Malaysian Journal of Microscopy 2025 21(2) 205-221

MALAYSIAN JOURNAL OF MICROSCOPY

Journal homepage: https://malaysianjournalofmicroscopy.org

RESEARCH ARTICLE

EXPLORING THE EFFECT OF DIFFERENT DILUENTS AND DILUTION RATIOS ON MICROALGAE GROWTH IN LANDFILL LEACHATE

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Abstract. The escalating discharge of landfill leachate presents significant environmental challenges, posing serious risks to ecosystems and public health. Leachate is characterised by its complex composition, high toxicity, and significant pollutant load, making its treatment and disposal a persistent issue for waste management systems worldwide. However, its nutrient-rich nature, including high concentrations of nitrogen, phosphorus, and trace elements, suggests it could be repurposed as a resource rather than merely treated as waste. This study explores the use of landfill leachate to culture *Chlorella sorokiniana*, focusing on its ability to support microalgae growth and enhance biomass productivity. Specifically, the study examines *Chlorella sorokiniana* growth in media derived from three different leachate treatment ponds (raw pond, Sequencing Batch Reactor Pond, and Dissolved Air Flotation Pond) and also investigates the growth of leachate in various diluents, including tap water, lake water, grey water, and rainwater. The best diluent is selected based on its characteristics and ability to support microalgae growth. It was subsequently used to dilute the leachate at concentrations of 25%, 50%, 75%, and 100% (v/v). Findings revealed that leachate from the raw pond provides the most suitable conditions for microalgae growth due to its balanced organic content, moderate suspended solids, low colour and suitable pH. Microalgae cultivated in this medium outperformed the other two ponds, achieving the highest specific growth rate (0.52 /day), cell division rate (0.75 divisions/day), and biomass productivity (14.17 mg/L/day). Analysis of diluents showed that tap water was the most favourable medium for microalgae cultivation, and when used to dilute leachate to 25%, it provided optimal growth by minimising inhibitory effects. These findings underscore the critical role of leachate composition, diluent selection, and dilution in optimising microalgae-based treatment technologies.

Keywords: Landfill leachate, *Chlorella sorokiniana*, diluent medium, biomass productivity, wastewater treatment.

Article Info

Received 26 February 2025
Accepted 23 May 2025
Published 4 December 2025

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ISSN: 1823-7010, eISSN: 2600-7444

1. INTRODUCTION

Rapid urbanization and population growth have significantly contributed to the escalation of waste generation, leading to severe environmental and health challenges. Landfills, a common method for disposing of municipal and industrial solid waste, accommodate approximately 95% of global solid waste [1]. Within these landfills, waste undergoes complex physical, chemical, and biological transformations influenced by factors such as organic matter decomposition, rainwater infiltration, and waste moisture content. These processes produce leachate, typically characterised as a highly contaminated liquid containing a diverse mix of organic matter, inorganic ions, heavy metals, and other pollutants [2].

The challenges posed by leachate management, given its complex and pollutant-rich composition, have spurred interest in innovative treatment and utilisation strategies. Rather than viewing leachate solely as a waste product, its nutrient-rich profile containing nitrogen, phosphorus, and trace elements presents an opportunity for sustainable applications. In this context, the use of leachate for microalgae cultivation offers a promising alternative [3]. Microalgae, particularly species like *C. sorokiniana*, have demonstrated the ability to thrive in wastewater environments, utilising these nutrients for growth while simultaneously producing valuable biomass. The biomass produced from microalgae holds significant potential as feedstock for biofuel production, animal feed, biofertilisers, and other high-value bioproducts. This could present an innovative solution to both waste management challenges and the demand for renewable resources [4].

However, the efficiency of microalgae growth is highly sensitive to the leachate's environmental conditions, particularly pollutant concentrations, nutrient balance, and light availability. Leachate from landfills contains a complex mixture of organic and inorganic compounds, heavy metals, and other contaminants that can inhibit the growth of microalgae. High concentrations of pollutants, such as ammonia, heavy metals, and salts, can be toxic, leading to decreased growth rates, altered metabolic pathways, and even cell death [5]. Additionally, excessive organic matter may create an oxygen-deficient environment, further stress the microalgae and limit their growth potential [6]. As a result, developing efficient dilution strategies is essential to mitigate these negative effects and create favourable conditions for microalgae to thrive [7].

Diluting leachate with suitable water sources reduces the concentration of harmful substances, but the dilution ratio must be optimised to balance contaminant reduction with maintaining adequate nutrient levels for growth. While previous studies have used synthetic media or distilled water for dilution, these approaches raise concerns about economic feasibility and scalability for industrial applications [8]. To address this limitation, the present study evaluates the suitability of alternative diluents, specifically tap water, grey water, rainwater, and lake water, for leachate dilution and examines various concentrations (25%, 50%, 75%, and 100% v/v) to determine optimal conditions as a growth medium for *C. sorokiniana* growth. This investigation not only seeks to enhance the growth of *C. sorokiniana* but also aims to provide sustainable and cost-effective solutions for integrating microalgae cultivation into waste management practices.

