levels of production and better water quality within the farms, as well as at the respective points of discharge (Martínez-Córdova et al., 2009; Martins et al., 2010). Apart from the cost involved, and of the potential environmental impact, the high rates of water exchange used to maintain an acceptable level of water quality within the culture system might be important points of entry for pathogenic organisms. Therefore, zero or limited water exchange systems should be considered as viable options (Cohen et al., 2005). Among these, biofloc technology systems (BFT) are considered highly efficient in intensive closed cultures, because they allow maintaining better water quality and limiting the organic load of effluent waters at the time of harvest. Adoption of this technology increases the efficiency of feed utilization, because organic and inorganic metabolites, as well as unused or partially used food, are recycled by microorganisms into microalgae and bacterial biomass, which tend to coalesce into flocculated material (bioflocs). This is used by the organisms in culture as a protein and lipid-rich food source (Animageech, 2007; Ballester et al., 2010), contributing between 18 and 29% to the total daily food consumption even in intensive shrimp cultures (Burford et al., 2004).

This flocculated material consists of an organic matrix on which thrive heterotrophic and autotrophic microorganisms (De Schryver et al., 2008; Ray et al., 2010). In particular, heterotrophic bacteria degrade organic residues, which are converted into new bacterial biomass. This is available as food for the next trophic levels, initiating the classical microbial loop (Azam et al., 1983). Regulation of their activity may be achieved through the addition of a carbon source, because in the pond environment organic carbon becomes limiting for bacteria growth, while dissolved nitrogen is usually present in excess, and is in fact the main source of deterioration of water quality (Emerenciano et al., 2013). Thus, this technology contributes significantly to the health of the industry, because by recycling nutrients it improves food use efficiency, reduces wastes and maintains good water quality and a healthy environment within the farm and in the surrounding water bodies (Stokstad, 2010). Although it appears that these heterotrophic communities may exert a controlling effect on pathogen growth (Defoirdt et al., 2007; Zhao et al., 2012; Aguilera-Rivera et al., 2014), there is insufficient information on the bacteria present in flocs. In this study we evaluated the effect of biofloc promotion on water quality, on shrimp growth and related production parameters, and identified the main groups and the dominant taxa in the heterotrophic communities present in experimental *Litopenaeus vannamei* closed cultures with, or without promotion of biofloc.

Materials and methods

On August 19, 2013, *L. vannamei* juveniles (1.0±0.3 g, wet weight), obtained from a local
shrimp farm, were stocked in six 1.2-m² high density polyethylene tanks, previously (one week) added a 10 cm-deep layer from the bottom sediment of the shrimp farm, since in Mexico most shrimp cultures are kept in earthen ponds, rather than in plastic-lined or concrete tanks. In all cases the initial biomass was 500 g m⁻². Bioflocs were started 18 days before the experiment in bioreactor tanks fertilized with 20 g of 35% protein shrimp feed and 20 g of cornmeal (MASECA® Gruma, S.A.B. de C.V., Mexico, 7.7% proteins, 70% carbohydrates, 4.6 lipids, C:N ratio 31.5:1). Vigorous aeration was maintained with a 1 HP Sweetwater blower (Aquatic Eco-Systems Inc., Apopka, FL, USA), distributed through PVC tubing to each tank, where fine bubbling was provided by Aerotube™ tubing (Water Management Technologies Inc., Baton Rouge, LA, USA).

Three days before stocking, the six experimental tanks received an initial 100 L inoculum of biofloc-containing water from the bioreactor and 900 L of filtered (Nytex) Mazatlán Bay seawater (35.3±0.1 salinity). In all tanks shrimps were fed ad libitum twice daily (08:00 and 18:00 h) using feeding trays and formulated 35% protein shrimp feed (Camaronina 35%, Purina Mexico, Cd. Obregon, Sonora, Mexico, 35% proteins, 8% lipids, 30% carbohydrates, C:N ratio 7:1:1).

