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ORIGINAL RESEARCH PAPER

Synergistic removal of organic and nutrients from landfill leachate using photobioreactor-cultivated microalgae-bacteria consortium

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ABSTRACT

BACKGROUND AND OBJECTIVES: The utilization of stabilization pond system for landfill leachate treatment is hindered by its requirement for expansive land areas and extended retention periods. Although the system effectively removes organic compounds, its ability to eliminate nutrients such as nitrogen and phosphorus is comparatively limited. Consequently, the leachate subjected to treatment often falls short of meeting the mandated standards for effluent quality. In response to this challenge, a research study was undertaken to investigate the potential of utilizing a consortium comprising microalgae and bacteria in the treatment of landfill leachate.

METHODS: The microalgae, bacteria, and leachate utilized in this study were sourced from a leachate treatment facility located at the Aceh regional domestic waste management unit in Blang Bintang, Aceh Besar, Indonesia. The two glass photobioreactors were operated batch-wise, where the first was provided with a combination of air and carbon dioxide, and the other was solely exposed to air. The pollutant removal efficacy in the leachate effluent was assessed through the measurements of chemical oxygen demand, ammonia, nitrate, nitrite, and phosphate concentrations. Subsequently, macroscopic identification of microalgae and bacteria species was also conducted.

FINDINGS: Utilizing a consortium of microalgae and bacteria has demonstrated efficacy in treating leachate, resulting in a notable reduction of contaminants within the effluent. The symbiotic association between microalgae and bacteria in the context of leachate waste treatment is evident. The bacteria's metabolic actions result in carbon dioxide emission, which subsequently serves as a substrate for the photosynthetic activities of the microalgae. The microalgae facilitate the transfer of oxygen, produced through photosynthesis, to the bacteria to support their metabolic processes. Therefore, introducing exogenous carbon dioxide to the consortium yields minimal discernible effects, given that the bacteria adequately fulfill the carbon dioxide requirements of the microalgae. This discovery enhances the efficacy of leachate treatment techniques by leveraging the utilization of pre-existing mixed cultures of microalgae and bacteria found in leachate facilities.

CONCLUSION: This study evaluated the microalgae-bacteria consortium's effectiveness in reducing leachate pollutants. The consortium exhibited a significant capability, achieving a 75 percent reduction in chemical oxygen demand and successfully eliminating a range of contaminants. Additionally, it demonstrated effective removal of nitrogen compounds such as ammonia, nitrate, and nitrite, with removal rates reaching 75 percent. Notably, the consortium showed a 99 percent removal rate for phosphate compounds. Even with the introduction of carbon dioxide, the pollutant removal remained consistently high, suggesting that the addition of carbon dioxide did not significantly influence the overall process.

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69

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4

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3

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INTRODUCTION

The issue of domestic solid waste management is still a prominent concern in Indonesia and other undeveloped or developing countries due to the absence of environmentally sustainable waste management practices (Ratnawati *et al.,* 2023; Samimi and Nouri, 2023). In 2020, domestic waste production in Indonesia was 67.8 million tons, with approximately 90 percent (%) in landfills. Regarding operational efficiency and fiscal considerations, landfilling is the simplest and most cost-effective method of solid waste disposal (Munawar and Fellner, 2013). The degradation of waste in landfills emits gases such as methane and carbon dioxide (CO_2), which intensify the issue of global warming. Furthermore, it generates by-products in the form of liquid leachate, potentially contaminating the surrounding groundwater and soil. Despite the availability and continuous development of multiple systems to use energy from landfill gas (Munawar and Fellner, 2013), extracting energy or products from landfill leachate remains exceedingly uncommon. Leachate is a liquid waste product originating when external fluids such as rainwater, surface runoff, and other liquids come into contact with landfill waste, causing dissolution and the washing away of dissolved substances. This includes organic materials stemming from the biological decomposition process. Generally, leachate is classified as hazardous liquid waste due to the elevated levels of biological oxygen demand (BOD), chemical oxygen demand (COD), ammonia (NH_3), and nitrogen (Zhu *et al*., 2019; Ahmed *et al*., 2018; Samimi and Shahriari Moghadam, 2018), with variations depending on the age of the landfill. It also contains toxic heavy metals (Xaypanya *et al*., 2018), necessitating treatment before being discharged into receiving water bodies. The prevalent treatment method in the country uses a pond system technology, with several stages such as collection, aerobic, and stabilization ponds. A key challenge posed by this pond treatment approach is the need for a relatively extended residence time, approximately spanning from 30 to 50 days, and a significant land area to accommodate the required facilities. This method effectively eliminates a significant portion of the organic matter and certain nutrients present in leachate. However, considerable nitrogen levels in the form of nitrate (NO_3) and phosphorus persist in the treated water, posing difficulties for bacterial nutrient removal. Consequently, the treated leachate frequently falls short of meeting the quality standards required for the release into water bodies, as stipulated by local environmental agencies. This phenomenon occurs due to excessive nitrogen content in the effluent, surpassing permissible limits. The discharge of surplus nutrient into natural water bodies poses the risk of inducing eutrophication in aquatic ecosystems, resulting in habitat degradation for aquatic flora and fauna due to the depletion of dissolved oxygen. Moreover, the integration of supplementary units for nutrient removal presents operational challenges. This is attributed to the low concentration of organics, leading to high investments and operating expenses. Due to the inherent simplicity and cost-effectiveness, stabilization ponds remain the preferred option for treating landfill leachate in most underdeveloped and developing countries (Arun *et al*., 2020; Amit *et al*., 2020). Recently, there has been an increasing scholarly focus on using microalgae for wastewater treatment, including leachate (Shahid *et al*., 2020; Aditya *et al*., 2022). This is because most wastewater contains nitrogen and phosphorus, with suitable growing conditions for microalgae growth. The approach can minimize the costs associated with removing nitrogen and phosphorus compounds (Kamyab *et al*., 2018) while generating valuable biomass as a by-product. The environmental problems of greenhouse gas emission are addressed by capturing CO_2 gas as the carbon source for growth (Srimongkol *et al*., 2022). Passing CO_2-rich gases across microalgae-based wastewater treatment systems proves to be a highly efficient method of extracting CO_2 and removing nutrients from liquid waste, simultaneously enhancing microalgae biomass growth at a minimal cost. This shows that the use of microalgae biomass offers significant economic and industrial prospects in serving as fundamental resources for the synthesis of medicinal compounds, food sources (Moejes and Moejes, 2017), energy production (Zaman *et al*., 2020) and other valuable products. In wastewater treatment, microalgae can be used to reduce pollutant concentrations and enhance biomass growth for potential conversion into economically viable products. Although the use of microalgae-bacteria consortium in wastewater treatment has gained significant attention globally, there is limited information on its application in treating landfill leachate in tropical countries. The use of cultures derived directly from leachate ponds can potentially reduce the acclimatization period for microalgae and bacteria, thereby enhancing efficiency. Sourcing cultures directly from leachate facilities ensures that the isolated species are highly adapted to the specific circumstances of the effluent requiring treatment. Consequently, this study aimed to investigate and validate the effectiveness of microalgae-bacteria consortium extracted from leachate treatment ponds in removing pollutants. The experiment focused on assessing the capability of the consortium to eliminate organic matter, nitrogen, and phosphate (PO_4) compounds from leachate collected from the treatment plant located at the Provincial Domestic Waste Management Unit in Blang Bintang, Aceh Besar District, Indonesia. This study was conducted at the Environmental Engineering Laboratory in the Chemical Engineering Department at Syiah Kuala University in Banda Aceh, Indonesia, in 2022. This study sought to enhance our understanding of the performance and potential of the consortium as a sustainable solution for addressing the intricate challenges associated with landfill leachate treatment in tropical regions.

