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Biogas quality and nutrient remediation in palm oil mill effluent through Chlorella vulgaris cultivation using a photobioreactor

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ABSTRACT

BACKGROUND AND OBJECTIVES: During this energy transition, research is being done to develop sustainable ways to support the shift to a decarbonized energy and production system. These ways include using renewable energy sources to promote circularity in products, green technologies, and safer procedures. Anaerobic digestion of palm oil mill effluent is a beneficial process for generating biogas, while the waste can also be utilized as fertilizer. The biogas can be further refined into biomethane, a valuable resource commonly used in transportation and power generation. The objective of this study is to examine the enhancement of biogas from Palm oil mill effluent and the elimination of sludge nutrients by utilizing microalgae Chlorella vulgaris. The microalgae will be cultivated in a modified photobioreactor to enhance the capture of carbon dioxide.

METHODS: The study utilized anaerobic batch reactor digesters. A modified photobioreactor, consisting of two columns separated by a membrane, was developed for the technological advancement of biogas upgrading, specifically for carbon dioxide capture and biogas upgrading. A technological gap in biogas upgrade technology innovation is filled by the improved photobioreactor. To optimize the bio-fixation of carbon dioxide from flue gas, it is essential to carefully select a suitable strain of microalgae that possesses both a strong ability to absorb carbon dioxide and a high tolerance to varying concentrations of this gas. By choosing the right strain, the efficiency of carbon dioxide removal can be significantly enhanced. Since Chlorella vulgaris microalgae have demonstrated this potential, they were chosen for this investigation. Microalgae also play a role in removing nutrients contained in the sludge.

FINDINGS: Numerous chemical and biological methods have been used to upgrade biogas. Results of biological upgrading of biogas from palm oil mill effluent have been reported, with carbon dioxide removal reaching 89 percent until the methane concentration of the biogas is upgraded to 84 percent. The highest biomass of 1,835 grams per liter was achieved by culturing the microalgae Chlorella vulgaris in laboratory-scale photobioreactors. In this study, the application of 15 percent volume per volume biogas with an optical density of 0.4 was found to be optimal for the growth of the microalgae. The cultivation period lasted for 14 days. The peak biomass production was observed due to the achievement of a remarkable 98 volume per volume efficiency in carbon dioxide removal, which subsequently led to a significant rise in methane content, reaching 60 percent. The enhanced biogas achieved a peak methane content of 98 percent, indicating a significant improvement in quality.

CONCLUSION: The findings of this study, conducted using a modified photobioreactor, indicate that Chlorella vulgaris demonstrated high efficacy in the removal of carbon dioxide, with a rate of up to 90 percent. Additionally, it exhibited remarkable performance in upgrading biogas derived from palm oil mill effluent, achieving a conversion rate of up to 98 percent. The optical density of microalgae at 0.4 played a crucial role in these processes. Furthermore, Chlorella vulgaris showcased its ability to effectively eliminate nutrient nitrogen, reaching a removal rate of 90 percent at an optical density of 0.2. Moreover, it demonstrated a phosphate removal rate of 80 percent at an optical density of 0.4.

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INTRODUCTION

Indonesia's palm oil sector is rapidly growing due to the country being the top global producer of palm oil. In 2021, Indonesia harvested 46.9 million tons of palm oil from 16.8 million hectares (ha) of oil palm farms. (Suardi *et al*., 2022). Waste of many kinds, particularly solid and liquid waste, is produced during the production of palm oil (Pascoal *et al*., 2021). Palm oil mill effluent (POME) is the primary liquid waste of the palm oil industry. Around 0.7 to 0.8 cubic meter (m^3) of palm oil mill effluent is produced for every tonne of fresh oil palm bunches. After processing, POME typically has a high temperature of between 70 and 800 degrees Celsius (°C), an potential of hydrogen (pH) of 4.56 to 4.98, a chemical oxygen demand (COD) of between 57,000 and 60,400 milligram per liter (mg/L), and a total suspended solids (TSS) of between 0.23 and 5.44 gram per liter (g/L) (Chia *et al*., 2020). POME has a rich source of organic matter, Volatile fatty acids (VFAs), hydrogen (H_2), carbon dioxide (CO_2), and essential nutrients (Table 1). Anaerobic digestion is facilitated by these components, which in turn stimulate microbial activity and result in the production of biogas. Organic matter serves as substrates for methane (CH_4) and CO_2 generation, while VFAs and H_2 are intermediates during this process. The presence of essential nutrients facilitates the growth and activity of microorganisms, thereby making palm oil mill effluent (POME) a valuable resource for generating renewable energy and managing waste in the palm oil industry. (Malik *et al*., 2020).

POME comprises a diverse range of nutrients, including both macro and micronutrients that are crucial for the growth and metabolic functions of microorganisms. (Nur and Buma, 2019). Among the macronutrients, POME typically contains significant levels of nitrogen, phosphorus, and potassium, ranging from 0.18 to 1.4 g/L for nitrogen, 0.094 to 0.13 g/L for phosphorus, and 1.28 to 1.92 g/L for potassium. These essential nutrients are crucial for the growth of microorganisms and for carrying out important biochemical reactions in biological treatment systems. Additionally, POME contains several micronutrients vital for enzymatic activities and cellular functions. These micronutrients include calcium (ranging from 0.27 to 0.40 g/L), iron (0.07 to 0.16 g/L), magnesium (0.25 to 0.34 g/L), manganese (0.021 to 0.004 g/L), zinc (0.0012 to 0.0018 g/L), and cobalt (0.04 to 0.06 g/L). Collectively, the diverse array of nutrients present in POME supports microbial communities and facilitates biodegradation processes, underscoring the potential for utilizing POME as a nutrient-rich substrate in various biotechnological applications. If high COD concentration is discharged into the sewage without proper treatment, it can have detrimental effects on aquatic life, leading to fish mortality and contamination of the food chain. Anaerobic digestion is considered the most efficient treatment method for waste discharges containing a high level of organic matter. Anaerobic degradation, also referred to as the fermentation of organic matter by anaerobic bacterial activity in the absence of free oxygen (O_2), is the transformation process that converts organic matter from suspended to dissolved form and generates biogás (Mahmod *et al*., 2020). Anaerobic digestion (AD) stands out as a highly sustainable and energy-efficient approach to bioenergy production, making it one of the most environmentally friendly methods available. Anaerobic waste treatment is a biochemical process that decomposes intricate organic substances into biogas, which is a sustainable energy source (Mulu *et al*., 2021). Anaerobic digestion offers several advantages compared to aerobic digestion. The anaerobic process requires less energy for aeration, reducing treatment costs. It also produces less sludge compared to the aerobic process. Moreover, most pollutants are converted into biogas, specifically methane gas, which can be used as an alternative energy source. A consortium of bacteria collaboratively engage in syntrophic interactions to fulfill their individual requirements while performing anaerobic processes. The bacterial consortium involved in the anaerobic process comprises the hydrolysis, acidification, acetogenesis, and methanogenesis processes. Anaerobic digestion entails a sequence of interconnected processes. The CO_2 content of biogas results in low combustion value. To increase the combustion value, the biogas purification method carried out uses microalgae *Chlorella* sp. utilizing the photosynthesis process carried out by microalgae. Biogas purification methods utilizing microalgae offer a cost-effective solution that effectively mitigates CO2 levels (Bosea *et al*., 2019). Over the past few decades, microalgae have garnered considerable attention as a viable replacement for traditional fossil fuels in various

Table 1: Characteristic of POME (Ahmad *et al*., 2019)

Parameters	Concentrations
COD (g/L)	15.0-100.0
BOD (g/L)	10.25-43.75
Total solid (g/L)	11.50-79.0
Total suspended solid (g/L)	5.0-54.0
Total volatile solid (g/L)	9.0-72.0
Total nitrogen (g/L)	0.18-1.4
Oil and grease (g/L)	0.13-18.0
Lignin (g/L)	4.7
Phenolics (g/L)	5.8
Pectin (g/L)	3.4
Carotene (g/L)	0.008
Calcium: Ca (g/L)	0.27-0.40
Cobalt: Co (g/L)	0.04-0.06
Iron: Fe (g/L)	0.07-0.16
Magnesium: Mg (g/L)	0.25-0.34
Manganese: Mn (g/L)	0021-0.004
Phosphorus: P (g/L)	0.094-0.13
Potassium: K (g/L)	1.28-1.92
Zinc: Zn (g/L)	0.0012-0.0018

