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Abstract

Wastewater-integrated microalgal cultivation offers a sustainable pathway to reduce biofuel production costs while simultaneously addressing nutrient-rich effluent management. In this study, matured compost leachate was systematically evaluated as a sole cultivation medium for *Desmodesmus* sp. under different dilution regimes, with emphasis on growth kinetics, wastewater remediation efficiency, lipid accumulation behavior, and biodiesel quality. *Desmodesmus* sp. successfully acclimatized to 100% undiluted matured compost leachate within four days and maintained stable mixotrophic growth without dilution or pre-treatment. Cultivation in undiluted leachate achieved a maximum biomass concentration of 2.69 ± 0.09 g L^{-1}, representing an approximately fourfold increase compared to Bold's Basal Medium. Concurrently, high treatment efficiencies were obtained, with chemical oxygen demand removal of 82.6%, total nitrogen reduction of 60–72%, and total phosphorus removal of 65–66%, confirming effective integration of biomass production with wastewater remediation. Lipid biosynthesis was strongly governed by nitrogen availability, with lipid concentration increasing from 0.32 g L^{-1} during exponential growth to 0.72 g L^{-1} under nitrogen-depleted stationary conditions. Fatty acid methyl ester profiling revealed a stress-induced shift toward saturated and monounsaturated fatty acids, accounting for 75.6% of total fatty acids and dominated by palmitic acid (C16:0). This compositional restructuring resulted in biodiesel properties characterized by a high cetane number of 64.5, low iodine value, and oxidative stability exceeding 30 h, meeting or surpassing international biodiesel quality benchmarks.

Keywords: *Desmodesmus* sp.; matured compost leachate; mixotrophic cultivation; biodiesel production; circular bioeconomy

1. Introduction

The rapid growth of the global population, accelerating urbanization, and expanding industrial activity are exerting unprecedented pressure on global energy systems, freshwater resources, and wastewater management infrastructures. Global population projections indicate an increase to approximately 9.7 billion by 2050, accompanied by an estimated 50% rise in global energy demand (Li & Li, 2023; Dodangodage et al., 2025; Holechek et al., 2022). Despite the increasing deployment of renewable energy technologies, fossil fuels continue to dominate the global energy mix, accounting

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for nearly 80% of primary energy consumption. This persistent dependence has resulted in severe environmental consequences, with global carbon dioxide (CO_2) emissions exceeding 35.8 Gt in 2023, thereby intensifying climate change, air pollution, and ecosystem degradation (Liu et al., 2024). Among these impacts, fossil fuel derived transportation fuels remain a major contributor to anthropogenic greenhouse gas emissions, underscoring the urgent need for sustainable, low-carbon liquid fuel alternatives (Shahzad & Iqbal Cheema, 2024).

Within this context, biodiesel has emerged as a promising renewable fuel due to its biodegradability, compatibility with existing diesel engines, and potential to reduce net greenhouse gas emissions. Global biodiesel consumption increased from approximately 2.2 million metric tons (MMT) in 2004 to 65.86 MMT in 2023 and is projected to reach nearly 75 MMT by 2030 (Awogbemi & Desai, 2025). However, the long-term sustainability of biodiesel production remains constrained by feedstock availability and cost. First-generation biodiesel derived from edible oils raises critical concerns related to food security, land-use change, and competition for agricultural resources, thereby motivating the development of non-food, third-generation biofuel pathways that decouple fuel production from arable land and conventional food systems (Rulli et al., 2016; Mhetras & Gokhale, 2025; W. Wang & Khanna, 2023).

Microalgae are widely regarded as one of the most promising third-generation biodiesel feedstocks owing to their rapid growth rates, high photosynthetic efficiencies, and superior lipid productivity compared to terrestrial oil crops (Casanova et al., 2023; Adewuyi, 2022; Behera et al., 2015; S. Zhang et al., 2022; Dodangodage et al., 2025; Kanwal et al., 2025). Under nutrient-replete conditions, most microalgal species accumulate approximately 10–30% lipids (w/w), while nutrient stress, particularly nitrogen limitation, can enhance lipid accumulation by two- to three-fold (Maltsev et al., 2023; Udayan et al., 2023; Adams et al., 2013). Several strains, including Desmodesmus, Chlorella, and Chlorococcum, have been reported to achieve lipid contents exceeding 45–60%. In addition to high lipid yields, microalgal biodiesel exhibits favorable physicochemical properties, such as a high cetane number, negligible sulfur content, and improved combustion performance, making it a technically attractive substitute for petroleum diesel (Mallick et al., 2012; Mathimani et al., 2023; Azarpour et al., 2022; Kumar et al., 2023).

Despite these advantages, the commercial deployment of microalgal biodiesel remains economically challenging. The reliance on synthetic growth media, such as BG-11 and Bold's Basal Medium, substantially increases production costs, with nutrient inputs alone accounting for approximately 30–50% of total cultivation expenses (Gu et al., 2023; Casanova et al., 2023; Kanwal et al., 2025; Maroušek et al., 2023). Techno-economic analyses consistently indicate that microalgal biodiesel production costs remain significantly higher than those of petroleum diesel, primarily due to elevated capital investment and operational expenditures. Consequently, the identification of low-cost, nutrient-rich alternative culture media has become a central research focus for improving the economic feasibility and scalability of microalgal biodiesel systems (Maroušek et al., 2023; Rafa et al., 2021; Patnaik & Mallick, 2021).

In parallel, inadequate wastewater management continues to pose a major global sustainability challenge. Approximately 42–45% of household wastewater worldwide is discharged without safe treatment, while much of the remaining fraction receives only partial treatment, resulting in nutrient pollution, eutrophication, and widespread degradation of aquatic ecosystems (Staff, 2025; UN-Water, 2025). High-strength wastewaters enriched with nitrogen, phosphorus, and biodegradable organic matter therefore represent both an environmental burden and an underutilized resource. From a circular bioeconomy perspective, such waste streams offer substantial opportunities for nutrient recovery and bioresource generation when integrated with biological treatment processes (Dodangodage, Kasturiarachchi, et al., 2025b).

Among these waste streams, matured compost leachate wastewater, generated during the later stages of organic waste composting following prolonged biological stabilization, has received comparatively limited research attention. Unlike fresh compost leachate, which is often characterized by extreme organic loading, high ammoniacal toxicity, and inhibitory effects on microbial growth,

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matured compost leachate exhibits a more stable chemical composition with reduced toxicity while retaining considerable concentrations of bioavailable nitrogen and phosphorus. These characteristics render matured compost leachate particularly suitable for biological treatment and valorization strategies, including microalgal cultivation (Naveen et al., 2015; Ahamad Sanadi et al., 2021).

The integration of microalgal cultivation with wastewater treatment represents a compelling circular bioeconomy pathway by enabling simultaneous nutrient removal, biomass production, and renewable fuel generation. Wastewater-based microalgal systems have demonstrated effective reductions in chemical oxygen demand (COD), total nitrogen (TN), and total phosphorus (TP) while producing lipid-rich biomass suitable for biodiesel conversion (Kadir et al., 2018; El-Sheekh et al., 2023). However, existing studies on compost-derived wastewaters have predominantly focused on treatment efficiency or biomass production in isolation, with limited emphasis on fuel-oriented lipid quality, fatty acid composition, and biodiesel property estimation. Moreover, systematic evaluations of microalgal performance under the elevated nitrogen and organic loading conditions characteristic of matured compost leachate remain scarce.

In this context, strain selection must prioritize not only high lipid accumulation but also tolerance to nutrient-rich and organically loaded environments. Desmodesmus sp. was selected in this study due to its demonstrated adaptability to wastewater conditions, resilience to high nutrient concentrations, and capacity for lipid accumulation. Previous investigations have reported biomass productivities ranging from 0.2 to 1.8 g L^{-1} for Desmodesmus spp., alongside removals of 70–99% TN, 60–95% TP, and 40–80% COD, with lipid contents between 20 and 45% (w/w) and fatty acid profiles suitable for biodiesel production (Ji et al., 2014a; Zheng et al., 2023; Ogbonna et al., 2025; Mandotra et al., 2016; C. Jia et al., 2025; Ji et al., 2014b).

Accordingly, this study investigates the sustainable valorization of matured compost leachate wastewater for microalgal biodiesel production using Desmodesmus sp. Optimal leachate dilution ratios were evaluated by simultaneously assessing growth kinetics, biomass productivity, and nutrient uptake performance, with COD, TN, and TP removal treated as primary functional outcomes. Harvested biomass was subsequently analyzed for total lipid content, and fatty acid profiles were determined through fatty acid methyl ester (FAME) analysis. Based on FAME composition, key biodiesel properties, including cetane number, iodine value, saponification value, and higher heating value, were estimated to evaluate fuel suitability in accordance with international biodiesel standards.

