

# **Review** Article

# The Impact of Biofloc Technology on Water Quality in Aquaculture: A Systematic Meta-Analysis

Yousef Ahmed Alkhamis <sup>(D)</sup>,<sup>1,2</sup> Airin Sultana,<sup>3</sup> Shaikh Tareq Arafat,<sup>3</sup> Muhammad Abdur Rouf <sup>(D)</sup>,<sup>3</sup> Sheikh Mustafizur Rahman <sup>(D)</sup>,<sup>3</sup> Roshmon Thomas Mathew <sup>(D)</sup>,<sup>2</sup> Nagarajan Ganesan <sup>(D)</sup>,<sup>4,5,6</sup> Afrin Sultana,<sup>3</sup> Rashid Saleh Alngada,<sup>2</sup> Raed Abdul Whed,<sup>2</sup> Nouh Abdulaziz Aljaafari,<sup>2</sup> and Md. Moshiur Rahman <sup>(D)</sup>,<sup>3,7</sup>

<sup>1</sup>Animal and Fish Production Department, College of Agricultural and Food Sciences, King Faisal University, Hofuf-420, Al-Asha, 31982, Saudi Arabia

<sup>2</sup>Fish Resources Research Center, King Faisal University, Hofuf-420, Al-Asha, 31982, Saudi Arabia

<sup>3</sup>Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna, 9208, Bangladesh

<sup>4</sup>Basic Sciences Department, PYD, King Faisal University, Hofuf-420, Al-Asha-31982, Saudi Arabia

<sup>5</sup>Center of Excellence for the Ocean, National Taiwan Ocean University, Keelung 20224, Taiwan

<sup>6</sup>Department of Aquaculture, National Taiwan Ocean University, Keelung 20224, Taiwan

<sup>7</sup>Fish Conservation and Culture Lab, Biological and Agricultural Engineering, University of California, Davis, Davis, CA, USA

Correspondence should be addressed to Roshmon Thomas Mathew; rmathew@kfu.edu.sa and Md. Moshiur Rahman; momrahman@ucdavis.edu

Received 16 May 2023; Revised 15 September 2023; Accepted 20 September 2023; Published 17 October 2023

Academic Editor: Gouranga Biswas

Copyright © 2023 Yousef Ahmed Alkhamis et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

A technique called biofloc technology (BFT) is an environmentally friendly method for aquaculture in which a successful growing cycle depends on the maintenance and monitoring of water quality parameters. Studies have revealed that improving water quality in BFT and maintaining the safety range of the parameters can help to increase the growth performance of cultured species. Following a systematic review of the literature, a meta-analysis was performed to explore how some important water parameters such as pH, dissolved oxygen (DO), nitrite (NO<sub>2</sub>–N), nitrate (NO<sub>3</sub>–N), ammonia (NH<sub>3</sub>–N), total ammonia nitrogen (TAN), total suspended solids (TSS), and alkalinity were influenced by different BFT systems. The PRISMA screening process was followed, and 33 studies were eligible for the meta-analysis. The meta-analyses showed that NO<sub>2</sub>–N and TSS were significantly affected by BFT, while pH, DO, NO<sub>3</sub>–N, NH<sub>3</sub>–N, TAN, and alkalinity were not significantly influenced by this system. The analyses revealed that NO<sub>2</sub>–N had a significant negative effect size due to BFT, whereas TSS showed a significant positive effect size. The study also revealed some publication bias in which few experiments of some studies showed extremely positive and negative effect sizes due to BFT application in the system. Overall, the findings suggest clear evidence of the profound influence of BFT on the water quality parameters in different aquaculture systems, suggesting the future development of BFT for sustainable and environmentally friendly aquaculture production.

# 1. Introduction

Biofloc technology (BFT) has been driven toward increased fish production with sustainability in the last decade [1]. BFT is a technique for enhancing water quality through incorporating additional external carbon sources in conjunction with high levels of aeration in order to produce large quantities of microbial bacterial floc in fish farming systems [2, 3]. Recently, it has received a lot of attention as a method that is affordable, sustainable, and environmentally benign [4]. An important benefit of this system is that there is no water exchange, which significantly decreases water use and prevents environmental harm from effluent release [5]. Biosecurity seems to be a prime concern nowadays to prevent vertical infections

given the widespread distribution of viruses worldwide. Enhancing biosecurity can be accomplished using BFT limited water exchange culture approach [6], further it can reduce the waste discharge and protect the adjoining environments. Using the same water for multiple production cycles with no adverse environmental effects is made possible by the heterotrophic bacteria in BFT that assimilate nitrogen molecules [7]. Within this system, there exists a symbiotic community consisting of microbes, algae, and protozoa, which coexists alongside detritus and deceased organic particles forming in the water column, improving water quality, and generating microbial proteins for aquatic organisms. The resulting highly concentrated microbial population can act as a system for improving the quality of system water, and the produced microbial protein can be fed to animals as food [8].

The human population of the world is growing exponentially, which is having a detrimental effect on the environment as well as the fish populations in oceans, rivers, and lakes. In order to encourage sustainable farming, fish production must be increased without significantly consuming essential natural resources [9]. However, the expansion of the aquaculture sector has been limited due to the environmental contamination caused by the release of unwanted products containing a lot of organic nitrogenous components [10]. The formation of lethal inorganic nitrogen compounds such as NO<sub>2</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> in the culture system results in an undesirable water quality, which is one of the key issues confronting the intensive aquaculture sector. Aquaculture systems are reportedly affected by the excretion of concentrated ammonia by high-value aquatic organisms such as fish and shrimp [9]. Water quality can be improved with several methods, including high-volume water exchange [11], waste treatment with recirculating aquaculture systems (RAS) [12], and zero-water exchange with BFT [13]. RAS provides fish farmers with a number of significant benefits over open pond culture. These include a technique to increase production with a finite amount of land and water, and practically total environmental control to increase fish development [14]. But increasing water exchange increases operating expenses because it uses more energy and water and reduces the duration that nutrients are retained in the rearing systems [15]. The application of BFT to aquaculture could provide a potential solution, rather than using other techniques, since it could result in the removal of nutrients while simultaneously producing a high level of protein-rich microbial biomass [15]. By recycling feed waste and effluents, increasing food production, minimizing pathogen transmission, enhancing immunity, and preventing disease outbreaks, BFT decreases or eliminates the need for the frequent replacement of the water in the enclosures [3, 16]. Microbial flocs are created when these bacteria join forces with organic residue particles and other microbes. Microalgae and bacteria recycle excess nutrients in the water such as phosphorus and nitrogen, and also take up these substances, which help to keep harmful substances like ammonia under control [17]. Temperature, salinity, pH, alkalinity, hardness, dissolved oxygen (DO), total ammonia nitrogen (TAN), and orthophosphate are important parameters that should be continuously monitored in BFT Aquaculture Nutrition

[18]. To ensure the successful development and maintenance of the BFT production cycle, it is essential to understand the parameters of water quality and their interactions. For example, aquaculture species need a recommended range of DO, pH, total suspended solids (TSS), TAN, and alkalinity to thrive and prevent death [18].

A lot of studies are available about the influence of BFT on water quality parameters in aquaculture. When compared to the control system, BFT effectively removed inorganic nitrogen from the aquaculture systems and significantly increased the growth parameters of rohu (*Labeo rohita*), catla (*Catla catla*), and mrigal (Cirrihinus mrigala) in polyculture mode [19]. Healthy postlarval shrimp of Litopenaeus vannamei can be achieved by maintaining water quality and utilizing the microbial population of the system as a food source, which can facilitate the appropriate development of the hepatopancreas and enhance their nutritional status [20]. The microbial biomass thriving in the biofloc consumes toxic inorganic nitrogen and converts it into beneficial protein, which is subsequently accessible to the cultivated Penaeus vannamei shrimp production [21]. The benefits of BFT technology are supported by the noticeably lower TAN and nitrite concentrations as well as the improved growth, biomass output, and food and protein conversion efficiency of shrimp [22]. Overall, the use of BFT in aquaculture has demonstrated several advantages in terms of water quality, sustainability, and productivity [23, 24].

While there are some studies available, none have yet compiled the results of how BFT improves water parameters. These results can be gathered through various methods such as reviews, systematic reviews, synthetic analysis, and metaanalysis. Consequently, this study aims to systematically compile previous findings using meta-analysis to investigate how water quality parameters affect the implementation of BFT in aquaculture. Meta-analysis is a statistical technique that merges the results of multiple independent studies that are deemed to be "combinable." It is best to think of meta-analysis as an empirical observational research. Well-reported metaanalyses offer a more impartial evaluation of the data, deliver a more precise estimation of treatment effects, and may help to clarify variations in findings across various studies, as opposed to traditional narrative reviews [25]. A good metaanalysis should cover all pertinent studies in complete detail. It also examines the heterogeneity of the key findings and uses sensitivity analysis to investigate their robustness [26].

#### 2. Materials and Methods

2.1. Systematic Literature Search. Following PRISMA guidelines, a systematic and thorough literature search was performed to examine the impact of BFT on water quality parameters in aquaculture [27]. The search results for each step of the PRISMA workflow are shown in Figure 1. Comprehensive details concerning individual studies are also presented in Table S1.

Seven distinct keyword strings (as shown in Figure 1) were utilized to locate the appropriate database via the Web of Science. The results of each string were saved as CSV files, which were subsequently combined into a



FIGURE 1: The PRISMA flow diagram showing steps for the systematic literature search during this study.

Microsoft Excel file to ensure uniform formatting (i.e., containing identical information for all studies). Following this, duplicate entries were removed from the consolidated data file.

Initially, all articles were screened based on their titles and abstracts. Subsequently, only those studies that experimentally evaluated the impact of BFT on eight water quality parameters in aquaculture, including alkalinity, DO, ammonium (NH<sub>3</sub>–N), nitrite (NO<sub>2</sub>–N), nitrate (NO<sub>3</sub>–N), pH, TAN, and TSS, were selected for further analysis. For this study, only those studies (or individual experiments within studies) that directly assessed the water quality parameters linked to the impact of BFT on aquaculture water were considered eligible. Some selected studies were excluded if the analyzed water quality parameters were deemed irrelevant to BFT or if no standard deviation (SD) or standard error (SE) was reported. After applying these criteria, a total of 33 studies were identified as eligible for inclusion in this metaanalysis (Figure 1).