Three tanks, selected at random were managed as BFT systems, adding daily to each tank an amount of cornmeal, equivalent to 50% of the shrimp feed supplied. The remaining three tanks received only shrimp feed and served as controls. Water lost through evaporation (approximately 6% weekly) was replaced with dechlorinated tap water to avoid salinity increases and there were no additional water changes or additions to the control and to the BFT cultures.

Water temperature, dissolved oxygen, pH and salinity were determined twice daily immediately before feeding, using an YSI 57 dissolved oxygen meter with temperature sensor (YSI Inc., Yellow Spring, OH, USA), a Hanna HI 98150 portable pH meter (Hanna Instruments, Woonsocket, RI, USA) and an Atago S/Mill-E refractometer (Atago Co. Ltd., Tokyo, Japan), respectively.

Water samples were obtained from each tank on the initial day, and at weekly intervals until the end of the experiment that lasted 21 days (August 19-September 9, 2013). Samples for nutrient analysis (1 L) in polyethylene bottles, were filtered through Whatman GF-C glass fiber filters. Filters and particle-free water were stored frozen until analysis according to Strickland and Parsons (1972) for P, PO₄³⁻, N-NH₄⁺ + NH₃ (TAN), N-NO₂⁻, N-NO₃⁻ and dissolved organic N concentrations; particulate N was determined as in Holm-Hansen (1968). Suspended solids were quantified gravimetrically, floc volume was recorded with Imhoff cones, and turbidity with the Secchi disc. The N contents of shrimp feed and of shrimp (initial and final) wet biomass, determined with the Kjeldahl method (AOAC, 2005), were 5.6 and 3.58%, respectively. Bacteriological samples were obtained on the same dates as the water samples, using pre-stereilized 20 mL test tubes that were capped and brought to the laboratory for immediate processing. After serial dilution (10⁻¹-10⁻⁵), 0.1 mL subsamples were plated in duplicate on different DIFCO media: Marine Agar for total marine bacteria, Tripticase Soy Agar with 2.5% NaCl (TSA) for total bacteria, TCBS Agar for presumptive Vibrio detection and Cetrimide and MacConkey Agar for Pseudomonas and total coliform bacteria, respectively (APHA, 1992).

After 48 h at 30±2 °C, colonies were counted to obtain the respective concentrations (CFU mL⁻¹). In the case of the final samples, bacterial colonies with different morphologies were replated, cross-streak purified and six colonies of each bacterial strain were stored at room temperature in a capped 1.5 mL Eppendorf tube with 1-mL anhydrous (99.8%) ethanol (ETOH).

Bacterial isolates were identified using morphological criteria after Gram staining. For specific identification, 50 ng µL⁻¹ of the DNA of ETOH-preserved strains were used for 16S ARN gene amplification with PCR, using the universal primers Forward 27f.1 (AGR GTT TGA TCM TGG CTC AG) and Reverse 1492R2 (94 °C/1' □ 56 °C/1' □ 72 °C/1') □ 72 °C/5' □ 4 °C/□. The amplification program was: 16S: 94 °C ‘ ’ 35 cycles (94 °C ‘ ’ 30 s 56 °C ‘ ’ 30 s 72 °C ‘ ’ 30 s) 72 °C ‘ ’ 4 °C ‘ ’ .

To identify the closest bacterial species, the sequences obtained were compared to the public databases GenBank® (BLAST) (http://blast.ncbi.nlm.nih.gov/Blastcg) and EzTaxon (http://www.ezbiocloud.net/eztaxon). The FASTA format of the sequences obtained, aligned with the multiple sequence program ClustalW (http://align.genome.jp), were used to obtain the respective phylogenetic trees. The molecular phylogeny was obtained using version 5.1 of the program MEGA (Tamura et al., 2011), using the Neighbor-Joining test (Saitou and Nei, 1987) with the p-distance method with 100 bootstrap repetitions, considering transitions and transversions. Programs BLAST (Altschul et al., 1997), and the procaryotic strain database EzTaxon (Chun et al., 2007) were used for phylogenetic relationships identification.

The number of strains of each species identified in each treatment served to calculate Shannon-Weaver’s diversity index $H_{w} = -\sum_{i} p_{i} \ln p_{i}$, where $n_{i}$ and $N$ are the number of strains of species $i$ and $N$ the total number of strains, respectively.

