



# Boosting aquaculture resilience through biofloc-forming Bacteria: A comparative approach

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## ABSTRACT

Maintaining optimal water parameters in a biofloc technology system is essential for ensuring the healthy growth, development, and survival of cultured aquatic organisms. Fluctuations in these parameters can negatively impact their health, growth performance, and overall survival rate. This study compares the effects of two biofloc-producing bacteria, *Bacillus cereus* and *Klebsiella pneumoniae*, on water quality. The study was conducted under sterile laboratory conditions using water with 10 ppt salinity, and water quality parameters were assessed at 24-h intervals. Both bacterial strains were capable of forming bioflocs, though their performance varied over time. *Bacillus cereus* showed fluctuations in pH (6.5–8.9), whereas *Klebsiella pneumoniae* maintained relative stability (6–7.4). Both treatments elevated total alkalinity, but *K. pneumoniae* caused a sharp increase from 150 ppm to 310 ppm, differing from previous observations. *B. cereus* maintained stable total hardness and dissolved oxygen (2–5 mg/L) but caused elevated turbidity (819 NTU) after floc disintegration. Overall, bioflocs produced by *B. cereus* were more consistent and exhibited properties better suited for sustainable aquaculture under controlled conditions compared to those formed by *K. pneumoniae*.

## 1. Introduction

The intensification of aquaculture has underscored the need for sustainable practices that balance productivity with environmental responsibility. According to FAO (2024), per capita consumption of aquatic animal food rose from 9.1 kg in 1961 to 20.7 kg in 2022, with demand continuing to increase annually. Meeting this growing demand exceeds the capacity of traditional methods, making intensive aquaculture approaches essential. In this context, biofloc technology (BFT) has emerged as a promising alternative.

BFT, driven by biofloc producing bacteria, enhances aquaculture sustainability by recycling waste nutrients into microbial biomass, reducing the need for water exchange, and strengthening biosecurity (Ahmed Alkhamis et al., 2023; Deswati et al., 2023). Evidence demonstrates that BFT improves water quality and animal performance by modulating nitrogenous compounds, pH, salinity, dissolved oxygen (DO), and total suspended solids (TSS), all of which are critical for aquatic health (Khanjani et al., 2022). Experimental studies further

support these claims: biofloc-producing bacteria and probiotics in BFT systems lowered nitrogen concentrations (NO<sub>2</sub>-N, NH<sub>4</sub>-N) and enhanced water quality, which translated into better fish and shrimp growth (Dewasti et al., 2022; Qiu et al., 2023). Similarly, Soliman and Tawab (2022) reported higher biofloc volumes and bacterial counts, leading to improved Nile tilapia growth performance. Nevertheless, results are not always consistent. For example, Kring et al. (2023) observed no significant differences in DO or salinity across substrate treatments, suggesting that BFT outcomes may vary with context.

Despite its promise, several research gaps remain. The specific roles of individual bacterial strains in sustaining water quality under diverse conditions are not fully understood (Mohammadi et al., 2024). Moreover, while carbon source manipulation (Rind et al., 2023) and probiotic supplementation (Ajamhasani et al., 2023) have been shown to influence water chemistry, standardized protocols for inoculum preparation, C/N ratio adjustment, and bacterial selection are still lacking, limiting reproducibility and scalability. In addition, most available studies focus on location- or species-specific systems, restricting broader applicability

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(Haung et al., 2022; Estante-Superio et al., 2025).

Addressing these gaps requires systematic evaluation of biofloc-producing bacterial strains and their direct influence on water quality regulation. Therefore, the objective of this study is to compare the effects of *Bacillus cereus* and *Klebsiella pneumoniae* on key water quality parameters under controlled aquaculture conditions, with the goal of advancing standardized, scalable, and sustainable biofloc-based aquaculture systems.

## 2. Materials and methods

### 2.1. Experimental design

#### 2.1.1. Imhoff cone

This experiment was performed in one liter of Borosilicate Glass Imhoff cone. The cones were washed, cleaned with chromic acid, and sterilized in a hot air oven at 180 °C for 42 min. The upper part of the Imhoff cone was sealed with aseptic aluminum foil.

