

# Removal of Organic and Nitrogen Compounds from Domestic Landfill Leachate by Microalgae

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**Abstract:** Water pollution by leachate has been widely reported in many regions in Indonesia. Landfill leachate contains high concentrations of organic and nitrogen, suitable for microalgae growth. Microalgae utilization for leachate treatment can be a promising alternative because it can simultaneously reduce pollutants and produce biomass. This study was conducted to identify microalgae that can grow well in leachate and examine mixed microalgae cultures' ability to reduce leachate pollutants. Microalgae obtained from a leachate treatment plant in Blang Bintang, Aceh Besar, were grown in a laboratory-scale photobioreactor. Microalgae identification was carried out morphologically using a microscope, while its performance in reducing pollutants was observed by COD, BOD, nitrate, and nitrite. The results showed that six microalgae species reduced leachate pollutants, namely *Synedra acus*, *Spirulina sp.*, *Euglena sp.*, *Trichocerca sp.*, *Paramecium sp.*, and *Closteriopsis longissima*, with *Euglena sp.* (40.63%) as the most abundant. The performance of mixed culture microalgae in this study showed promising results. COD, BOD, nitrate, and nitrite removal of 76.26%, 75.48%, 74.86%, and 73.52%, respectively, was observed during the experiment. As microalgae can grow well in leachate, in addition to reducing pollutants, such a treatment system can be integrated to produce biofuels and other bioproducts from microalgae biomass.

**Keywords:** wastewater; landfill leachate; microalgae; organic; nitrogen.

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## 1. Introduction

The present municipal solid waste (MSW) infrastructure in low- and middle-income nations will be severely strained as the urban population grows and garbage production per capita rises. The issue in most of these countries is to improve garbage collection and solid waste management systems, including the treatment approach at the final disposal site (landfill) to reduce greenhouse gas (GHG) emissions and other adverse impacts resulting from the waste. On the other hand, high-income countries can boost trash recovery by promoting reuse and recycling and upstream efforts to reduce waste at the source.

A similar case is also faced by Indonesia, as the MSW is still one of the most severe environmental issues that most cities in the country have to tackle. The Government of Indonesia issued an Act no. 18/2008 regarding Waste Management as a guideline for the local government to handle the MSW. The Act stipulated that five years after the issuance of the Act, the local governments, either city or regency, must have a final disposal processing site

for the MSW designed and operated according to established standards and criteria and comply with environmental regulations. In addition, the Act open dumping site for MSW is not acceptable, and such site and practice must be closed and stopped 2013. Unfortunately, even up to early 2021, many big, medium, and small cities still operate open dumping landfills.

The landfill's waste can be categorized into solid, gas, and liquid wastes. The biological degradation process of solid waste in the landfill will produce gases such as methane and carbon dioxide, which negatively impact global warming [1]. Liquid waste produced by a landfill, commonly called leachate, is a liquid that arises from the natural decomposition of organic matter in contact with liquids, chemicals, and rainwater that enters the landfill. Leachate is categorized as hazardous liquid waste because it contains high concentrations of organic compounds as measured by BOD, COD, and nitrogen pollutants as determined by ammonia, nitrite, and nitrate. In some cases, leachate may contain hazardous metals [2-5]. Poor leachate management will negatively impact the quality of ground and surface water and the health of the community living in the vicinity of the landfill. For example, Tangahu *et al.* [6] reported that surface and well waters around the Jabon landfill in Sidoarjo, East Java, Indonesia, have been contaminated by organic and nitrogen compounds at an alarming level because their concentrations have exceeded the allowable limit. Ulfani *et al.* [7] also reported that leachate produced from the Kampung Jawa landfill in Banda Aceh of the Province of Aceh had spread into the river around the landfill. Although many similar cases occur in other parts of Indonesia, the above information is sufficient to identify that leachate is a liquid waste that must be treated before being discharged into water bodies to prevent the environment and water bodies from contamination [8].