2. MATERIALS AND METHODS

2.1 Landfill Leachate and Diluent Water Collection

Landfill leachate samples were obtained from the Jeram Sanitary Landfill (3°11'20'' N,101°21'50″ E), located in Selangor, Malaysia. The effluent was collected from three ponds, namely the Raw Pond, Sequencing Batch Treatment (SBR) pond and Dissolved Air Flotation (DAF) pond as shown in Figure 1. The SBR process is an activated sludge process designed to operate in a true batch mode. There are usually five cycles to complete a true batch: filling, aeration, anoxic, settling and separation [9]. The DAF process, on the other hand, involves separating suspended solids (SS) from fluids by transferring the particles to the surface of the fluid [10]. The samples from the three ponds were collected independently in the plastic container. The collected samples were stored in a 4 °C cold room to maintain their chemical characteristics [11].

Meanwhile, the diluents used in the study were tap water, grey water, rainwater and lake water. Tap water was sourced directly from a municipal supply line, while the greywater was collected from residential wastewater sources, comprising wastewater from sinks, showers, and laundry activities. Rainwater was collected directly during rainfall using clean, wide-mouthed containers placed in an open area to avoid contamination from nearby structures or vegetation and the lake water was collected from Seksyen 7 Lake, located in Shah Alam, Selangor, Malaysia (3.0670° N, 101.4933° E). It is a man-made recreational lake located in a residential area. Surrounded by landscaped parks, walking trails, and community facilities.

(a) Raw pond (b) SBR pond

(c) DAF pond

Figure 1: The leachate treatment plant at Jeram Landfill consisting of (a) raw leachate collection pond, (b) SBR pond and (c) DAF pond

2.2 Landfill Leachate and Diluent Characteristics

The physicochemical parameters of the water samples were analysed using standardised methods. Chemical oxygen demand (COD), Total Nitrogen (TN) and Total Phosphorous (TP) measurement were carried out following HACH DR 2800 spectrophotometer manual. Biochemical Oxygen Demand (BOD) was determined by the 5-day BOD test [12]. The colour analysis was carried out by following platinum-cobalt standard method 8025 using HACH DR 2800 spectrophotometer. The TSS level in the water samples was determined using APHA 2540-D gravimetric method. Finally, the pH and turbidity were measured using HACH pH meter (HQ40D) and HACH 2100 AN portable turbidimeter respectively.

2.3 Microalgae Preparation

The microalgae isolate used in this study is *Chlorella sorokiniana* UKM3 (*C. Sorokiniana*) with the NCBI accession number KP262477. The isolate was obtained from Palm oil mill effluent (POME) sources, collected from Sime Darby Palm Oil East Mill (2°53’85’’N,101°26’10.8’’E). The culture was maintained at room temperature (25 °C) and exposed to 7000 lx intensity for 24 hours. Each flask was aerated for 24 hours using an aquarium air pump [13]. The cultures were maintained for 14 days, and samples were collected every 48 hours to measure microalgae growth rate performance. The samples were analyzed using a TESCAN VEGA3 SEM-EDX operated at an accelerating voltage of 15 kV, as shown in Figure 2. Prior to imaging, the samples were sputter-coated with an ultra-thin 5 nm layer of gold (Au).

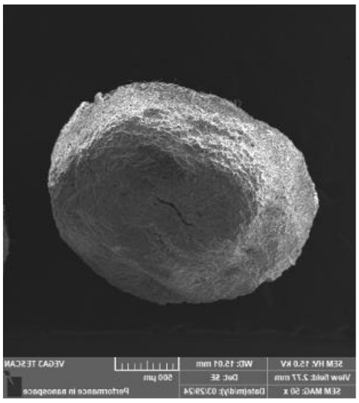

Figure 2: SEM image of *C. Sorokiniana*

2.4 Experimental Setup

The initial stage of the study involved the characterization of wastewater samples from each pond and the diluents. The microalgae were then cultivated in leachate from the three different ponds, as shown in Figure 3(a), using a 20% (v/v) inoculum, as illustrated in Figure 3(b), in 1 L of culture medium [14]. Based on the results, the pond that produced the best microalgae growth was selected for further analysis.

(a) (b)

Figure 3: (a) The leachate samples from raw pond, Sequencing Batch Reactor Pond (SBR) and Dissolved Air Flotation Pond (DAF) and (b) Microalgae inoculum

Subsequently, the characteristics of four different diluents were analysed to identify the most suitable option for enhancing leachate conditions and supporting rapid microalgae growth. The evaluation focused on nutrient concentrations, pH, and SS as these factors directly influence microalgae growth rates. The growth patterns of microalgae in each diluent were also established to support the characterization data and determine which provided the best growth kinetics.

In the final stage, the study investigated the optimum dilution conditions by testing the selected diluent with leachate concentrations of 25%, 50%, 75%, and 100% (v/v) The microalgae were cultured in 1 L of culture medium using 20% inoculum size[15], and their performance was assessed based on growth parameters and biomass yield, which served as key indicators of cultivation success.

