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Performance of indigenous bacterial consortia for bioremediation of domestic wastewater

Sukmawati[1,2], Agus Bintara Birawida[3*], Ambeng[4], Anwar Daud[3], Irwan Ridwan Rahim[5], Erniwati Ibrahim[3], Shaharuddin Mohd Sham[6]

[1] Department of Public Health, Doctoral Student Public Health Faculty, Hasanuddin University, 90245, Makassar, Indonesia
[2] Department of Public Health, Al Asyariah Mandar University, 91311, Polewali Mandar, West Sulawesi, Indonesia
[3] Department of Environmental Health, Public Health Faculty, Hasanuddin University, 90245, Makassar, Indonesia
[4] Department of Biology, Faculty of Mathematics and Natural Sciences, Hasanuddin University, 90245, Makassar, Indonesia
[5] Department of Civil Engineering, Faculty of Engineering, Hasanuddin University, 90245, Makassar, Indonesia
[6] Department of Environmental and Occupational Health, Faculty of Medicine and Health Sciences, University Putra Malaysia, Malaysia
* Corresponding authot's email: agusbirawida@unhas.ac.id

ABSTRACT

Domestic wastewater pollution poses significant environmental challenges, particularly in small island communities where centralized treatment systems are limited. This study evaluated the performance of an indigenous six-strain bacterial consortium (*Aeromonas veronii*, *Enterobacter sichuanensis*, *Klebsiella pneumoniae*, *Bacillus albus*, *Escherichia fergusonii*, and *Pseudomonas aeruginosa*) for domestic wastewater treatment under controlled laboratory conditions. The consortium was applied at inoculum concentrations of 0%, 5%, 10%, and 15% and incubated for 2–8 days. Removal efficiencies of total suspended solids (TSS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammonia (NH_3), and oil-grease were determined using standard analytical procedures (SNI 2005–2019). Two-way ANOVA indicated that both inoculum concentration and incubation period significantly affected pollutant removal efficiency ($p < 0.05$). The highest treatment performance was achieved at 15% inoculum after 8 days, with removal efficiencies of 68.75% (TSS), 52.01% (BOD), 42.94% (COD), 80.70% (NH_3), and 98.75% (oil-grease). Removal efficiency increased proportionally with microbial dosage and exposure duration, suggesting cooperative microbial degradation processes. These findings suggest that indigenous multi-strain bacterial consortia can serve as a feasible biological alternative for decentralized domestic wastewater treatment systems in small island environments

Keywords: bioremediation, indigenous bacteria, domestic wastewater, TSS, BOD, COD, NH_3, oil-grease removal.

INTRODUCTION

Domestic wastewater discharge represents a major environmental concern due to its high content of suspended solids, biodegradable organic matter, nutrients, and lipids. Key parameters such as total suspended solids (TSS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammonia (NH_3), and oil-grease are widely used to assess wastewater pollution levels and treatment performance (Pallarès et al., 2021; Dölle & Lex, 2022; Novita et al., 2022). In many developing regions, a significant proportion of domestic wastewater is discharged without adequate treatment, contributing to the deterioration of receiving water bodies.

In Indonesia, rapid population growth and urbanization pose significant challenges to domestic wastewater management. Approximately 51–53% of domestic wastewater is discharged directly into the environment without adequate treatment (Widhiawati et al., 2024; Yogaswara & Mulyana, 2024). In urban areas, nearly 61% of wastewater from bathrooms and laundry is released into open drains or rivers, while in rural areas approximately 38% is disposed of without treatment(Widyarani et al., 2022). These discharge patterns contribute to eutrophication, dissolved oxygen depletion, and associated public health risks in receiving coastal waters.

Conventional wastewater treatment technologies, including physicochemical coagulation, filtration, and chemical oxidation, are often energy-intensive and costly to implement in small or decentralized systems (Abbas et al., 2023; Alquwaizany et al., 2021). These limitations necessitate alternative treatment approaches that are operationally simple, cost-effective, and environmentally sustainable.

Bioremediation using microbial processes has emerged as a promising strategy for organic and nutrient removal in wastewater systems. Indigenous microorganisms, naturally adapted to local environmental conditions, may exhibit enhanced survival and metabolic performance compared to exogenous strains (Shah et al., 2025). In particular, multi-strain bacterial consortia have been reported to improve degradation efficiency through complementary metabolic pathways and cooperative substrate utilization (L. Liu et al., 2022; Y. Zhang et al., 2022). Although microbial consortia have been widely investigated for industrial effluents and laboratory-simulated wastewater, studies focusing on indigenous bacterial consortia for domestic wastewater treatment in small island environments remain limited. Environmental variability, including fluctuating salinity and temperature, may influence microbial degradation kinetics and overall treatment efficiency in such settings.