To the best of the authors' knowledge, this is the first study to simultaneously assess biomass productivity, nutrient removal dynamics, fatty acid composition, and biodiesel fuel property estimation for Desmodesmus sp. cultivated in matured compost leachate wastewater. The findings demonstrate an integrated circular bioeconomy approach aligned with the United Nations Sustainable Development Goals SDG 6, SDG 7, and SDG 12 (Sayago et al., 2024; Kanchanamala Delanka-Pedige et al., 2021; Dodangodage, Kasturiarachchi, et al., 2025a; Rajendran et al., 2024).

2. Method

2.1. Wastewater Collection and Pre-Treatment

Matured compost leachate wastewater was collected from the Karadiyana municipal solid waste dumping facility, South Colombo, Sri Lanka. Samples were collected in sterile polyethylene containers, transported to the laboratory under ice-cooled conditions, and processed within 24 h of collection. To remove suspended solids and particulate matter, the leachate was filtered through a 0.45 µm nylon membrane filter (Millipore). The clarified filtrate was subsequently used as the experimental growth medium.

Preliminary screening experiments were conducted to identify the optimal leachate dilution ratio for the cultivation of Desmodesmus sp. Based on observed growth performance, undiluted leachate (100% v/v) was selected for subsequent experiments. The initial pH of the wastewater (less than 3.3) was adjusted to 6.8 using 1 M NaOH to ensure physiological suitability for microalgal

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growth. Bold's Basal Medium (BBM) was employed as the control medium. All culture media were sterilized by autoclaving at 121 °C for 20 min prior to inoculation.

2.2. Wastewater Characterization

Initial physicochemical characterization of the matured compost leachate was conducted in accordance with APHA standard methods. All measurements were performed in triplicate (n = 3). The following parameters were analyzed (Association, 1926):

pH: measured using a calibrated benchtop pH meter

Nitrate (NO_3^-–N): determined by UV absorbance at 220 nm with correction at 275 nm

Phosphate (PO_4^{3-}–P): quantified using the molybdenum blue method at 880 nm

COD: Determined by the open reflux titrimetric method with potassium dichromate digestion.

Measured nutrient concentrations were compared with Sri Lankan national discharge standards (Central Environmental Authority, 2022).

2.3. Microalgal Strain and Inoculum Preparation

A pure culture of *Desmodesmus* sp. was obtained from the Pro Green Laboratory, University of Moratuwa, Sri Lanka. Pre-cultures were maintained in BBM containing standard nitrate concentrations under controlled laboratory conditions (25 ± 2 °C, continuous illumination at 150 μmol photons m^{-2} s^{-1} using cool-white LED lighting, and aeration at 0.5 vvm with sterile-filtered air).

Cells were harvested during the exponential growth phase by centrifugation at 4000 rpm for 20 min and subsequently inoculated into experimental media at an initial biomass concentration of 0.30 g L^{-1}. Cultivation experiments were conducted in 2 L laboratory-scale glass photobioreactors with a working volume of 1.5 L. Each reactor was equipped with three-port GL45 screw caps to facilitate aeration, sampling, and pressure compensation. Aeration ports were fitted with 0.45 μm polytetrafluoroethylene (PTFE) membrane filters to ensure sterility and minimize evaporative losses. Illumination was provided using cool-white LED strips under a 12:12 h light–dark photoperiod (Naseema Rasheed et al., 2023; Mattos et al., 2012; Nicodemou et al., 2024).

2.4. Experimental Setup and Cultivation Conditions

2.4.1. Screening of Wastewater Dilution Factors

Screening experiments were conducted using four dilution ratios of matured compost leachate wastewater (25%, 50%, 75%, and 100% v/v), prepared using distilled water. Cultures were grown in 500 mL Erlenmeyer flasks containing a working volume of 400 mL. BBM (400 mL) served as the control medium. The initial pH of all treatments was adjusted to 6.8 using NaOH.

Cultures were incubated at 25 ± 2 °C under a 12:12 h light–dark cycle, continuously aerated, and illuminated at an intensity of 150 μmol photons m^{-2} s^{-1} using cool-white LED lighting. Biomass growth was monitored at 48 h intervals by measuring optical density at 680 nm (OD_{680}) and by dry weight determination.

2.4.2. Main Cultivation Experiment

Based on the screening results, undiluted matured compost leachate (100% v/v) was selected for the main cultivation experiment, as it supported high biomass productivity without the need for freshwater dilution. The selected light intensity (150 μmol photons m^{-2} s^{-1}) was retained, as it provided an optimal balance between growth performance and energy input.

Batch cultivation was performed in 2 L sterilized glass photobioreactors with a working volume of 1.5 L using undiluted leachate and BBM as the control. Cultures were maintained at 25 ± 2 °C under a 12:12 h light–dark cycle, aerated with sterile-filtered air (BOYO pumps, 0.45 μm filters), and illuminated using cool-white LED lighting. Light intensity was periodically verified using a quantum

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sensor (LI-COR LI-250A). The BBM control was cultivated under identical conditions to ensure experimental comparability (Dodangodage, Kasturiarachchi, et al., 2025b).

2.5. Analytical Procedures

2.5.1. Biomass Concentration

Microalgal growth was monitored every 48 h. Optical density at 680 nm (OD_{680}) was measured using a UV–Vis spectrophotometer (Shimadzu UV-1800). Biomass concentration was determined gravimetrically by filtering 5 mL culture aliquots through pre-dried and pre-weighed glass microfiber filters (Hyundai GF/C, 47 mm diameter, 1.2 µm pore size)(Dodangodage et al., 2025). Filters were oven-dried at 60 °C for 24 h prior to weighing.

Biomass concentration (DW_n, g L^{-1}) was calculated as:

$$\textbf{Dry Weight } (DW_n) = \frac{W_f - W_i}{V} \text{ ---------} (1)$$

where W_f is the final weight of the filter with biomass, W_i is the initial filter weight, and V is the volume of the filtered culture sample.

2.5.2. Nutrient and COD Removal

Nitrate, phosphate, COD, and pH were measured at 2-day intervals. Culture samples (5 mL) were centrifuged at 10,000 × g for 5 min, and the supernatant was filtered through 0.22 µm nylon syringe filters. Nitrate and phosphate concentrations were analyzed as described in Section 2.2, while pH was measured using a calibrated pH probe (Hanna HI98194)(Dodangodage et al., 2025).

Removal efficiency (RE, %) was calculated as:

$$\textbf{(RE)} = \frac{C_i - C_f}{C_i} \times 100 \text{ ----------------------------} (2)$$

where C_i and C_f represent the initial and final concentrations (mg L^{-1}), respectively.

2.5.3. Analytical Validation

All analytical measurements were conducted in triplicate. UV–Vis analyses were calibrated using standard solutions of potassium nitrate, KH_2PO_4, and potassium hydrogen phthalate. Calibration curves demonstrated strong linearity ($R^2 \geq 0.996$). Instrumental blanks were analyzed with each batch to minimize baseline drift.

2.6. Biomass Harvesting

At the end of the 20-day cultivation period, microalgal biomass was harvested by centrifugation at 4000 rpm for 20 min (Eppendorf 5810R). The biomass was washed twice with sterile distilled water and oven-dried at 60 °C until constant weight was achieved (Potts, 2000; Dodangodage, Kasturiarachchi, et al., 2025b).

2.7. Total Lipid Extraction and Quantification

Microalgal biomass was harvested on the 20th day of cultivation by centrifugation at 4500 × g for 10 min, followed by oven drying at 60 °C for 24 h to obtain constant dry weight. Lipid extraction was performed using a modified Bligh and Dyer protocol [27]. Briefly, 10 mg of dried biomass was homogenized with a chloroform–methanol mixture (1:1, v/v) to facilitate lipid solubilization. Phase separation was subsequently induced by adding 0.8% (w/v) NaCl solution, resulting in a final solvent ratio of chloroform/methanol/0.8% NaCl = 10:10:9 (v/v/v).

The lower chloroform phase containing the extracted lipids was carefully transferred into pre-dried and pre-weighed centrifuge tubes and evaporated under a gentle stream of nitrogen gas. Residual solvent was removed by oven drying at 60 °C until constant mass was achieved. The total

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lipid content was quantified gravimetrically and expressed as a percentage of dry biomass (Lee et al., 2013; DuBois et al., 1956; J. Jia et al., 2015).

2.8. Fatty Acid Methyl Ester (FAME) Preparation and Fatty Acid Profiling

Total lipids extracted as described in Section 2.7 were dissolved in chloroform, and 0.5 mg of heptadecanoic acid (C17:0) was added to each sample as an internal standard prior to solvent evaporation under nitrogen. Fatty acid transesterification was conducted by adding 5 mL of 1% (v/v) sulfuric acid in methanol, followed by incubation in a 50 °C water bath overnight, as previously reported.

