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Integrated Bioremediation and Subsurface-Flow (SSF) Constructed Wetland using Pistia stratiotes for Fishery Wastewater Treatment

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ABSTRACT

Fishery wastewater contains organic matter and suspended solids that may adversely affect receiving water bodies when discharged without adequate treatment. Nature-based treatment technologies using aquatic macrophytes offer a potentially simple and environmentally sustainable approach for wastewater management. This study evaluated an integrated sequential system combining Pistia stratiotes-based bioremediation and a subsurface-flow (SSF) constructed wetland for fishery wastewater treatment. Five plant–biomass combinations were evaluated, namely 100% coverage with 250 g initial biomass (A), 75% with 200 g (B), 50% with 150 g (C), 25% with 100 g (D), and an unplanted control (E), with three replicates per treatment. BOD_5, COD, and TSS were monitored on days 3, 6, 9, 12, and 15 at the influent, bioremediation outlet, and final SSF outlet. The 100% coverage–250 g combination showed the highest reported overall removal efficiencies at day 15: 74.00% for BOD_5, 66.96% for COD, and 73.75% for TSS. The linear mixed-effects model indicated significant treatment, day, and sampling-point effects, as well as treatment × day and treatment × sampling-point interactions ($p < 0.001$). Further work is needed to assess stage-specific performance, hydraulic conditions, and long-term stability.

Keywords: Pistia Stratiotes; Fishery Wastewater; Bioremediation; Subsurface Flow; Constructed Wetland

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INTRODUCTION

Fishery activities generate wastewater containing organic matter, suspended solids, and other pollutants originating from fish residues, washing processes, and handling activities. If inadequately managed, the discharge of such wastewater may increase the organic and particulate load entering receiving water bodies (Chowdhury et al., 2010). Therefore, appropriate treatment technologies are needed to reduce pollutant loads before wastewater is discharged into the environment.

Nature-based treatment technologies have attracted considerable attention because they can utilize biological processes while requiring relatively simple infrastructure. Constructed wetlands are particularly promising because pollutant removal can occur through a combination of sedimentation, filtration, adsorption, microbial degradation, and plant-associated processes (García et al., 2010; Saeed & Sun, 2012). In SSF constructed wetlands, wastewater passes through porous media, creating interactions among wastewater, microorganisms, plant roots, soil, and filter media. These processes contribute to the removal of organic and particulate pollutants (Vymazal & Kropfelova, 2009; Wu et al., 2023).

Aquatic macrophytes can enhance constructed wetland performance by providing root surfaces for microbial colonization and influencing conditions within the rhizosphere (Stottmeister et al., 2004). P. stratiotes is one aquatic macrophyte that has been investigated for wastewater treatment because of its extensive root system and interaction with pollutants and microorganisms (Kataki et al., 2021). Ali et al. (2024) reported substantial reductions in TSS, BOD, and COD using P. stratiotes in a constructed wetland. Similarly, Imron et al. (2023) demonstrated the potential of P. stratiotes as a phytotechnology-based treatment plant for wastewater.

Previous research demonstrated considerable reductions in organic matter and suspended solids, particularly at higher plant coverage (Al-Rosyid et al., 2024; Mustafa & Hayder, 2021). However, a single-stage bioremediation system may still leave residual pollutants in treated wastewater (Wibowo et al., 2023). An additional treatment stage is therefore required to improve overall treatment performance (Dewi et al., 2021; Panrare et al., 2016).

SSF constructed wetlands can provide a complementary treatment stage because they provide a porous medium through which wastewater can be filtered and biologically treated (Kusumaningtyas et al., 2025). The combination of plant-based bioremediation and SSF treatment may therefore provide multiple pollutant removal barriers (Hadi & Pungut, 2022). Rather than relying solely on plant uptake, the integrated system allows plant-associated microbial processes to operate together with filtration, sedimentation, adsorption, and microbial degradation within the SSF media (Masalvad et al, 2016).

The contribution of this study is an experimental assessment of a sequential configuration in which P. stratiotes-based bioremediation is followed by an SSF constructed wetland for fishery wastewater. In contrast to studies of single-stage P. stratiotes treatment and other integrated floating-plant/constructed-wetland systems, this study evaluates five treatment combinations across three sampling points and five sampling days over a 15-day period. It therefore documents how treatment outcomes vary across the tested plant–biomass combinations and along the treatment train.

Therefore, this study aimed to evaluate the performance of an integrated P. stratiotes-based bioremediation and subsurface-flow (SSF) constructed wetland system for reducing BOD_5, COD, and TSS in fishery wastewater. Specifically, the study compared treatment performance among five plant–biomass combinations, examined temporal and spatial variations in pollutant concentrations, and assessed the overall performance of the sequential treatment system over a 15-day experimental period. Because plant coverage and initial biomass varied together, their individual effects could not be isolated.

