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The effectivity of organic matter removal in poultry slaughterhouse wastewater by *Bacillus cereus* TD5B

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ABSTRACT

Poultry slaughterhouse wastewater (PSW) is a highly polluted effluent with a significant organic load, posing environmental challenges for treatment. This study investigates the potential of *Bacillus cereus* TD5B to grow and reduce organic matter in PSW. Laboratory-scale experiments were performed to assess the growth of *Bacillus cereus* TD5B in a PSW-containing medium and to evaluate the effectiveness of this bacterial strain in reducing key parameters, including biological oxygen demand (BOD_5), chemical oxygen demand (COD), total solids (TS), total volatile solids (TVS), total suspended solids (TSS), total dissolved solids (TDS), and ammonium concentration. *Bacillus cereus* TD5B exhibited growth in both liquid and solid mediums containing PSW. The highest bacterial growth in liquid medium occurred in T1 (25% PSW), while in solid medium, T4 (100% PSW) showed the highest growth ($5.1 \pm 0.8 \times 10^7$ CFU/mL). The survival ability of *Bacillus cereus* TD5B following incubation in a medium supplemented with nutrients and PSW at varying concentrations was confirmed through viability assays and was evidenced by the expansion of colony diameter, with the largest colony diameter observed in T2 (50% PSW). *Bacillus cereus* TD5B effectively decomposed organic matter, significantly reducing several parameters assessed. The highest performance was observed in T4 (100% PSW), which achieved removal effectivity of 92.54 ± 5.53% for BOD_5, 51.28 ± 14.84% for COD, 27.40 ± 6.66% for TS, 70.53 ± 14.84% for TVS, 88.49 ± 4.51% for TSS, 22.45 ± 7.30% for TDS, and 63.38 ± 21.48% for ammonium. These findings suggest that *Bacillus cereus* TD5B holds promise as a bioremediation agent for treating high-content organic wastewater, such as PSW. Further research is recommended to optimize the conditions for bacterial growth and enhance performance in large-scale applications.

Keywords: bioremediation, wastewater, organic matter, biological treatment, *Bacillus cereus*.

INTRODUCTION

High level of poultry meat consumption, particularly chicken, has resulted in an increased demand for slaughtering activities in poultry processing plants. Consequently, the heightened production of poultry meat has led to rise in poultry slaughterhouse wastewater (PSW) (Njoya et al., 2019). Large volumes of wastewater are generated during the poultry processing procedures of slaughtering, scalding, carcass rinsing, evisceration, equipment cleaning, and sanitation. A diverse variety of materials including blood, excreta, microbiological pollutants, undigested feed, and tissue residues are present in PSW (Atchala et al., 2025). PSW exhibits high levels of various pollutants, with a BOD_5 value reaching up to 2875 mg/L (Rinquest et al., 2019), COD value reaching 15,000 mg/L (Aziz et al., 2019), a total solids (TS) concentration of approximately 1594 mg/L (Debik and Coskun, 2009), a total volatile solids (TVS) value of 1286 mg/L (Debik and Coskun, 2009), total suspended solids (TSS) of 5462 mg/L (Yaakob et al., 2018), total dissolved solids (TDS) of 1138 mg/L (Basitere et al., 2019), and ammonium concentration of around 215 mg/L (Basitere et al., 2019). PSW contains substantial amounts of

organic matter, which fosters the growth of oxygen-consuming bacteria and other microbes in receiving water bodies, which can lead to eutrophication, driven by the elevated nutrient levels in the wastewater (Ng et al., 2022).

The management of wastewater from poultry slaughterhouses is a critical environmental concern. Kanafin et al. (2022) emphasized that waste treatment has become a pressing issue in the meat-based food sector due to increasing consumer awareness of environmental impacts, necessitating more vigorous regulatory enforcement. In line with this, Philipp et al. (2021) highlighted the growing attention toward environmental sustainability and circular economy practices in industrial systems, which are expected to be applicable to poultry slaughterhouses across various scales. The technical application of PSW reuse has emerged as a key area of ongoing research and development. Conventional wastewater treatment methods, such as chemical and physical processes, tend to be cost-prohibitive. Additionally, these methods depend on high energy from thermal power plants, which are environmentally detrimental. Chemical treatments can negatively impact soil and water quality due to the remaining chemical substances in the sludge (Kothari et al., 2024). In contrast, biological treatment methods, encompassing aerobic and anaerobic processes, offer numerous advantages over traditional techniques (Aziz et al., 2019). These systems are particularly offer notable advantages owing to their capacity to treat a wide spectrum of contaminants and nutrients, and they can generate energy and chemicals while efficiently removing organic matter, thus enhancing wastewater quality and minimizing environmental impact (Wu and Yin, 2020). Previous studies have explored various biological approaches for the treatment of PSW. Bunraksa et al. (2020) conducted research that employed purple nonsulfur bacteria for PSW, resulting in the production of both microbial biomass containing plant growth-promoting bacteria and effluents abundant in phyto-stimulatory substances. Mukandi et al. (2024) investigated the utilization of *Bacillus megaterium* which demonstrated flocculant properties for the treatment of PSW. Activated sludge was applied by Rajab et al. (2017) in an combined aerobic and anaerobic sequencing batch reactor system for PSW treatment. Park et al. (2023) further emphasized the promising potential of utilizing bacteria or bacterial-derived enzymes for bioremediation or bioconversion of waste. The use of single microorganisms in various applications remains an active area of research.