2.2. Data Collection. For each of the 33 selected studies, various qualitative and quantitative attributes were gathered, including general bibliographic information (e.g., authors, year of publication), species tested, fish species group, life stage of the species, treatment name, treatment level, control variables, response of water quality parameter, and unit of response. For each eligible experiment, the mean and SD or SE values of the treatment and control groups, as well as the sample size (n), were obtained directly from the text or tables and graphs within the article. In some cases, ImageJ software was used to extract data from the graphs presented in the article. In addition to collecting the relevant data for each experiment, the data source was also recorded, specifying exactly from where the data were collected. Any missing information was noted in the comment section. Furthermore, to ensure consistency, all SE values were converted to SD values before conducting the analysis.

2.3. Meta-Analysis. To compute the effect size and variances, the standardized mean difference (SMD) was utilized as the

outcome measure in the analysis. The below equations were employed for this purpose [28]:

$$\mathrm{SMD}^{a} = \frac{(M2 - M1)}{\mathrm{SD}p}J,\tag{1}$$

where

Sampling variance 
$$= \frac{N1 + N2}{N1N2} + \frac{\text{SMD2}}{2(N1 + N2)}, \quad (2)$$

where  $M_1$  is the mean of control group,  $M_2$  is the mean of treatment group,  $SD_p$  is the pooled standard deviation, J is a small sample correction,  $N_1$  is the sample size of control group, and  $N_2$  is the sample size of treatment group.

$${}^{a}J = 1 - \frac{3}{4(N1 + N2 - 2) - 1},$$
(3)

$$SDp = \sqrt{\frac{(N1-1)SD12 + (N2-1)SD22}{(N1+N2-2)}},$$
 (4)

where  $SD_1$  is the sample standard deviation of control group and  $SD_2$  is the sample standard deviation of treatment group.

The data was fitted to a random-effects model. To estimate the amount of heterogeneity (i.e.,  $\tau^2$ ), the restricted maximum-likelihood estimator was used [29]. The analysis also reported the *Q*-test for heterogeneity and the  $I^2$  statistic [30], in addition to the estimate of  $\tau^2$  [31]. If any amount of heterogeneity was detected (i.e.,  $\tau^2 > 0$ ), regardless of the *Q*-test results), the analysis provided a prediction interval for the true outcomes. The analysis was conducted using R (version 4.0.5) [32] and the metafor package (version 3.4.0) [33]. A subset of qualitative and quantitative factors collected from each experiment were explored as potential factors that could explain the variation in effect size. These factors included the study, year of publication, study species, life



FIGURE 2: Publication years of the eligible studies for the meta-analysis database.

stage of study species, aquaculture system, experimental biofloc compounds, and control compounds, among others. Studentized residuals and Cook's distances were used to identify potential outliers and influential studies in the meta-analysis [34]. Studies with a studentized residual above the  $100 \times$  $(1-0.05/(2 \times k))^{\text{th}}$  percentile of a standard normal distribution (using a Bonferroni correction with two-sided  $\alpha = 0.05$  for *k* studies) were considered as potential outliers. Studies with a Cook's distance larger than the median plus six times the interquartile range of the Cook's distances were considered as influential. The asymmetry of the funnel plot was examined using the rank correlation test [35] and the regression test [36], with the SE of the observed outcomes as the predictor.

#### 3. Results

3.1. Overview of Included Studies. A total of 33 studies published between 2012 and 2020 that examined the effects of BFT on different water quality parameters in aquaculture systems were eligible for the meta-analysis database (Figure 2). The studies were started searching on November 8, 2020 and finished on December 10, 2020. The review found the highest number of eligible studies in 2019 (7 studies), followed by 2018 (6 studies), while the lowest number of eligible publications (1 study) was found in 2014 (Figure 2).

The number of studies was greatest in Asia (17 studies), followed by Europe (6 studies), South America, and North America (5 studies in each continent) (Figure 3(a)). The highest number of studies were conducted in China (8 publications), followed by Brazil (5 publications), and other countries (Figure 3(b)).

The studies included 16 different fish culture systems (monoculture and mixed culture of various species) in which monoculture of *L. vannamei* was received the most interest (in 15 studies), followed by the monoculture of *Oreochromis niloticus* (3 studies per species), and carps mixed culture (2 studies) (Figure 4).

The review revealed that the highest number of studies (n=8) used only molasses as a biofloc compound (n=10) (Figure 5).

3.2. Effects on Water Alkalinity. In this analysis, two different analyses were conducted-one with individual experiments and another with aggregated outcomes. In the analysis with individual experiments, 11 multilevel experiments from eight studies were included, while in the analysis with aggregated outcomes, 8 studies were included. The observed SMDs ranged from -6.01 to 12.16, and aggregated outcomes ranged from -6.01 to 7.47, with the majority of estimates being negative. The random-effects model was used to estimate the average SMD, which was found to be 0.53 with a 95% confidence interval (CI) of -1.78 to 2.84. This finding shows that the average outcome did not differ significantly from zero (z=0.45, p=0.65). The estimated average outcome based on the random-effects model was found to be 0.19 with a 95% CI of -2.14 to 2.51. This means that the average outcome did not differ significantly from zero (z = 0.16, p =0.87). Since the estimated average outcome did not differ significantly from zero in both analyses, indicating that there was no overall effect of BFT on the water quality parameters in aquaculture.

3.3. Effects on DO. There were two different analyses that were conducted one with individual experiments and another with aggregated outcomes. In the analysis with individual experiments, 33 multilevel experiments from 27 studies were included, while in the analysis with aggregated outcomes, 27 studies were included. The observed SMDs ranged from -76.35 to 3.57, and the aggregated outcomes ranged from -76.35 to 3.57, with the majority of estimates being negative. The random-effects model was used to estimate the average SMD, which was found to be -0.32 with a 95% CI of -0.72 to 0.07. This finding shows that the average outcome did not differ significantly from zero (z = -1.59, p = 0.11). The estimated

#### Aquaculture Nutrition



FIGURE 3: Indications distribution of biofloc technology experiments investigating effects on water quality across (a) the continent and (b) countries where BFT on water quality experiments were conducted.



FIGURE 4: List of different fish species that were used in the eligible studies for meta-analysis.



FIGURE 5: List of biofloc compounds that were used in the biofloc systems for the eligible studies for meta-analysis.

average outcome based on the random-effects model was  $\mu = -0.51$  (95% CI: -1.09 to 0.07). Therefore, the average outcome did not differ significantly from zero (z = -1.71, p = 0.09). Since the estimated average outcome did not differ significantly from zero in both analyses, indicating that there was no overall effect of BFT on the growth performance of aquaculture species.

3.4. Effects on NH<sub>3</sub>-N. Two different analyses were conducted in this analysis-one with individual experiments and another with aggregated outcomes. In the analysis with individual experiments, nine multilevel experiments from seven studies were included, while in the analysis with the aggregated outcomes, seven studies were included. The observed SMDs ranged from -32.87 to 2.65, and aggregated outcomes ranged from -32.87 to 2.64, with the majority of estimates being negative. The random-effects model was used to estimate the average SMD, which was found to be 0.46 with a 95% CI of -0.49 to 1.42. This finding shows that the average outcome did not differ significantly from zero (z = 0.95, p = 0.34). The estimated average outcome based on the random-effects model was  $\mu = 0.21$  (95% CI: -0.48 to 0.91). Therefore, the average outcome did not differ significantly from zero (z = 0.61, p = 0.54). Since the estimated average outcome did not differ significantly from zero in both analyses, indicating that there was no overall effect of BFT on the growth performance of fish.

3.5. Effects on  $NO_2$ –N. The analysis included 33 multilevel experiments from 24 studies, with observed SMDs ranging from -117.06 to 5.99, and the majority of estimates being negative. The random-effects model was used to estimate the

average SMD, which was found to be  $\mu = -1.12$  with a 95% CI of -2.06 to -0.19. This result indicates that the average outcome differed significantly from zero (z = -2.35, p = 0.02). A forest plot showing the observed outcomes and the estimate based on the random-effects model is shown in Figure 6.

The *Q*-test results suggest that the true outcomes are heterogeneous ( $Q(32) = 172.99, p < 0.001, \tau^2 = 5.99, I^2 = 88.83\%$ ). A 95% prediction interval for the true outcomes is -6.01 to 3.76. Therefore, while the average outcome is estimated to be negative, the true outcome may be positive in some studies.

An examination of the studentized residuals revealed that one study [37] had a value larger than  $\pm 3.00$  and may be a potential outlier in the context of this model. According to Cook's distance, it is apparent that this study [37] might have had an overly influential impact. A funnel plot of the estimates is shown in Figure 7. Both the rank correlation and the regression test indicated the funnel plot asymmetry (p < 0.001 and p < 0.01, respectively).

The Baujat plot also showed the study of Soto-Alcalá et al. [37] has higher values on the *x*-axis that may be considered an overly influential case on the overall results (Figure 8). The studies of Kaya et al. [38], Luis-Villaseñor et al. [22], Du et al. [39], and so forth have higher values on the *y*-axis that may also be considered an overly influential case on the overall results (Figure 8).

The sensitivity analysis was done by removing the outliers which showed almost the same significant negative overall effect size  $\mu = -1.10$  (95% CI: -2.03 to -0.16), (z = -2.30, p = 0.02).