The resulting fatty acid methyl esters (FAMEs) were extracted into n-hexane and analyzed using gas chromatography with flame ionization detection (GC–FID) equipped with a DB-23 capillary column (60 m × 0.25 mm × 0.15 μm). The injector temperature was maintained at 250 °C, with an injection volume of 1 μL and a 1:50 split ratio. Helium was used as the carrier gas at a constant pressure of 230 kPa(Breuer et al., 2013)(Ma et al., 2016).

The oven temperature program consisted of an initial hold at 50 °C for 1 min, followed by an increase at 25 °C min^{-1} to 175 °C, then ramped at 4 °C min^{-1} to 230 °C and held for 5 min. The detector temperature was set at 280 °C, with hydrogen, air, and helium flow rates of 40 mL min^{-1}, 450 mL min^{-1}, and 30 mL min^{-1}, respectively. Fatty acids were identified and quantified using a 37-component FAME standard mixture, which was used for calibration.

2.9. Biodiesel Property Estimation Based on Fatty Acid Composition

The physicochemical properties of biodiesel derived from microalgal lipids were estimated theoretically based on the fatty acid methyl ester profile, using established empirical correlations (Islam et al., 2013)(Mondal et al., 2021).

The iodine value (IV) was calculated as:

Iodine value (IV) = $\sum_{i=1}^{n} 254N\, D_i/M_i$ --(3)

The kinematic viscosity (ν) was determined using:

Kinematic viscosity (ν) = $exp(\sum_{i=1}^{n} w_i\, ln\,(\eta_i))$ ---------------------------------------(4)

where: $\eta_i = -12.503 + 2.496ln\,(M_i) - 0.178N$

The higher heating value (HHV) was estimated as:

High heating value (HHV) = $\sum_{i=1}^{n} w_i\, \delta_i$ ---(5)

where:

δi=46.19−(1794/Mi)−0.21N

The density (ϱ) of biodiesel was calculated using:

Density (ϱ) = $\sum_{i=1}^{n} w_i\, \rho_i$ ---(6)

where:

ϱi=0.8463−(0.49/Mi)+0.0118N

The saponification value (SV) was calculated as:

Saponification value (SV) = $\sum_{i=1}^{n} 560N/\,M_i$ -----------------------------------(7)

The cetane number (CN) was estimated using:

Cetane number (CN) = $46.3 + \left(\frac{5458}{SV}\right) - 0.225IV$ ---------- (8)

Finally, oxidation stability was determined as:

Oxidation stability = $(117.9295/X) + 2.5905$ -------------------------------(9)

where D_i represents the weight percentage of the i^{th} fatty acid, w_i is its corresponding weight fraction, M_i is the molecular weight, and N denotes the number of double bonds. X corresponds to the combined weight percentage of linoleic and linolenic acids.

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2.10. Statistical Analysis

All experiments were performed in triplicate (n=3). Statistical significance was assessed using one-way analysis of variance (ANOVA) with a 95% confidence interval ($p < 0.05$), implemented using the Data Analysis Toolpak in Microsoft office 365.

3. Results

3.1. Characterization of Matured Compost Leachate Wastewater

The physicochemical characteristics of the raw matured compost leachate collected from the Karadiyana dump site are summarized in Table 1. The leachate exhibited a slightly acidic to near-neutral pH (6.83 ± 0.15) and a dark brown, turbid appearance, indicating the presence of dissolved humic substances and stabilized organic matter.

The organic load was considerable, with a Chemical Oxygen Demand (COD) of 1365.89 ± 48.19 mg L^{-1}. However, when compared to fresh compost or landfill leachates—which can exhibit COD levels exceeding 10,000–50,000 mg L^{-1}—this value confirms the matured nature of the effluent, reflecting partial biological stabilization during composting.

Nutrient analysis revealed elevated concentrations of nitrogen and phosphorus, classifying the leachate as a high-strength wastewater. Total Nitrogen (TN) and Total Phosphorus (TP) concentrations were 735.29 ± 17.87 mg L^{-1} and 20.87 ± 1.20 mg L^{-1}, respectively. The resulting N:P ratio (~35:1) deviates substantially from the Redfield ratio (16:1), indicating a phosphorus-limited nutrient balance. Despite this imbalance, the high nutrient content highlights the potential of this waste stream as a nutrient-rich cultivation medium, provided the microalgal strain can tolerate the associated turbidity and ammoniacal stress.

Table 1. Physicochemical characterization of raw matured compost leachate wastewater used in the study (n = 3).

Parameter	Unit	Value (Mean ± SD)
pH	-	6.83 ± 0.15
Chemical Oxygen Demand (COD)	mg L^{-1}	1365.89 ± 48.19
Total Nitrogen (TN)	mg L^{-1}	735.29 ± 17.87
Total Phosphorus (TP)	mg L^{-1}	20.87 ± 1.20
Appearance	-	Dark brown, Turbid

3.2. Effect of Leachate Dilution on Microalgal Growth (Screening Phase)

The growth response of *Desmodesmus* sp. was evaluated under varying leachate dilutions (25%, 50%, 75%, and 100% v/v) and compared with a BBM control over a 20-day cultivation period. One-way ANOVA revealed statistically significant differences in growth performance among treatments, based on both optical density ($F_{4,10} = 33.62$, $p < 0.001$) and dry cell weight ($F_{4,10} = 82.82$, $p < 0.001$).

As shown in Figure 1, cultures grown in higher leachate concentrations (75% and 100%) exhibited a distinct lag phase during the first four days, whereas lower dilutions and the control showed earlier growth initiation. Following this acclimatization phase, rapid exponential growth was observed in all leachate treatments.

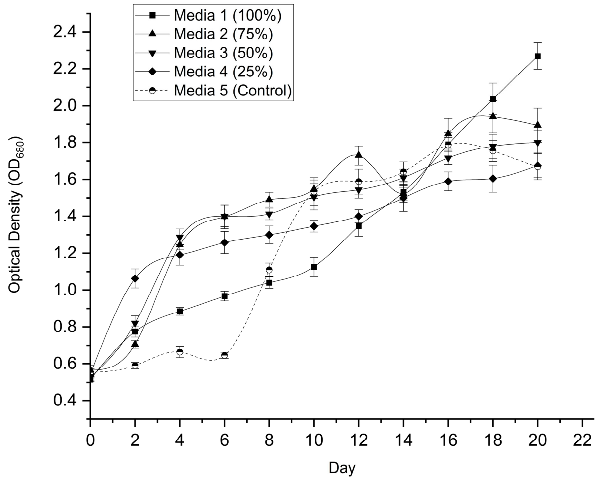

Figure 1. Growth profiles of *Desmodesmus sp.* under varying leachate dilutions (25%, 50%, 75%, 100% v/v) compared with BBM control over 20 days, measured as optical density.

By Day 20, the undiluted leachate (100%) achieved the highest optical density (2.269 ± 0.072), which was significantly higher than both the BBM control (1.668 ± 0.070) and the 75% dilution (1.894 ± 0.003) ($p < 0.05$). Biomass measurements corroborated these trends (Figure 2), with the 75% leachate yielding the highest dry biomass (2.025 ± 0.089 g L^{-1}). Importantly, the undiluted leachate also produced substantially higher biomass (1.567 ± 0.072 g L^{-1}) than the BBM control (1.063 ± 0.048 g L^{-1}).

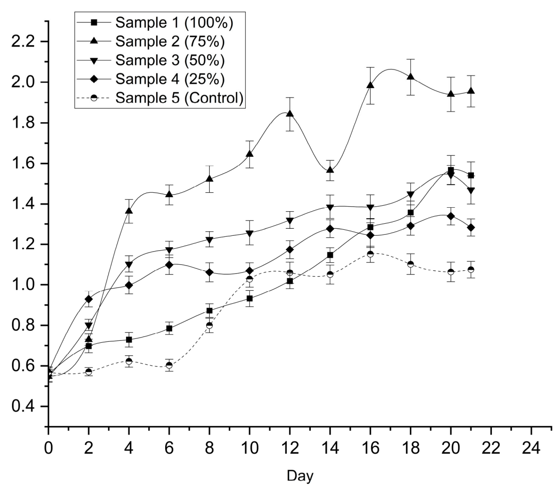

Figure 2. Dry biomass accumulation of *Desmodesmus sp.* across different leachate dilutions and BBM control after 20 days of cultivation.

Based on these results, the 100% leachate condition was selected for subsequent experiments. Although the 75% dilution produced marginally higher biomass, the elimination of freshwater dilution in the 100% condition represents a more sustainable and industrially relevant option, particularly given its ~47% higher biomass yield compared to the control medium.

