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RESEARCH

Optimising the control factors of a smart biofloc aquaculture system for the sustainable production of Pacific whiteleg shrimp, *Litopenaeus vannamei*

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Abstract
Despite the growth in aquaculture, there are uncertainties over its sustainability as the global population is projected to hit 9.6 million in 2050 with an estimated demand gap of 21.6 million metric tonnes. Prominent among the challenges is the sub-optimal performance of culture systems. There is a need to improve the systems using scientific approaches for optimum production. This research optimised the control factors of a traditional biofloc system using water quality conditions for the setup of an indoor multi-techno aquaculture system (IMTAS). IMTAS is a fully automated culture system controlled by internet of things (IOT). Taguchi L9 design and grey relational analysis (GRA) were used to determine the optimum levels of three control factors, namely stocking density, carbon-to-nitrogen ratio (C/N ratio), and probiotic dosage. Water quality parameters were measured as response factors over a culture period of 12 weeks. During a Taguchi single-response optimisation, the signal-to-noise ratio (S/N ratio) identified optimum levels for total ammonia nitrogen in run 7 (0.309), nitrite, phosphate and pH in run 1 (7.929, 17.960 and 17.588 respectively), dissolved oxygen in run 6 (15.265), electrical conductivity and temperature in run 5 (93.861 and 29.227), total dissolved solids in run 2 (88.986) and salinity in run 4 (30.972). The Taguchi-based GRA further consolidated these levels to determine the optimum stocking density as 200 shrimp.m^{-3}, C/N ratio of 15 and probiotic application dosage of 14 ml.l^{-1}. Probiotic was evaluated as the most substantial control factor that enhanced the performance of this smart culture system.

Keywords Biofloc technology · Taguchi method · Signal-to-noise ratio · Grey relational analysis · Principal component analysis

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Extended author information available on the last page of the article

Introduction

Due to severe adverse environmental effects, wild capture is no longer a sustainable source of aquatic products for the growing world population. This has brought aquaculture under increasing pressure to provide this demand with an associated need to improve culture systems for optimum production. Aquaculture systems are considered efficient by their ability to promote the growth, health and biosafety of the cultured organisms as well as enhance the general operational efficiency. The control factors in these systems are deliberate adjustments of key inputs and rates that maintain good conditions such as water quality for higher production (Kaleem & Bio Singou Sabi 2021). Biofloc aquaculture systems are closed systems which typically operate as zero-water discharge systems. Maintaining low concentrations of organic toxins, especially ammonia, from unconsumed feed and faecal discharge has remained the major challenge in these systems (Suantika et al. 2018).

The whiteleg shrimp, *Litopenaeus vannamei*, is one of the most important species in the global fishery and aquaculture industries. The shrimp aquaculture industry has contributed significantly to the global economy, particularly in middle- and low-income countries. It has been reported that *L. vannamei*, *Penaeus monodon* and *Macrobrachium rosenbergii* contributed to the annual global shrimp aquaculture production of 6.0 million tonnes in 2018, with an increase to 6.55 million tonnes in 2019 increased to 6.55 million tonnes in 2019, estimated at USD 40.67 billion (FAO 2019). The economic value of the shrimp aquaculture industry has been vast over the past decade, with an export value of billions of dollars per year. *L. vannamei* is widely distributed and cultivated worldwide, owing to its economic significance in the aquaculture industry. Its several bio-economic advantages make it a commercially important species compared to other shrimp species (Gao et al. 2021; Yu et al. 2022). Gyan et al. (2022) confirmed a connection between the growth performance and survival rates of the organism and the intestinal microbiota that enhances nutrient digestibility, adaptation to the environment and resistance to diseases.

Biofloc aquaculture systems, operating as closed, zero-water discharge systems, offer a sustainable solution by recycling nutrients and minimising environmental impact. However, maintaining low concentrations of organic toxins, particularly ammonia from unconsumed feed and faecal discharge, remains a major challenge (Suantika et al. 2018). Aeration settings, typically delivering 0.5–2 $m^3.h^{-1}$ via aerotubes, ensure dissolved oxygen levels (>5 $mg.l^{-1}$) for shrimp respiration and floc suspension, as inadequate aeration can lead to anoxic conditions (Han et al. 2024). Floc volume maintained between 10 and 15 $ml.l^{-1}$ is known to balance nutrient recycling to prevent oxygen depletion (Debnath et al 2025). Water quality conditions regulate nearly all physiological processes in cultured aquatic animals, directly affecting growth performance and disease resistance (Elayaraja et al. 2020). In biofloc systems, inadequate knowledge of optimal control factors such as stocking density (AftabUddin et al. 2020;), carbon-to-nitrogen (C/N) ratio (Azhar et al. 2020) and bacterial inoculum dosage (Kasan et al. 2017) has led to poor yields and water quality issues (Zahra and Mateen 2024). Optimising these factors is critical to enhancing system performance.

Optimisation is the multidisciplinary technique for configuring the control settings of systems for best performance. Taguchi optimisation which is primarily used in engineering design is recently adopted in various fields to develop systems and processes for high-output at least cost options (Kavadias and Triantafyllou 2021; Louvros et al. 2022). The Taguchi approach is a tool suitable for selecting the most compelling factors from a large spectrum of considerations that affect the optimality of machines and processes (Mahla et al. 2022). It is a systematic process for identifying factors targeted for improvement of operational efficiency and resource utilisation. Categorising these factors as control or noise factors aids the appropriation of resources and efforts on the factors. The data-driven approach of the Taguchi method provides data on the quantitative impact of various combinations of control factors on the results (Sun and Kainz 2023).Accordingly, Taguchi method minimises the impact of noise factors by optimising control factors and reducing variability for more consistent and predictable system outputs.