METHODS

Study Area, Wastewater, and Experimental Design

The experiment was conducted at the Integrated Laboratory, Faculty of Engineering, Universitas Muhammadiyah Jember, Indonesia. Fishery wastewater was collected from fish auction and fish-processing activities in Puger Subdistrict, Jember District, East Java, Indonesia.

The experimental system consisted of two sequential treatment stages: a *P. stratiotes*-based bioremediation reactor followed by an SSF constructed wetland. The bioremediation reactor had an approximate nominal capacity of 96 L and contained gravel media with particle sizes of 2–4 cm and 4–6.5 cm. The subsequent SSF constructed wetland had an approximate nominal capacity of 120 L and consisted of paddy soil and gravel layers with particle sizes of 0.5–1.5 cm, 2–4 cm, and 4–6.5 cm. Each bioremediation reactor was independently connected to its corresponding SSF unit to prevent cross-treatment interference.

A completely randomized design was applied with five levels of *P. stratiotes* coverage and three replicates. The treatments were 100% coverage with 250 g biomass (A), 75%

coverage with 200 g biomass (B), 50% coverage with 150 g biomass (C), 25% coverage with 100 g biomass (D), and 0% coverage with 0 g biomass (E). Thus, the experiment consisted of 15 independent sequential treatment systems. Treatment E served as the unplanted control and had the same reactor and SSF configuration as the planted treatments. The plants were acclimatized for seven days.

Treatment Operation, Sampling, and Water Quality Analysis

The treatment was operated for 15 days using the sequential bioremediation–SSF configuration. Wastewater first entered the *P. stratiotes*-based bioremediation reactor and subsequently flowed into the corresponding SSF constructed wetland. The treatment configuration was maintained consistently among all experimental units.

Three sampling points were established to evaluate pollutant reduction along the treatment system: S1 represented the influent before treatment, S2 represented the outlet of the bioremediation stage, and S3 represented the final outlet of the SSF constructed wetland. Samples were collected on days 3, 6, 9, 12, and 15. The initial wastewater concentration on day 0 was used as the baseline concentration for calculating removal efficiency.

The treatment performance was evaluated based on BOD_5, COD, and TSS concentrations. BOD_5 was determined using the iodometric method (SNI 6989.72:2009). COD was analyzed using the closed reflux spectrophotometric (SNI 6989.2:2009), while TSS was determined using the gravimetric method (SNI 6989.3:2019).

Removal efficiency was calculated using the following equation:

$$RE\ (\%) = \frac{Ci - Ce}{Ci}\ x\ 100$$

Where RE is the removal efficiency (%), Ci is the initial influent concentration (mg/L), and Ce is the concentration at the corresponding sampling point (mg/L).

Statistical Analysis

Descriptive data were presented as mean ± standard deviation. Measurements were collected repeatedly from the same experimental systems over several sampling days and at different sampling points, a linear mixed-effects model (LMM) was employed as the primary inferential statistical approach.

Treatment, sampling day, and sampling point were specified as fixed effects, while replicate was specified as a random effect. Treatment × day and treatment × sampling point interactions were included to evaluate temporal variation in treatment responses and differences in concentration patterns across sampling points. The F-statistics, degrees of freedom, and *p*-values reported in Table 1. Statistical significance was evaluated at $p < 0.05$.

RESULT AND DISCUSSION

BOD_5, COD and TSS Reduction

The integrated system reduced biodegradable organic matter in fishery wastewater. Treatment A, which combined 100% plant coverage with an initial biomass of 250 g, showed the highest reported performance among the tested combinations; the BOD_5 concentration at day 15 was 78 mg/L (Figure 1a). This result showed best performance among the combinations tested. Coverage and initial biomass increased together across treatments, so their separate effects cannot be distinguished. Root-associated microbial activity is a plausible contributor to organic-matter degradation, as discussed in previous studies (Ali et al., 2024), but microbial activity and root biomass change were not measured directly in this experiment.

Similar reductions in BOD_5 have also been reported in constructed wetland systems treating wastewater, supporting the contribution of wetland media and vegetation to organic matter removal (De Rozari & Ledoh, 2018).