#### 2.1.2. Aeration setup

An aquarium air pump (Venus Aqua AP-338 Air Aquarium Pump –100 cm) with a capacity of  $4 \times 10$  L/min was supplied via aeration. An air-water transparent silicon tube (internal diameter, 2.5 mm) was used to supply air into the Imhoff cones. The aeration pipe was adequately weighted to ensure proper submersion within the water.

#### 2.1.3. Biofloc water

Biofloc water (BW) was formulated by diluting clean, uncontaminated seawater (32–35 PPT) with distilled water to achieve a salinity of 10 ppt (Saeedi and Chapara, 2024). Salinity was measured using a digital salinity meter (AR 8012) to ensure accurate determination of salt concentration. Next, the BW water was sterilized in an autoclave at a temperature of 121 °C and a pressure of 15 lbs. for a duration of 15 min. Subsequently, a volume of one liter of 10 ppt BW was transferred into sterilized Imhoff cones in duplicates. The alkalinity of the water with a salinity of 10 ppt was modified to a range of 150–200 ppm using the APHA 2320 B titration method with  $\text{NaHCO}_3$  (sodium bicarbonate) (Martin, et al., 2017) in accordance with the criteria set by APHA (2012) (Saeedi and Chapara, 2024).

#### 2.1.4. Temperature

Room temperature of 30 °C  $\pm$  2 °C was maintained throughout the experiment.

#### 2.1.5. Light

During the incubation period, no light was provided to the experimental setup to prevent the growth of photoautotrophic organisms.

#### 2.1.6. Sterilization

All the Imhoff cones, glassware, and equipment were washed with tap water, cleaned with chromic acid, and then sterilized in a hot air oven at 180 °C for 42 min (Fraise Adam et al., 2013; Hancock, 2013). The liquids and media were sterilized in an autoclave at a temperature of 121 °C and a pressure of 15 pounds for a duration of 15 min.

#### 2.1.7. Inoculum

Two bacteria that successfully produced biofloc were chosen for this experiment, *Bacillus cereus* and *Klebsiella pneumoniae*, whose codes were KBF-103 and KBF-111, respectively. The experiment was performed for 96 h in a laboratory environment, and Imhoff cones were replicated.

Bacterial cultures that demonstrated successful biofloc generation were used as the inoculum. Cultivation was initiated by an overnight incubation on nutrient agar plates containing 3 % NaCl. Next, a portion of a recently cultivated culture that had matured for 15–18 h was introduced into 10–15 mL of nutrient broth with a sodium chloride concentration ranging from 0.5 to 3 %. The culture was stirred until it

achieved a specified level of cloudiness between 0.5 and 0.7 nm at 600 nm, as measured using a spectrophotometer (Go Direct SpectroVis Plus Spectrophotometer), showing the presence of bacterial growth and activity. The activated bacteria were then used as an inoculum to start the biofloc production process and to assess the water parameters (Saeedi and Chapara, 2024).

Every day, a volume–25–30 mL of BSM (Biofloc Standard Medium (BSM) was added to the Imhoff cones. The sample was subjected to ocular inspection at 24-h intervals to observe the formation of bioflocs. This was achieved by briefly halting the aeration process for 30 min. The volume of flocs generated was quantified. Aeration was used to prolong the incubation period and 25–30 mL BSM was added for 96 h (Saeedi and Chapara, 2024).

### 2.2. Physicochemical analysis of water

#### 2.2.1. Physical parameters

Initial physical characteristics, such as temperature, color, odor, salinity, conductivity, and the total amount of dissolved substances, were recorded every 24 h using a BLE-C600 Smart Bluetooth 7 in 1 pH TDS EC ORP SALY S.G Temperature PH Metre. Turbidity was also assessed at 24-h intervals using a portable turbidity meter (ISO 7027), model M10.