At the end of the experiment all shrimps were counted and weighed. Survival (S) and specific growth rates (SGR) were calculated as in Hernández et al. (2013), with the equations:

\[ S = 100 \left( \frac{N_{f}}{N_{i}} \right) \quad \text{and} \quad \text{SGR} = 100 \left( \ln M_{f} - \ln M_{i} \right) t^{-1} \]

where $N_{i}$ and $N_{f}$ are final and initial number of organisms of each tank, $M_{i}$ and $M_{f}$ are the individual final and initial wet weights, respectively, and $t$ = duration of the experiment (days).

Food conversion and protein efficiency ratios FCR and PER were calculated as:

\[ \text{FCR} = \frac{FS}{(B_{r} - B_{s})} \quad \text{and} \quad \text{PER} = \frac{(B_{r} - B_{s})}{PS} \]

FS and PS = total food and total amount of biomass protein supplied to each tank, ($B_{r} - B_{s}$) = increase in total biomass in each tank (all data in grams).

The mean final values of survival, individual weight and total biomass, FCR, PER, SGR calculated for the BFT and control tanks were compared with t or Mann Whitney tests depending on the results of Kolmogorov-Smirnov and Fisher’s F tests. Daily mean water characteristics and weekly mean bacteria and nutrient concentrations were compared using t tests for paired observations or non parametric Wilcoxon tests, after ln and arcsine square root transformation for bacterial counts and for data in percentage, respectively. All tests were performed with a confidence level =0.05 (Zar, 1996).

**Results**

Temperature, salinity, pH and dissolved oxygen concentrations ranged from 32 to 34°C, 35.0 to 35.4 psu, 7.5 to 7.8 and 3.7 to 4.0 mg L⁻¹, respectively, and there were no significant differences between the mean values calculated for BFT and control cultures. The mean concentrations of P-PO₄³⁻ and of all inorganic dissolved N species (TAN, N-NO₂⁻, N-NO₃⁻) were significantly lower in the BFT than in the control cultures, whereas dissolved and particulate organic N (DON and PON, respectively), suspended solids, biofloc volumes and turbidity were higher in the BFT tanks (Table 1).
Mean survival was similar in both treatments, but the individual final weight and all remaining zootechnical indicators (total biomass harvested, increase in biomass, SGR, amount of shrimp feed supplied, FCR and PER) were significantly better in the BFT than in the control cultures (Table 2).

Bacterial concentrations varied widely and, although at the time of harvest the mean numbers of total and marine bacteria (TSA and Marine Agar, respectively) were higher in the BFT (13.5±3.3x10⁶ and 0.8±0.1x10⁶ CFU mL⁻¹) than in the control cultures (4.2±2.5x10⁶, 0.4±0.1x10⁶ CFU mL⁻¹), there were no significant differences between the mean values calculated with the concentrations determined at weekly intervals throughout the experiment (Table 3).

The samples obtained in the final day yielded 58 Gram- and 2 unidentified Gram+ bacterial strains, 26 of which from the control and 34 from the BFT cultures. Vibrionaceae, followed by Enterobacteriaceae were the dominant families in the BFT and in the control cultures, and the remaining strains pertained to Alteromonadaceae and Micrococcaceae in the BFT, and Pseudoalteromonadaceae, Rhodobacteriaceae and Alteromonadaceae in the control cultures, respectively.

According to the numbers of strains isolated and of the species detected in the two culture systems, the bacteria community was more diverse in the control (26 strains representing 14 taxa: H=2.36) than in the BFT treatment (10 taxa out of 32 strains, H=1.89) (Tables 4 and 5).

**Discussion**

Temperature and salinity remained within acceptable limits for *L. vannamei* culture (Audelo-Naranjo et al., 2011), given the wide upper salinity and thermal tolerance of this species (Betts and Vinateau, 2009; Kumu et al., 2010). In spite of the high biological load, aeration was sufficient to maintain oxygen at 65 to 69% saturation levels (Gilbert et al., 1968), which were not modified by the additional load of cornmeal added in the BFT tanks.