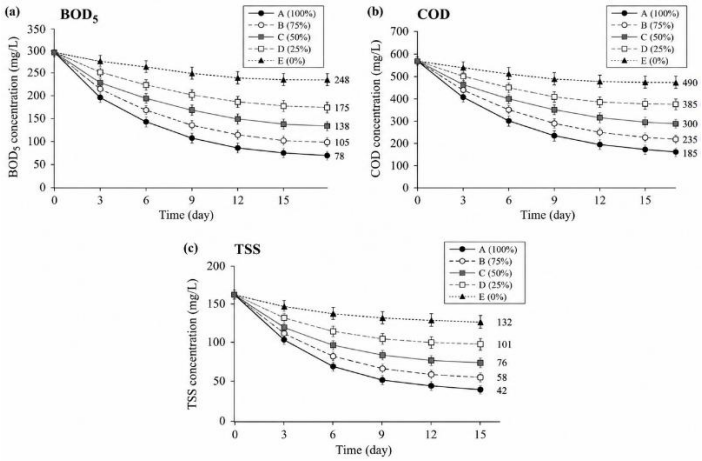


Figure 1. (a) BOD_5 Reduction; (b) COD Reduction and (c) TSS Reduction.

COD followed a pattern similar to BOD_5. In treatment A, COD decreased from 560 mg/L to 185 mg/L (Figure 1b), equivalent to 66.96% removal. Root surfaces may support microbial colonization and associated organic-matter transformation (Imron et al., 2023). The observed reduction reflects the performance of the complete sequential system and cannot be assigned solely to plant-associated processes.

The SSF stage brought wastewater into contact with soil and gravel media. These substrates may contribute

through physical filtration and particle retention, sorption of some constituents, and provision of surfaces for biofilm development (García et al., 2010; Saeed & Sun, 2012). The present experiment did not quantify these mechanisms separately. Accordingly, the contribution of the SSF unit is discussed as a plausible additional treatment barrier rather than as an independently quantified mechanism.

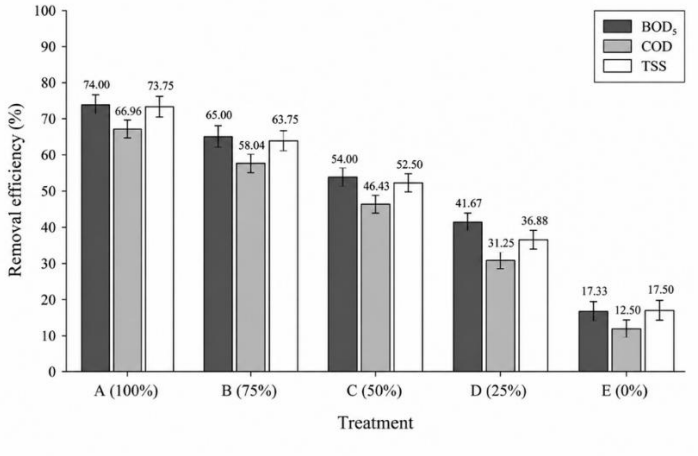


Figure 2. Removal Efficiency of BOD_5, COD and TSS.

The day-15 values estimated from the plotted treatment means show a consistent descriptive difference between the planted treatments and the unplanted control (E). For BOD_5, concentrations were approximately 78, 105, 138, and 175 mg/L in treatments A–D, compared with 248 mg/L in E. Thus, concentrations in A–D were about 170, 143, 110, and 73 mg/L lower than the control, equivalent to approximately 68.5%, 57.7%, 44.4%, and 29.4% lower, respectively. For COD, the corresponding concentrations were approximately 185, 235, 300, and 385 mg/L, compared with 490 mg/L in E; the differences were 305, 255, 190, and 105 mg/L, or about 62.2%, 52.0%, 38.8%, and 21.4% below the control. For TSS, concentrations were approximately 42, 58, 76, and 101 mg/L, compared with 132 mg/L in E; the differences were 90, 74, 56, and 31 mg/L, or about 68.2%, 56.1%, 42.4%, and 23.5% below the control.

The removal-efficiency reports overall efficiencies of 74.00%, 65.00%, 54.00%, and 41.67% for BOD_5; 66.96%, 58.04%, 46.43%, and 31.25% for COD; and 73.75%, 63.75%, 52.50%, and 36.88% for TSS in treatments A–D, while the unplanted control E achieved 17.33%, 17.50%, and 17.50% for BOD_5, COD, and TSS, respectively. Compared with E, treatment A therefore exceeded the control removal efficiency by 56.67 percentage points for BOD_5, 54.46 percentage points for COD, and 56.25 percentage points for TSS. The pattern supports better overall performance in the planted treatment combinations under the conditions tested, but it does not isolate the effect of plant presence from all other processes or establish statistical significance for each pairwise comparison. Because coverage and initial biomass increased together across A–D, these comparisons interpreted as differences among treatment combinations.