MATERIALS AND METHODS

Leachate, bacteria, and microalgae used in this study were obtained directly from a leachate treatment pond managed by Aceh Regional Waste Management Agency in Blang Bintang, Aceh Besar, Indonesia. Moreover, using resources directly from the actual treatment ponds ensured the experimental conditions were similar to real-world circumstances. By sourcing components from the pond system, this study aimed to generate results and data representing leachate properties in the tropics.

Microalgae-bacteria culture and analysis

The bacterial culture was sourced from the aerated lagoon located at the Provincial Domestic Waste Management Unit in Blang Bintang. Subsequently, the bacterial culture was isolated before its application in the photobioreactor experiment through the pour method, using nutrient broth media (Merck). After the implementation of the isolation protocol, the bacterial culture was subjected to a controlled incubation period for two days in an incubator at a temperature of 28 degrees Celsius (°C) (Tighiri and Erkurt, 2019).

Microalgae samples were also obtained from the Regional Waste Management Unit at the final stabilization pond of the landfill leachate treatment plant, where the treated leachate is ready for discharge into the environment. Microalgae was subjected to cultivation in Erlenmeyer flasks using BG-11 medium for 14 days. In the cultivation period, the light intensity maintained a level of 2,600 lux, operating under a 12-hour light-dark cycle (Emalya *et al*., 2023). Microalgae and bacteria species were identified using conventional methods by examining individual cell morphology or colony characteristics. Due to the reliability of the established phenotypic parameters, the characterization and identification of the microalgae-bacteria consortium were conducted using a light microscope (Wolfe) at a magnification of 400x. The collected data were cross-referenced with a comprehensive reference compendium designed explicitly for identifying microalgae and bacteria (Holt, 1994). This procedural measure facilitated a thorough analysis and verification of the results, guaranteeing precision and dependability. Microalgae-bacteria abundance was calculated based on the procedure used in Effendi *et al*. (2016) with slight modifications as expressed in Eq. 1.

$$N = F\,x\,\frac{Vt}{Vs}\,x\,\frac{1}{Vd} \tag{1}$$

Where *N* is the total abundance of biota in individuals per liter (ind./L), *F* is the number of observed biota in individuals (ind.), *Vt* is the volume of filtered water in liters (L), *Vs* is the volume of filtered water sample in liters (L), and *Vd* is the volume of the sample filtered in liters (L). The results of microalgae-bacteria consortium analysis and abundance are presented in Table 3.

Landfill leachate sampling

The landfill leachate used in this study was sourced from the latest stabilization lagoon of the Regional Waste Management Unit. This selection was based on the consideration that the strength of leachate from the collection pond would require a longer period for the microalgae-bacteria consortium to acclimate. A cumulative quantity of 20 liter (L) landfill leachate was obtained and carefully preserved in sanitized containers before being transported to the laboratory. Subsequently, the sample was securely stored in a freezer at approximately a temperature of 4°C. Leachate from the stabilization pond had BOD and COD concentrations of 117 milligrams per liter (mg/L) and

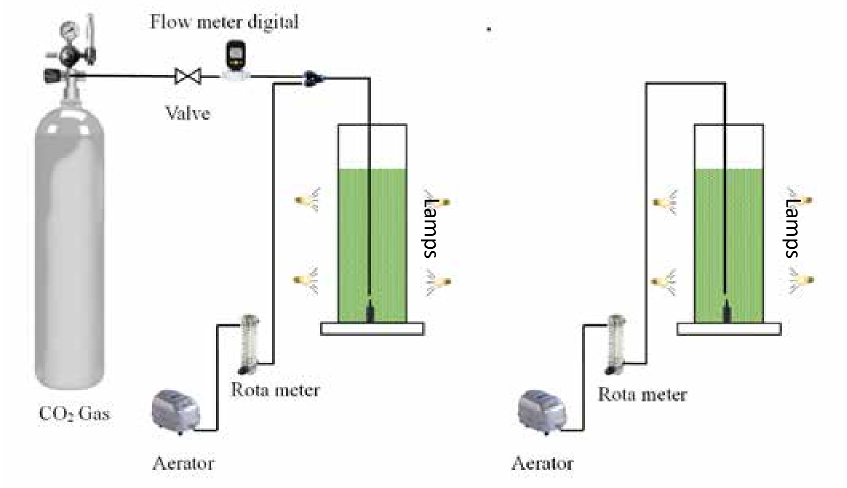

Fig. 1: Experimental photobioreactors arrangement

350 mg/L, containing 243 mg/L NH_3, 294 mg/L NO_3, and 337 mg/L nitrite (NO_2). The initial concentrations of iron (Fe), manganese (Mn), and zinc (Zn) before discharge were 6.4 mg/L, 1.8 mg/L, and 6.8 mg/L, respectively. Before operating the photobioreactor, the concentrations of pollutants in leachate were reassessed. The concentrations of COD, NH_3, NO_3, NO_2, and PO_4 at the start of photobioreactor operation were recorded as 233 mg/L, 29 mg/L, 63 mg/L, 85 mg/L, and 1.157 mg/L, respectively.