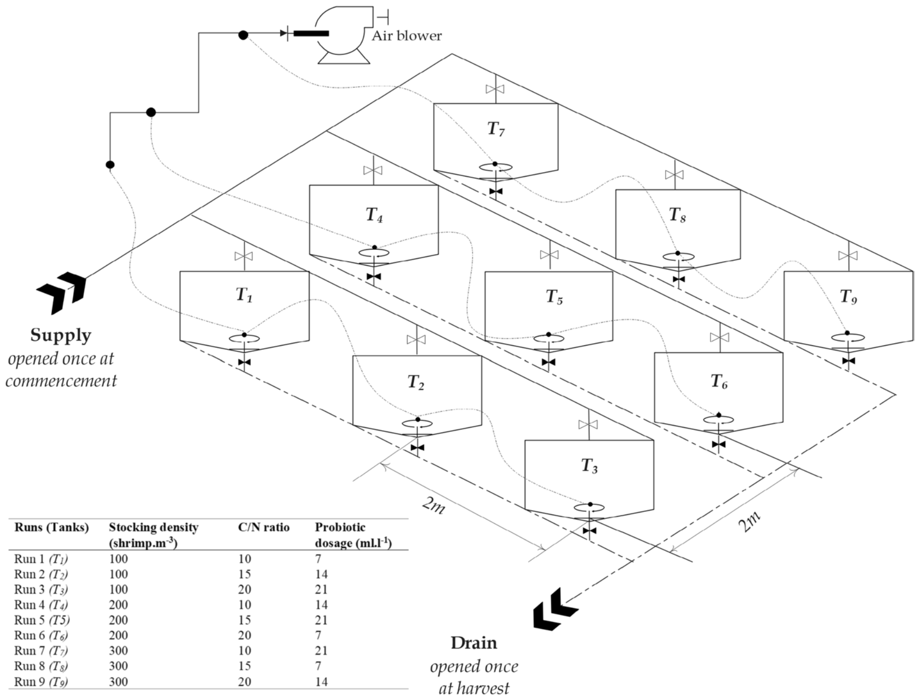
The use of Taguchi optimisation in aquaculture systems and technology, specifically system design, is relatively scanty. Ighodalo et al. (2020) developed a fish feed pelletiser machine where the die thickness, number of die holes, shaft speed and feed rate were optimised in an L9 orthogonal Taguchi array. Mahalakshmi and Ganesan (2009) also applied this technique to evaluate aquaculture development policies. This study optimised critical control factors in a traditional biofloc aquaculture system to establish optimal settings for an Indoor Multi-Techno Aquaculture System (IMTAS), developed for the sustainable production of Pacific whiteleg shrimp, *L. vannamei*. A Taguchi L9 orthogonal array, grey relational analysis (GRA) and principal component analysis (PCA) were employed to systematically determine the optimal levels of these factors, using key water quality parameters to enhance system performance. The optimised control factor levels are to be applied to configure IMTAS, which integrates Internet of Things (IoT) technology for real-time monitoring and control.

Materials and methods

Experimental set-up and design

The experiment was conducted from March 14 to June 5, 2023, at the indoor hatchery of the Institute of Tropical Aquaculture and Fisheries (AKUATROP), Universiti Malaysia Terengganu. The experiment was set up using nine circular high-density polyethylene (HDPE) tanks (3 m^3, 2 m diameter, 1 m height) arranged in three parallel series of three tanks each. The tanks were connected by 3-inch PVC pipes under zero-water discharge conditions in a traditional biofloc system. Figure 2 shows a side view of the tank design, including the water column (1 m depth), sediment base and aeration system. Each tank had one central aerotube (50-mm circular diffuser membrane) connected to a regenerative blower (4.5 $m^3.h^{-1}$ total capacity). The aerotube was positioned vertically at the centre of the tank (0.2 m above sediment) to deliver 0.5 $m^3.h^{-1}$ vertical circular current. This ensured floc suspension, uniform oxygenation (> 5 mg.l^{-1}) and minimum sediment disturbance.

The Taguchi L9 design was used to maintain C/N ratio at 10, 15, and 20, stocking densities at 100, 200, and 300 shrimp.m^{-3} and probiotic weekly dosages at 7 ml.l^{-1}, 14 ml.l^{-1} and 21 ml.l^{-1}. The levels for each of these control factors were adopted based on previous levels reported by Ferreira et al. (2016) for stocking density, Xu and Pan (2013) for C/N ratio and Amjad et al. (2022) for amount of probiotic. The L9 orthogonal array was generated using Minitab 19th edition. Nine experimental units (run) were accordingly assigned to nine tanks, as shown in Fig. 1.



Runs (Tanks)	Stocking density (shrimp.m^{-3})	C/N ratio	Probiotic dosage (ml.l^{-1})
Run 1 *(T_1)*	100	10	7
Run 2 *(T_2)*	100	15	14
Run 3 *(T_3)*	100	20	21
Run 4 *(T_4)*	200	10	14
Run 5 *(T5)*	200	15	21
Run 6 *(T_6)*	200	20	7
Run 7 *(T_7)*	300	10	21
Run 8 *(T_8)*	300	15	7
Run 9 *(T_9)*	300	20	14

Fig. 1 Experimental layout of a traditional biofloc system for the parameter setting of IMTAS

Stocking and feeding

Post larvae, PL of *L. vannamei*, were obtained from a local supplier in Pahang, Malaysia. The stock passed the stress test performed on the PLs upon arrival (87%), which measured the mortality of 100 PLs placed in 1000 ml of swirling water for 30 min. The PLs, aged 20 days, were stocked each tank based on the quantities stated in Fig. 1. They were fed twice daily with commercial Blanca® shrimp feed (36% crude protein) at a feeding rate of 5% body weight based on levels specified in each run. Sampling was performed weekly to monitor shrimp growth and water quality. To monitor shrimp growth, 10 samples of *L. vannamei* were randomly sampled from each tank using a fine-mesh dip net (1 mm mesh size) to minimise stress. They were weighed individually (to 0.01 g precision) using a digital balance (Shimadzu, BL-320H) and measured for total length (tip of rostrum to telson, to 0.1 mm) with digital calipers. Average weekly weight and length were calculated to assess growth rates.

Preparation of inoculum and molasses application

Rapid BFT™ was used as a bacteria inoculum in this study. One thousand milliliters of deionised water was mixed with 40.25 g of Zobell Marine Broth 2216 to dissolve the pellets. The mixture was autoclaved for 15 min at a pressure of 15 lbs and temperature of 121 °C. A streak from a pure culture of *Bacillus tropicus* was introduced into the broth and kept in an incubator shaker (Sartorius, CERTOMAT® IS) for 24 h before use. Sugar beet molasses (carbon, approximately 50% on a dry matter basis) was used as the carbon source during the experiment. The amount of molasses applied was adjusted weekly based on the progressive change in the body weight of the cultured shrimp. Molasses and probiotic applications were discontinued at week 5 upon floc maturation.

Water quality monitoring

Water quality was evaluated by collecting 500 mL samples from three tank locations (centre, wall, mid-depth) using a Van Dorn sampler. Temperature (°C), dissolved oxygen (mg.l^{-1}), electrical conductivity (µS/cm), total dissolved solids (mg.l^{-1}), salinity (ppt) and pH were assessed using a YSI multiparameter analyser (Professional Plus). UV spectrophotometer (Shimadzu, UV-1800) was used to measure the total ammonia nitrogen (TAN), nitrite, and phosphate. TAN was measured at a wavelength of 640 nm via the phenol-hypochlorite method as described in Liang et al. (2016). Nitrite was measured at a wavelength of 543 nm using the sodium nitrate method outlined by Imorwitz and Keliher (1984), while phosphate was measured using the method described by Shyla et al. (2011) at a 680-nm wavelength.

Parameter optimisation using Taguchi-grey relational analysis method

Taguchi single-response optimisation was used to determine the optimum levels of the response factors, while grey relational analysis (GRA) was used to synchronically analyse the impact of all the response factors to determine the treatment (run) with optimum performance. Principal component analysis (PCA) on the other hand was used to establish the comparative impact of each response factor.