Bacillus cereus TD5B is an aerobic, motile, rod-shaped, rapid-growing, Gram-positive bacterium capable of spore formation and has a partial gene sequence 99% similar to *Bacillus cereus* NC740T (AP007209) (Fitriyanto et al., 2021). This strain, isolated from soil near a layer chicken farm, exhibits a high tolerance to polluted environments, particularly ammonia, and has the ability to produce protease with a maximum enzyme activity of 57.765 U/mg protein. Their ability to synthesize protease offers potential advantages in various sectors, including the leather tanning industry, as a dehairing and bating agent (Fitriyanto et al., 2014). The production of both extracellular protease (Fitriyanto et al., 2014) and extracellular keratinase (Fitriyanto et al., 2022) further supports its application in the management of waste from poultry slaughterhouses. Research on the potential of this strain as an aerobic bioremediation agent for PSW has not yet been conducted. This study will evaluate the growth of *Bacillus cereus* TD5B in a medium including PSW and its capacity to reduce the organic waste in PSW. The findings are expected to provide insights into the feasibility of utilizing *Bacillus cereus* TD5B for the aerobic treatment of PSW, contributing to the advancement of effective and sustainable waste management.

METHODS

Bacterial growth profile measurement in liquid medium

The growth profile of *Bacillus cereus* TD5B was established using a 100 mL liquid medium with the composition stated in Table 1. The diluted nutrition broth (T0) was used as a control. The liquid medium with various PSW concentrations was subjected to autoclave sterilization at 121 °C and 15 psi for 15 min. The sterilized liquid medium was permitted to cool and continued with the addition of 2% (v/v) *Bacillus cereus* TD5B. The mixture was then incubated at ambient temperature for 48 h on a rotary shaker at 120 rpm. The bacterial growth assay in liquid medium is based on the methodology of Winarti et al. (2018) with minor modifications. Bacterial growth was

Table 1. Medium composition

Composition	Control (T0)		Treatments			
	GP	BPE	T1	T2	T3	T4
PSW (%)	0	100	25	50	75	100
Dilluted nutrient broth (10^{-1}) (%)	100	0	75	50	25	0

Note: GP is the control for measuring the growth profile, while BPE is the control for measuring bioremediation potential effectivity.

monitored every 3 h utilizing a Shimadzu UV-1601PC spectrophotometer at a wavelength of 600 nm. The data were recorded, and a bacterial growth curve was generated afterward. The procedure for assessing bacterial growth is based on Murthy et al. (2014), with minor adjustments.

Bacterial viability assay

The viability of bacterial cells was evaluated by inoculating 1 µL of bacteria, previously cultured in liquid medium control (T0) and liquid medium supplemented with PSW (T1, T2, T3, and T4) for 24 and 48 h onto nutritional agar medium. Five bacterial colonies were created on a single petri dish by dropping 1 µL of bacteria from T0, T1, T2, T3, and T4 onto a solid medium. The inoculated solid medium was incubated at 30 °C and monitored every 24 h for 3 days. The bacterial viability assay procedure was conducted according to Fitriyanto et al. (2022) with minor adjustments.

Bacterial growth profile measurement in solid medium

The liquid medium with different PSW concentrations for growth profile measurement (Table 1) was added with 1.5% agar powder and was sterilized in an autoclave at 121 °C and 15 psi for 15 min. After sterilization, the medium was aseptically poured into petri dishes and left to solidify under sterile conditions. *Bacillus cereus* TD5B from a pre-culture with a 10^{-5} dilution (100 µL) was inoculated using the spread plate method. Incubation of the plates was then conducted at 30 °C for 72 h. Colonies of Bacillus cereus TD5B grown on the solid medium were counted employing an automatic colony counter. The counts were recorded, and only results with colony numbers between 30 and 300 were considered. The data obtained were then used to calculate the bacterial count in CFU/mL using the Equation 1 according to Njoku et al. (2015).

$$Colony\ forming\ unit\ (CFU/ml) = \frac{Total\ colonies}{Culture\ inoculated\ (ml) \times dilution\ factor} \quad (1)$$

Bioremediation potential effectivity measurement

To observe bioremediation effectifity, 3 L samples were prepared following the composition outlined in Table 1. The 100% PSW treatment (T0) without *Bacillus cereus* TD5B supplementation, served as the control group for the bioremediation potential effectivity measurement. The liquid medium was sterilized in an autoclave at 121 °C and 15 psi for 15 min. After sterilization, the medium was allowed to ambient temperature, and 2% (v/v) *Bacillus cereus* TD5B was added. A 1 L subsample was collected for initial assessment prior to aeration, whereas the remaining 2 L underwent aeration treatment. The bioremediation procedure was conducted over 4 days utilizing a laboratory-scale aerator tank. Subsequent to aeration, the samples were let to settle for one day. Samples from both pre-aeration and post-aeration were analyzed for BOD_5, COD, TS, TVS, TSS, TDS, and ammonium concentration. The test findings were utilized to determine the percentage reduction or effectivity of the aeration process. The bioremediation potential effectivity, expressed as a percentage reduction, was determined using the Equation 2 stated by Nikuze et al. (2020).

$$Reduction\ (\%) = \frac{Value\ before\ aeration - Value\ after\ aeration}{Value\ before\ aeration} \times 100\% \quad (2)$$