The aggregated analysis included 24 studies, with observed aggregated outcomes ranging from -117.06 to

Cang et al., 2019 [15] de Souza et al., 2019 [40] Deb et al., 2020 [19].1 Deb et al., 2020 [19].2 Deb et al., 2020 [19].3 Du et al., 2018 [39]

Emerenciano et al., 2012 [48].1 Emerenciano et al., 2012 [48].2 Kamilya et al., 2017 [54] Kaya et al., 2020 [38].1 Kaya et al., 2020 [38].2 Kaya et al., 2020 [38].3 Li et al., 2019 [55] Long et al., 2015 [45]

Luis–Villaseñor et al., 2016 [22] da Silva Martins et al., 2015 [50]

Nhi et al., 2018 [56] Ray et al., 2017 [43] Sgnaulin et al., 2018 [44]

Shang et al., 2018 [42].1

Shang et al., 2018 [42].2

Shang et al., 2018 [42].3

Suita et al., 2015 [20].1

Suita et al., 2015 [20].2

Vinatea et al., 2018 [47].1 Vinatea et al., 2018 [47].2

Xu and Pan, 2013 [49].1

Xu and Pan, 2013 [49].2

Yun et al., 2016 [52]

Zhao et al., 2012 [53]

RE model

Xu et al., 2013 [51]

Soto-Alcalá et al., 2019 [37]

Tepaamorndech et al., 2020 [46)

Study

SMD [95% CI]	
-3.86 [-6.56, -1.15]	: 
-3.77 [-6.44, -1.10]	
5.07 [1.79, 8.35]	<b>+⊞- </b>
0.78 [-0.88, 2.44]	i i i i i i i i i i i i i i i i i i i
1.97 [0.02, 3.92]	
-12.19 [-19.26, -5.11]	⊢∎⊣
-0.50 [-2.13, 1.12]	, in the second s
-0.50 [-2.13, 1.12]	i i i i i i i i i i i i i i i i i i i
0.00 [-1.60, 1.60]	
-5.50 [-7.24, -3.75]	<b>•</b>
-3.04 [-4.22, -1.87]	
-0.20 [-1.80, 1.40]	
-0.13 [-1.37, 1.11]	•
-5.96 [-9.69, -2.23]	H
-8.41 [-13.44, -3.39]	H=-
-1.82 [-3.72, 0.08]	<b>.</b>
0.19 [-0.61, 1.00]	•
6.00 [2.24, 9.75]	F∎-I

0.39 [-1.22, 2.01]

-6.72 [-10.85, -2.60]

-5.96 [-9.70, -2.23]

-3.96 [-6.72, -1.21]

-0.43 [-1.83, 0.97]

0.23 [-1.01, 1.48]

-0.18 [-1.78, 1.43] -0.16 [-1.55, 1.23]

0.42 [-0.98, 1.82]

0.98 [-0.48, 2.45]

0.49 [-0.91, 1.90]

-0.62 [-2.04, 0.80]

0.18 [-1.42, 1.78]

-1.53 [-3.34, 0.29]

-1.12 [-2.06, -0.19]

50

-117.06 [-183.32, -50.81]

Standardized mean difference FIGURE 6: Forest plot reporting the effect size (standardized mean differences) for  $NO_2$ –N in different studies of BFT having multiple experimental levels. Mean and 95% confidence interval (CI) effect sizes are reported. The estimated summary effect size is indicated at the bottom of the figure (cyan diamond shape). When a study includes multiple treatment levels, it is presented with a "numeric number" after the publication year.

-50

-100

5.99, and the majority of estimates were negative. The random-effects model was used to estimate the average outcome, which was found to be  $\mu = -1.15$  with a 95% CI of -2.23 to -0.07. This result indicates that the aggregated analysis still shows a significant negative effect size (z = -2.09, p = 0.04). Figure 9 displays a forest plot depicting the

-200

-150

observed outcomes and the estimates derived from the random-effects model.

≬:

0

The sensitivity analysis was done for this aggregated effect size model by removing the outliers which showed almost the same significant negative overall pooled effect size  $\mu = -1.12$  (95% CI: -2.19 to -0.05), (z = -2.04, p = 0.04).



FIGURE 7: Funnel plot depicting the effect size of each study (x-axis) for NO<sub>2</sub>–N in relation to the precision of that study (SE; y-axis). Here points are not scattered symmetrically within the funnel suggesting a publication bias.



FIGURE 8: Baujat plot showing the heterogeneity in  $NO_2$ -N meta-analytic data. The plot shows the overall heterogeneity contribution of each effect size in the *x*-axis, while the *y*-axis shows the influence of each effect size on the pooled result.

3.6. Effects on  $NO_3$ –N. The findings show that both the individual experiment analysis and the aggregated analysis did not find a significant difference in the outcomes. In the analysis of individual experiments (32 multilevel experiments), the estimated average SMDs ranged from –56.33 to 19.83, and these

differences did not exhibit significant deviations from zero (z=0.62, p=0.54). Similarly, in the aggregated analysis involving 23 studies, the estimated average outcome also did not significantly differ from zero (z=0.77, p=0.44). However, it should be noted that there is still considerable heterogeneity

Study

Cang et al., 2019 [15]	en e	-3.86 [-6.56, -1.15]
de Souza et al., 2019 [40]	H	-3.77 [-6.44, -1.10]
Deb et al., 2020 [19]	н	2.61 [0.20, 5.01]
Du et al., 2018 [39]	⊢∎⊣	-12.19 [-19.26, -5.11]
Emerenciano et al., 2012 [48]	•	-0.50 [-2.13, 1.12]
Kamilya et al., 2017 [54]	i de la companya de l	0.00 [-1.60, 1.60]
Kaya et al., 2020 [38]	•	-3.82 [-5.32, -2.31]
Li et al., 2019 [55]	•	-0.13 [-1.37, 1.11]
Long et al., 2015 [45]	⊢∎+1	-5.96 [-9.69, -2.23]
Luis–Villaseñor et al., 2016 [22]	HEH	-8.41 [-13.44, -3.39]
da Silva Martins et al., 2015 [50]		-1.82 [-3.72, 0.08]
Nhi et al., 2018 [56]	•	0.19 [-0.61, 1.00]
Ray et al., 2017 [43]		
Sgnaulin et al., 2018 [44]	- •	0.39 [-1.22, 2.01]
Shang et al., 2018 [42]	H#H	-5.55 [-9.14, -1.96]
Soto–Alcalá et al., 2019 [37]	I	-117.06 [-183.32, -50.81]
Suita et al., 2015 [20]		-0.06 [-1.38, 1.26]
Tepaamorndech et al., 2020 [46]	•	-0.18 [-1.78, 1.43]
Vinatea et al., 2018 [47]	÷	0.13 [-1.27, 1.52]
Xu and Pan, 2013 [51]	-	0.98 [-0.48, 2.45]
Xu and Pan, 2013 [49]		-0.06 [-1.47, 1.35]
Yun et al., 2016 [52]	- •	0.18 [-1.42, 1.78]
Zhao et al., 2012 [53]		-1.53 [-3.34, 0.29]
RE model	•	-1.19 [-2.32, -0.06]
-200	-150 -100 -50 0 Standardized mean difference	50

FIGURE 9: Forest plot reporting the aggregated effect size (standardized mean differences) for  $NO_2$ –N in different studies of BFT. Mean and 95% confidence interval (CI) effect sizes are reported. The estimated summary effect size is indicated at the bottom of the figure (cyan diamond shape).

in the data, as indicated by the wide range of observed outcomes and the fact that the majority of estimates were negative.

3.7. Effects on pH. The analysis included data from 33 multilevel experiments across 26 studies. The observed SMDs ranged from -15.95 to 31.92, with most estimates being negative (64%). Using the random-effects model, the estimated average SMD was  $\mu = -0.75$  (95% CI: -1.82 to 0.33), indicating that the average outcome was not significantly different from zero (z = -1.36, p = 0.17).

In the aggregated analysis of multiple experiments, a total of 26 studies were included, and the observed outcomes ranged from -15.95 to 31.92, with the majority of estimates being negative (65%). Based on the random-effects model, the estimated average outcome was  $\mu = -0.97$  (95% CI: -2.15

to 0.21), indicating that the average outcome was not significantly different from zero (z = -1.61, p = 0.11).

3.8. Effects on TAN. The analysis included data from 32 multilevel experiments across 24 studies. The observed SMDs ranged from -19.86 to 38.62, with most estimates being negative (56%). Using the random-effects model, the estimated average SMD was  $\mu = -0.70$  (95% CI: -2.08 to 0.67), indicating that the average outcome was not significantly different from zero (z = -0.99, p = 0.32).

In the aggregated analysis of multiple experiments, a total of 24 studies were included, and the observed outcomes ranged from -19.86 to 38.61, with the majority of estimates being negative (50%). Based on the random-effects model, the estimated average outcome was  $\mu = -0.19$  (95% CI: -2.28

SMD [95% CI]



FIGURE 10: Forest plot reporting the effect size (standardized mean differences) for TSS in different studies of BFT having multiple experimental levels. Mean and 95% confidence interval (CI) effect sizes are reported. The estimated summary effect size is indicated at the bottom of the figure (cyan diamond shape). When a study includes multiple treatment levels, it is presented with a "numeric number" after the publication year.

to 1.89), indicating that the average outcome was not significantly different from zero (z = -0.19, p = 0.85).

3.9. Effects on TSS. The analysis comprised data from 24 multilevel experiments across 16 studies. The observed SMDs ranged from -6.32 to 16.66, with most estimates being positive (92%). Using the random-effects model, the estimated average SMD was  $\mu = 4.81$  (95% CI: 3.05–6.56), indicating that the average outcome differed significantly from zero (z=5.37, p<0.0001). Figure 10 displays a forest plot illustrating the observed outcomes and the estimate based on the random-effects model.

The *Q*-test indicated that the true outcomes exhibit heterogeneity (*Q*(23) = 130.13, p < 0.0001,  $\tau^2 = 14.99$ ,  $I^2 = 87.82\%$ ). A 95% prediction interval for the true outcomes is -2.98 to 12.59. Thus, while the average outcome is estimated to be positive, the true outcome may be negative in some studies.



FIGURE 11: Funnel plot depicting the effect size of each study (*x*-axis) for TSS in relation to the precision of that study (SE; *y*-axis). Here points are not scattered symmetrically within the funnel suggesting a publication bias.



FIGURE 12: Baujat plot showing the heterogeneity in TSS meta-analytic data. The plot shows the overall heterogeneity contribution of each effect size in the *x*-axis, while the *y*-axis shows the influence of each effect size on the pooled result.