It was hypothesized that indigenous bacterial consortia isolated from Barrang Lompo Island exhibit adaptive metabolic capabilities that enhance the biodegradation of organic, nitrogenous, and lipid pollutants in domestic wastewater. Therefore, this study aimed to evaluate the effects of inoculum concentration (0–15%) and incubation period (2–8 days) on the removal efficiency of TSS, BOD, COD, ammonia, and oil-grease under controlled laboratory conditions.

METHODS AND ANALYSIS

Study area and wastewater sampling

Domestic wastewater samples were collected from a household discharge channel on Barrang Lompo Island, Makassar, Indonesia (119°19′48″ E; 5°02′48″ S). The island represents a densely populated small island settlement with limited centralized wastewater treatment infrastructure. Sampling was conducted during peak domestic discharge hours (08:00–10:00). Approximately 20 L of composite wastewater were collected in sterile polyethylene containers and transported under cooled conditions (4 °C) to the laboratory within 4 h for analysis.

Isolation and preparation of indigenous bacterial consortium

Indigenous bacterial strains were isolated from environmental samples collected near the wastewater discharge area using serial dilution followed by spread plate and streak plate techniques on nutrient agar. Distinct colonies were purified through repeated streaking until morphologically uniform colonies were obtained. Representative colony morphology during purification is shown in Figure 2.

Six bacterial strains were identified and selected for consortium preparation: *Aeromonas veronii*, Enterobacter sichuanensis, Klebsiella pneumoniae, *Bacillus albus*, *Escherichia fergusonii*, and *Pseudomonas aeruginosa*. Each strain was cultured in nutrient broth at 30 ± 2 °C for 24 h prior to application. Bacterial suspensions were adjusted to approximately 10^8 CFU/mL (OD600 ≈ 0.8). Equal volumes of each strain suspension were combined to form the indigenous bacterial consortium.

Experimental setup and incubation

Batch contact experiments were conducted using sterile polyethylene containers containing domestic wastewater. The bacterial consortium was applied at concentrations of 0% (control), 5%, 10%, 15% Each treatment was performed in triplicate. Following inoculation, the mixtures were manually homogenized to ensure uniform microbial distribution. Samples were incubated under static conditions at room temperature (25–27 °C) for 2, 4, 6, and 8 days. Containers were loosely capped to allow passive atmospheric oxygen exchange.

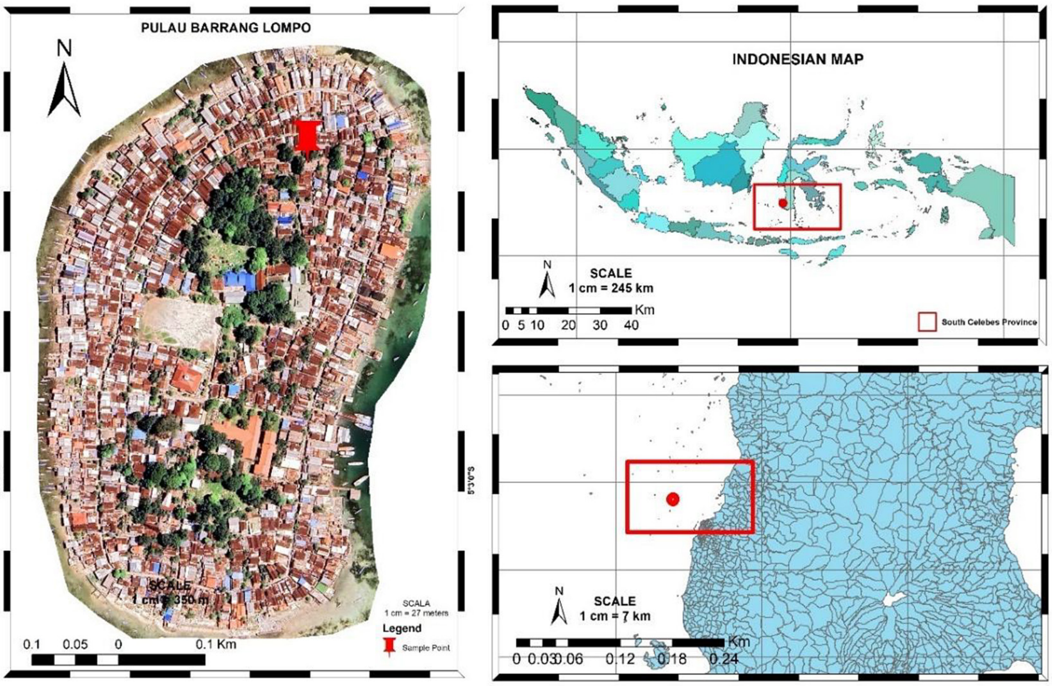

Figure 1. Location of the domestic wastewater sampling site on Barrang Lompo Island, Makassar, Indonesia (119°19′48″ E; 5°02′48″ S). The sampling point is indicated in red. The map illustrates the geographical position of the island within South Sulawesi Province