Experimental Setup

A total of two photobioreactors were operated in batch mode with distinct aeration conditions. Photobioreactors represent a category of bioreactors designed to utilize light as an energy source for cultivating phototrophic microorganisms, including microalgae, cyanobacteria, and purple non-sulfur bacteria. The first photobioreactor was performed by introducing a combination of air and pure CO_2 gas, with CO_2 gas constituting 2% of the total airflow. Meanwhile, the second photobioreactor was exclusively supplied with air at a rate of 1 L/min. Photobioreactors were fabricated using glass material, with dimensions of 40 centimeters (cm) in height and 10 cm in diameter, yielding a combined volume of 2,500 milliliters (mL). The schematic representation is presented in Fig. 1. Vertically installed two fluorescent lamps (8 Watt) emitting a light intensity of 2,600 lux served as the light source, adhering to a 12:12 light-dark cycle regulated by a digital timer socket. Moreover, photobioreactors featured provisions including an aerator, cylinders containing pure CO_2 gas, and a gas mixer to blend air and CO_2. The mixture obtained was supplied to the reactor through a sparger positioned at the lower section of photobioreactors. For the inoculation process, a consortium of microalgae and bacteria was introduced into photobioreactors, constituting 20% of the volume, with an initial concentration of 28.645 mg/L.

Analytical procedure

Daily potential of hydrogen (pH) measurements were conducted in the experimental period using a pH meter (HANNA Instrument HI9813-6). All leachate samples were passed through centrifugation (TOMY LC-121) at 4000 rpm for 10 minutes for water quality analysis. Subsequently, the supernatant was collected to assess ammonia, nitrate, nitrite, phosphate, and COD concentrations using an ultraviolet-visible (UV-Vis) spectrophotometer (Shimadzu Spectrophotometer UV-1800). Ammonia concentration was quantified using the salicylate method (HACH Method 10031), with a measurement range spanning from 0.4 to 50 mg/L and a wavelength of 655 nanometers (nm). Nitrate concentration was measured with the cadmium reduction method (HACH Method 8029), comprising a range of 0.3 to 30 mg/L and wavelength at 500 nm. Furthermore, nitrite concentration was determined through the ferrous sulfate technique (HACH Method 8153), with method validity for concentrations ranging from 2 to 250 mg/L at a wavelength of 515 nm. For phosphate concentration, the estimation was in the range of 0 to 12.5 mg/L. The measurement protocol included transferring 5 mL of the supernatant into a 25 mL volumetric flask, which was then filled to the mark with mineral-free water. The measurement was conducted using the ultraviolet (UV) persulfate oxidation method, specifically following HACH Method 8007, at a wavelength of 880 nm. COD concentration assessments were accomplished using reactor digestion and calorimetry methods (HACH Method 8000), ranging from 20 to 1500 mg/L, with a wavelength of 620 nm. When the measured concentration surpasses or deviates from the required range, dilutions are performed on the supernatant, ensuring the concentration is consistent with the designated measurement range.

RESULTS AND DISCUSSION

The landfill leachate treatment plant in Blang Bintang, overseen by the Regional Waste Management Unit and spanning an area of 206 hectares (ha) in Aceh Besar District, has been in operation since 2014. In its initial implementation stage, the landfill was conceived to accommodate domestic solid wastes without the capacity for sorting or handling. These wastes were indiscriminately dumped without additional treatment, reflecting a lack of appropriate facilities. Despite recent efforts involving waste compaction and soil cover on the site, the landfill primarily receives waste from Banda Aceh and Aceh Besar District. On average, approximately 252 tons of household and commercial waste is generated daily, accumulating a total of 92.2 thousand tons of domestic waste in 2022. Table 1 presents an overview of the attributes pertaining to the solid waste deposited at Blang Bintang landfill for the entire duration of 2022. Among the various types of solid waste deposited, food waste constitutes the most considerable quantity and proportion, followed by wood trash. These waste types and proportions show similarities to the garbage composition data found in the study conducted by Qonitan *et al*. (2021) on other prominent cities in Indonesia. The data showed that food waste was the predominant material disposed of in landfills in various urban regions of the country. Farahdiba *et al.* (2023) also identified food waste, plastic, and wood as the primary sources of solid waste, significantly impacting landfill waste accumulation. Similarly, Ma *et al*. (2022) reported substantial food waste in landfills, considerably contributing to the increased concentration of organic compounds in leachate. Nanda and Berruti (2021) reported that waste type significantly influenced leachate quality, including age, geography, climate, and landfill management.

Landfill leachate characteristics

Earlier research has indicated that the characteristics and composition of landfill-generated leachate undergo changes based on maturity (Malovanyy *et al*., 2022). Leachate from landfills is typically categorized into three age-related groups: young (<5 years), medium (5-10 years), and old (>10 years). When examining key pollution indicators for each age group, the average COD transitioned from over 15,000 mg/L in young landfills to below 3,000 mg/L in older counterparts. Ammonium nitrogen (NH_4-N) exhibited an increasing trend with landfill age, ranging from approximately 500-1,000 mg/L in young landfills to about 1,000-3,000 mg/L in older ones. While real-world measurements may vary from these averages, a discernible pattern of specific alterations emerges as leachates age. Analysis of BOD and COD measurements revealed a gradual decline in the concentration of organic pollutants in landfill leachate over time. Simultaneously, levels of

Table 1: Municipal solid waste composition in Blang Bintang landfill

Waste component	Composition (%)
Food waste	32.4
Wood	20.6
Paper and cardboard	3
Plastics	4.8
Metals	4.6
Textiles	6.8
Rubber	13.2
Glass	4.75
Others	9.85

Table 2: Comparison of landfill leachate characteristics of this study with other records