The current leachate treatment system using pond technology has been known to provide several disadvantages, requiring spacious land for its facilities, high investment and operational costs, and requires periodic control [9]. Furthermore, heavy metal and nitrogen remediation in biological leachate treatment does not achieve maximum results and necessitates additional processing for the sludge produced. The treated leachate still contains pollutants above the threshold limit in many cases. The utilization of microalgae to overcome the above problems can be a promising alternative. In addition to their capability to utilize organics and nutrients in the wastewaters for their growth, microalgae biomass has high economic and industrial potential as raw materials for producing medicines, healthy foods, chemicals, biofuels, electricity, animal feed, and many more [10-13]. Many studies have also reported that microalgae could offer solutions to current environmental problems, such as reducing greenhouse gases and removing metal ions from the surrounding medium [14, 15]. Pereira *et al.* [16] looked into *Chorella vulgaris'* ability to reduce ammonia, nitrate, and phosphate in biologically treated leachate from three different sanitary landfills in Portugal. The study reported that ammonia was successfully removed in the range of 22-100 percent, the phosphate was eliminated 38-100 percent, and nitrate was found to be removed by 0-27 percent. Furthermore, microalgae have been reported to be capable of fixing carbon dioxide via photosynthesis, absorbing heavy metals, and removing excess nutrients at a low cost [17].

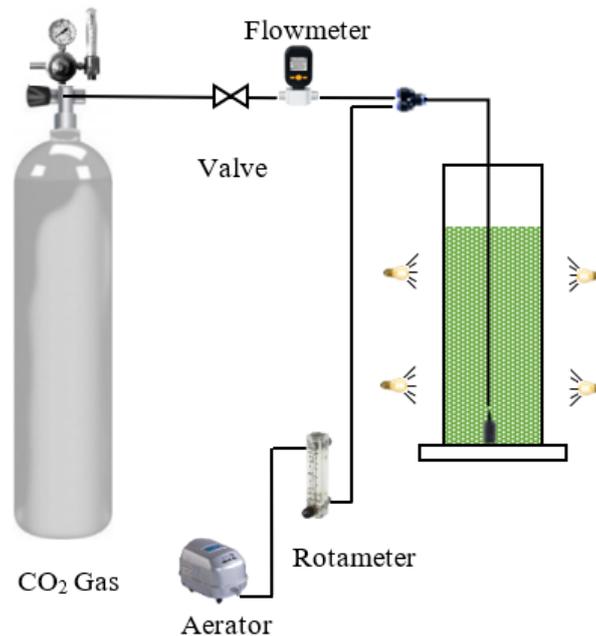
Several studies related to the use of microalgae for wastewater treatment have been widely presented [2, 15, 18-20], but most of these studies utilized pure culture microalgae, which were then cultivated in wastewater. The fact is that in the leachate, as evident in its treatment ponds, a mixed culture of microalgae grows well together with other microbes responsible for the removal of pollutants, such as bacteria and yeast. However, research related to using the mixed microalgae culture to treat leachate waste pollutants has not been widely

carried out. This paper reports the results of a study of the performance of mixed culture microalgae in the treatment of contaminants in leachate

## 2. Materials and Methods

### 2.1. Sample collection and microalgae cultivation.

Leachate and microalgae were collected from the stabilization pond of landfill leachate treatment facility at the Aceh Regional Waste Management Area in Blang Bintang (5°31'0.87"N 95°28'18.81"S), Aceh Besar, the Province of Aceh, Indonesia. The leachate sample was collected from the pond's surface at a depth of 30 cm and placed in a container before being transported to the laboratory. Mixed culture microalgae samples were collected from the surface of the stabilization pond, using a plankton net with a mesh size of 35  $\mu\text{m}$  which was pulled horizontally by towing method for 2 minutes. The microalgae samples were then poured into bottles with a volume of 500 mL. A mixed culture of microalgae was used in the present study. However, before being used for research, a 10 mL microalgae water sample was transferred to a 250 mL sterilized BG-11 liquid medium in an Erlenmeyer for 14 days of cultivation [21]. The light-dark cycle was set for 12 hours, with a light intensity of 2600 lux as measured using a lux meter. Microalgae growth was measured daily using an optical density method using a spectrophotometer at a wavelength of 680 nm. The photobioreactor was then inoculated with 500 mL (20% of total leachate volume) of microalgae.



