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# Treatment of soy sauce wastewater using integrated algal biofilm and bio membrane system for clean water and nutrient recovery

Novirina Hendrasarie[1*], Dedin Finatsiyatull Rosida[2], Muchammad Tamyiz[3]

[1] Department of Environmental Engineering, Faculty of Science, UPN Veteran Jawa Timur, Rungkut Madya Street, Surabaya, Indonesia
[2] Department of Food Technology, Faculty of Science, UPN Veteran Jawa Timur, Rungkut Madya Street, Surabaya, Indonesia
[3] Department of Environmental Engineering, Faculty of Science, Sunan Ampel State Islamic University Surabaya, Ahmad Yani Street, Surabaya, Indonesia
* Corresponding author's e-mail: novirina@upnjatim.ac.id

## ABSTRACT

Soy sauce production generates wastewater with high concentrations of organic pollutants and recalcitrant colorants, posing significant challenges for conventional treatment technologies. This study evaluated an integrated treatment process designed to enhance pollutant removal and recover clean water through the combination of an algae-based biofilm system and a bio-membrane unit. The system comprises an Anoxic-Oxic moving bed biofilm reactor (MBBR) equipped with Kaldness K3 carriers that support native microbial consortia and algae biofilms to promote biodegradation as well as color reduction. Treatment performance was assessed using COD, $BOD_5$, total suspended solids (TSS), total dissolved solids (TDS), total nitrogen (TN), total phosphate (TP), and color intensity as key indicators. The results showed that filling the media to 40% and HRT of 38 hours produced the best overall performance, MBBR achieved removal efficiencies of 89.67% COD, 88.87% $BOD_5$, 80% TSS, 57.85% TDS, 83.65% TN, 82.76% TP, and 94.35% color. The ultrafiltration process further improves the quality of wastewater from the MBBR process. The results of the ultrafiltration process meet Indonesia's non-potable clean water quality standards in accordance with Government Regulation No. 22/2021. These findings highlight the potential of integrated systems as a sustainable and efficient solution for treating high-concentration industrial food wastewater while supporting resource recovery.

**Keywords:** soy sauce wastewater, Anoxic-Oxic MBBR, algal-based biofilm, ultrafiltration membrane, clean water, resource reuse.

## INTRODUCTION

Soy sauce manufacturing generates large volumes of high strength wastewater containing elevated levels of organic matter, nitrogen, phosphorus, salts, and pigments, which, if untreated, can cause serious water pollution and eutrophication. Several recent studies have explored biological and membrane-based approaches for its treatment. For instance, Biffa et al. (2020); Qian et al. (2023) investigated algal biofilm formation mechanisms in soy sauce wastewater, revealing the potential of algal consortia for pollutant removal. Similarly, Hu et al. (2024) demonstrated a rotating algal biofilm (RAB) system that achieved high removal efficiencies of COD, ammonium, and phosphorus from soy sauce wastewater. Kim et al. (2023) successfully cultivated *Chlorella* sp. HS2 using soy sauce factory effluent, emphasizing the feasibility of phytoremediation for nutrient rich industrial wastewater. In contrast, Zhang et al. (2021); Ke Xu et al. (2024); Yang et al. (2024) applied a biomimetic dynamic membrane to remove color and COD from soy sauce wastewater, while Goh et al. (2022); Toor and Nghiem (2022); Ende et al. (2024); and Lu et al. (2025) reviewed the potential of algal-membrane photobioreactors for nutrient recovery from various industrial effluents.

Despite these advances, most existing studies have focused either on algal-based remediation or on membrane filtration alone, without fully integrating both technologies into a single treatment platform. Moreover, few studies have addressed the challenges posed by the high salinity, dark color, and complex organic composition of soy sauce wastewater, which can inhibit algal growth and exacerbate membrane fouling. Research specifically addressing nutrient recovery and clean-water reuse from soy sauce wastewater through an integrated algal biofilm–bio membrane system remains limited. Therefore, this study aimed to develop and evaluate a novel integrated algal biofilm and bio membrane system for the efficient treatment of soy sauce wastewater while enabling nutrient recovery and promoting circular resource utilization.

Accordingly, this study aimed to develop and evaluate an integrated algal biofilm–moving bed biofilm reactor (MBBR) for the treatment of soy sauce wastewater, focusing on producing high-quality reclaimed water suitable for reuse within soy sauce manufacturing processes and on recovering algal biomass as an agricultural bio stimulant. The integration of algal biofilm and bio membrane units is designed to achieve synergistic performance, where the algal biofilm contributes to nutrient assimilation and oxygen production, while the bio membrane enables efficient solid–liquid separation and water purification. Unlike previous studies that independently examined algal or membrane-based treatment systems (Clagnan et al 2024; Hu et al., 2024; Josivaldo et al. 2024; Qian et al., 2023), this research introduced a circular and sustainable approach that simultaneously achieves wastewater reclamation and nutrient valorization. The harvested algal biomass is envisioned to be utilized as a natural bio stimulant, enhancing soil fertility and crop productivity, thereby reinforcing the principles of circular bioeconomy within the food processing sector.

## RESEARCH METHODS

### Wastewater source and characterization

The raw soy sauce wastewater exhibited typical features of high-strength industrial effluents with a dark brown color and strong odor. Initial characterization showed Dissolved Oxygen 0.50–0.78 mg/L, COD values exceeding 2371.2–2564.6 mg $L^{-1}$, $BOD_5$ around 826.88–1021.44 mg $L^{-1}$, TN between 58.8–70 mg $L^{-1}$, TSS between 280–340 mg $L^{-1}$, TDS 2280–2291 mg $L^{-1}$, TP around 3.82–4.20 mg $L^{-1}$ and color 2245–2337PtCo. The salinity and pigment concentration were relatively high due to the fermentation and soybean hydrolysis processes. These characteristics confirmed the need for an integrated biological and membrane-based system capable of handling complex organic loads and pigment molecules. The physicochemical profile was consistent with previous reports for soy sauce effluents (Qian et al., 2023; Hu et al., 2024; Zhang et al., 2021; Hendrasarie et al., 2021).