The significantly higher mean individual final weight, growth rate and biomass yield, as well as the better FCR and PER confirm the twin advantages of BFT technology with organic carbon (OC) addition (Gao et al., 2012; Xu et al., 2013; Megahed and Mohamed, 2014). The first is the low TAN and N-NO₂ concentrations (Da Silva et al., 2013; Liu et al., 2014), because the additional OC maintains an appropriate C:N ratio for bacterial transformation of these toxic N compounds into single cell protein (Ebeling et al., 2006; Asaduzzaman et al., 2008). This was made evident by the significantly lower concentrations of dissolved inorganic N and P species, which were used for bacterial and microalgae growth, as shown by the concentrations of suspended particulate organic N (PON), which were higher in the BFT than in the control tanks.

The second advantage is the direct or indirect use of PON by the cultured organisms. In this case, this allowed a >22% higher mean yield in the BFT cultures, even if the amount of food added was lower by close to 7%. In traditional open systems, the percentage of protein-N added as feed that is converted into shrimp biomass generally lies between 20 and 25% (Piedrahita, 2003; Crab et al., 2007) whereas

### Table 1. Mean concentrations (±standard deviation) of inorganic dissolved nitrogen species, dissolved and particulate organic nitrogen, dissolved P-PO₄, total suspended solids, mean floc volume, turbidity determined in the biofloc technology and control cultures of *L. vannamei* juveniles.

|                | BFT                                                                 | Control                                                              |
|----------------|----------------------------------------------------------------------|----------------------------------------------------------------------|
| TAN, mg L⁻¹    | 0.266±0.019ᵃ                                                         | 0.424±0.015ᵇ                                                        |
| N-NH₃, mg L⁻¹  | 0.011±0.007ᵃ                                                         | 0.020±0.007ᵇ                                                        |
| N-NO₂⁻, mg L⁻¹ | 0.156±0.026ᵇ                                                         | 0.452±0.03ᵇ                                                         |
| N-NO₃⁻, mg L⁻¹ | 0.949±0.027ᵇ                                                         | 1.617±0.098ᵇ                                                        |
| DON, mg L⁻¹    | 0.710±0.437ᵇ                                                         | 0.150±0.089ᵇ                                                        |
| P-PO₄⁻, mg L⁻¹ | 0.327±0.077ᵇ                                                         | 0.523±0.045ᵇ                                                        |
| SST, mg L⁻¹    | 400.8±30.4ᵇ                                                          | 129.6±14.9ᵇ                                                        |
| Floc volume, mL L⁻¹ | 16.8±1.5ᵇ                                                          | 3.4±0.7ᵃ                                                            |
| Turbidity, cm  | 18.2±1.5ᵇ                                                          | 58.2±2.0ᵇ                                                          |

**BFT**, biofloc technology; **DON**, dissolved organic N; **PON**, particulate organic N. ᵃDifferent letters indicate significant difference between data in the same row (t tests, α=0.05, a-b). ᵇDifferent letters indicate significant difference between data in the same row (t tests for paired observations, α=0.05, a-b).

### Table 2. Mean values (± standard deviation) of zootechnical indicators determined in the biofloc technology and control cultures of *L. vannamei* juveniles.

|                | BFT                                                                 | Control                                                              |
|----------------|----------------------------------------------------------------------|----------------------------------------------------------------------|
| Survival, %    | 70.0±3.0ᵃ                                                           | 68.3±2.2ᵇ                                                          |
| FIW, g         | 4.0±0.1ᵇ                                                           | 3.6±0.1ᵇ                                                          |
| SGR, % day⁻¹   | 1.40±0.1ᵇ                                                          | 0.92±0.10ᵇ                                                          |
| BH, g          | 1409.7±73.7ᵇ                                                       | 1243.1±55.1ᵇ                                                        |
| BG, g m⁻³      | 909.7±73.7ᵇ                                                       | 743.1±55.1ᵇ                                                        |
| FS, g          | 1279.3±3.1ᵇ                                                       | 1378.0±3.6ᵇ                                                        |
| FCR            | 1.41±0.11ᵇ                                                          | 1.86±0.13ᵇ                                                          |
| PER            | 1.99±0.16ᵇ                                                          | 1.51±0.11                                                            |

