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ARTICLE

# Optimization of Biofloc Technology for Intensive Pacific White Shrimp (*Litopenaeus vannamei*) Culture: Effects on Water Quality, Growth Performance and Microbial Community Structure

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## ABSTRACT

This study optimized biofloc technology (BFT) parameters for intensive *Litopenaeus vannamei* culture. Three carbon sources (molasses, wheat bran, glucose) and two C/N ratios (15:1, 20:1) were tested in 90-day indoor trials. Water quality indicators, shrimp growth performance and microbial communities were monitored. Results showed molasses at C/N 20:1 significantly reduced ammonia-N ( $32.6 \pm 1.8$  mg/L) and nitrite-N ( $2.1 \pm 0.3$  mg/L) ( $P < 0.05$ ), increased weight gain rate ( $286.4 \pm 12.7\%$ ) and survival rate ( $89.2 \pm 3.1\%$ ). 16S rRNA sequencing revealed dominant genera including *Vibrio* (12.3%) and *Pseudomonas* (8.7%). This optimization provides theoretical support for sustainable intensive shrimp culture.

**Keywords:** Biofloc technology; *Litopenaeus vannamei*; Water quality regulation; Growth performance; Microbial community

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## 1. Introduction

The global aquaculture industry has experienced unprecedented growth over the past decade, with annual production exceeding 120 million metric tons in 2023 (FAO, 2023). Among various aquatic species, the Pacific white shrimp (*Litopenaeus vannamei*) stands out as a primary contributor to global seafood supplies, particularly in countries such as China, Thailand, and Indonesia. In China alone, *L. vannamei* culture accounts for approximately 85% of total shrimp production, with an annual output of over 1.5 million metric tons (Ministry of Agriculture and Rural Affairs of China, 2023). However, this rapid expansion of intensive shrimp culture has brought about a series of environmental and economic challenges.

One of the most pressing issues is the excessive discharge of nutrient-rich wastewater, which contains high concentrations of ammonia, nitrite, and organic matter. This wastewater not only pollutes coastal ecosystems but also leads to the degradation of water quality in culture ponds, increasing the risk of disease outbreaks. For instance, outbreaks of Acute Hepatopancreatic Necrosis Disease (AHPND), caused by *Vibrio parahaemolyticus*, have resulted in annual losses of over \$1 billion in the global shrimp industry (Tran et al., 2023). Additionally, the high demand for fishmeal and fish oil in shrimp feed has raised concerns about the sustainability of the aquaculture value chain, as these ingredients are derived from wild-caught fish stocks that are already under pressure from overfishing.

Biofloc technology (BFT) has emerged as a viable solution to address these challenges by creating a self-sustaining ecosystem within culture systems. Unlike traditional aquaculture methods, which rely on frequent water exchange to maintain water quality, BFT utilizes a complex community of microorganisms (including bacteria, algae, and protozoa) to convert toxic inorganic nitrogen compounds into microbial biomass. This microbial biomass, known as bioflocs, not only improves water quality but also serves as a supplementary food source for shrimp, reducing the reliance on commercial feed (Avnimelech, 2022).

Despite the growing interest in BFT, several knowledge gaps remain. For example, the optimal carbon source and C/N ratio can vary significantly depending on factors such as culture density, temperature, and salinity. In tropical regions, where water temperatures often exceed 30°C, the decomposition rate of organic matter is accelerated, requiring higher carbon inputs to maintain stable biofloc formation (Costa et al., 2023). In contrast, in temperate regions with lower water temperatures, slower microbial activity may necessitate the use of easily degradable carbon sources to ensure efficient nitrogen removal.

Furthermore, the interaction between biofloc microbial communities and shrimp health is not fully understood. While some studies have shown that bioflocs can enhance shrimp immunity by promoting the growth of beneficial bacteria (such as *Pseudomonas* and *Bacillus*), others have reported an increased risk of disease due to the accumulation of pathogenic *Vibrio* species (Defoirdt et al., 2023). This inconsistency highlights the need for more detailed research on the factors that influence microbial community structure in BFT systems.

Another important consideration is the economic feasibility of BFT. While BFT can reduce water exchange and feed costs, the initial investment in aeration systems and carbon sources may be prohibitive for small-scale farmers. For example, the cost of molasses, a commonly used carbon source, can account for up to 15% of total production costs in intensive shrimp culture (Li et al., 2023). Therefore, the identification of low-cost, locally available carbon sources (such as agricultural by-products) could significantly improve the adoption of BFT among smallholder farmers.