### Integrated algal biofilm in moving bed biofilm reactor–bio membrane reactor setup

The experimental system consisted of three main components:
1) Anoxic MBBR, grown with native microbial biofilm from soy sauce wastewater, for initial organic degradation and denitrification
2) Oxic MBBR, grown with algal biofilm, where Chlorella cells are immobilized for aerobic oxidation and nutrient assimilation, also grown with indigenous microbes, then compared the optimization of both in degrading pollutants,
3) Biological membrane filtration module using ultrafiltration, equipped with polyvinylidene fluoride (PVDF) membranes (pore size 0.01–0.1 μm).

The wastewater was treated in a biofilter membrane integration system, consisting of three anoxic MBBRs arranged in series, each with a volume of 30 L, which use a consortium of native microbes as pollutant degrading agents, attached to Kaldness K1 media. The wastewater then flows into two oxic MBBRs arranged in series, each with a volume of 30 L, which use algae (treatment 1) to compare their effectiveness in degrading pollutants with a native microbial consortium (treatment 2) attached to Kaldness K1 media as a pollutant degrading agent. For algae, lighting (12:12 hour light-dark cycle) and aeration are maintained to promote algal photosynthesis. The integrated design allows the algal biofilm to pretreat wastewater through nutrient absorption and oxygenation, reducing clogging and improving membrane performance.

## Reactor configuration and operation

The system was operated in continuous mode for 39 days with hydraulic retention times (HRT) of 14, 26, and 38 hours. Each reactor had a working volume of 30 L and was filled with 20%,40% and 60% (v/v) suspended carrier media to promote biofilm attachment. The reactors were operated in batch recirculation mode under constant mixing. The temperature was maintained at 27–31 °C, and the light regime followed a 12:12 h light–dark cycle with continuous aeration at 70 L min$^{-1}$ to promote oxygen transfer and algal photosynthesis. For the microbial system, aeration supported nitrifying activity in the oxic reactor. The pH naturally increased from 4.0 to approximately 6.8 during the process due to $CO_2$ uptake and metabolic activity.

Two treatment systems were tested separately under identical hydraulic and environmental conditions:

- Algal biofilm system: using *Chlorella* sp. as the dominant phototrophic organism.
- Indigenous microbial biofilm system: dominated by *Lactobacillus* (in anoxic stages) and *Nitrosomonas* (in the oxic stage).

Samples were taken periodically every 1–3 days from the influent and effluent for analysis. Seven replicates were taken. Parameters including COD, $BOD_5$, TSS, TDS, TN, TP, and color were measured to evaluate pollutant removal. Permeate flow and transmembrane pressure (TMP), flux, and permeate quality were monitored to assess membrane performance and fouling behavior. In an enhanced MBBR system, MLSS needs to be between 2000 and 5000 mg/L (Pratap et al; 2024; Kamilya et al; 2023; Hendrasarie and Zarfandi, 2023).

## Algal strain and cultivation conditions

After treatment, the algal biofilm was carefully removed from the medium, washed, and dried at 40 °C. The dried biomass was ground into a fine powder and analyzed for total nitrogen (Kjeldahl method), total phosphorus, and chlorophyll content. Water extracts from the algal biomass were prepared following the method of Ronga et al. (2019) to obtain liquid bio-stimulants. Germination and growth promotion tests were conducted using amaranth seeds (*Amaranthus* spp.) to evaluate the bio stimulant potential of the isolated algal biomass.

Fresh biomass of *Chlorella* sp. was obtained in paste form. EM4 (effective microorganisms 4), containing lactic acid bacteria (*Lactobacillus* sp.), yeasts (*Saccharomyces* sp.), photosynthetic bacteria (*Rhod pseudomonas* sp.), and fermentation fungi (*Aspergillus* and *Penicillium*), was used as the microbial inoculum. Brown sugar served as the carbon source. *Amaranthus hybridus* L. was selected as the test plant due to its rapid physiological response to bio stimulants.

The following (Figure 1) biofilm algal and endogenous microbes attached to the growth medium in MBBR. The dark gray indigenous microbial biofilm adheres to the Kaldness media, while the dark green algal biofilm indicates that the biofilm is ready to reduce contaminants in soy sauce wastewater. Both biofilms predominantly adhere to the Kaldness cavity and are thinly attached to the Kaldness surface. This is due to the movement of the media during the treatment process.