**BFT**, biofloc technology; **FIW**, final individual weight; **SGR**, specific growth rate; **BH**, biomass harvested; **BG**, biomass gain; **FS**, shrimp feed supplied (Camaronina 37%); **FCR**, food conversion ratio; **PER**, protein efficiency ratio. ᵃDifferent letters indicate significant difference between data in the same row (t tests, α=0.05, a-b). The BFT systems received also 640 g of cornmeal.

### Table 3. Mean bacteria concentrations (±standard deviation) determined in the biofloc technology and control cultures of *L. vannamei* juveniles.

|                | BFT                                                                 | Control                                                              |
|----------------|----------------------------------------------------------------------|----------------------------------------------------------------------|
| Marine Agar, 10⁶ CFU mL⁻¹ | 8.159±2.990ᵃ                                                        | 19.890±6.019ᵇ                                                        |
| TSA, 10⁶ CFU mL⁻¹          | 4.621±2.068ᵃ                                                        | 3.613±1.277ᵇ                                                        |
| Vibrio, 10⁵ CFU mL⁻¹       | 1.579±0.375ᵃ                                                        | 1.101±0.251ᵇ                                                        |
| Coliforms, 10⁶ CFU mL⁻¹    | 1.008±0.274ᵃ                                                        | 3.292±0.142ᵇ                                                        |
| Pseudomonas, 10⁶ CFU mL⁻¹  | 0.915±0.259ᵇ                                                        | 1.042±0.865ᵇ                                                        |

ᵃEqual letters indicate lack of significant difference between data in the same row (t tests for paired observations, α=0.05, a-b). ᵇBFT, biofloc technology; TSA, Trypticase Soy Agar.
in a closed system at least part of the protein-N is recycled by microalgae and microorganisms, which increases the percentage of N converted in shrimp biomass. Since the mean N content of shrimp biomass was 3.6%, the close to 910 g of shrimp biomass gained in our BFT cultures represented 45.7% of the protein-N added as feed, which is 9% higher than the 34.7% recycled into the 743 g of shrimp biomass gained in the control cultures.

According to Teichert-Coddington et al. (1999), the impact on the environment represented by nutrient discharges may be considerably reduced by a short residence time in a settling pond. This would be a further advantage of BFT cultures in which, apart from the significantly lower concentration of dissolved P, approximately 38.7% of the nitrogen present in the culture water was in the form of easily settleable particulate N, in comparison to the 22.7% determined in the control cultures. Therefore, a settling pond with a short residence time would allow a lower environmental impact of BFT cultures at the time of harvest.

The coefficients of variation of bacterial concentrations on marine and TS agar indicated a slightly higher variability in the BFT than in the control cultures. Concentrations were in the same order of magnitude than the mean value (5.43x10^6 CFU mL^-1) found by Kim et al. (2014) in BFT-managed shrimp cultures, but one order of magnitude lower than those determined in BFT-managed shrimp cultures by Burford et al. (2004), although in that case bacteria were counted with the epifluorescence technique which yields higher values than the method used in this work (Maki and Remsen, 1981).

The unsanitary conditions of the source of the raw seawater used in this work (Alonso-Rodríguez et al., 2000) are the most probable explanation for the high numbers of coliforms, Pseudomonas and Vibrio colonies. The latter were two orders of magnitude higher than those determined by Moreira de Souza et al. (2014) in a similar experiment, in which Vibrio concentrations were significantly lower and less variable in the BFT cultures, which does not correspond to the results of our experiment.

One of the claimed advantages of BFT cultures is the low occurrence of pathogenic events or of high pathogen loads in comparison with traditional cultures, possibly because of interspecific interactions, or of competition for substrate or essentials nutrients between pathogens and biofloc-forming microorganisms (Cabrera et al., 2010; Emerenciano et al., 2013). This effect may be particularly evident when bioflocs are enriched with probiotic bacteria, which Krummenauer et al. (2014) found effective in increasing survival and growth of Vibrio parahaemolyticus-infected L. vannamei in a BFT culture system.