**Figure 1.** Experimental arrangement of microalgae photobioreactor.

### 2.2. Photobioreactor arrangement.

Figure 1 illustrates the photobioreactor employed in this study. It was made of cylindrical glass with a height of 40 cm and a diameter of 10 cm with an effective volume of 2500 mL. Two fluorescent lamps were installed close to the photobioreactor as a light source to provide an intensity of 2600 lux and set automatically to give 12 hours light and 12 hours dark. The photobioreactor was also equipped with an aerator to circulate air and supply carbon dioxide. The airflow rate was set constant at 1 L/min, while the carbon dioxide gas flow rate

was fixed at 2% of the airflow rate. The overall arrangement of the experiment is presented in Figure 1.

### 2.3. Analytical procedure.

During the experimental period, the growth of microalgae was observed daily by optical density method using a spectrophotometer (Shimadzu UV-1800) at a wavelength of 680 nm. A total of 5 mL of a mixture of leachate and microalgae was centrifuged (TOMY LC-121) at a rotation speed of 5000 rpm for 5 minutes. The supernatant was separated for COD, nitrate, and nitrite measurements. The pellet was added with 5 mL of distilled water, and then the concentration was measured using a spectrophotometer. Identification of the microalgae was carried out at the end of the experiment. The isolated microalgae were recognized and morphologically examined using a 400x magnification light microscope (Wolfe). The images were compared with the morphological characters in the microalgae identification book [22]. The microalgae abundance in the leachate waste was calculated according to the APHA standard formulation as presented in Equation 1 [23].

$$N = F \times \frac{V_t}{V_s} \times \frac{1}{V_d} \dots\dots\dots (Eq. 1)$$

where: N= Total abundance of microalgae (ind/L), Vd= Volume of the filtered sample (L), Vt= Volume of the filtered water (L), Vs= Volume of the analyzed water (L), F= Number of biota found (inds).

The ability of mixed culture microalgae in reducing pollutants was evaluated through COD, BOD, nitrate, and nitrite parameters. COD, nitrate, and nitrite measurements were carried out every day, while BOD analyses were carried out every five days. Supernatant from microalgae concentration measurement was used to measure COD, nitrate, and nitrite. Leachate supernatant was diluted ten times using distilled water. COD concentration was determined using the reactor digestion and calorimetry method (HACH Method 8007) and a spectrophotometer at a wavelength of 620 nm. Measurements of nitrate concentrations (HACH Method 8029) and nitrite (HACH Method 8153) were also performed spectrophotometrically at wavelengths of 500 nm and 515 nm, respectively. Meanwhile, the BOD concentration was analyzed in duplicate using sensors (VELP Scientifica) for five days.

## 3. Results and Discussion

### 3.1. Leachate characteristics.

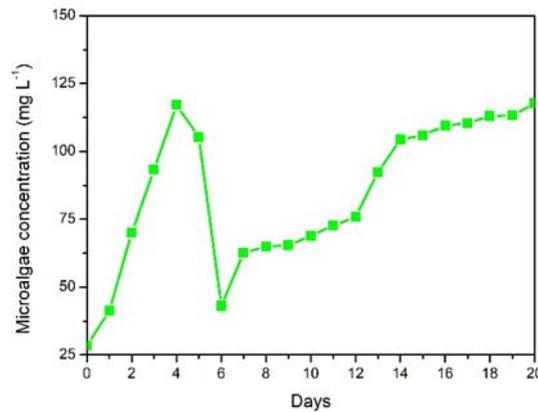
The quantity and quality of leachate are influenced by several factors such as waste composition, season, humidity, pH, intensity, and age of landfill [5]. The leachate effluent quality standard in Indonesia is directed in the Regulation of the Minister of Environment and Forestry of the Republic of Indonesia Number P.59/2016. The effluent standard under this regulation is presented in the last column of Table 1, while the result of the leachate analysis is shown in the second column of Table 1. The leachate used in this study was taken from the last stabilization pond of the leachate treatment facility at the Aceh Regional Solid Waste Management treatment facilities. The processed leachate leaving the last stabilization pond will flow through the channel and eventually enter a water body. The leachate analysis shows that the COD concentration of leachate from the last pond in the leachate treatment system was still relatively high, greater than 1000 mg/L, which does not meet the quality standards set by the Government of Indonesia through the regulation mentioned above. This result indicates that