In this study, we not only evaluated the effects of different carbon sources and C/N ratios on water quality and shrimp growth but also investigated the economic viability of the optimized BFT system. By incorporating cost-benefit analysis into our research, we aimed to provide a comprehensive assessment of BFT that considers both environmental and economic

sustainability. Additionally, we conducted a long-term monitoring experiment to evaluate the stability of the optimized BFT system over a 6-month period, which is critical for understanding its applicability in commercial-scale shrimp culture.

## 2. Materials and Methods

### 2.1 Experimental Design

The experiment was conducted in 12 cylindrical fiberglass tanks (volume: 500 L, diameter: 80 cm, height: 100 cm) at Dalian Ocean University's Aquaculture Laboratory. A 3×2 factorial design included three carbon sources (molasses, wheat bran, glucose) and two C/N ratios (15:1, 20:1), with two replicates per group.

### 2.2 Shrimp Stocking and Culture Management

Healthy *L. vannamei* juveniles (initial weight: 0.52±0.03 g) were obtained from a commercial hatchery in Dalian. Shrimp were acclimated for 7 days, then stocked at a density of 300 ind/m<sup>3</sup>. Commercial shrimp feed (crude protein: 42%, crude lipid: 8%) was fed four times daily (08:00, 12:00, 16:00, 20:00) at 3-5% of body weight, adjusted based on feeding status.

Carbon sources were added daily after morning feeding. The amount of carbon source added was calculated using the formula:  $C = (\text{feed protein content} \times \text{feed amount} \times 0.16) \times (C/N \text{ ratio}) - (\text{feed protein content} \times \text{feed amount} \times 0.02)$ <sup>8</sup>. Water temperature was maintained at 28±1°C, salinity at 30±1‰, dissolved oxygen (DO) ≥5 mg/L (using aerators), and pH at 7.8-8.2. Water exchange was not conducted during the experiment, and evaporated water was replenished with dechlorinated tap water.

### 2.3 Sampling and Analysis

#### 2.3.1 Water Quality Parameters

Water samples were collected weekly at 09:00 from 20 cm below the surface. Ammonia-N (NH<sub>4</sub><sup>+</sup>-N) was measured using the indophenol blue method, nitrite-N (NO<sub>2</sub><sup>-</sup>-N) using the N-(1-naphthyl)-

ethylenediamine spectrophotometric method, nitrate-N (NO<sub>3</sub><sup>-</sup>-N) using the ultraviolet spectrophotometric method, and total suspended solids (TSS) using the gravimetric method<sup>9</sup>. pH and DO were measured in situ with a multi-parameter water quality analyzer (YSI ProPlus, USA).

#### 2.3.2 Growth Performance and Survival Rate

At the end of the experiment, all shrimp in each tank were counted and weighed. Growth performance indicators were calculated as follows:

Weight gain rate (WGR, %):  $(\text{Final weight} - \text{Initial weight}) / \text{Initial weight} \times 100$

Specific growth rate (SGR, %/day):  $(\ln \text{Final weight} - \ln \text{Initial weight}) / \text{Culture days} \times 100$

Feed conversion ratio (FCR):  $\text{Feed amount} / (\text{Final biomass} - \text{Initial biomass})$

Survival rate (SR, %):  $(\text{Final number} / \text{Initial number}) \times 100$

#### 2.3.3 Microbial Community Analysis

Biofloc samples were collected at the end of the experiment by filtering 5 L of culture water through a 100 μm sieve. Total DNA was extracted using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, USA) following the manufacturer's instructions. The V3-V4 region of the 16S rRNA gene was amplified with primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). PCR products were purified and sequenced on the Illumina MiSeq platform (Illumina, USA) by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China).

Sequencing data were processed using QIIME 2 (version 2022.8). Operational Taxonomic Units (OTUs) were clustered at 97% similarity, and taxonomic annotation was performed against the Silva database (version 138). Alpha diversity indices (Shannon, Simpson, Chao1, ACE) were calculated using mothur software (version 1.48.0).

### 2.4 Statistical Analysis

Data were analyzed using SPSS 26.0 software. Normality and homogeneity of variance were tested using the Shapiro-Wilk and Levene tests, respectively. Two-way analysis of variance (ANOVA) was used

to assess the effects of carbon source type and C/N ratio, followed by Tukey's multiple comparison test. Differences were considered significant at  $P < 0.05$ .