However, although in this work the pathogenic Vibrio parahaemolyticus and Photobacterium damselae (Vandenberghhe et al., 1999) were isolated only from the control cultures, the most common species from the BFT tanks was V. rotiferianus, which has been associated with mass mortalities of the penaeid shrimp Fenneropenaeus chinensis (Zhang et al., 2014).

Among the non-pathogenic species detected in the BFT cultures, Vibrio natriegens deserves attention because it can utilize with high efficiency different carbon and nitrogen sources (Austin et al., 1978), and because of its ability to increase the number of ribosomes in order to achieve high rates of protein synthesis (Aiyar et al., 2002). This explains its high growth rate (Coyer et al., 1996; Maida et al., 2013), and suggests that it might be able to outcompete pathogenic bacteria in the carbon and nutrient-rich BFT environment.

A second strain that deserves attention is Vibrio hepatarius, which was isolated from the control cultures, because it is known to alleviate viral (white spot virus) infection and, when administered in mixture with other probiotics, confers resistance to Vibrio harveyi and promotes L. vannamei growth (Balcazar et al., 2006).

### Conclusions

Addition of organic carbon to closed intensive shrimp culture increased heterotrophic bacterial activity, which resulted in low TAN and N-NO2 concentrations, increased food util-

### Table 4. Bacteria strains isolated in the biofloc technology cultures of L. vannamei juveniles.

| Strain     | Species                  | Class                             | Family                        |
|------------|--------------------------|-----------------------------------|-------------------------------|
| AM2-3-12   | Vibrio rotiferianus      | Gammaproteobacteria               | Vibrionaceae                  |
| AM4-2-14   | Vibrio rotiferianus      | Gammaproteobacteria               | Vibrionaceae                  |
| AM4-2-18   | Vibrio rotiferianus      | Gammaproteobacteria               | Vibrionaceae                  |
| AM4-2-3    | Vibrio rotiferianus      | Gammaproteobacteria               | Vibrionaceae                  |
| AM4-2-8    | Vibrio rotiferianus      | Gammaproteobacteria               | Vibrionaceae                  |
| AM4-3-11   | Vibrio rotiferianus      | Gammaproteobacteria               | Vibrionaceae                  |
| AM4-3-16   | Vibrio rotiferianus      | Gammaproteobacteria               | Vibrionaceae                  |
| AM4-3-7    | Vibrio rotiferianus      | Gammaproteobacteria               | Vibrionaceae                  |
| TC4-3-22   | Vibrio rotiferianus      | Gammaproteobacteria               | Vibrionaceae                  |
| TC4-3-23   | Vibrio rotiferianus      | Gammaproteobacteria               | Vibrionaceae                  |
| TS2-3-35   | Vibrio rotiferianus      | Gammaproteobacteria               | Vibrionaceae                  |
| TS4-3-23   | Vibrio rotiferianus      | Gammaproteobacteria               | Vibrionaceae                  |
| TS4-1-1    | Vibrio mytili            | Gammaproteobacteria               | Vibronaceae                   |
| TS4-1-6    | Vibrio mytili            | Gammaproteobacteria               | Vibronaceae                   |
| TS4-2-15B  | Vibrio mytili            | Gammaproteobacteria               | Vibronaceae                   |
| TS4-3-10   | Vibrio mytili            | Gammaproteobacteria               | Vibronaceae                   |
| TS4-2-24   | Vibrio communis          | Gammaproteobacteria               | Vibronaceae                   |
| TC4-3-12   | Vibrio communis          | Gammaproteobacteria               | Vibronaceae                   |
| AM4-3-9    | Vibrio natriegens        | Gammaproteobacteria               | Vibronaceae                   |
| TS4-2-19   | Photobacterium jeanii    | Gammaproteobacteria               | Vibronaceae                   |
| TS4-1-8    | Photobacterium jeanii    | Gammaproteobacteria               | Vibronaceae                   |
| TS4-1-2    | Photobacterium gaetulicola| Gammaproteobacteria               | Vibronaceae                   |
| TC4-2-18   | Photobacterium leiognathi| Gammaproteobacteria               | Vibronaceae                   |
| Mac4-1-10  | Proteus mirabilis        | Gammaproteobacteria               | Enterobacteriaceae            |
| Mac4-1-4   | Proteus mirabilis        | Gammaproteobacteria               | Enterobacteriaceae            |
| Mac4-1-8   | Proteus mirabilis        | Gammaproteobacteria               | Enterobacteriaceae            |
| Mac4-2-7   | Proteus mirabilis        | Gammaproteobacteria               | Enterobacteriaceae            |
| Mac4-3-1   | Proteus mirabilis        | Gammaproteobacteria               | Enterobacteriaceae            |
| Mac4-3-5   | Proteus mirabilis        | Gammaproteobacteria               | Enterobacteriaceae            |
| TS4-2-BIS  | Marinobacter gaseognensis| Gammaproteobacteria               | Alteromonadaceae              |
| AM2-3-18   | Marinobacter gaseognensis| Gammaproteobacteria               | Alteromonadaceae              |
Table 5. Bacteria strains isolated in the control cultures of L. vannamei juveniles.