2.5 Microalgae Growth Measurement

The procedure to determine the microalgae growth rate was based on the biomass dry weight (BDW). About 10 mL of microalgae sample was collected and filtered by using pre-weighted dried blank Whatman GF/C 47 mm glass microfiber filter paper. Then, the microfilter paper was dried at 80 ℃ for 48 hours before being weighed again. Before measuring growth, the microalgae biomass underwent two washes with distilled water to eliminate any SS from the leachate. The BDW was calculated as per Equation (1) [16]:

$$Biomass\ Dry\ Weight, BDW\ in \left(\frac{mg}{L}\right) = \frac{x_f - x_0}{sample\ volume\ (L)} \tag{1}$$

where x_f = final weight of filter paper (mg), while x_0 = initial weight of filter paper (mg).

The specific growth rate (μ) of the microalgae was computed from the logarithmic growth using Equation (2) [17]:

$$Specific\ Growth\ Rate, \mu(day^{-1}) = \frac{Ln\ (x_2 - x_1)}{t_2 - t_1} \tag{2}$$

where x_1 = biomass dry weight in time t_1 (mg), x_2 = biomass dry weight in time t_2 (mg), t_1 = time at x_1 (day), and t_2 = time at x_2 (day).

The cell division time, *D'*, was calculated in Equation (3) [17]:

$$Cell\ division, D'(day^{-1}) = \frac{\mu}{\ln 2} \tag{3}$$

Total biomass and biomass productivity were determined using Equation (4) and Equation (5), respectively [18]:

$$Total\ biomass\ production \left(\frac{mg}{l}\right) = x_2 - x_1 \tag{4}$$

$$Biomass\ productivity \left(\frac{\frac{mg}{L}}{d}\right) = \frac{x_2 - x_1}{t_{x2} - t_{x1}} \tag{5}$$

where x_1 = biomass dry weight at the beginning of the cultivation, x_2 = biomass dry weight at the end of the cultivation, t_{x1} = time at x_1 (day), and t_{x2} = time at x_2 (day).

3. RESULTS AND DISCUSSION

3.2 Characterisation of Leachate from Different Ponds

The present study aims to enhance microalgae growth under various leachate treatment ponds, diluents, and dilution ratios. Initially, the characteristics of different ponds were evaluated to determine their suitability for microalgae cultivation. Key parameters, including pH, turbidity, SS, colour and nutrient content, were measured and are summarised in Table 1.

Table 1: Characterisation of leachate from different ponds

Parameter (mg/L)	Raw pond	Sequencing Batch Reactor (SBR) Pond	Dissolved Air Flotation (DAF) Pond
BOD	324.43	68.01	48.15
COD	2390.37	1990.42	1718.91
TN	1166.21	591.37	285.28
TP	96.83	80.39	34.80
SS	60.11	260.25	180.15
Turbidity	118.24	365.86	340.34
pH	8.52	5.07	4.61
Colour (Pt.co)	3929.45	8360.13	8310.03

The characteristics of leachate from the three ponds reflect the effects of different treatment stages, which are the sequence of raw pond, SBR Pond, and DAF Pond on leachate quality and its suitability for microalgae cultivation. The raw pond contains untreated leachate with a high BOD of 324.43 mg/L, indicating abundant biodegradable organic matter that can serve as a substrate for microalgae. After treatment, BOD values decrease markedly in SBR and DAF at 68.01 mg/L and 48.15 mg/L, respectively, showing the effectiveness of the SBR and DAF processes in reducing easily degradable organic compounds.

COD decreases across the treatment processes, starting at 2390.37 mg/L in the raw pond, reducing to 1990.42 mg/L in the SBR pond, and further to 1718.91 mg/L in the DAF pond. For microalgae cultivation, these COD values have mixed implications. The biodegradable portion of COD provides a carbon source that facilitates microalgae metabolism and promotes biomass growth [19]. However, excessive COD can promote bacterial proliferation, which depletes dissolved oxygen during organic matter degradation. This creates oxygen-depleted conditions that hinder microalgae growth and biomass accumulation [6]. In general, overhigh initial COD concentration (over 2000 mg/L) was shown to suppress the growth rates of microalgae [20]. However, a study by Shaari et. al. [5] reported than *Chlorella* sp. able to survive up to 4500 mg/L COD level albeit lower than the cell density compared to 2500 mg/L COD concentration.

TN and TP levels are considerably higher in the raw pond compared to the treated ponds. In the raw pond, TN is measured at 1166.21 mg/L and TP at 96.83 mg/L. In the SBR pond, TN decreases by almost 50%, dropping to 591.37 mg/L, and TP decreases to 80.39 mg/L, reflecting a 17% removal. This finding aligns with the study by Anna Tałałaj et. al.[21], which reported reductions of 86.4% and 46.7% in TN from young and old leachate respectively, after SBR treatment. Further decreases are observed in the DAF pond, where TN drops to 285.28 mg/L, marking a total reduction of 75.5% from the raw pond, and TP decreases to 34.80 mg/L, representing a 64.1% total reduction. The reduction in TN and TP concentrations in the SBR and DAF ponds can be attributed to the specific treatment processes. In the SBR system, biological nitrification and denitrification processes occur, which convert ammonia nitrogen (NH_3) into nitrate (NO_3) and ultimately reduce nitrogen levels [22]. TP removal in the SBR is also achieved through biological uptake by microorganisms, particularly during the aeration phase when microbes assimilate phosphorus for growth [23]. The DAF system further reduces nutrient levels by removing SS, organic matter, and some dissolved nutrients through coagulation and flotation. The coagulation process in DAF helps bind pollutants to form larger particles, which are then removed by flotation [24]. These combined biological and physical processes in the SBR and DAF systems effectively lower the concentrations of TN and TP compared to the raw pond.