Parameters	Blang Bintang Aceh (this study)	Other parts of Indonesia[1]	Southeast Asia[2]
pH	7.95	7.6–8.5	7.4–8.46
Temperature (°C)	28.5	25.8–29.3	27.5–31
BOD (mg/L)	241	423–4,700	84–2,073
COD (mg/L)	4,177	143.5–4,000	590–2,920
NH_3 (mg/L)	1,708	0.196–11,324	7.5–850
NO_3 (mg/L)	625	3.7–638.8	3.2–20
NO_2 (mg/L)	27.5	0.117	22
PO_4 (mg/L)	14.97	[3)]	[3)]
Fe (mg/L)	14.82	0.6–1.8	1.447–12.81
Mn (mg/L)	5.94	0.4	0.001–0.3
Zn (mg/L)	9.7	0.06	0.2

[1)] Isnadina *et al*. (2019); Irfa'I *et al*. (2016); Yusmartini and Setiabudidaya (2013); Sukma and Widiadnyana (2020)
[2)] Xaypanya *et al*. (2018); Mohd-Salleh *et al*. (2020); Galarpe and Parilla (2012); Radzuan *et al*. (2005); Foul and Aziz (2009)
[3)] Data not available

NH_4-N and the pH of leachate tended to rise with the aging process. This suggests that as landfill leachate matures, there is a decrease in organic pollutants alongside an increase in ammonium levels and pH. In the current study, the analyzed leachate from the Blang Bintang landfill falls into the intermediate category within this aging classification. The relationship between leachate age and the BOD to COD ratio holds significant importance in evaluating the leachate's potential for biological treatment, as highlighted by Siracusa *et al*. (2020). Notably, the BOD to COD ratio tends to decrease as leachate ages, primarily due to the diminishing presence of biologically degradable organic components, as assessed by BOD. The observed decline in the BOD to COD ratio indicates a reduction in easily degradable organic matter over time, posing challenges for biological treatment methods. Understanding this intricate relationship is crucial for developing effective waste management strategies and refining leachate treatment procedures. Table 2 provides a comprehensive analysis of the characteristics exhibited by leachate obtained from the collection pond at the landfill leachate treatment plant within the Provincial Domestic Waste Management Unit in Blang Bintang, Aceh Besar Regency, Indonesia. This analysis is compared to leachate gathered from other locations in Indonesia and Southeast Asia. The leachate from the Blang Bintang landfill shares numerous similarities with leachate from other landfill sites across Indonesia and Southeast Asia, likely attributable to the shared tropical climate of these regions. Notably, the metal composition in the Blang Bintang leachate significantly differs from that of other areas, showing notably elevated levels of Zn, Mn, and Fe. This variation can be attributed to the heterogeneous composition of domestic solid waste at the Blang Bintang landfill, characterized by a lack of an adequate segregation mechanism. The COD and BOD concentrations in the treated leachate fell within the range observed in leachates from Indonesia and Southeast Asia.

The utilization of pond-based technology remains predominant in the landfill leachate treatment plant at the Provincial Domestic Waste Management Unit in Blang Bintang, Aceh. Initially, leachate from the landfill cell is collected and stored in a collection pond during the treatment process. The leachate characteristics presented in Table 2 are based on samples taken from this collection pond. Subsequently, the leachate undergoes treatment in an anaerobic pond to eliminate organic molecules. It is then directed to an anoxic pond with the primary objective of nitrogen removal, followed by transfer to an aeration pond to reduce concentrations of organic compounds and nitrogen. The final phase involves treatment within the stabilization pond. After undergoing a sequence of treatment steps, BOD and COD concentrations were measured at 117 mg/L and 350 mg/L, respectively. The concentrations of NH_3, NO_3 and NO_2 were recorded at 243 mg/L, 294 mg/L, and 337 mg/L, respectively. Initial concentrations of Fe, Mn, and Zn metals before leachate discharge into the aquatic environment were measured at 6.4 mg/L, 1.8 mg/L, and 6.8 mg/L, respectively. Despite the leachate undergoing previous biological processes before reaching the final pond, the BOD and COD levels had decreased, yet the BOD/

COD ratio indicated that their levels remained high. Even after getting the last pond, the BOD/COD ratio was greater than 0.5, suggesting that the leachate could undergo further biological treatment. Regarding the leachate effluent standard, the Government of Indonesia sets discharge into water bodies through the Minister of Environment and Forestry Regulation Number P.59/2016. The guidelines stipulate that treated leachate's maximum allowable COD and BOD are 300 mg/L and 150 mg/L, respectively. Although the leachate from Blang Bintang was essentially ready for discharge, COD and BOD were still four times higher than the allowable standard. While factors such as geographical location, climate, and waste composition can affect the leachate treatment process, the inability to meet the effluent standard highlights a weakness in the stabilization pond technology.

Distribution of microalgae and bacteria

The microalgae and bacteria in this study were meticulously identified through microscopic examination, utilizing a magnification of 400x. The comprehensive breakdown of the various types of microalgae and bacteria and their respective population sizes is presented in Table 3. Notably, a significant proportion of the identified bacteria belonged to the phylum cyanobacteria, characterized by their gram-negative characteristics. In contrast, the microalgae exhibited a broader taxonomic diversity, indicating a wider variety of microalgae species within the study sample. The most abundant bacteria identified in this experiment was *Centritractus belenophorus*. This bacterial type is characterized by its long and cylindrical shapes, featuring spines at both ends. The cell length and spine dimensions of *Centritractus belenophorus* ranged from 40-90 µm and 15-32 µm, respectively. Deže *et al*. (2020) explored the potential of microalgae and cyanobacteria communities as co-substrates for biogas production, with *Centritractus belenophorus* being one of the identified species in this community. The 42-day experiment yielded approximately 64% methane gas production from the microalgae and cyanobacteria community, with total biogas and volatile solid (VS) yields reaching 421.40 and 383.34 mL/g, respectively.