Signal-to-noise (S/N) ratio is a statistical measure used in the Taguchi method to evaluate the performance and robustness of a system or process by comparing the desired output (signal) to unwanted variability (noise) caused by uncontrollable factors. A higher S/N ratio indicates better performance, as it reflects a stronger desired effect with minimal variability. The S/N ratio was set based on the impact and trend of desirability established for each response factor. 'small for the better' was set for TAN referenced to the report of Mansour et al. (2022), nitrite according to Addo et al. (2023) and phosphate based on Prates et al. (2023). On the other hand, 'the larger the better' was set for DO in reference to Whangchai et al. (2022), Nayak et al. (2023) for EC, Carvalho et al. (2023) for TDS, Holanda et al. (2022) for salinity, Brito et al. (2018) for pH and Blancaflor and Baccay (2022) for temperature.

The S/N ratio for the 'smaller was the better' was computed from the formula

$$S/N\ ratio = (-10)^{*} log_{10}\left(\frac{1}{n}\right)\sum_{1=1}^{n}\chi_{ij}^{2} \tag{1}$$

While the S/N ratio for the 'larger was the better' was calculated from the relationship

$$S/N\ ratio = (-10)^{*} log_{10}\left(\frac{1}{n}\right)\sum_{1=1}^{n}\frac{1}{\chi_{ij}^{2}} \tag{2}$$

where:

n is the number of replicates, and

χ_{ij} is the response factor (water quality variables).

Accordingly, the GRA of TAN, nitrite and phosphate which are desired at low concentrations were calculated in the established S/N ratios in single-response optimisation using the relationship

$$\chi_j^*(p) = \frac{\chi_j(p) - min\chi_j(p)}{max\chi(p) - min\chi_j(p)} \tag{3}$$

Dissolved oxygen, electrical conductivity, total dissolved solids, salinity, pH and temperature are preferred at higher values for the optimum performance of the biofloc aquaculture system. The GRA for these parameters was therefore computed from the equation

$$\chi_j^*(p) = \frac{max\chi(p) - \chi(p)}{max\chi_j(p) - \min\chi_i(p)} \tag{4}$$

where:

$\chi_j^*(p)$ are the calculated grey relational values of each run,
p is the number of response factors (the nine water quality variables in this research) in 9 stepwise sequence $\chi_j(p)$ and.
$j = 1,2,3\ldots,9$ represents the L9 orthogonal array.

$Max\chi(p)$ and $min\chi(p)$ represented the maximum and minimum values of the computed p^{th} S/N ratios.

Normalisation was performed to test the optimality in the performance of the grey relational values which were placed at a maximum of 1 (Peterson 2021). Furthermore, the deviation sequence of the normalised values was computed as absolute values of the differences between a normalised value at a run $\chi_0^*(p)$ referential to the sequence $\chi_j^*(p)$ in the relationship

$$\Delta_{0j}(p) = |\chi_0^*(p) - \chi(p)| \tag{5}$$

The grey relational coefficients, GRCs, were then calculated using the formula

$$\xi\left(\chi_j^*(p), \chi_0^*(p)\right) = \frac{\Delta_{min}(p) + \zeta\Delta_{max}(p)}{\Delta_{0j}(p) + \Delta_{max}(p)} \tag{6}$$

Although the distinguishing coefficient, ζ, ranges between 0 and 1, this research set ζ at 0.5, based on the criteria considered by Qazi et al. (2020) and Sarikaya and Güllü (2015).

The GRCs computed from the response factors obtained in this research were subjected to principal component analysis, PCA, in a response matrix (Eq. 7)

$$\chi = \begin{vmatrix} \chi_1(1) & \chi_1(2) & \chi_1(k) \\ \chi_2(1) & \chi_2(2) & \chi_2(k) \\ \cdots & \cdots & \cdots \\ \cdots & \cdots & \cdots \\ \chi_j(1) & \chi_j(2) & \chi_j(k) \end{vmatrix} \tag{7}$$

where:

$\chi_r(p)$ represents the GRC of each response factor is explained by $r = 1,2,\ldots,$
j is the experimental run and $p = 1,2,\ldots,$
k is the number of response factors (water quality parameters).

A coefficient of correlation matrix was generated from this experiment with run (j) and parameters (k) as follows.

$$\vartheta_{jl} = \left(\frac{Cov(\chi_r(p), \chi_r(l))}{\sigma_{\chi r}(p) * \sigma_{\chi r}(l)} \right) r = 1, 2, \ldots k; l = 1, 2, \ldots, k \tag{8}$$

Where the covariance of $\chi_r(p)$ and $\chi_r(l)$ is represented by $Cov(\chi_r(p), \chi_r(l))$ and their standard deviations are $\sigma_{\chi r}(p)$ and $\sigma_{\chi r}(l)$, respectively.

The eigenvalues and eigenvectors were then calculated from the resultant array from the formula.

$$\left(\varrho - \lambda_k l_j\right) V_{pk} = 0 \tag{9}$$

From the matrix ϱ, the eigenvalues (λ_k) and eigenvectors V_{pk} were used to calculate the uncorrelated principal components from the relationship.

$$Z_{jk} = \sum_{i=1}^{n} \chi(p) * V_{pk} \tag{10}$$

The Z_{jk} represents the kth principal component which is arranged in descending order with eigenvalues to indicate the level of variance among the response factors.

GRGs were also calculated from the GRCs and eigenvectors using Eq. (11), using the relationship.

$$\gamma_i\left(\chi_0^*, \chi_j^*\right) = \frac{1}{n} \sum_{p=1}^{n} \xi(\chi_j^*(p), \chi_0^*(p)) \tag{11}$$

where:

$\gamma_i\left(\chi_0^*, \chi_j^*\right)$ is the GRG of the j^{th} experimental run and.
N is the number of response factors.

Results

Single-response optimisation of response factors

Water quality data obtained during the experimental period were computed into means of response factors as presented in Table 1. The phosphate levels showed minimal fluctuations across the runs. Run 2 had the highest phosphate concentration at 0.135 mg.l^{-1}, while Run 1 recorded the lowest phosphate concentration at 0.126. mg.l^{-1} Among the runs, Run 6 recorded the highest DO concentration of 5.79 mg.l^{-1}, whereas the lowest DO level was observed in Run 5 (5.31 mg.l^{-1}). Run 5 had the highest EC value of 49,321.93 µS.cm^{-1}, while Run 8 recorded the lowest EC (46,920.24 µS.cm^{-1}). The TDS values also varied, with Run 7 recording the highest TDS value at 30,330.73 mg.l^{-1} and Run 8 having the lowest TDS value at 27,239.61 mg.l^{-1}. Salinity levels also varied, with the highest value recorded in Run 4 at 35.36 ppm and the lowest in Run 2 at 33.76 ppm.