The raw pond has lower turbidity at 118.24 NTU and SS at 60.11 mg/L, indicating a simpler composition of untreated leachate. In contrast, the SBR and DAF ponds show high turbidity and SS levels with 365.86 NTU and 340.34 NTU for turbidity, and 260.25 mg/L and 180.15 mg/L for SS, respectively. These elevated values can be attributed to the biological and mechanical processes occurring in each treatment stage. In line with this finding, Olisa et. al. [25] reported that the increase in colour during subsequent treatments at Jeram Landfill was attributed to the higher concentration of organic matter generated during the treatment processes. In the SBR pond, for instance, the mixed fill phase involves the blending of influent with biomass, initiating microbial degradation of organic matter. This biological activity often results in the release of colour-causing substances, turning the water yellow, brown, or even black [26,27] In the DAF pond, the increase in turbidity and SS may be attributed to the sampling technique, as the present study used surface samples that likely captured higher concentrations of floating solids, scum, unseparated particles, and microbubbles [28]. Correspondingly, colour intensity is highest in the SBR and DAF ponds, with values exceeding 8300 Platinum Cobalt (Pt.Co.), whereas the raw pond exhibits moderate colouration at 3929 Pt.Co.

The pH varies notably across the ponds, with the raw pond maintaining a slightly alkaline level at 8.50, likely due to the absence of treatment-induced chemical shifts. In contrast, the SBR and DAF ponds exhibit more acidic conditions, with pH values ranging from 5.03 to 4.59. This shift can be explained by treatment processes such as nitrification in the SBR pond, where alkalinity is consumed during the biological conversion of ammonia to nitrate, leading to a drop in pH when insufficient buffering capacity is present [29]. A similar trend is observed in the DAF pond, where the introduction of coagulants such as $Al_2(SO_4)_3$ contributes to a slight reduction in pH. As a result, the treated ponds show pH levels converging toward slightly acidic ranges, from 4.8 to 5.7 [30]. This pH parameter plays a critical role in determining the suitability of each pond for microalgae cultivation, as these organisms generally thrive within a pH range of 6 to 8.

Overall, the leachate from the raw pond presents the most nutrient-rich conditions for microalgae cultivation, with moderate turbidity, lowest colour level and a pH suitable for microalgae growth. The treated leachates from the SBR and DAF ponds, while showing reductions in BOD and COD, present challenges such as high turbidity, acidic pH, and intense colour. These parameters, resulting from the biological and chemical treatment processes, may hinder light penetration and impact the photosynthetic efficiency of microalgae. Therefore, the raw pond leachate appears to offer the most balanced and favorable conditions for microalgae cultivation.

3.2 Microalgae Growth in Landfill Leachate from Different Ponds

Figure 4 illustrates the growth profile of the *C. sorokiniana* in raw, SBR and DAF ponds, respectively. Raw pond exhibited a notable lag phase during the initial cultivation period, where dry weights remained low as the microalgae adapted to their new environment. Starting from day 4, microalgae grown in landfill leachate from raw pond began to enter the exponential phase, demonstrating a rapid increase in biomass accumulation. In contrast, microalgae growth in the SBR treated leachate remained consistently low throughout the cultivation period, with dry weight values fluctuating slightly but showing no clear upward trend. This suggests that the microalgae experienced either prolonged adaptation or growth inhibition under these conditions, with no evident transition into the exponential phase. Meanwhile, microalgae growth in landfill leachate taken from the DAF pond showed a declining pattern over time. The absence of any growth trend and the declining biomass indicate that the conditions in the DAF treated sample may have been unfavorable for microalgae production.