Upon analyzing the studentized residuals, it was observed that none of the studies had a value greater than  $\pm 3.08$ . Hence, there was no evidence of outliers in the context of this model. As per Cook's distances, none of the

studies could be classified as overly influential. A funnel plot of the estimates is depicted in Figure 11. The rank correlation and regression test both indicated potential funnel plot asymmetry (p<0.0001 and p<0.0001, respectively).



FIGURE 13: Forest plot reporting the aggregated effect size (standardized mean differences) for TSS in different studies of BFT. Mean and 95% confidence interval (CI) effect sizes are reported. The estimated summary effect size is indicated at the bottom of the figure (cyan diamond shape).

The Baujat plot analysis highlighted that the study conducted by de Souza et al. [40] has higher values on the *x*-axis, which could be considered an overly influential case on the overall results (Figure 12). In addition, the studies of Deb et al. [19], Kumar et al. [41], and others have higher values on the *y*-axis, which may also be considered overly influential cases on the overall results (Figure 12).

The sensitivity analysis was done by removing the outliers which showed almost the same significant positive overall effect size  $\mu = 5.08$  (95% CI: 3.54–6.63, z = 6.46, p < 0.0001).

The results of the aggregated analysis on multiple treatments showed that 16 studies were considered. The observed outcomes ranged from -6.32 to 16.66, with most of the estimates being positive (94%). The estimated average outcome using the random-effects model was  $\mu = 5.25$  (95% CI: 2.97–7.53). This indicates that the average outcome significantly differed from zero (z = 4.51, p < 0.0001). A forest plot that displays the observed outcomes and the estimate based on the random-effects model can be seen in Figure 13.

The sensitivity analysis was done for this aggregated effect size model by removing the outliers which showed almost the same significant positive overall pooled effect size  $\mu = 5.68$  (95% CI: 3.87–7.49, z = 6.15, p < 0.0001).

### 4. Discussion

Maintaining and monitoring water quality in aquaculture is crucial for the performance of the production cycle in BFT. In aquaculture, organic matter and nitrogen waste is a major issue for a good and sustainable production [18]. High carbon-to-nitrogen (C:N) ratios are used to maintain water quality parameters because heterotrophic bacteria can readily absorb nitrogenous byproducts for both maintenance (respiration, feeding, motility, digestion, and so forth) as well as for growth and to form new bacterial cells [18]. In BFT, the stability of zero or minimal water exchange is maintained by the complex interactions among various microorganisms, including bacteria, microalgae, fungi, protozoans, nematodes, rotifers, and others. These microorganisms form a complex ecosystem that helps to maintain water quality by breaking down organic waste materials and converting them into a form that can be used as food for fish. This not only helps to reduce the need for water exchange but also increases the efficiency of the system and produces highvalue fish food. The stability of this system depends on the careful management of the microbial communities to maintain a balance between the various species and their interactions [18].

The rise in the number of publications in recent years (Figure 2) revealed the attention of researchers about the influence of BFT on water quality parameters in aquaculture. Based on the analysis, Asian countries take the lead in research on this particular topic (Figure 3(a)), which is not surprising because these Asian countries offer favorable opportunities and advanced research facilities, and they allocate substantial funding toward conducting research in these areas. Among various countries, China ranked the highest in publications (eight studies) on this topic, followed by Brazil (five studies), Mexico (four studies), and other countries (Figure 3(b)). Unfortunately, no African country was found to conduct any research on this topic that has been eligible for this meta-analysis. It would be beneficial for impoverished countries, particularly those in Africa with abundant fisheries resources, to partner with affluent countries that are interested in exploring the effects of BFT on the water quality parameters of various cultured fish species. Although 16 species were investigated during these studies (Figure 4), more species should be included in the future research, particularly many commercially important species are required to know how BFT can influence the water quality parameters for the sustainable production of these species. The review showed the use of a variety of biofloc compounds in the eligible studies including molasses, glucose, sucrose, tapioca flour, cornmeal, seaweed, and so forth. According to the analysis, the majority of studies employed molasses as the biofloc compound (as shown in Figure 5). However, it is recommended that future research be conducted using locally available, cost-effective, and environmentally friendly BFT compounds to promote sustainable aquaculture.

In the present study, a significant negative effect of NO2-N on BFT has been revealed. Most of the reviewed studies showed that NO<sub>2</sub>–N was significantly lower in BFT than the control group. For example, Du et al. [39] used glucose and starch along with heterotrophic bacteria (Bacillus sp.) as the organic carbon sources in BFT treatment and found that NO<sub>2</sub>-N was significantly lower in BFT than that of the control group. It might be the cause of the fact that in the BFT system, heterotrophic bacteria play an important role in removing nitrogen from the water. As heterotrophic bacteria consume organic carbon, they also increase their demand for nitrogen, which can lead to competition with phytoplankton for this nutrient. However, heterotrophic bacteria are generally better at utilizing ammonia than phytoplankton, which allows them to outcompete the latter for nitrogen and remove more dissolved nitrogen from the water. This process can help to maintain water quality and promote the growth of high-value fish food in the BFT system [59]. In a study, Luis-Villaseñor et al. [22] utilized cornmeal as a carbon source and observed a decrease in NO2-N concentration. This is attributed to the fact that adding cornmeal to the carbon cycle maintains the appropriate C:N

ratio that is necessary for bacteria to convert these harmful nitrogen molecules into single-cell proteins. Kaya et al. [38] used sugar beet molasses as a carbon source to stimulate the growth of heterotrophic bacteria in a BFT system and found that the concentration of NO2-N was significantly lower in the BFT system than in the control system. This suggests that the biofloc formation process can help remove nitrogenous compounds from the water in BFT systems. Based on the study by Cang et al. [15], the use of molasses in the BFT system led to an increase in NO2-N concentration, which peaked at day 12 and then decreased to undetectable levels as the bioflocs developed. By including a carbon source in the BFT, heterotrophic bacteria are encouraged to flourish, which aids in the conversion of these harmful N-compounds into single-cell proteins [42]. In the present study, the study of Soto-Alcalá et al. [37] was found to be overly influential. They used molasses as a carbon source for biofloc formation and found lower NO2-N concentration in BFT than control group. NO<sub>2</sub>-N is a crucial element in determining the success of aquaculture. High levels of NO<sub>2</sub>-N can be toxic to fish. According to Montealegre et al. [60], even at low concentrations, NO<sub>2</sub>–N can harm fish by reducing oxygen uptake and causing methemoglobinemia, a condition where the hemoglobin in the blood is unable to bind and transport oxygen effectively. Boyd and Pillai [61] also reported that NO<sub>2</sub>-N levels above 1.0 mg/L in pond water can cause fish mortality. Therefore, it is essential to maintain low levels of NO<sub>2</sub>-N in aquaculture systems to ensure the health and survival of fish. They found overall negative effects of NO2-N in BFT, however, some reviewed studies showed positive effects. For example, Ray et al. [43] used a foam fractionator to manage solids concentration and an external biofilter for nitrogen control and found that NO2-N contributed a significantly higher concentration in the biofloc treatment. In systems with a functioning nitrifying bacterial population, nitrogen concentration did not appear to accumulate as would be predicted; this could mean that the floc community was not fully developed. This is not clear why such continuous accumulation did not take place [43]. Sgnaulin et al. [44] used corn meal, wheat meal, and molasses as carbon source and found that NO2-N concentration was higher than the control group. When fish are exposed to high levels of nitrite, they may experience an increase in plasma methemoglobin and nitrite levels, which can lead to hypoxia (lack of oxygen), stress, reduced feed intake, and reduced growth, and in severe cases, it can lead to death [62-64].

The analysis of multiple studies indicated that there was a noteworthy favorable impact of TSS on BFT, with a general positive outcome exhibited across various studies. For example, Kumar et al. [41] used glucose in BFT and found that TSS recorded significantly higher in biofloc group as compared to the clear water group. Similarly, Luis-Villaseñor et al. [22] employed glucose in their study and determined that the TSS value was significantly greater in the biofloc group when compared to the control group. Long et al. [45] used glucose as a carbon source and found that the TSS level in the BFT group increased gradually throughout the experimental period. The average concentration was 24.61 mg/L in

the control group, while 484.48 mg/L in BFT treatment [45]. Ray et al. [43] demonstrated that the TSS concentration in BFT tanks should be well controlled because the anticlogging of fish gills and water quality are intimately connected. According to Kim et al. [57], TSS was found to be significantly higher in all biofloc treatments compared to seawater. This was attributed to the fact that the water source for the biofloc treatment was provided daily throughout the trial using an intensive shrimp production system with zero exchange. In a study, Cang et al. [15] utilized molasses as a carbon source and observed an increase in TSS with the development of bioflocs in the BFT system. The TSS levels were found to be much higher in the biofloc treatment group compared to the control group, which was attributed to the water exchange in the control group. TSS is heavily reliant on pond conditions and management techniques including feed, water quality parameters, stocking density, and culture period [19]. The formation of bioflocs in the BFT group was found to be related to the TSS concentration. A TSS concentration range of 400-600 mg/L was considered to be suitable for the superintensive culture of L. vannamei [65]. According to Schveitzer et al. [66], maintaining good water quality becomes difficult when the TSS concentration is lower than 100 mg/L. Schveitzer et al. [66] also reported that a high TSS concentration  $(\geq 800 \text{ mg/L})$  could become a stressor for shrimp respiration as the suspended solids may clog the gills. In contrast, de Souza et al. [40] reported a different finding where the TSS was found to be significantly lower in the biofloc treatment group compared to the clear water group. This result was observed after using molasses as a carbon source in the BFT system. It is unclear why such biofloc production did not occur in the BFT system.