#### 2.2.2. Chemical parameters

The titration method of APHA (2012) was used to measure chemical parameters, such as total alkalinity, total hardness, calcium hardness, and magnesium hardness. Total ammonia nitrogen (TAN), nitrite, sulfate, and phosphate levels were measured using a portable mini-spectrophotometer (The Go Direct SpectroVis Plus Spectrophotometer). Additionally, the pH and DO concentrations were assessed every 24 h using a portable DO meter (RCYAGO Dissolved Oxygen Metre, Soikoi brand). The experiment was replicated. An appropriate amount of water sample was placed in 50 mL centrifuge tubes between 9:00 to 10:00 AM for ammonia, nitrate, sulfate, and phosphate tests. The mixture was then centrifuged at 2500 rpm for 10–15 min. The supernatant was transferred to fresh tubes, and the pellet was discarded.

The dataset obtained from the experiment was analyzed using Microsoft Excel 365. The average and average errors were computed in Excel to quantitatively represent the central tendency and variability of the dataset. Furthermore, a graphical representation was generated using Excel to visualize trends and relationships within the dataset. The graph is meticulously plotted to illustrate the experimental findings effectively.

## 3. Results

Water parameters are important for the growth of microorganisms, particularly bacteria, because they directly or indirectly affect the culture's growth rate and metabolic activity. Factors, such as temperature, pH, and nutrient levels, directly influence these activities within a culture.

Flocs are clusters of bacterial aggregates suspended in water. Table 1. shows the increase in biofloc concentration from 0 ml at the start to 35 ml at 48 h and then a decrease back to 0 mL; in *Bacillus Cereus*, likewise, flocs were 0 ml at 24 h and 25 ml at 48 h, 45 ml in 72 h and 40 ml at 96 h in *Klebsiella pneumoniae*. Both suggest the aggregation and subsequent settling of suspended particles, which could indicate changes in water quality or management practices. In addition, a consistent salinity level of approximately 10 ppt throughout the observation period indicates stability in the salt content of the water.

Fig. 1 shows pH dynamics of *Bacillus cereus* and *Klebsiella pneumoniae* over time. *Bacillus cereus* started at pH 8.5, declined to 6.5 at 72 h, and rose again to 8.9 at 96 h, indicating marked fluctuations (6.5–8.9). In contrast, *Klebsiella pneumoniae* maintained relative stability, ranging from 6.0 to 7.45.

**Table 1**  
Water parameters assessment comparison of *Bacillus* spp. (KBF-103) and *Klebsiella* spp. (KBF-111) bacteria strains.

S. N	Water Parameter	0 h		24 h		48 h		72 h		96 h	
		KBF-103	KBF-111	KBF-103	KBF-111	KBF-103	KBF-111	KBF-103	KBF-111	KBF-103	KBF-111
1	Flocs (ml/L)	0	0	22.5	0	32.5	25	22.5	45	0	40
2	Salinity (ppt)	10	10	10	10	10	10	10	10	10	10
3	Temperature	28.15	32.3	29.5	31.6	28.9	32	29.5	31.7	30	31.9
4	pH	8.5	6	7.8	6.5	7	6.65	6.5	7.2	8.9	7.45
5	ORP (mV)	-128	-65	-116	220.5	-266	220.5	-318	206.5	-304	180.5
6	TDS (ppm)	1670	7840	3345	7445	2330	7710	3790	7195	3400	7285
7	Specific gravity	1.003	1.003	1.003	1.003	1.003	1.003	1.003	1.003	1.003	1.003
8	Alkalinity (ppm)	262.5	150	250	180	232.5	195	247.5	240	280	310
9	Total Hardness (ppm)	2000	1900	2000	1900	2000	1900	2000	1900	2000	1800
10	Ca. Hardness (ppm)	270	200	250	200	250	190	220	190	210	170
11	Mg. Hardness (ppm)	1730	1700	1800	1700	1750	1700	1790	1700	1790	1655
12	Amount of Ca ion (ppm)	108	80	100	80	100	76	88	76	84	68
13	Amount of Mg Ion (ppm)	484	476	504	476	488.8	478.6	497.2	478.6	501.2	449.4
14	Ammonia (ppm)	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD
15	Nitrate (ppm)	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD
16	Sulphate (ppm)	605	800	657	710	560	700	619	680	619	675
17	Phosphate (ppm)	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
18	Turbidity (NTU)	8.9	46	16.85	268	85.5	203	356.5	303	819	544
19	D-O (mg/L)	5.15	4.55	2.25	0.69	1.75	0.86	1.15	0.76	5.32	3.4

UD = Undetected.