### *Biochemical transformations during fermentation*

Fermentation induced several key biochemical reactions enhancing nutrient bioavailability and chemical stability:

- Polysaccharide hydrolysis

Fungal amylases catalyzed the hydrolysis of algal starch into fermentable monosaccharides:

$$(C_6H_{10}O_5)_n + nH_2O \xrightarrow{\text{amylase}} nC_6H_{12}O_6 \quad (1)$$

- Lactic acid fermentation

*Lactobacillus* sp. converted glucose into lactic acid via homofermentative pathways, reducing pH and inhibiting spoilage organisms:

$$C_6H_{12}O_6 \rightarrow 2C_3H_6O_3 \quad (2)$$

- Alcoholic fermentation

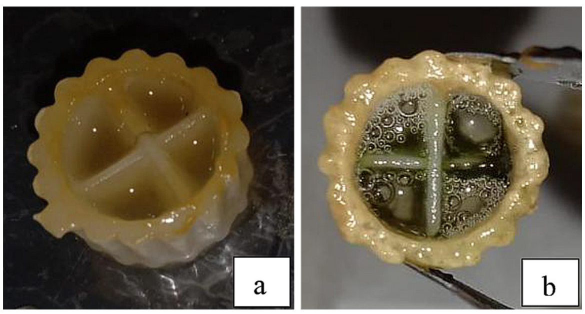


**Figure 1.** (a) *Indigenous microbe* attached to the media (b) microalgal *Chlorella* sp. attached to the media

Yeasts fermented glucose into ethanol and $CO_2$, contributing to the breakdown of organic matter:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \quad (3)$$

- Proteolysis

Proteases produced by filamentous fungi and EM4 microbes hydrolyzed algal proteins into free amino acids:

$$\text{Protein} + H_2O \xrightarrow{\text{protease}} \text{amino acids} \quad (4)$$

These reactions collectively enhanced the concentration of assimilable compounds (simple sugars, amino acids, organic acids, and pigments), improving the physiological activity of the biostimulant.

#### *Bio stimulant preparation*

Following fermentation, the suspension was filtered to remove coarse solids. The clarified liquid was stored at 4–10 °C and diluted to 10–20 $mL \cdot L^{-1}$ prior to application. Physicochemical parameters (pH, Odor, and color) were recorded as stability indicators.

#### *Plant cultivation and experimental design*

Seeds were sown directly into 20 × 20 cm pots filled with a soil–sand–compost mixture (2:1:1, v/v). Plants were grown under full sunlight and watered regularly. A completely randomized design (CRD) with three replicates was used, and pot positions were rearranged periodically to minimize microenvironmental variation. No additional fertilizers were applied.

#### *Harvest and growth measurements*

The bio stimulant was applied as a foliar spray twice weekly for four weeks, beginning 7–10 days after sowing. Plants were harvested 35 days after sowing. Growth parameters included plant height, number of expanded leaves, maximum leaf width, stem diameter (measured at the base using a caliper), and primary root length. The data were subjected to one-way ANOVA, and differences among treatments were evaluated using Tukey's test at $p < 0.05$.

### Analytical methods

All analytical measurements were performed in triplicate. COD and $BOD_5$ were determined using standard dichromate and respirometry methods, respectively. TN and TP were measured using spectrophotometric methods (Hach DR 3900). Color and turbidity were measured using a UV–Vis spectrophotometer at 465 nm and a nephelometer, respectively. Statistical analyses were performed using SPSS v26.0, and significance was tested using one-way ANOVA ($p < 0.05$).

### Performance evaluation

The overall performance of the integrated system was evaluated based on:

- Pollutant removal efficiency (%) for COD, $BOD_5$, TN, TP, TSS, TDS, and color by comparing MBBR under oxic conditions, algal biofilm with microbial indigenous biofilm
- Water quality index (WQI) of the water compared with industrial water reuse standards;
- Bio stimulant activity of algal biomass extracts on seed germination and shoot/root elongation.

These parameters were used to assess both the environmental and resource-recovery potential of the integrated algal biofilm–bio membrane system.

## RESULTS AND DISCUSSION

### Performance evaluation of algal and microbial biofilm systems

This section presents the performance evaluation of the algal (*Chlorella* sp.) and indigenous microbial (*Lactobacillus–Nitrosomonas*) biofilm systems operated in a three-stage MBBR configuration consisting of two anoxic and one oxic reactor. The comparative assessment focuses on the removal of organic matter, nutrients, solids, and color at the hydraulic retention time (HRT) of 14, 26, and 38 hours. This subsection provides a general comparison of system performance in terms of organic, nutrient, and color removal to establish the basis for detailed analysis in the following sections.

On the basis of the overall removal efficiencies, organic matter degradation was identified as a key indicator of biological activity within the biofilm systems. Therefore, the subsequent analysis focused on the reduction of chemical oxygen demand (COD) and biochemical oxygen demand ($BOD_5$) to evaluate the metabolic capacity of each biofilm type.

*Organic matter removal (COD and BOD$_5$)*

The algal biofilm system exhibited higher COD and BOD$_5$ removal compared to the indigenous microbial biofilm, indicating enhanced organic oxidation efficiency. This improvement reflects the contribution of photosynthetically generated oxygen and the adsorption of dissolved organics within the algal matrix, as illustrated in Figure 2.

The organic matter removal performance of both systems increased consistently with longer hydraulic retention time (HRT), with the best results obtained at 38 h. Under these conditions, the indigenous microbial biofilm achieved COD and BOD$_5$ removals of 89.67% and 88.87%, corresponding to residual concentrations of 264.92 mg/L and 113.69 mg/L, respectively.

In contrast, the *Chlorella* sp. – microbial biofilm system demonstrated superior removal efficiencies of 91.65% COD and 91.35% BOD$_5$, with effluent concentrations of 214.14 mg/L and 88.35 mg/L, respectively. This improvement reflects the additional oxygen supply generated through algal photosynthesis, which enhances the aerobic degradation rate of organic pollutants. Furthermore, extracellular polymeric substances (EPS) secreted by *Chlorella* sp. facilitated the adsorption of soluble organics and supported stable biofilm attachment on Kaldness media.