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3.3. Biomass Production in Main Cultivation Experiment

Growth kinetics of *Desmodesmus* sp. were further evaluated in undiluted matured compost leachate and BBM over a 24-day batch cultivation period. Optical density profiles (Figure 3) revealed distinct growth patterns between the two media. The leachate culture exhibited rapid early growth, reaching a plateau around Day 14 (OD ≈ 3.04), whereas the BBM control showed a more gradual increase, ultimately reaching a higher OD by Day 24 (4.372 ± 0.156).

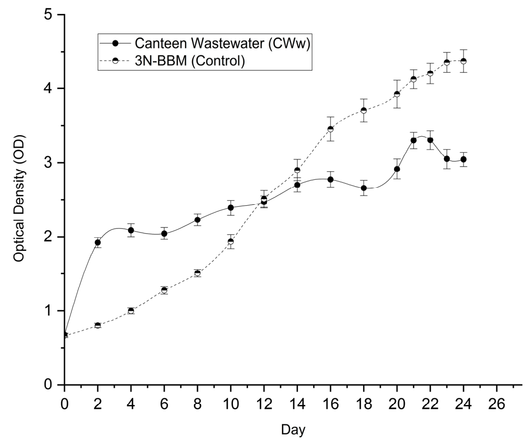

Figure 3. Optical density-based growth kinetics of *Desmodesmus sp.* in undiluted matured compost leachate and BBM control over a 24-day batch cultivation period.

Given the potential influence of wastewater turbidity and cellular morphology on optical measurements, biomass productivity was assessed using dry cell weight (DCW). As shown in Figure 4, DCW values in the leachate culture were consistently and significantly higher ($p < 0.001$) than those in the control throughout the cultivation period. The maximum biomass concentration in leachate reached 2.692 ± 0.093 g L^{-1} on Day 22, compared to only 0.668 ± 0.031 g L^{-1} in the BBM control.

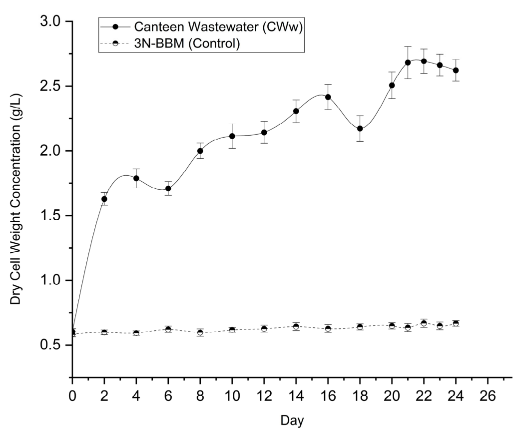

Figure 4. Dry cell weight (DCW) of *Desmodesmus sp.* in matured compost leachate versus BBM control during 24 days of batch cultivation.

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Overall, cultivation in matured compost leachate resulted in approximately fourfold higher biomass accumulation relative to synthetic media, confirming its suitability as an effective growth substrate for high-density algal cultivation.

3.4. Nutrient Removal Efficiency

3.4.1. Total Nitrogen (TN) Removal

Nitrogen is a critical macronutrient for microalgal protein synthesis and growth. The temporal variation of Total Nitrogen (TN) concentration in the matured compost leachate and the BBM control is presented in Figure 5.

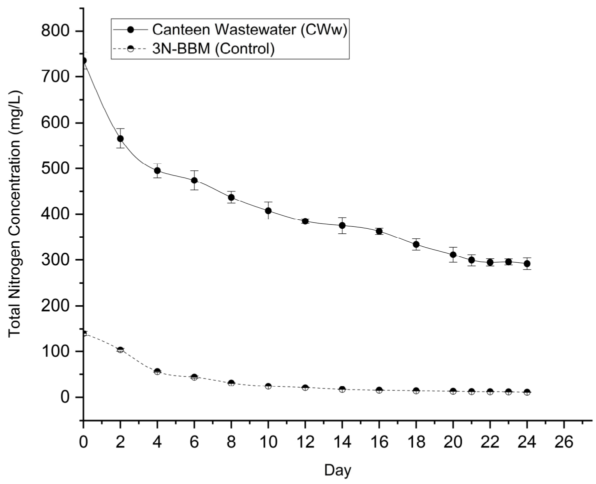

Figure 5. Total nitrogen (TN) removal by *Desmodesmus sp.* from matured compost leachate and BBM control over the cultivation period.

The initial TN concentration in the matured compost leachate was recorded at 735.29 ± 17.87 mg L^{-1}, significantly higher than the synthetic control (139.37 ± 4.42 mg L^{-1}), confirming the nitrogen-rich nature of the compost effluent. Despite this high initial load, Desmodesmus sp. demonstrated efficient nitrogen uptake capability.

A rapid depletion of nitrogen was observed during the first 4 days of cultivation, where the TN concentration in the leachate dropped sharply to 495.20 ± 16.43 mg L^{-1}. Statistical analysis confirms this initial reduction was highly significant ($p < 0.01$), corresponding to a removal of approximately 32.6% of the initial load. This correlates with the rapid exponential growth phase observed in the biomass data (Section 3.2). Following this initial surge, the removal rate stabilized but continued steadily throughout the cultivation period.

By the end of the experiment (Day 24), the TN concentration in the leachate had decreased to 291.75 ± 12.12 mg L^{-1}. This represents a statistically significant total reduction ($p < 0.001$) relative to the initial concentration, achieving a removal efficiency of 60.32%. In comparison, the control medium showed a depletion from 139.37 mg L^{-1} to 10.82 mg L^{-1}, achieving a removal efficiency of 92.2%. Although the percentage removal was higher in the control due to the lower initial starting concentration, the total mass of nitrogen removed in the leachate system (−443.5 mg L^{-1} removed) was substantially higher than in the control (−128.5 mg L^{-1} removed). This high nutrient removal capacity highlights the potential of Desmodesmus sp. to bioremediate high-strength nitrogenous wastewaters while converting the excess nutrients into valuable biomass.

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3.4.2. Total Phosphorus (TP) Removal

Phosphorus is a vital nutrient for microalgal metabolism, primarily involved in energy transfer (ATP synthesis) and nucleic acid formation. The removal of Total Phosphorus (TP) from the matured compost leachate and BBM control was monitored throughout the cultivation period, as shown in Figure 6.

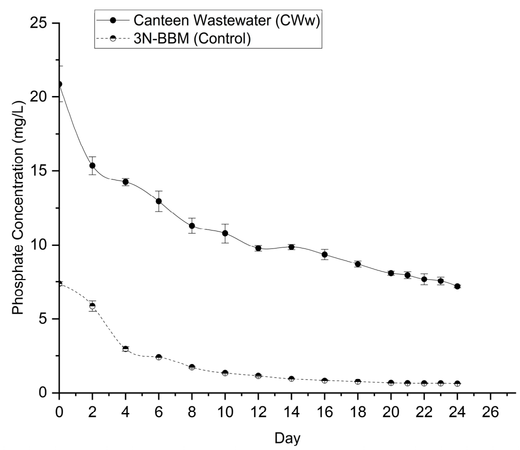

Figure 6. Total phosphorus (TP) removal by *Desmodesmus sp.* from matured compost leachate and BBM control during 24-day cultivation.

The matured compost leachate initially contained a TP concentration of 20.87 ± 1.20 mg L^{-1}, which is typical for organic-rich effluents and significantly higher than the control medium (7.37 ± 0.14 mg L^{-1}). The removal trend followed a pattern similar to nitrogen uptake, characterized by a rapid reduction during the initial and rapid growth phases (Days 0–6). During this first week, the TP concentration in the leachate decreased to 12.96 ± 0.69 mg L^{-1}, indicating active assimilation by the algae during cell division.

By the end of the 24-day cultivation period, the TP concentration in the leachate had significantly decreased to 7.18 ± 0.09 mg L^{-1} ($p < 0.001$), corresponding to a removal efficiency of 65.57%. In contrast, the BBM control achieved a higher removal efficiency of 91.54%, with the final concentration dropping to 0.62 ± 0.02 mg L^{-1}.

While the percentage removal was superior in the synthetic control, the absolute mass of phosphorus removed was significantly higher in the wastewater system. *Desmodesmus* sp. removed approximately 13.69 mg L^{-1} of phosphorus from the leachate, compared to only 6.74 mg L^{-1} in the control. This robust phosphorus uptake confirms that matured compost leachate provides bioavailable phosphate that can be effectively recovered by microalgal biomass, thereby reducing the eutrophication potential of the effluent before discharge.