BOD_5 measurement

The BOD_5 was assessed according to the Indonesian National Standard (SNI, 2009). Four Winkler bottles were prepared and labeled A1, A2, B1, and B2. The sample was placed into bottles A1 and A2 until overflowing, while the diluent solution was similarly added to bottles

B1 and B2. Each container was carefully sealed to prevent air bubbles and then agitated multiple times. Bottles A2 and B2 were incubated at 20 °C for 5 days. The dissolved oxygen (DO) levels in bottles A1 and B1 were promptly assessed with a calibrated DO meter, and the findings were documented as A1 and B1. Furthermore, the dissolved oxygen levels in bottles A2 and B2 were assessed and documented. The BOD_5 value was calculated using the Equation 3.

$$BOD_5\ (mg/L) = \frac{(A_1 - A_2) - \left(\frac{B_1 - B_2}{V_B}\right) \times V_C}{P} \quad (3)$$

where: A_1 – DO value of the test sample prior to incubation (mg/L), A_2 – DO value of the test sample after 5 days of incubation (mg/L), B_1 – DO value of the blank before incubation (mg/L), B_2 – DO value of blank after 5 days of incubation (mg/L), VB – volume of the microbial suspension (mL) in the DO blank bottle, VC – volume of the microbial suspension (mL) in the test sample bottle, P – ratio of the test sample volume and total volume.

COD measurement

The COD was measured following the Indonesian National Standard (SNI, 2019). An erlenmeyer flask containing boiling stones was filled with a 5 mL sample, followed by 10 mL of 0.25 N $K_2Cr_2O_7$ and 15 mL of an $AgSO_4$-H_2SO_4, and blended thoroughly. The sample was heated for 2 h under a reflux with cooling water circulating through the condenser. After the heating, the interior of the condenser was flushed with 40 mL of distilled water. A total of three drops of a ferroin indicator were carefully added to the sample prior to titration, which was then carried out using 0.25 N FAS solution until the color changed from greenish blue to brick red. The volume of titrant utilized was documented as Vc (mL). A blank measurement was conducted using distilled water, and the volume of titrant utilized for the blank was noted as Vb (mL). The COD value was determined using Equation 4.

$$COD\ (mg/L) = \frac{(V_b - V_c) \times N \times 8000}{Sample\ volume\ (ml)} \quad (4)$$

where: Vb – the amount of FAS solution required for the blank titration (mL), Vc – the amount of FAS solution used in the sample titration (mL), N – the normality of the FAS solution.

TS and TVS measurement

The TS and TVS were evaluated following the Indonesian National Standard (SNI, 2005). The crucible used for the TS determination was dried in an oven at 105 °C for 60 min and weighed. The result was documented as A1 (g). The crucible was then heated in a furnace at 550 °C for 60 min and was reweighed, and the result is documented as A2 (g) for the TVS calculation. The sample was homogenized, and 5 mL was transferred into the crucible using a pipette. The crucible containing the sample was dried in the oven at 105 °C for 5 h and followed by weighing. The measurement was documented as B (g). The TS value was calculated using Equatgion 5.

$$TS\ (mg/L) = \frac{(B - A_1) \times 10^6}{Sample\ volume\ (ml)} \quad (5)$$

where: B – weight of the crucible containing samples after the oven at 105 °C (g), A_1 – initial crucible without samples after the oven at 105 °C (g).

The TVS value was evaluated by extending the TS value testing protocol. The crucible and sample, which had been pre-dried in an oven at 105 °C, were then heated in a furnace at 550 °C for 60 min and weighed. The weight was documented as C (g). The TVS value was further calculated using Equation 6.

$$TVS\ (mg/L) = \frac{(C - A_2) \times 10^6}{Sample\ volume\ (ml)} \quad (6)$$

where: C – weight of the crucible containing samples after the furnace at 550 °C (g), A_2 – initial crucible without samples after the furnace at 550 °C (g).

TSS and TDS measurement

The TSS were evaluated following the Indonesian National Standard (SNI, 2019). The filter paper used for sample filtration was initially rinsed with deionized water using a filtration apparatus. The filter paper was placed in a crucible and was heated in an oven at 105 °C for one hour. Following the drying process, the crucible containing the filter paper was cooled in a desiccator and subsequently weighed. The obtained weight was recorded as W_0 (g). Prior to sample filtering, the filter paper was pre-hydrated with a minimal quantity of deionized water. The sample was

homogenized with a magnetic stirrer, and 20 mL of the homogenate was pipetted for filtering. The material was then filtered, and the filter paper was rinsed three times with 10 mL of deionized water each time. The filter paper was then returned to the crucible and dried in an oven at 105 °C for one hour. It was then cooled in a desiccator and weighed to determine its constant mass. The result was documented as W_1 (g). The TSS value was determined using Equation 7.

$$TSS\ (mg/L) = \frac{(W_1 - W_0) \times 10^6}{Sample\ volume\ (ml)} \quad (7)$$

where: W_1 – weight of the crucible and filter paper containing solids (g), W_0 – initial crucible and filter paper weight (g).