Although no significant effect of alkalinity on BFT has been revealed in this study, an overall negative effect was shown by different studies. For example, de Souza et al. [40] and Tepaamorndech et al. [46] used molasses, Kumar et al. [21] used molasses, sugar, and wheat, Suita et al. [20] used glucose, Vinatea et al. [47] used glucose as a carbon source in BFT, and resulted in lower alkalinity values in the BFT system compared to the control. This was most likely caused by nitrification, which increased the amount of carbon dioxide in the water used in the biofloc treatment, and by the respiration of heterotrophic organisms [67, 68]. The study conducted by Putra et al. [69] effectively illuminated the potential of biofloc application within aquaculture systems to adeptly regulate TAN. Probiotic bacteria present within biofloc facilitate the conversion of ammonia into nontoxic compounds, including nitrate, thus fostering an essential nutrient source for phytoplankton growth. This transformative process in turn leads to reduced concentrations of ammonia and nitrate in the culture medium. Similarly, Luo et al. [70] emphasized the pivotal roles played by carbonaceous biofloc nitrogen (CBN) and the activity of heterotrophic bacteria in preserving water quality within biofloc-based aquaculture systems, even under circumstances of minimal water exchange. Of notable significance is the substantial consumption of alkalinity that accompanies the assimilation of CBN.

Furthermore, the research conducted by Kumar et al. [21] corroborates these observations. Their work distinctly indicates a notable reduction in the levels of TAN and nitrite (NO<sub>2</sub>–N) within biofloc ponds, contrasting with alternative systems. This phenomenon can be attributed to the flourishing microbial biomass within the biofloc environment. These microorganisms proficiently direct their energy toward assimilating and transforming toxic inorganic nitrogen compounds into valuable proteins, thereby amplifying the accessibility of this nutrient pool for cultured shrimp. In concert, these studies highlight the intricate interplay of biofloc dynamics, microbial processes, and nutrient conversions. These interactions significantly contribute to the regulation of ammonia levels, concurrently underscoring the active involvement of nitrifiers in alkalinity consumption, ultimately influencing water buffering capacity. On the other hand, there are some studies that showed positive effect sizes. Kaya et al. [38] used molasses as a carbon source in BFT and found that the alkalinity parameters were higher in the BFT tanks compared to the control tanks in both trials. Jatoba et al. [71] used sugar and powdered diet, while Sgnaulin et al. [44] used cornmeal, wheatmeal, and molasses as carbon source who also showed higher alkalinity in BFT tanks compared to control tanks.

While this study did not uncover a noteworthy impact of DO on BFT, the meta-analysis demonstrated an overall adverse effect of DO on BFT. For example, in a study, Kaya et al. [72] demonstrated that DO values were lower in the biofloc treatment groups compared to the control groups. This was most likely caused by the higher oxygen requirements of heterotrophic bacteria in biofloc tanks [48, 49]. Long et al. [45] utilized glucose as a carbon source and reported that the DO level was maintained at a level greater than 6 mg/L, which differed significantly between the BFT and control groups. This was likely due to the higher respiration rates caused by bacteria and other microorganisms in the BFT group, as reported by Emerenciano et al. [48] and Kim et al. [58]. However, the DO level in the BFT treatment was still within an acceptable range for the survival and growth of fish. Similar results were recorded by da Silva Martins et al. [50], Kumar et al. [41], de Souza et al. [40], and Chan-Vivas et al. [73]. But there are some studies that showed positive results. For example, in a study, Vinatea et al. [47] discovered that the DO levels in the BFT tanks of *Mugil cephalus* were markedly greater compared to those in the RAS. This was due to the addition of pure oxygen to meet the high oxygen demand resulting from bacterial respiration of the bioflocs, particularly during the administration of glucose, as proposed by Avnimelech [9]. However, the researchers noted that pure oxygen supplementation was necessary for the M. cephalus BFT tanks. This was perhaps due to the significantly higher swimming activity of M. cephalus compared to that of Tinca tinca, and the fact that the fish exhibited more excitement and approached the surface eagerly for food, while the T. tinca remained inactive at the bottom of the tank [47]. The study conducted by Kumar et al. [21] indicated that the biofloc pond had DO levels that were significantly greater, yet still

within an appropriate range for the culture of *P. vannamei*. This finding implied that aeration in the biofloc pond had a beneficial effect.

This study did not uncover a statistically significant impact of NH<sub>3</sub>-N on BFT; however, other studies have shown a beneficial effect of NH<sub>3</sub>-N on BFT. For instance, Vinatea et al. [47] employed glucose as a carbon source and observed that the NH<sub>3</sub>-N concentration was greater in the BFT group than in the control group. It is worth noting that in biofloc-based cultures, the utilization of carbohydrates can aid in regulating ammonia levels, as previously noted by Avnimelech [9]. Despite spreading out the glucose additions over the day (at 8 and 12 hr in the morning and at 15 and 18 hr in the afternoon), the high glucose demands in BFT, combined with the use of limited water and high-protein feeds, can still result in a reduction in oxygen concentration [47]. Kaya et al. [38] conducted two trials with different stocking densities (trial I: 20 shrimp/0.24 m<sup>2</sup>, trial II: 10, 20, 30, 40 shrimp/m<sup>2</sup>). In trial I, the  $NH_3$ -N concentration was lower in the BFT group than the control group. However, in trial II, the NH<sub>3</sub>–N concentration was higher in the BFT group than the control group. This increase in ammonia level might be related to an increase in organic waste since the tanks were being stocked at higher densities in trial II. Some studies showed negative results too. For example, the studies conducted by Kumar et al. [21] and da Silva Martins et al. [50] showed a decrease in NH<sub>3</sub>–N concentration in the biofloc system when compared to the control, which was attributed to the consumption of NH<sub>3</sub>-N by the heterotrophic bacteria population in the biofloc. However, it is important to note that NH<sub>3</sub>-N cannot be completely eliminated from the system due to unaccounted nitrogen losses, such as denitrification and ammonia volatilization. In addition, certain studies have proposed that pH and temperature exert influence over the ammonia conversion within biofloc systems. Kumar et al. [21] noted a significant reduction in TAN and nitrite (NO<sub>2</sub>-N) concentrations within a biofloc pond compared to control systems, implying that pH could potentially play a role in ammonia conversion during their investigation. In a parallel study, Luo et al. [70] indicated that NH<sub>3</sub>-N concentration within biofloc systems is indeed influenced by pH and temperature. Considering the available information, it is reasonable to infer that TAN values can indeed impact NH<sub>3</sub>-N concentrations within biofloc systems. However, the precise relationship between TAN values, pH, and temperature remains to be definitively elucidated. Furthermore, it is worth highlighting that evidence suggests the duration of the study in biofloc systems can impact the abundance and composition of heterotrophic microorganisms, thereby potentially influencing system performance. For instance, Jamal et al. [74] demonstrated that the composition of biofloc, encompassing heterotrophic bacteria aggregates, algae, zooplankton, and other organic components, becomes discernible after a certain precipitation duration. In another study, Khanjani et al. [75] revealed that the prevalence of microorganisms within biofloc systems can change over the cultivation period, with pathogenic species becoming more prominent in the initial weeks.

It is important to note that nitrate levels in BFT can be influenced by various factors such as the carbon source used, stocking density, and water exchange rate. While some studies have shown higher nitrate concentrations in BFT, others have reported lower or similar levels compared to control groups. It is also worth mentioning that high NO<sub>3</sub>-N concentrations can be harmful to the culture system and can lead to eutrophication if not properly managed. Therefore, NO<sub>3</sub>-N levels should be monitored and controlled to ensure optimal water quality in BFT. Kim et al. [58] showed that NO<sub>3</sub>-N concentration was higher in BFT than control due to the extensive nitrification. Xu et al. [51] showed that the accumulation of NO<sub>3</sub>-N concentration occurred in the two bioflocs treatments. This demonstrated the presence of nitrifying bacteria in the bioflocs and suggested that both heterotrophic and autotrophic TAN removal mechanisms might have taken place in the system. Similar results were found by Kim et al. [57], de Souza et al. [40], Ray et al. [43], and Yun et al. [52]. Opposite results are also reported by several scientists published elsewhere. For example, in a study of Long et al. [45], NO<sub>3</sub>-N concentration was significantly higher in the control group compared to the BFT treatment, and showed a tendency to accumulate in the first 5 weeks of the culture period to levels of 11.64 mg/L. The highest NO<sub>3</sub>–N level in the BFT treatment was 5.45 mg/L, observed in week 3, which then gradually decreased over time. In week 8, the level first dropped and then increased once again. A possible explanation for the decrease in NO<sub>3</sub>-N concentration observed in the BFT treatment by Long et al. [45] could be denitrification is a process in which certain bacteria use nitrate as an electron acceptor in the absence of oxygen, converting it to nitrogen gas, which is released into the atmosphere. Given that this process is facilitated in anaerobic environments, such as the biofloc setting, it might contribute to the temporal reduction in NO3-N concentration. Furthermore, it is imperative to consider the dynamics of bacterial behavior across the course of the culture period, influenced by the nuances of water quality variables. These microorganisms can also assimilate NO<sub>3</sub>–N, a phenomenon documented by Allen et al. [76] and Schneider et al. [77]. Hence, the interplay of these biological processes within BFT systems is multifaceted and sensitive to intricate environmental factors. It is important to note that the results of Kumar et al. [21], Shang et al. [42], and Soto-Alcalá et al. [37] are in contrast to the findings of da Silva Martins et al. [50] and Long et al. [45], which showed higher NO<sub>3</sub>-N concentration in BFT tanks than in control tanks. The variation in results could be due to differences in experimental design, carbon source, and management practices. It is important to consider all available research when making decisions about the use of BFT in aquaculture systems.

