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Research Article

Sustainable Cultivation of *Desmodesmus armatus* SAG276.4d using Leachate as a Growth Supplement for Simultaneous Biomass Production and CO_2 Fixation

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ABSTRACT. Microalgae cultivation has been identified to be highly beneficial for the production of valuable biomass. The recent worldwide interest is to cultivate microalgae in wastewater to replace the use of expensive commercial media. Microalgae can utilize nutrients from the wastewater for their biomass growth, which is useful as feedstock in many products. Interestingly, microalgae cultivation is also capable of reducing a greenhouse gas due to absorption of carbon dioxide (CO_2) during photosynthesis. This study was conducted to study the growth of microalgae using leachate as a nutrient supplement. The scope of the research involved the cultivation of freshwater microalgae, *Desmodesmus armatus*, in the synthetics medium with various percentages of leachate under different light exposures. The growth parameters such as the specific growth rate, biomass productivity, and cell division time were used to evaluate the microalgae growth performance. The amount of CO_2 absorbed during the cultivation was determined based on the total biomass production. The highest growth rate of 0.423/day was achieved using a 5% leachate medium under 12 h light duration, and the highest carbon fixation of 1.317 g CO_2/L/day was calculated using a culture supplemented with 5% leachate with 24 h light period. The high presence of nutrients in the leachate has contributed to the growth of the microalgae; thus, it has great potential as an alternative growth medium to support biomass production and subsequently help to mitigate global warming.

Keywords: Microalgae; biomass; *Desmodesmus armatus*; leachate; biomass production; CO_2 fixation

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1. Introduction

Microalgae are one of the best photosynthetic microorganisms that can be found in various earth ecosystems. They have been subjected to growing attention around the world, due to their great potential in various applications (Chen *et al*. 2020). Their biomass contained compounds of high biotechnological interest useful for biofuel, vitamins, protein, cosmetic, and health food production (Tsolcha *et al*. 2017). Besides, microalgae-based systems have been theoretically proved to be an environment-friendly approach to overcoming the problem of carbon dioxide (CO_2) emissions (Song *et al*. 2020). Photosynthetic reaction by the microalgae uses light as a source of energy and converts it into chemical energy. Through this, a large amount of CO_2 is consumed, thus reducing the impending threat of global climate change that is mainly attributed to CO_2 release to the atmosphere (Comitre *et al*. 2021).

Microalgae are commonly cultivated in a commercial medium that contains all necessary nutrients needed for microalgae growth. Bold's Basal Medium (BBM) is a freshwater microalgae medium that has been widely used to grow a variety of green microalgae cultures (Wong *et al*. 2017). Preparation of a commercial medium that requires expensive chemicals is one of the major challenges facing large-scale cultivation of microalgae (Leite *et al*. 2019). The application of microalgae in wastewater has been recognized as one of the most feasible ways of sustainable cultivation (Hawrot-Paw *et al*. 2020). Wastewater typically contains nutrients such as carbon (C), phosphorus (P) and nitrogen (N) required for microalgae growth (Naveen *et al*. 2017). Microalgae have the ability to adsorb nutrients that are readily available in the wastewater for their cell generation. Recent studies have exhibited successful application of microalgae cultivation using wastewater from various sources, such as municipal (Sasongko *et al*. 2018), aquaculture (Hawrot-Paw *et al*. 2020), swine (Chen *et al*. 2020), textile (Mubashar *et al*. 2020), brewery (Song *et al*. 2020), dairy (Hemalatha *et al*. 2019) and agricultural (Syafaini *et al*. 2021) wastewater.

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Previous studies have explored the viability of microalgae cultivation using landfill leachate (Chang *et al*. 2018; Khanzada 2020; Quan *et al*. 2020). Leachate is characterized by high concentration of nutrients that could be useful for microalgae cultivation (de Souza *et al*. 2021). However, the microalgae growth could be retarded by its dark colored nature and elevated nutrient load that could reach a toxicity level (Hernández-garcía *et al*. 2019). Hence, leachates were often mixed with distilled water, commercial medium, seawater or other low concentration wastewater (Nair *et al*. 2019). It is also important to provide sufficient lighting to further improve the culture conditions (Nawaz *et al*. 2020). Despite numerous experiments on microalgae cultivation using leachate, some aspects of the process are not well studied. Limited information is available on the combined effect of different leachate percentages and photoperiods towards microalgae growth. Among the most recent studies, only Nair *et al*. (2019) reported on the carbon fixation rate of microalgae cultivated in leachate.

Therefore, the present study aimed to investigate the optimum percentage of leachate in the culture medium to promote high microalgae biomass growth and simultaneously diminish the release of CO_2 into the atmosphere. A mixture of commercial medium and leachate could reduce cultivation costs without significantly impeding light penetration. It can also eliminate the toxicity effect induced by a high concentration of organic and inorganic compounds in the leachate. This study supports the hypothesis that microalgae growth using leachate as a nutrient supplement is possible by avoiding the inhibitory level induced by turbidity and toxicity effect at higher leachate concentrations. For this purpose, freshwater microalgae Desmodesmus sp. were selected based on literature reviews that highlighted their potential for wastewater cultivation and production of valuable biomass (Eze *et al*., 2018; Hernández-García *et al*., 2019; Sarfraz *et al*., 2021). In addition, the cultures were also exposed to different photoperiods to determine the highest biomass production, thus increasing the carbon fixation rate.

2. Materials and Methods

2.1 Wastewater collections

The leachate was obtained from Jeram Sanitary Landfill, Selangor, Malaysia. The collected samples were collected in 20 L labeled containers and immediately stored in a 4°C cold room to maintain its chemical characteristics..

2.2 Microalgae strain and culture conditions

The microalgae species used in this study is Desmodesmus armatus SAG276.4d (hereafter Desmodesmus) that was obtained from culture collection of algae at the University of Göttingen, Germany. The microalgae were cultured in BBM as a control experiment. The preparation for BBM is as described by Ding *et al.* (2016).