| Strain     | Species                     | Class                   | Family                  |
|------------|-----------------------------|-------------------------|-------------------------|
| AM1-2-13   | Vibrio rotiferianus         | Gammaproteobacteria     | Vibrionaceae             |
| AM1-3-10Bis| Vibrio rotiferianus         | Gammaproteobacteria     | Vibrionaceae             |
| AM1-3-5    | Vibrio rotiferianus         | Gammaproteobacteria     | Vibrionaceae             |
| TCI-3-6    | Vibrio rotiferianus         | Gammaproteobacteria     | Vibrionaceae             |
| TS1-3-23   | Vibrio rotiferianus         | Gammaproteobacteria     | Vibrionaceae             |
| TS1-3-5    | Vibrio rotiferianus         | Gammaproteobacteria     | Vibrionaceae             |
| TCI-3-4    | Vibrio neptunius            | Gammaproteobacteria     | Vibrionaceae             |
| TS1-1-4    | Vibrio communis             | Gammaproteobacteria     | Vibrionaceae             |
| AM1-2-15   | Vibrio paraahemolyticus     | Gammaproteobacteria     | Vibrionaceae             |
| TCI-3-14   | Photobacterium damselae     | Gammaproteobacteria     | Vibrionaceae             |
| Mac1-2-2   | Proteus mirabilis           | Gammaproteobacteria     | Enterobacteriaceae       |
| Mac1-2-6   | Proteus mirabilis           | Gammaproteobacteria     | Enterobacteriaceae       |
| Mac1-2-9   | Proteus mirabilis           | Gammaproteobacteria     | Enterobacteriaceae       |
| Mac1-3-3   | Proteus mirabilis           | Gammaproteobacteria     | Enterobacteriaceae       |
| AM1-2-1P   | Pseudalteromonas spongiae   | Gammaproteobacteria     | Pseudalteromonadaceae    |
| AM1-2-2P   | Pseudalteromonas spongiae   | Gammaproteobacteria     | Pseudalteromonadaceae    |
| AM1-3-10P  | Pseudalteromonas spongiae   | Gammaproteobacteria     | Pseudalteromonadaceae    |
| AM1-2-20   | Silicibacter lacuscaerulensis| Alphaproteobacteria     | Rhodobacteraceae         |
| AM1-2-19   | Silicibacter lacuscaerulensis| Alphaproteobacteria     | Rhodobacteraceae         |
| TS2.3-25   | unidentified Gram +         |                         |                         |
| TC2.3-27   | unidentified Gram +         |                         |                         |

lization efficiency and better growth rate and biomass yield, with the added advantage of a lower level of environmental impact, because of the reduction in nutrient and organic matter discharges. The lower nutrient concentrations and the higher protein efficiency ratio confirmed the direct biofloc consumption by cultured shrimp, which resulted in similar bacterial concentrations in the two types of culture systems.

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