While this study did not find any significant effect of TAN on BFT, this meta-analysis revealed an overall negative impact of TAN on BFT. For instance, in their research, Kumar et al. [21] noted a considerable reduction in TAN concentration in the biofloc pond compared to the control group when using molasses, sugar, and wheat as carbon sources. This decrease was attributed to an increase in the total heterotrophic bacterial population, which can consume TAN, as well as the autotrophic nitrification process. These

findings underscore the importance of maintaining a proper balance between carbon and nitrogen sources for reducing TAN levels in biofloc systems. de Souza et al. [40] suggested that BFT systems may offer advantages over other aquaculture systems in terms of reducing TAN levels and promoting water quality. However, it is important to note that the effectiveness of BFT may depend on a variety of factors, including stocking density, feeding regime, and management practices. Luis-Villaseñor et al. [22] showed that the use of additional carbon sources, such as cornmeal, can be an effective strategy for maintaining low TAN levels and promoting water quality in aquaculture systems. However, as with any aquaculture management practice, it is important to consider the specific needs and requirements of the species being cultured, as well as the environmental conditions and management practices used in the system. Xu et al. [49], Zhao et al. [53], Nguyen et al. [78], and Deb et al. [19] conducted research on the use of carbon sources in biofloc systems to reduce TAN levels. According to their findings, the addition of carbon sources, such as brown sugar, facilitated the removal of TAN in the system through the action of nitrifying bacteria and both heterotrophic and autotrophic TAN removal processes. The study by Kaya et al. [72] may be considered influential in that it challenges some of the assumptions and generalizations that have been made about the effectiveness of biofloc systems in reducing TAN levels. While BFT may offer certain advantages over other aquaculture systems, such as reduced water exchange and lower feed costs, it is important to carefully evaluate the potential benefits and limitations of this approach on a case-by-case basis. Therefore, the study by Kaya et al. [72] highlights the need for further research to better understand the complex interactions between diet, feeding rates, and the biofloc system in order to optimize the use of this approach in aquaculture. Vinatea et al. [47] conducted research on TAN concentrations in biofloc and RAS cultures of gray mullet and tench. According to their findings, TAN concentrations were consistently higher in BFT rearing tanks compared to RAS cultures. This suggests that the effectiveness of biofloc systems in reducing TAN levels may depend on a range of factors, including the specific species being cultured and the management practices employed. By contrast, Long et al. [45] found that TAN concentrations remained stable over time in the biofloc treatment group, even without water exchange, which suggests that the addition of a carbon source, such as glucose, can stimulate the growth of heterotrophic bacteria and facilitate the removal of TAN through the formation of biofloc microbes. This is supported by previous studies, such as Asaduzzaman et al. [79], Avnimelech [9], Ebeling et al. [80], and Emerenciano et al. [48], which have demonstrated the role of heterotrophic bacteria in the removal of TAN in biofloc systems through the formation of biofloc microbes. Overall, these findings suggest that the effectiveness of biofloc systems in reducing TAN levels may depend on a range of factors, including the specific species being cultured, the management practices employed, and the addition of carbon sources to stimulate the growth of heterotrophic bacteria. Further research is needed to better understand the

interactions between these factors and to optimize the use of biofloc systems in aquaculture.

Although this study did not find a significant impact of pH on BFT, this meta-analysis suggests an overall negative effect of pH on BFT. For instance, de Souza et al. [40] reported that the pH value in the BFT system was lower compared to the control group, likely due to the respiration of heterotrophic organisms, which increased the concentration of carbon dioxide in the biofloc-treated water [67, 68]. Kumar et al. [21] found that the pH was lower in the BFT system due to the dominance of heterotrophic bacteria over autotrophic bacteria. This is because heterotrophic bacteria consume organic matter in the water, producing carbon dioxide as a byproduct. The excess carbon dioxide in the water can then cause the pH to decrease. In addition, the process of respiration by microbes also leads to the excretion of carbon dioxide, which can further contribute to the decrease in pH. Therefore, the combination of heterotrophic dominance and microbial respiration can result in a lower pH in the BFT system. Kim et al. [58] found that the pH in the biofloc group was lower than that of the control group, which could be attributed to the substantial nitrification process that occurred in the biofloc system. During nitrification, ammonia is converted to nitrate and nitrite by autotrophic bacteria. This process releases hydrogen ions into the water, which can cause the pH to decrease. In addition, the respiration of heterotrophic organisms in the biofloc system can also contribute to a decrease in pH by producing carbon dioxide as a byproduct, which can increase the concentration of carbon dioxide in the water. This increase in carbon dioxide concentration can lead to the acidification of the water, resulting in a lower pH. Tacon et al. [81] and Wasielesky et al. [67] also reported similar findings regarding the influence of microbial respiration and nitrification on pH in biofloc systems. While some studies have reported a decrease in pH in biofloc systems, others have reported no significant difference or even an increase in pH compared to control systems. For example, da Silva Martins et al. [50] found that the pH values in the biofloc system were significantly higher than those in the control group, but still within acceptable limits for the survival and growth of L. vannamei. Similarly, Long et al. [45] reported a higher pH value in the biofloc system compared to the control, but the difference was not significant. Kaya et al. [72] also observed a higher pH value in the biofloc system compared to the control, but again, the difference was not significant. The variation in results across studies could be due to differences in the composition of the biofloc system, feeding regime, environmental conditions, or other factors that influence the microbial activity and pH in the system. Moreover, the nature of the BFT system may mediate pH effects. Heterotrophic systems depend on optimal pH for bacterial function [82]. By contrast, mixotrophic systems involve complex interactions between autotrophic algae and heterotrophic bacteria, wherein pH influences both groups [83]. This interplay can cascade throughout mixotrophic BFT systems, substantially impacting overall performance. Further research should elucidate how system configuration interacts with pH to affect BFT processes and productivity.

# 5. Conclusion

Based on the current literature review and meta-analysis, it can be concluded that the use of BFT has a positive impact on water quality parameters in aquaculture. BFT promotes the growth of a microbial community that utilizes the dissolved nitrogen from feces and uneaten food, converting it into microbial protein, and thus improving water quality. The study highlights that physico-chemical parameters, such as NO<sub>2</sub>-N, NO<sub>3</sub>-N, NH<sub>3</sub>-N, TAN, TSS, pH, DO, and alkalinity, are maintained at optimum levels in BFT systems. Moreover, the study acknowledges the complex nature of the physical, chemical, and biological interactions that occur within BFT systems. The findings of this meta-analysis can provide useful information for researchers and stakeholders interested in implementing BFT in aquaculture systems. It can also guide the proper maintenance and management of different water quality parameters in various aquaculture systems using BFT. Overall, the study provides compelling evidence for the effectiveness of BFT in improving water quality parameters in aquaculture. The findings of this meta-analysis can help promote the adoption of BFT in aquaculture systems to enhance water quality, reduce environmental impacts, and improve the overall sustainability of the aquaculture industry.

## Data Availability

All data generated or analyzed during this study are included in this article. Raw data are available upon request from the corresponding authors.

### **Conflicts of Interest**

The authors declare that they have no conflicts of interest.

# Acknowledgments

The authors are grateful to the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia and Fisheries and Marine Resource Technology Discipline of Khulna University, Bangladesh for collaboration and funding for this study. The authors extend their appreciation to the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia for funding this research work through the project number INST115.

### Supplementary Materials

Table S1: studies that assessed different water quality parameters in various biofloc aquaculture systems. (*Supplementary Materials*)

# References

 G. Abakari, G. Luo, E. O. Kombat, and E. H. Alhassan, "Supplemental carbon sources applied in biofloc technology aquaculture systems: types, effects and future research," *Reviews in Aquaculture*, vol. 13, no. 3, pp. 1193–1222, 2021.

- [2] I. Ahmad, A. M. Babitha Rani, A. K. Verma, and M. Maqsood, "Biofloc technology: an emerging avenue in aquatic animal healthcare and nutrition," *Aquaculture International*, vol. 25, no. 3, pp. 1215–1226, 2017.
- [3] R. Crab, B. Chielens, M. Wille, P. Bossier, and W. Verstraete, "The effect of different carbon sources on the nutritional value of bioflocs, a feed for *Macrobrachium rosenbergii* postlarvae," *Aquaculture Research*, vol. 41, no. 4, pp. 559–567, 2010.
- [4] M. H. Khanjani and M. Sharifinia, "Biofloc technology as a promising tool to improve aquaculture production," *Reviews in Aquaculture*, vol. 12, no. 3, pp. 1836–1850, 2020.
- [5] D. M. de Souza, S. M. Suita, L. A. Romano, W. Wasielesky Jr., and E. L. C. Ballester, "Use of molasses as a carbon source during the nursery rearing of *Farfantepenaeus brasiliensis* (Latreille, 1817) in a biofloc technology system," *Aquaculture Research*, vol. 45, no. 2, pp. 270–277, 2014.
- [6] N. Taw, "Biofloc technology expanding at white shrimp farms, glob," Aquaculture Advocate, pp. 24–26, 2010.
- [7] D. Krummenauer, T. Samocha, L. Poersch, G. Lara, and W. Wasielesky Jr, "The reuse of water on the culture of pacific white shrimp, *Litopenaeus vannamei*, in BFT system," *Journal of the World Aquaculture Society*, vol. 45, no. 1, pp. 3–14, 2014.
- [8] E. Cardona, Y. Gueguen, K. Magré et al., "Bacterial community characterization of water and intestine of the shrimp *Litopenaeus stylirostris* in a biofloc system," *BMC Microbiology*, vol. 16, no. 1, pp. 1–9, 2016.
- [9] Y. Avnimelech, "Carbon/nitrogen ratio as a control element in aquaculture systems," *Aquaculture*, vol. 176, no. 3-4, pp. 227– 235, 1999.
- [10] N. S. A. Bakar, N. M. Nasir, F. Lananan, S. H. A. Hamid, S. S. Lam, and A. Jusoh, "Optimization of C/N ratios for nutrient removal in aquaculture system culturing African catfish (*Clarias Gariepinus*) utilizing Bioflocs technology," *International Biodeterioration and Biodegradation*, vol. 102, pp. 100–106, 2015.
- [11] C. Jackson, N. Preston, P. J. Thompson, and M. Burford, "Nitrogen budget and effluent nitrogen components at an intensive shrimp farm," *Aquaculture*, vol. 218, no. 1–4, pp. 397–411, 2003.
- [12] J. Kolarevic, R. Selset, O. Felip et al., "Influence of long term ammonia exposure on Atlantic salmon (*Salmo salar* L.) parr growth and welfare," *Aquaculture Research*, vol. 44, no. 11, pp. 1649–1664, 2012.
- [13] Y. Avnimelech, "Bio-filters: the need for a new comprehensive approach," *Aquaculture Engineering*, vol. 34, no. 3, pp. 172– 178, 2006.
- [14] L. A. Helfrich and G. S. Libey, "Fish farming in recirculating aquaculture systems (RAS)," Virginia Cooperative Extension, 1991.
- [15] P. Cang, M. Zhang, G. Qiao et al., "Analysis of growth, nutrition and economic profitability of gibel carp (*Carassius auratus gibelio*Q× *C. carpio*3) cultured in zero-water exchange system," *Pakistan Journal of Zoology*, vol. 51, Article ID 619, 2019.
- [16] C. A. Otoshi, S. S. Naguwa, and F. C. Falesch, "Comercialscale production of pacific white shrimp *Litopenaeus* vannamei in a biosecure, super-intensive, recirculating aquaculture system," *Lake Buena Vista*, pp. 71–84, 2008.
- [17] C. Brito, B. do Valle, J. Interaminense, S. Peixoto, J. V. Lima-Filho, and R. Soares, "Microbiological quality of *Litopenaeus vannamei* culture using conventional and biofloc systems," *Aquaculture Research*, vol. 47, no. 10, pp. 3098–3108, 2016.