The experiments were conducted in the batch mode under controlled temperature of 25°C ± 2°C and exposed to 7,000 lux intensity under 0 h, 12 h and 24 h photoperiods. Three different percentages of leachate were used in BBM, namely 5%, 10% and 15% (v/v). Each flask was aerated for 24 h continuously at 1 L/min using air compressor, as illustrated in Fig. 1.

The inoculum size was maintained at 10% (v/v) for each experiment. The cultures were maintained for a period of 8 days, and a sample was collected daily at approximately 9 am. By sampling at roughly the same time on each occasion, the microalgae growth performance and biomass production can be directly compared over time. Table 1 summarizes the experimental setups.

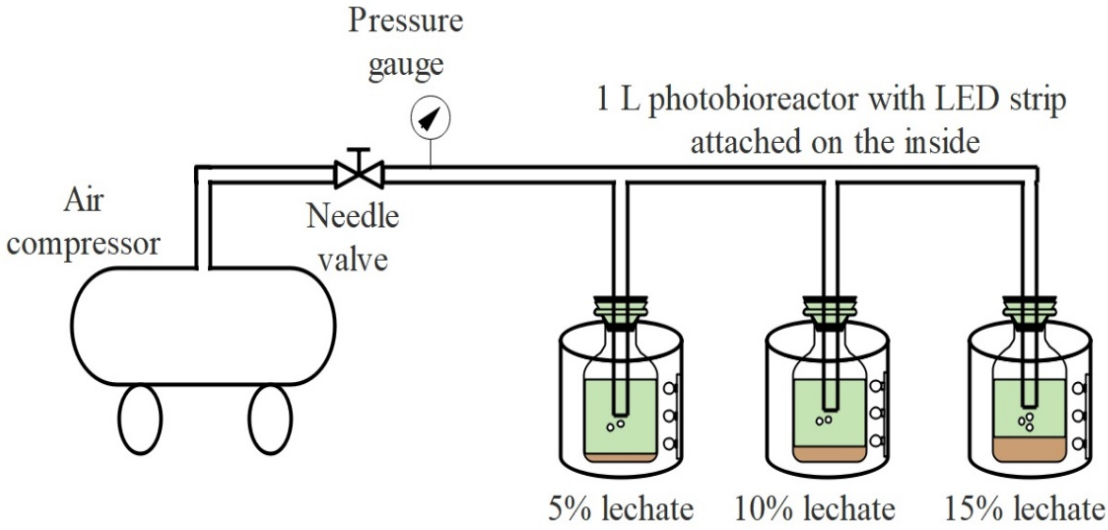

Fig 1 Schematic diagram of microalgae cultivation using leachate as a nutrient supplement

Table 1
Experimental setup using different percentages of leachate

No.	Microalgae (mL)	Medium (mL)	
		BBM (mL)	Leachate
1	100	850	50 mL (5%)
2	100	800	100 mL (10%)
3	100	750	150 mL (15%)

2.3 Nutrient content in leachate

The chemical characteristic of leachate and BBM were examined to compare the concentration of major nutrients. A leachate sample is widely known to have high nutrient concentration; thus, the samples were appropriately diluted prior to the analysis.

2.3.1. Chemical Oxygen Demand (COD)

COD concentration in POME was determined using a Hach COD High-Range Reagent (cat. no.: 212595). 2 mL of a diluted sample was added into the reagent vials with a 45-degree angle and gently inverted twice. Another set of reagent vial was added, with 2 mL of distilled water as a blank sample. Both reagent vials were digested in a pre-heated DRB200 reactor at 150°C for 120 min. After digestion, the vials were inverted while still warm around 120°C and let to cool to room temperature. The COD concentration was measured using the Hach DR 2800 spectrophotometer using 435 COD HR program.

2.3.2. Total Nitrogen (TN)

Determination of TN concentration was done using a Hach TN-High Range Kit (2714100). The content of total Nitrogen Persulfate Reagent Powder Pillow was added to HR Total Nitrogen Hydroxide Digestion Reagent Vials, capped tightly and mixed vigorously for 30 s. 0.5 mL of organic-free water was added to the vial for the blank preparation. The sample preparation was a repeated by adding the 0.5 mL sample. The reagent vials were placed in the DRB200 reactor preheated to 105°C for 30 min. Then, the vials were removed from the reactor and let to cool to room temperature. Next, the content of Total Nitrogen (TN) Reagent Powder A was added to the vial containing the digested blank or sample, capped tightly and mixed for 30 s. The TN Reagents B Powder Pillow was then added into the vials, mixed vigorously for 15 s. 2 mL of digested samples (or reagent blank) were transferred into TN Reagent C vials using a pipet. The solution was mixed by inverting the vials for ten times. After 5 min reaction, the vial was placed to the adapter of the Hach DR 2800 spectrophotometer to obtain the TN reading.

2.3.3. Total Phosphorus (TP)

Determination of Total Phosphorus was carried out with Hach TP Reagent using Molybdovanadate through the Acid Persulfate Method. The analysis started by pre-heating the DRB200 reactor at 150°C. 5 mL of the diluted sample was added to TP test vial. The content of Potassium Persulfate Powder Pillow was added to the reagent vials, capped tightly and mixed vigorously to dissolve all the powder added. The vials were then placed into the reactor for 30 min at 150°C. The digested vials were let to cool to room temperature before 2 mL of 1054 N of the Sodium Hydroxide Standard Solution was added. Next, 0.5 mL of the Molybdovanadate Reagent was added to the vials using a polyethylene dropper and mixed well. The mixture was set to have a reaction time of 7 min before TP values of the vials were measured using the HACH Spectrophotometer DR 2800.