Table 1 Mean response factors in a traditional biofloc system for the parameter setting of IMTAS

Run	Factors/levels			Water Quality Parameters								
	Stocking density (shrimp.m^{-3})	C/N ratio	Probiotics dosage (ml.l^{-1})	TAN (mg.l^{-1})	Nitrite (mg.l^{-1})	Phosphate (mg.l^{-1})	DO (mg.l^{-1})	EC (µS.cm^{-1})	TDS (mg.l^{-1})	Salinity (ppm)	pH	Temp (°C)
Run 1	100	10	7	1.005	0.401	0.126	5.45	46,302.64	26,155.39	34.16	7.57	28.74
Run 2	100	15	14	1.046	0.497	0.135	5.77	48,949.95	28,137.91	33.76	7.60	28.65
Run 3	100	20	21	1.030	0.500	0.135	5.60	47,792.67	27,676.41	34.22	7.51	28.85
Run 4	200	10	14	1.053	0.458	0.132	5.73	46,721.74	28,559.59	35.36	7.42	28.62
Run 5	200	15	21	1.035	0.431	0.128	5.31	49,321.93	28,753.21	35.04	7.55	28.92
Run 6	200	20	7	1.040	0.488	0.135	5.79	47,193.56	29,151.84	34.99	7.52	28.71
Run 7	300	10	21	0.965	0.459	0.130	5.33	47,666.87	30,330.73	35.26	7.52	28.69
Run 8	300	15	7	1.110	0.543	0.131	5.77	46,920.24	27,239.61	34.55	7.35	28.71
Run 9	300	20	14	1.299	0.476	0.129	5.75	45,485.32	28,852.07	35.25	7.52	28.66

Run 5 featured the highest pH value at 7.60, while Run 8 had the lowest pH of 7.35. Run 5 recorded the highest temperature at 28.92 °C, while the lowest was observed in Run 4 at 28.62 °C.

The signal-to-noise ratios (S/N ratio) were calculated the mean response factors and presented in Table 2. Run 7 had the best performance (S/N ratio 0.30) for TAN. The optimum S/N ratio for nitrate was observed in Run 1 (7.92). Run 1 yielded the highest S/N ratio (17.96) for phosphate. The optimised S/N ratio for DO was achieved in Run 6 (15.26). EC optimisation led to the highest S/N ratio in Run 5 (93.86), while 89.63 was recorded as the highest for TDS. Run 4 achieved the highest S/N ratio for salinity at 30.97, whereas pH was in Run 2 (17.62). Temperature had Run 5 with the highest S/N ratio at 29.22.

Multi-response optimisation of response factors

Table 3 presents the grey relational analysis (GRA) of signal-to-noise (S/N) ratios for water quality parameters in a biofloc aquaculture system. GRA consolidates varying quality characteristics of response variables namely total ammonia nitrogen (TAN), nitrite and phosphate for smaller-the-better and dissolved oxygen (DO) and electrical conductivity (EC), total dissolved solids (TDS), salinity, pH and temperature for larger-the-better. This is to facilitate the ranking of the nine experimental runs into a single grey relational grade (GRG). The ranges of the S/N ratio were salinity (62.58), temperature (70.81), TAN (2.58), nitrite (2.63), phosphate (0.57), DO (0.75), EC (0.70), TDS (1.28) and pH (15.79).

The normalised signal-to-noise (S/N) ratios for water quality parameters in a biofloc aquaculture system are presented in Table 4. The normalisation scaled S/N ratios to a 0–1 range to allow uniform comparison of the various response factors essential for computing grey relational coefficients and grades for the multi-response optimization. The lowest and highest normalised values were recorded as follows: TAN (1.00 in Run 7, 0.00 in Run 9), nitrite (1.00 in Run 1, 0.00 in Run 8), phosphate (1.00 in Run 1, 0.00 in Run 6), DO (1.00 in Run 6, 0.00 in Run 5) and EC (1.00 in Run 5, 0.00 in Run 9). Also, TDS recorded 1.00 in Run 7 and 0.00 in Run 1, salinity (1.00 in Run 4, 0.00 in Run 2), pH (1.00 in Run 2, 0.00 in Run 8) and temperature (1.00 in Run 5, 0.00 in Run 4).

Table 5 presents the deviation sequences of normalised signal-to-noise (S/N) ratios for water quality parameters. The deviation sequence quantifies the dispersion from the optimal performance established S/N ratio to enable computation of grey relational. Deviation sequences showed substantial trends and variations across parameters where the optimal deviations were recorded for TAN at Run 7, nitrite at Run 1, phosphate at Run 1 and DO at Run 6. Also, EC had the highest deviation at Run 5, TDS at Run 7, salinity at Run 4, pH in Run 2 and temperature in Run 5.

The grey relational coefficients (GRCs) for water quality parameters are presented in Table 6. GRCs are derived from deviation sequences using a distinguishing coefficient (where 0.5 was used in this research) to quantify the relational degree between each normalised response and the ideal sequence. The values expressed the proximity to optimal performance that facilitated aggregation into the grey relational grade (GRG) for multi-response ranking and optimization. TAN had the highest GRC (1.00) at Run 7, nitrite at Run 1, phosphate at Run 6 and DO at Run 6. In addition, EC recorded the highest GRC in Run 5, TDS in Run 7, salinity in Run 4 and pH in Run 2, while temperature was in Run 5. Notable trends showed Run 7 recording highest GRCs in TAN and TDS, Run 1 in nitrite and phosphate and Run 5 in EC and temperature.

Table 2 S/N ratio in the single-response optimisation of the response factors in a traditional biofloc system for the parameter setting of IMTAS

Run	Factors/levels			Water Quality Parameters								
	Stocking density (shrimp. m^{-3})	C/N ratio	Probiotics dosage (ml. l^{-1})	TAN ($mg.l^{-1}$)	Nitrite ($mg.l^{-1}$)	Phosphate ($mg.l^{-1}$)	DO ($mg.l^{-1}$)	EC ($\mu S.cm^{-1}$)	TDS ($mg.l^{-1}$)	Salinity (ppm)	pH	Temp (°C)
Run 1	100	10	7	−0.04	7.92*	17.96*	14.72	93.31	88.35	30.67	17.58	29.17
Run 2	100	15	14	−0.39	6.07	17.40	15.23	93.79	88.98	30.56	17.62*	29.14
Run 3	100	20	21	−0.25	6.02	17.40	14.97	93.58	88.84	30.68	17.51	29.20
Run 4	200	10	14	−0.44	6.78	17.56	15.17	93.39	89.11	30.97*	17.41	29.13
Run 5	200	15	21	−0.30	7.31	17.86	14.51	93.86*	89.17	30.89	17.56	29.22*
Run 6	200	20	7	−0.34	6.22	17.38	15.26*	93.47	89.29	30.87	17.53	29.16
Run 7	300	10	21	0.30*	6.76	17.69	14.53	93.56	89.63*	30.94	17.53	29.15
Run 8	300	15	7	−0.90	5.29	17.64	15.23	93.42	88.70	30.76	17.32	29.16
Run 9	300	20	14	−2.27	6.44	17.81	15.20	93.15	89.20	30.94	17.52	29.14
Optimum				$S_{L3}C_{L1}A_{L3}$	$S_{L1}C_{L1}A_{L1}$	$S_{L1}C_{L1}A_{L1}$	$S_{L2}C_{L3}A_{L1}$	$S_{L2}C_{L2}A_{L3}$	$S_{L3}C_{L1}A_{L3}$	$S_{L2}C_{L1}A_{L2}$	$S_{L1}C_{L2}A_{L2}$	$S_{L2}C_{L3}A_{L3}$
Criteria				*smaller*	*smaller*	*smaller*	*Larger*	*Larger*	*Larger*	*Larger*	*Larger*	*Larger*