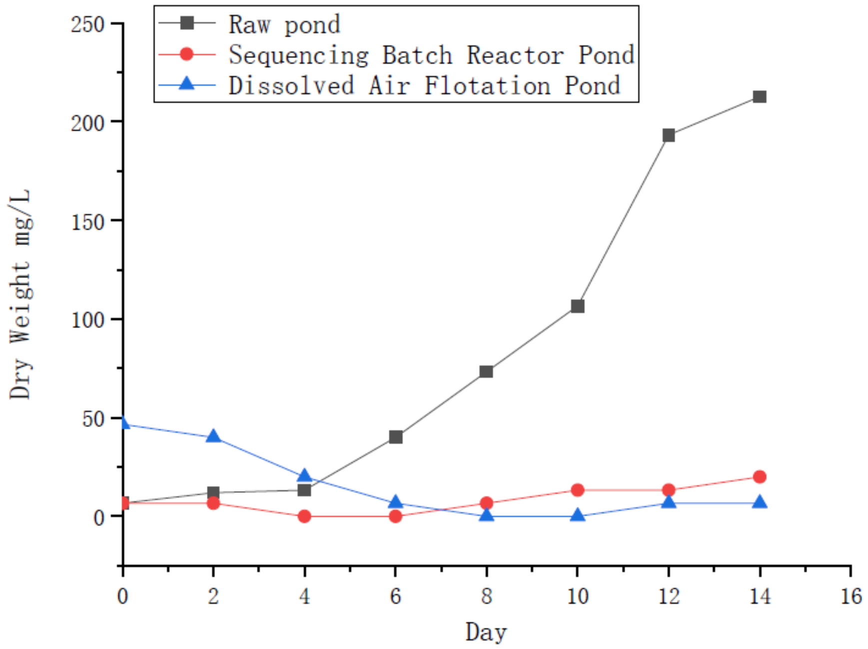

Figure 4: Growth profile of *C. Sorokiniana* in different leachate ponds

Based on the results, it can be suggested that although the raw pond leachate had high COD and TN levels, these concentrations remained within the tolerable range for *C. Sorokiniana*. This indicates that nutrients alone were not the limiting factor for its growth. Instead, the primary constraints on growth in the SBR and DAF pond appear to be physicochemical parameters, particularly pH, SS, and turbidity. The growth performance was further evaluated based on key parameters summarised in Table 2, including specific growth rate (μ), cell division rate, biomass productivity, and total biomass productivity. These parameters provided a comprehensive evaluation of how the different leachates influenced microalgae growth, reproduction, and biomass accumulation. The specific growth rate, μ, is a critical indicator of how efficiently microalgae grow and proliferate over time.

Table 2: Growth parameters for microalgae in different leachate treatment ponds

Growth Parameters	Raw pond	Sequencing Batch Reactor (SBR) Pond	Dissolved Air Flotation (DAF) Pond
Specific growth rate, μ	0.52	0.15	N/A
Cell division (/day)	0.75	0.22	N/A
Biomass productivity (mg/L/day)	14.17	0.95	- 2.85
Total biomass production (mg/L)	206.21	13.33	- 40.00

The microalgae in the raw pond demonstrated the best growth performance, with a μ of 0.52/day, remarkably higher than that of the other two ponds. In contrast, the SBR pond exhibited a lower growth rate at 0.15/day, and the DAF pond was unable to support any microalgae growth. This difference in growth rates correlated with the composition of the leachates in these ponds. Raw pond leachate provided an abundant nutrient and had a lower concentration of turbidity and pollutants, thus facilitating faster microalgal growth. In addition, as shown in Table 1, the pH of approximately 8.52 in the raw pond appears to be favorable for microalgae cultivation. This finding corresponds with a study by Yadav et. al. [31], which revealed that a slightly alkaline pH (7.5–8.5) was the preferred pH range for the growth of *Chlorella* sp. In another study, Huang et. al. [32] found that *C. sorokiniana* achieved its highest biomass concentration in a medium with a pH of 8.5. In contrast, the acidic conditions of 5.07 and 4.61 in the SBR and DAF, respectively, created an environment where microalgae were unable to grow. The cell division rate reflects the ability of microalgae to reproduce, which is the frequency of cell division within a given time period. Raw pond exhibited the highest cell division rate, with 0.75 divisions per day, markedly higher than SBR Pond at 0.22 divisions/day, while DAF pond was unable to undergo notable cell division. This trend is consistent with the growth rate analysis, which indicates a higher growth rate with faster cell division.

It was also observed that the leachate from the raw pond provided the most optimum conditions for *C. sorokiniana* growth, as demonstrated by the substantial increase in biomass over time. Biomass productivity reflects the rate at which microalgae accumulate biomass. In this experiment, the raw pond demonstrated the highest biomass productivity at 14.17 mg/L/day, substantially outperforming the SBR pond, which recorded only 0.95 mg/L/day. This disparity can be attributed to variations in light availability across the different ponds. Specifically, the leachate from the SBR and DAF ponds exhibited elevated levels of colour and SS, that likely impeded light penetration which a critical factor for photosynthetic activity. Since photosynthesis is the primary mechanism by which microalgae convert light energy into chemical energy to build biomass, any reduction in light availability directly limits biomass accumulation [33]. The negative effect of turbidity on microalgae growth was statistically significant ($p < 0.05$), as indicated by the results from experiments conducted at 300 NTU by Greses et. al. [34]. In their experiments, chlorophyll content was lower compared to those conducted at 50 and 175 NTU. These parameters are crucial for microalgae cultivation, as elevated turbidity, SS, and colour intensity can hinder light penetration through the medium, directly impacting the photosynthetic efficiency and growth of microalgae. The DAF Pond exhibited negative productivity (-2.85%), where the biomass concentration decreased from 46.67 mg/L at the initial culture stage to 6.67 mg/L at the end of cultivation period.

Total biomass productivity reflects the total biomass accumulated by microalgae over the entire experimental period. The results showed that the raw pond had the highest total biomass productivity, reaching 206.21 mg/L, indicating that the leachate from this pond provided the most favorable conditions, leading to rapid growth and considerable biomass accumulation. In contrast, the SBR pond had a total biomass productivity of only 13.33 mg/L. These results highlight the importance of leachate composition in determining the effectiveness of microalgae cultivation and suggest that leachates from different sources can vary greatly in their ability to support microalgae growth.