The TDS were assessed following the Indonesian National Standard (SNI, 2005). The filter paper was positioned on the filtration apparatus and rinsed with 20 mL of deionized water. The rinse was discarded, and the filter paper was utilized to assess the TSS value. The dish was dried for one hour at 105 °C in the oven then was allowed to cool in a desiccator. An analytical balance was used to measure the weight of the dish. The result of the weighing was recorded as A1 (g). A 20 mL sample was pipetted into a filtration apparatus with a vacuum and filter paper. The filtration was then performed. Upon completion of the filtration process, the filter paper was rinsed three times with distilled water, 10 mL for each rinse. The rinsed water and filter results were placed in a pre-prepared dish and evaporated until dry. The dish containing the dissolved solids was heated at 105 °C for 18 h in an oven. The dish was then allowed to cool in a desiccator, followed by weighing. The results of the weighing are presented as B (g). The TDS value was determined using Equation 8.

$$TDS\ (mg/L) = \frac{(B - A_1) \times 10^6}{Sample\ volume\ (ml)} \quad (8)$$

where: A_1 – weight of the empty dish after the 105 °C oven (g), B – weight of the dish and soluble solids after the 105 °C oven (g).

Ammonium measurement

Ammonium content was determined using the Nessler method, as detailed by Anupong et al. (2022), with minor modifications. The procedure comprises two phases: the establishment of a standard curve and the determination of ammonium concentrations. The standard curve was generated from a standard solution of $(NH_4)_2SO_4$ at concentrations of 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 mg/L, each measured at 9.8 mL. A total of 200 µL of a mixture of Nessler solutions A and B was added to each $(NH_4)_2SO_4$ concentration in a dark condition. The mixture was vortexed and allowed to stand for 10 min in the absence of light. A spectrophotometer was then used to measure absorbance at 570 nm. The standard curve was created using the absorbance data, producing a regression equation for measuring ammonium content.

Furthermore, the ammonium content in the sample was analyzed. A 1 mL sample was centrifuged at 5000 rpm for 5 min. Afterwards, 100 µL of the supernatant was pipetted into a test tube and was added with 9.7 mL of distilled water. A total of 200 µL of a mixture of Nessler solutions A and B was then added in a dark environment. The sample was vortexed and kept at rest under dark conditions for 10 min. A spectrophotometer was then used to quantify the absorbance at 570 nm. The absorbance measurements were utilized to determine ammonia concentrations using the regression equation derived from the established standard curve.

Statistical analysis

Each treatment was conducted in triplicate. The data from the bioremediation potential effectivity measurement samples were analyzed using one-way ANOVA based on a completely randomized design (CRD). Bacterial growth data and bacterial viability were analyzed descriptively in a liquid medium. Duncan's multiple range test (DMRT) was conducted to determine significant differences among treatment means when initial analysis indicated variability (Steel et al., 1997).

RESULTS AND DISCUSSION

Bacterial growth profile measurement in liquid medium

Bacillus cereus TD5B growth profile was evaluated to determine the ability to survive in the environment of PSW. The cell growth rate is important in establishing the start-up time of PSW treatment systems in practical applications,

necessitating an examination of the bacteria's growth parameters (Cao et al., 2024). The cell growth profile over 48 h, shown in Figure 1, indicated that *Bacillus cereus* TD5B exhibited growth under all treatments. From the treatments of T0 (control), T1 (25% PSW), T3 (75% PSW), and T4 (100% PSW) exhibited fluctuating growth patterns. The most significant cell growth occurred in treatment T1 (25% PSW), continued by T2 (50% PSW), T3 (75% PSW), and T0 (control), while the least growth was observed in T4 (100% PSW). *Bacillus cereus* TD5B showed a lag phase from 0 to 3 h, a log phase from 3 to 21 h, and subsequently entered the stationary phase.

Increasing growth occurred as *Bacillus cereus* TD5B has the ability to utilize the nutrients in the liquid medium for growing and cultivation, resulting in decreasing nutritional availability. The low nutrient concentration causes a decrease in bacterial growth due to cellular mortality. The remaining cells then employed the deceased one to facilitate the re-growth in the same batch. Maier et al. (2009) stated that deceased cells may occur lysis and function as a regenerated source of nutrients. Development that derives nutrients from deceased cells is termed endogenous metabolism, which may occur during the bacterial growth cycle. The changes in the growth profile of *Bacillus cereus* TD5B were ascribed to disparities in wastewater concentrations, which affected its growth performance.

The existence of organic matter and other materials in wastewater helps facilitate optimal bacterial development when their availability aligns with the microorganism's unique requirements. An imbalance in availability may impede growth. This conclusion corresponds with Ehrenberg et al. (2013), who indicated that nutrient composition substantially influences bacterial growth rates, with superior-quality nutrients facilitating more rapid growth than inferior-quality options. The noted brief lag phase and exponential growth for up to 21 h further suggest that *Bacillus cereus* TD5B demonstrates rapid growth traits, aligning with the findings of Fitriyanto et al. (2014). The findings underscore the adaptability and efficacy of *Bacillus cereus* TD5B in nutrient-dense conditions, positioning it as a viable choice for wastewater bioremediation applications.