In this experiment, the second most abundant bacteria identified was *Gloeocapsa* sp., a member of the cyanobacteria phylum. Cyanobacteria have recently gained attention in the biomedical field due to their metabolites exhibiting antibacterial, antifungal, antiviral, anticancer, and antiplasmodial properties (Gacheva *et al*., 2013). Additionally, *Gloeocapsa* sp. is reported to have the potential to remediate heavy metals. Raungsomboon *et al*. (2008) reported that *Gloeocapsa* sp. can still grow at lead (II) (Pb^{2+}) concentrations of 0-20 mg/L, but the efficiency of Pb^{2+} metal removal decreases. At a low concentration of Pb^{2+} (2 mg/L), *Gloeocapsa* sp. can remove metal Pb^{2+} up to 100%. Besides lead (Pb) metal, *Gloeocapsa* sp. has been reported to remove other metals such as Zn, cadmium (Cd), and copper (Cu) (Pokrovsky *et al*., 2008). Another identified bacteria is *Microcystis* sp., which can produce the microcystin poison if it multiplies, potentially causing harm to plants and animals and making its presence in fresh waters less desirable. However, *Microcystis* sp. was reported to absorb nitrogen and phosphate (Xie *et al*., 2003). When it overgrows or blooms, *Microcystis* sp. can be used as a raw material for urea production, absorbing nitrogen and assimilating carbon from the environment (Krausfeldt *et al*., 2019). *Anabaena* sp., another type of bacteria identified in this experiment belonging

Table 3: Types and abundance of microalgae-bacteria consortium in leachate

Phylum	Genus	Species	Abundance (%)
Bacteria			
Ochrophyta	*Centritractus*	*Centritractus belenophorus*	28.14
Cyanobacteria	*Gloeocapsa*	*Gloeocapsa* sp.	25.97
Cyanobacteria	*Microcystis*	*Microcystis* sp.	24.24
Cyanobacteria	*Anabaena*	*Anabaena* sp	21.65
Microalgae			
Euglenazoa	*Euglena*	*Euglena sp.*	40.63
Cyanobacteria	*Spirulina*	*Spirulina sp.*	15.62
Bacillariophyta	*Synedra*	*Synedra acus*	14.38
Chlorophyta	*Closteriopsis*	*Closteriopsis longissima*	13.75
Ciliata	*Paramecium*	*Paramecium sp.*	12.5
Rotifera	*Trichocerca*	*Trichocerca sp.*	3.12

to cyanobacteria, has a cell size of about 6-10 µm. *Anabaena* sp. is known for its ability to fix nitrogen from the air and can potentially remove heavy metals. The biomass *Anabaena* sp. has been reported to remove up to 80% of Cd metal, with a maximum biosorption capacity of 162 mg Cd per gram of dry *Anabaena* sp. biomass (Clares *et al*., 2015). Additionally, *Anabaena* sp. has been reported to contribute to carbon dioxide removal (Chiang *et al*., 2011; Fernández *et al*., 2012). During the course of this study, *Euglena* sp. exhibited the highest prevalence among microalgae, followed by *Spirulina* sp., *Synedra acus*, *Closteriopsis longissima*, *Paramecium* sp., and *Trichocerca* sp., which were the subsequent most frequently identified species. *Euglena* sp. has garnered attention in the scientific community for its potential application in biofuel production, as highlighted in studies by Erfianti *et al.* (2023) and Indahsari *et al*. (2022). Additionally, *Euglena* sp. has demonstrated efficacy in reducing pollutants in wastewater, as supported by research conducted by Khatiwada *et al*. (2020) and Chiellini *et al*. (2020). In a study by Mahapatra and Chanakya (2013), *Euglena* sp. was investigated for cleaning domestic wastewater and generating biofuel, showing significant efficacy in removing 98% of ammonium and 92% of total organic carbon within eight days, with a substantial lipid content of 24.6% (w/w). The study underscores the potential of microalgae for dual purposes in wastewater treatment and biofuel production. *Spirulina* sp. was observed as the second most frequently occurring microalgae in this study, following *Euglena* sp. *Spirulina* species are acknowledged for their substantial protein content, as extensively documented by Khan *et al*. (2005). This high protein content makes *Spirulina* sp. suitable for human consumption, and studies by Jin *et al*. (2020), Mohadi *et al*. (2020), and Casazza *et al*. (2016) have highlighted its application as a dietary supplement for improving overall health. *Spirulina* sp. has also demonstrated resilience to and mitigation of the impacts of heavy metal pollution, further emphasizing its ecological importance and potential applications in industries and health-related fields.

Changes in the pH medium

Fig. 2 shows the variation in leachate pH during this experiment. The initial pH of the leachate in photobioreactors with and without CO_2 was 7.6 and 8.6, respectively. On the first day, the pH with the addition of CO_2 dropped to 7.1 but increased to 9.3 on the seventh day and was stabilized at the end of this experiment (9.379 ± 0.067). Meanwhile, the pH of the photobioreactor without CO_2 addition leachate tended to be stable from day 0 to day 20, which was around 9.28 ± 0.18. The pH of the medium is a factor affecting the growth of the microalgae-bacteria consortium (Khan *et al*., 2018). In this study, the identified microalgae-bacteria consortium had an optimum pH of 7-9. Changes in leachate pH in both photobioreactors were still in the optimum range for the microalgae-bacteria consortium. The most significant shift in pH occurred on the first day of the photobioreactor with the addition of CO_2 gas (Sutherland *et al*. 2014). This phenomenon was attributed to the occurrence of carbon fixation in the photosynthesis process,

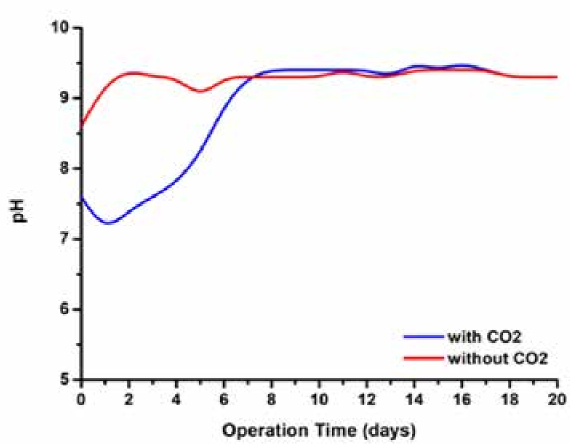

Fig. 2: Changes in the pH medium in the photobioreactor

facilitating the accumulation of OH^- ions in the medium (Shahid *et al*., 2020). The increase in pH in photobioreactors, attributed to the presence of CO_2, was controlled by the metabolic activity of microalgae. Sayadi *et al.* (2016) stated that an increase in pH value also showed a rise in microalgae growth. The observed elimination of pollutants in this investigation was not significantly affected by the variations in leachate pH, as shown in Figs. 3 and 4. Although there was an initial reduction in pH due to the addition of CO_2, the removal of COD, ammonia, nitrate, nitrite, and phosphates persisted. Both photobioreactors showed minimal variations in the reduction of pollutant concentrations due to a decrease in pH levels, which remained in the ideal range for the specific microalgae consortium found in this study. Consequently, the metabolic activity of microalgae persisted, facilitating the continuous elimination of pollutants in leachate.