3.4.3. Chemical Oxygen Demand (COD) Removal

Chemical Oxygen Demand (COD) serves as a primary indicator of the organic matter content in the wastewater. Unlike the inorganic BBM control, which contains negligible organic carbon, the matured compost leachate presented a high initial organic load, necessitating the evaluation of carbon removal efficiency. The temporal reduction of COD in the leachate culture is illustrated in Figure 7.

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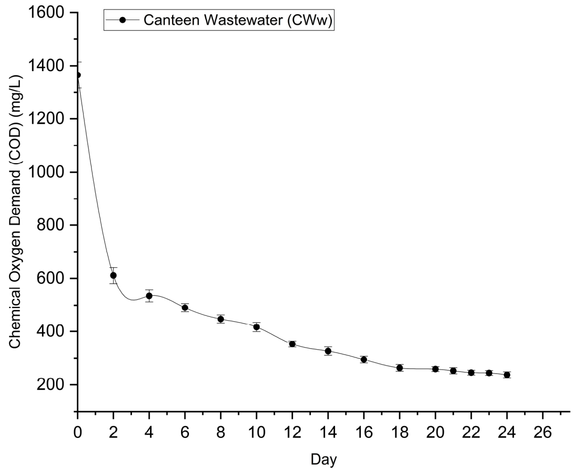

Figure 7. Chemical Oxygen Demand (COD) reduction in matured compost leachate during cultivation of *Desmodesmus sp.*

The initial COD of the matured compost leachate was recorded at 1365.89 ± 48.19 mg L^{-1}. A precipitous drop in COD was observed within the first 48 hours of cultivation, where the concentration decreased to 611.23 ± 30.48 mg L^{-1}. This corresponds to a removal of nearly 55% of the total organic load in just 2 days. This rapid consumption of organic carbon strongly suggests that Desmodesmus sp. switched to a mixotrophic growth mode, utilizing the dissolved organic compounds as an exogenous carbon source to fuel the rapid biomass production observed in the early growth phase (Section 3.2).

Following this initial rapid assimilation phase, the COD removal rate stabilized, decreasing gradually as the readily biodegradable organic fraction was exhausted. By Day 24, the final COD concentration reached 237.43 ± 10.95 mg L^{-1}, representing a statistically significant reduction ($p < 0.001$) from the initial value.

The overall COD removal efficiency achieved was 82.62%. This high removal efficiency indicates that Desmodesmus sp. is highly effective at mineralizing the organic pollutants present in matured compost leachate. The final COD levels are well within the discharge limits for many industrial effluents, demonstrating the dual capability of this system for both biomass generation and effective organic wastewater treatment.

3.4.4. pH Evolution During Cultivation

The variation of pH in the culture medium serves as an indirect proxy for microalgal metabolic activity, particularly photosynthetic carbon fixation. The pH profiles of the matured compost leachate and control cultures are presented in Figure 8.

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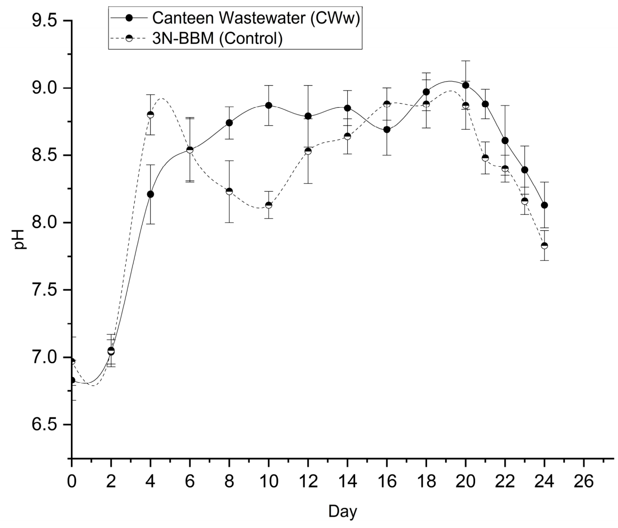

Figure 8. pH evolution of matured compost leachate and BBM control during the cultivation of *Desmodesmus sp.*

Both media started with a near-neutral pH (6.83 ± 0.15 for leachate and 6.97 ± 0.18 for control). As cultivation progressed, a distinct alkalinization trend was observed in both conditions. In the matured compost leachate, the pH rose significantly, reaching a maximum value of 9.02 ± 0.18 by Day 20. A similar trend was observed in the control, which peaked at 8.88 ± 0.18.

This substantial increase in pH is attributed to the photosynthetic consumption of dissolved inorganic carbon. As *Desmodesmus* sp. actively assimilates dissolved CO_2 and bicarbonates (HCO_3^-) during the light period, the equilibrium of the carbonate system shifts, leading to the consumption of hydrogen ions (H^+) and a subsequent rise in hydroxyl ions (OH^-). The fact that the pH remained consistently high (>8.5) from Day 6 to Day 20 confirms that the culture was maintaining robust photosynthetic activity throughout the exponential growth phase.

It is worth noting that sustaining a high pH (approx. 9.0) in wastewater treatment systems offers secondary benefits. Alkaline conditions are known to promote ammonia stripping (volatilization of free ammonia) and can induce the precipitation of heavy metals and phosphates. Furthermore, high pH levels create a harsh environment for enteric pathogens and coliforms, potentially enhancing the disinfection quality of the treated effluent. Towards the end of the cultivation (Days 22–24), a slight decline in pH was observed (dropping to 8.13 in leachate), likely signaling the onset of the stationary phase where cellular respiration and organic acid release began to counterbalance photosynthetic carbon uptake.

3.5. Lipid Production and Accumulation

The viability of microalgal biodiesel depends not only on biomass growth but critically on the lipid yield per unit volume of culture. To evaluate this, lipid weight (g) and lipid concentration (g L^{-1}) was quantified during the transition from the exponential to the stationary phase (Days 18–23), as presented in Figure 9 and 10.

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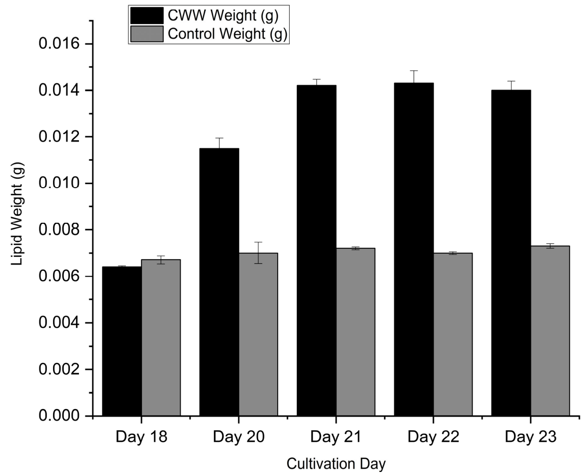

Figure 9. Lipid accumulation (g) of *Desmodesmus sp.* in matured compost leachate and BBM control from Day 18 to Day 23.

Interesting dynamics were observed regarding the timing of lipid accumulation. At Day 18, there was no statistically significant difference ($p > 0.05$) in lipid concentration between the matured compost leachate (0.320 ± 0.002 g L^{-1}) and the BBM control (0.334 ± 0.009 g L^{-1}). This indicates that during the active growth phase, metabolic energy was primarily directed toward cellular division and biomass synthesis rather than lipid storage.

However, a marked shift occurred as the culture entered the stationary phase. In the matured compost leachate cultures, lipid concentration surged rapidly between Day 18 and Day 22. The lipid yield more than doubled during this 4-day window, peaking at a maximum concentration of 0.715 ± 0.027 g L^{-1} on Day 22. This accumulation coincides with the depletion of nitrogen observed in Section 3.3.1. It is well-established that under nitrogen-limited conditions, *Desmodesmus* sp. shifts its metabolic pathway from protein synthesis to triglyceride accumulation as an energy storage mechanism.

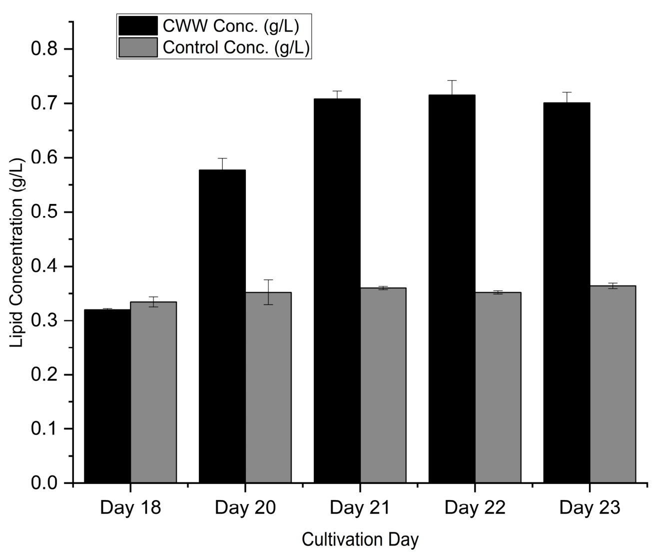

Figure 10. Lipid concentration (g L^{-1}) of *Desmodesmus sp.* in matured compost leachate versus BBM control during stationary phase (Days 18–23).