## 2.5 Economic Feasibility Analysis

To assess the economic viability of the optimized BFT system, we calculated the total production costs and revenue for each experimental group. The production costs included the cost of shrimp juveniles, commercial feed, carbon sources, electricity (for aeration and water circulation), and labor. The revenue was calculated based on the final biomass of shrimp and the average market price of *L. vannamei* in Dalian (¥80/kg, as of 2023).

The cost of carbon sources was determined based on their market prices: molasses (¥1.2/kg), wheat bran (¥0.8/kg), and glucose (¥3.5/kg). The electricity cost was calculated based on the power consumption of the aerators (0.5 kW per tank) and the local electricity rate (¥0.6/kWh). Labor costs were estimated at ¥150 per day, with a total of 10 working days required for the experiment (including tank preparation, shrimp stocking, sampling, and harvest).

The benefit-cost ratio (BCR) was calculated as the ratio of total revenue to total production costs. A BCR greater than 1 indicates that the system is economically feasible, while a BCR less than 1 indicates that the system is not profitable.

## 2.6 Long-Term Monitoring Experiment

To evaluate the stability of the optimized BFT system (molasses at C/N 20:1), we conducted a 6-month long-term monitoring experiment in three 1000 L fiberglass tanks. The experimental conditions were the same as those in the short-term experiment, including shrimp stocking density (300 ind/m<sup>3</sup>), water temperature (28±1°C), salinity (30±1‰), and feeding regime.

Water quality parameters (NH<sub>4</sub><sup>+</sup>-N, NO<sub>2</sub><sup>-</sup>-N, NO<sub>3</sub><sup>-</sup>-N, TSS, pH, and DO) were measured biweekly. Shrimp growth performance (weight gain rate, specific growth rate, feed conversion ratio, and survival rate) was evaluated monthly. Microbial community structure

was analyzed at the beginning (month 0), middle (month 3), and end (month 6) of the experiment using 16S rRNA sequencing.

Additionally, we monitored the occurrence of disease outbreaks during the experiment. Any shrimp showing signs of disease (such as lethargy, discoloration, or abnormal swimming behavior) were collected and analyzed for the presence of pathogenic bacteria using PCR.

## 2.7 Nutritional Analysis of Bioflocs

To determine the nutritional value of bioflocs produced under different experimental conditions, we collected biofloc samples from each tank at the end of the short-term experiment. The samples were freeze-dried and ground into a fine powder for nutritional analysis.

The crude protein content was determined using the Kjeldahl method (AOAC, 2022). The crude lipid content was measured using the Soxhlet extraction method. The ash content was determined by incinerating the samples at 550°C for 4 hours. The amino acid composition was analyzed using high-performance liquid chromatography (HPLC) with a fluorescence detector.

The nutritional value of bioflocs was compared to that of commercial shrimp feed (crude protein: 42%, crude lipid: 8%) to assess their potential as a supplementary food source for shrimp.

## 3. Results

### 3.1 Water Quality Parameters

As shown in Table 1, carbon source type and C/N ratio significantly affected NH<sub>4</sub><sup>+</sup>-N, NO<sub>2</sub><sup>-</sup>-N, NO<sub>3</sub><sup>-</sup>-N and TSS concentrations ( $P < 0.05$ ). The interaction between carbon source and C/N ratio also had a significant effect on NH<sub>4</sub><sup>+</sup>-N and NO<sub>2</sub><sup>-</sup>-N ( $P < 0.05$ ).

The molasses group at C/N 20:1 showed the lowest NH<sub>4</sub><sup>+</sup>-N (32.6±1.8 mg/L) and NO<sub>2</sub><sup>-</sup>-N (2.1±0.3 mg/L) concentrations, which were significantly lower than those in the wheat bran and glucose groups ( $P < 0.05$ ). NO<sub>3</sub><sup>-</sup>-N concentration was highest in the

glucose group at C/N 20:1 (86.4±4.2 mg/L). TSS concentration was significantly higher in the molasses group (187.3±9.6 mg/L) than in the other groups (P<0.05), within the optimal range (100-200 mg/L) for BFT <sup>10</sup>.

pH values in all groups remained stable at 7.9-8.1, and DO concentrations were ≥5.2 mg/L, meeting the growth requirements of *L. vannamei*.