Microalgae-bacteria consortium interaction in pollutant removal

The majority of microalgae applications for wastewater treatment focused on removing nitrogen and phosphorus compounds while generating biomass (Hernández-García *et al*., 2019). Microalgae require CO_2 for photosynthesis, and therefore, combining microalgae wastewater treatment with industrial CO_2 capture is common. However, if CO_2 levels are insufficient, additional CO_2 may be needed, resulting in higher costs. An alternative approach is the combination of microalgae with bacteria, requiring oxygen and producing CO_2 during metabolism that microalgae can supply through photosynthesis. This symbiotic microalgae-bacteria relationship offers excellent benefits for wastewater remediation. Each organism plays a distinct role in remediating wastewater in the microalgae-bacteria consortium. Bacteria contribute by enhancing the removal of organic carbon compounds, supplying vitamins and hormones that stimulate microalgal growth, and providing CO_2 to microalgae. Meanwhile, microalgae supply oxygen to bacteria, improve nitrogen and phosphorus elimination efficiency, and generate valuable biomass (Fallahi *et al*., 2021). The mechanism of wastewater pollutant removal relies on the symbiotic interaction between the microalgae and bacteria. Bacteria metabolize degradable organics and some nitrogen/phosphorus compounds, producing CO_2 and phytohormones that facilitate microalgal growth. Through photosynthesis, the microalgae consume the CO_2 and inorganic nutrients, generating oxygen for bacterial reuse and increased algal biomass. This microalgae-bacteria interplay forms resilient cell flocs able to withstand environmental disturbances (Jiang *et al*., 2021).

COD removal

Fig. 3 illustrates the reduction in COD concentration within the photobioreactor. The initial COD concentration in the leachate at the start of the experiment was measured at 233 mg/L. Throughout the investigation, the COD concentration in the photobioreactor displayed a consistent pattern, steadily decreasing. This trend was observed both in the presence and absence of CO_2, with final concentrations measured separately at 55 mg/L

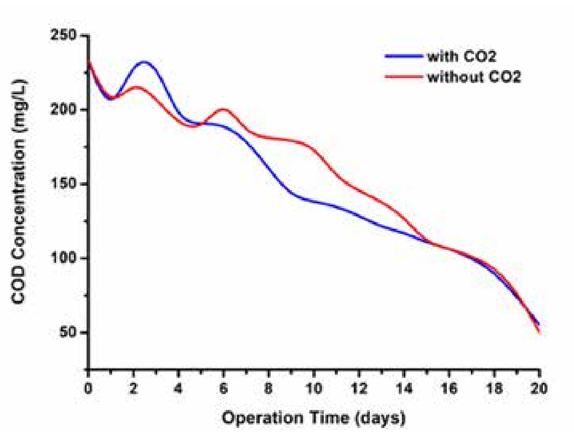

Fig. 3: COD elimination in the photobioreactors

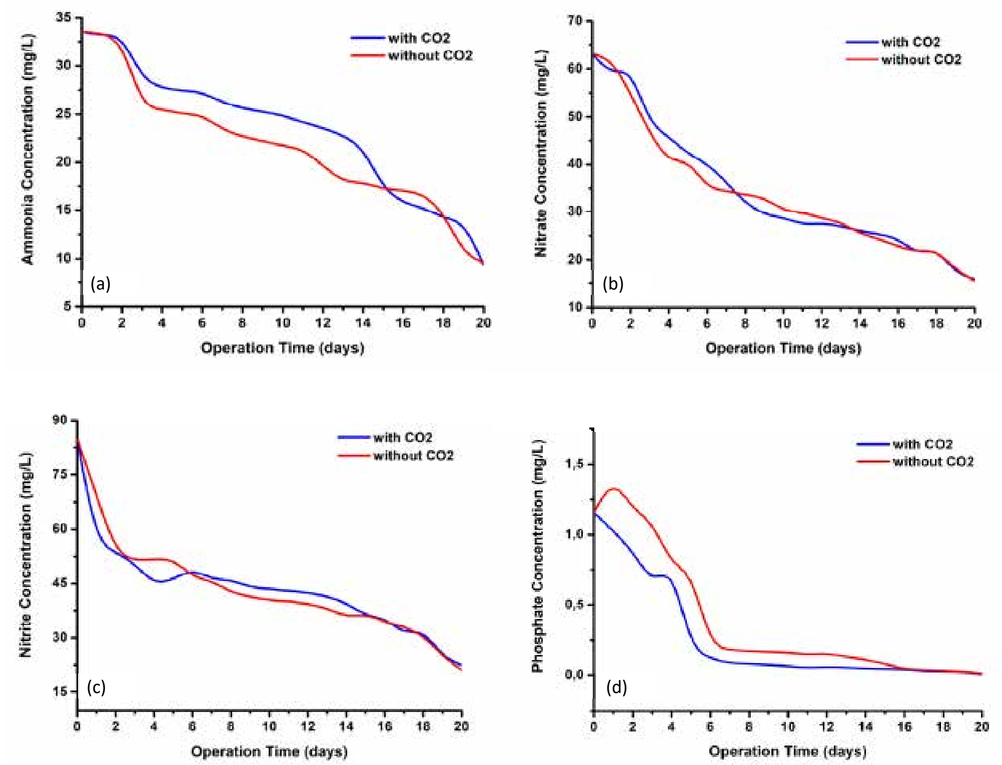

Fig. 4: Changes in (a) ammonia, (b) nitrate, (c) nitrite, and (d) phosphate concentration during the course of the experiment