3.3 Characteristics of Diluents and Their Impact on Microalgae Growth

Table 3 illustrates the characteristics of the four diluents, namely tap water, grey water, rainwater, and lake water, which might reveal their varying potential to enhance microalgae growth when combined with raw leachate.

From the results, it can be observed that tap water exhibits excellent quality, with low organic content, evidenced by a BOD of only 0.2 mg/L, and a negligible COD of 0 mg/L. It also has low turbidity (0.98 NTU) and nutrient levels, with TN at 6.6 mg/L and TP at 0.02 mg/L. These factors, combined with a slightly acidic pH of 6.54, make tap water ideal for creating a clean, controlled environment conducive to microalgae growth. Tap water is generally clean due to treatment processes that remove impurities and meet safety standards.

In contrast, grey water has a high organic content, with a BOD of 6.87 mg/L and a COD of 120 mg/L. It also contains notable levels of TN (13.7 mg/L) and TP (39.3 mg/L). Furthermore, its elevated turbidity (89.47 NTU) and strong colour intensity (1276.67 Pt.co) suggest a complex composition that may impact its suitability for microalgae growth, particularly in terms of light penetration.

The high variability in grey water composition is due to its origin in household activities, including washing, cooking, and cleaning, which contribute organic matter, detergents, and suspended particles.

Table 3: Characteristics of diluents for the dilution of raw leachate

Parameter (mg/L)	Tap water	Grey water	Rainwater	Lake
BOD	0.21	6.87	2.71	3.01
COD	0.00	120.21	7.22	11.09
TN	6.61	13.71	6.30	15.50
TP	0.02	39.34	1.59	1.03
SS	6.67	131.11	6.67	20.00
Turbidity	0.98	89.47	4.40	27.97
pH	6.54	6.62	7.99	6.78
Colour (Pt.co)	13.3	1276.67	56.33	161.33

Rainwater, on the other hand, has a slightly alkaline pH of 7.99, low turbidity (4.4 NTU), and light colour intensity (56.33 Pt.co), demonstrating excellent transparency. The nutrient levels, with TN at 6.3 mg/L and TP at 1.59 mg/L, strike a balance that supports photosynthesis and nutrient uptake. The clean quality of rainwater is attributed to its direct collection from open spaces, which minimises contamination during sample collection.

Meanwhile, lake water shows higher nutrient concentrations, with TN reaching 15.5 mg/L and TP at 1.03 mg/L, which could support growth. However, its higher turbidity (27.97 NTU) and darker colour (161.33 Pt.co) may hinder light penetration, reducing photosynthetic efficiency. These differences arise because lake water typically accumulates runoff from surrounding land, introducing organic matter, sediments, and nutrients, contributing to its higher turbidity and colour.

Figure 5 illustrates the growth pattern of microalgae in the respective diluents, as indicated by their dry weight measurements.

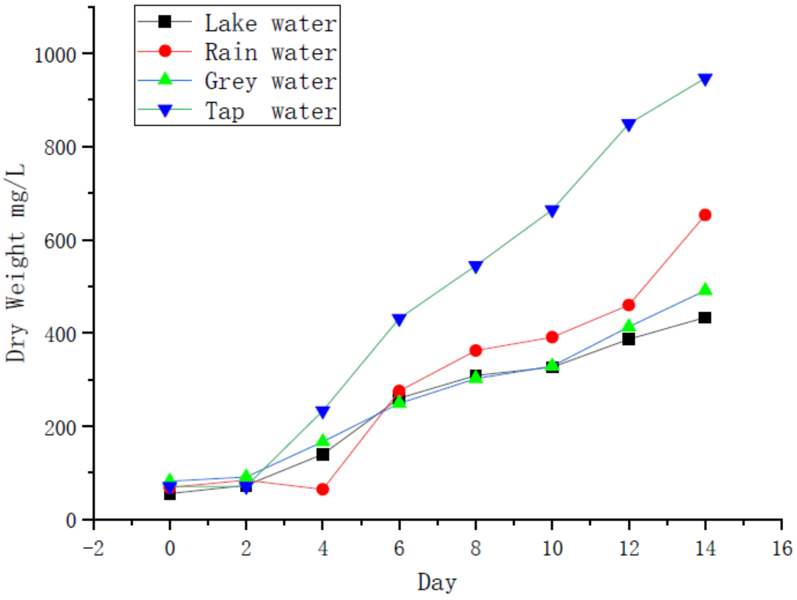

Figure 5: Microalgae growth in different mediums

Based on the observed data, tap water supported the most substantial growth, with a consistent and rapid increase after day 4, culminating in the highest biomass of approximately 1000 mg/L by day 14. This result aligns with tap water's characteristics, including low turbidity (0.98 NTU) and low colour intensity (73.3 Pt.co), which promote optimal light penetration, along with a neutral pH (6.54) and balanced nutrient levels that support growth. Rainwater also demonstrated favorable growth, surpassing grey and lake water. Its slightly alkaline pH (7.99), low turbidity, and sufficient nutrient content provided a conducive environment for microalgae proliferation, resulting in biomass levels nearing 700 mg/L by day 14.