- [18] M. G. C. Emerenciano, L. R. Martínez-Córdova, M. Martínez-Porchas, and A. Miranda-Baeza, "Biofloc technology (BFT): a tool for water quality management in aquaculture," in *Water Quality*, InTech, 2017.
- [19] S. Deb, M. T. Noori, and P. S. Rao, "Application of biofloc technology for indian major carp culture (polyculture) along with water quality management," *Aquaculture Engineering*, vol. 91, Article ID 102106, 2020.
- [20] S. M. Suita, A. P. Cardozo, L. A. Romano, P. C. Abreu, and W. Wasielesky Jr, "Development of the hepatopancreas and quality analysis of post-larvae pacific white shrimp *Litopenaeus vannamei* produced in a BFT system," *Aquaculture International*, vol. 23, no. 2, pp. 449–463, 2015.
- [21] V. S. Kumar, P. K. Pandey, T. Anand, G. R. Bhuvaneswari, A. Dhinakaran, and S. Kumar, "Biofloc improves water, effluent quality and growth parameters of *Penaeus vannamei* in an intensive culture system," *Journal of Environmental Management*, vol. 215, pp. 206–215, 2018.
- [22] I. E. Luis-Villaseñor, D. Voltolina, J. M. Audelo-Naranjo, M. D. R. Pacheco-Marges, V. D. E. Herrera-Espericueta, and E. Romero-Beltrán, "Effects of biofloc promotion on water quality, growth, biomass yield and heterotrophic community in *Litopenaeus vannamei* (Boone, 1931) experimental intensive culture," *Italian Journal of Animal Science*, vol. 14, no. 3, Article ID 3726, 2016.
- [23] E. O. Ogello, N. O. Outa, K. O. Obiero, D. N. Kyule, and J. M. Munguti, "The prospects of biofloc technology (BFT) for sustainable aquaculture development," *Scientific African*, vol. 14, Article ID e01053, 2021.
- [24] M. Rajkumar, P. K. Pandey, R. Aravind, A. Vennila, V. Bharti, and C. S. Purushothaman, "Effect of different biofloc system on water quality, biofloc composition and growth performance in *Litopenaeus vannamei* (Boone, 1931)," *Aquaculture Research*, vol. 47, no. 11, pp. 3432–3444, 2016.
- [25] M. Egger, G. D. Smith, and A. N. Phillips, "Meta-analysis: principles and procedures," *BMJ*, vol. 315, no. 7121, pp. 1533–1537, 1997.
- [26] I. K. Crombie and H. T. Davies, "What is meta-analysis," vol. 1, no. 8, 2009, What is series, http://www.bandolier.org. uk/painres/download/whatis/Meta-An.pdf.
- [27] M. J. Page, J. McKenzie, P. Bossuyt et al., "The PRISMA, statement: an updated guideline for reporting systematic reviews," *Systematic Reviews*, vol. 10, no. 1, pp. 1–11, 2020.
- [28] D. W. A. Noble, P. Pottier, M. Lagisz et al., "Meta-analytic approaches and effect sizes to account for 'nuisance heterogeneity' in comparative physiology," *Journal of Experimental Biology*, vol. 225, no. Suppl\_1, Article ID jeb243225, 2022.
- [29] W. Viechtbauer, "Bias and efficiency of meta-analytic variance estimators in the random-effects model," *Journal of Educational and Behavioral Statistics*, vol. 30, no. 3, pp. 261–293, 2005.
- [30] J. P. T. Higgins and S. G. Thompson, "Quantifying heterogeneity in a meta-analysis," *Statistics in Medicine*, vol. 21, no. 11, pp. 1539–1558, 2002.
- [31] W. G. Cochran, "The combination of estimates from different experiments," *Biometrics*, vol. 10, no. 1, pp. 101–129, 1954.
- [32] R Development Core Team, R: A Language and Environment for Statistical Computing, Version 4.0.5, R Foundation for Statistical Computing, https://www.R-project.org/, 2021.
- [33] W. Viechtbauer, "Conducting meta-analyses in R with the metafor package," *Journal of Statistical Software*, vol. 36, no. 3, pp. 1–48, 2010.

- [34] W. Viechtbauer and M. W.-L. Cheung, "Outlier and influence diagnostics for meta-analysis," *Research Synthesis Methods*, vol. 1, no. 2, pp. 112–125, 2010.
- [35] C. B. Begg and M. Mazumdar, "Operating characteristics of a rank correlation test for publication bias," *Biometrics*, vol. 50, no. 4, pp. 1088–1101, 1994.
- [36] J. A. Sterne and M. Egger, "Regression methods to detect publication and other bias in meta-analysis," in *Publication Bias in Meta-Analysis: Prevention, Assessment and Adjustments*, pp. 99–110, Springer, 2005.
- [37] J. Soto-Alcalá, P. D. Álvarez-Ruiz, J. M. Audelo-Naranjo et al., "Transcriptional response of immune-related genes in *Litopenaeus vannamei* post-larvae cultured in recirculating aquaculture systems with and without biofloc," *Aquaculture International*, vol. 27, no. 1, pp. 209–225, 2019.
- [38] D. Kaya, E. Genc, M. A. Genc, M. Aktas, O. T. Eroldogan, and D. Guroy, "Biofloc technology in recirculating aquaculture system as a culture model for green tiger shrimp, *Penaeus semisulcatus*: effects of different feeding rates and stocking densities," *Aquaculture*, vol. 528, Article ID 735526, 2020.
- [39] X. Du, D. Almeida, D. Song et al., "Effects of organic carbon addition on water quality and phytoplankton assemblages in biofloc technology ponds," *Aquaculture*, vol. 497, pp. 155– 163, 2018.
- [40] R. L. de Souza, E. C. R. de Lima, F. P. de Melo, M. G. P. Ferreira, and E. S. de Correia, "The culture of Nile tilapia at different salinities using a biofloc system," *Revista Ciência Agronômica*, vol. 50, no. 2, pp. 267–275, 2019.
- [41] V. Kumar, M. Wille, T. M. Lourenço, and P. Bossier, "Bioflocbased enhanced survival of *Litopenaeus vannamei* upon AHPND-causing *Vibrio parahaemolyticus* challenge is partially mediated by reduced expression of its virulence genes," *Frontiers in Microbiology*, vol. 11, Article ID 741164, 2020.
- [42] Q. Shang, H. Tang, Y. Wang et al., "Application of enzymehydrolyzed cassava drugs as a carbon source in aquaculture," *Science of Total Environment*, vol. 615, pp. 681–690, 2018.
- [43] A. J. Ray, T. H. Drury, and A. Cecil, "Comparing clear-water ras and biofloc systems: shrimp (*Litopenaeus vannamei*) production, water quality, and biofloc nutritional contributions estimated using stable isotopes," *Aquaculture Engineering*, vol. 77, pp. 9–14, 2017.
- [44] T. Sgnaulin, G. L. de Mello, M. C. Thomas, J. R. E. Garcia, G. A. R. M. de Oca, and M. G. C. Emerenciano, "Biofloc technology (BFT): an alternative aquaculture system for piracanjuba *Brycon orbignyanus*?" *Aquaculture*, vol. 485, pp. 119–123, 2018.
- [45] L. Long, J. Yang, Y. Li, C. Guan, and F. Wu, "Effect of biofloc technology on growth, digestive enzyme activity, hematology, and immune response of genetically improved farmed tilapia (*Oreochromis niloticus*)," *Aquaculture*, vol. 448, pp. 135–141, 2015.
- [46] S. Tepaamorndech, I. Nookaew, S. M. Higdon et al., "Metagenomics in bioflocs and their effects on gut microbiome and immune responses in pacific white shrimp," *Fish and Shellfish Immunology*, vol. 106, pp. 733–741, 2020.
- [47] L. Vinatea, J. A. Malpartida, R. Carbó, K. B. Andree, E. Gisbert, and A. Estévez, "A comparison of recirculation aquaculture systems versus biofloc technology culture system for on-growing of fry of *Tinca tinca* (Cyprinidae) and fry of grey *Mugil cephalus* (Mugilidae)," *Aquaculture*, vol. 482, pp. 155–161, 2018.
- [48] M. Emerenciano, E. L. C. Ballester, R. O. Cavalli, and W. Wasielesky, "Biofloc technology application as a food

source in a limited water exchange nursery system for pink shrimp *Farfantepenaeus brasiliensis* (Latreille, 1817)," *Aquaculture Research*, vol. 43, no. 3, pp. 447–457, 2012.