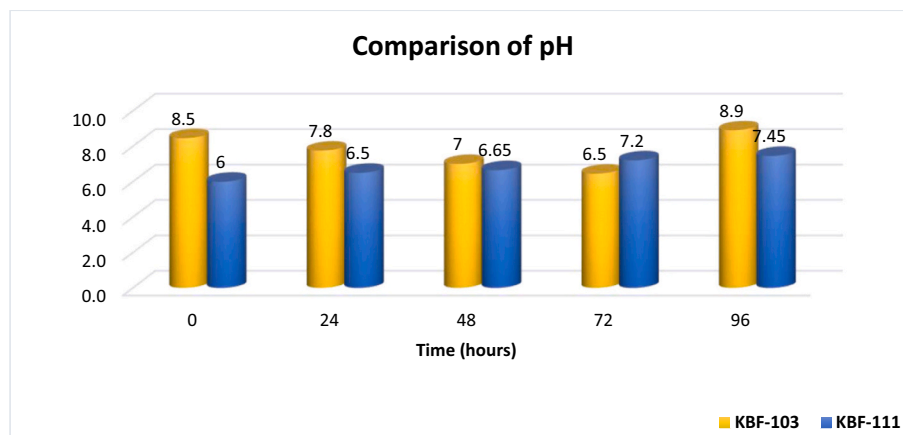


Fig. 1. pH variation of *Bacillus* spp. (KBF-103) and *Klebsiella* spp. (KBF-111) over 96 h.

The fluctuation in the ORP values over the observation period indicates changes in the redox potential of the water, which could influence the availability of oxygen and activity of microorganisms. As illustrated in Fig. 2 *Klebsiella pneumoniae* at 0 h shows -65 mV, but there was a continuous fluctuation between 220 and 180.5 mV. However, *Bacillus cereus* was negative, starting at -128, -116, -266, -318, and -304, for 24, 48, 72, and 96 h, respectively. Therefore, a significant difference was observed between the *Klebsiella pneumoniae* and *Bacillus cereus* ORP measurements.

The fluctuation in TDS levels indicates changes in the composition of dissolved substances in the water over time. They were not steady, decreasing, or continuously increasing in either *Bacillus cereus* or *Klebsiella pneumoniae*. Overall, TDS showed a continuous decrease in *Klebsiella pneumoniae* but a fluctuation in *Bacillus cereus*.

The consistent specific gravity of 1.003 throughout the observation period suggests stability in the density of the water in *Bacillus cereus* and *Klebsiella pneumoniae*. There was no change, and was 1.003 in both the samples.

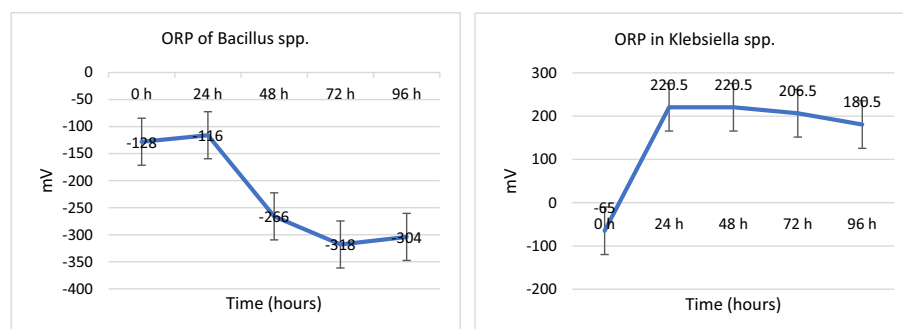


Fig. 2. The ORP of water in *Bacillus* spp. and *Klebsiella* spp.