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In contrast, the control cultures displayed a stagnant lipid profile, with concentrations fluctuating marginally around 0.350–0.360 g L^{-1} throughout the stationary phase. Consequently, by Day 22, the matured compost leachate yielded approximately 2.03 times more lipid per liter compared to the synthetic control ($p < 0.001$). This superior volumetric lipid productivity confirms that matured compost leachate not only supports higher biomass densities but effectively triggers lipid accumulation, making it an excellent feedstock for scalable biodiesel production.

3.6. Fatty Acid Profiling and Biodiesel Characterization

3.6.1. Fatty Acid Methyl Ester (FAME) Composition

The quality and performance of microalgal biodiesel are intrinsically governed by the fatty acid profile of the parent oil. The FAME composition of *Desmodesmus* sp. cultivated in matured compost leachate was determined via GC-MS analysis (Table 3).

The raw chromatograms revealed that the crude lipid extract contained significant quantities of non-saponifiable lipids, primarily stigmasterol (RT 29.24 min), which accounted for approximately 38% of the raw peak area. For biodiesel characterization, the profile was normalized to include only fatty acid methyl esters. The analysis revealed a unique profile dominated by saturated fatty acids (SFAs), specifically medium-chain fatty acids. The most abundant components were Palmitic acid (C16:0) at 26.95% and Lauric acid (C12:0) at 25.78%. Notably, the presence of short-chain fatty acids such as Caprylic (C8:0) and Capric (C10:0) acid was also detected, which significantly influences the fuel's flow properties.

This profile contrasts with previous studies on *Desmodesmus* sp. cultivated in synthetic media (Pozzobon et al., 2020), which typically report profiles dominated by polyunsaturated C18 fatty acids. The shift towards shorter, saturated chains (C12–C16) in this study is likely a stress response to the complex organic matrix of the compost leachate, favoring the accumulation of energy-dense saturated lipids.

Table 3. Fatty acid profile of *Desmodesmus* sp. biomass cultivated in matured compost leachate (normalized % of total FAMEs).

Fatty Acid	**Carbon Structure**	**Relative Abundance (%)**
Caprylic acid	C8:0	4.55
Capric acid	C10:0	3.86
Lauric acid	C12:0	25.78
Myristic acid	C14:0	9.39
Palmitic acid	C16:0	26.95
Stearic acid	C18:0	5.11
Oleic acid	C18:1	20.46
Linoleic acid	C18:2	3.90
Total Saturated	-	75.64
Total Unsaturated	-	24.36

3.6.2. Estimation of Biodiesel Fuel Properties

The theoretical fuel properties were calculated based on the FAME composition and compared against ASTM D6751-12 and EN 14214:2012 standards (Table 4).

The Iodine Value (IV) was calculated to be 25.45 g I_2/100 g, which is drastically lower than the EN maximum limit of 120. This indicates exceptional oxidative stability, as confirmed by the predicted Oxidative Stability time of >30 hours (significantly surpassing the ASTM requirement of 3 hours). This stability is attributed to the low content of polyunsaturated fatty acids (only 3.90%), preventing rapid fuel degradation.

The Saponification Value (SV) was calculated to be 228.5 mg KOH g^{-1}. This relatively high value reflects the abundance of low-molecular-weight fatty acids (C8–C12). Consequently, the Cetane

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Number (CN) was estimated at 64.5. This value significantly exceeds the minimum requirements for both ASTM (>47) and EN (>51) standards, indicating that the fuel possesses superior ignition quality and would result in smoother engine combustion with minimal knocking.

Crucially, the presence of medium-chain fatty acids (C12:0, C14:0) balanced the high viscosity typically associated with saturated palmitic acid. The predicted Kinematic Viscosity was 3.42 $mm^2 s^{-1}$, which falls squarely within the ASTM D6751 standard range (1.9–6.0 $mm^2 s^{-1}$). Unlike long-chain saturated fats that can cause injector clogging, this viscosity profile suggests excellent atomization properties while maintaining sufficient lubricity for fuel system components. Therefore, the biodiesel derived from this feedstock meets key international standards for use as a standalone fuel or a premium blending stock.

Table 4. Predicted fuel properties of *Desmodesmus* sp. biodiesel compared to international standards.

Biodiesel Property	Unit	This Study (Leachate)	ASTM D6751	EN 14214
Iodine Value	g I_2/100g	25.45	-	< 120
Kinematic Viscosity	mm^2/s	3.42	1.9–6.0	3.5–5.0
Higher Heating Value	MJ/kg	38.36	-	-
Density	g/cm^3	0.85	-	0.86–0.90
Saponification Value	mg KOH/g	**228.5**	-	-
Cetane Number	-	**64.5**	> 47	> 51
Oxidative Stability	h	32.86	> 3	> 8

4. Discussion

The ability of *Desmodesmus* sp. to sustain growth in 100% matured compost leachate without dilution represents a notable advance for wastewater-integrated microalgal cultivation. High-strength organic effluents are typically characterized by elevated ammoniacal nitrogen and organic loads that necessitate dilution or pre-treatment to prevent culture failure. Among these inhibitory constituents, ammonium nitrogen (NH_4^+-N) is widely recognized as a primary toxicant in anaerobic and compost-derived wastewaters due to its direct interference with cellular metabolism and photosynthetic processes (López-Rosales et al., 2022).

Within this context, the four-day lag phase observed under undiluted leachate conditions should be interpreted as a physiological acclimatization period rather than growth inhibition. Previous kinetic studies have demonstrated a strong correlation between wastewater toxicity intensity and lag-phase duration (λ), with extended lag phases reflecting metabolic reprogramming rather than cell mortality (Khanzada & Övez, 2017) (López-Rosales et al., 2022). During this acclimation phase, free ammonia (NH_3), which readily diffuses across cellular membranes, disrupts photosystem II by uncoupling electron transport and destabilizing thylakoid membranes, resulting in transient suppression of photosynthetic efficiency(Metin & Altınbaş, 2024)(Drath et al., 2008).

In response to ammonium stress, microalgal cells activate detoxification and nitrogen assimilation pathways, particularly the ATP-dependent GS/GOGAT cycle, enabling conversion of toxic ammonium into amino acids and structural biomass. The subsequent recovery and proliferation of *Desmodesmus* sp. therefore indicate successful metabolic restructuring and restoration of photosynthetic functionality. This adaptive response supports the feasibility of gradual acclimatization strategies for stable microalgal operation in high-strength effluents without dilution, offering clear economic and operational advantages for large-scale wastewater treatment systems (Guo et al., 2025; López-Rosales et al., 2022; Ji et al., 2014a; J. Wang et al., 2019).

Following acclimatization, the rapid biomass accumulation observed confirms the energetic advantage of mixotrophic metabolism under compost leachate conditions. Unlike strict photoautotrophy, which is constrained by CO_2 mass-transfer limitations and photon availability, mixotrophy enables simultaneous utilization of light energy and organic carbon substrates (COD) through respiratory metabolism. This dual metabolic strategy promotes energetic coupling between chloroplasts and mitochondria, facilitating ATP and reducing-equivalent exchange (NADPH) and

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lowering the energetic cost of biomass synthesis (Yan et al., 2024; Z. Zhang et al., 2021; Bo et al., 2021; López-Rosales et al., 2022; Tossavainen et al., 2017).

Consistent with this framework, the pronounced growth surge recorded aligns with literature reporting 8–12-fold increases in biomass productivity under mixotrophic conditions relative to autotrophic controls. From a kinetic standpoint, the specific growth rate under mixotrophy can be approximated as the additive contribution of autotrophic and heterotrophic metabolic fluxes. This advantage allows *Desmodesmus* sp. to effectively exploit the biodegradable organic matter present in matured compost leachate, overcoming photosynthetic limitations imposed by the wastewater matrix and explaining the high biomass yields achieved (Eze et al., 2018; Cheng et al., 2013; de Souza et al., 2021; Yun et al., 2021; López-Rosales et al., 2022).

Sustained growth in optically dense leachate is particularly noteworthy, as wastewater systems commonly exhibit elevated background turbidity (K_bg) due to humic substances, suspended solids, and chromophoric organic matter. Such conditions intensify light attenuation, restrict the photic zone, and often induce severe photolimitation, with turbidity levels exceeding 90–100 NTU reported to cause biomass stagnation or collapse in conventional photobioreactors (Segredo-Morales et al., 2023; Otim et al., 2021; Benner et al., 2022; López-Rosales et al., 2022).