studies (Tan *et al*. 2022). The effects of light intensity and CO_2 supply on microalgal development, bio-fixation efficiency, and nutrient elimination have been the subject of numerous investigations (Ali *et al*., 2023). The effects of CO_2 concentrations ranging from 1 to 20 percent (%) on *Chlorella vulgaris* (*C. vulgaris*) in household wastewater were assessed by Liu *et al*. (2023). The primary objective of the current study is to delve into the anaerobic processes exhibited by photosynthetic bacteria and microalgae, which give rise to the generation of CO2. Variations in the raw materials used in anaerobic digestion may indicate an uneven composition of the biogas produced. The utilization of POME as the biogas substrate in this study was based on its inclusion of methanogenic bacteria, which served as the source for biogas production. Compared to CO_2, methane (CH_4) has a larger heat capacity (Yong *et al*., 2023). CO_2 may have an impact on the quality of biogas, which could lead to incomplete combustion (Paulauskas *et al*., 2023). An efficient purification process is necessary to remove CO2 gas from biogas. In this study, various parameters such as biomass, density, biogas concentration, temperature, pH, and COD were measured to investigate the purification process. Microalgae possess a fundamental composition and exhibit remarkable efficiency in photosynthesis, enabling them to endure and flourish in extreme conditions such as elevated salinity levels, severe temperatures, and exposure to heavy metals and nutrients. Due to its benefits of being sustainable, low-carbon, and promising, numerous attempts have been made to investigate an integrated system that reconciles the upgrading of biogas with slurry treatment from anaerobic digestion (Zhang *et al*, 2022) which is because of the aforementioned advantages (Rodero *et al*., 2020). Various methods have been employed to document the enhancement or rise in biogas and biomethane production, along with the utilization of traditional, emerging, or advanced technologies for the purification, enrichment, and refining of biogas. It is advised to grow *Chlorella* sp. in both high and low light levels. A low proportion of CO_2 can prevent microalgae growth, according to Cantera *et al*. (2021). If the CO_2 percentage is more than 15%, it offers enough support for the growth of microalgae to balance their carbon intake, a high concentration of CO_2 is required (Bai *et al*.,2021). Numerous chemical and biological methods have been used to enhance the quality of biogas. Results of biological upgrading of biogas from POME have been reported, with CO_2 removal reaching 89% until the CH_4 concentration of the biogas is upgraded to 84%. The microalgae *C. vulgaris* is grown in laboratory-scale photobioreactors (Hajinajaf *et.al*., 2022). Conventionally, physicochemical techniques are used to remove CO_2 and other contaminants like sulfuric acid from biogas in order to enhance its

quality (Ramanathan *et al*., 2020). Physical separation techniques include, among others, membrane separation, pressure swing adsorption, and cryogenic separation; chemical techniques include CO_2 absorption using solvents or mineral carbonation (Kapoor *et al*., 2019). Despite being commercialized, these technologies still have notable drawbacks. These include energy penalties, which account for 3-6% of the energy content of biogas, and high costs, reaching up to 30% of the overall cost of upgraded biogas (Ahmed *et al*., 2021). Negative emission technologies are essential given the urgency of achieving the Paris Agreement's targets to prevent climate change risks, according to the European Academies Science Advisory Council (EASAC) (Erickson and Brase, 2019). Utilizing bioenergy in conjunction with carbon capture and reuse is a method to decrease the carbon footprint of biogas systems (Bosea *et al*., 2019). Microalgae cultivation and the biological enhancement of biogas seem to be a practical and efficient approach for generating supplementary revenue and valuable commodities (Ooi *et al*., 2023). Although the causes for the microalgae's biological methane consumption are yet unknown, the majority of them that were chosen for biogas upgrading can survive the typical CH_4 levels in biogas (Ruiz-Ruiz *et al*., 2020). Glover and Besley (2021) found that when cultured in methane concentrations of 0%, 50%, and 100%, *Nannochloropsis gaditana* (wild type) showed no effect on biomass concentrations and growth rates. POME integration with microalgae production has provided a long-term solution for reducing contaminants in wastewater and final effluents from POME. This potential to contribute to an alternate culture medium for microalgae development requires more investigation (Resdi *et al*., 2016). POME as a nutrient-rich media promoted *Chlorella pyrenoidosa* growth and significantly reduced the levels of organic and inorganic contaminants (Kamyab *et al*., 2018). Microalgae possess the ability to store lipids and retain the essential nutrients found in wastewater, thereby aiding in the process of wastewater treatment. The presence of adequate light and essential nutrients like nitrogen and phosphorus are crucial factors for the growth of microalgae (Kamyab *et al*., 2019). Three microalgal strains demonstrated adaptation in the presence of 50% CH_4 and 50% CO_2, similar to the composition of biogas: *Chlorella* sp., *C. protothecoides*, and marine *Chlorella* sp. The marine strain showed strong growth and CO_2 removal (Srinuanpan *et al*., 2019). The state of the art in this study is that biological approaches are considered a promising alternative, owing to their economic competitiveness and enhanced environmental sustainability. This approach relies on the utilization of microbial communities, which can be effectively employed even in limited settings. This study stands out for its innovative approach, utilizing a customized photobioreactor to enhance the efficiency of CO_2 absorption by microalgae. The cultivation of microalgae often involves the use of a single-column photobioreactor, however, this research utilizes a two-column photobioreactor separated by a membrane. The incorporation of membranes represents a sophisticated approach to streamline the process of harvesting microalgae biomass. This particular design sets itself apart from prior studies on POME biogas upgrading. Since *C. vulgaris* microalgae have demonstrated this potential (Handayani *et al*., 2020), they were chosen for this investigation. Phycoremediation, or the use of microalgae to remediate wastewater, has three mechanisms: biosorption, bioaccumulation, and biodegradation. Biosorption involves the use of a biological material as a sorbent to passively absorb and concentrate contaminants from water. Bioaccumulation and biosorption mechanisms are fundamentally separate processes; quantifying biosorbed and bioaccumulated contaminants is difficult since the two systems are dynamically interchangeable (Tang *et al*., 2020). Biodegradation is an extremely effective method for removing pollutants from waste as it decomposes complex substances into basic and harmless chemical components. Bioaccumulation and biosorption involve the use of microorganisms as biological filters to concentrate pollutants and separate them from the water, unlike phytoremediation. Microalgae collect contaminants in addition to nutrients and microelements (Song *et al*., 2019). Microalgae's POME remediation method comprises biodegradation mechanisms. This study hypothesis is that after biogas upgrading by *C. vulgaris*, the final biomethane created can be used as a direct substitute when CH_4 levels exceed 96%, which is the same as natural gas. Furthermore, *C. vulgaris* effectively eliminates the nitrogen and phosphate nutrient content present in

the sludge. The modified photobioreactor used in this study contains two chambers, although photobioreactors typically only have one. The modification creates two columns in the photobioreactor: one for CO_2 capture and the other for biogas upgrading, and it is positioned in the center of the reactor that collects CO_2 from biogas, separated by a membrane. The enhanced photobioreactor effectively bridges the technological divide in biogas upgrade technology innovation. An appropriate strain of microalgae with a high capacity to absorb CO_2 and a high tolerance to CO_2 concentrations should be chosen to maximize the bio-fixation of CO_2 from flue gas. This study aims to improve POME through biogas generation and recover sludge nutrients using *Chlorella vulgaris* in a specialized photobioreactor to increase CO_2 sequestration. The efficiency of CO_2 elimination was enhanced by improving CO_2 absorption in an adapted photobioreactor. The research was conducted at The Study Center for Sustainable Production System and Life Cycle Assessment in Serpong, South Tangerang, Banten, Indonesia from 2023-2024.

MATERIALS AND METHODS

POME Characterisation

Analysis of chemical and physical characteritics of POME was done in the Chemical Analytic Laboratory, National Research and Innovation Agency's laboratory at Serpong, South Tangerang, Banten, Indonesia. The analyzed characteristics of POME include fiber, fat, ash, nitrogen, phosphate, phenol, potassium, sulphate, ammonia, Fe, COD, BOD, suspended solids, carbon to nitrogen (C/N) ratio, and pH.

Microalgae and POME

Chlorella vulgaris was collected at The Research Center for Sustainable Production System and Life Cycle Assessment, National Research and Innovation Agency's laboratory at Serpong, South Tangerang, Banten, Indonesia. The media used to cultivate *C. vulgaris* is Bold Basal medium, a nutrient-rich culture medium often used for microalgae cultivation (Dani *et al.*, 2021). Cells were incubated for 6 days in a 2 L Erlenmeyer flask with aeration and constant light conditions (75 transpiration micromolar per square meter per second (tμmol/m^2s). The microalga was cultivated under controlled conditions at a temperature of 20 ± 2 degrees Celsius to ensure optimal growth and development. The POME samples were taken from the palm oil sector in Cikasungka Bogor, West Java, which was packed in a container and then transported to the laboratory to be preserved at 5°C to keep the POME from degrading.