Grey water, despite its high nutrient content, exhibited slower growth. This limitation was likely due to its high turbidity (89.47 NTU) and high organic content, which may have inhibited light penetration and introduced competition. Consequently, its dry weight reached approximately 500 mg/L by the end of the period. Likewise, lake water, with its high turbidity (27.97 NTU) and darker colour (161.33 Pt.co), likely experienced reduced photosynthetic efficiency despite its higher nitrogen levels. It exhibited the lowest microalgae growth, achieving around 400 mg/L by day 14.

Based on the characterization and growth patterns, it can be concluded that tap water is the most suitable medium for microalgae growth due to its clean quality, low turbidity, and balanced nutrient profile. Grey water, with its high organic content and turbidity, and lake water, with its elevated turbidity, may present challenges for effective growth.

3.4 Microalgae Growth in Diluted Leachate at Different Ratios

Tap water was selected as the diluent to test the growth of microalgae under different mixing ratios of diluent and leachate. Figure 6 illustrates the growth profile of microalgae under different leachate concentrations. The graph shows that the lowest leachate concentration (25%) resulted in faster microalgae growth without a notable lag phase. This trend can be attributed to better light penetration in the culture medium. Additionally, the COD and BOD content might fall into the optimum range for microalgae growth, providing sufficient organic carbon sources and energy.

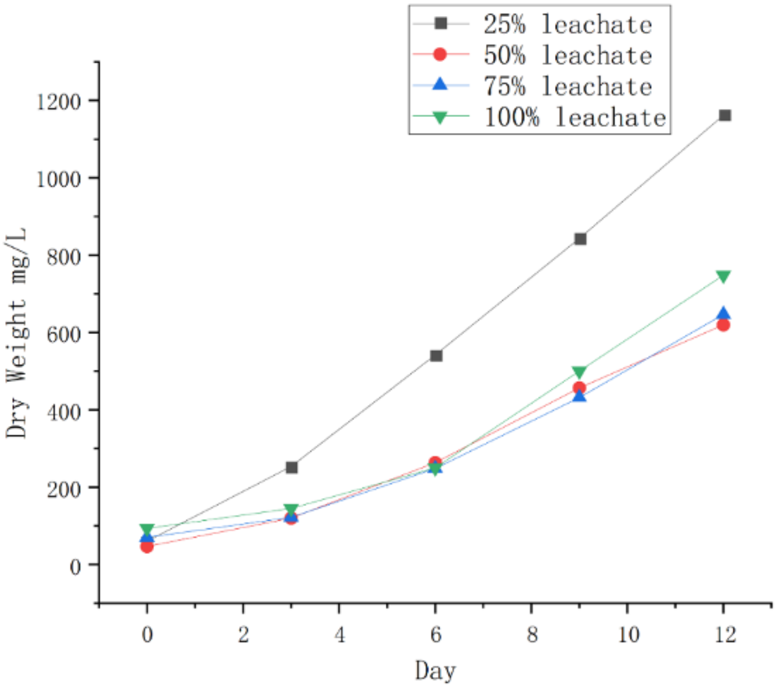

Figure 6: Microalgae growth in different leachate concentrations diluted using tap water

The moderate concentrations of essential nutrients, such as TP and TN, also satisfy the basic requirements for microalgae growth, enabling efficient biosynthesis and growth. Saleem et. al. [35] and Mohan Singh et. al. [36] observed similar effects, where microalgae growth proceeded linearly without a stationary phase when leachate was diluted. As the leachate concentration increased to 50%, the growth trend of microalgae changed, with a slower growth was observed from day 0 to day 3. A comparable pattern was noted at higher concentrations of 75% and 100%. This can be primarily attributed to the excessively high COD and BOD content, which led to rapid oxygen depletion and the formation of an anoxic or anaerobic environment, unfavorable for microalgae growth [37]. Furthermore, high concentrations of essential nutrients, such as TP and TN, may have exerted toxic effects on microalgae, damaging their cell structures and inhibiting metabolic activities. Similar effects on microalgae growth were reported by Hernández-García et. al. [38], who worked with *Scenedesmus* in a high concentration of TN at 1566 mg/L and observed inhibited growth. The salinity and presence of other harmful substances in the high-concentration leachate could also adversely affect microalgae growth. The growth of the microalgae was also monitored using growth parameters, as summarized in Table 4.

Table 4: Growth parameter of microalgae in different leachate ratios diluted with tap water

Growth Parameters	25% leachate	50% leachate	75% leachate	100% leachate
Specific growth rate (/day)	0.76	0.71	0.70	0.69
cell division(/day)	1.10	1.02	1.01	0.995
Biomass productivity (mg/L/day)	91.81	58.33	48.06	54.58
Total Biomass productivity (mg/L)	1101.67	576.67	573.33	655.00