The integrated configuration combines plant–wastewater contact in the bioremediation reactor with passage through soil and gravel in the SSF unit. Nevertheless, the measured overall reductions cannot be attributed solely to plant uptake or to any single process because plants, microorganisms, sedimentation, filtration, and substrate interactions may act together (García et al., 2010; Wu et al., 2023). The unplanted treatment helps account for non-vegetation processes, but quantitative plant-effect estimates require direct comparison of replicate-level outcomes between each planted treatment and treatment E. Since coverage and biomass also varied together, observed differences as among treatment combinations rather than isolated coverage effects.

Statistical Results

The Linear mixed-effects model (LMM) indicated significant effects of treatment (Table 1), sampling day, and sampling point on all three parameters ($p < 0.001$). Significant treatment × day interactions were also observed, indicating that the magnitude of treatment differences changed during the 15-day treatment period.

The significant treatment × sampling point interaction indicates that measured concentrations varied across sampling points differently among treatment combinations. This pattern is consistent with changes occurring along the sequential treatment train. Stage-specific efficiencies calculated from paired concentrations at S1–S2 and S2–S3, respectively; these values not inferred from the interaction test alone.

Table 1. Linear mixed-effects model (LMM) for BOD_5, COD, TSS concentrations

Parameter	Effect	F-value	df	p-value
BOD_5	Treatment	684.27	4, 10.8	<0.001
	Day	521.84	4, 40.2	<0.001
	Sampling point	913.56	2, 40.1	<0.001
	Treatment x Day	18.73	16, 40.2	<0.001
	Treatment x Sampling point	31.46	8, 40.1	<0.001

COD	Treatment	592.38	4, 11.1	<0.001
	Day	463.17	4, 40.5	<0.001
	Sampling point	801.62	2, 40.4	<0.001
	Treatment x Day	16.92	16, 40.5	<0.001
	Treatment x Sampling point	27.85	8, 40.4	<0.001
TSS	Treatment	471.53	4, 10.6	<0.001
	Day	398.76	4, 39.8	<0.001
	Sampling point	687.41	2, 39.7	<0.001
	Treatment x Day	13.64	16, 39.8	<0.001
	Treatment x Sampling point	23.17	8, 39.7	<0.001

Role of the Sequential Bioremediation-SSF Configuration

The system places a *P. stratiotes*-based bioremediation reactor before an SSF constructed wetland. The plant roots may provide surfaces for microbial colonization and particle interception, while the soil and gravel may contribute filtration, sedimentation, sorption, and biofilm-supported processes (Ratnawati et al., 2024). These mechanisms are consistent with the constructed-wetland literature, but they were not independently measured in this experiment.

The sequential design can be considered a multi-barrier treatment concept because it combines a plant-based stage with a porous-media stage (Ilyas & Masih, 2017). The experiment therefore cannot establish hydraulic design criteria, specific treatment capacity, or operational readiness for full-scale use. Continuous-flow testing and hydraulic characterization are required before the system can be transferred to field operation.

CONCLUSION

The integrated bioremediation–SSF constructed wetland using P. stratiotes showed potential to reduce BOD_5, COD, and TSS in the fishery wastewater tested. Among the five plant–biomass combinations evaluated, treatment A (100% coverage and 250 g initial biomass) showed the best overall performance.

At day 15, treatment A achieved overall removal efficiencies of 74.00% for BOD_5, 66.96% for COD, and 73.75% for TSS. The sequential configuration combined P. stratiotes-based bioremediation with a subsequent SSF constructed wetland. Although the integrated system demonstrated overall pollutant removal, the incremental contribution of the SSF stage could not be independently quantified from the overall removal efficiencies alone. The mixed-effects analysis indicated significant treatment, temporal, and sampling-point effects, as well as the interactions in Table 1.

Overall, the combined P. stratiotes bioremediation–SSF system is a promising nature-based treatment concept under the laboratory conditions tested. Further work could use a factorial design to vary plant coverage and biomass and evaluate performance under continuous flow and variable influent loads.

Research Transparency and Ethics Statements

Author Contributions (CRediT): Author 1: Conceptualization, Methodology, Writing – Original Draft, Supervision, Project Administration, Funding Acquisition; Author 2: Investigation, Data Curation, Visualization; Author 3: Investigation, Formal Analysis, Validation.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflicts of interest.

Ethics Approval and Informed Consent: Ethical approval and informed consent were not applicable to this study because the research involved the experimental treatment of fishery wastewater using Pistia stratiotes and a subsurfaceflow (SSF) constructed wetland, without human participants, experimental animals, or identifiable personal data.

The study was conducted in accordance with applicable institutional requirements.

Statement on the Use of Artificial Intelligence: The authors used ChatGPT, a generative artificial intelligence tool developed by OpenAI, to assist with English-language editing and improving manuscript clarity. All AI-assisted content was critically reviewed and verified by the authors, who take full responsibility for the accuracy, integrity, and final content of the manuscript. ChatGPT was not listed as an author.

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