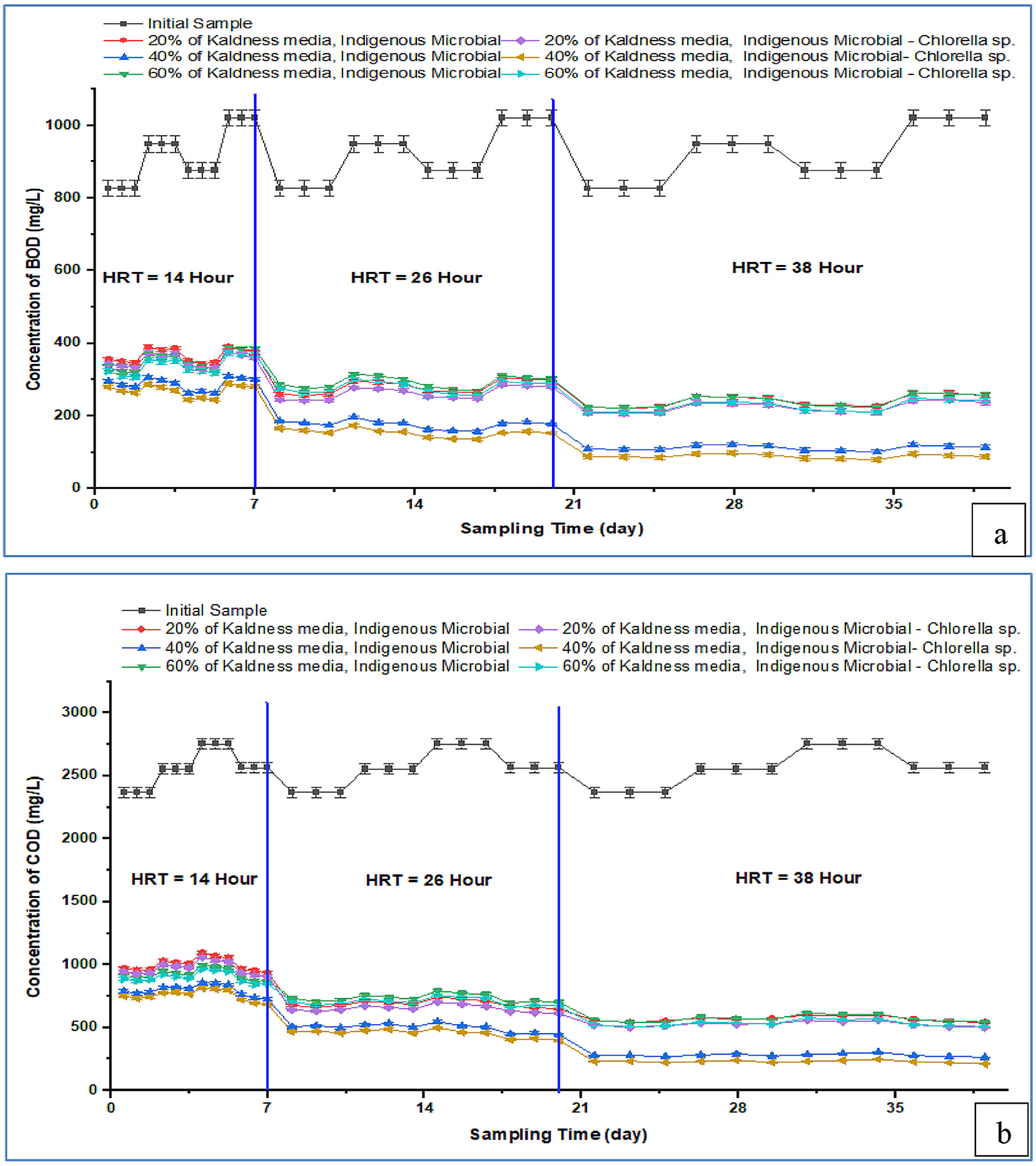


**Figure 2.** Effect of hydraulic retention time (HRT) on BOD$_5$ (a) and COD (b) removal efficiency in microbial and algal biofilm systems. Data represent mean ± SD (n = 7)

The trend across all HRT conditions (14, 26, and 38 h) shows that longer retention time promoted greater substrate–biofilm interaction, allowing more complete oxidation of organic matter. These findings are consistent with the previous reports that link algal–bacterial consortia to enhanced organic carbon removal due to synergistic oxygen and nutrient exchange (e.g., Xu et al. 2024; Li et al., 2022; Hendrasarie and Fadilah,2022; Hendrasarie and Pratama, 2023). Following the analysis of organic matter removal, nutrient removal performance was examined to understand how nitrogen and phosphorus dynamics were influenced by biofilm composition and reactor configuration.

*Nutrient removal (total nitrogen and total phosphate)*

Both systems showed substantial nutrient removal; however, the algal biofilm achieved notably higher efficiencies. This subsection discussed the assimilation of nitrogen and phosphorus into algal biomass and the synergistic interactions between phototrophic and heterotrophic processes that enhanced nutrient recovery, as illustrated in Figure 3.

Nutrient removal also improved with increasing HRT. At 38 h, the indigenous microbial system achieved total nitrogen (TN) and total phosphate (TP) removals of 83.65% and 82.76%, with residual concentrations of 11.45 mg/L and 0.65 mg/L, respectively.