Alkalinity measures the capacity of water to resist the pH changes caused by acids. It appears that alkalinity increases slightly over time. Fig. 3 shows alkalinity gradually increased over time. The alkalinity level was initially measured at 262.5 ppm at 0 h, which then declined to 250 ppm at 24 h and dropped to 232.5 ppm at 48 h. After 48 h, alkalinity demonstrated a further increase, and by 72 h, the level reaches 247.5 ppm. There was a considerable increase in alkalinity between 72 h and 96 h, with the highest level of 280 ppm observed at 96 h in *Bacillus cereus*. In Addition, the alkalinity of *Klebsiella pneumoniae* did not show any decline and appeared to continuously increase. At 0 h, the alkalinity was 150, 180, 195, 240, and 310 ppm at 48, 72, and 96 h, respectively. It appears that the alkalinity of *Klebsiella pneumoniae* increases more than that of *Bacillus cereus*.

The total hardness indicates the dissolved calcium and magnesium ions concentration in water. For *Bacillus cereus*, the total hardness at 0 h was 2000 ppm and remained constant for 96 h. The total hardness of *Klebsiella pneumoniae* was 1900 ppm from 0 to 72 h but only decreased to 1800 ppm at 96 h.

Fluctuations in calcium hardness indicate changes in the concentration of calcium ions over time. Table 1 shows that the calcium ion concentration in *Klebsiella pneumoniae* was 80 ppm at 0 h and 24 h, but decreased at 48–72 h to 76 ppm and finally to 68 ppm at 96 h. Similarly, the amount of calcium ions in *Bacillus cereus* decreased continuously. At 0 h, the measurements were 108 ppm and decreased at 24 and 48 h to 100 ppm and 88 and 84 ppm at 72 and 96 h, respectively. Similar to calcium hardness, the variation in magnesium hardness levels suggests changes in magnesium ion concentration over time. The concentrations of Mg in *Bacillus cereus* at 0, 24, 48, 72, and 96 h were 484, 504, 488.8, 497.2, and 501.2 respectively. Concentrations of *Klebsiella pneumoniae* were 476, 476, 479, 479, and 449 ppm, over a span of 0 to 96 h respectively.

The absence of detectable ammonia throughout the observation period suggests good water quality with respect to nitrogen compound levels in *Klebsiella pneumoniae* and *Bacillus cereus*. Nitrate is another nitrogen compound that affects the water quality and aquatic life. However, the results showed that low concentrations undetected from 0 to 96 h indicated either low levels or efficient removal of nitrate from the samples by bacteria.

The sulfate concentration varied over time in *Bacillus cereus* and *Klebsiella pneumoniae*, suggesting dynamic alterations in the water chemistry. The *Bacillus cereus* biofloc water sample did not show a drop, except at 48 h, to 560 ppm. However, *Klebsiella pneumoniae* showed a continuous decrease in sulfate concentration from 0 to 96 h. At 0 h, the sulfate concentration was 800 ppm, which was similar to 710, 700, 680, and 690 ppm at 24, 48, 72, and 96 h, respectively. These variations can be affected by factors such as the ambient conditions, microbial activity,

and chemical interactions.

Table 1.1 shows that initial turbidity is very low, only showing 46 NTU for *Klebsiella pneumoniae* and 8.9 NTU for *Bacillus cereus*. The turbidity for *Klebsiella pneumoniae* shows 268, 203, 303 and 544 NTU at 24 h, 48 h, 72 h and 96 h, respectively. Likewise, the turbidity of *Bacillus cereus* also increased: 16.9, 85.5, 365.5 and 819 NTU at 24 h, 48 h, 72 h, and 96 h, respectively. The significant increase in turbidity from 8.9 NTU at the start to 819 NTU at 96 h indicates a deterioration in water clarity, likely due to increased suspension of bacterial growth.

Dissolved oxygen (DO) is crucial for the survival of aquatic organisms and aerobic bacteria. At initiation, the DO concentration was between 4.5 and 5 mg/L in *Klebsiella pneumoniae* and *Bacillus cereus*, respectively. However, both *Bacillus cereus* and *Klebsiella pneumoniae* showed decreased DO levels over time, from 5.1 mg/L initially to 1.15 mg/L at 72 h, suggesting a decline in oxygen availability, which could biofloc producing bacteria. However, both increased at 96 h. Overall, the detailed interpretation of these water parameters provides insights into water quality dynamics in aquaculture systems over the observation period, highlighting trends and potential areas for further investigation or management intervention.