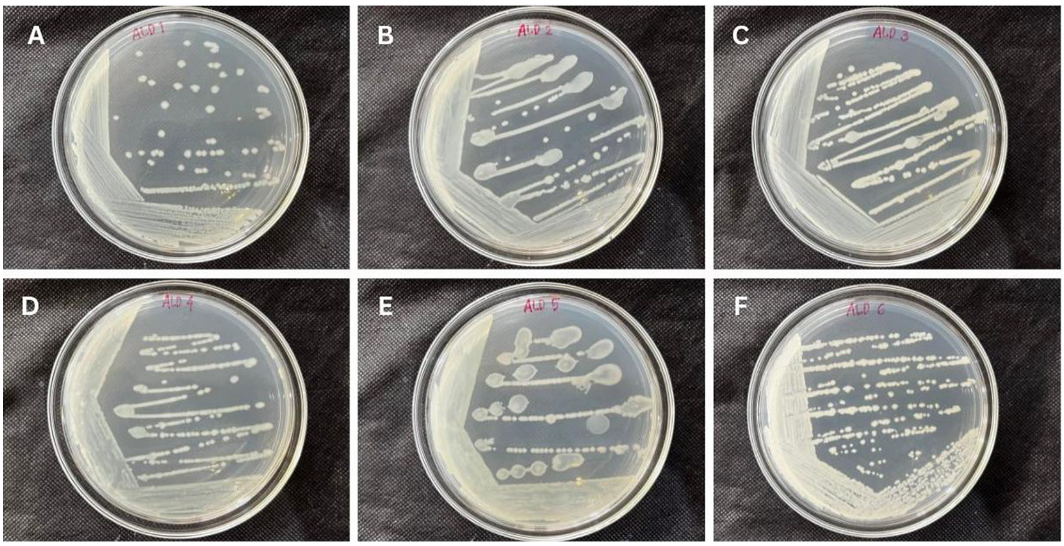

Figure 2. Representative colony morphology of indigenous bacterial isolates during purification on nutrient agar using the streak plate method. Panels (A–F) correspond to purified isolates (ALD1–ALD6) prior to consortium preparation.

No mechanical aeration or external carbon supplementation was applied. Representative documentation of the batch contact experiments at early and final incubation stages is presented in Figure 3.

Water quality analysis

The following parameters were analyzed according to Indonesian National Standards (SNI): (1) total suspended solids (TSS) – measured by the gravimetric method (SNI 06-6898.3-2019); (2) biochemical oxygen demand (BOD_5) - 5-day incubation method (SNI 6989.72-2009); (3)