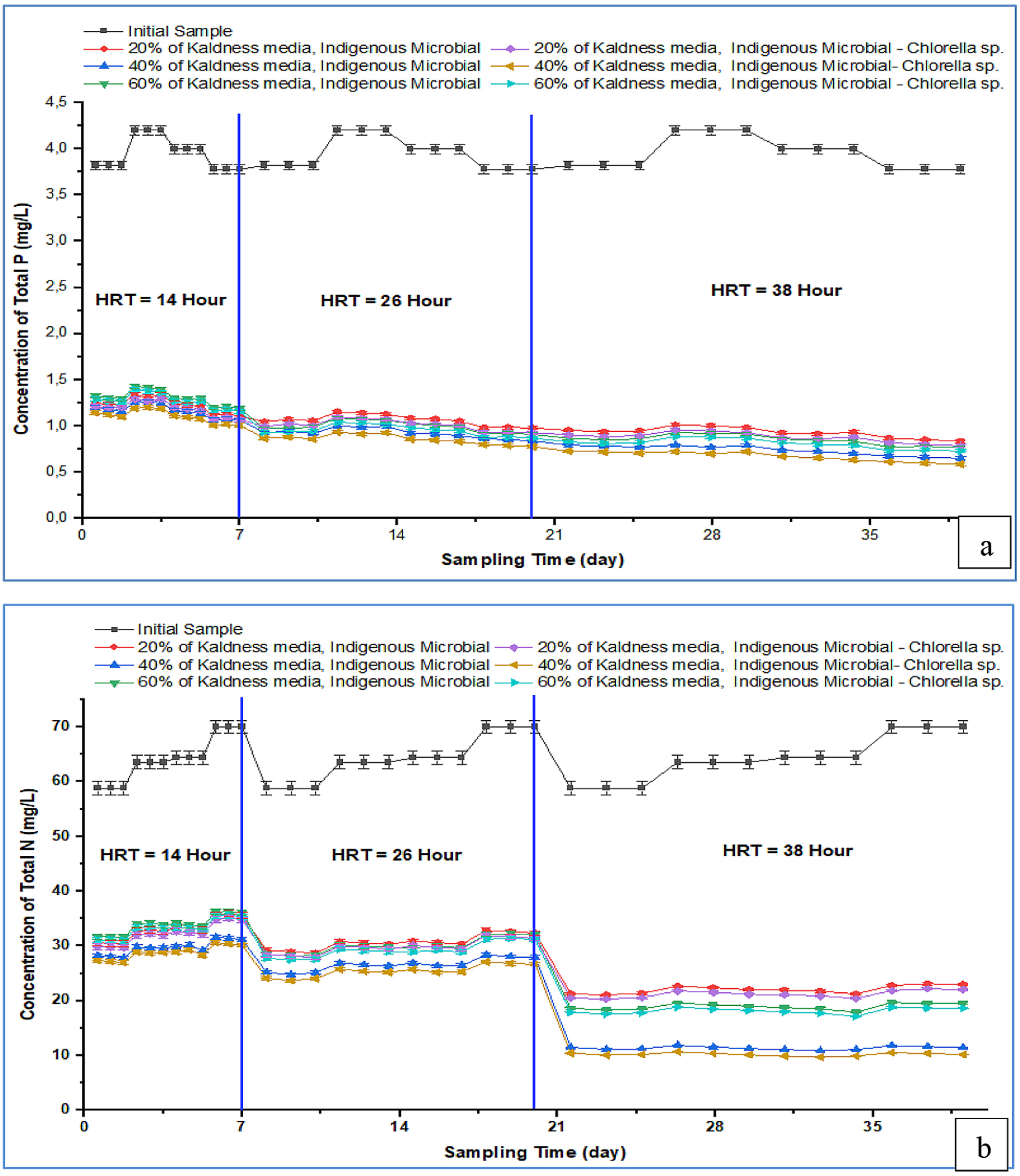


**Figure 3.** Comparative removal efficiencies of total phosphate (TP) (a), total nitrogen (TN) (b), at optimal HRT (38 h) for microbial and algal biofilm systems