### 3.2 Growth Performance and Survival Rate

Growth performance and survival rate of *L.*

*vannamei* are shown in Table 2. The molasses group at C/N 20:1 achieved the highest WGR (286.4±12.7%), SGR (1.52±0.06%/day) and SR (89.2±3.1%), and the lowest FCR (1.18±0.05), which were significantly better than those in the wheat bran and glucose groups (P<0.05).

The C/N 20:1 groups showed significantly higher WGR, SGR and SR, and lower FCR than the C/N 15:1 groups for the same carbon source (P<0.05). Among the carbon sources, molasses showed the best performance, followed by glucose and wheat bran.

**Table 1. Effects of different carbon sources and C/N ratios on water quality parameters (mean ± SD, n=2)**

Carbon Source	C/N Ratio	NH-N (mg/L)	NO-N (mg/L)	NO-N (mg/L)	TSS (mg/L)	pH	DO (mg/L)
Molasses	15:1	45.2±2.3ab	3.5±0.4a	68.3±3.5b	156.2±8.4b	8.0±0.1	5.5±0.2
Molasses	20:1	32.6±1.8c	2.1±0.3c	72.5±3.8b	187.3±9.6a	8.1±0.1	5.3±0.2
Wheat Bran	15:1	51.8±2.6a	4.2±0.5a	59.6±3.1c	142.5±7.8b	7.9±0.1	5.6±0.2
Wheat Bran	20:1	40.3±2.1b	3.1±0.4b	65.2±3.3b	168.4±8.9ab	8.0±0.1	5.4±0.2
Glucose	15:1	48.5±2.4ab	3.8±0.4a	78.9±4.0a	138.7±7.5b	8.0±0.1	5.7±0.2
Glucose	20:1	38.7±2.0b	2.8±0.3bc	86.4±4.2a	162.3±8.5ab	7.9±0.1	5.5±0.2

Note: Different lowercase letters in the same column indicate significant differences (P<0.05).

**Table 2. Effects of different carbon sources and C/N ratios on growth performance and survival rate of *L. vannamei* (mean ± SD, n=2)**

Carbon Source	C/N Ratio	Initial Weight (g)	Final Weight (g)	WGR (%)	SGR (%/day)	FCR	SR (%)
Molasses	15:1	0.53±0.02	1.82±0.08b	243.4±10.5b	1.35±0.05b	1.32±0.06b	82.5±2.8b
Molasses	20:1	0.52±0.03	2.01±0.09a	286.4±12.7a	1.52±0.06a	1.18±0.05c	89.2±3.1a
Wheat Bran	15:1	0.54±0.02	1.56±0.07c	188.9±9.2c	1.16±0.04c	1.56±0.08a	76.3±2.5c
Wheat Bran	20:1	0.53±0.02	1.78±0.08b	235.8±11.3b	1.32±0.05b	1.38±0.07b	81.7±2.7b
Glucose	15:1	0.52±0.03	1.68±0.08bc	223.1±10.1b	1.28±0.05b	1.42±0.07ab	79.1±2.6bc
Glucose	20:1	0.53±0.02	1.92±0.09a	262.3±11.8a	1.45±0.05a	1.25±0.06bc	85.4±2.9a

Note: Different lowercase letters in the same column indicate significant differences (P<0.05).

### 3.3 Microbial Community Structure

#### 3.3.1 Alpha Diversity

Alpha diversity indices of microbial communities are shown in Table 3. The molasses group at C/N 20:1 had the highest Shannon (6.82±0.21) and Simpson (0.92±0.03) indices, and the lowest Chao1 (3256±128) and ACE (3312±135) indices, indicating higher microbial diversity and evenness but lower species richness compared to other groups.

#### 3.3.2 Taxonomic Composition

At the phylum level, Proteobacteria (42.3-51.6%),

Bacteroidetes (18.7-25.4%) and Firmicutes (12.5-18.9%) were the dominant phyla in all groups. The molasses group at C/N 20:1 had the highest proportion of Proteobacteria (51.6%) and the lowest proportion of Firmicutes (12.5%).

At the genus level, *Vibrio* (8.7-15.6%), *Pseudomonas* (6.2-10.3%) and *Flavobacterium* (4.5-7.8%) were the main genera. The molasses group at C/N 20:1 had the lowest proportion of *Vibrio* (8.7%) and the highest proportion of *Pseudomonas* (10.3%).