the leachate processing system in the Aceh Regional Solid Waste Management has not been running well. Several factors, including, but not limited to, the aeration system in the aerobic pond not working properly, the leachate residence time in various ponds not being long enough, and the COD input of leachate being already high due to the landfill's young age [24].

**Table 1.** Leachate characteristics from the Aceh Regional Solid Waste Management treatment facilities.

Parameter	Analytical result	Effluent standard[22]
pH	8.9	6–9
Temp (°C)	29.8	NA
BOD (mg/L)	620	150
COD (mg/L)	1,213	300
NH <sub>3</sub> (mg/L)	338.85	NA
NO <sub>3</sub> (mg/L)	289.8	NA
NO <sub>2</sub> (mg/L)	489	NA
PO <sub>4</sub> (mg/L)	17.25	NA
Fe (mg/L)	3.285	NA
Mn (mg/L)	5.94	NA
Zn (mg/L)	2.87	NA
Cu (mg/L)	9.23	NA

NA = Not available



**Figure 2.** Growth of mixed culture of microalgae in landfill leachate.

### 3.2. Microalgae growth in landfill leachate.

Microalgae growth was observed every day for up to 20 days. The reactor temperature during the experiment ranged from  $28.0 \pm 0.5^\circ\text{C}$  with a pH of  $9.3 \pm 0.1$ . A study by Chowdury *et al.* [25] stated that the optimal temperature for microalgae growth is in the range of  $20\text{--}35^\circ\text{C}$ . Temperatures that are too low will inhibit the growth rate of microalgae, while temperatures that are too high can potentially reduce photosynthetic efficiency in microalgae [26, 27]. The concentration of mixed culture microalgae on the first day of the experiment was 28.65 mg/L. Algae concentration increased significantly until the fifth day (105.33 mg/L), then dropped drastically to 43.15 mg/L on the sixth day. The drastic decrease in concentration occurred due to the shock load [28] caused by the addition of fresh leachate to replace the leachate taken for BOD testing. The environmental conditions in the reactor were favorable for the growth of mixed culture algae during the first five days. However, when fresh leachate was added, the mixed culture microalgae had to re-adapt to the new environment. As shown in Figure 2, the mixed culture microalgae successfully acclimated to the new environment condition over the following days, characterized by an increasing concentration of mixed culture microalgae until the last day of the experiment (117.654 mg/L).