and 50 mg/L. This observation aligns with the initial assumption made in this paper's introduction, suggesting that the microalgae and bacteria cultures cultivated in the leachate treatment pond possess resistance to the leachate's characteristics, facilitating a rapid acclimatization process. During the COD removal process, the concentration of the microalgae-bacteria consortium incubated in the photobioreactor increased. The initial consortium concentration at the start of the experiment was 28.645 mg/L. After 20 days, the concentrations reached 117.65 mg/L and 109.40 mg/L in the reactors with and without CO_2 addition, respectively. Consequently, to achieve approximately 75% COD removal, a minimum of 80.8 mg of microalgae-bacteria consortium per liter was required. This COD removal occurred through assimilation by the identified microalgae and bacteria species listed in Table 3. In comparison to previous studies, this study's 75% COD removal is comparable to the 77.14-81% efficiency reported by Tighiri and Erkut (2019), who also utilized a microalgae-bacteria consortium for leachate pollutant elimination. This consistency suggests that the COD removal results obtained here are in line with prior research using similar mixed microalgal-bacterial approaches for treating landfill leachate. In a recent laboratory study, Chang *et al*. (2023) tested a microalgae-bacteria consortium for treating dairy manure wastewater and achieved a COD elimination efficiency of 68.4 to 76.8% for organic matter removal. While slightly lower than the 75% COD removal obtained in this study, it further supports the reliable and promising capability of the microalgae-bacteria consortium in eliminating COD from various wastewater streams. In summary, this study contributes to the growing body of evidence showcasing the efficacy of the microalgae-bacteria consortium in COD removal from diverse wastewater sources.

The interaction between oxygen and carbon dioxide transfer within a microalgae-bacteria consortium is a crucial metabolic process. During this interaction, microalgae engage in photosynthesis, producing oxygen, which is then utilized by bacteria to oxidize organic compounds. Simultaneously, carbon dioxide is released as a by-product of bacterial metabolism, serving as the carbon source for microalgae (Oviedo *et al*., 2022). This collaborative interaction results in no significant difference in pollutant degradation performance between photobioreactors with and without CO_2 addition. This is because the carbon needs of microalgae are met through bacterial activity, marking a notable departure from photobioreactors relying solely on microalgae. The introduction of carbon dioxide in a system exclusively dependent on microalgae significantly influences their cultivation efficacy. Unlike microalgae-bacteria consortia, carbon supply in these systems depends on the culture medium and externally provided CO_2, as highlighted by Chaudhary et al. (2020) in their laboratory-scale study on *Chlorella vulgaris* in wastewater treatment photobioreactors supplied with either CO_2 (5% v/v) or regular air (0.03% CO_2 v/v). Their findings showed that photobioreactors with CO_2 addition achieved a 7% greater COD reduction and increased nitrogen removal rates by up to 16%. The microalgae-bacteria consortium has also been reported to reduce pollutant concentrations such as COD, ammonia, nitrate, nitrite, and phosphate (Fito and Alemu, 2019; Rossi *et al*., 2020). The results of this investigation demonstrate comparable COD removal outcomes between photobioreactors with and without CO_2 addition. Throughout the experiment, the COD concentration consistently decreased in both conditions, irrespective of the presence or absence of carbon dioxide. These findings align with the results reported by Thongpinyochai and Ritchie (2014), indicating a consistent and gradual decrease in COD levels over time. Thongpinyochai and Ritchie (2014) aimed to examine the potential of *Chlorella vulgaris*, a green microalgae species, in reducing COD concentrations in leachate. In the present experimental setting, *Chlorella vulgaris* achieved a COD elimination percentage of 51.05%, signifying its significant role in reducing COD levels within this specific environmental context.

Nitrogen and phosphate removal

Nitrogen removal in traditional sewage treatment relies heavily on the nitrification process, which requires oxygen. Mechanical aeration is commonly used to fulfill this oxygen requirement. However, implementing a microalgae-bacteria consortium can potentially reduce the need for substantial mechanical aeration. The microalgae consortium contributes by providing oxygen for nitrifying bacteria to carry out nutrient assimilation in wastewater, as demonstrated by Jia and Yuan (2016). Moreover, the nitrogen removal process within this consortium is not solely dependent on bacterial activity; it is also significantly influenced by the assimilation capacity of the microalgal biomass, as noted in the study by Oviedo *et al*. (2022). Fig. 4 illustrates the removal of nitrogen-phosphate compounds. The initial ammonia concentration in the photobioreactor was 29 mg/L. On the last day of the experiment, the photobioreactor's ammonia concentration with CO_2 and without CO_2 addition was 9.36 mg/L and 9.585 mg/L, respectively. The percentage of ammonia removal with the addition of CO_2 was more significant than that without CO_2, namely 72.09% and 71.42%, respectively. In comparison to the research conducted by Chang *et al*. (2023), the present study demonstrates a comparatively diminished efficacy in eliminating ammonia. Chang *et al*. (2023) documented a noteworthy achievement in the field of wastewater treatment, specifically in the context of dairy manure wastewater, with an ammonia removal efficiency reaching as high as 99%. The observed disparity underscores the significance of considering the intricacy and structure of the treatment system to achieve the most favorable outcomes in removing specific pollutants, such as ammonia, in wastewater treatment research. The initial concentration of nitrate was 63 mg/L, and there was no significant difference in nitrate and nitrite reduction between photobioreactors supplemented with and without CO_2 gas. The concentration of nitrate at the experiment's conclusion was 15.875 mg/L and 15.568 mg/L, respectively, for the photobioreactor with CO_2 and without CO_2, corresponding to the removal percentages of 74.86% and 75.35%, respectively. The initial concentration of nitrite in the photobioreactor was 85 mg/L. The removal of nitrite in the photobioreactor with and without the addition of CO_2 gas at the end of the experiment was 73.52% and 75.12%, respectively. At the start of the investigation, the phosphate concentration was 1.157 mg/L. Phosphate concentration decreased significantly on the seventh day in both photobioreactors. On the following days, the phosphate concentration continued to decline to 0.008 mg/L and 0.012 mg/L, respectively, for photobioreactors with added CO_2 and without CO_2, corresponding to the removal percentage of 99.29% and 98.94%, respectively.