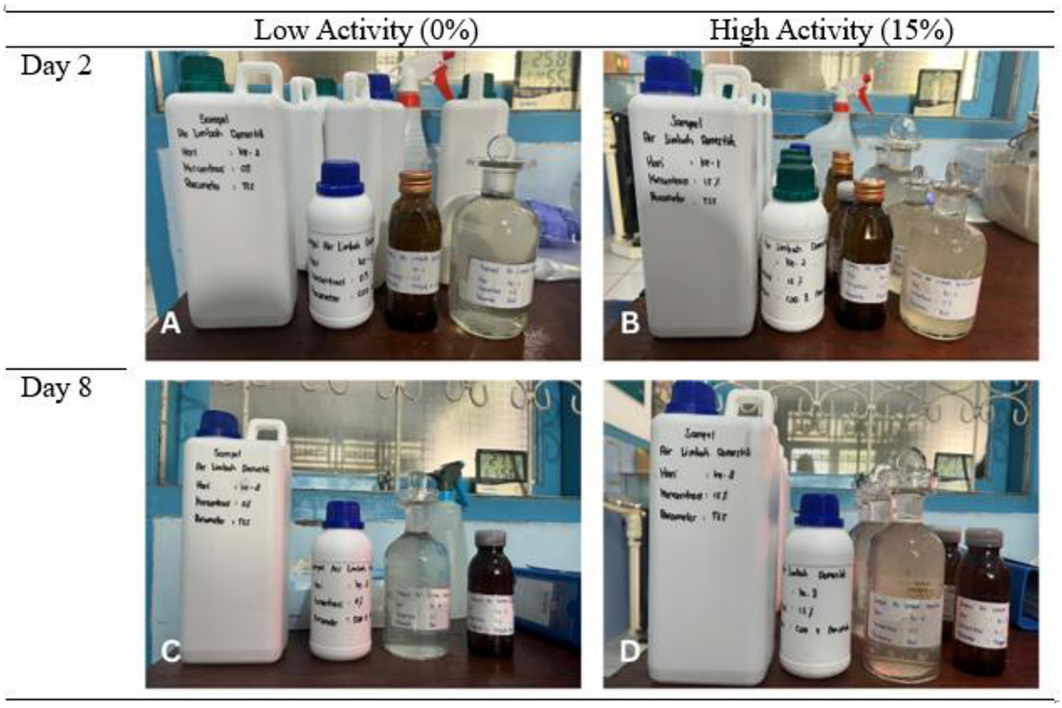

Figure 3. Representative photographs of domestic wastewater during batch treatment with indigenous bacterial consortium. (A) Day 2, 0% (control); (B) Day 2, 15% inoculum; (C) Day 8, 0% (control); (D) Day 8, 15% inoculum. Increased clarity and visible sedimentation were observed in the 15% treatment after 8 days of incubation

chemical oxygen demand (COD) – closed reflux method (SNI 6989.2-2019); (4) ammonia (NH_3) – phenate method (SNI 06-6989.30-2005); and (5) oil-grease: measured by the gravimetric method. Pollutant removal efficiency (%) was calculated according to Equation (1):

$$Removal\ efficiency\ (\%) = \frac{C_0 - C_t}{C_0} \times 100 \quad (1)$$

Statistical analysis

All experiments were conducted in triplicate (n = 3), and results are presented as mean ± standard deviation. Data normality was assessed using the Shapiro-Wilk test, and homogeneity of variances was evaluated using Levene's test. Two-way analysis of variance (ANOVA) was performed to determine the effects of inoculum concentration (0%, 5%, 10%, and 15%) and incubation time (2, 4, 6, and 8 days) on pollutant removal efficiency (TSS, BOD, COD, ammonia, and oil-grease). Interaction effects between inoculum concentration and incubation time were also examined. When significant differences were detected ($p < 0.05$), post hoc comparisons were performed using Tukey's Honestly Significant Difference (HSD) test to identify differences between treatment groups. Statistical significance was established at $\alpha = 0.05$. All statistical analyses were conducted using SPSS version 26 (IBM Corp., USA). Graphical visualizations were prepared using RStudio (version 4.3.0).

Ethical considerations

This study was approved by the Health Research Ethics Committee of the Faculty of Public Health, Hasanuddin University (approval number: 151024093071).

RESULTS

The effects of inoculum concentration and incubation time on pollutant removal efficiency were evaluated using two-way ANOVA (Table 1). For TSS removal, both concentration (F = 160.78; $p < 0.001$) and incubation time (F = 16.04; p = 0.001) showed statistically significant effects. Similarly, COD removal was significantly influenced by concentration (F = 16.81; $p < 0.001$) and incubation time (F = 17.82; $p < 0.001$). For ammonia, concentration exerted a strong effect (F = 114.09; $p < 0.001$), while incubation time showed a moderate but significant influence ($F = 4.90$; $p = 0.028$). BOD removal was also significantly affected by concentration ($F = 8.22$; $p = 0.006$) and incubation time ($F = 4.55$; $p = 0.033$). In contrast, oil-grease removal was primarily influenced by concentration ($F = 28.37$; $p < 0.001$), whereas incubation time did not show a statistically significant effect ($F = 1.31$; $p = 0.330$).

The interaction effect between inoculum concentration and incubation time was also examined. Although both factors contributed to treatment performance, inoculum concentration consistently exhibited higher F-values across parameters, indicating a stronger statistical influence on pollutant removal efficiency under the tested conditions.

Removal efficiency trends over time

The temporal evolution of pollutant removal efficiency under different inoculum concentrations is presented in Figure 4. Across all parameters, removal efficiency increased with increasing bacterial consortium concentration. The 15% inoculum consistently demonstrated the highest removal performance over the incubation period. TSS removal efficiency reached approximately 68–69% at the highest concentration by day 8. COD removal increased progressively, reaching values above 40% under 15% inoculum conditions Ammonia removal exhibited high efficiency across concentrations, exceeding 80% at higher inoculum levels. Oil-grease removal showed rapid improvement at higher concentrations, approaching nearly complete removal under 10–15% inoculum.