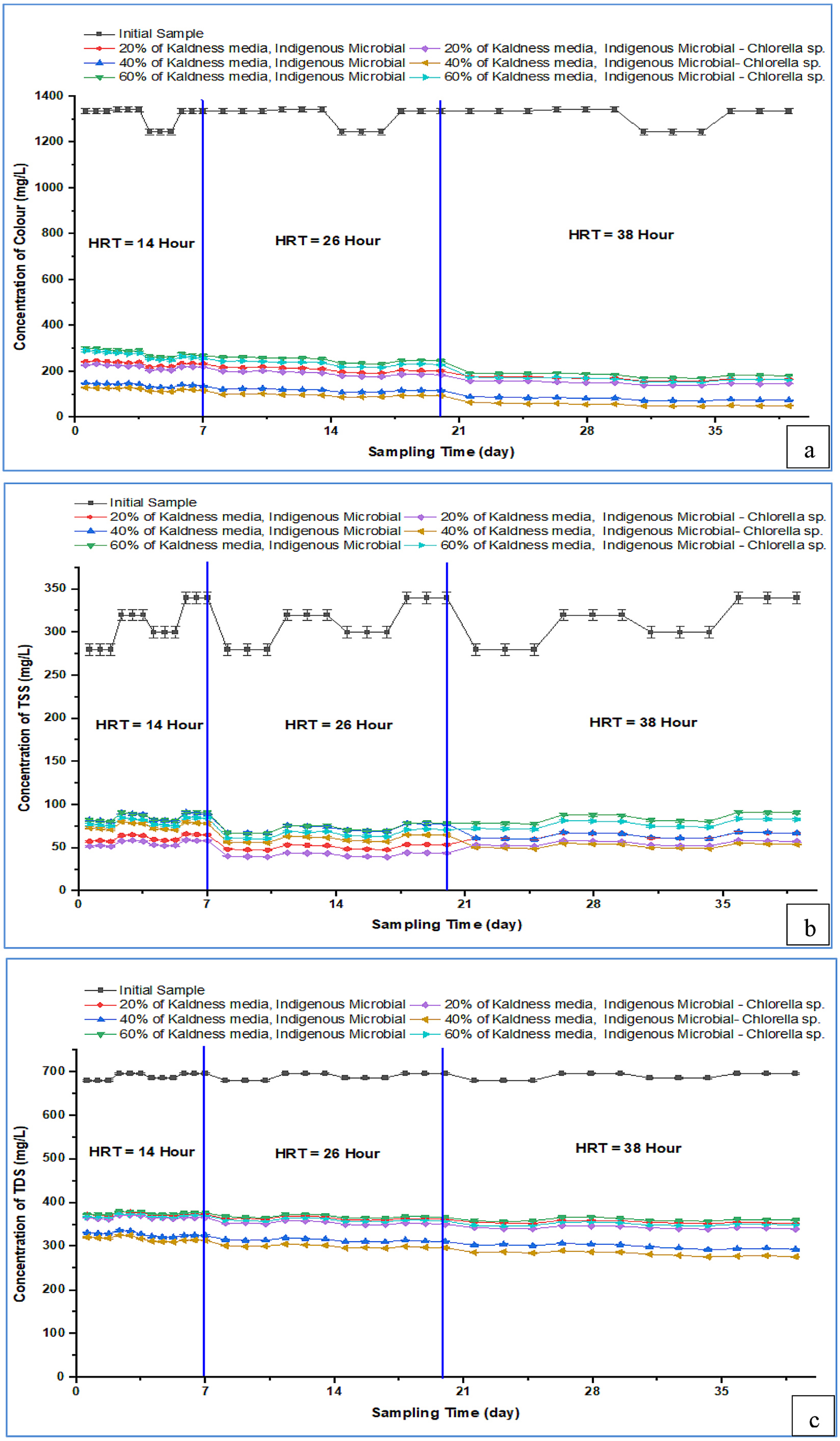


**Figure 4.** Pollutant reduction: (a) color, (b) total suspended solids, (c) total dissolved solids

Meanwhile, the *Chlorella* sp.–based system reached slightly higher TN and TP removals of 85.47% and 84.41%, corresponding to effluent concentrations of 10.17 mg/L and 0.59 mg/L. The improvement can be attributed to nutrient uptake by algal biomass and enhanced nitrification–denitrification due to oxygen release from photosynthesis in the oxic MBBR.

Additionally, the gradual increase in pH (from 4.0 to around 6.8) during algal treatment likely promoted phosphate precipitation as insoluble phosphates. The synergistic metabolic relationship between *Chlorella* sp., *Lactobacillus*, and *Nitrosomonas* provided a stable nitrogen and phosphorus transformation pathway, resulting in higher overall nutrient recovery compared to the bacterial biofilm alone.

Beyond organic and nutrient removal, the effectiveness of each biofilm system in reducing suspended and dissolved solids, as well as color, was assessed to determine the overall improvement in effluent quality.

### *Solids and color removal*

The algal biofilm system demonstrated superior removal of total suspended solids (TSS), total dissolved solids (TDS), and color compared to the microbial biofilm. These improvements are attributed to algal extracellular polymeric substances (EPS) that enhance flocculation, sedimentation, and photodegradation of colored compounds typical of soy sauce wastewater (Figure 4).

The removal of suspended solids, dissolved solids, and color followed similar trends, with the algal biofilm system consistently outperforming the microbial biofilm. At 38 h HRT, the *Chlorella* sp. system achieved 84% TSS, 60% TDS, and 96% color removal, whereas the microbial biofilm achieved 80% TSS, 58% TDS, and 94% color reduction.

The superior performance of the algal system in solids removal can be linked to the production of EPS and oxygen bubbles during photosynthesis, which enhanced floc formation and sedimentation. The high color removal efficiency (up to 96%) demonstrates the ability of *Chlorella* sp. to degrade and adsorb melanoidin pigments commonly found in soy sauce effluent. Light-driven photolysis and oxidative reactions likely contributed to breaking down chromophore compounds, producing clearer effluent compared to the microbial-only reactor**.**

The continuous aeration (70 L/min) and 12:12 h light-dark cycle maintained ideal mixing and photosynthetic activity, leading to stable solids and color removal during all seven replicate runs at each HRT.

Since pH and temperature play crucial roles in both microbial and algal metabolisms, their variation during treatment was monitored to assess system stability and its correlation with pollutant removal efficiency.

### *pH and dissolved oxygen dynamics*

During operation, both systems maintained stable conditions within the optimal range for biological activity. The algal biofilm showed a more pronounced pH increase due to $CO_2$ uptake during photosynthesis, which further supported nutrient precipitation and organic degradation (Table 1).

The pH values increased significantly from the influent (pH 5.3) to the effluent across all reactor configurations, indicating efficient biochemical transformation within the MBBR system. Reactors integrated with *Chlorella* sp. exhibited a more distinct pH elevation compared to those containing only indigenous microorganisms. This trend aligns with the well-established role of photosynthetic $CO_2$ uptake in reducing carbonic acid concentrations and subsequently increasing pH (Spormann and Iglesias-Rodriguez, 2019; Maity et al., 2021). Elevated pH in microalgal-assisted systems may further promote nutrient precipitation and improve degradation of organic matter, consistent with the behavior of algal–bacteria

**Table 1.** Characteristics of average pH and DO values in MBBRs

| Parameter | Influent | Effluent in clarifier | | | | | |
|---|---|---|---|---|---|---|---|
| | | 20% media, *Indigenous Microbial* | 40% media, *Indigenous Microbial* | 60% media, *Indigenous Microbial* | 20% media, *Indigen Microbial - Chlorella sp.* | 40% media, *Indigen Microbial - Chlorella sp.* | 60% media, *Indigen Microbial - Chlorella* sp. |
| pH | 5.3 | 6.9 | 7.2 | 6.9 | 6.3 | 6.7 | 6.3 |
| DO (mg/L) | 0.2 | 5.4 | 5.2 | 5.4 | 5.8 | 5.8 | 5.6 |

consortia reported in previous studies (Nwoba et al., 2022; Hendrasarie et al., 2024).

