JEE Journal of Ecological Engineering

Journal of Ecological Engineering, 2026, 27(3), 249–258
https://doi.org/10.12911/22998993/213526
ISSN 2299–8993, License CC-BY 4.0

Received: 2025.10.05
Accepted: 2025.11.22
Published: 2026.02.01

Dairy waste management: Optimizing biogas slurry dilution for microalgae *Euglena* sp. cultivation

Windri Riansyah[1], Ambar Pertiwiningrum[1*], Muhlisin[2], Dhomas Indiwara Prana Jhouhanggirs[1]

[1] Department of Animal Products Technology, Faculty of Animal Science, Gadjah Mada University, Indonesia
[2] Department of Animal Nutrition and Feed, Faculty of Animal Science, Gadjah Mada University, Indonesia
* Corresponding author's e-mail: artiwi@mail.ugm.ac.id

ABSTRACT

This research critically explores the application of biogas slurry (bioslurry) as a culture medium for *Euglena* sp. to assess its viability in microalgae cultivation. A series of bioslurry dilutions (0%, 10%, 20%, and 30%) were evaluated for their influence on growth rate, biomass production, and lipid content of *Euglena* sp. The experiment was conducted in a 5 L laboratory scale photobioreactor with 3 controlled repetitions for 16 days. The 10% bioslurry dilution exhibited favorable nutrient ratios, with C/N and P/N values of 2.73 ± 0.17 and 0.58 ± 0.03, respectively, while the 20% dilution showed increased C/N and reduced P/N ratios of 5.30 ± 0.51 and 0.37 ± 0.07. Among the tested conditions, the 10% dilution achieved the highest growth performance, with a peak cell density of $26 \times 10^4 \pm 6.75$ cells/mL, though it remained lower than the control (0% bioslurry) at $169 \times 10^4 \pm 5.14$ cells/mL. Conversely, the 20% and 30% dilutions significantly hindered growth, with peak densities of $20 \times 10^4 \pm 2.08$ cells/mL and $24 \times 10^4 \pm 7.02$ cells/mL, respectively ($p>0.05$). The highest lipid content was observed in the control medium (0% bioslurry) at 22.67 ± 2.52 mg/L, followed by 10%, 20%, and 30% dilutions with lipid concentrations of 8.00 ± 1.00 mg/L, 7.33 ± 1.53 mg/L, and 7.67 ± 1.15 mg/L, respectively. These results underscore the potential of a 10% bioslurry dilution as a practical strategy for enhancing *Euglena* sp. cultivation, offering a sustainable pathway to valorize biogas production residues within a circular agricultural economy framework.

Keywords: bioslurry, dilution, *Euglena* sp., growth rate, microalgae.

INTRODUCTION

Dairy farming is a vital agricultural activity that produces high-economic-value milk products. However, alongside its benefits, dairy farming also generates substantial waste, including liquid, solid, and gaseous forms. Liquid dairy waste primarily comprises 99.9% liquid (a mixture of urine, water, and feces) and 0.1% solid suspension, which contains both organic and inorganic materials (Hidayatullah et al., 2005). This liquid waste is often used solely for cleaning and sanitation purposes (Lumina, 2018), leaving its potential for further utilization untapped.

The use of liquid livestock waste, particularly from dairy cows, to produce biogas has emerged as an environmentally friendly alternative energy solution. Beyond biogas generation, the by-product, known as bioslurry, has demonstrated significant potential as a culture medium for microalgae cultivation. Utilizing bioslurry for microalgae culture provides dual benefits by recycling biogas waste while reducing eutrophication in aquatic ecosystems. The nutrients in bioslurry provide a food source for biological agents involved in the treatment of liquid waste without relying on chemical agents. Simultaneously, microalgae cultivation contributes to greenhouse gas mitigation as microalgae utilize CO_2 during photosynthesis. This integration presents an efficient method for nutrient recovery and CO_2 fixation within a single process. Additionally, microalgae provide renewable resources for biofuel production after extraction and conversion stages (Judd et al., 2017).

Several studies have highlighted the capabilities of *Euglena* sp. to reduce organic compounds in liquid waste while enhancing biomass production (Tossavainen et al., 2018). For instance, *Euglena* sp. has been shown to reduce nitrogen concentrations by 98% and phospor by 85% (Kuroda et al., 2018; Mahapatra et al., 2013). Research by Asiandu et al. (2022) further emphasizes that nutrient-rich liquid waste, such as tofu wastewater containing essential elements like C, N, and P, can significantly boost *Euglena* sp. metabolism. Such methods present cost-effective, efficient, and sustainable cultivation strategies (Rubiyatno et al., 2021).