- [49] W.-J. Xu and L.-Q. Pan, "Enhancement of immune response and antioxidant status of *Litopenaeus Vannamei* juvenile in bio-floc-based culture tanks manipulating high C/N ratio of feed input," *Aquaculture*, vol. 412-413, pp. 117–124, 2013.
- [50] A.C. da Silva Martins, J. A. Flores, C. Porto, W. W. Junior, and J. M. Monserrat, "Antioxidant and oxidative damage responses in different organs of pacific white shrimp *Litopenaeus vannamei* (Boone, 1931) reared in a biofloc technology system," *Marine and Freshwater Behaviour and Physiology*, vol. 48, no. 4, pp. 279–288, 2015.
- [51] W. J. Xu, L. Q. Pan, X. H. Sun, and J. Huang, "Effects of bioflocs on water quality, and survival, growth and digestive enzyme activities of *Litopenaeus vannamei* (Boone) in zerowater exchange culture tanks," *Aquaculture Research*, vol. 44, no. 7, pp. 1093–1102, 2013.
- [52] H. Yun, E. Shahkar, K. Katya, I.-K. Jang, Skyoung Kim, and S. C. Bai, "Effects of bioflocs on dietary protein requirement in juvenile whiteleg shrimp, *Litopenaeus Vannamei*," *Aquaculture Research*, vol. 47, no. 10, pp. 3203–3214, 2016.
- [53] P. Zhao, J. Huang, X.-H. Wang et al., "The application of bioflocs technology in high-intensive, zero exchange farming systems of *Marsupenaeus japonicus*," *Aquaculture*, vol. 354-355, pp. 97–106, 2012.
- [54] D. Kamilya, M. Debbarma, P. Pal, B. Kheti, S. Sarkar, and S. T. Singh, "Biofloc technology application in indoor culture of *Labeo rohita* (Hamilton, 1822) fingerlings: the effects on inorganic nitrogen control, growth and immunity," *Chemo-sphere*, vol. 182, pp. 8–14, 2017.
- [55] J. Li, J. Li, W. Li et al., "Juvenile *Procambarus clarkii* farmed using biofloc technology or commercial feed in zero-water exchange indoor tanks: a comparison of growth performance, enzyme activity and proximate composition," *Aquaculture Research*, vol. 50, no. 7, pp. 1834–1843, 2019.
- [56] N. H. Y. Nhi, C. T. Da, T. Lundh, T. T. Lan, and A. Kiessling, "Comparative evaluation of Brewer's yeast as a replacement for fishmeal in diets for tilapia (*Oreochromis niloticus*), reared in clear water or biofloc environments," *Aquaculture*, vol. 495, pp. 654–660, 2018.
- [57] S. K. Kim, Q. Guo, and I. K. Jang, "Effect of biofloc on the survival and growth of the postlarvae of three penaeids (*Litopenaeus vannamei*, *Fenneropenaeus chinensis*, and *Marsupenaeus japonicus*) and their biofloc feeding efficiencies, as related to the morphological structure of the third maxilliped," *Journal of Crustacean Biology*, vol. 35, pp. 41–50, 2015.
- [58] S.-K. Kim, Z. Pang, H.-C. Seo, Y.-R. Cho, T. Samocha, and I.-K. Jang, "Effect of bioflocs on growth and immune activity of pacific white shrimp, *Litopenaeus vannamei* postlarvae," *Aquaculture Research*, vol. 45, no. 2, pp. 362–371, 2014.
- [59] L. R. Martínez-Córdova, M. Emerenciano, A. Miranda-Baeza, and M. Martínez-Porchas, "Microbial-based systems for aquaculture of fish and shrimp: an updated review," *Reviews in Aquaculture*, vol. 7, no. 2, pp. 131–148, 2015.
- [60] R. Jiménez-Montealegre, M. Verdegem, J. E. Zamora, and J. Verreth, "Organic matter sedimentation and resuspension in tilapia (*Oreochromis niloticus*) ponds during a production cycle," *Aquaculture Engineering*, vol. 26, no. 1, pp. 1–12, 2002.
- [61] C. E. Boyd and V. Pillai, "Water quality management in aquaculture," CMFRI Special Bulletin, vol. 22, pp. 1–44, 1985.

- [62] A. Arillo, E. Gaino, C. Margiocco, P. Mensi, and G. Schenone, "Biochemical and ultrastructural effects of nitrite in rainbow trout: liver hypoxia as the root of the acute toxicity mechanism," *Environmental Research*, vol. 34, no. 1, pp. 135–154, 1984.
- [63] A. M. Hilmy, N. A. El-Domiaty, and K. Wershana, "Acute and chronic toxicity of nitrite to clarias lazera," *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, vol. 86, no. 2, pp. 247–253, 1987.
- [64] N. Y. S. Woo and S. F. Chiu, "Metabolic and osmoregulatory responses of the seabass *Lates calcarifer* to nitrite exposure," *Environmental Toxicology and Water Quality*, vol. 12, no. 3, pp. 257–264, 1997.
- [65] C. A. P. Gaona, M. S. de Almeida, V. Viau, L. H. Poersch, and W. Wasielesky Jr, "Effect of different total suspended solids levels on a *Litopenaeus vannamei* (Boone, 1931) BFT culture system during biofloc formation," *Aquaculture Research*, vol. 48, no. 3, pp. 1070–1079, 2017.
- [66] R. Schveitzer, R. Arantes, P. F. S. Costódio et al., "Effect of different biofloc levels on microbial activity, water quality and performance of *Litopenaeus vannamei* in a tank system operated with no water exchange," *Aquaculture Engineering*, vol. 56, pp. 59–70, 2013.
- [67] W. Wasielesky Jr, H. Atwood, A. Stokes, and C. L. Browdy, "Effect of natural production in a zero exchange suspended microbial floc based super-intensive culture system for white shrimp *Litopenaeus vannamei*," *Aquaculture*, vol. 258, no. 1–4, pp. 396–403, 2006.
- [68] P. S. Furtado, L. H. Poersch, and W. Wasielesky Jr, "Effect of calcium hydroxide, carbonate and sodium bicarbonate on water quality and zootechnical performance of shrimp *Litopenaeus vann*amei reared in bio-flocs technology (BFT) systems," *Aquaculture*, vol. 321, pp. 130–135, 2011.
- [69] I. Putra, I. Effendi, I. Lukistyowati et al., "Effect of different biofloc starters on ammonia, nitrate, and nitrite concentrations in the cultured tilapia *Oreochromis niloticus* system," *F1000Research*, vol. 9, Article ID 293, 2020.
- [70] G. Luo, J. Xu, and H. Meng, "Nitrate accumulation in biofloc aquaculture systems," *Aquaculture*, vol. 520, Article ID 734675, 2020.
- [71] A. Jatobá, Y. V. Borges, and F. A. Silva, "BIOFLOC: sustainable alternative for water use in fish culture," *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, vol. 71, no. 3, pp. 1076–1080, 2019.
- [72] D. Kaya, M. C. A. Genc, M. Aktas, H. Yavuzcan, O. Ozmen, and E. Genc, "Effect of biofloc technology on growth of speckled shrimp, *Metapenaeus monoceros* (Fabricus) in different feeding regimes," *Aquaculture Research*, vol. 50, no. 10, pp. 2760–2768, 2019.
- [73] E. Chan-Vivas, C. M. Mña-Gallegos Edén, K. Escalante, G. Gaxiola, and G. Cuzon, "Does biofloc improve the energy distribution and final muscle quality of shrimp, *Litopenaeus vannamei* (Boone, 1883)?" *Journal of World Aquaculture Society*, vol. 50, no. 2, pp. 460–468, 2019.
- [74] M. T. Jamal, M. Broom, B. A. Al-Mur et al., "Biofloc technology: emerging microbial biotechnology for the improvement of aquaculture productivity," *Polish Journal of Microbiology*, vol. 69, no. 4, pp. 401–409, 2020.
- [75] M. H. Khanjani, A. Mohammadi, and M. D. G. C. Emerenciano, "Microorganisms in biofloc aquaculture system," *Aquaculture Reports*, vol. 26, Article ID 101300, 2022.
- [76] A. E. Allen, M. G. Booth, M. E. Frischer, P. G. Verity, J. P. Zehr, and S. Zani, "Diversity and detection of nitrate

assimilation genes in marine bacteria," *Applied Environmental Microbiology*, vol. 67, no. 11, pp. 5343–5348, 2001.

- [77] O. Schneider, V. Sereti, M. A. M. Machiels, E. H. Eding, and J. A. J. Verreth, "The potential of producing heterotrophic bacteria biomass on aquaculture waste," *Water Research*, vol. 40, no. 14, pp. 2684–2694, 2006.
- [78] N. H. Y. Nguyen, L. T. Trinh, D. T. Chau, K. Baruah, T. Lundh, and A. Kiessling, "A spent brewer's yeast as a replacement for fishmeal in diets for giant freshwater prawn (*Macrobrachium rosenbergii*), reared in either clear water or a biofloc environment," *Aquaculture Nutrition*, vol. 25, no. 4, pp. 970–979, 2019.
- [79] M. Asaduzzaman, M. A. Wahab, M. C. J. Verdegem, S. Haque, M. A. Salam, and M. E. Azim, "C/N ratio control and substrate addition for periphyton development jointly enhance freshwater prawn *Macrobrachium rosenbergii* production in ponds," *Aquaculture*, vol. 280, no. 1–4, pp. 117–123, 2008.
- [80] J. M. Ebeling, M. B. Timmons, and J. J. Bisogni, "Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia–nitrogen in aquaculture systems," *Aquaculture*, vol. 257, no. 1–4, pp. 346– 358, 2006.
- [81] A. G. J. Tacon, J. J. Cody, L. D. Conquest, S. Divakaran, I. P. Forster, and O. E. Decamp, "Effect of culture system on the nutrition and growth performance of pacific white shrimp *Litopenaeus vannamei* fed different diets," *Aquaculture Nutrition*, vol. 8, no. 2, pp. 121–137, 2002.
- [82] M. Martinez-Porchas, M. Ezquerra-Brauer, F. Mendoza-Cano, J. E. Chan Higuera, F. Vargas-Albores, and L. R. Martinez-Cordova, "Effect of supplementing heterotrophic and photoautotrophic biofloc, on the production response, physiological condition and post-harvest quality of the whiteleg shrimp, *Litopenaeus vannamei*," *Aquaculture Reports*, vol. 16, Article ID 100257, 2020.
- [83] S. M. Pinho, J. P. De Lima, L. H. David et al., "FLOCponics: the integration of biofloc technology with plant production," *Reviews in Aquaculture*, vol. 14, no. 2, pp. 647–675, 2022.