Dissolved oxygen (DO) concentrations rose markedly from 0.2 mg/L in the influent to 5.2–5.8 mg/L in the effluent, demonstrating stable aerobic conditions across all media configurations. The reactors incorporating *Chlorella* sp. produced slightly higher DO levels, indicating an oxygen-producing contribution from photosynthetic activity, which has been documented in integrated microalgae-based treatment systems (Su et al., 2012; Posadas et al., 2017). The elevated DO levels may enhance aerobic microbial pathways and overall pollutant removal efficiency, aligning with performance characteristics commonly observed in both conventional and hybrid MBBR systems (Ødegaard, 2016; Hendrasarie et al., 2022).

Overall, the integration of algal biofilm improved reactor stability through increases in pH and DO, reinforcing the potential benefits of microalgae–bacteria interactions in optimizing biological wastewater treatment processes.

### *Statistical analysis*

One-way analysis of variance (ANOVA) revealed significant differences ($p < 0.05$) in removal efficiencies between the algal and microbial biofilm systems for all parameters. The algal biofilm achieved particularly higher significance levels for color and phosphorus removal ($p < 0.01$), confirming its superior treatment performance and operational consistency. Repeated trials demonstrated consistent results (RSD < 5%), validating the reliability of the MBBR performance and the stability of the algal biofilm process.

Overall, the results demonstrate that the integration of *Chlorella* sp. into the biofilm system significantly enhanced pollutant removal across all tested parameters. The improvement was most evident at an HRT of 38 h, confirming that sufficient contact time, optimal light conditions, and photosynthetic activity play critical roles in achieving effective treatment of high-strength industrial wastewater.

## Pollutant removal efficiency and water quality improvement

To evaluate the performance of the two post-treatment configurations, namely the Indigenous Microbial Biofilm–UF system and the Algal Biofilm–UF system, a comparative analysis was conducted based on key water quality parameters. The parameters assessed included organic matter (COD and $BOD_5$), suspended and dissolved solids (TSS and TDS), nutrients (total nitrogen and total phosphate), color, and pH. The results of membrane ultrafiltration treatment were evaluated using the Indonesian non-potable clean water quality standards as required by Government Regulation No. 22/2021. Table 2 summarizes the influent and effluent concentrations for each treatment system, along with the corresponding removal efficiencies.

Overall, the results of the study show that both systems, namely Indigenous Microbial Biofilm–UF and Algal Biofilm–UF, are capable of producing effluent with a quality that meets river water quality standards as stipulated in Indonesian Government Regulation No. 22 of 2021. Although both systems perform well, the Algal Biofilm–UF system consistently provides higher removal efficiency for organic parameters, suspended solids, dissolved solids, nutrients, and color. The

**Table 2.** Comparison of treatment using algal biofilm-UF with indigenous microbial biofilm-UF

| Parameter | MBBR–indigenous biofilm(anoxic)-indigenous biofilm (oxic) (influent of UF, $C_0$) | Effluent UF (indigenous biofilm, $C_e$) | % Removal (indigenous) | MBBR–indigenous biofilm(anoxic)-algal biofilm (oxic) (influent UF, $C_0$) | Effluent UF (algal biofilm, $C_e$) (Ce) | % Removal (algal biofilm) | Standard |
|---|---|---|---|---|---|---|---|
| COD (mg/L) | 264.92 | 4.37 | 98.35 | 214.14 | 1.78 | 99.17 | 10 |
| BOD (mg/L) | 113.69 | 0.98 | 99.14 | 88.35 | 0.64 | 99.28 | 2 |
| TSS (mg/L) | 67.08 | 3.00 | 96.12 | 53.99 | 1.00 | 98.15 | 40 |
| TDS (mg/L) | 293.36 | 62.00 | 78.87 | 276.31 | 52.86 | 80.87 | 300 |
| Total nitrogen (mg/L) | 11.45 | 1.08 | 90.56 | 10.17 | 0.86 | 91.54 | 15 |
| Total phosphate (mg/L) | 0.84 | 0.07 | 91.63 | 0.59 | 0.04 | 93.21 | 0,2 |
| Color (Pt Co) | 75.54 | 7.00 | 90.73 | 49.87 | 4.00 | 91.98 | 15 |
| pH | 7.20 | 7.10 | - | 7.20 | 7.10 | - | 6–9 |

effluent produced by the Algal Biofilm–UF system showed COD, $BOD_5$, TSS, total nitrogen, total phosphate, color, and pH values that were well below the maximum limits required, confirming its ability to produce high-quality effluent.

These findings confirm that the integration of algal biofilm with ultrafiltration not only improves the final polishing process but also ensures that the effluent quality meets regulatory requirements for discharge into surface water bodies in accordance with the Indonesian Government Regulation No. 22/2021. Thus, the Algal Biofilm–UF configuration can be considered a superior post-treatment technology for improving wastewater quality prior to discharge or reuse.