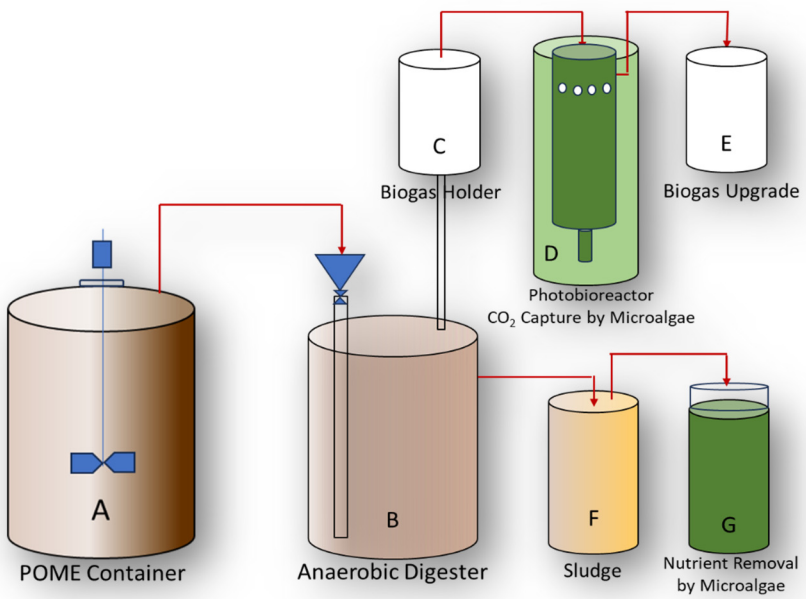
Anaerobic POME digestion

For this study, two anaerobic batch reactor digesters with a combined working volume of 10 liters were utilized. Fig. 1 shows the representation system of the biogas reactors and photobioreactor. The initial digester served as a control, while the subsequent digester was referred to as a treatment digester. In the treatment digester, POME was utilized as a substrate along with a biogas bacterial starter, whereas in the control digester, POME was solely employed as a substrate. The digester was equipped with a mechanical agitation mechanism, which was programmed to operate at 120 rpm for a duration of 10 minutes every 6 hours.. The temperature outside the digester was maintained at 30 ± 1°C. By adding 1 M sodium hydroxide (NaOH) as needed, the pH was kept between 6.5 and 7. Before being used in the *C. vulgaris* culture medium, the sludge digest was gathered and stored in a container. Prior to reaching the microalgae in the photobioreactor, the methane produced from the biogas process was first collected in the two digesters connected to the biogas holder. Daily monitoring of temperature and pH was conducted until a decrease in biogas production was observed. The biogas production was quantified with a manometer, while gas chromatography was utilized to analyze the composition of the biogas.

Microalgae cultivation

The experiments were conducted using a modified transparent photobioreactor (PBR) that was advanced and had a usable volume of 0.9 m3. The photobioreactor was divided into two columns by a membrane with a capacity of 0.45 m3, as shown in Fig. 1). Throughout the duration of the experiment, the PBR remained illuminated at a constant intensity of 4800 lux for both eight and sixteen-hour intervals, obviating the requirement for supplementary lighting. A specific growth medium tailored for the cultivation of microalgae was employed, resembling the basal media typically utilized in laboratory settings. The entire process extended over a period of thirty days to monitor the progression and maturation of

(a)



(b)

Fig.1: a) Photograph of the experimental setup: 1. Anaerobic bath digester, 2. Floating gas holder, 3. Photobioreactor, 4. Upgrade biogas holder, 5. Effluent/sludge, 6. Nutrient remove. b) Schematic diagram of process system biogas upgrade and nutrient removal by microalgae.

Table 2: Treatment variables of biogas supply to microalgae

No.	Code of variables	Biogas (%) (B)	Optical density of microalgae (M)
1	B0M1	0	0.2
2	B5M1	5	0.2
3	B10M1	10	0.2
4	B15M1	15	0.2
5	B0M2	0	0.4
6	B5M2	5	0.4
7	B10M2	10	0.4
8	B15M2	15	0.4
9	B0M3	0	0.6
10	B5M3	5	0.6
11	B10M3	10	0.6
12	B15M3	15	0.6

the microalgae. The principal aim of the research was to assess the effects of methane produced by the POME anaerobic digester on the growth of microalgae. Regular monitoring of the microalgae's development due to the carbon dioxide absorption from biogas supply was conducted through optical density (OD) measurements during the initial two weeks of growth. Gravimetric techniques were employed to evaluate the biomass of *C. vulgaris*. The material was homogenized using a centrifuge running at 40 revolutions per minute (rpm). The cells were obtained through filtration and repeatedly cleaned with deionized water. The filter paper containing cells was inspected prior to the drying process.

Biogas supply, and initial density of microalgae

The farmed microalgae in the PBR were given a mixture of air aeration containing around 5%, 10%, and 15% volume per volume (v/v) and biogas containing CO_2 and CH_4. The starting microalgae density was used at 0.2, 0.4, and 0.6, and CO_2 was utilized as a carbon source for microalgae photosynthesis. For the first four days of this investigation, CO_2 was constantly injected using a sparger. Then, for the next fourteen days, CO_2 was gradually added one hour every day. As a result, Table 2 lists the twelve variables for this investigation, a combination of biogas concentration and optical density of microalgae. The gas chromatography technique was employed to determine the composition of the biogas through monitoring. The composition of the biogas was measured both before and after the cultivation. CH_4, CO_2, and various other gases make up biogas. On the gasmeter, however, the readings are limited to CO_2 and CH_4.

Biogas upgrading

The ability of CO_2 fixation by microalgae in PBR is generally measured based on the CO_2 uptake efficiency and biomass production rate of microalgae in FBR. CO_2 uptake efficiency by microalgae in FBR can be determined based on the difference between CO_2 entering the FBR system and CO_2 leaving the system, which is expressed by the formula: Where: E = Uptake efficiency (%) CO_2 inlet = CO_2 entering the FBR system CO_2 outlet = CO_2 leaving the FBR system, using Eq. 1 (Huy *et al.* (2018).

$$E = \frac{CO_{2\,inlet} - CO_{2\,outlet}}{CO_{2\,inlet}} \times 100\% \quad (1)$$

Where, the upgraded biogas is calculated by increasing the CH_4 content in the biogas outlet volume from the FBR.

Nutrient removal of sludge

The high nutrient content of sludge obtained from POME biogas digestion made it an ideal choice for microalgae cultivation. The microalgae utilized in this study were derived from the preceding culture for biogas CO2 capture, which exhibited the most favorable growth conditions. There was only a single air aeration provided by the air compressor after the CO2 was turned off in order to avoid the settling of microalgae. The initial density of microalgae was utilized in the meantime to improve the recovery of nutrients from digests. An assessment was conducted to compare the role of microalgae by detecting nutrient removal from sludge in the absence of

microalgae. With a pH meter (Hanna HI 98107), the temperature and pH of the water were tested every day. To calculate the density of microalgae using spectrophotometry at λ = 680 nm. On a daily basis, a sample of mixed liquor was procured from the photobioreactor as well as from each flask. TSS analysis was conducted using the Indonesian National Standards Standard Methodology. Indonesian National Standards were used to measure ammonia (NH_3-N), nitrate (NO_3-), nitrite (NO_2), and total Kjeldahl nitrogen (TKN) and total phosphorus (TP) in the same samples. In spectrophotometry, a calibration curve was utilized to track the daily growth of microalgae. Each cultivation and measurement was done in triplicate, and the results are presented as the average ± standard deviation. SPSS version 16.0 was used to conduct a one-way analysis of variance (ANOVA) and Tukey's multiple comparisons test. For every test, the significance threshold that was employed was 0.05. This study utilized the ANOVA method to evaluate the efficacy of nutrient removal under varying CO2 concentrations and initial microalgae densities.

Statistical analysis

The measurement of Total Kjeldahl Nitrogen (TKN) and total phosphorus (TP) was conducted on samples obtained from a photobioreactor without undergoing filtration. This analysis allowed for the determination of nitrogen and phosphorus levels in the microalgae and suspended particles within the sample, as well as the concentrations of TKN and TP. To guarantee the quantity of actual TKN and TP in the treatment's effluent, the author has therefore removed the TKN and TP concentration without the use of microalgae. The concentration of TKN and TP was ascertained through the utilization of an author-derived computation derived from the reduction of nitrogen and phosphorus in samples containing microalgal content. The nitrogen and phosphorus levels of microalgae in Indonesia were determined through the utilization of the Standard Method. The nitrogen and phosphorus content were calculated by employing Eqs. 2 and 3 (Ermis and Altinbas, 2019).

$$TKN\ (mg\ L^{-1}) = TKN_{samples} - (10.33\% \times TSS_{algae} \times 1000) \quad (2)$$

$$TP\ (mg\ L^{-1}) = TP_{samples} - (0.96\% \times TSS_{algae} \times 1000) \quad (3)$$

The nutrient removal determined using the calculation was as Eq. 4 (Saidu *et al*. 2017).

$$E = \frac{S_1 - S_2}{S_1} \times 100\% \quad (4)$$

RESULTS AND DISCUSSION

POME characteristics

The physical and chemical properties of POME are summarized in Table 3. In general, the chemical properties (BOD and COD) of POME are suitable for the production of biogas. Moreover, POME is acidic, with a pH of 4-4.5, so we needed to adjust the pH to 7 to optimize the biogas production. The ratio of C/N in the POME was observed at only 0.42, which is much