**Table 2.** Microalgae species and their abundance in the landfill leachate

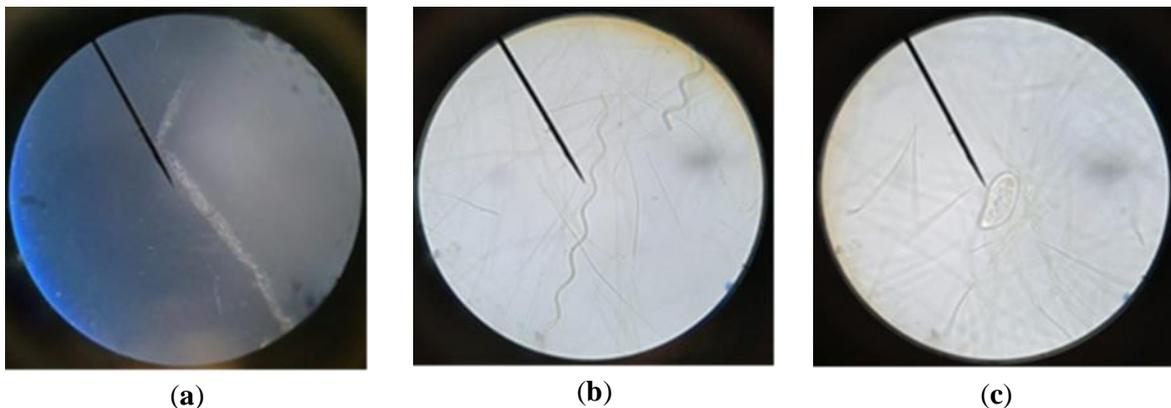
Class	Genus	Species	Abundance(%)
Bacillariophyceae	<i>Synedra</i>	<i>Synedra acus</i>	14,38
Cyanophyceae	<i>Spirulina</i>	<i>Spirulina sp.</i>	15,62
Euglenophyceae	<i>Euglena</i>	<i>Euglena sp.</i>	40,63
Monogonata	<i>Trichocerca</i>	<i>Trichocerca sp.</i>	3,12
Oligohymenophorea	<i>Paramecium</i>	<i>Paramecium sp</i>	12,5
Trebouxiophyceae	<i>Closteriopsis</i>	<i>Closteriopsis longissima</i>	13,75

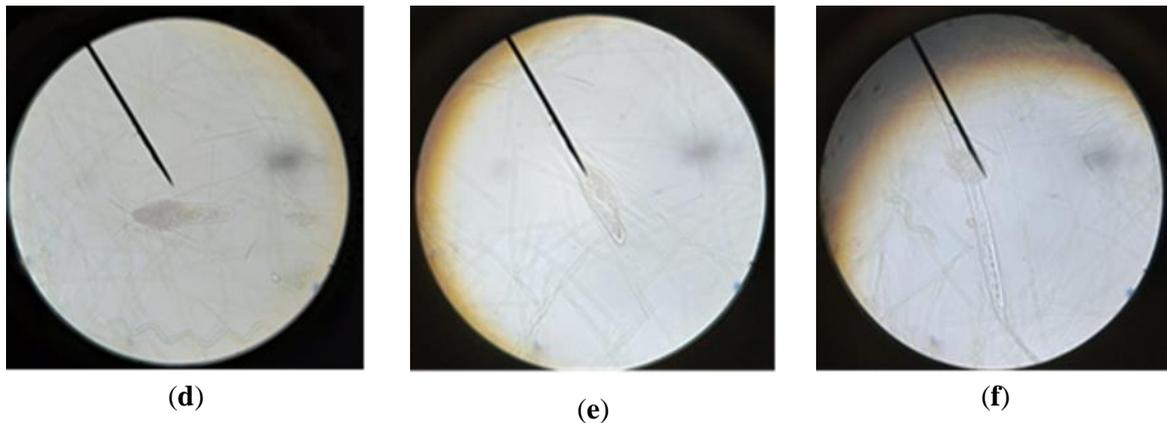
3.3. Microalgae distribution and their abundance.

The results of species identification and percentage of microalgae abundance are presented in Table 2. Six microalgae species identified were *Synedra acus*, *Spirulina sp.*, *Euglena sp.*, *Trichocerca sp.*, *Paramecium sp.*, and *Closteriopsis longissima*. Figure 3 shows the morphologically identified microalgae species using a microscope with 400x magnification. The leachate's highest abundance of microalgal culture was *Euglena sp.*, followed by *Spirulina sp* and *Synedra acus*. *Euglena* is a highly versatile microbe and can survive in harsh environments [29]. Chiellini *et al.* [30] investigated the resistance of freshwater microalgae (*Chlorella sp.*, *Chlorogonium sp.*, and *Euglena sp.*) to heavy metals (Cu, Zn As (III), Fe, and Ni) at concentrations ranging from 0.5 to 15 mM. According to the study's findings, only *Euglena sp.* is capable of tolerating all of the metals examined at all doses. *Euglena* includes animal-like protists having flexible cell walls that can change shape to become elongated. The size of *Euglena* is very small, around 0.05 mm. Red and green *Euglena* are widely found in ponds or lakes. They usually exist in fresh or brackish water and can survive at high carbon dioxide concentrations. *Euglena* can produce lipids [31], further utilized as a food source, beauty products, and biofuels [32, 33].