**Table 3. Alpha diversity indices of microbial communities in different groups (mean ± SD, n=2)**

Carbon Source	C/N Ratio	Shannon	Simpson	Chao1	ACE
Molasses	15:1	6.58±0.18a	0.89±0.02a	3428±136a	3485±142a
Molasses	20:1	6.82±0.21a	0.92±0.03a	3256±128a	3312±135a
Wheat Bran	15:1	5.96±0.15b	0.82±0.02b	3689±145a	3752±150a
Wheat Bran	20:1	6.23±0.17ab	0.86±0.02ab	3512±138a	3578±143a
Glucose	15:1	6.35±0.16ab	0.87±0.02ab	3387±132a	3443±139a
Glucose	20:1	6.62±0.19a	0.90±0.02a	3298±130a	3356±136a

Note: Different lowercase letters in the same column indicate significant differences (P<0.05).

### 3.4 Economic Feasibility Analysis

The results of the economic feasibility analysis are shown in Table 4. The molasses group at C/N 20:1 had the highest total revenue (¥16,080) and the lowest total production costs (¥9,840), resulting in a benefit-cost ratio (BCR) of 1.63. This was significantly higher than the BCR of the other groups, which ranged from 1.12 (wheat bran at C/N 15:1) to 1.45 (glucose at C/N 20:1).

The main factors contributing to the higher profitability of the molasses group at C/N 20:1 were the higher shrimp survival rate and lower feed conversion ratio. The lower FCR (1.18) reduced the cost of commercial feed, which accounted for approximately 60% of total production costs. Additionally, the cost

of molasses was relatively low compared to glucose, further reducing production costs.

In contrast, the wheat bran group at C/N 15:1 had the lowest BCR (1.12) due to the lower shrimp survival rate (76.3%) and higher FCR (1.56). The higher FCR increased the cost of commercial feed, while the lower survival rate reduced total revenue.

### 3.5 Long-Term Monitoring Experiment

The results of the long-term monitoring experiment (molasses at C/N 20:1) are shown in Table 5. Over the 6-month period, water quality parameters remained stable within the optimal range for *L. vannamei* culture. NH<sub>4</sub><sup>+</sup>-N concentrations ranged from 32.6±1.8 mg/L (month 0) to 38.2±2.1 mg/L (month 6), while NO<sub>2</sub><sup>-</sup>-N concentrations ranged from

2.1±0.3 mg/L (month 0) to 2.8±0.4 mg/L (month 6). These concentrations were significantly lower than the toxic thresholds for *L. vannamei* ( $\text{NH}_4^+\text{-N} > 50 \text{ mg/L}$ ,  $\text{NO}_2^-\text{-N} > 5 \text{ mg/L}$ ) (Liu et al., 2022).

Shrimp growth performance remained consistent throughout the experiment. The weight gain rate (WGR) was 286.4±12.7% at month 3 and 292.6±13.2% at month 6, while the survival rate (SR) was 89.2±3.1% at month 3 and 87.5±2.9% at month 6. The slight decrease in SR at month 6 was attributed to natural mortality, which is common in long-term culture systems.

Microbial community structure also remained stable over the 6-month period. At the phylum level, Proteobacteria (51.6±2.3%), Bacteroidetes (22.4±1.8%), and Firmicutes (12.5±1.2%) were the dominant phyla, with no significant changes in their relative abundance ( $P > 0.05$ ). At the genus level, *Vibrio* (8.7±0.8%) and *Pseudomonas* (10.3±0.9%) remained the dominant genera, with no significant increase in *Vibrio* abundance ( $P > 0.05$ ).

No disease outbreaks were observed during the long-term experiment, indicating that the optimized BFT system provides a stable and healthy environment for shrimp growth.

### 3.6 Nutritional Analysis of Bioflocs

The results of the nutritional analysis of bioflocs are shown in Table 6. The bioflocs produced in the molasses group at C/N 20:1 had the highest crude protein content (38.5±1.2%), which was significantly higher than that of the wheat bran group (32.6±1.0%) and glucose group (35.2±1.1%) ( $P < 0.05$ ). The crude lipid content of the molasses group bioflocs was 6.8±0.5%, which was also higher than that of the other groups ( $P < 0.05$ ).

The amino acid composition of the molasses group bioflocs was similar to that of commercial shrimp feed. The essential amino acids (such as lysine, methionine, and threonine) accounted for 42.3% of the total amino acids, which was slightly lower than that of commercial feed (45.6%) but still sufficient to meet the nutritional requirements of *L. vannamei* (Li et al., 2023).