Under mixotrophic regimes, however, the organic turbidity characteristic of compost leachates provides a functional advantage. Rather than acting solely as an optical barrier, particulate and dissolved organic compounds serve as metabolizable carbon sources. By reducing reliance on photon penetration, *Desmodesmus* sp. was able to sustain growth even within light-limited reactor zones, effectively bypassing the classical shadowing effects that inhibit obligate photoautotrophs in turbid wastewater environments (Wu et al., 2025; Licata et al., 2025; López-Rosales et al., 2022).

In addition, the high biomass density achieved during cultivation likely enhanced tolerance to ammonia toxicity. Dense algal cultures are known to buffer toxic shocks more effectively than dilute inocula, as collective assimilation and diffusion gradients reduce intracellular exposure to inhibitory compounds. This density-dependent resistance plausibly contributed to the sustained stability of the culture under prolonged exposure to elevated ammonium concentrations (Collos & Harrison, 2014). Beyond sustaining biomass growth, the same physiological and metabolic mechanisms underpinning mixotrophic proliferation directly governed organic matter attenuation, thereby linking culture stability to wastewater treatment performance.

The high chemical oxygen demand (COD) removal efficiency achieved in this study (82.6%) demonstrates the strong capacity of *Desmodesmus* sp. to remediate organic-rich matured compost leachate. This value exceeds those commonly reported for *Desmodesmus* spp. cultivated in high-strength agro-industrial or landfill leachates, where COD reductions typically range from 36% to 70%, depending on wastewater composition and operational conditions (López-Rosales et al., 2022;(Ahmed et al., 2017a). Such performance underscores the suitability of matured compost leachate as an effective substrate for integrated algal wastewater treatment.

COD removal in microalgal systems occurs through multiple concurrent mechanisms, including (i) direct assimilation of dissolved organic carbon under mixotrophic metabolism, (ii) respiratory oxidation of organic substrates, and (iii) adsorption or entrapment of organic matter on algal cell surfaces and extracellular polymeric substances (EPS) (Debeni Devi et al., 2022). Among these pathways, mixotrophic assimilation is widely recognized as the dominant mechanism when readily biodegradable organic compounds are present (Yun et al., 2021). The rapid COD decline observed during the exponential growth phase in this study strongly supports the predominance of this biological assimilation pathway.

The superior COD removal can be further attributed to the distinct composition of matured compost leachate, which differs fundamentally from fresh leachate and many industrial effluents (Doyle et al., 2022). Its organic fraction is dominated by partially stabilized, low-molecular-weight compounds such as volatile fatty acids and humic degradation products, which exhibit higher bioavailability than recalcitrant industrial organics. This compositional characteristic provides a

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mechanistic explanation for the enhanced COD removal observed relative to untreated landfill leachates (López-Rosales et al., 2022).

Beyond algal assimilation, the observed COD removal efficiency is consistent with functional algal–bacterial cooperation under non-axenic cultivation conditions. In such systems, microalgae supply dissolved oxygen via photosynthesis, supporting aerobic bacterial degradation of organic matter, while heterotrophic bacteria mineralize organics into CO_2 that sustains algal photosynthesis and mixotrophic growth (Ríos et al., 2018). This synergistic carbon exchange has been widely reported to enhance COD removal beyond levels achievable by axenic algal cultures alone; Yun et al., 2021).

In addition, non-sterile conditions promote EPS secretion by both algae and bacteria, enhancing bio-flocculation and physical entrapment of suspended organic particulates. This process contributes further COD reduction through aggregation and sedimentation, particularly under high-strength wastewater conditions (Debeni Devi et al., 2022). Collectively, biological assimilation, microbial synergy, and EPS-mediated physical removal explain the high COD attenuation achieved.

Following organic carbon removal, total nitrogen (TN) removal reached approximately 60–72%, indicating effective nitrogen attenuation despite the high initial concentration (~800 mg L^{-1}). In algal-based systems, nitrogen removal is primarily driven by biological assimilation into proteins, nucleic acids, chlorophyll, and other cellular constituents (Song et al., 2024); (Salbitani & Carfagna, 2021). As biodegradable organic carbon was progressively depleted through mixotrophic assimilation and microbial oxidation, nitrogen transformation emerged as the next dominant regulatory process within the system.

Under ammonium-rich conditions, *Desmodesmus* sp. preferentially assimilates reduced nitrogen forms due to their lower energetic demand relative to nitrate reduction. This metabolic preference explains the rapid decline in ammoniacal nitrogen following acclimatization and aligns with reports of enhanced ammonium uptake in *Desmodesmus* cultivated in high-strength wastewaters(Wan et al., 2018)(Olofsson et al., 2019).

However, biological uptake alone does not fully account for nitrogen removal in unbuffered algal systems. The increase in culture pH to 9.02 created favorable conditions for ammonia stripping, whereby ammonium ions (NH_4^+) shift to volatile free ammonia (NH_3) and are lost to the atmosphere. Experimental and thermodynamic studies indicate that at pH values between 9.0 and 9.5, approximately 10–30% of total ammoniacal nitrogen may be removed via volatilization (Ahmed et al., 2017b);(Liu et al., 2023)). While this abiotic loss does not contribute to biomass nitrogen recovery, it likely reduced free-ammonia toxicity and supported sustained algal growth (López-Rosales et al., 2022).

Phosphorus removal reached approximately 65–66%, consistent with values reported for algal systems operating under alkaline conditions. Phosphorus uptake occurs through assimilation into phospholipids, nucleotides, ATP, and intracellular polyphosphate storage via luxury uptake mechanisms (Martínez et al., 2018). In parallel with nitrogen attenuation, phosphorus dynamics were strongly influenced by the evolving physicochemical environment created by algal metabolism

In addition to biological assimilation, alkaline-induced precipitation contributed significantly to phosphorus removal. Photosynthetic CO_2 consumption increased culture pH, promoting the formation of insoluble phosphate minerals. In compost leachate matrices containing calcium and magnesium, phosphate precipitation may occur as calcium phosphate, magnesium phosphate, or struvite ($MgNH_4PO_4 \cdot 6H_2O$) (Shaikh et al., 2024)(Gutiérrez et al., 2015). Previous studies indicate that at pH values above 8.5, physicochemical precipitation can account for 40–60% of total phosphorus removal when Ca^{2+} and Mg^{2+} are sufficient (Tirok & Scharler, 2014). Accordingly, phosphorus reduction in this study resulted from combined metabolic assimilation and mineral precipitation rather than biomass growth alone.

Nitrogen availability is a primary regulator of carbon partitioning in microalgae, governing the balance between biomass formation and lipid accumulation. Under nitrogen-replete conditions, assimilated carbon is preferentially directed toward protein synthesis, chlorophyll production, and

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active cell division(Fakhry & El Maghraby, 2015;Wang et al., 2019). When nitrogen becomes limiting, however, the synthesis of nitrogen-containing macromolecules is constrained, forcing excess fixed carbon to be redirected toward neutral lipid storage, predominantly as triacylglycerols (TAGs)(Adams et al., 2013;López-Rosales et al., 2022).

This regulatory shift was clearly reflected in the two-stage lipid production pattern observed in the present study. During the exponential growth phase, lipid concentration remained relatively low (0.320 g L^{-1} at Day 18), coinciding with sufficient nitrogen availability to sustain rapid biomass accumulation. In contrast, lipid content more than doubled during the stationary phase (0.715 g L^{-1} at Day 22), temporally aligned with near-complete nitrogen depletion from the compost leachate. This decoupling of biomass growth and lipid synthesis provides strong empirical support for the nitrogen starvation hypothesis, wherein lipid biosynthesis functions primarily as a survival-oriented carbon storage strategy rather than a growth-linked process (López-Rosales et al., 2022).

At the mechanistic level, nitrogen starvation suppresses protein and nucleic acid synthesis, redirecting photosynthetically fixed carbon flux toward fatty acid and TAG biosynthesis. Previous studies have shown that under nitrogen-limited conditions, *Scenedesmus/Desmodesmus* species can channel more than 90% of newly synthesized lipids into neutral lipid pools, forming dense intracellular lipid droplets that enhance survival during prolonged nutrient scarcity (López-Rosales et al., 2022). Accordingly, the stationary phase observed in this system represents a critical "starvation window," during which metabolic flux was actively diverted toward lipid storage rather than cellular proliferation(Yang et al., 2014;Sulochana & Arumugam, 2020).