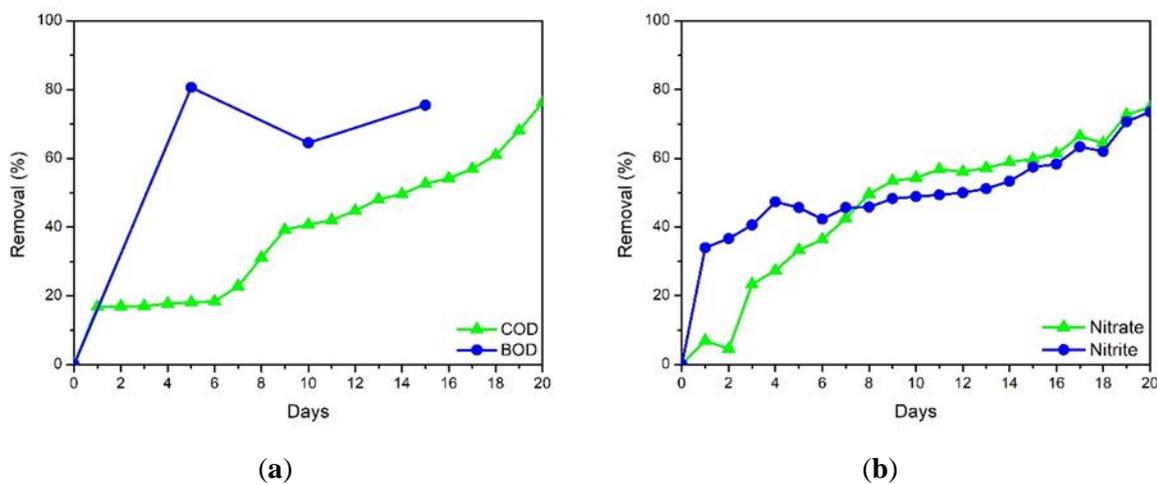
Microalgae *Spirulina sp.*, like *Euglena sp.*, can also remove heavy metals from wastewater. Mohadi *et al.* [34] investigated the growth of *Spirulina sp.* in BG-11 medium supplemented with Cd(II) metal. According to their findings, *Spirulina sp.* grows reasonably well on BG-11 medium with a maximum Cd concentration of 3 mg/L. *Spirulina sp.* has been shown to produce significant protein levels, lipid, phycocyanin, and the fatty acid C18:3n3 (Omega-3) in wastewater, making it appropriate for use as a dietary raw material.

*Synedra* is one of the diatom species that live in fresh and sea waters and can survive in extreme environmental conditions [35]. *Synedra* has the ability to store food reserves in the form of an insoluble polymer, enabling it to survive at low concentrations of phosphate and nitrogen. Research conducted by Andrade *et al.* [36] suggested that *Synedra acus* can be an anti-cancer drug.





**Figure 3.** Microalgae species identified in the landfill leachate (a) *Synedra acus*, (b) *Spirulina sp.*, (c) *Euglena sp.*, (d) *Trichocerca sp.*, (e) *Paramecium sp.*, and (f) *Closteriopsis longissima* (400x magnification)



**Figure 4.** The percentage removals of (a) organic and (b) nitrogen from landfill leachate by microalgae.

### 3.4. Pollutants removal.

#### 3.4.1. Organics.

The removal percentage of COD and BOD is presented in Figure 4(a). The COD and BOD concentrations of leachate at the beginning of the experiment were 233,035 mg/L and 155 mg/L, respectively. The decrease in BOD concentration fluctuated due to the addition of fresh leachate on the fifth day. Meanwhile, the COD removal showed a positive linear trend until the last day of the experiment. On the last day of the investigation, the COD concentration was reduced to 76.26% (55.32 mg/L), and the removal percentage of BOD was 75.48% (38 mg/L). The results obtained in this study are in agreement with the experiments conducted by Thongpinyochai *et al.* [37]; however, their investigation focused on the ability of pure microalga *Chlorella vulgaris* to reduce COD and BOD in leachates. Their results showed that *Chlorella vulgaris* grew well in leachate diluted by 30% using tap water and the removal percentage of BOD and COD was 52.78% and 51.05%, respectively.