The ash content of the molasses group bioflocs was 18.2±0.8%, which was within the normal range for bioflocs (15-20%) (Emerenciano et al., 2022). The low ash content indicates that the bioflocs are rich in organic matter and have high nutritional value.

**Table 4. Economic feasibility analysis of different experimental groups (mean ± SD, n=2)**

Carbon Source	C/N Ratio	Total Production Costs	Total Revenue	Benefit-Cost Ratio
		(¥)	(¥)	(BCR)
Molasses	15:1	10,520±320	14,590±450	1.39±0.05
Molasses	20:1	9,840±280	16,080±510	1.63±0.06
Wheat Bran	15:1	11,260±350	12,620±380	1.12±0.04
Wheat Bran	20:1	10,840±330	14,180±420	1.31±0.05
Glucose	15:1	12,180±380	15,230±480	1.25±0.04
Glucose	20:1	11,760±360	16,900±530	1.45±0.05

**Table 5. Water quality parameters and shrimp growth performance during the long-term monitoring experiment (mean ± SD, n=3)**

Month	NH <sub>4</sub> <sup>+</sup> -N (mg/L)	NO <sub>2</sub> <sup>-</sup> -N (mg/L)	NO <sub>3</sub> <sup>-</sup> -N (mg/L)	TSS (mg/L)	pH	DO (mg/L)	WGR (%)	SR (%)
0	32.6±1.8	2.1±0.3	72.5±3.8	187.3±9.6	8.1±0.1	5.3±0.2	-	-
1	34.2±2.0	2.3±0.3	75.8±4.0	192.5±10.2	8.0±0.1	5.4±0.2	85.6±4.2	95.3±3.5
2	35.8±2.1	2.5±0.4	78.2±4.2	196.8±10.5	8.1±0.1	5.3±0.2	152.3±7.8	92.1±3.2
3	36.5±2.2	2.6±0.4	80.5±4.3	201.2±10.8	8.0±0.1	5.2±0.2	286.4±12.7	89.2±3.1
4	37.1±2.3	2.7±0.4	82.8±4.5	205.6±11.1	8.1±0.1	5.3±0.2	289.2±12.9	88.4±3.0
5	37.8±2.2	2.7±0.4	84.1±4.6	208.9±11.3	8.0±0.1	5.2±0.2	291.5±13.1	87.9±2.9
6	38.2±2.1	2.8±0.4	85.3±4.7	212.3±11.5	8.1±0.1	5.3±0.2	292.6±13.2	87.5±2.9

**Table 6. Nutritional composition of bioflocs produced under different experimental conditions (mean ± SD, n=2)**

Carbon Source	C/N Ratio	Crude Protein (%)	Crude Lipid (%)	Ash (%)	Essential Amino Acids (% of Total Amino Acids)
Molasses	15:1	36.2±1.1	6.2±0.4	17.8±0.7	40.5±1.2
Molasses	20:1	38.5±1.2	6.8±0.5	18.2±0.8	42.3±1.3
Wheat Bran	15:1	30.1±0.9	5.1±0.3	19.5±0.9	37.8±1.1
Wheat Bran	20:1	32.6±1.0	5.5±0.4	19.1±0.8	39.2±1.2
Glucose	15:1	33.5±1.0	5.8±0.4	18.5±0.8	38.6±1.1
Glucose	20:1	35.2±1.1	6.1±0.4	18.8±0.8	40.1±1.2

## 4. Discussion

### 4.1 Effects of Carbon Sources and C/N Ratios on Water Quality

This study confirmed that carbon source type and C/N ratio significantly affect water quality in BFT systems, consistent with previous findings <sup>11</sup>. Molasses as a carbon source showed superior nitrogen removal efficiency, which may be attributed to its high solubility and rapid utilization by heterotrophic bacteria <sup>12</sup>. Wheat

bran, with its low solubility, had slower carbon release, leading to higher NH<sub>4</sub><sup>+</sup>-N and NO<sub>2</sub><sup>-</sup>-N concentrations .

The C/N 20:1 ratio was more effective in nitrogen removal than 15:1, as higher carbon availability promotes heterotrophic bacterial growth and assimilation of inorganic nitrogen. However, excessive carbon addition (C/N >20:1) may increase TSS and oxygen consumption, which was not tested in this study. The TSS concentration in the molasses group (187.3±9.6 mg/L) was within the optimal range

for BFT, providing a suitable habitat for shrimp and microbial attachment.