S stocking density, C=C/N ratio, *A* amount of probiotic and subscripts L1–L3 are levels 1–3, respectively

*The optimised SN ratio in parameter

Table 3 Grey relational analysis of signal-to-noise ratios of the response factors in a traditional biofloc system for the parameter setting of IMTAS

Run	Factors/levels			Water quality parameters								
	Stocking density (shrimp.m^{-3})	C/N ratio	Probiotics dosage (ml.l^{-1})	TAN (mg.l^{-1})	Nitrite (mg.l^{-1})	Phosphate (mg.l^{-1})	DO (mg.l^{-1})	EC (µS.cm^{-1})	TDS (mg.l^{-1})	Salinity (ppm)	pH	Temp (°C)
Run 1	100	10	7	−0.04	7.92	17.96	14.72	93.31	88.35	30.67	17.58	29.17
Run 2	100	15	14	−0.39	6.07	17.40	15.23	93.79	88.98	30.56	17.62	29.14
Run 3	100	20	21	−0.25	6.02	17.40	14.97	93.58	88.84	30.68	17.51	29.20
Run 4	200	10	14	−0.44	6.78	17.56	15.17	93.39	89.11	30.97	17.41	29.13
Run 5	200	15	21	−0.30	7.31	17.86	14.51	93.86	89.17	30.89	17.56	29.22
Run 6	200	20	7	−0.34	6.22	17.38	15.26	93.47	89.29	30.87	17.53	29.16
Run 7	300	10	21	0.30	6.76	17.69	14.53	93.56	89.63	30.94	17.53	29.15
Run 8	300	15	7	−0.90	5.29	17.64	15.23	93.42	88.70	30.76	17.32	29.16
Run 9	300	20	14	−2.27	6.44	17.81	15.20	93.15	89.20	30.94	17.52	29.14
Criteria				*smaller*	*smaller*	*smaller*	*Larger*	*Larger*	*Larger*	*Larger*	*Larger*	*Larger*
Min				*−2.27*	*5.29*	*17.38*	*14.51*	*93.15*	*88.35*	*−88.92*	*−48.71*	*−121.69*
Max				*0.30*	*7.92*	*17.96*	*15.26*	*93.86*	*89.63*	*−26.34*	*−32.91*	*−50.87*
Range				*2.58*	*2.63*	*0.57*	*0.75*	*0.70*	*1.28*	*62.58*	*15.79*	*70.81*

Table 4 Normalisation of signal-to-noise ratios response factors in a traditional biofloc system for the parameter setting of IMTAS

Run	Factors/levels			Water quality parameters								
	Stocking density (shrimp.m^{-3})	C/N ratio	Probiotics dosage (ml.l^{-1})	TAN (mg.l^{-1})	Nitrite (mg.l^{-1})	Phosphate (mg.l^{-1})	DO (mg.l^{-1})	EC (µS.cm^{-1})	TDS (mg.l^{-1})	Salinity (ppm)	pH	Temp (°C)
Run 1	100	10	7	0.8	1.00	1.00	0.28	0.22	0.00	0.25	0.87	0.40
Run 2	100	15	14	0.72	0.29	0.03	0.95	0.90	0.49	0.00	1.00	0.11
Run 3	100	20	21	0.78	0.27	0.04	0.61	0.61	0.38	0.29	0.63	0.76
Run 4	200	10	14	0.70	0.56	0.31	0.88	0.33	0.59	1.00	0.29	0.00
Run 5	200	15	21	0.76	0.76	0.82	0.00	1.00	0.63	0.80	0.81	1.00
Run 6	200	20	7	0.74	0.35	0.00	1.00	0.45	0.73	0.77	0.68	0.30
Run 7	300	10	21	1.00	0.55	0.54	0.03	0.57	1.00	0.94	0.68	0.22
Run 8	300	15	7	0.52	0.00	0.45	0.96	0.38	0.27	0.49	0.00	0.29
Run 9	300	20	14	0.00	0.43	0.74	0.92	0.00	0.66	0.93	0.67	0.15

Table 5 Deviation sequence of the normalised SN ratios response factors in a traditional biofloc system for the parameter setting of IMTAS

Run	Factors/levels			Water quality parameters								
	Stocking density (shrimp.m^{-3})	C/N ratio	Probiotics dosage (ml.l^{-1})	TAN (mg.l^{-1})	Nitrite (mg.l^{-1})	Phosphate (mg.l^{-1})	DO (mg.l^{-1})	EC (µS.cm^{-1})	TDS (mg.l^{-1})	Salinity (ppm)	pH	Temp (°C)
Run 1	100	10	7	0.13	0.00	0.00	0.71	0.78	1.00	0.74	0.12	0.59
Run 2	100	15	14	0.27	0.70	0.96	0.04	0.09	0.50	1.00	0.00	0.88
Run 3	100	20	21	0.21	0.72	0.95	0.38	0.38	0.61	0.70	0.36	0.23
Run 4	200	10	14	0.29	0.43	0.68	0.11	0.66	0.40	0.00	0.70	1.00
Run 5	200	15	21	0.23	0.23	0.17	1.00	0.00	0.36	0.20	0.18	0.00
Run 6	200	20	7	0.25	0.64	1.00	0.00	0.54	0.26	0.23	0.31	0.69
Run 7	300	10	21	0.00	0.44	0.45	0.96	0.42	0.00	0.06	0.31	0.77
Run 8	300	15	7	0.47	1.00	0.54	0.04	0.61	0.72	0.50	1.00	0.70
Run 9	300	20	14	1.00	0.56	0.25	0.07	1.00	0.33	0.06	0.32	0.84

Table 6 Grey relational coefficients response factors in a traditional biofloc system for the parameter setting of IMTAS

Run	Factors/levels			Water quality parameters								
	Stocking density (shrimp.m^{-3})	C/N ratio	Probiotics dosage (ml.l^{-1})	TAN (mg.l^{-1})	Nitrite (mg.l^{-1})	Phosphate (mg.l^{-1})	DO (mg.l^{-1})	EC (µS.cm^{-1})	TDS (mg.l^{-1})	Salinity (ppm)	pH	Temp (°C)
Run 1	100	10	7	0.78	1.00	1.00	0.41	0.39	0.33	0.40	0.80	0.45
Run 2	100	15	14	0.64	0.41	0.34	0.92	0.84	0.49	0.33	1.00	0.36
Run 3	100	20	21	0.69	0.40	0.34	0.56	0.56	0.44	0.41	0.58	0.67
Run 4	200	10	14	0.63	0.53	0.42	0.80	0.42	0.55	1.00	0.41	0.33
Run 5	200	15	21	0.67	0.68	0.74	0.33	1.00	0.58	0.71	0.72	1.00
Run 6	200	20	7	0.66	0.43	0.33	1.00	0.47	0.65	0.68	0.61	0.41
Run 7	300	10	21	1.00	0.53	0.52	0.34	0.54	1.00	0.89	0.61	0.39
Run 8	300	15	7	0.51	0.33	0.47	0.92	0.44	0.40	0.49	0.33	0.41
Run 9	300	20	14	0.33	0.46	0.66	0.86	0.33	0.59	0.87	0.60	0.37

Evaluation of the comparative impact of the response factors and integration to single-metric grade

The eigenvalues and eigenvectors of the PCA for the response variables are presented in Table 7. PC1 had the highest eigenvalue of 0.14, which explained 35.20% of the total variance in the system. PC2, PC3 and the subsequent components explained the decreasing amounts of variance. DO was observed to have the highest variability recorded in PC1 (0.522), whereas TDS had the least variability (0.035).