#### 4. Discussion

This study provides critical insights into the dynamics of water quality parameters in biofloc systems, emphasizing how microbial activity, nutrient availability, and system management shape floc stability and environmental conditions.

The observed increase in floc volume from 0 mL to 35 mL within 48 h, followed by a decline to 0 mL, reflects the natural cycle of aggregation and settling of suspended particles. This dynamic underscores the ever-changing nature of biofloc systems, where microbial processes and resource availability continuously influence floc stability. Sedimentation plays a crucial role in maintaining water transparency and preventing excessive accumulation of suspended solids.

Temperature strongly influences the physiology of cultured organisms, microbial metabolism, and dissolved oxygen (DO) levels. Elevated temperatures can reduce DO availability (Hostins et al., 2015; Hostins et al., 2015) and disrupt metabolic pathways, reproduction, and behavior (Narum et al., 2013; Kim et al., 2017). However, in this study, water temperature remained within the optimal range, supporting bacterial growth and sustaining biofloc composition without causing stress to the microbial or aquatic communities.

The pH responses of *Bacillus cereus* and *Klebsiella pneumoniae* highlight the pivotal role of microbial metabolism in shaping system chemistry. *B. cereus* showed greater fluctuations, indicating acidifying metabolic shifts, while *K. pneumoniae* maintained a steadier trajectory.

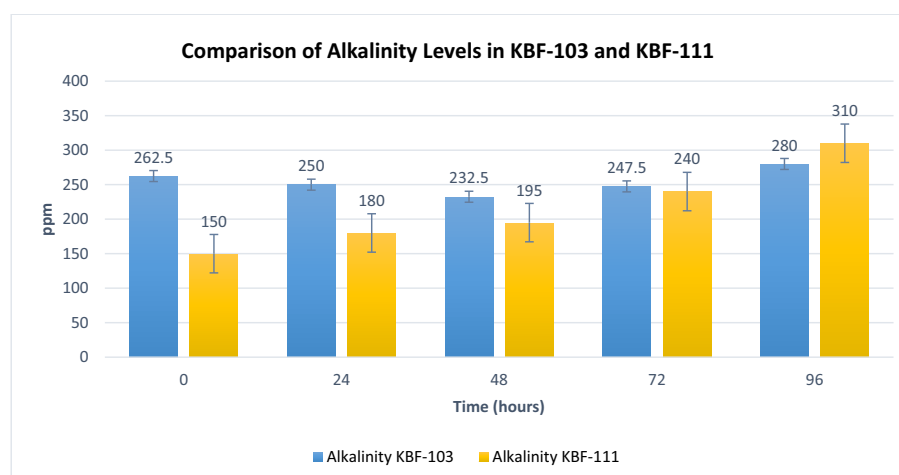


Fig. 3. Alkalinity of *Bacillus* spp. (KBF-103) and *Klebsiella* spp. (KBF-111) over 96 h.

Since pH directly affects nutrient availability, enzymatic activity, and bacterial growth, stability within 7–8.5 is essential for biofloc efficiency (Deswati Suyani et al., 2019; Sanchez-Estrada et al., 2018). Flocculation typically peaks near neutral pH but declines under more acidic or alkaline conditions (Jayaprakash et al., 2023). The observed pH decline after 24–48 h likely reflects bacterial acid production, with alkalinity increases insufficient to fully buffer the system. Notably, alkalinity rose significantly in both treatments, with *K. pneumoniae* nearly doubling from 150 to 310 ppm, exceeding levels typically consumed by heterotrophic bacteria (Ebeling et al., 2006). Maintaining alkalinity above 100 mg CaCO<sub>3</sub>/L is critical (Crab et al., 2009), and levels up to 300 mg CaCO<sub>3</sub>/L can enhance nitrification and growth (Furtado et al., 2015). Regular supplementation with NaHCO<sub>3</sub> may therefore be required to stabilize pH in operational systems.