2.3.4. Microalgae growth measurement in BBM and leachate

The growth of microalgae was determined based on the biomass dry weight(BDW). For this purpose, 10 mL of a microalgae sample was harvested and filtered using pre-weighted blank Whatman GF/C, a 47 mm glass microfiber filter paper. The filter paper that contained samples was then dried at 100 ºC for 24 h before being weighed again. Prior to growth measurement, the microalgae biomass was washed twice with distilled water to remove any suspended solid from the leachate. The biomass dry weight was calculated based on Eq. (1):

$$BDW\ (mg/L) = \left(x_f - x_0\right) \Big/ sample\ volume(L), \quad (1)$$

where x_f = final weight of filter paper (mg), and x_0 = initial weight of the filter paper (mg)

2.5 Determination of the growth parameters

From the logarithmic growth, the specific growth rate, μ of the microalgae was calculated using Eq. (2) (Danshvar *et al.* 2018):

$$Specific\ growth\ rate, \mu = \frac{Ln\,(x_2 - x_1)}{t_2 - t_1}, \quad (2)$$

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 using Eq. (3) (Borowitzka and Moheimani 2013):

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

Next, the biomass productivity and the total biomass was calculated using Eq. (4) and Eq. (5), respectively (Koo *et al.* 2017):

$$Biomass\ productivity\ (mg/L/d) = \frac{x_2 - x_1}{t_{x2} - t_{x1}} \quad (4)$$

$$Total\ biomass\ production\ (mg/L) = x_2 - x_1, \quad (5)$$

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).

2.6. Estimation of the CO_2 fixation rate

The amount of CO_2 absorbed during the cultivation period was used to determine the efficiency of CO_2 fixation by the microalgae.

The CO_2 fixation by microalgae was calculated based on the balanced photosynthesis chemical reaction represented by Eq. (6) (Bajunaid *et al.* 2019):

$$4CO_2 + nutrient + H_2O + light \rightarrow 4CO_{0.48}H_{1.83}N_{0.11}P_{0.01} + 3\left(\frac{1}{2}\right)O_2. \quad (6)$$

Using this theoretical formula, the amount of CO_2 assimilated by microalgae can be estimated at a ratio of 1.882 of the total biomass production during the cultivation period, as indicated by Eq. (7):

$$CO_2\ fixation\ rate(g\ CO_2/L/day) = 1.882 \text{ x total biomass production} \quad (7)$$

3. Results and Discussions

3.1 Nutrient content in leachate

Table 2 presents physicochemical characteristics of leachate and BBM used in this study. Notably, the main nutrients required for microalgae biomass production, which are C, N and P, were the main composition in the BBM solution.

Importantly, analysis revealed that these important nutrients were also available in leachate to support optimum microalgae growth. The concentration of TP was found at 225 mg/L, which is more than four-fold the concentration in BBM. A significant higher concentration of nitrogen sources was also found in the leachate with 2,350 mg/L, as compared to 41.2 mg/L in BBM.

The leachate is also considered to be high-strength wastewater with a COD value reaching 13,000 mg/L. It signifies the presence of concentrated biodegradable organic load that could be utilized by microalgae as a carbon source. Although not measured in this study, leachate at Jeram landfill also contains macronutrients such as Cd, Cr, Zn, and Cu as reported by Syazwani *et al.* (2019) and Hussein *et al.* (2020). These trace elements are essential for stimulating optimum growth of the microalgae. By contrast, pH value of 8.52 for leachate was higher than BBM. Although pH value exceeded the neutral pH value, it was still within the suitable range for optimum microalgae growth, which is up to pH 11.5 (Sakarika and Kornaros 2016). Thus, results from the present study indicate the potential of leachate to supply important nutrients for microalgae growth, with their utilization for simultaneous biomass production and CO2 fixation being relatively novel.

Table 2
Chemical characteristics of leachate and the standard growth medium (BBM)

Chemical Characteristic	Leachate (mg/L)	BBM
Total phosphorus, TP	225	53.4
Total nitrogen, TN	2,350	41.2
Biochemical oxygen demand, BOD	2,865	N/A
Chemical oxygen demand, COD	13,000	N/A
pH	8.25	7.0

Table 3
Growth parameters for microalgae growth in BBM under different light durations

Growth parameters (in BBM)	Light duration		
	0 h	12 h	24 h
Growth rate, (μ /day)	−0.268	0.440	0.400
Cell division per day, D'	−0.387	0.635	0.577
Biomass productivity (g/L/day)	−0.003	0.049	0.100
Total biomass production (g/L)	−0.040	0.390	0.800

3.2. Microalgae growth in BBM

The result for microalgae growth in BBM, which served as control experiment in this study, is presented in Table 3. The culture under 0 h light exposure was unable to sustain growth where negative values were observed for all growth parameters. The negative growth rate obtained using the specific growth rate formula indicates the decay of microalgae cell concentration. These negative growth rates were also reported by Huesemann *et al*., (2016), Roleda *et al*. (2013) and Wu *et al*., (2017) in their respective studies on microalgae growth under various culture conditions.

Because they are photosynthetic microorganisms, the growth of microalgae is largely dependent upon the rate of photosynthesis. Photosynthetic efficiency is mostly decided on illumination conditions among other factors such as carbon dioxide concentration, temperature, pH, and nutrients (Singh and Singh 2015; Xing *et al*. 2019). A similar result was reported by Eze *et al*. (2018) where they found that the metabolism for the *Desmodesmus sp*. growth was predominantly photoautotrophic.

The highest growth rate, μ, was found, under the 12 h light period, to be 0.440/day, as compared to 0.400/day from the 24 h light cultivation. This finding indicates that continuous lighting would not be necessarily the best condition for microalgae. A balanced ratio of light and dark period is more important for optimum growth. This result was supported by Barra *et al*. (2014) and Borowitzka and Vonshak (2017), who suggested that dark respiration may be required by the microalgae to relax and repair the damage to the photosystems that naturally occur during high irradiances. Nevertheless, the highest biomass productivity of 0.1 g/L/day was achieved by cultivation using 24 h light. This culture duly recorded the highest total biomass production of 0.8 g/L as the microalgae were able to continuously harvest the light for photosynthesis activity to produce biomass.