Bacterial cell viability assay

The viability assessment of *Bacillus cereus* TD5B was performed to determine its survival following cultivation in a liquid medium with different concentrations of PSW. Viability was evidenced by the capacity of colonies to enlarge over time (Kaur and Sethi, 2012). A visual investigation of the bacterial colonies shown in Figure 2 revealed that *Bacillus cereus* TD5B successfully grew on nutrient agar after being cultivated in a liquid medium containing nutrients and PSW at varying concentrations for 24 and 48 h. The bacterial growth was distinctly evident from the progressive enlargement of the colony's diameter, which extended from day 1 to day 3, signifying

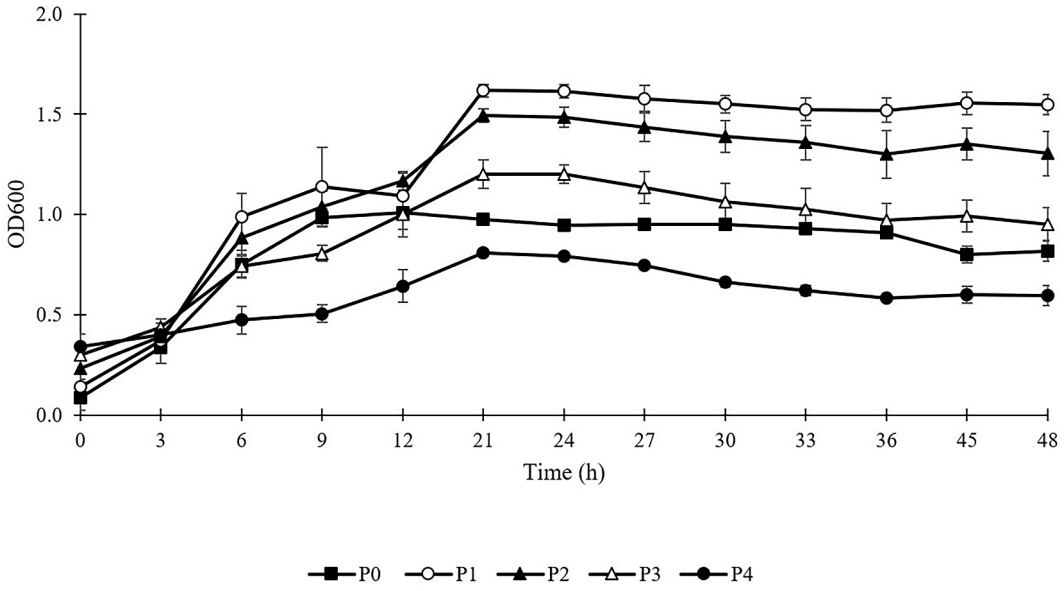

Figure 1. Growth profile of *Bacillus cereus* TD5B in liquid medium over 48 h

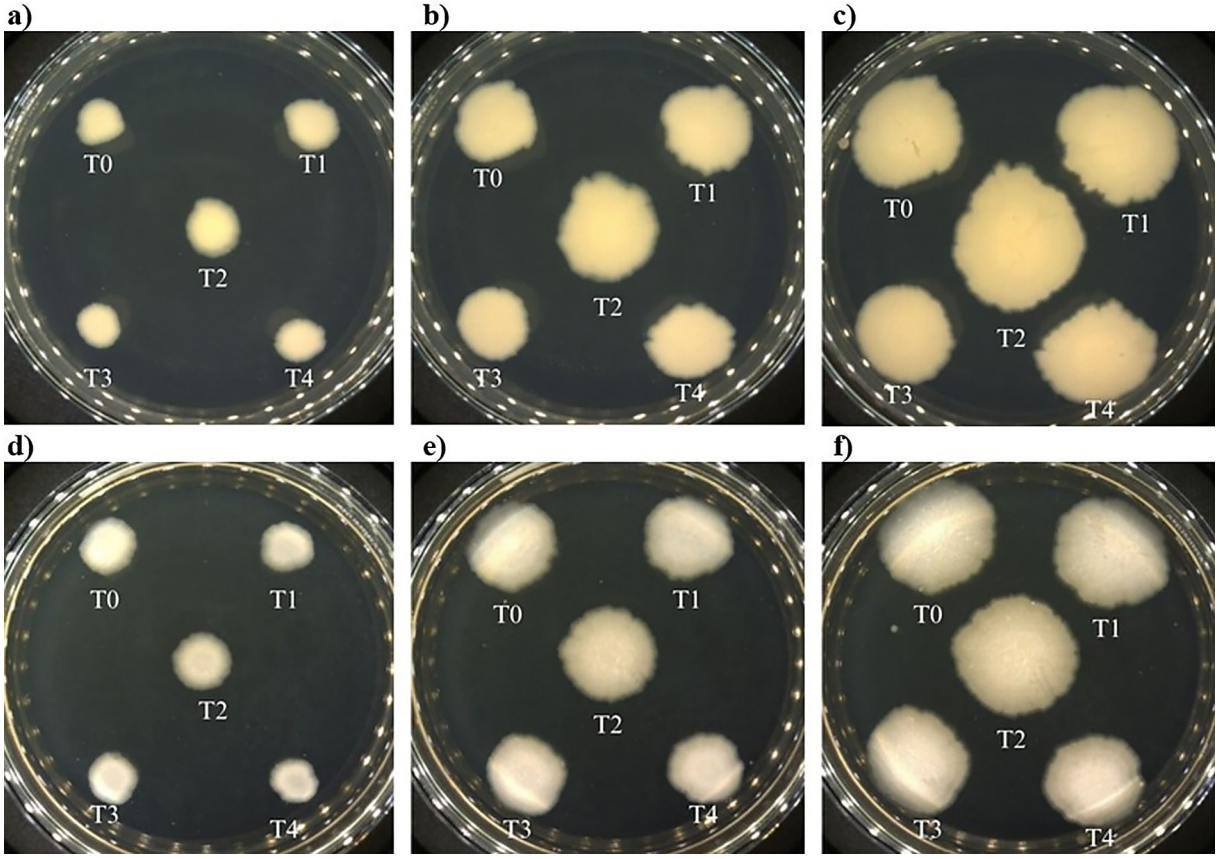

Figure 2. Cell viability of *Bacillus cereus* TD5B at: (a) 24 h day 1 (b) 24 h day 2 (c) 24 h day 3 (d) 48 h day 1 (e) 48 h day 2 (d) 48 h day 3