Table 3: POME chemical and physical characteristics

Parameter		Range before anaerobic digestion	Range after 20 days anaerobic digestion
Protein	g/L	3.9 - 5.0	2.1 – 3.2
Carbohydrate	g/L	22.2 - 28.4	10.3 – 16.2
Fat	g/L	5.92-6.51	3.74-4.21
Fiber	g/L	0.9-2.1	0.6-1.7
Ash	g/L	6.43–8.32	4.13–6.12
Nitrogen	%	0.0394	0.0194
Phosphate	mg/L	38.072	58.146
Phenol	mg/L	≤0.0001	≤0.0001
Potassium	g/L	1.359	2.543
Sulphate	g/L	1.102	2.201
Ammonia	g/L	0.121	2.231
Fe	mg/L	0.193	0.287
COD	g/L	50 – 60	0,4 – 0.6
BOD	g/L	16 – 35	0.13 – 0.21
Suspendid solid	g/L	15 – 30	0.21 – 0.38
C/N ratio		0.42	1.32
pH		4-4.5	6.2-7.0

lower than the C/N ratio for biogas production (in the range of 20-30); therefore, cow feces (10%) were added as a bacterial starter to increase the C/N ratio. The biogas-producing components found in POME are rich in carbohydrates, lipids, and proteins. (Alvionita *et al*., 2019). The biogas processing of the POME-cow feces mixture via anaerobic digestion using a digester tank produced 89% CH_4 and 11% CO_2. In general, the incorporation of cow manure and pH adjustment were discovered to enhance the C/N ratio. After undergoing anaerobic digestion, the effluent from POME, also referred to as sludge, exhibits a reduction in BOD and COD. Conversely, there is an increase in nutritional phosphate and ammonia, while nitrogen levels decrease. As a result, the C/N ratio experiences an elevation. Similar research on anaerobic digestion conducted by Widyowanti *et al*. (2021) found that the nutrients present in POME sludge rose following anaerobic digestion, except of nitrogen, which dropped. The remaining high-nutrient content is then used as fertilizer.

Growth and biomass production of microalgae

The biogas system upgrade commences by generating biogas through POME digestion. Biogas fed to *C. vulgaris* microalgae at concentrations of 5%, 10%, and 15% (v/v) and at optical densities of 0.2, 0.4, and 0.6 produced significantly different microalgae growth at OD 0.6. The presence of light is of utmost significance in the cultivation of photosynthetic microalgae, as it directly influences their growth and overall productivity. Cultivation of *C. vulgaris* microalgae in this study only provided light at 4800 lux at open room temperature. Light is the primary energy input for photosynthetic microalgae, hence it must be maximized for optimal production. Excessive light exposure, especially in conjunction with less-than-ideal temperature or increased oxygen levels, can negatively impact the photosynthetic process (Chowdhury *et al*., 2020). Fig. 2 shows the observation findings of microalgae growth.

Microalgal biomass production increased with biogas application up to 15% (v/v) at OD 0.2 to 0.4. An increase in OD of 0.6 with the same biogas feeding showed a decrease in biomass. An elevation in OD leads to a higher demand for CO2, despite the absence of an increase in CO2 supply, resulting in slower growth compared to OD 0.2 and 0.4. Biomass production is presented in Fig. 3a. The graph of biomass productivity per day shows an increase with the given treatment (Fig. 3b).

The outcomes of the biomass growth rate trials were compared to a study (Gabrielyan *et al*., 2022) that had comparable objectives, instruments, and

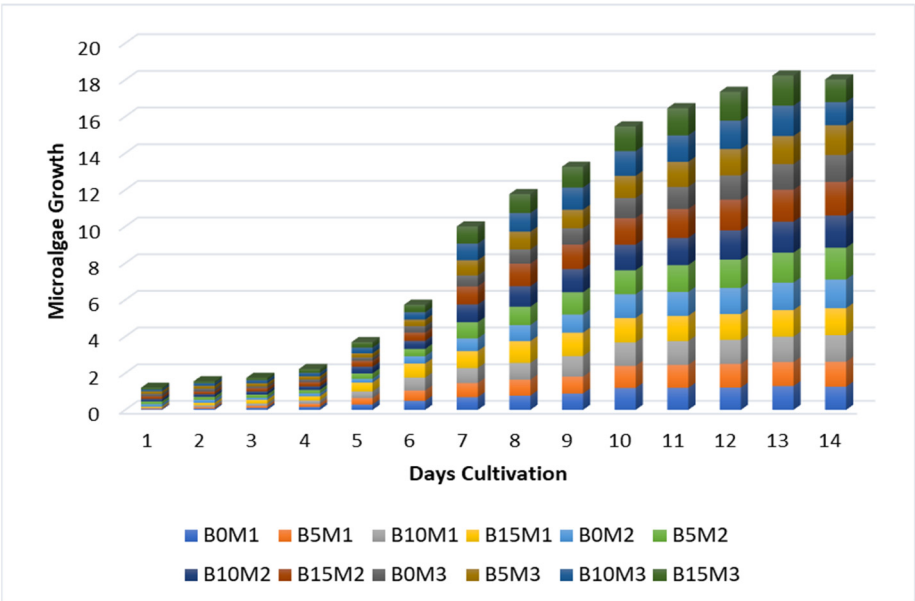


Fig. 2: Microalgae growth in 14 days of cultivation with biogas treatment and microalgae density

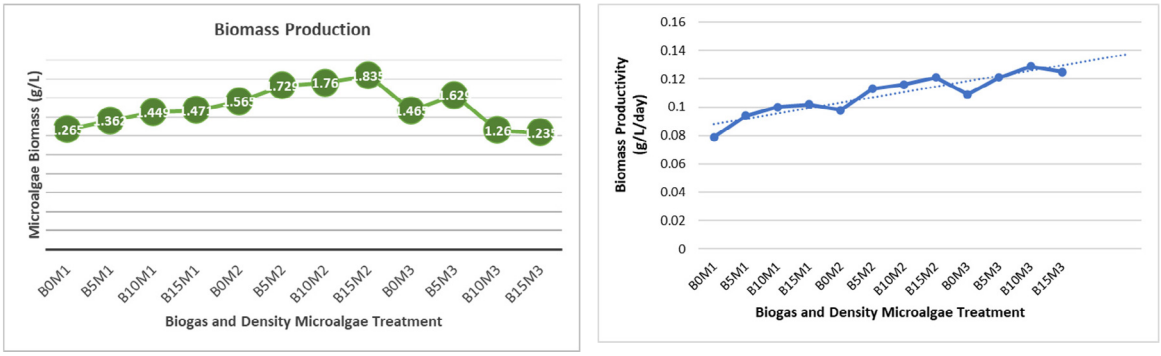


Fig. 3: (a) Microalgae biomass production on day 14 of cultivation, the end of the observation day; (b) Biomass productivity at increasing biogas feeding and microalgae OD over 14 cultivation periods

procedures and that used *Chlorella sorokiniana* IPPAS C-1 as a research subject. The cultivation process was conducted in 5 and 18 L flat-panel photobioreactors with a light-emitting diode (LED) illumination system that had an intensity of 900 µmol quanta·m−2·s−1, and a temperature of 35.5 ± 0.5 °C. *Chlorella sorokiniana's* productivity was estimated based on varying CO2 concentrations and the ventilation coefficient of the gas-air mixture. The CO2 level in the gas-air mixture ranged from 1 to 4% lower than that of the trial, with growth rate data only available for the initial three days of the experiment. The greatest growth rate of 1.51 ± 0.07 g/L wt% per day was achieved at a concentration of CO_2 = 1.5%, but the biomass growth rates were more significant than in this experiment. Thus, the biomass density was measured after a three-day period under varying CO2 concentrations of 1.5%, 2%, and 4%, resulting in a range of 4-5 g·L−1 wt%. Throughout the experiment, an increase in CO2 levels of up to 15% did not result in a decrease in the growth rate. Rather, the biomass density continued to remain stable and consistent during the 14-day trial period. An examination of the data suggests the possibility of enhancing the cultivation process to boost the growth rate of Chlorella microalgae biomass and subsequently enhance the absorption of CO2. The increased absorption efficiency of microalgae in response to high CO2 concentrations underscores the importance of this adaptation process. Based on random mutation and natural selection, Zhang *et al.*, (2021) demonstrated that adaptation enhances the phenotype of microalgae. The microalgae displayed diverse reactions to increased CO2 concentrations and exhibited varying speeds of adjustment to these levels during the tests. Therefore, at all CO_2 concentrations, *C. vulgaris* displayed an almost consistent growth rate. Research on CO_2 bio-fixation by *Chlorella* sp with *Scenedesmus* sp has been reported by Handayani *et al.* (2023). The microalgae species used were a consortium of *Chlorella* sp. and Scenedesmus sp. Five levels of carbon dioxide were provided to microalgae cultures: 0%, 5.5%, 6.2%, 8.1%, and 10.3%. The variables that were monitored were CO2 absorption, efficiency of absorption, and the production of microalgal biomass. The results showed that the CO_2 sequestration efficiency by indigenous microalgae reached 0%, 9.2%, 98.8%, 96.2%, and 93.2% with average CO_2 level loadings of 0%, 5.2%, 6.2%, 8.1%, and 10.3%, respectively. *Chlorella* sp. exhibited greater tolerance to high levels of CO_2 concentration than *Scenedesmus* sp. (Handayani *et al.*, 2023). Scenedesmus sp. and Chlorella sp. microalgae were utilized in combination. Microalgae cultures were exposed to five different concentrations of carbon dioxide: 0%, 5.5%, 6.2%, 8.1%, and 10.3%. Several characteristics were observed, including the generation of microalgal biomass, absorption efficiency, and CO2 uptake. The findings demonstrated that native microalgae had CO_2 sequestration efficiencies of 0%, 9.2%, 98.8%, 96.2%, and 93.2%, respectively, with average CO_2 level loadings of 0%, 5.2%, 6.2%, 8.1%, and 10.3%. *Scenedesmus* sp. was less tolerant of high CO_2 concentrations than *Chlorella* sp. (Handayani *et al.*, 2023). The CO_2 uptake efficiency of *Tetraselmis* sp. microalgae using 100,000-litre scale FBR technology