Fig. 2 illustrates the growth profile of microalgae for control medium BBM under three different light exposures. The growth profile for microalgae under 0 h light exposure indicates a declining pattern that indicates that microalgae failed to undergo the growth process. It has been reported that some microalgae species such as *Chlorella* sp. are capable of growing in darkness through the heterotrophic mode. Through this metabolism, the microalgae consume external carbon source such as glucose instead of light energy for their growth. Therefore, it can be suggested that *Desmodesmus* sp. is not suitable for the heterotrophic growth mode in a leachate environment, and it totally depends on light availability for its growth.

This result is in contrast with previous studies conducted by Altenhofen *et al*. (2018) and Ogbonna *et al*. (2019) that reported successful growth of Desmodesmus under a heterotrophic condition. This is due to different culture conditions they studied. The first work used sterilized vinasse wastewater under constant stirring at 3.0 × g and aeration at 1 VVM (volume air/ volume medium/min), whereas the Ogbonna *et al*. (2019) used acclimatized inoculum, which improves the tolerance of microalgae in the dark. As for 12 h and 24 h light exposures, the growth profile for microalgae was increased throughout 8 days of cultivation. There was no stationary phase in the profile which indicated that there was no delay in the growth of microalgae. The characteristic of BBM that is clear from any turbidity and other microorganism contributed to rapid growth of the microalgae. At the end of the culture period, highest biomass accumulation was recorded by 24 h culture, followed by the 12 h culture.

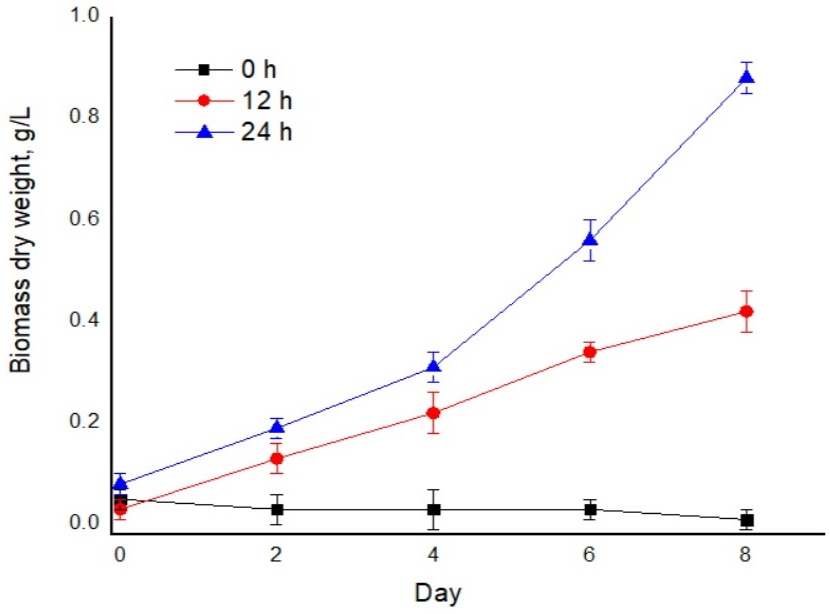

Fig. 2 Microalgae growth curve in BBM under different light periods

Table 4
Microalgae growth under different leachate percentages and light exposures

Growth parameters	Lighting period (h)								
	0:24 (Light: Dark)			12:12 (Light: Dark)			24:0 (Light: Dark)		
	Leachate percentage (% v/v)								
	5%	10%	15%	5%	10%	15%	5%	10%	15%
Growth rate, μ	-0.068	-0.097	-0.085	0.423	0.324	0.228	0.347	0.397	0.249
Cell division *D'*(/day)	-0.097	-10.26	-0.123	0.610	0.468	0.329	0.500	0.573	0.359
Biomass (g/L/day)	-0.003	-0.020	-0.003	0.044	0.045	0.051	0.087	0.074	0.078
Total Biomass (g/L)	-0.020	-0.038	-0.020	0.350	0.360	0.410	0.700	0.590	0.620