## Membrane performance and fouling control

The main advantage of integrating algal biofilms with membrane systems is the observed reduction in membrane fouling. The presence of biofilms enhances oxygen release and microbial balance, which limits the accumulation of extracellular polymeric substances (EPS) and surface clogging. Permeate flow remained stable at 80–90% of the initial rate after several cycles, while transmembrane pressure (TMP) increased only moderately. Compared to standalone membrane operation, the integrated system showed a 30–40% improvement in flow rate stability. These results support the hypothesis that biofilm activity reduces fouling by decreasing organic load and promoting a self-regulating microbial layer on the membrane surface (Toor and Nghiem, 2022; Riechelman et al., 203; Xu Shiling et al., 2024).

## Bio stimulant of algal biomass

Algal biofilms showed dense green growth throughout the treatment period. The average dry biomass yield harvested was [1.5 g $L^{-1}$], containing 3–4% total nitrogen, 1–1.5% phosphorus, 3–3.5% potassium, and high chlorophyll-a content (~18 mg $g^{-1}$ DW). This composition indicates that the biomass still contains significant nutritional value, making it suitable for use as a bio-based fertilizer or bio stimulant. Table 3 shows the results of applying bio stimulants to *Lactuca sativa* plants.

Effect of bio stimulant concentration derived from *Chlorella* sp. on the growth parameters of *Amaranthus* sp. (a) plant height, (b) number of leaves, and (c) root length at 35 days after treatment. Data represent means of three replicates. Error bars are omitted for clarity. The results indicate a positive dose response relationship, with the highest bio stimulant concentration (100%) significantly improving all parameters compared to control (0%).

Water-extractable compounds from the biomass – particularly amino acids, phytohormones, and micronutrients – are known to promote plant growth and stress tolerance. The recovered nutrients thus represent a value-added output, transforming the wastewater into a resource stream rather than a disposal burden (Kim et al., 2023; Goh et al., 2022; Madan et al., 2022; Lu et al., 2025).

Germination bioassays using *Lactuca sativa* seeds indicated that algal extracts stimulated root and shoot elongation by 8–10% compared to control samples. This positive effect is attributed to the presence of auxin-like and cytokinin-like compounds naturally produced by *Chlorella* species during wastewater assimilation. Such findings demonstrate that the recovered algal biomass is not merely a byproduct but a functional bio stimulant that can enhance agricultural productivity, aligning with circular bioeconomy principles (Ronga et al., 2019; Guo et al., 2024; Gupta et al., 2024).

Each variation was observed in terms of plant height, number of leaves, leaf width, stem diameter, and root length after 35 days of planting. The results showed that with the addition of 50% and 100% biostimulants, there was an increase in spinach growth, as shown in Figure 5.

## Mechanistic insights and system synergy

The improved treatment performance can be attributed to the synergistic interaction between biofilm metabolism and membrane filtration. The algal biofilm facilitated nutrient uptake and in

**Table 3.** The average results of applying bio stimulants to *Amaranthus* sp. plants

| Treatment | Plant height (cm) | Leaf number | Leaf width (cm) | Stem size (cm) | Root length (cm) |
|---|---|---|---|---|---|
| 0% | 29.67 | 33 | 3.70 | 0.66 | 11.33 |
| 50% | 35.83 | 41 | 4.40 | 0.70 | 13.2 |
| 100% | 38.33 | 44 | 4.97 | 0.77 | 14.07 |


**Figure 5.** Comparison of the growth of *Amaratus* sp. plants without biostimulants (0%), with 50% biostimulants, and with 100% biostimulants

situ oxygen production through photosynthesis, which enhanced aerobic degradation by heterotrophic microorganisms. This, in turn, reduced the organic burden on the membrane and minimized fouling. Simultaneously, the membrane unit ensured the retention of active biomass and produced clarified permeate water suitable for reuse. Such complementary functionality reflects a self-sustaining treatment ecosystem, where the algal membrane integration improves both environmental quality and resource recovery potential.

## CONCLUSIONS

The results showed that the integration of *Chlorella* sp. into anoxic-oxic moving bed biofilm reactor (MBBR) system significantly improves the treatment performance of high-concentration soy sauce wastewater. The algal biofilm system consistently achieved higher removal efficiencies for all measured parameters compared to the native microbial biofilm, with optimal performance observed at a hydraulic retention time (HRT) of 38 hours and filling the media to 40%. Specifically, the algal biofilm achieved removal efficiencies of 91.65% COD, 91.35% $BOD_5$, 85.47% total nitrogen, 84.41% total phosphate, 84.12% TSS, 60.30% TDS, and 96.27% color. The improved efficiency of the algal biofilm can be attributed to photosynthetic oxygen production, nutrient assimilation into algal biomass, photodegradation of colored compounds, and the formation of extracellular polymeric substances (EPS) that facilitate bio-flocculation. The gradual increase in pH during algae treatment also promotes nutrient precipitation, contributing to overall water quality improvement. A subsequent ultrafiltration process further polished the effluent, resulting in final concentrations of 1.78 mg $L^{-1}$ COD; 0.64 mg $L^{-1}$ $BOD_5$; 1.00 mg $L^{-1}$ TSS; 52.86 mg $L^{-1}$ TDS; 0.86 mg $L^{-1}$ TN; 0.04 mg $L^{-1}$TP; 4.00 Pt.Co color units, and a pH of 7.10. Notably, the ultrafiltration effluent meets the Indonesian non-potable clean water quality standards as required by Government Regulation No. 22/2021. These findings demonstrate that algal biofilm systems offer a sustainable and resource-recovery alternative to conventional microbial biofilms for industrial wastewater treatment. In addition to achieving high pollutant removal, this system enables nutrient recycling and biomass formation, aligning with circular economy and clean water recovery goals.

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