The potential for large-scale cultivation of *Euglena* sp. is immense, given its ability to produce high-value natural products applicable in various industries (Kottuparambil et al., 2019). Its high lipid accumulation capacity during biomass production can be integrated with pollutant removal from liquid waste, thus supporting environmental sustainability (Mahapatra et al., 2013). Applications of microalgae include food, feed, chemicals, cosmetics, and pharmaceuticals. While macroalgae cultivation is prominent in Asian countries like China, Indonesia, and Japan, large-scale systems are also being developed in Western nations, such as the United States, Canada, and parts of Europe, for open-sea farming (Chynoweth, 2002). Among these, *Euglena* sp. stands out as a highly promising and widely used microalgae. this study aims to evaluate the potential of diluted bioslurry as a culture medium for cultivating *Euglena* sp., a microalga with diverse applications across various industries. The research seeks to address the challenge of managing biogas bioslurry liquid waste while exploring the viability of *Euglena* sp. cultivation for utilization in health, feed, food, and pharmaceutical sectors.

MATERIALS AND METHODS

Bioslurry preparation

The bioslurry dilution method is a crucial step in preparing an optimal growth medium for microalgae. Dilution processes of 10%, 20%, and 30% as many as 3 repititions were carried out to reduce the concentration of dissolved solids and decrease turbidity, thereby improving light penetration and the availability of essential nutrients for microalgae. The bioslurry was then sterilized to eliminate biological contaminants. The control medium used was Cramer Myers (CM) as 0% Bioslurry. The nutrient content of the Cramer Myers medium (CM) is shown in Table 1.

Bioslurry characterization

Bioslurry characterization was conducted to determine the nutrient content at various dilution levels used in the study. Parameters analyzed included total nitrogen (TN), total phosphorus (TP), and C/N and P/N ratios, using procedures based on Standard Methods. TN analysis was performed using the Kjeldahl Method (SNI: 2803, 2010), which involves sample decomposition with H_2SO_4 to produce ammonium sulfate $(NH_4)_2SO_4$, followed by distillation with NaOH and titration to determine TN concentration. TP analysis used ammonium molybdate and ammonium vanadate reagents mixed in specific ratios, with phosphorus concentration measured using a UV-VIS spectrophotometer at a wavelength of 882 nm, according to the ISRIC method (2002). The C/N and P/N ratios were calculated based on the comparison of carbon and phosphorus content to TN levels in the samples.

Euglena sp. growth rate

The growth rate was determined by daily cell count measurements using the Neubauer Improved Haemocytometer (Zhang et al., 2020). A total of 900 µL of microalgae sample was mixed with 100 µL of 70% alcohol in a 1 mL microtube for preservation. The sample was then loaded into a haemocytometer (1 mm size) and observed under a microscope.

Table 1. Cramer Myers (CM) composition used for *Euglena* sp. cultivation

Component	Unit	Volume
$(NH_4)_2SO_4$	mg/L	1000
KH_2PO_4	mg/L	1000
$MgSO_4.7H_2O$	mg/L	200
$CaCl_2.2H_2O$	mg/L	20
Jejak logam 10.000x	µL/L	100
$Na_2MoO_4.2H_2O$ 10.000x	µL/L	100
Vitamin B1 50.000x	µL/L	20
Vitamin B12 40.000x	µL/L	25

Biomass measurement of *Euglena* sp.

The dry weight of biomass was measured every three days over a 16-day period using the centrifugation method. For each replicate, 10 mL of the sample was transferred into a 15 mL conical tube and centrifuged at 4000 rpm for 10 minutes. The supernatant was separated using filter paper, and the residual biomass was dried in an oven at 30 °C until a constant weight was achieved. The dry biomass was then weighed using an analytical balance. (Wu et al., 2021).

Lipid content analysis

Lipid content was determined using the Bligh and Dryer method (1959). A 10 mL culture sample was centrifuged at 4000 rpm for 15 minutes, and the resulting pellet was collected. The pellet was mixed with 2 mL methanol and 1 mL chloroform, homogenized using a vortex mixer, and centrifuged again under the same conditions. The separated lower phase, containing lipids, was collected and transferred into a petri dish. The chloroform was evaporated in an oven at 40 °C, leaving the total lipid, which was calculated by the weight difference of the empty and lipid-containing petri dishes.

RESULT AND DISCUSSION

Characteristic of diluted bioslurry

The characterization of the bioslurry used in this study is presented in Table 2, while Figure 1 illustrates the nutrient reduction across different bioslurry dilutions. The bioslurry demonstrated the presence of essential nutrients, including C/N and P/N ratios, that are critical for fulfilling the physiological requirements for microalgae growth. Optimal C/N ratios are crucial for microalgae cultivation, as imbalances can adversely affect growth. Excessively high or low C/N ratios result in nutrient limitations, with excessive C/N causing restricted growth due to surplus carbon supply, while low C/N ratios can lead to ammonia inhibition (Malolan et al., 2020).