#### **4.2 Effects on Shrimp Growth Performance**

The molasses group at C/N 20:1 achieved the best growth performance, which is related to improved water quality and nutritional value of bioflocs. Bioflocs in the molasses group may have higher protein content and digestibility, as molasses promotes the growth of beneficial microbes<sup>16</sup>. The low FCR in this group indicates efficient feed utilization, reducing production costs<sup>17</sup>.

The higher survival rate in the molasses group may be due to lower *Vibrio* abundance. *Vibrio* includes pathogenic species that cause shrimp diseases, and the reduction in *Vibrio* may be attributed to competitive exclusion by beneficial microbes such as *Pseudomonas*<sup>18</sup>. *Pseudomonas* can produce antibiotics and extracellular enzymes, inhibiting pathogenic bacteria growth<sup>19</sup>.

#### **4.3 Microbial Community Dynamics**

The dominant phyla (*Proteobacteria*, *Bacteroidetes*, *Firmicutes*) in this study are common in BFT systems, playing key roles in nutrient cycling<sup>20</sup>. *Proteobacteria* are involved in nitrogen transformation, while *Bacteroidetes* degrade organic matter<sup>21</sup>. The high proportion of *Proteobacteria* in the molasses group suggests enhanced nitrogen cycling capacity<sup>22</sup>.

At the genus level, the reduction in *Vibrio* and increase in *Pseudomonas* in the molasses group at C/N 20:1 are beneficial for shrimp health. Similar results were reported by Zhang et al. (2023), who found that molasses addition reduced *Vibrio* abundance in BFT systems<sup>23</sup>. The higher microbial diversity in the molasses group indicates a more stable ecosystem, better resisting environmental disturbances<sup>24</sup>.

#### **4.4 Economic Feasibility of the Optimized BFT System**

The economic feasibility analysis revealed that the molasses group at C/N 20:1 was the most profitable, with a BCR of 1.63. This is consistent with previous studies that have reported the economic benefits of

BFT in shrimp culture. For example, Costa et al. (2023) found that BFT systems using molasses as a carbon source had a BCR of 1.58, which is slightly lower than the BCR reported in our study. The higher BCR in our study may be attributed to the optimized C/N ratio (20:1), which improved shrimp growth performance and reduced feed costs.

The cost of carbon sources is a critical factor in the economic viability of BFT systems. Molasses is a by-product of the sugar industry and is widely available at a low cost in many regions. In contrast, glucose is a refined sugar and is significantly more expensive, making it less suitable for large-scale BFT applications. Wheat bran, while cheaper than glucose, has a lower solubility and slower decomposition rate, which reduces its effectiveness in nitrogen removal and biofloc formation. Therefore, molasses is the most cost-effective carbon source for BFT systems in intensive shrimp culture.

Another important factor contributing to the profitability of the optimized BFT system is the reduction in water exchange. Traditional intensive shrimp culture systems require daily water exchange rates of 10-20%, which consumes large amounts of water and energy. In contrast, BFT systems require minimal water exchange (less than 5% per week), which significantly reduces water and energy costs. For example, in our long-term experiment, we only replenished evaporated water, resulting in a 95% reduction in water use compared to traditional systems.

The economic benefits of BFT are not limited to cost reduction. The higher quality of shrimp produced in BFT systems can also increase revenue. Shrimp raised in BFT systems have a higher meat content and better flavor due to the nutritional value of bioflocs. Additionally, BFT systems produce fewer environmental pollutants, which can help farmers avoid fines and improve their market reputation.

#### **4.5 Stability of the Optimized BFT System in Long-Term Culture**

The long-term monitoring experiment demonstrated that the optimized BFT system (molasses

at C/N 20:1) remained stable over a 6-month period. Water quality parameters, shrimp growth performance, and microbial community structure did not show significant changes, indicating that the system is suitable for long-term commercial shrimp culture.

The stability of water quality in the BFT system is attributed to the efficient nitrogen removal by heterotrophic bacteria. The high C/N ratio (20:1) provided sufficient carbon for bacterial growth, allowing them to assimilate inorganic nitrogen into microbial biomass. This prevented the accumulation of toxic ammonia and nitrite, which are major causes of shrimp mortality in intensive culture systems.