can reach 80% (Hajinajaf *et.al*., 2022). Microalgal Carbon Capture Storage (CCS) technology has garnered significant interest due to the potential for further processing the biomass products obtained for various applications. Culture pond and FBR technologies have been widely applied in producing food and feed supplement products (de Oliveira *et. al.,* 2022) cosmetics, and medicinal raw materials (Zhuang *et.al*., 2022). According to the findings of a techno-economic analysis of the complete biomass production process (excluding additional processing), it was determined that the cost of producing one kilogram (kg) of dry weight biomass in a 100 he area was $3.72 USD. (Ruiz *et al*., 2020). The findings of the study suggest that utilizing biomass for the production of pigments can be economically feasible, however, reducing operational costs will be necessary for it to remain competitive in the production of food and chemicals. Therefore, considering the techno-economic perspective, it is logical to utilize microalgae carbon capture storage (CCS) technology alongside biomass conversion into valuable products and continuous efforts to reduce production expenses. The data presented in Fig. 4 indicates that gas production commenced on the third day, contrasting with the seventh day for the digester that was not restarted. Likewise, the peak in natural gas output occurred sooner. (Santoso *et al*., 2023).

Biogas production

Fig. 4 displays the coefficients of y =-0.916 X^2 + 27.03 X - 35, R^2 = 0.9408 for values ranging from 20 to 180, the volume of gas produced per day (cm^3), daily gas production (days), pH, temperature, R^2, and trendline equations for biogas output per day in a restarted digester. The R^2 score was close to one, indicating that the trendlines can accurately predict future outcomes. It is worth highlighting that due to the early commencement of gas production, the trendline was captured throughout the entire period. Consequently, when a digester is established using slurry from a previous digestion, the generation of gas initiates at an earlier stage. The reason behind this is that the microorganisms necessary for the process to continue are already in motion and were merely eliminated by the introduction of oxygen. Version 1: The time delay occurred solely due to the depletion of oxygen by the aerobic bacteria present in the digester. Once the oxygen is completely used up, the production of biogas reaches its peak capacity. Since

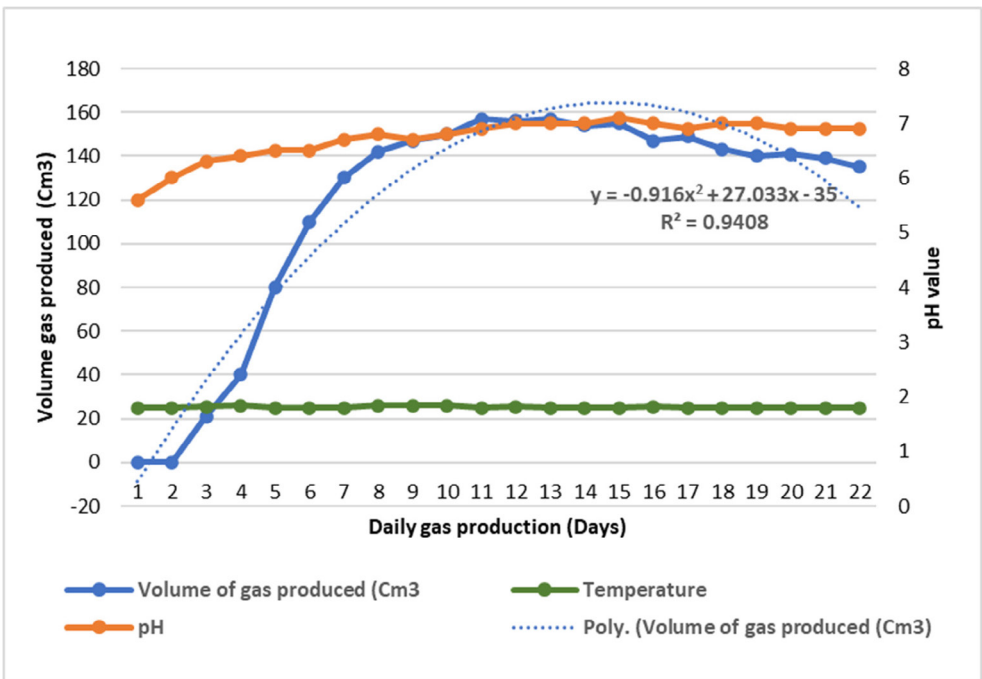


Fig. 4: Trendline of biogas daily production, pH, and temperature.

the digester has a high concentration of bacteria, the consumption of oxygen happens at a faster rate, resulting in a shorter time period. Hence, the bacteria that have been developed earlier are simply provided with substrates to carry out their tasks. They function at a higher speed owing to their larger population, thereby reducing the operational time of the digester. The increase in gas volume can be attributed to the effortless growth of the population, as they are already established. Consequently, there are a greater number of bacteria that will work on the substrate, resulting in the release of a larger amount of gas during the process.

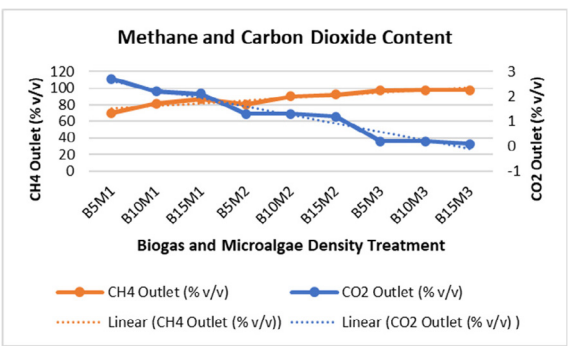
Biogas upgrading

Biogas produced from Anaerobic POME digestion for 20 days produced CH_4, CO_2, and other gases with percentages of 77.86, 22.08, and 0.05%. The outlet biogas composition from the best treatment B15M3 produced CH_4, CO_2, O_2, and other gases with presentations of 98, 0.1, 0.05, and 1.85% respectively (Table 4). Biogas typically has a composition that ranges from 50–75% methane, 25–65% carbon dioxide, and 1%–5% hydrogen sulfide (Mulu *et al.*, 2021).

Based on the Fig. 5a, it can be seen that during the biogas purification period, the CO_2 content decreased

Table 4: The composition of POME biogas inlet and outlet from the best microalgae cultivation

No.	Biogas composition	Inlet (% v/v) Before	Outlet (% v/v) After
1	CH_4	77.86 ± 0.2	98 ± 0.2
2	CO_2	22.08 ± 0.1	0.1 ± 0.02
3	Other gas	0.05 ± 0.01	0.05 ± 0.001
4	O_2		1.85 ± 0.1



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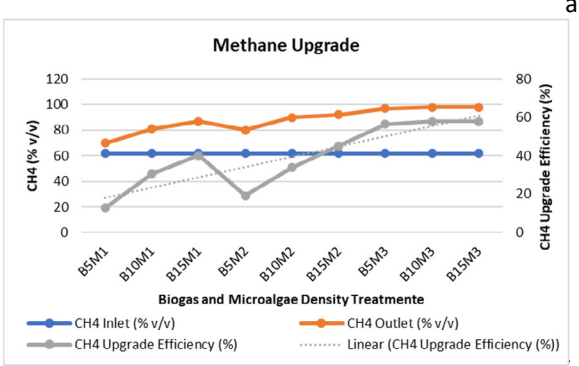