sustained growth during incubation. A disparity in colony diameter was noted among treatments, with T2 (50% PSW) exhibiting the biggest diameter and T3 (75% PSW) the lowest, at 24 and 48 h of viability. The bacterial cell viability test findings between 24 and 48 h indicated a variation in the coloration of the colonies produced. By 24 h, the colonies were yellowish, but by 48 h, they seemed paler or tended to be white.

Variations in colony diameters indicate that PSW concentration influences bacterial growth potential. Reduced colony size may signify diminished growth rates or stress that impedes the growth of bacteria (Tavichakorntrakool et al., 2017). The color alterations observed during 24 and 48 h indicate the adaptation of *Bacillus cereus* TD5B to the PSW-enriched environment. Alterations in colony morphology, including differences in color, size, form, and texture, may signify phenotypic adaptability. These modifications are essential for bacterial survival in the face of environmental stresses. Phenotypic and genotypic variety within bacterial communities boosts tolerance to environmental fluctuations and facilitates survival (Sousa et al., 2013).

Bacterial growth profile assessment on solid medium

The findings demonstrate a substantial difference in the colony counts of *Bacillus cereus* TD5B ($P < 0.05$). The results indicated that the colony numbers in treatments T0 (control), T3 (75% PSW), and T4 (100% PSW) significantly differed from those in T1 (25% PSW) and T2 (50% PSW). The maximum colony count was recorded in T1 (25% PSW), at $(13 \pm 5.7) \times 10^7$ CFU/mL, whereas the minimum was noted in T3 (75% PSW), at $(4.4 \pm 1.9) \times 10^7$ CFU/mL (Table 2).

The nutrient availability in T1 (25% PSW) and T2 (50% PSW) corresponded with the bacterial requirements, facilitating optimal growth. As the concentration of wastewater in the medium escalated, the number of bacterial colonies diminished due to the inadequate supplementation of nutrient broth, which proved insufficient to fulfill the bacterial requirements. PSW alone is insufficient to fulfill the bacterial nutritional requirements. Furthermore, the organic matter in PSW is more intricate than that in nutrient broth, necessitating a longer duration for utilization (Kaskote et al., 2024; Madeira

Table 2. Total colonies of *Bacillus cereus* TD5B in PSW containing solid medium

Treatments	Total colonies (×10^7 CFU/mL)
T0	5.4 ± 0.5^a
T1	1.3 ± 5.7^b
T2	1.2 ± 0.9^b
T3	4.4 ± 1.9^a
T4	5.1 ± 0.8^a

Note: mean ± standar deviation. Values sharing same letters differ non-significantly (P > 0.05).

et al., 2023). Nutrient availability in T0 (control) was negligible due to the absence of PSW, leading to diminished bacterial growth. The measurements of bacterial growth in both liquid and solid medium exhibited comparable tendencies, indicating that *Bacillus cereus* TD5B can endure on medium containing PSW, necessitating concentration adjustments for optimal development.

Bioremediation potential effectivity

The effectivity of the bioremediation process was evaluated based on the reduction of variables that represent the quality of wastewater, including BOD_5, COD, TS, TVS, TSS, TDS, and ammonium content (Table 3). Water quality indicators, including BOD_5, COD, TS, TVS, TSS, and TDS, are essential to determine the status of aquatic ecosystems and the effectiveness of wastewater treatment methods (Johnson and Mehrvar, 2020; Misman et al., 2023; Razif, 2022).

Biological oxygen demand (BOD_5) and chemical oxygen demand (COD)

The BOD_5 quantifies the dissolved oxygen necessary for microorganisms to decompose organic matter aerobically (Tchobanoglous et al., 2014). In contrast, COD assesses the total oxygen demand for the chemical oxidation of organic and certain inorganic substances in wastewater (Mongioví et al., 2024).The BOD_5 value after the bioremediation process decreased significantly ($P < 0.05$). The degradation percentage in BOD_5 for T1 (25% PSW) and T2 (50% PSW) significantly differed from T0 (control), T3 (75% PSW), and T4 (100% PSW). The reduction in BOD_5 indicates that organic matter was degraded during bioremediation. The COD value was found to decrease. However, the reduction percentage did not significantly differ ($P > 0.05$). A reduction in BOD_5 and COD signifies the breakdown of organic matter during bioremediation. Incorporating *Bacillus cereus* TD5B and aeration decreased BOD_5 and COD in PSW. The decrease in organic content transpires when bacteria convert complex organic molecules into simpler forms, a process facilitated by ongoing oxygen supply through aerators (Raza et al., 2023).