The covariances of the response factors are shown in the PCA biplot (Fig. 2). Temperature and nitrite had similar covariances and an inverse trend to DO, TDS and salinity. Similarly, EC and TAN had reverse covariances with phosphate and pH. EC and TAN showed the closest impacts in the aquaculture system.

Table 8 shows the weighted grey relational grades and PCA ranking of the response factors. Run 1 obtained a GRG of 0.534 and a PCA ranking of 9. Conversely, Run 2 had a comparatively higher GRG of 0.651 and held a PCA ranking of 4. Run 3 recorded a lower GRG of 0.483 and a PCA ranking of 8, while Run 4 achieved a high GRG of 0.762, signifying a strong correlation among the response factors and secured a relatively higher PCA ranking of 2. Run 5 had a GRG of 0.577 and ranked 6th in capturing the system variability. Run 6 obtained the highest GRG of 0.705 and ranked 1 to represent the highest correlation among the response factors and capture the variability in the system. Run 7 had a GRG of 0.490 and a PCA ranking of 7, while Run 8 achieved a comparatively higher GRG of 0.680 and a PCA ranking of 3. Finally, Run 9 recorded a GRG of 0.648 and secured a PCA ranking of 5. The GRGs and PCA rankings collectively provided a comprehensive evaluation of the performance of each parameter setting within the system, with Run 6 recording the best GRG and PCA rankings.

Table 7 Eigenvectors of response factors in a traditional biofloc system for the parameter setting of IMTAS

Run	Principal components								
	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Eigenvalue	0.145	0.077	0.075	0.047	0.034	0.028	0.003	0.001	0.000
Proportion	0.352*	0.187	0.182	0.115	0.084	0.068	0.009	0.003	0.000
Cumulative	0.352	0.538	0.720	0.835	0.919	0.987	0.997	1.000	1.000
Eigenvectors									
TAN	−0.197	0.155	−0.231	0.158	0.461	−0.655	−0.316	−0.271	0.208
Nitrite	−0.314	−0.139	0.103	0.551	0.456	0.155	0.534	0.213	0.069
Phosphate	0.290	−0.362	0.596	−0.022	0.160	0.075	−0.057	−0.442	0.446
DO	0.522	−0.001	−0.633	−0.137	0.229	0.176	0.352	−0.158	0.275
EC	−0.231	0.165	0.208	−0.755	0.487	0.026	0.220	0.050	−0.134
TDS	0.035	0.546	0.232	0.005	0.379	−0.355	0.516	−0.045	0.329
Salinity	0.388	0.504	0.209	0.145	0.301	0.206	−0.363	0.485	0.176
pH	0.261	−0.488	0.037	−0.156	−0.022	−0.529	0.098	0.608	0.091
Temp	−0.482	−0.089	−0.195	−0.192	−0.171	0.245	−0.179	0.227	0.716

*The highest proportional contribution to variations in the system

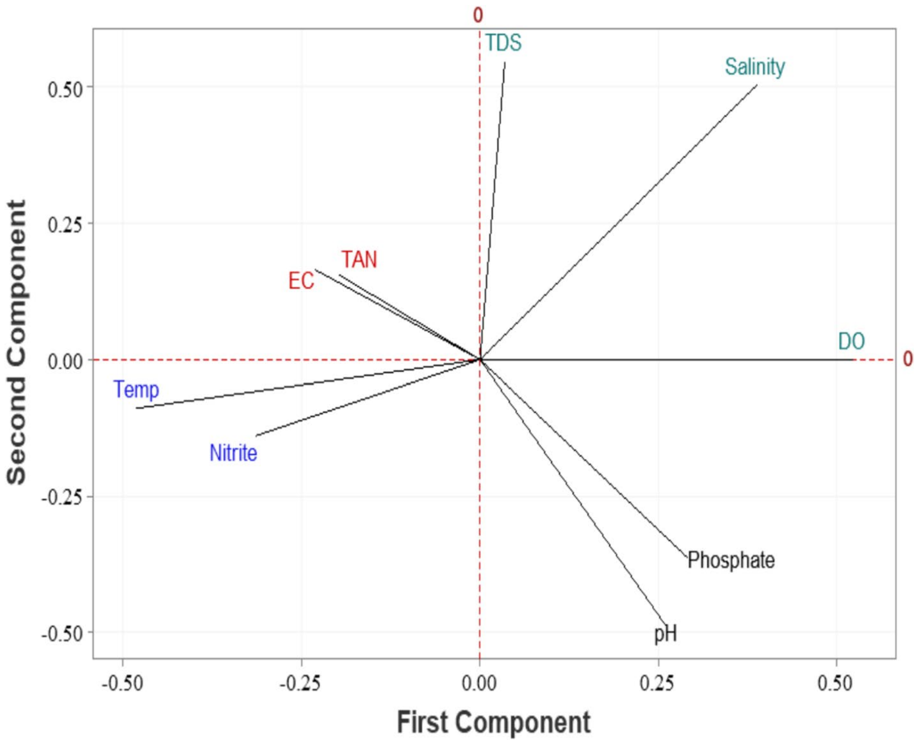


Fig. 2 PCA biplot of response factors in a traditional biofloc system for the parameter setting of IMTAS

Determination of optimum control-factor combinations

The GRGs calculated from GRA and PCA in Table 8 were further subjected to the conventional Taguchi analysis with 'larger-the-better' as the option of S/N ratio to establish the respective levels of each control factor that produced the optimum GRGs as presented in Fig. 3.

The 3D plot presented in Fig. 4 shows an amalgamated GRG of 0.687 which represents the optimum performance of the system based on water quality conditions. The respective impact of the control factors showed that the stocking density (0.580) had the least effect on the overall performance of the system. In contrast, the probiotic dosage (0.635) had the greatest influence on the response factors. Based on the water quality conditions obtained from this experiment, 200 shrimp.m^{-3} was considered the optimum level of stocking density, while the C/N ratio and probiotic dosage were 15 and 14 ml.l^{-1}, respectively.