Parameter-specific performance patterns

The parameter-specific removal dynamics are shown in Figure 5. TSS and COD removal exhibited gradual increasing trends with both concentration and incubation time. BOD removal demonstrated an initial decrease followed by recovery at higher concentrations. Ammonia removal showed consistent enhancement with increasing inoculum concentration, reaching maximal efficiency by Day 8. Oil-grease removal displayed concentration-dependent behavior, with limited improvement at 0% but substantial enhancement at ≥10% inoculum.

Comparative removal intensity

A heatmap representation (Figure 6) summarizes removal efficiencies across all treatments and incubation times. Higher removal intensities (indicated by warmer colors) were predominantly associated with 10% and 15% inoculum concentrations. The heatmap visually confirms the statistical findings that concentration was the primary determinant of treatment performance.

Table 1. Two-way ANOVA results for the effects of inoculum concentration and incubation time on pollutant removal efficiency

Parameter	Factor	*df*	*F*	*p*-value
TSS	Concentration	3	160.78	<0.001
	Incubation time	3	16.04	0.001
	Model (overall)	6	88.41	<0.001
BOD	Concentration	3	8.22	0.006
	Incubation time	3	4.55	0.033
	Model (overall)	6	6.39	0.007
COD	Concentration	3	16.81	<0.001
	Incubation time	3	17.82	<0.001
	Model (overall)	6	17.32	<0.001
Ammonia	Concentration	3	114.09	<0.001
	Incubation time	3	4.90	0.028
	Model (overall)	6	59.50	<0.001
Oil-grease	Concentration	3	28.37	<0.001
	Incubation time	3	1.31	0.330
	Model (overall)	6	14.84	<0.001

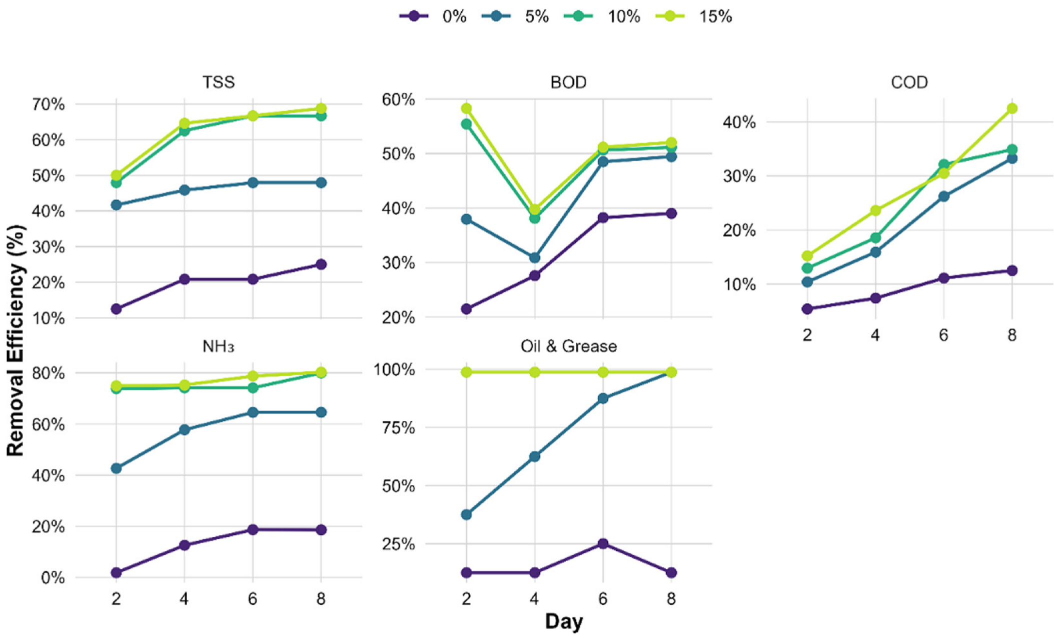

Figure 4. Trends of pollutant concentrations (BOD, COD, NH_3, oil-grease, and TSS) in domestic wastewater during incubation (2–8 days) at different bacterial inoculum concentrations (0%, 5%, 10%, and 15%)