This study exhibited a greater reduction in BOD_5 and a lesser reduction in COD relative to the research conducted by Oktafani et al. (2019), which attained BOD_5 and COD reductions of 72.23% and 72.83%, respectively, utilizing a granular activated sludge-sequencing batch reactor (GAS-SBR) for 2 h. The BOD_5 decrease in treatments T0, T3, and T4 was analogous to the results of Aziz et al. (2018), who reported a reduction of over 90% in BOD_5 employing submerged fibers in an attached growth sequential batch reactor. Nonetheless, the COD reduction observed in our investigation was inferior to the > 90% established by Aziz et al. (2018). The findings indicate bioremediation with *Bacillus cereus* TD5B is more efficacious in diminishing BOD_5 than COD. The restricted capacity of *Bacillus cereus* TD5B

Table 3. PSW bioremediation reduction effectivity by *Bacillus cereus* TD5B

Variables (mg/L)	Reduction effectivity (%)				
	T0	T1	T2	T3	T4
BOD_5	95.81 ± 3.19^b	70.97 ± 11.06^a	78.48 ± 8.12^a	93.70 ± 3.10^b	92.54 ± 5.53^b
COD^{ns}	61.71 ± 21.20	51.11 ± 17.85	58.79 ± 7.37	43.16 ± 9.27	51.28 ± 14.84
TS	15.94 ± 2.40^a	51.07 ± 7.12^c	51.10 ± 6.48^c	41.05 ± 1.66^c	27.40 ± 6.66b
TVS^{ns}	43.93 ± 13.74	72.30 ± 12.16	77.20 ± 11.99	73.80 ± 9.93	70.53 ± 14.84
TSS	73.18 ± 17.37^c	16.39 ± 3.76^a	38.80 ± 19.06^{ab}	69.24 ± 27.67^{bc}	88.49 ± 4.51^c
TDS	11.93 ± 7.77^a	48.62 ± 5.34b	43.95 ± 7.22^b	36.49 ± 6.32^b	22.45 ± 7.30^a
Ammonium	26.13 ± 39.31^c	-285.55 ± 247.08^{ab}	-353.87 ± 61.95^a	-97.52 ± 121.50^{bc}	63.38 ± 21.48^c

Note: mean ± standar deviation. Values sharing same letters differ non-significantly (P > 0.05).

to decompose non-biodegradable organic materials is demonstrated by a COD decrease of less than 65%. COD denotes the aggregate organic matter, encompassing biodegradable and non-biodegradable components (Qi et al., 2021).

Total solid and total volatile solid

TS are the residue left in a container following the complete evaporation of water at a temperature of 103 °C to 105 °C. In contrast, total volatile solids (TVS) are the solids that volatilize and burn entirely during ashing at 500 ± 50 °C. TVS is presumed to denote the concentration of organic solids in wastewater. The TS value in each treatment decreased after the bioremediation process. The reduction percentage in TS values showed a significant difference ($P < 0.05$). According to the DMRT post hoc test, the reduction percentage in TS values between T1 (25% PSW), T2 (50% PSW), and T3 (75% PSW) was not significantly different. However, the reduction percentage in T0 (control) and T4 (100% PSW) significantly differed from the other treatments. A decrease in TVS values was observed. However, there was no significant difference ($P > 0.05$) in the reduction percentage of TVS values. The decrease in TS and TVS values indicates the efficacy of wastewater treatment methods (Tchobanoglous et al., 2014). Elevated amounts of organic solids in wastewater can cause environmental harm by reducing dissolved oxygen concentrations, disrupting aquatic ecosystems, and posing health hazards to humans, animals, and plants (Ngobeni et al., 2022).

The decrease in TS and TVS noted in this study is attributed to the capacity of *Bacillus cereus* TD5B to decompose solids in wastewater by transforming organic matter into simpler substances, including CO_2, hydrogen, H_2O, and energy, which are employed for its growth and reproduction (Salishcheva et al., 2023). Furthermore, sedimentation after the aeration procedure facilitated decreased solid content (Abood et al., 2022; Rebosura et al., 2021). The rising bacterial biomass during aeration further increases the solid content, requiring sedimentation. During aeration, bacteria generate flocs that enhance the separation of particles by sedimentation (Li et al., 2020). The decrease in TS across all treatments in this investigation was smaller than the 85% reported by Shih and Kozink (1980) utilizing an ultrafiltration system and the 90.5% noted by Fatima et al. (2023) using a sequential membrane process that incorporated ultrafiltration, forward osmosis, and reverse osmosis. The decrease in TVS noted in this investigation exhibited no significant variations among treatments. It was inferior to the 85.3% reduction that Fatima et al. (2023) employed the identical sequential membrane technique.

Total suspended solid and total dissolved solid

TSS denote particle debris suspended in water that can negatively impact aquatic ecosystems. Increased TSS levels elevate water turbidity, obstructing sunlight penetration and affecting photosynthesis in aquatic ecosystems. Moreover, TSS may exacerbate eutrophication by adding nutrients that promote excessive algal proliferation (Fernández Del Castillo et al., 2022). TDS refer to the overall concentration of both organic and inorganic materials dissolved in water, encompassing cationic elements such as magnesium, calcium, sodium, and potassium, along with anionic elements such as carbonates, bicarbonates, nitrates, sulfates, and chlorides (Pushpalatha et al., 2022). Increased TDS levels can intensify toxicity by raising salinity, modifying ionic composition, and introducing harmful ions. Such conditions may result in diminished biodiversity, alterations in aquatic community structure, the exclusion of sensitive species, and acute and chronic consequences at different life stages (Weber-Scan and Duffy, 2007). In this study, the TSS value decreased, and the reduction percentage showed a notable difference ($P < 0.05$). The reduction percentage in TSS for T0 (control) showed no meaningful variation from T3 (75% PSW) and T4 (100% PSW) but significantly different from T1 (25% PSW) and T2 (50% PSW). The TDS value also decreased, and the reduction percentage showed a notable variation ($P < 0.05$). The reduction percentage in TDS for T0 (control) showed no meaningful difference from T4 (100% PSW) but exhibited a notable variation from T1 (25% PSW), T2 (50% PSW), and T3 (75% PSW).