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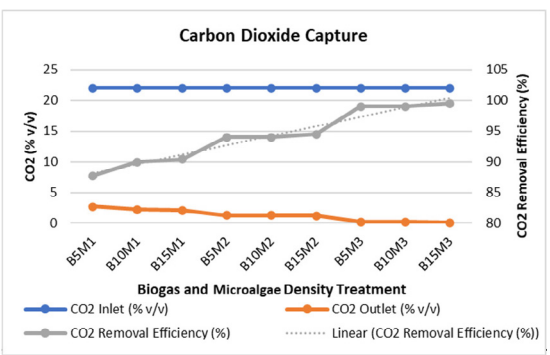

c

Fig. 5: (a) Increased CO_2 removal followed by CH_4 upgrading at increased microalgal density; (b) CH_4 efficiency upgrade on increasing microalgae density; (c) CO_2 removal efficiency at increased microalgal density.

accompanied by an increase in CH_4 content in all treatments. The CO_2 content in the biogas inlet of 77.86% in treatment B15M3 when first channeled to the FBR decreased to 0.1% on day 14. While the CH_4 content in biogas treatment B15M3 increased with an inlet content of 77.86% on the first day channeled to 98% on day 14. The effectiveness of microalgae *C vulgaris* in absorbing CO2 in biogas through photosynthesis is demonstrated by the production of O2 as a by-product. The percentages of methane contained in the biogas before and after treatment with *C. vulgaris*, with carbon dioxide accounting for the remaining amount illustrated in Fig. 5b and Fig. 5c. It is evident that the amount of methane exiting the microalgal growth system consistently exceeded the percentage that entered, leading to a decrease in the concentration of carbon dioxide. Methane concentrations of above 90% were consistently reported in B10M2, B15M2, B5M3, B10M3, and B15M3, reaching a maximum of 98%. The rise in microalgae density observed with the biogas concentration treatment indicates an enhancement in biogas upgrade. This improvement in biogas upgrade can be attributed to the capture of carbon dioxide by microalgae. CO_2 capture increases along with the increase in microalgae density and reaches maximum capture with 99% efficiency, and CH_4 upgrade shows maximum efficiency with 60%.

Various exceptional reviews have discussed the potential of combining anaerobic digestion with microalgal cultivation (Zhang *et al*., 2021). A fantastic floating range of 62% to 98% for the microalgal cultivation-based biogas upgrading CO_2 removal efficiency. For instance, to upgrade biogas with 90.6 ± 0.7% of CH_4, 0.9 ± 0.8% of CO_2, 0 ± 0% of H_2S, and 8.6 ± 0.1% of N_2 + O_2 under 2.1 of the liquid to biogas ratio (L/G) and 370 liters per hour (L/h) of biogas flowrate, Rodero *et al*. (2020) used an outdoor HRAP cultured with algal-bacterial. The calorific value of biogas was significantly enhanced by elevating the CH4 concentration and utilizing CO2 captured from biogas to promote the growth of microalgae as a carbon source. Sufficient adjustments can transform biomethane to closely resemble pure gas. It has been proposed that *Chlorella* sp. played a vital role in effectively removing CO2 from biogas. Renowned for their courage, these microalgal strains exhibit extraordinary resilience in the face of challenging environments. Furthermore, it is worth delving into the correlation between PBR operation mode, parameter settings, and the efficiency of CO2 removal. A high-quality cultivation apparatus is necessary to boost the CO2 content of biogas even further. Oleaginous microalgae produce lipids and increase methane content by absorbing CO_2 from biogas. The capacity of several microalgae to develop and synthesize lipids using CO_2 in biogas was tested. The best strain of *Chlorella* sp. was marine because it could both use 50% v/v CO2 in air and biogas (50% v/v CO_2 in methane) to capture CO_2 and produce lipids (Dani *et al*., 2021). Approximately 25,000 kg of palm oil mill effluent (POME) is derived from the production of one ton of crude palm oil. This POME can then be converted into approximately 70 cubic meters of biogás (Aziz *et al*., 2020). Consequently, the conversion of one ton of POME can yield an estimated 28.13 cubic meters of biogas. By considering the calorific value of 21.5 megajoules per cubic meter, the EC biogas quantity was calculated to be 6 kilowatt-hours per cubic meter (Khalid *et al*., 2019). However, the creation of biogas from POME has the potential to upset the biogeochemical cycle and release significant volumes of CH_4 into the atmosphere if improperly stored and treated (Safieddin Ardebili, 2020). Studies conducted previously suggest that the involvement of POME components in methanogenic processes may lead to the production of biogas with a methane concentration exceeding 50% and a yield surpassing 0.8 liters per gram (Nitamakwuavan and Abd Rahim, 2022). Over the past fifteen years, up-flow anaerobic sludge blanket (UASB), up-flow anaerobic sludge fixed-film (UASFF), continously stirred reactor (CSTR), membrane anaerobic system (MAS), and integrated anaerobic–aerobic bioreactor (IAAB) and continously stirred reactor (CSTR) have been the POME bioreactor technologies that have been explored the most. In-depth assessments of the benefits and constraints of these technologies have already been given (Aziz *et al*., 2020). Among these technologies, the up-flow anaerobic sludge blanket (UASB) and continuous stirred tank reactor (CSTR) are the most commonly utilized for commercial applications. The CSTR stands out for its superior mixing capabilities, ease of use, and straightforward design (Mahmod *et al*., 2020). Similar to UASB, its noteworthy benefits include yielding CH_4 and producing high-quality effluent (Yong *et al*., 2023).

Table 5: Nutrient removal efficiency

Variable	Efficiency (%)				
	Nitrogen	Phosphate	Nitrate	Nitrite	Ammonia
B5M1	94 ± 2	87 ± 1.7	93 ± 5	88 ± 2	11 ± 2
B10M1	92 ± 2	82 ± 4.5	91 ± 5	88 ± 2	17 ± 1
B15M1	90 ± 3	81 ± 3.2	87 ± 5	88 ± 2	18 ± 2
B5M2	83 ± 8	79 ± 7.6	93 ± 5	687 ± 28	138 ± 7
B10M2	82 ± 6	79 ± 1.8	90 ± 5	687 ± 28	147 ± 28
B15M2	81 ± 3	81 ± 2.1	84 ± 5	687 ± 28	130 ± 12
B1	78 ± 2.9	41 ± 6.1	93 ± 0.3	93 ± 3.3	55 ± 11.6
B2	77 ± 1.8	48 ± 6.7	91 ± 0.8	91 ± 5.2	49 ± 10.0
B3	80 ± 2.9	44 ± 6.1	87 ± 0.8	87 ± 2.7	62 ± 8.90

Nutrient removal

The utilization of palm oil mill effluent sludge as a nutrient source was explored through the application of microalgae. Nutrient removal contained in the sludge is used for microalgae cultivation from the best CO_2 capture treatment results, namely B5M1, B10M1, B15M1, B5M2, B10M2, and B15M2 (Table 5). Additionally, a nutrient removal analysis was carried out on sludge without the presence of microalgae to compare its effectiveness in removing nutrients. The measured biogas effluent contained nitrogen, phosphate, nitrate, nitrite, and ammonia as the nutrients under consideration. Measurements were conducted every three days until the completion of the observation period. Statistical analysis showed nitrogen removal efficiency of 81-94%, while phosphate removal efficiency of 79-87%. Nitrogen and phosphate removal from sludge without microalgae treatment showed lower efficiencies of 76-80% for nitrogen removal and 41-48% for phosphate removal. The data in Table 3 illustrates the effectiveness of nutrient removal, including nitrogen, phosphate, nitrate, nitrite, and ammonia. The findings indicate a reduction in nitrogen and phosphate levels.

The main nitrogen compounds in effluents were NH_3, NO_2-, and NO_3-. Incorporating biogas concentration into the microalgae density did not demonstrate any substantial impact on the removal of nitrogen and phosphate. Nitrogen removal at microalgae concentration 0.2 density showed above 90% removal efficiency, while at microalgae concentrationof 0.4 density showed below 90% removal efficiency (Fig. 6a). In comparison to the removal of phosphate, the density of microalgae was less than 80% at a concentration of 0.2. Conversely, when the microalgae concentration reached 0.4, the removal efficiency surpassed 80% (Fig. 6b). Nutrient removal in sludge processed without microalgae showed lower removal efficiency. Nitrogen removal showed efficiency below 80% (Fig. 7a), and phosphate removal showed efficiency below 50% (Fig. 7b). The presence of microalgae plays a significant role due to the symbiotic relationship between heterotrophic bacteria found in POME and microalgae. Waste serves as a nutrient for microalgae, which use enzymes to break down pollutants. The nitrogen and phosphorus present in waste are utilized by microalgae as carbon sources (Farahdiba *et al*., 2020). Observing the attributes of POME, it is evident that there are nitrate compounds that microalgae can absorb directly to support their nutritional requirements for growth. Ammonia will be converted first through the process of nitrification into the form of nitrate compounds that can finally be absorbed by microalgae (Hanurawaty *et al*., 2022). Nitrate removal in microalgae is initiated by the plasma membrane within the microalgae, which absorbs nitrate and stores it in the cytoplasm. Subsequently, the stored nitrate is converted into protein and chlorophyll through the assistance of the enzyme nitrate reductase (Ali *et al*., 2022). Phosphate reduction can occur in microalgae through adsorption and assimilation processes, where organic phosphate will be reduced to orthophosphate by phosphatase enzymes on the cell surface. Calcium ions play a crucial role as a component of microalgae cell walls, facilitating the binding of phosphate and calcium monohydrogen phosphate. These bound compounds are subsequently utilized by microalgae for their