The substantially higher lipid yield achieved in compost leachate cultures relative to the BBM control further highlights the importance of carbon availability and the C:N ratio in regulating lipid biosynthesis. Wastewater-grown cultures reached a final lipid concentration of 0.715 g L^{-1}, more than twice that of the BBM control (0.352 g L^{-1}), despite comparable cultivation durations. This enhancement is directly attributable to the presence of biodegradable organic carbon in the matured compost leachate, which supported mixotrophic metabolism (Yun et al., 2021).

Under mixotrophic conditions, microalgae simultaneously utilize inorganic CO_2 and organic carbon substrates, reducing reliance on light-driven carbon fixation while increasing intracellular carbon availability. The high chemical oxygen demand (COD) of the compost leachate therefore created a high C:N ratio environment, which is widely recognized as a key driver of lipid accumulation. Once nitrogen becomes limiting, excess carbon cannot be incorporated into biomass and is instead diverted toward fatty acid and TAG synthesis (López-Rosales et al., 2022).

Consistent experimental evidence demonstrates that organic carbon supplementation significantly enhances lipid productivity compared to purely inorganic media. Carbon-supplemented systems consistently exhibit higher TAG accumulation than nitrogen-rich systems, confirming that lipid induction is not driven by nitrogen limitation alone but is strongly modulated by carbon availability (Yun et al., 2021). In the present study, the biodegradable organic fraction of the matured compost leachate effectively functioned as a heterotrophic substrate, sustaining carbon flux into lipid biosynthesis beyond levels achievable in the BBM control.

Beyond nutrient ratios, the complex chemical matrix of compost leachate imposed multifactorial environmental stress, including elevated ammonium concentrations, residual organic acids, trace metals, salinity, and pH fluctuations. Such stressors are well documented to intensify lipid induction responses in microalgae by activating protective metabolic pathways (Shaari et al., 2021; Alkhamis et al., 2022). Under stress conditions, microalgal cells downregulate protein synthesis and redirect metabolic energy toward fatty acid and TAG formation. Lipid droplets function as inert carbon sinks that reduce intracellular redox pressure, prevent over-reduction of the photosynthetic electron transport chain, and protect cellular components from oxidative damage. This stress-induced metabolic reprogramming explains why wastewater-grown *Desmodesmus* sp. accumulated substantially more lipids than cultures grown in nutrient-balanced BBM, where minimal physiological stress favored continued growth over carbon storage (Shi et al., 2020; Zienkiewicz et al., 2016; Wu et al., 2012).

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The use of matured compost leachate was particularly advantageous in this regard. Compared with fresh leachate, matured leachate exhibits reduced acute toxicity while retaining sufficient nutrient and organic carbon levels to support initial biomass formation. This enabled an optimal cultivation trajectory consisting of an early growth phase supported by available nutrients, followed by rapid nutrient depletion that triggered stress-induced lipid accumulation. Such a naturally phased cultivation strategy mimics two-stage lipid production intrinsically, eliminating the need for artificial stress induction techniques and thereby improving process simplicity and economic feasibility (Apandi et al., 2020; Bomer & Leverett, 2024; Sriram & Seenivasan, 2015).

Qualitatively, this environmental stress induced a pronounced metabolic shift toward saturated fatty acids (SFAs), which accounted for 75.6% of total fatty acids and were dominated by palmitic acid (C16:0) (Vimali et al., 2022). Although this profile contrasts with the PUFA-rich compositions typically reported for algae cultivated in clean, nutrient-replete media, extensive literature confirms that such a shift represents a predictable stress-induced metabolic adaptation. Nutrient limitation and chemical stress inherent to compost leachate environments are well known to trigger lipid reprogramming, where biosynthesis shifts away from membrane-bound polar lipids toward neutral storage triacylglycerols (TAGs) (Arguelles et al., 2019).

This mechanism is further reinforced by the "membrane hardening" hypothesis, whereby microalgae reduce fatty acid unsaturation to decrease membrane fluidity and permeability under hostile environmental conditions. Increased SFA content acts as a defensive mechanism against osmotic and chemical stress by limiting the diffusion of toxic ions into the cell (López-Rosales et al., 2022). Comparable shifts toward SFA-dominated lipid profiles have been reported under salinity and wastewater stress, confirming that the dominance of C16:0 observed in this study reflects a physiologically rational adaptation rather than aberrant metabolism (De Bhowmick et al., 2025; Chen et al., 2022).

From a fuel chemistry perspective, this reduction in PUFA content represents a substantial technical advantage. Polyunsaturated fatty acids are highly susceptible to oxidative degradation, leading to gum formation and reduced fuel stability. Consequently, the high oxidative stability (>30 h) predicted for the biodiesel derived in this study is directly attributable to its elevated SFA fraction and low iodine value, effectively enhancing long-term storage stability without the need for antioxidant additives (Huang et al., 2022; Amran et al., 2022).

The elevated saturation level also translated into superior ignition behavior, as reflected by the high cetane number of 64.5. This value exceeds those reported for most conventional vegetable oil biodiesels, including rapeseed, soybean, and palm oil (Inambao, 2020). Furthermore, compared with biodiesel produced via direct chemical transesterification of landfill-derived oils (Cetane Index ~55.4), the algal biodiesel produced here exhibited markedly superior ignition quality, underscoring the value of microalgal bioconversion for leachate valorization (Mekonnen et al., 2024).

Regarding flow properties, the predicted kinematic viscosity was approximately 3.42 $mm^2 s^{-1}$. This value falls squarely within the ASTM D6751 limits (1.9–6.0 $mm^2 s^{-1}$), driven by the balancing effect of medium-chain fatty acids (Lauric C12:0) offsetting the heavier Palmitic acid fractions. This indicates that the fuel possesses adequate lubricity for injection systems without requiring viscosity modifiers. While the high saturation level inherently limits cold-flow performance, this leachate-derived biodiesel is optimally positioned for applications in warm climates or stationary energy systems—such as agricultural machinery and backup generators—where oxidative stability is paramount (Calijuri et al., 2025; Velásquez-Orta et al., 2024).

From a sustainability perspective, the ability of *Desmodesmus* sp. to grow directly in 100% matured compost leachate aligns strongly with circular bioeconomy principles. Nutrient recycling, carbon diversion from mineralization, and the elimination of freshwater dilution reduce environmental impact and improve resource efficiency. Future research should focus on optimizing reactor operation, microbial community dynamics, and downstream biomass valorization to further enhance productivity and techno-environmental benefits.

5. Conclusions

This study provides compelling evidence that matured compost leachate can be directly valorized as both a cultivation medium and nutrient source for microalgal biodiesel production using *Desmodesmus* sp. Sustained growth in 100% undiluted leachate following a short acclimatization phase demonstrates the robustness of the strain and confirms that dilution, sterilization, or synthetic nutrient supplementation are not prerequisites for stable operation. This represents a significant advancement for wastewater-integrated algal bioprocesses, where high-strength effluents typically impose severe biological constraints.

Stable mixotrophic metabolism enabled simultaneous biomass generation and efficient wastewater remediation, achieving substantial reductions in COD (82.6%), total nitrogen, and total phosphorus. Organic carbon assimilation, algal–bacterial synergy, and physicochemical nutrient removal mechanisms collectively governed treatment performance, underscoring the functional coupling between biological resilience and pollutant attenuation. The naturally evolving cultivation trajectory—comprising acclimation, rapid biomass accumulation, and nutrient-depletion-driven stress—effectively mimicked a two-stage production strategy without external process manipulation.

Nitrogen depletion emerged as the principal trigger for metabolic reprogramming toward lipid accumulation, resulting in significantly enhanced lipid yields relative to the inorganic control medium. The presence of biodegradable organic carbon and a high C:N ratio further intensified lipid biosynthesis under mixotrophic conditions. These findings confirm that lipid induction in wastewater-grown microalgae is governed by the combined effects of nutrient limitation, carbon availability, and environmental stress rather than nitrogen starvation alone.

Fatty acid profiling revealed a pronounced shift toward saturated and monounsaturated fatty acids, yielding biodiesel with high cetane number (64.5), low iodine value, and excellent oxidative stability. The predicted kinematic viscosity (~3.42 $mm^2\ s^{-1}$) falls well within international biodiesel standards (ASTM D6751), driven by the balancing effect of medium-chain fatty acids. Consequently, the resulting fuel possesses excellent ignition quality and storage stability, making it particularly advantageous for applications in warm climates and stationary energy systems.

Finally, this work establishes matured compost leachate as a low-cost, nutrient-rich feedstock capable of supporting high-performance microalgal cultivation while delivering tangible wastewater treatment benefits. By converting a problematic waste stream into biomass and energy carriers, the proposed system aligns strongly with circular bioeconomy principles and sustainable resource management. Future studies should focus on pilot-scale validation, reactor configuration optimization, microbial community control, and comprehensive life cycle and techno-economic assessments to accelerate translation toward industrial implementation.

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