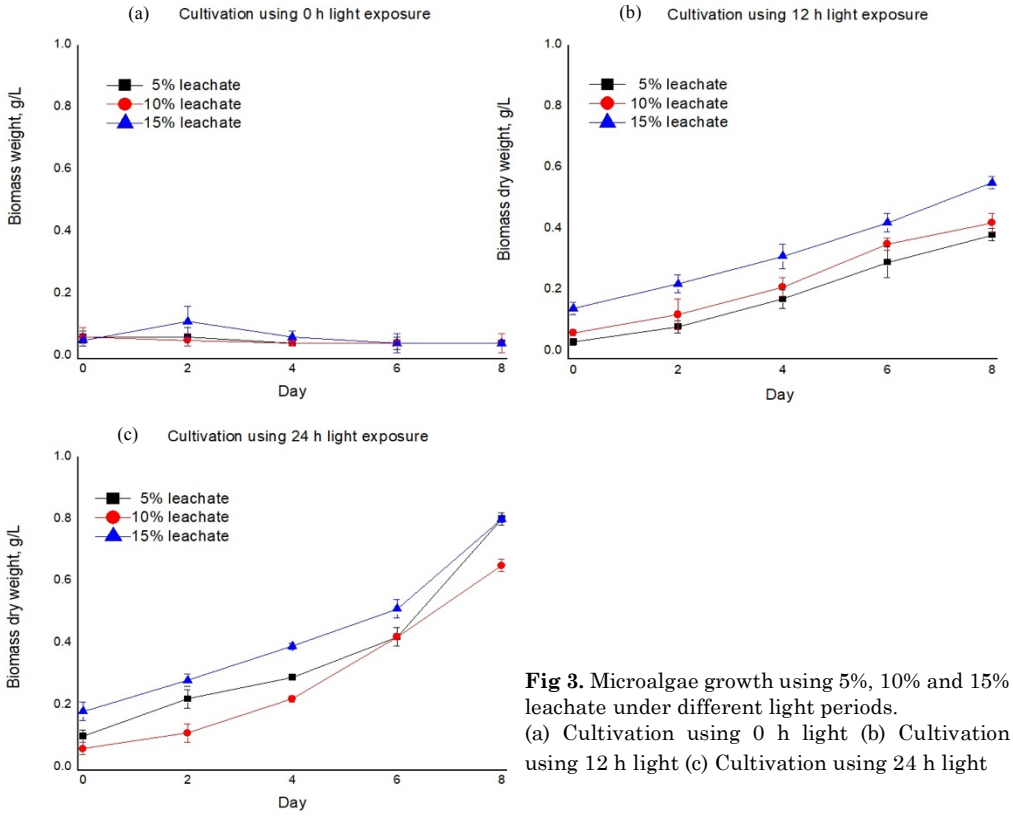

Fig 3. Microalgae growth using 5%, 10% and 15% leachate under different light periods.
(a) Cultivation using 0 h light (b) Cultivation using 12 h light (c) Cultivation using 24 h light

3.3.Microalgae growth in BBM supplemented by leachate

Table 4 presents the result for cultivation using different percentages of leachate and in three different durations of light. Similarly to growth in BBM, culture under 0 h exposure was unable to grow in all percentages of leachate. The highest growth rate, μ, of 0.423/day was achieved in 5% leachate with 12 h light, as compared to 0.347/day in the same percentage but under 24 h lighting. Remarkably, the growth rate was comparable with microalgae growth in the BBM medium that achieved the highest μ of 0.440/day under 12 h lighting.

The microalgae cell division per day is another indicator for growth performance. A high division rate indicates that the cells are dividing rapidly; this corresponds to a higher specific growth rate. Accordingly, the fastest cell division of 0.610/day in this study is shown by culture with the highest μ (12 h light with 5% leachate).

It was slightly slower than cell division in BBM of 0.635/day in the 12 h light culture but faster than 0.577 in the 24 h light BBM culture. Results from this study reveal that the optimum light exposure for the microalgae was the 12 h duration. This finding is important to provide the most conducive condition for microalgae growth and to eliminate the cost for unnecessary power supply by continuous illumination.

The addition of 15% leachate resulted in lower growth rates of 0.228/day and 0.249/day, respectively, for 12 h and 24 h lighting. This might be attributed to a higher concentration of the nitrogen source, such as ammoniacal nitrogen induced by the addition of more leachate into the medium. It was reported that ammoniacal nitrogen above 210 mg/L could have an inhibitory effect on microalgae by decreasing the electron transport rate for photosynthetic activity (Park *et al.* 2010; Li *et al.* 2019). On the other hand, higher amount of leachate might increase the turbidity of the culture, thus reducing light penetration.

Nevertheless, more biomass accumulation was observed in cultures with more leachate added as a supplement. Cultures that were supplied with 15% leachate produced the highest biomass of 0.051 g/L/day and 0.078 g/L/day for 12 h and 24 h lighting, respectively.

By contrast, when BBM was used, the highest biomass productivity for 12 h was 0.049 g/L/day, which was lower than those achieved with the leachate supplement. Notably, the determined biomass productivity was comparable and even higher than that in some previous studies using leachate as a growth medium. For instance, Sforza *et al*. (2015) reported that 0.07 g/L/day biomass was produced by *Acutodesmus obliquus* utilizing nutrients from leachate, whereas Pereira *et al*. (2016) obtained 0.020 to 0.049 g/L/day of productivity for *C. vulgaris* in various leachate compositions. Another study by Hernández-García *et al*. (2019), using *Desmodesmus* spp., produced 0.039 g/L/day biomass when grown in a mixture of leachate and municipal wastewater.

The concentration of leachate has an impact on the amount of nutrients in the medium. A significantly high nutrient content in leachate increases the amount of nutrients available for microalgae assimilation for conversion into biomass. A finding of the study indicates that application of leachate as a nutrient supplement could have an economic benefit for microalgae-based technology. Although highest biomass productivity and total biomass was achieved in BBM in the 24 h culture, the use of continuous lighting coupled with utilization of a full-BBM medium means that it is less desirable from the economic perspective. The chemical cost of preparing the standard medium could be reduced by recycling of leachate.

Fig. 3 illustrates the growth profile for cultivation of microalgae under three different light exposures supplemented with a leachate medium. As illustrated in Fig. 3(a), the profile curve indicates neither growth increment nor biomass production for the culture under 0 h light. The cultivation was failed by the absence of photosynthesis activity because no biomass can be produced without light supply. Although for the 15% leachate medium shows a brief increment on day 2, the microalgae cannot sustain without any photosynthesis activity which leads to premature death phase. Significantly improved growth curves were observed for 12 and 24 h light cultures as illustrated in Fig. 3(b) and Fig. 3(c) respectively. For both cultures, no lag phase was observed with a steep increase from the beginning of the culture period. This indicates that microalgae can adapt well in the medium with added leachate.