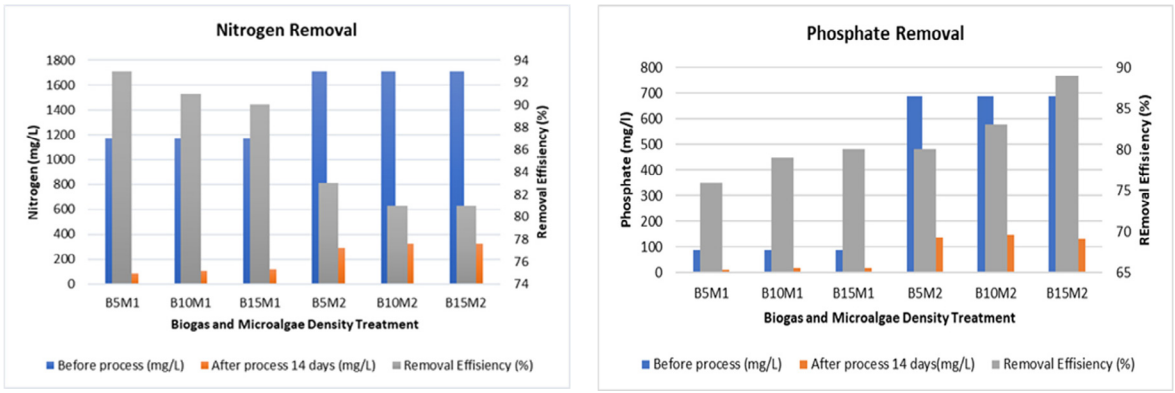

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Fig. 6: Removal efficiency of nitrogen and phosphate at increased microalgal density.

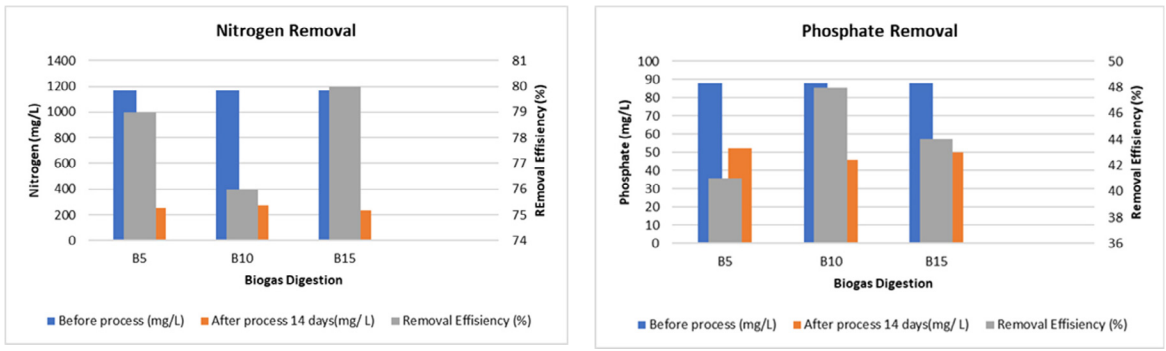

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Fig. 7: Removal efficiency of nitrogen and phosphate without microalgal culture.

cellular metabolism (Calijuri *et al*., 2022). According to Nur and Buma's (2019), POME is a rich source of micronutrients, carbon, nitrogen, phosphorus, and other critical elements that are necessary for the growth of algae. But POME's quality may still be improved to maximize its potential for supporting the growth of particular algae species that reduce pollution and provide useful biomass. The study has emphasized the potential to enhance the quality of POME by reducing various indices such as COD, BOD, lipids, proteins, and tannins Nur (2021). It is possible to improve POME's availability and accessibility of vital nutrients by reducing these elements (Ahmad *et al*., 2019). Enhanced nutrient availability can be particularly beneficial for cultivating specific algae strains aimed at eliminating pollutants and generating valuable biomass (Low *et al*., 2021). Dilution plays a crucial role in reducing the likelihood of pH extremes in the initial wastewater. Failure to do so may impede the growth of microalgae due to the presence of drastic pH fluctuations. By diluting the wastewater, various problems such as turbidity, coloring, and high pH levels can be effectively addressed, while simultaneously controlling COD levels and fostering the growth of microalgae.

Microalgae are considered to be promising candidates for a variety of biotechnological applications across multiple industries. Their ability to thrive in diverse settings, coupled with their effective utilization of solar power and capacity to generate eco-friendly biomass, highlights their promise in supporting sustainable solutions and cutting-edge products. Wastewater treatment with microalgae is a sustainable technique that can lower pollution (You *et al*., 2022). The pre-treatment of POME is of utmost importance as it enhances light penetration and

establishes the perfect conditions for the flourishing of microalgae (Samsudin *et al*., 2019). For this, several methods have been used, including coagulation and adsorption. The research conducted by Mahmod *et al*. (2020) describes the utilization of rice straw starch in the coagulation process. The utilization of microalgae for POME treatment proves to be a remarkably economical solution due to the substantial biomass generated by microalgae thriving in POME. This biomass holds immense potential as a significant supplier of biofuel and various valuable commodities (Nur and Buma, 2019). Research has shown that other species, such as *Chlorococcum oleofaciens* (Tan *et al*., 2022), *Scenedesmus* sp. (Udaiyappan *et al*., 2021), and locally isolated microalgae strains, such as *Chlorella sorokiniana* UKM2, *Coelastrella* sp UKM4, and *Chlorella pyrenoidosa* UKM7 (Ding *et al*., 2020) are effective in breaking down pollutants in POME. The core purpose of systemic integration is to effectively combine biogas upgrading and sludge purification, while also optimizing the utilization of microalgal biomass. This integrated approach holds promise for reducing environmental harm and maximizing economic advantages. A multitude of features are incorporated into the integrated system, which leverages microalgal culture technology: I) using biogas slurry cyclically to grow microalgae and remove nutrients from the slurry; II) effectively eliminating CO_2 content and raising the calorific value of biogas; III) creating useful biomass; IV) avoiding the possibility of nutrient runoff and anaerobic digestion-induced eutrophication of waterbodies. Additionally, the biodiesel and/or biogas generated could help maintain the smooth operation of the integrated system. Food products, animal feed, pharmaceuticals, biopolymers, bioplastics, and various chemicals are among the valuable bioproducts that can be derived from microalgae biomass (Calijuri *et.al*., 2022). Multi-stage cultivation model is recommended to use this model for upgrading biogas and treating sludge (Marín *et al*., 2019). Due to the diverse conditions of microalgae growth and lipid/starch accumulation, such as operation duration, high-rate retention time (HRT), Photosynthetically active radiation (PAR), and organic loading rate, this mode can effectively modify operating parameters (Rowan *et al*., 2022). When compared to batch and semi-continuous cultivation, multi-stage cultivation shows a higher likelihood of efficiently increasing biomass production, enhancing the purification of biogas slurry, and upgrading biogas simultaneously. It is possible to maximize microalgal biomass, lessen adverse environmental effects, and increase the total economic benefit by integrating microalgal production with slurry treatment for biogas upgrading. It is advisable to utilize the algal-bacteria symbiosis in the integrated cultivation technique, as it has shown superior performance in both nutrient removal efficiency and algal growth when co-cultivating microalgae with fungi or bacteria.

Comparison to other results

Table 6 summarizes different experiments involving the utilization of various microalgae species for biogas production and CO_2 removal efficiency. The first row indicates the use of *Chlorella vulgaris*, Ganoderma lucidum, and endophytic bacteria in a biogas system sourced from a pig farm. After purification, the biogas contained 64.21% methane (CH_4) and 32.78% carbon dioxide (CO_2), with an upgraded CH_4/biogas ratio of 80-82% and a CO_2 removal efficiency of 53-63%. The optimal conditions for cultivation involved maintaining a temperature of 25 ± 1°C over a period of 10 days, with a light intensity of 200 µmol·m−2·s−1 and a 12-hour light/12-hour dark cycle, supplemented with strigolactone analogs. Similarly, consortia of microalgae and bacteria were utilized in a wastewater treatment plant (WWTP), resulting in biogas composition of 63.7% CH_4, 33.7% CO_2, 0.45% O_2, and 1.59% N2, with a remarkable CO_2 removal efficiency of 97% and a CH_4 enrichment efficiency of 91%. This process involved supplementation of sodium hydrogen carbonate ($NaHCO_3$) and natrium carbonate (Na_2CO_3) during cultivation under high-rate algal ponds (HRAP) outdoor conditions. Another investigation incorporated both Chlorella vulgaris and activated sludge in an anaerobic digester system, successfully producing desulfurized biogas with a methane concentration of 62.87% and achieving a CO2 removal effectiveness of more than 60%. The optimal condition included an initial inoculum of 0.74 g/L microalgae and 3.74 g/L total suspended solids (TSS) of activated sludge, supplemented with strigolactone analogs. Lastly, this study focused solely on *Chlorella vulgaris* in anaerobic digestion of POME yielded highly efficient CO_2 removal (98%) and CH_4 enrichment (98%), with optimal pH conditions maintained between 6.8-7.2. These experiments highlight the potential of microalgae-based systems