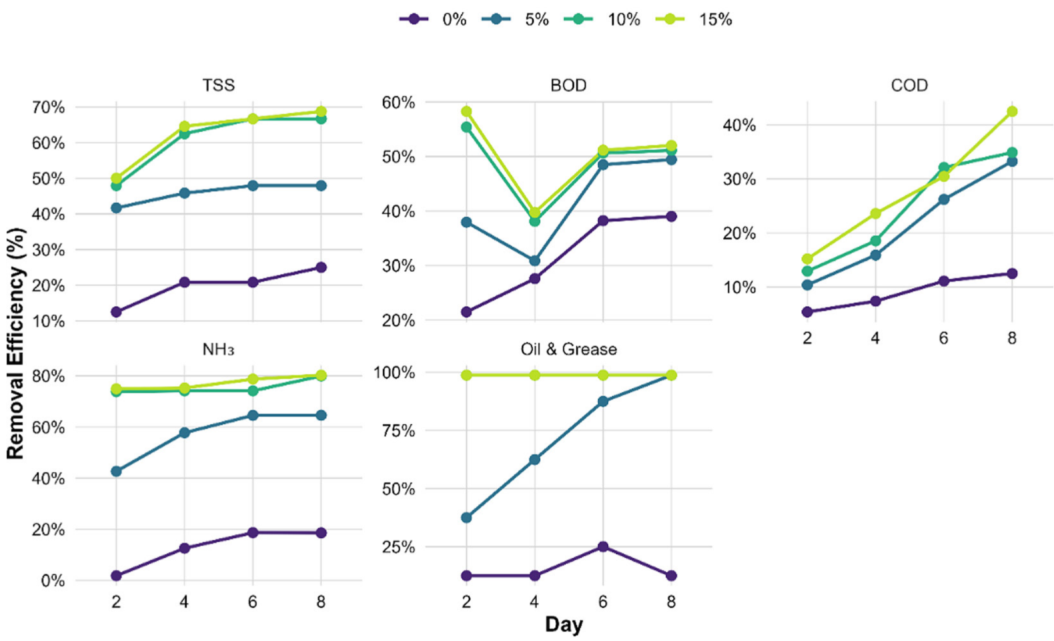

Figure 5. Removal efficiency of bacterial consortia on TSS, BOD, COD, NH_3, and Oil-Grease at different inoculum concentrations (0%, 5%, 10%, 15%) across incubation periods (2, 4, 6, and 8 days)

DISCUSSION

Overall effect of inoculum concentration and incubation time

The present study demonstrates that inoculum concentration and incubation time significantly influenced pollutant removal efficiency, as confirmed by two-way ANOVA results (Table 1). Concentration had a statistically significant effect on all parameters ($p < 0.01$), while incubation time significantly affected TSS, BOD, COD, and ammonia ($p < 0.05$), but not oil-grease removal.

The statistical results indicate that inoculum concentration was the primary determinant

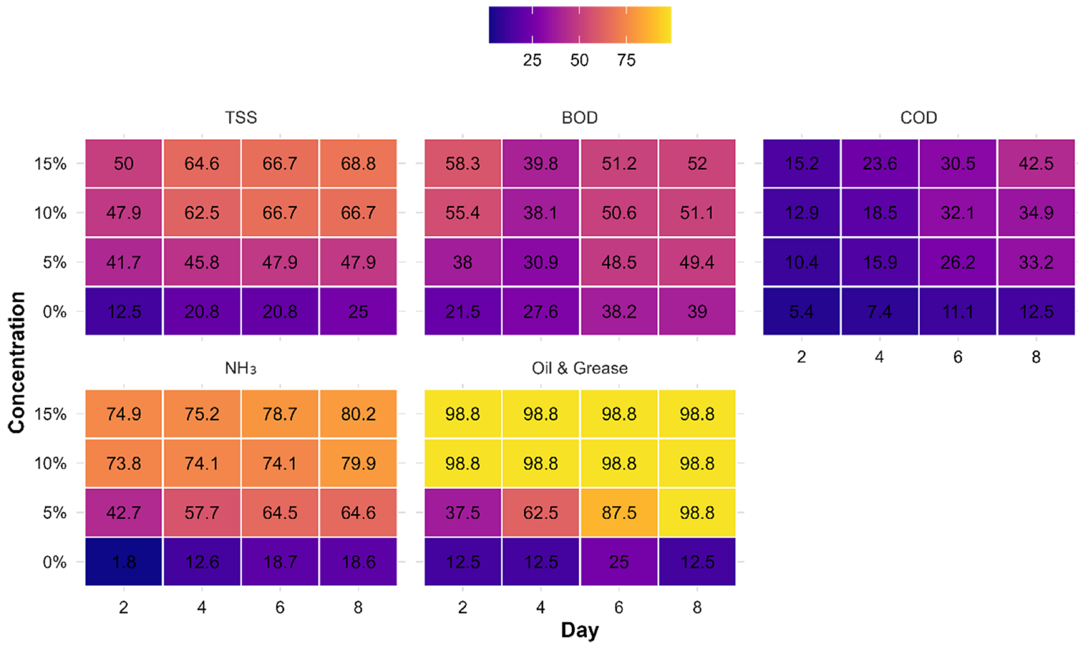

Figure 6. Heatmap visualization of the removal efficiencies (%) of TSS, BOD, COD, NH_3, and oil-grease by bacterial consortia at varying inoculum concentrations and incubation periods

of treatment performance across all measured pollutants, as reflected by consistently higher F-values compared with incubation time (Table 1). This finding indicates that microbial biomass availability governs substrate conversion capacity under batch conditions.