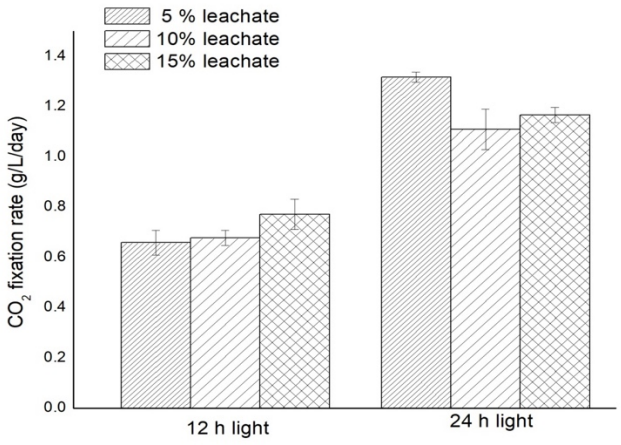

Fig 4. Microalgae fixation rates under different light periods

3.4. CO_2 fixation by the microalgae

Microalgae fixation rate is directly proportionate to the amount of total biomass produced. As illustrated in Fig. 4, the maximum CO_2 fixation of 1.317 g CO_2/L/day was achieved in the culture with 5% leachate and under 24 h light. In this culture, the continuous lighting and added nutrient from the leachate support longer photosynthesis activity that consumes CO_2.

At higher leachate concentrations of 10% and 15%, reduced light penetration due to the turbidity of the leachate might slightly hinder the photosynthesis process. As for the 12 h culture, shorter light exposure means reduce photosynthesis activity by the microalgae. The highest CO_2 fixture was 0.772 g CO_2/L/day, which was higher than CO_2 fixation under the same light duration in BBM, 0.734 g CO_2/L/day. This result was higher than that obtained by Jacob-Lopes *et al*. (2009), who achieved 0.562 g/L/day when they cultured *Aphanothece microscopica Nägeli* in a photobioreactor using a synthetic medium with a 12 h photoperiod. In another study by Hariz *et al*. (2018), 0.1208 g CO_2/L/day fixation rate was obtained under RSM optimum conditions using Chlorella sp. cultivated in a palm oil mill effluent. The present study indicates that utilization of leachate for nutrient supply could provide a more viable option for large-scale CO_2 biofixation. The cost for the chemical medium could be reduced; thus providing a more cost-effective microalgae culture system for long-term sustainability.

The outcome of this work has established the possibility of using leachate as a nutrient supply for Desmodesmus cultivation. However, the present study demonstrates that the microalgae tolerance level was considerably low at the 5% leachate concentration. Higher percentage of leachate utilized could further reduce the cost for the commercial medium. Therefore, subsequent studies should focus on enhancing the tolerance of the microalgae to maximize leachate concentration in the culture medium. One possible approach is through the adaptation process, by gradually exposing the microalgae to higher leachate concentrations. In addition, the optimum condition on the light periods should also be determined in future studies. This is important for achieving high biomass production under the most desirable economic condition

4. Conclusion

This study demonstrated that addition of leachate as a nutrient supplement for *Desmodesmus* cultivation was a feasible alternative for reducing usage of a commercial medium. Microalgae assimilated the nutrient in the leachate for their growth, provided that the leachate was not added at an inhibiting concentration. Notably, the findings indicated that the final biomass yield was dependent on the photoperiods. Continuous light supply produced the highest biomass yield. This suggests that biomass production correlates with light availability rather than only nutrient concentration to achieve higher photosynthetic activity. Next, the CO2 fixation rate was found to be directly proportionate to the total biomass accumulation. This indicates that microalgae assimilated inorganic carbon as carbon source for biomass productivity. The present study validated viability of

leachate recycling for microalgae cultivation to produce high value biomass and efficient for CO2 fixation..

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References

Altenhofen, M., Henrique, G., & Brito, C. (2018). Heterotrophic growth of green microalgae. Journal of Chemical Technology & Biotechnology, 92(3), 573–579.

Bajunaid, H., Sobri, M., Haiza, N., Yasin, M., Ba-abbad, M. M., Irma, N., & Mohd, N. (2019). Journal of Water Process Engineering Potential of the microalgae-based integrated wastewater treatment and CO2 fixation system to treat Palm Oil Mill Effluent (POME) by indigenous microalgae ; Scenedesmus sp . and Chlorella sp, 32. https://doi.org/10.1016/j.jwpe.2019.100907

Barra, L., Chandrasekaran, R., Corato, F., & Brunet, C. (2014). The challenge of ecophysiological biodiversity for biotechnological applications of marine microalgae. Marine Drugs, 12(3), 1641–1675. https://doi.org/10.3390/md12031641

Borowitzka, M. A., & Moheimani, N. R. (2013). Algae for Biofuels and Energy. Vol. 5, Springer. https://doi.org/10.1007/978-94-007-5479-9

Borowitzka, M. A., & Vonshak, A. (2017). Scaling up microalgal cultures to commercial scale. European Journal of Phycology, 52(4), 407–418. https://doi.org/10.1080/09670262.2017.1365177

Chang, H., Quan, X., Zhong, N., Zhang, Z., Lu, C., Li, G., Cheng Z., & Yang L. (2018). High efficiency nutrients reclamation from landfill leachate by microalgae Chlorella vulgaris in membrane photobioreactor for bio-lipid production. Bioresource Technology, 266, 374–381. https://doi.org/10.1016/j.biortech.2018.06.077

Chen, C. Y., Kuo, E. W., Nagarajan, D., Ho, S. H., Dong, C. Di, Lee, D. J., & Chang, J. S. (2020). Cultivating Chlorella sorokiniana AK-1 with swine wastewater for simultaneous wastewater treatment and algal biomass production. Bioresource Technology, 302(November 2019), 122814. https://doi.org/10.1016/j.biortech.2020.122814

Comitre, A. A., Vaz, B. da S., Costa, J. A. V., & Morais, M. G. de. (2021). Renewal of nanofibers in Chlorella fusca microalgae cultivation to increase CO2 fixation. Bioresource Technology, 321. https://doi.org/10.1016/j.biortech.2020.124452