In this study, the 30% diluted bioslurry exhibited higher concentrations of nitrogen, organic carbon, phosphorus, potassium, and C/N ratios compared to the 20% and 10% dilutions. These variations in nutrient content among the bioslurry media are attributable to differences in dilution concentrations during the preparation of the culture media. The results confirm that nutrient availability in the bioslurry medium plays a pivotal role in influencing the growth and metabolism of *Euglena* sp. The findings emphasize the importance of selecting an appropriate bioslurry dilution to optimize nutrient balance for effective microalgae cultivation. This finding aligns with Heifetz et al. (2000), which indicates that the provision of high concentrations of organic carbon can inhibit microalgal growth. The increase in organic carbon concentration raises ATP demand for carbon assimilation, which in turn limits carbon fixation through photosynthesis, resulting in inhibited microalgae growth. Additionally, bioslurry at a 30% concentration exhibited higher levels of nitrogen, organic carbon, phosphorus, potassium, and a higher C/N ratio compared to bioslurry at 10% and 20% concentrations. These differences in nutrient content can be attributed to the varying degrees of bioslurry dilution applied in this experiment.

The study examines the nutrient removal efficiency of bioslurry used as a growth medium for microalgae under various dilution levels. The percentage of nutrient removal was evaluated to determine the optimal dilution for microalgal

Table 2. Nutrient composition of bioslurry at different dilution levels

Parameter (mg/L)	Diluted bioslurry					
	10%		20%		30%	
	Before	After	Before	After	Before	After
Nitrogen	55.34 ± 0.06[a]	42.77 ± 0.04[b]	85.8 ± 0.06[a]	69.03 ± 0.06[bc]	127.85 ± 0.10[a]	85.15 ± 0.10[c]
C organic	149.43 ± 0.47[a]	116.60 ± 0.22[b]	379.2 ± 0.29[a]	365.93 ± 0.30[bc]	560.81 ± 0.83[a]	367.51 ± 0.75[c]
Fosfor	28.75 ± 0.08[a]	25.00 ± 0.03[b]	30.26 ± 0.06[a]	25.83 ± 0.02[bc]	32.81 ± 0.20[a]	25.416 ± 0.17[c]
C/N	2.70 ± 0.12[a]	2.73 ± 0.14[c]	4.42 ± 0.06[a]	4.57 ± 0.04[b]	4.39 ± 0.18[a]	4.32 ± 0.24[bc]
P/N	0.52 ± 0.13[a]	0.58 ± 0.08[b]	0.35 ± 0.13[a]	0.37 ± 0.05[c]	0.26 ± 0.08[a]	0.30 ± 0.013[d]

Note: [a,b,c,d] different superscripts on the same row indicate significant differences.

growth and nutrient uptake. Among the tested dilutions, the 10% bioslurry demonstrated the highest overall nutrient removal efficiency. This indicates that microalgae were able to thrive and perform effective nutrient uptake at this concentration. The findings highlight the potential of 10% bioslurry dilution as a promising option for maximizing nutrient removal in microalgal cultivation systems.

The use of *Euglena* sp. in wastewater treatment is associated with its high metabolic flexibility and ability to absorb both inorganic and organic substances, thereby reducing their concentrations in water (Kotoula et al., 2020). The absorption of inorganic and organic compounds in bioslurry concentrations provides the nutrients required for microalgae metabolism. A 10% bioslurry concentration demonstrated more optimal growth of *Euglena* sp. compared to 20% and 30% bioslurry concentrations due to its higher C/N ratio. However, excessively high C/N ratios lead to the death phase in *Euglena* sp. As the C/N ratio increases, the turbidity of the wastewater used also increases. Turbidity can serve as an indicator of pollution, as higher turbidity is more likely to indicate the presence of contaminants in the water (Ramadhani et al., 2016).

This research examines the dilution of bioslurry, a waste product with potential as a nutrient source for microalgae cultivation. Metal contamination in cow dung is generally low because it is not a chemical waste, particularly after dilution has already occurred. This underscores the significance of dilution in preparing biogas bioslurry as a culture medium for microalgae, as it effectively reduces the concentration of pollutants. Diluting bioslurry with water decreases the levels of each contaminant in the wastewater. The primary objective of initial dilution treatment is to minimize or eliminate toxic substances from the wastewater (Vidyawati and Fitrihidajati, 2019).