#### 3.4.2. Nitrogen.

Nutrients are important factors for the cell metabolism and growth of microalgal biomass. Most of them require macronutrients such as carbon (C), nitrogen (N), and phosphorus (P) [38-40]. Shashirekha *et al.* [39] investigated the utilization of *Scenedesmus*

*obliquus* in the remediation of sugar factory wastewater by modifying the C-N-P ratio. The study results showed that the biomass yield of *Scenedesmus obliquus* was directly proportional to the percentage of C-N-P removal and succeeded in reducing the BOD and COD of sugar factory effluent by up to 80%. They also reported that the treated wastewater contained nitrogen and phosphorus below the detectable limit, reaching a removal rate of most probably around 99.0%. A similar trend was observed in the present study when inspecting Figures 4 (a) and 4 (b). The carbon uptake denoted by COD removal and nitrogen utilization by microalgae demonstrated a similar trend, signifying that the mixed culture of microalgae was consuming the carbon and nitrogen for their growth and metabolism.

Given that the microalgae biomass C:N:P ratio is 100:14:2, only primary treatment wastewater comes close to this proportion as in the case of sugar factory effluent [39], with the others having a surplus of N and P or shortage of C. Such analysis demonstrates that microalgae grown in the substrates of carbon limited require additional carbon in the form of CO<sub>2</sub> or bicarbonate to complete the assimilation of N and P in the culture medium. Even if the wastewater concentration does not require CO<sub>2</sub>, injection of CO<sub>2</sub> is still necessary to maintain the system's pH. While growing, microalgae take carbon and nitrogen from the wastewater, causing the pH to rise to a level of 10.0 or higher. Both bacteria and microalgae are harmed by high pH levels, limiting their performance and growth rate and their ability to remove pollutants from the wastewater. The leachate was taken from the last stabilization pond in the present study. Although the COD was numerically still high enough, the leachate lacked carbon content because the nitrogen concentration was significantly high, more than 1100 mg/L, as shown in Table 1. However, the removal of both carbon and nitrogen was stable because the experimental reactor was continuously supplied with CO<sub>2</sub> to maintain the pH below 10.0, which was observed at around 9.3 on average.

The nitrate and nitrite concentrations in this experiment were measured every day until the last day of the experiment. The initial nitrate and nitrite concentrations were 63.14 mg/L and 84.89 mg/L, respectively. Figure 4(b) shows the removal percentage of nitrate and nitrite. The removal of nitrate and nitrite shows the same trend, increasing linearly positively. On the last day of the experiment, the removal efficiency of nitrate and nitrite was 74.58% and 73.52%, respectively. Research conducted by Olguin [41] indicated that the level of nitrogen removal by *Euglena sp.* was significantly high, with around 74.86% nitrate reduction. This finding is in line with the results obtained from this study.

#### 4. Conclusions

The results showed that microalgae could be utilized to remove organics and nitrogen pollutants from leachate and, at the same time, produce their biomass. The highest concentration of microalgae, 117.654 mg/L, in the photobioreactor was achieved on the last day of the experiment at a temperature of 28±0.5°C and an operating pH of 9.3±0.1. Six microalgae species were identified as capable of reducing the contaminants in the leachate, including *Synedra acus*, *Spirulina sp.*, *Euglena sp.*, *Trichocerca sp.*, *Paramecium sp.*, and *Closteriopsis longissima*. The removal percentage of COD, BOD, nitrate, and nitrite by mixed culture microalgae was 76.26%, 75.48%, 74.58%, and 73.52%, respectively. From the results of this experiment, mixed culture microalgae can simultaneously remove organic and nitrogenous substances from leachates.

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## Conflicts of Interest

The authors declare no conflict of interest.

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