Discussion

The Taguchi method L9 orthogonal array used in this research provided a reliable and efficient framework for optimising water quality parameters in IMTAS for the production of *L. vannamei*. Single-response optimization identified distinct optimum levels that

Table 8 Weighted grey relational grades and PCA ranking response factors in a traditional biofloc system for the parameter setting of IMTAS

Run	Factors/levels			Water quality parameters									PCA ranking		
	Stocking density (shrimp. m^{-3})	C/N ratio	Probiotics dosage (ml. l^{-1})	TAN ($mg.l^{-1}$)	Nitrite ($mg.l^{-1}$)	Phosphate ($mg.l^{-1}$)	DO ($mg.l^{-1}$)	EC ($\mu S.cm^{-1}$)	TDS ($mg.l^{-1}$)	Salinity (ppm)	pH	Temp (°C)	Eigen-vector	GRG	Rank
Run 1	100	10	7	0.786	1.000	0.536	0.411	0.391	0.333	0.400	0.552	0.856	0.039	0.534	9
Run 2	100	15	14	0.648	0.415	0.340	0.921	0.842	0.497	0.586	0.552	0.842	0.099	0.651	4
Run 3	100	20	21	0.695	0.409	0.343	0.566	0.562	0.447	0.333	0.526	1.000	0.084	0.483	8
Run 4	200	10	14	0.631	0.536	0.421	0.809	0.428	0.552	1.000	0.333	0.652	0.272	0.762	2
Run 5	200	15	21	0.679	0.680	0.744	0.333	1.000	0.581	0.815	0.354	0.741	0.053	0.577	6
Run 6	200	20	7	1.000	0.531	0.333	1.000	0.479	0.651	0.885	0.483	0.499	0.001	0.705	1*
Run 7	300	10	21	0.664	0.435	0.522	0.341	0.543	1.000	0.789	0.494	0.615	0.151	0.490	7
Run 8	300	15	7	0.515	0.333	1.000	0.925	0.448	0.408	0.803	1.000	0.333	0.068	0.680	3
Run 9	300	20	14	0.333	0.469	0.661	0.866	0.333	0.597	0.832	0.433	0.563	0.232	0.648	5

*The run with the best performance

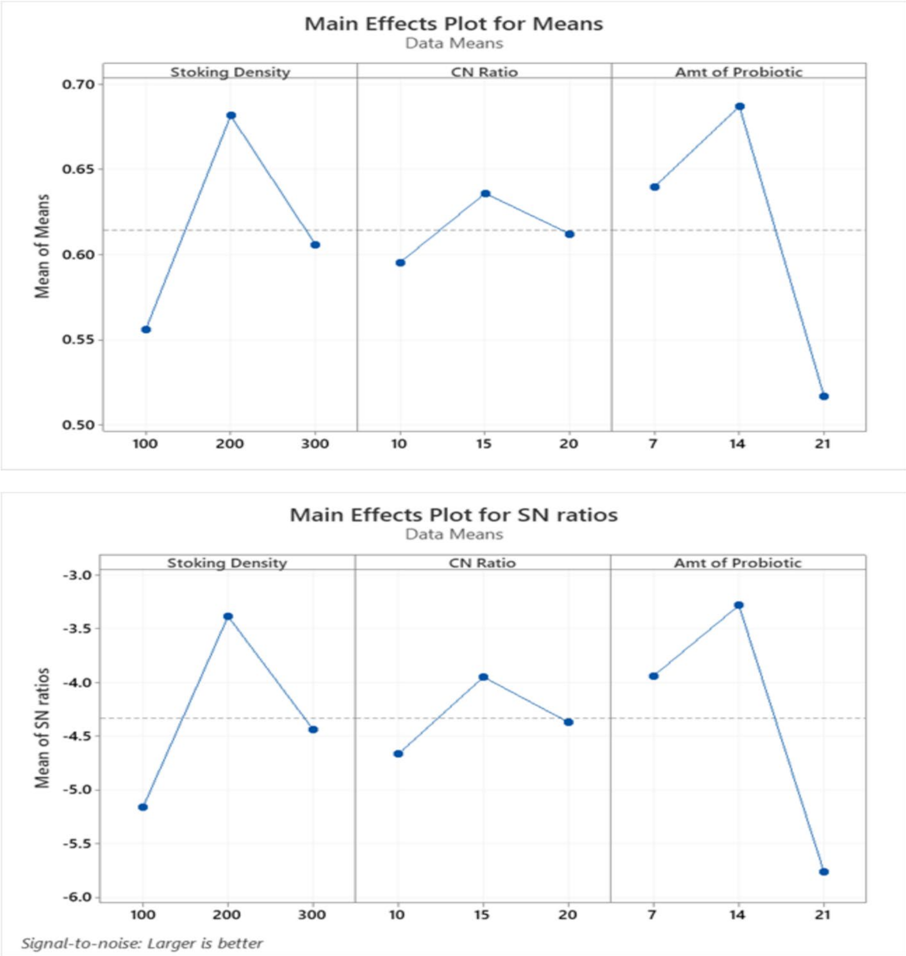


Fig. 3 Means and S/N ratios of the GRGs of the response factors in a traditional biofloc system for the parameter setting of IMTAS

revealed adjustments driven by microbial community in floc and dynamics in the water quality conditions of the system. These adjustments identified by S/N ratios provide the ability of Taguchi methodology in quantifying factor contributions needed for the optimisation of IMTAS. Taguchi methodology has been reported to achieve significant nitrogen removal efficiency in aquaculture systems (Arici et al. 2025). Taguchi method has been used to determine new-generation of neonicotinoid in Pacific white shrimp (Omar et al. 2025). Taguchi-based GRA optimised pond aeration systems to achieve fourfold performance increases and sixfold cost reductions by prioritising gate opening which represents its versatility in multi-variable challenges. Compared to full factorial designs and response surface methodology, Taguchi-GRA often achieves a cost-savings approach to identify these optimum levels. Maduekwe and Oke (2021) reported that this approach saves 65–80% of experimental cost comparable to full factorial design.

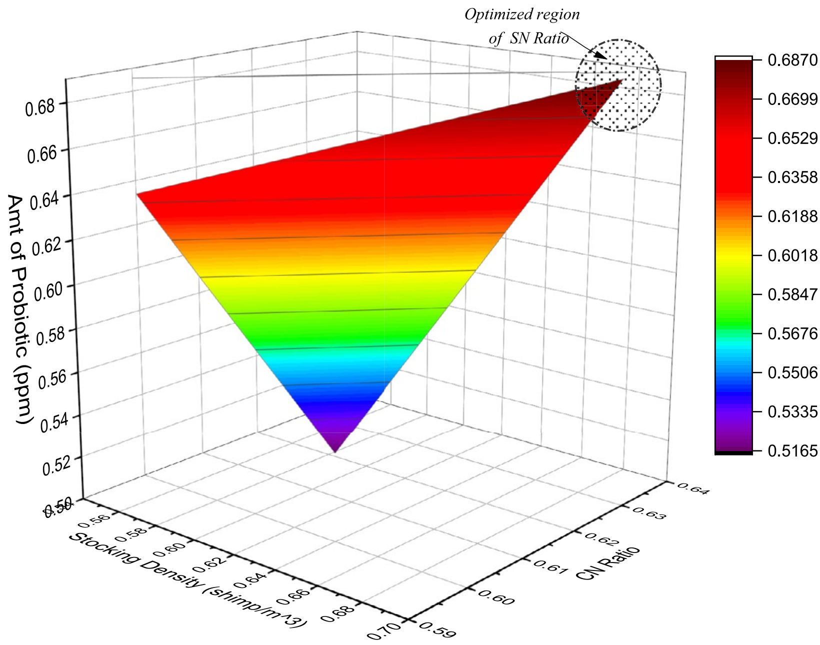


Fig. 4 3D plot of the GRGs of the response factors in a traditional biofloc system for the parameter setting of IMTAS