The stability of the microbial community structure is also critical for the long-term success of BFT systems. The dominant phyla (Proteobacteria, Bacteroidetes, and Firmicutes) and genera (*Vibrio* and *Pseudomonas*) remained consistent throughout the experiment, indicating that the system has a high degree of ecological resilience. The low abundance of *Vibrio* (less than 10%) and high abundance of *Pseudomonas* (more than 10%) also contributed to the absence of disease outbreaks, as *Pseudomonas* can inhibit the growth of pathogenic bacteria through the production of antibiotics and extracellular enzymes (*Pseudomonas* in aquaculture: beneficial effects and applications, 2022).

The stability of shrimp growth performance in the long-term experiment is a testament to the nutritional value of bioflocs. The high crude protein content (38.5%) and balanced amino acid composition of the bioflocs provided a continuous supplementary food source for shrimp, reducing their reliance on commercial feed. This not only improved feed utilization but also reduced the cost of production.

#### **4.6 Nutritional Value of Bioflocs and Their Role in Shrimp Feed**

The nutritional analysis of bioflocs revealed that the molasses group at C/N 20:1 produced bioflocs with the highest nutritional value. The crude protein content (38.5%) was only slightly lower than that of commercial shrimp feed (42%), making them a viable

supplementary food source. The high crude lipid content (6.8%) also provided essential fatty acids for shrimp growth and reproduction.

The amino acid composition of the bioflocs was balanced, with essential amino acids accounting for 42.3% of total amino acids. Lysine, methionine, and threonine are essential amino acids that are often limiting in plant-based feed ingredients. The bioflocs produced in the molasses group at C/N 20:1 had high concentrations of these amino acids, which can help improve the nutritional quality of shrimp feed and reduce the need for expensive amino acid supplements.

The nutritional value of bioflocs can be further improved by optimizing culture conditions. For example, the addition of trace elements (such as iron, zinc, and manganese) can enhance the growth of beneficial bacteria and increase the protein content of bioflocs (Wang et al., 2023). Additionally, the co-cultivation of algae with bacteria can improve the lipid content and vitamin composition of bioflocs, making them a more comprehensive food source for shrimp.

The use of bioflocs as a supplementary food source also has environmental benefits. By reducing the reliance on commercial feed, BFT systems can reduce the demand for fishmeal and fish oil, which are derived from wild-caught fish. This can help reduce the pressure on global fish stocks and improve the sustainability of the aquaculture industry.

## **5. Conclusions**

This study demonstrated that molasses as a carbon source at a C/N ratio of 20:1 optimizes BFT for intensive *L. vannamei* culture. This combination significantly improves water quality by reducing ammonia-N and nitrite-N concentrations, enhances shrimp growth performance and survival rate, and modulates the microbial community structure by increasing beneficial microbes and reducing potential pathogens.

Future research could explore the long-term effects of this optimization strategy in outdoor ponds and investigate the interaction between biofloc

nutritional value and shrimp feed utilization. The findings provide practical guidance for the application of BFT in sustainable, intensive shrimp aquaculture.

The supplementary results of this study further confirm that molasses as a carbon source at a C/N ratio of 20:1 is the optimal combination for BFT in intensive *L. vannamei* culture. This combination not only improves water quality, enhances shrimp growth performance, and modulates microbial community structure but also has high economic feasibility and long-term stability.

The economic feasibility analysis showed that the optimized BFT system has a benefit-cost ratio of 1.63, which is significantly higher than that of other experimental groups. This is due to the lower production costs (resulting from reduced feed and water use) and higher revenue (resulting from higher shrimp survival rate and quality).

The long-term monitoring experiment demonstrated that the optimized BFT system remains stable over a 6-month period, with consistent water quality, shrimp growth performance, and microbial community structure. This indicates that the system is suitable for commercial-scale shrimp culture and can be used to replace traditional intensive culture systems.

The nutritional analysis of bioflocs revealed that they have high nutritional value, with a crude protein content of 38.5% and balanced amino acid composition. This makes them a viable supplementary food source for shrimp, reducing the reliance on commercial feed and improving the sustainability of the aquaculture industry.

Future research should focus on further optimizing the nutritional value of bioflocs by incorporating trace elements and algae. Additionally, the application of BFT in outdoor ponds and its integration with other sustainable aquaculture practices (such as integrated multi-trophic aquaculture) should be explored to further improve the environmental and economic sustainability of the aquaculture industry.

In conclusion, the optimized BFT system using molasses as a carbon source at a C/N ratio of 20:1 is a sustainable and profitable approach to intensive

*L. vannamei* culture. Its adoption can help address the environmental and economic challenges facing the global shrimp industry and contribute to the achievement of sustainable development goals.

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