According to the experimental data, the 25% leachate concentration had the highest growth rate, μ, of 0.76/day, while the growth rate decreased progressively with increasing leachate concentration. The sample with 50% leachate concentration exhibited a lower μ of 0.71/day, which further decreased to 0.70/day and 0.69/day for 75% and 100% leachate, respectively. This trend indicates a negative correlation between the microalgae growth rate and leachate concentration. Higher leachate concentrations may inhibit microalgae proliferation by introducing harmful substances such as salts, organic pollutants, or heavy metals. The 25% leachate concentration, with the highest growth rate, suggests that lower leachate concentrations are more conducive to microalgae growth. This observation aligns with previous studies on microalgae cultivation using Jeram landfill leachate. For instance, a study by Nordin et. al. [39] tested various microalgae species, including *Chlorella sp.*, and reported species selected were able to survive up to 20% (v/v) concentration of leachate obtained from the Jeram landfill. Another study was later conducted by Nordin et. al. [40], using different concentrations of Jeram landfill leachate ranging from 10% to 30%. They reported that the highest μ of *Chlorella sp.* cultured in landfill leachate was observed at 15%, with μ = 0.75/day, while at 25%, μ was 0.70/day—almost identical to the present study's result at 25% (μ = 0.76/day). Another study conducted using Jeram landfill leachate was reported by Fuad et. al. [41], in which *Desmodesmus armatus* achieved μ values of 0.423, 0.324, and 0.228 using 5%, 10%, and 15% leachate, respectively. This observation aligns with the findings of Shaari et. al. [5], who reported that *Chlorella* cell density substantially decreased with increasing leachate concentration. In their study, leachate samples collected from a pre-treated effluent pool showed the highest cell density in 5% diluted leachate, followed by 10%, with the lowest observed in 15% dilution. These findings demonstrate optimal growth at lower concentrations, whereas higher concentrations may exert inhibitory effects which is consistent with the present study.

The results showed that the cell division rate for the 25% leachate concentration was 1.10/day, which was slightly higher than the rates observed in the other samples. As the leachate concentration increased, the cell division rate decreased. For 50% and 75% leachate concentrations, the rates were down to 1.02/day and 1.0/day, respectively, indicating a slight inhibition of cell division activity. At 100% leachate concentration, the cell division rate dropped further to 0.995/day, the lowest observed, suggesting that higher leachate concentrations may inhibit cell division. Biomass productivity was highest at the 25% leachate concentration, with a value of 91.81 mg/L/day, noticeably higher than the other concentrations. It has been observed that biomass productivity decreased with 50% and 75% concentrations, producing 58.33 mg/L/day and 48.06 mg/L/day respectively. A contrasting result was reported by de Souza et al. [42], who found that an 80% leachate concentration produced the highest biomass productivity at 421 mg/L/day. However, it is important to note that their study used treated leachate following secondary treatment, which likely reduced the nutrient concentration compared to raw leachate. Interestingly, for the 100% leachate concentration, biomass productivity slightly increased to 54.58 mg/L/day. This outcome, which deviates from the decreasing biomass trend observed for 50% and 75% concentrations, could be explained by residual leachate remaining on the microalgae biomass during dry weight measurement. The higher concentration of 100% leachate could lead to more residual leachate being retained on the biomass, affecting the dry weight measurement. Nonetheless, the overall trend supports the conclusion that higher leachate concentrations negatively impact microalgae growth.

Meanwhile, the total biomass produced by microalgae over the entire culture period was highest at 25% leachate concentration, yielding 1101.67 mg/L, indicating the most robust growth. As the leachate concentration increased, total biomass production decreased, with 50% and 75% concentrations producing 576.67 mg/L and 573.33 mg/L, respectively. In contrast, the 100% leachate concentration showed a slight increase in total biomass to 655.0 mg/L, consistent with the trend observed in biomass productivity. The growth characteristics of microalgae are closely related to the leachate and nutrient concentrations in their environment. At low concentrations, microalgae efficiently utilise the nutrients in the culture medium for growth. However, as the leachate concentration increases, changes in nutrient availability and the emergence of toxic effects begin to impair microalgae growth, resulting in slower growth rates. These findings highlight the importance of optimising leachate concentration to maximise microalgae biomass production while minimising inhibitory effects, offering a sustainable approach to resource recovery and wastewater treatment.

4. CONCLUSIONS

The leachate from the raw pond provides the most suitable conditions for microalgae growth, with optimal nutrient levels, moderate SS, and neutral pH, resulting in the highest specific growth rate (0.52 1/day), cell division rate (0.75 divisions/day), and biomass productivity (14.17 mg/L/day). In contrast, leachates from SBR and DAF ponds, with higher colour and SS level combined with slight acidic condition, show reduced growth rates, cell division rates, and biomass productivity. A characterization study revealed that tap water is suitable for microalgae growth due to its clean quality and available nutrients. The study demonstrates that low concentrations of 25% of leachate diluted using tap water promote the best microalgae growth, while higher concentrations inhibit growth. This study highlights the importance of leachate composition in optimising microalgae cultivation. The findings can be applied to enhance the efficiency of microalgae-based systems for environmental and industrial applications.

Acknowledgements

The authors are grateful to the Faculty of Civil Engineering, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia for all the facilities and financial support in preparation of this manuscript.

Author Contributions

Meng Di performed the experiments, analysed the data, and wrote the manuscript. Azianabiha A Halip Khalid supervised the project and assisted in preparing the final version of the manuscript. Nazlina Haiza Mohd Yasin and Gong Tao Ding contributed to the analysis and interpretation of the results.

Disclosure of Conflict of Interest

The authors have no disclosures to declare

Compliance with Ethical Standards

The work is compliant with ethical standards

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