Run 6 (200 shrimp.m^{-3}, C/N 20, 7 ml.l^{-1} probiotic) emerged as the optimum configuration by achieving the highest grey relational grade (GRG, 0.705) and PCA rank (1). The integration of Taguchi with grey relational analysis (GRA) improved on the single-response optimization by holistically balancing multiple response parameters essential for the best performance of IMTAS. Multi-response optimization via Taguchi-GRA analysis usually weighs the relative importance of parameters to for the overall performance of a system (Ashebir et al. 2022). Low stocking density improves water quality by reducing nitrogen and phosphorus build-up thereby optimising nitrite and phosphate levels for sustainable aquaculture. Studies across species such as shrimp, tilapia and black carp show that lower densities enhance specific growth rate and survival and better yield performance (Mugwanya et al 2022). However, higher density yields greater total biomass despite potential nutrient accumulation. This is essential for biofloc system which utilises the biomass for floc formation. Balancing stocking density is therefore essential to achieve both water quality and production goals. TAN generated in the run with high stocking density (Run 1–3) was minimised by accelerating nitrification. Chattaraj et al. (2024) reported that these dynamics cause a decline in DO arising from the competition between the microbial community and the cultured shrimp. Conversely, low stocking optimised nitrite and phosphate to minimise nutrient build-up. Deswati et al. (2022) however noted that this usually limit the production capacity of a biofloc system. Accordingly, efficient nitrification is known to minimise TAN even at high density as observed in this research.

C/N ratio above 10 in biofloc technology enhances nitrogen assimilation by promoting heterotrophic floc formation (Li et al 2023). This research adopted C/N ratio above 10 in all the run. The high C/N ratio recorded in this study promoted the formation of heterotrophic floc responsible for the assimilation of nitrogenous waste into microbial protein. This process enhances nutrient recycling and stabilises phosphate levels in biofloc systems (Dilmi et al. 2021). Heterotrophic microorganisms efficiently utilise organic carbon to assimilate toxic nitrogen compounds to improve nitrogen removal and system stability at high ratios. This shift favours heterotrophic nitrification and denitrification over ammonia oxidation by reducing the dominance of autotrophic ammonia-oxidising bacteria (Farooq et al 2022). The increase in C/N ratio promotes the proliferation of heterotrophic bacteria to form stable anoxic microzones that support anaerobic ammonia-oxidising bacteria. Consequently, high C/N ratio in this research was observed to enhance nitrogen removal efficiency in the culture systems.

This study also established that probiotic dosage had the most substantial effect on the system performance. The low probiotic dose was enough to sustain microbial balancing to boost nitrification and suppress harmful pathogens such as *Vibrio* sp. It is also known that the beneficial microbes in floc improves growth and feed conversion ratio (FCR) (Hartono and Barades 2022). Akange et al. (2024) employed PCA to determine an optimal probiotic dosage in *L. vannamei* biofloc systems. Probiotic dosage of 14 $ml.l^{-1}$ presented higher contributory effect (11.03) on growth and water quality in the nature where one-way ANOVA showed limited differentiation. This corroborates Taguchi multi-response optimization where precise control factors are critically evaluated for ecological and economic viability. The optimum probiotic dosage (14 $ml.l^{-1}$) determined through Taguchi analysis indicates more productive effects at this level probably due to suitability of the microbial level to the ammonia levels in the system. This is needed for balancing efficacy and cost sustainability of IMTAS. Luo et al. (2025) earlier identified the role of *Bacillus* sp. in enhancing nitrification and denitrification by upregulating genes (amoC_B, hao, nirS, nosZ). This was observed in the reduced TAN and nitrite concentrations recorded in during the research. Akange et al. (2025) reported that a C/N ratio of 20 and 7 $ml.l^{-1}$ probiotic dosage treatment yielded higher lauric, myristic and stearic acids and a rich amino acid profile, ideal for *L. vannamei*. Freeze-drying was also noted to preserve more essential amino acids and fatty acids than oven-drying.

Temperature was observed to have an inverse correlation with DO due to reduced solubility which boosts nitrification. Moloantoa et al. (2022) however noted the risk of denitrification dominance microbes under low DO which potentially result in nitrate losses. Similarly, the strong correlation between EC and TAN identified EC as a reliable proxy for ionic nitrogen compounds (Wang et al. 2024). However, non-nitrogen ions such as Na^+ are known to interfere this balancing (Li et al. 2025). Abakari et al. (2022) established that optimum DO levels play critical role of DO in shrimp respiration and microbial activity in biofloc systems. Stable EC and TDS values reflect balanced ionic conditions for enhancing microbial floc stability as confirmed by Nayak et al. (2023) and Carvalho et al. (2023). The salinity and pH levels agreed with ranges ideal for shrimp growth (Holanda et al. 2022; Brito et al. 2018), while temperature levels supported floc formation (Blancaflor and Baccay 2022). These findings corroborate Hu et al. (2024) who reported the need for water quality management for the sustainability of the floc.

Conclusion

This study optimised control factors in a traditional biofloc system to configure IMTAS for sustainable production of *L. vannamei*. A total of 200 shrimp.m^{-3}, a C/N ratio of 15, and a 14 ml.l^{-1} probiotic dosage were identified as the optimal settings of the control factors tested. Single-response optimization identified the best performance for total ammonia nitrogen in Run 7 (S/N ratio 0.30), nitrite and phosphate in Run 1 (7.92 and 17.96), dissolved oxygen in Run 6 (15.26), electrical conductivity and temperature in Run 5 (93.86 and 29.22), total dissolved solids in Run 2 (88.99) and salinity in Run 4 (30.97). Multi-response optimization using Taguchi-GRA and PCA ranked Run 6 highest (GRG 0.705), with probiotic dosage exerting the greatest influence on system performance by enhancing nitrification and microbial balance. These settings considered water quality as crucial condition regulating shrimp growth and system sustainability of IMTAS. The Taguchi-GRA approach achieved methodical optimization using least resource. Future research should validate these control factors in large-scale IMTAS and explore additional control and response factors to further improve the efficiency of biofloc shrimp production.

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Data availability Data will be made available on request.

Declarations

Competing interest The authors declare no competing interests.

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