Table 6: Comparison of biogas upgrading using microalgae

Microalgae species	Biogas source	Upgraded CH_4/ biogas	CO_2 removal efficiency	Optimal condition	Sources
Chlorella vulgaris, Ganoderma lucidum, and endophytic bacteria	Biogas pig farm. Purified biogas: 64.21% CH_4, 32.78% CO_2	80-82%	53-63%	Cultivation: 25 ± 1°C, period of 10 days, intensity of 200 µmol·m−2·s−1, and 12 h light/12 h dark+ strigolactone analogs	(Liu *et al*., 2023)
Consortia of microalgae and bacteria	WWTP: 63.7% CH_4, 33.7% CO_2, 0.45% O_2 and 1.59% N_2	91%	97%	Supplementation of $NaHCO_3$ and Na_2CO_3 in the cultivation at HRAP outdoor condition.	(Méndez *et al*., 2022)
C. vulgaris + activated sludge	Anaerobic digester plant. Desulfurization: 62.87 % CH_4, 33.62% CO_2	88-89%	> 60%	0.74 g/L initial inoculum of microalgae and 3.74 g/L TSS activated sludge + strigolactone analogs	(Zhang *et al.*, 2021)
C. vulgaris	Anaerobic digestion, POME	98%	98%	Optimum pH 6.8-7.2	This study

in promoting sustainable biogas production and CO2 mitigation across a range of waste treatment scenarios. The results of this study using a modified photobioreactor showed that *Chlorella vulgaris* was effective in removing carbon dioxide up to 90 percent and upgrading biogas up to 98 from palm oil mill effluent biogas by optical density microalgae 0.4, and was able to remove nutrient nitrogen up to 90 percent at optical density 0.2 and phosphate 80 percent at optical density 0.4. with optical density microalgae 0.4, and able to remove nutrient-nitrogen up to 90 percent at optical density 0.2 and phosphate 80 percent at optical density 0.4.

The production of biogas and organic manure from POME is inherently sustainable due to several key factors (Nur and Buma, 2019). Firstly, by employing anaerobic digestion to convert POME into biogas, the process effectively mitigates greenhouse gas emissions, particularly methane, a potent contributor to climate change. This approach is consistent with global initiatives aimed at reducing the impact of climate change and encourages the adoption of renewable energy alternatives. Additionally, biogas generated from POME serves as a viable alternative energy source, thereby reducing reliance on fossil fuels and contributing to energy security. Additionally, the anaerobic digestion process results in the production of biogas and organic manure containing vital nutrients necessary for soil fertility and plant development. Utilizing this organic manure in agricultural fields improves soil quality, increases crop productivity, and reduces the need for synthetic fertilizers, thereby encouraging sustainable farming methods. This comprehensive strategy for managing palm oil mill effluent (POME) encompasses the principles of environmental responsibility, energy conservation, and agricultural viability. It represents a noteworthy advancement towards the adoption of circular economy principles and the promotion of sustainable development.

CONCLUSION

Microalgae have biological traits such as high photosynthetic efficacy and a basic structure, allowing them to survive and even develop in adverse environments such as high salinity, nutritional and heavy metal stress, and harsh temperatures. Various endeavors have been undertaken to examine an

integrated system that harmonizes biogas upgrading with sludge treatment from anaerobic digestion, as a consequence of the aforementioned benefits, which are sustainable, low-carbon, and promising. Microalgae are single-cell tiny phytoplankton species, with over 40,000 different species found thus far. Unfortunately, only a few algae species, such as *C. vulgaris*, have an exceptional ability to defend radical environments even when subjected to severe pollution hazards. The primary standards guiding the choice of microalgae include rapid growth rate and strong resistance against it. The application of POME biogas to microalgae cultivation showed an increase in growth and biomass production. The application of 15%v/v biogas with OD of 0.4 microalgae cultured for 14 days produced the highest biomass of 1,835 g/L. Enhancing the optical density of microalgae by 0.6 using 10 and 15% biogas did not result in higher biomass production. This is due to the fact that a higher OD level requires more CO2, but without a corresponding increase in CO2 supply, the growth rate is slower compared to OD levels of 0.2 and 0.4. The peak biomass production was achieved when the CO2 removal efficiency reached 98% and the CH4 content efficiency increased to 60%. This means that the upgraded biogas reached the highest CH4 content of 98%, while the other treatments had 90% CH_4. The results of this study showed that *C. vulgaris* was able to perform CO_2 removal and biogas upgrading from biogas produced from POME with an optimum microalgae OD of 0.4. *C. vulgaris* also showed the ability to remove Nitrogen and Phosphate nutrients. Nitrogen removal at microalgae concentration 0.2 showed removal efficiency above 90%, while at microalgae concentration 0.4 showed removal efficiency below 90%. In comparison to the elimination of phosphate, when the microalgae concentration is below 80% at 0.2 OD, the removal efficiency is less than 80%. However, when the microalgae concentration reaches 0.4 OD, the removal efficiency exceeds 80%. Utilizing POME for biogas production offers an alternative and sustainable approach to reduce greenhouse gas (GHG) emissions from POME, while also offering economic advantages. The expansion of oil palm plantations in Indonesia is driving up palm oil production and the generation of palm oil mill effluent (POME). This could lead to higher greenhouse gas emissions and exacerbate the issue of global warming.

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T. Handayani prepared research and experimental design, conducted the experiments, data analysis and interpretation, wrote a draft manuscript, and edited. I. N. Djarot prepared and conducted experiments, wrote a draft manuscript and edited. N. Widyastuti performed the literature review, wrote a draft manuscript and edited it. F. D. Arianti prepared the methodology, conducted experiments, wrote a draft manuscript and edited. M. D. Pertiwi conducted experiments, data analysis and interpreted, and wrote a draft manuscript. A. Rifai performed project administration, prepared methology and software, data curation and visualization. A. I. Sitomurni prepared research, data curation and visualization, and manuscript editing. M. M. A. Nur the corresponding author, conducted experiments, formal analyses, funding acquisition, and manuscript editing. R. Nurmala Dewi prepared the methodology, data curation and visualization; N. Nuha performed the experiment, project administration, and manuscript editing. J. Hariyanti performed data analysis and interpretation. D. Pinardi prepared methodology, data analysis and interpretation, writing a draft manuscript. Y. Suryana performed the literature review, manuscript preparation, and editing. A. Aziz conducted research, methodology, and software. T. Rochmadi conducted research, methodology and software, data analysis. E. Syamsudin prepared methodology, software, data analysis and interpretation. P. A. Lomak prepared funding acquisition, project administration, and manuscript preparation. A. Hadi prepared funding acquisition, project administration, and manuscript writing. E. Yuniastuti conducted the experiments, data analysis and interpretation, wrote a draft manuscript and edited it. N. A. Putri performed the literature review and manuscript preparation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest 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
^{o}C	Degree Celsius
AD	Anaerobic digestion
ANOVA	Analysis of Variance
BOD	Biological oxygen demand
CCS	Carbon capture storage
CH_4	Methane
C/N	Carbon per nitrogen
CO_2	Carbon dioxide
COD	Chemical oxygen demand
C. vulgaris	*Chlorella vulgaris*
CSTR	Continously Stirred Reactor
EASAC	European Academies Science Advisory Council
EC	Electrical conductivity
GHG	GreenHouse gas
g/L	Gram/liter
k/g	Kilogram
ha	hectare
HRAP	High-rate algal ponds
HRT	High-rate retention time
IAAB	Integrated anaerobic–aerobic bioreactor
L/G	Liquid to biogas ratio
L/h	Liters per hour
LED	Light-emitting diode
PBR	Photobioreactor
pH	Potential hydrogen
m^3	cubic meter
M^2S	Mass flow rate
MAS	Membrane anaerobic system
N	Nitrogen
Na_2CO_3	Natrium carbonate
$NaHCO_3$	Sodium hydrogen carbonate
NaOH	Sodium hydroxide
NH_3	Ammonia
NO_2	Nitrite
NO_3	Nitrate
OD	Optical density
PAR	Photosynthetically active radiation
POME	Palm oil mill effluent
rpm	revolutions per minute
tμmol/m^2s	transpiration micromolar per square meter per second
TKN	Total Kjeldahl nitrogen
TP	Total phosphorus

UASB	Up-flow anaerobic sludge blanket
UASFF	Up-flow anaerobic sludge fixed-film
USD	Unite Staes dollar
v/v	Volume per volume
WWTP	Wastewater treatment plant

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