Daneshvar, E., Santhosh, C., Antikainen, E., & Bhatnagar, A. (2018). Microalgal growth and nitrate removal efficiency in different cultivation conditions: Effect of macro and micronutrients and salinity. Journal of Environmental Chemical Engineering, 6(2), 1848–1854. https://doi.org/10.1016/j.jece.2018.02.033

de Souza, L., Lima, A. S., Matos, Â. P., Wheeler, R. M., Bork, J. A., Vieira Cubas, A. L., & Moecke, E. H. S. (2021). Biopolishing sanitary landfill leachate via cultivation of lipid-rich Scenedesmus microalgae. Journal of Cleaner Production, 303, 127094. https://doi.org/https://doi.org/10.1016/j.jclepro.2021.127094

Ding, G. T., Yaakob, Z., Takriff, M. S., Salihon, J., & Abd Rahaman, M. S. (2016). Biomass production and nutrients removal by a newly-isolated microalgal strain Chlamydomonas sp in palm oil mill effluent (POME). International Journal of Hydrogen Energy, 41(8), 4888–4895. https://doi.org/10.1016/j.ijhydene.2015.12.010

Eze, V. C., Velasquez-Orta, S. B., Hernández-García, A., Monje-Ramírez, I., & Orta-Ledesma, M. T. (2018). Kinetic modelling of microalgae cultivation for wastewater treatment and carbon dioxide sequestration. Algal Research, 32(March), 131–141. https://doi.org/10.1016/j.algal.2018.03.015

Hariz, H. B., Takriff, M. S., Ba-Abbad, M. M., Mohd Yasin, N. H., & Mohd Hakim, N. I. N. (2018). CO2 fixation capability of Chlorella sp. and its use in treating agricultural wastewater. Journal of Applied Phycology, 1–11. https://doi.org/10.1007/s10811-018-1488-0

Hawrot-Paw, M., Koniuszy, A., Gałczynska, M., Zajac, G., & Szyszlak-Bargłowicz, J. (2020). Production of microalgal biomass using aquaculture wastewater as growth medium. Water (Switzerland), 12(1). https://doi.org/10.3390/w12010106

Hemalatha, M., Sravan, J. S., Min, B., & Venkata Mohan, S. (2019). Microalgae-biorefinery with cascading resource recovery design associated to dairy wastewater treatment. Bioresource Technology, 284(March), 424–429. https://doi.org/10.1016/j.biortech.2019.03.106

Hernández-García, A., Velásquez-Orta, S. B., Novelo, E., Yáñez-Noguez, I., Monje-Ramírez, I., & Orta Ledesma, M. T. (2019). Wastewater-leachate treatment by microalgae: Biomass, carbohydrate and lipid production. Ecotoxicology and Environmental Safety, 174(March), 435–444. https://doi.org/10.1016/j.ecoenv.2019.02.052

Hernández-garcía, A., Velásquez-orta, S. B., Novelo, E., Yáñez-noguez, I., Monje-ramírez, I., & Orta, M. T. (2019). Ecotoxicology and Environmental Safety Wastewater-leachate treatment by microalgae : Biomass , carbohydrate and lipid production. Ecotoxicology and Environmental Safety, 174(August 2018), 435–444. https://doi.org/10.1016/j.ecoenv.2019.02.052

Huesemann, M., Crowe, B., Waller, P., Chavis, A., Hobbs, S., Edmundson, S., & Wigmosta, M. (2016). A validated model to predict microalgae growth in outdoor pond cultures subjected to fl uctuating light intensities and water temperatures. ALGAL, 13, 195–206. https://doi.org/10.1016/j.algal.2015.11.008

Hussein, M., Yoneda, K., Mohd-zaki, Z., Amir, A., & Othman, N. (2020). Heavy metals in leachate, impacted soils and natural soils of different landfills in Malaysia: An alarming threat. Chemosphere, 267. https://doi.org/10.1016/j.chemosphere.2020.128874

Jacob-Lopes, E., Scoparo, C. H. G., Lacerda, L. M. C. F., & Franco, T. T. (2009). Effect of light cycles (night/day) on CO2 fixation and biomass production by microalgae in photobioreactors. Chemical Engineering and Processing: Process Intensification, 48(1), 306–310. https://doi.org/10.1016/j.cep.2008.04.007

Jumaah, M. A., Othman, M. R., & Yusop, M. R. (2016). Characterization of Leachate from Jeram Sanitary landfill - Malaysia, International Journal of ChemTech Research, 9(08), 571–574.

Khanzada, Z. T. (2020). Phosphorus removal from land fi ll leachate by microalgae. Biotechnology Reports, 25, e00419. https://doi.org/10.1016/j.btre.2020.e00419

Koo, C. G., Woo, M. H., Yury, N., Lam, M. K., & Lee, K. T. (2017). Dual Role of Chlorella vulgaris in Wastewater Treatment for Biodiesel Production: Growth Optimization and Nutrients Removal Study. Journal of the Japan Institute of Energy, 96(8), 290–299. https://doi.org/10.3775/jie.96.290

Leite, L. de S., Hoffmann, M. T., & Daniel, L. A. (2019). Microalgae cultivation for municipal and piggery wastewater treatment in Brazil. Journal of Water Process Engineering, 31(March), 1–7. https://doi.org/10.1016/j.jwpe.2019.100821

Li, X., Li, W., Zhai, J., Wei, H., & Wang, Q. (2019). Effect of ammonium nitrogen on microalgal growth, biochemical composition and photosynthetic performance in mixotrophic cultivation. Bioresource Technology, 273(November 2018), 368–376. https://doi.org/10.1016/j.biortech.2018.11.042

Mubashar, M., Naveed, M., Mustafa, A., Ashraf, S., Shehzad Baig, K., Alamri, S., Siddiqui, M.H., Zabochnicka-Świątek, M., Szota, M. and Kalaji, H.M. (2020). Experimental Investigation of Chlorella vulgaris and Removal of Heavy Metals from Textile Wastewater. Water, 12(11), 3034.