The observed higher percentage of nitrate removal without the addition of CO_2 gas, as noted in this experiment, aligns with findings by Sutherland *et al*. (2014), who reported that the concentration of nitrate is lower without the addition of CO_2. The results of this experiment further corroborate the nitrogen-phosphate removal efficacy discussed by Olguin (2012), emphasizing the relatively high nitrogen removal rate exhibited by *Euglena* sp. It is essential to recognize that the efficiency of nitrogen and phosphorus removal can be influenced by various factors, including the composition of the medium and environmental conditions such as initial nutrient concentration, light intensity, nitrogen/phosphorus ratio, light/dark cycle, and algae species (Aslan and Kapdan, 2006). Based on this experiment's findings, it can be inferred that employing a consortium of microalgae and bacteria holds considerable potential as a viable and environmentally sound strategy for pre-treating leachate wastewater originating from a landfill in the Aceh region. However, it is imperative to acknowledge that the efficacy of employing a microalgae-bacteria consortium for leachate treatment could be contingent upon various elements, including the distinct attributes of the leachate, the configuration of the treatment system, and pertinent environmental restrictions in the given locality. Further research and initial inquiries may be needed to refine the process and ensure adherence to regulatory standards. In conclusion, this study's findings indicate that utilizing a consortium consisting of microalgae and bacteria exhibits promise as a feasible approach for the treatment of landfill leachate. This methodology demonstrates the ability to decrease operational expenses while effectively mitigating environmental issues related to landfill leachate.

Application of microalgae-bacteria consortium in landfill leachate treatment

Experimentally, using a microalgae-bacteria consortium demonstrated the successful removal of COD, ammonia, nitrate, nitrite, and phosphate from Blang Bintang landfill leachate. However, translating this technology into real-world applications for leachate treatment necessitates additional time and comprehensive investigations. Further testing is imperative, particularly in the areas of microalgae harvesting methods from leachate, assessment of microalgal lipid content cultivated on leachate, and the design of integrated treatment systems. While the application of microalgae-bacteria consortium promises to be efficient, effective, and economical, challenges must be addressed. Some bacteria exhibit parasitic tendencies towards microalgae, thereby diminishing both biomass quality and quantity. In the diverse microbiomes present in leachate, other organisms such as fungi, protozoa, or zooplankton may directly or indirectly inhibit microalgal growth, as highlighted by Jiang *et al*. (2021). A comprehensive and in-depth research approach is necessary to tackle these challenges. Furthermore, exploring the potential of harnessing microalgae consortium for biofuel production from leachate could significantly contribute to the sustainability of the treatment process. Although the microalgae-bacteria consortium exhibits promise for leachate treatment, the successful real-world application requires further optimization and a holistic understanding of the intricate dynamics involved. The ongoing research and refinement efforts are essential for realizing the full potential of this innovative approach in addressing the complex challenges posed by landfill leachate.

CONCLUSION

The effectiveness of pollutant degradation in landfill leachate was assessed through experimental investigations utilizing a consortium of microalgae and bacteria in two distinct photobioreactors, with and without the inclusion of CO_2 gas supplementation. The experimental results showcased the remarkable efficiency of the consortium consisting of microalgae and bacteria. The study's findings indicated a substantial decrease in COD by 75%, suggesting that the employed approach is highly effective in removing organic compounds from landfill leachate. Additionally, the consortium demonstrated significant efficacy in eliminating various nitrogen compounds, including ammonia, nitrate, and nitrite, with removal rates reaching as high as 75%. Remarkably, it also exhibited outstanding efficacy in eliminating phosphate compounds, achieving an impressive removal rate of 99%. One of the microalgae species identified in this study was *Euglena* sp., known for its remarkable ability to remove nitrogenous and phosphoric compounds. Surprisingly, regardless of the presence or absence of CO_2, the elimination of pollutants continued to exhibit a high level of efficacy, indicating that the inclusion of CO_2 did not substantially influence the overall pollutant removal process. Several factors may potentially elucidate the underlying causes for this occurrence. A mutually beneficial connection known as synergism is commonly observed in wastewater treatment processes incorporating microalgae and bacteria. Microalgae engage in photosynthesis, generating oxygen as a by-product, which becomes advantageous for bacteria relying on aerobic metabolic activities. Conversely, bacteria have the capacity to supply microalgae with organic carbon molecules, serving as nourishing sustenance. This symbiotic relationship allows for the recycling of carbon within the system. As these organisms undergo growth and reproduction, they progressively amass carbon within their biomass. The carbon can subsequently be extracted and utilized as a final product, such as biomass, for producing biofuels, mitigating the need for additional CO_2. Notably, the utilization of microalgae and bacteria in the wastewater treatment process capitalizes on their inherent capacity to metabolize carbon dioxide and the carbon compounds naturally present in the wastewater. This approach enhances the sustainability and environmental friendliness of the process by eliminating the requirement for an external source of CO_2 gas.

AUTHOR CONTRIBUTIONS

N. Emalya conceived, designed and conducted the experiment, sample collection and analytical measurements and prepared the manuscript draft. E. Munawar contributed to the design of the experiment and analytical measurements. Suhendrayatna supervised the first author, contributed to data analysis and examined the paper draft. Tarmizi supervised the first author and contributed to setting the experimental arrangements and data analysis. Y. Yunardi, the corresponding author, supervised the first author, conceived the research idea, designed the experiment, interpreted the results and finalized the manuscript.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

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PUBLISHER'S NOTE

ABBREVIATIONS

%	Percent
µm	Micrometer
^0C	Degrees Celsius
BG-11	Blue-green medium
BOD	Biological oxygen demand
Cd	Cadmium
cm	Centimeter
CO_2	Carbon dioxide
COD	Chemical oxygen demand
Cu	Copper
Fe	Iron
ha	Hectare
Ind.	Individuals
Ind./L	Individuals per liter
L	Liter
L/min	Liter per minute
mg	Milligram
mg/L	Milligram per liter

mL	Milliliter
mL/g	Milliliter per gram
Mn	Manganese
NH_3	Ammonia
NH_4-N	Ammonium nitrogen
nm	Nanometer
NO_2	Nitrite
NO_3	Nitrate
Pb	Lead
Pb^{2+}	Lead (II)
pH	Potential of hydrogen
PO_4	Phosphate
rpm	Revolutions per minute
UV	Ultraviolet
UV-Vis	Ultraviolet-visible
VS	Volatile solid
v/v	Volume concentration
w/w	Weight concentration
Zn	Zinc

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