DO is essential for aerobic bacterial growth and floc formation. A sharp decline from 5 to 0.6 mg/L coincided with peak bacterial growth, consistent with previous studies reporting rapid oxygen depletion in active biofloc systems (Hwihy et al., 2021; Pérez-Fuentes et al., 2016). Interestingly, DO rebounded to 3–4 mg/L by 96 h, likely due to bacterial death-phase dynamics reducing oxygen demand. Such fluctuations highlight the dual role of bioflocs as oxygen consumers through respiration and oxygen stabilizers through organic matter assimilation. Parallel changes in oxidation-reduction potential (ORP) further underscore shifts in redox balance, reflecting differences in microbial activity and oxygen availability between *B. cereus* and *K. pneumoniae* systems.

Variations in hardness (total, calcium, and magnesium) and ion concentrations indicate ongoing changes in the ionic balance of the culture medium. These fluctuations directly influence alkalinity, pH buffering, and nutrient availability, thereby affecting microbial growth and water stability. Importantly, phosphate levels did not accumulate, preventing disruptions in the N:P ratio that could trigger cyanobacterial blooms (Emerenciano et al., 2022; Smith, 1983). This favorable outcome aligns with reports of phosphate removal through microbial assimilation (Ray and Lotz, 2014; Liu et al., 2018).

Ammonia and nitrate were not detected throughout the study, suggesting effective microbial assimilation and conversion into microbial biomass. This aligns with previous findings that heterotrophic bacteria and protozoa regulate nitrogen cycling by continuously transforming toxic ammonia into safer compounds within biofloc technology systems (Sigeo, 2005; Khanjani et al., 2022; Wang et al., 2016; Ma and Wang, 2023). Such efficient nitrogen removal supports water quality stability and reduces the risk of toxic accumulation.

Sulfate, present in the culture medium, can serve as an electron acceptor for sulfate-reducing bacteria (SRB), contributing to anaerobic respiration and organic matter breakdown. While not directly measured here, the role of SRB highlights the importance of sulfate cycling in sustaining microbial diversity and system functionality.

Overall, these findings emphasize that successful biofloc management requires careful monitoring of interconnected parameters including floc aggregation, temperature, pH, alkalinity, DO, ORP, ionic composition, and nutrient dynamics. By balancing microbial metabolism with environmental stability, biofloc technology can sustain water quality and enhance the resilience of aquaculture systems.

## 5. Conclusion

*Bacillus cereus* exhibited wider pH variations, indicating potential shifts in acidity, whereas *Klebsiella pneumoniae* maintained a stable pH. But both organisms' pH is suitable for the BFT. Both bacteria increased total alkalinity, with *Klebsiella pneumoniae* showing a significant rise. Though it's not out of the acceptable range but this needs further investigation into the mechanisms driving alkalinity dynamics in biofloc systems. Fluctuations in oxidation-reduction potential values, with *Bacillus cereus* remaining negative above 100 mV, highlight the redox dynamics influencing oxygen availability and microbial activity. *Bacillus cereus* maintained a more stable total hardness than *Klebsiella*

*pneumoniae*. TAN was below the accepting range which is another positive point for both biofloc producing bacteria. Fluctuations in DO concentration suggest complex interactions between microbial respiration, organic matter decomposition, and environmental conditions. *Bacillus cereus* maintained higher DO levels than *Klebsiella pneumoniae*. Overall, *Bacillus cereus* showed better for early floc formation, pH stability, total hardness stability, and DO levels, though it also caused a significant turbidity increase after floc deformation.

### 5.1. Limitation

This study was conducted in a controlled laboratory setting, which may not accurately reflect the field conditions. Although this study provides valuable insights into the behavior of subject matter under ideal conditions, it may not directly apply to situations in which external factors, such as weather or environmental conditions, can significantly impact the outcomes. Future studies should be conducted in a more realistic field setting to overcome this limitation, in which researchers can better control and monitor various factors that may influence the results. This would help ensure that the findings are more generalizable to real-world applications and provide more accurate predictions of how the subject matter may behave under different conditions.

### CRedit authorship contribution statement

**Khadem Hussain Saeedi:** Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Manjulatha Chapara:** Writing – review & editing. **Jane Polyn P. Bejoc:** Writing – review & editing. **S.K.K.A. Perera:** Writing – review & editing.

### Declaration of competing interest

The authors declare that they have no identifiable financial, non-financial, professional, or personal conflicts of interest that could have influenced the outcomes of this study.

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### Data availability

Data will be made available on request.

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