Nair, A. T., Senthilnathan, J., & Nagendra, S. M. S. (2019). Application of the phycoremediation process for tertiary treatment of landfill leachate and carbon dioxide mitigation. Journal of Water Process Engineering, 28(January), 322–330. https://doi.org/10.1016/j.jwpe.2019.02.017

Naveen, B. P., Mahapatra, D. M., Sitharam, T. G., Sivapullaiah, P. V., & Ramachandra, T. V. (2017). Physico-chemical and biological characterization of urban municipal landfill leachate. Environmental Pollution, 220, 1–12. https://doi.org/10.1016/j.envpol.2016.09.002

Nawaz, T., Rahman, A., Pan, S., Dixon, K., Petri, B., & Selvaratnam, T. (2020). A Review of Landfill Leachate Treatment by Microalgae: Current Status and Future Directions. Processes, 8(4). https://doi.org/10.3390/pr8040384

Ogbonna, I. O., Okpozu, O. O., Ikwebe, J., & Ogbonna, J. C. (2019). Utilisation of Desmodesmus subspicatus LC172266 for simultaneous remediation of cassava wastewater and accumulation of lipids for biodiesel production. Biofuels, 10(5), 657–664. https://doi.org/10.1080/17597269.2018.1426164

Park, J., Jin, H. F., Lim, B. R., Park, K. Y., & Lee, K. (2010). Ammonia removal from anaerobic digestion effluent of livestock waste using green alga Scenedesmus sp. Bioresource Technology, 101(22), 8649–8657. https://doi.org/10.1016/j.biortech.2010.06.142

Pereira, S. F. L., Gonçalves, A. L., Moreira, F. C., Silva, T. F. C. V., Vilar, V. J. P., & Pires, J. C. M. (2016). Nitrogen removal from landfill leachate by microalgae. International Journal of Molecular Sciences, 17(11), 1–14. https://doi.org/10.3390/ijms17111926

Quan, X., Hu, R., Chang, H., Tang, X., & Huang, X. (2020). Enhancing microalgae growth and land fi ll leachate treatment through ozonization. Journal of Cleaner Production, 248, 119182. https://doi.org/10.1016/j.jclepro.2019.119182

Roleda, M. Y., Slocombe, S. P., Leakey, R. J. G., Day, J. G., Bell, E. M., & Stanley, M. S. (2013). Bioresource Technology Effects of temperature and nutrient regimes on biomass and lipid production by six oleaginous microalgae in batch culture employing a two-phase cultivation strategy. Bioresource Technology, 129, 439–449. https://doi.org/10.1016/j.biortech.2012.11.043

Sakarika, M., & Kornaros, M. (2016). Effect of pH on growth and lipid accumulation kinetics of the microalga Chlorella vulgaris grown heterotrophically under sulfur limitation. Bioresource Technology, 219, 694–701. https://doi.org/10.1016/j.biortech.2016.08.033

Sasongko, N. A., Noguchi, R., Ito, J., Demura, M., Ichikawa, S., Nakajima, M., & Watanabe, M. M. (2018). Engineering study of a pilot scale process plant for microalgae-oil production utilizing municipal wastewater and flue gases: Fukushima pilot plant. Energies, 11(7). https://doi.org/10.3390/en11071693

Sforza, E., Khairallah Al Emara, M. H., Sharif, A., & Bertucco, A. (2015). Exploitation of urban landfill leachate as nutrient source for microalgal biomass production. Chemical Engineering Transactions, 43, 373–378. https://doi.org/10.3303/CET1543063

Singh, S. P., & Singh, P. (2015). Effect of temperature and light on the growth of algae species: A review. Renewable and Sustainable Energy Reviews, 50, 431–444. https://doi.org/10.1016/j.rser.2015.05.024

Song, C., Hu, X., Liu, Z., Li, S., & Kitamura, Y. (2020). Combination of brewery wastewater purification and CO2 fixation with potential value-added ingredients production via different microalgae strains cultivation. Journal of Cleaner Production, 268, 122332. https://doi.org/10.1016/j.jclepro.2020.122332

Syafaini, A., Sobri, M., Haiza, N., & Yasin, M. (2021). Microalgae acclimatization in industrial wastewater and its effect on growth and primary metabolite composition. Algal Research, 53(June 2020), 102163. https://doi.org/10.1016/j.algal.2020.102163

Syazwani, N., Rahman, B. A., & Ahmad, N. (2019). Removal of Pollutants from Landfill Leachate Using Physicochemical Technique. International Journal of Civil Engineering, 0(0), 0. https://doi.org/10.1007/s40999-019-00396-4

Tsolcha, O. N., Tekerlekopoulou, A. G., Akratos, C. S., Aggelis, G., Genitsaris, S., Moustaka-Gouni, M., & Vayenas, D. V. (2017). Biotreatment of raisin and winery wastewaters and simultaneous biodiesel production using a Leptolyngbya-based microbial consortium. Journal of Cleaner Production, 148, 185–193. https://doi.org/10.1016/j.jclepro.2017.02.026

Wong, Y., Ho, Y., & Ho, K. (2017). Growth Medium Screening for Chlorella vulgaris Growth and Lipid Production. Journal of Aquaculture & Marine Biology, 6(1). https://doi.org/10.15406/jamb.2017.06.00143

Wu, K.-C., Yau, Y.-H., & Ho, K.-C. (2017). Capability of microalgae for local saline sewage treatment towards biodiesel production. {IOP} Conference Series: Earth and Environmental Science, 82, 12008. https://doi.org/10.1088/1755-1315/82/1/012008

Xing, R., Ma, W., Shao, Y., Cao, X., Chen, L., & Jiang, A. (2019). Factors that affect the growth and photosynthesis of the filamentous green algae, Chaetomorpha valida, in static sea cucumber aquaculture ponds with high salinity and high pH. PeerJ, 7, e6468–e6468. https://doi.org/10.7717/peerj.6468.