The observed increase in removal efficiency with higher inoculum concentration suggests that microbial density plays a critical role in substrate conversion capacity. Increased biomass concentration may enhance enzymatic activity and improve substrate–microorganism contact frequency. Similar improvements in degradation efficiency with increasing microbial population density have been reported in previous studies (Zhang et al., 2020; Liu et al., 2023), where complementary metabolic interactions within microbial consortia were identified as contributing factors.

The progressive improvement in removal performance over incubation time indicates active microbial adaptation and growth during treatment. Indigenous microorganisms are generally adapted to local environmental conditions, which may improve survival and metabolic stability under fluctuating wastewater characteristics (Shah et al., 2025). However, the magnitude of improvement varied among pollutants, suggesting different degradation kinetics and metabolic pathways.

Total suspended solids removal

The maximum TSS removal efficiency reached 68.75% at 15% inoculum concentration after eight days of incubation. Statistical analysis confirmed that both concentration and incubation time significantly affected TSS removal ($p < 0.01$).

This removal range is comparable to previous reports indicating approximately 60% TSS reduction after six days of microbial treatment (Zabermawi et al., 2022). Similar TSS reduction patterns have been observed in textile wastewater treatment using microbial consortia (Bashir et al., 2023; Dhameliya, 2024).

TSS reduction in biological systems is generally associated with microbial flocculation and biodegradation of particulate organic matter. Floc formation enhances sedimentation of suspended particles and promotes aggregation processes. Environmental parameters such as temperature and pH can influence microbial growth and floc structure stability. The recorded incubation temperature range (25–27°C) falls within the optimal growth range for mesophilic bacteria, including Pseudomonas aeruginosa and Bacillus albus (Annisa et al., 2021; Ibrahim et al., 2024).

The significant interaction between concentration and time suggests that TSS removal was not solely a function of microbial presence, but of sufficient biomass accumulation and exposure duration to promote aggregation and sedimentation processes.

Biochemical oxygen demand removal

BOD removal reached 52% at the highest inoculum concentration. Two-way ANOVA indicated significant effects of both concentration ($p = 0.006$) and incubation time ($p = 0.033$).

The reduction in BOD reflects microbial oxidation of biodegradable organic matter. Increasing inoculum concentration likely enhanced enzymatic hydrolysis and aerobic respiration processes. Previous studies have reported higher BOD removal (80–85%) under optimized environmental conditions using microalgae–bacteria consortia (Foladori et al., 2018; L. Liu et al., 2022). Differences between the present study and those reports may be attributed to wastewater composition, reactor configuration, and environmental control.

Species such as Pseudomonas and Klebsiella are known to contribute to organic matter degradation through extracellular enzyme production (Mhedhbi et al., 2020). Environmental parameters, particularly temperature (28–32 °C) and pH 7–8, have been reported to enhance BOD removal efficiency (Khakimova et al., 2022; Mostafa et al., 2022), which is consistent with the operational conditions applied in this study.

The moderate removal efficiency observed in this study indicates that while the indigenous consortium effectively degraded readily biodegradable fractions, longer retention times or controlled aeration may further enhance BOD reduction in practical applications.

Chemical oxygen demand removal

COD removal increased with both inoculum concentration and incubation time, reaching 42.5% under the highest treatment conditions. Statistical analysis confirmed significant effects of concentration and incubation time ($p < 0.001$).

COD represents both biodegradable and partially recalcitrant organic compounds; therefore, lower removal efficiency compared with optimized systems is expected. Microbial consortia often demonstrate enhanced degradation performance due to metabolic cooperation and enzymatic diversity (Zhang et al., 2020; Bôto et al., 2021).

Comparable COD removal efficiencies have been reported using bacterial consortia such as Enterobacter cloacae and Pseudomonas otitis (Zabermawi et al., 2022). Higher removal ranges (78.5–99.03%) have been documented under optimized laboratory conditions with controlled aeration and nutrient balance (Nwajuaku & Agunwamba, 2023; Farhami et al., 2025; Velu DK, et al., 2025).

The lower COD removal observed here likely reflects the complexity of untreated domestic wastewater and the absence of mechanical aeration, indicating that oxygen availability may have limited oxidative degradation rates.

Ammonia removal

Ammonia concentration decreased from 50.05 mg/L to 9.66 mg/L under the highest treatment condition. Both concentration ($p < 0.001$) and incubation time ($p = 0.028$) significantly influenced ammonia removal.