The introduction of *Bacillus cereus* TD5B during aeration significantly diminished TSS and TDS by breaking down complex organic molecules into simpler forms, which were then assimilated as nutrients for bacterial metabolism (Salishcheva et al., 2023; Wu et al., 2021). The TSS reduction observed in this study for T1 (25% PSW), T3 (75% PSW), and T4 (100% PSW)

surpassed the 60% documented by Audelia et al. (2023) utilizing a granular activated sludge-sequencing batch reactor (GAS-SBR) yet fell short of the 99% reduction attained by Bingo et al. (2021) with a laboratory-scale integrated multi-stage PSW treatment system, which included an aerobic pre-treatment tank, an expanded granular sludge bed (EGSB) bioreactor, and a submerged ultrafiltration (UF) membrane. The diminished TSS reduction percentages in treatments T1 (24% PSW) and T2 (50% PSW) can be ascribed to the favorable proliferation of *Bacillus cereus* TD5B, supported by nutrition supplementation and the constrained wastewater load. The excellent growth led to enhanced biomass during aeration, which diminished the effectivity of organic matter breakdown due to the accumulation of suspended biomass, as indicated by the TSS measurements (Aziz et al., 2019). The decrease in TDS noted in this study surpassed the sub-5% reduction documented by Sardari et al. (2018) utilizing only electrocoagulation. However, their findings were analogous to those of integrating electrocoagulation with ultrafiltration. Nonetheless, the TDS decrease in this investigation was inferior to the 61.43% documented by Baddour et al. (2016) utilizing an aerobic moving bed biofilm reactor. Treatments T1 (25% PSW), T2 (50% PSW), and T3 (75% PSW) demonstrated superior TDS reductions relative to other treatments due to augmented bacterial growth. The enhanced bacterial activity enabled increased consumption of dissolved solids as organic substrates for bacterial metabolism, significantly reducing TDS levels (Pushpalatha et al., 2022).

Ammonium content

Based on the results of ammonium content, it was found that the reduction percentage in ammonium levels showed a notable difference ($P < 0.05$). However, the reduction percentage for T1 (35% PSW), T2 (50% PSW), and T3 (100% PSW) was negative because ammonium contents increased. Ammonium reduction only occurred in T0 (control) and T4 (100% PSW). The highest reduction percentage was 63.38 ± 21.48% in T4 (100% PSW). The changes in ammonium levels suggest that *Bacillus cereus* TD5B effectively promoted nitrification and denitrification throughout the aeration process. Nitrification entails the oxidation of ammonium to nitrite and then to nitrate, whereas denitrification converts nitrite and nitrate into dinitrogen gas emitted into the atmosphere (Rodelas, 2021; Rout et al., 2017). Furthermore, ammonium reduction may occur because of its use as a nitrogen source to facilitate bacterial proliferation (Shen et al., 2020). Despite the noted decrease in ammonium concentrations, the efficacy in this study was inferior to that of previously documented techniques, including the constructed wetland system, which attained an 80% reduction (Ramdani et al., 2019), and the anaerobic-anoxic-aerobic combined reactor, which achieved an 84% reduction (Lopes et al., 2022). Treatments T1 (25% PSW), T2 (50% PSW), and T3 (75% PSW) exhibited an increase in ammonium concentration, likely attributable to heightened bacterial metabolic activity and cell lysis, which release nitrogen into the medium (Tchobanoglous et al., 2014). The results validate the findings of Kim et al. (2005), emphasizing the capacity of heterotrophic *Bacillus* strains to execute nitrification and denitrification in aerobic environments. The results indicate that incorporating sequential anaerobic, anoxic, and aerobic processes within a single reactor may improve ammonium removal efficacy during PSW bioremediation with *Bacillus cereus* (Lopes et al., 2022). Future research should investigate integrated methodologies to enhance overall therapy efficacy.

CONCLUSIONS

The findings of this study indicate that *Bacillus cereus* TD5B successfully decreases organic matter and ammonium levels in PSW by aeration over a four-day duration. Variations in nutritional and PSW concentrations considerably affected most metrics, except for TVS, which exhibited no significant differences among treatments. Treatment T4 (100% PSW) had the best removal effectivity, attaining 92.54 ± 5.53% for BOD_5, 51.28 ± 14.84% for COD, 27.40 ± 6.66% for TS, 70.53 ± 14.84% for TVS, 88.49 ± 4.51% for TSS, 22.45 ± 7.30% for TDS, and 63.38 ± 21.48% for ammonium. The incorporation of nutrients affected the growth of *Bacillus cereus* TD5B, hence influencing its bioremediation efficacy both positively and negatively. Although adequate nutrition supply augmented bacterial activity and facilitated the breakdown of organic matter and ammonium in PSW, excessive bacterial proliferation resulted in heightened biomass, potentially diminishing the overall treatment effectiveness. The findings

underscore the efficacy of *Bacillus cereus* TD5B as a bioremediation agent for treating PSW, especially under optimal aeration conditions.

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