Ammonia removal in mixed bacterial systems typically involves heterotrophic nitrification and aerobic denitrification pathways. Indigenous bacterial communities may facilitate nitrogen transformation through synergistic metabolic interactions.

Ammonia removal efficiencies exceeding 99% have been reported under controlled incubation and neutral pH conditions (Jasmin et al., 2024; Bhambri et al., 2025). Mixed cultures containing Enterobacter and Klebsiella species have also demonstrated enhanced nitrogen transformation capacity (Y. Zhang et al., 2022). Biofilm-based microbial systems have similarly shown effective ammonia removal in wastewater treatment applications (Petrilli et al., 2024).

The strong statistical effect of inoculum concentration ($F = 114.09$) suggests that nitrogen transformation capacity was highly dependent on microbial biomass density, reinforcing the role of consortium structure in nitrogen removal efficiency.

Oil and grease removal

Oil-grease removal exceeded 98% under all treatment conditions. Two-way ANOVA indicated that inoculum concentration significantly influenced oil-grease removal ($p < 0.001$), while incubation time did not show a significant effect ($p = 0.330$).

The rapid reduction suggests strong lipolytic activity within the consortium. Species such as

Pseudomonas aeruginosa and Aeromonas veronii are known to produce biosurfactants that enhance oil emulsification and biodegradation (Aktar et al., 2022; Wu et al., 2023). Other genera, including Bacillus and Enterobacter, may support hydrocarbon degradation by stabilizing microenvironmental conditions (Li et al., 2023; Mnif et al., 2023).

Similar oil-grease removal efficiencies exceeding 95% have been reported in previous wastewater bioremediation studies (Adetunji & Olaniran, 2021; Zabermawi et al., 2022). The non-significant effect of incubation time indicates that oil-grease degradation occurred predominantly during early treatment stages, suggesting rapid enzymatic hydrolysis of lipid fractions.

Implications for wastewater treatment in island communities

The findings indicate that indigenous bacterial consortia can reduce multiple pollutant parameters under controlled laboratory conditions. The observed treatment efficiency supports the feasibility of applying locally adapted microbial systems in decentralized wastewater management contexts.

Indigenous microorganisms may offer operational advantages in environments where infrastructure is limited, as they are pre-adapted to local climatic and wastewater characteristics (Shah et al., 2025). Biological treatment using microbial consortia may represent an alternative to physicochemical processes, particularly in small island or resource-limited settings.

However, scaling from batch laboratory systems to field-scale applications requires further investigation, including hydraulic retention time optimization, aeration control, and long-term stability assessment.

Engineering implications for decentralized wastewater treatment

The observed removal efficiencies under controlled laboratory conditions indicate that indigenous bacterial consortia may be applicable for decentralized wastewater treatment systems, particularly in small island or resource-limited settings. The consistent improvement in removal performance with increasing inoculum concentration suggests that system optimization can be achieved through controlled biomass dosing strategies.

The consistent statistical significance of inoculum concentration across parameters suggests that system optimization can be achieved through controlled biomass dosing strategies rather than solely increasing hydraulic retention time.

The significant influence of incubation time on TSS, BOD, COD, and ammonia removal further indicates that hydraulic retention time (HRT) is a critical design parameter for potential field implementation. Under the tested conditions, an incubation period of 6–8 days yielded stable and comparatively higher removal efficiencies for most parameters.

Given the tropical temperature range (29–32.5 °C) during the experiment, the system operated within a favorable range for mesophilic bacterial activity. This suggests potential compatibility with passive or low-energy treatment units in warm-climate regions, where temperature control is not required.

However, translation to engineering-scale applications requires validation under continuous-flow conditions, evaluation of long-term microbial stability, and assessment of operational robustness before full-scale implementation can be recommended.

CONCLUSIONS

This study evaluated the performance of an indigenous bacterial consortium for domestic wastewater treatment under controlled batch conditions. Two-way ANOVA confirmed that inoculum concentration significantly affected the removal efficiency of all evaluated parameters, while incubation time significantly influenced TSS, BOD, COD, and ammonia removal. The highest treatment performance was observed at 15% inoculum concentration after 8 days of incubation, achieving removal efficiencies of 68.75% (TSS), 52.01% (BOD), 42.94% (COD), 80.70% (ammonia), and 98.75% (oil-grease). These findings indicate that microbial dosage plays a dominant role in pollutant removal efficiency, while incubation duration contributes to performance stabilization for most parameters. Under laboratory conditions, the indigenous multi-strain consortium demonstrated the capacity to reduce organic, nitrogenous, and lipid pollutants in domestic wastewater. Further investigation under continuous-flow and pilot-scale conditions is required to evaluate long-term operational stability and field applicability.

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