Growth performance of *Euglena* sp. in diluted bioslurry

The study demonstrated that *Euglena* sp. could survive in all diluted bioslurry samples. This indicates the organism's adaptability to varying concentrations of nutrients present in the bioslurry. The growth rates of *Euglena* sp. varied significantly across different bioslurry treatments over the 16-day cultivation period, reflecting the influence of nutrient availability on microalgal growth and cell development. The highest growth rate was observed in the synthetic CM medium, achieving a maximum cell density of $169 \times 10^4 \pm 5.14$ cells/mL, showcasing the effectiveness of CM as an optimized growth medium for *Euglena* sp. However, notable growth was also achieved in the 10% bioslurry dilution, with a maximum cell density of $48 \times 10^4 \pm 5.02$ cells/mL. This suggests that diluted bioslurry at 10% concentration provides sufficient nutrients to support substantial growth, making it a viable alternative to synthetic media for cultivating *Euglena* sp. In contrast, growth rates in 20% and 30% bioslurry dilutions were lower, with maximum cell densities of $20 \times 10^4 \pm 2.08$ cells/mL and $24 \times 10^4 \pm 7.02$ cells/mL, respectively. The reduced growth at higher bioslurry concentrations could be attributed to nutrient imbalances or the potential presence of inhibitory substances at these higher concentrations,

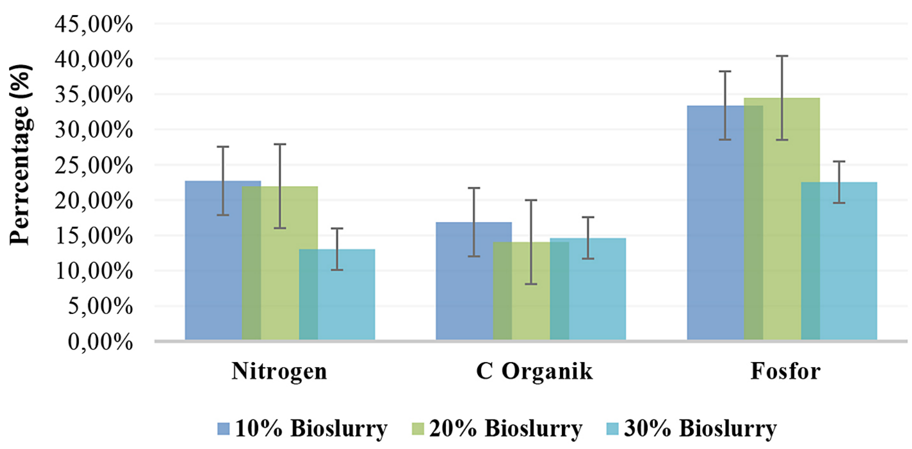

Figure 1. Nutrient removal efficiency of different dilution levels bioslurry through microalgae *Euglena* sp. cultivation

such as ammonia or heavy metals, which may impede cell division and overall growth (Figure 2).

The growth rate productivity of microalgae is influenced by light intensity and nutrient dilution levels, both of which play crucial roles in the photosynthesis process that supports biomass formation (Virama, 2015). Additionally, the decline in growth rate with increased bioslurry addition corresponds to its turbidity. Biogas by-products contain suspended solids or insoluble solids in water, which may be in floating, suspended, or settled forms. These compounds exist in both organic and inorganic forms. The dissolved solids contribute to water turbidity (Chotimah, 2010). Increased turbidity can limit the diversity of microalgae, as their growth declines with higher nutrient levels in the water. This suggests that turbidity and nutrient availability are significant factors affecting microalgal growth (Effendi et al., 2016). High turbidity can obstruct sunlight penetration into the water body, thereby inhibiting the photosynthesis process in microalgae (Wisha et al., 2016).

Euglena sp. can absorb organic compounds from heterotrophic environments through the process of phagocytosis. The surface of *Euglena* sp. cells forms vesicles to capture food particles. This microalga effectively absorbs nitrogen (N) and phosphorus (P) contained in wastewater. However, when the amount of wastewater absorbed exceeds the capacity for N and P uptake, the absorption process becomes less effective. Excessively high wastewater concentrations can inhibit microalgal growth. As noted by Elystia et al. (2020), the addition of tofu wastewater above 40% reduced the growth rate of *Scenedesmus* sp. due to the high wastewater concentration causing stress on the cells.

The most promising results were observed with the 10% bioslurry treatment, although the primary focus of this study was on the economic and environmental implications. This research highlights the potential of how *Euglena* sp. for bioremediation of biogas bioslurry, offering a cost-effective alternative to expensive synthetic media. Using waste media provides two key advantages: (1) generating microalgae biomass that can serve as a food source, and (2) reducing the need for synthetic nutrients by harnessing the nutrients already present in waste (Budiyono et al., 2014). In the 10% bioslurry treatment, the highest growth rate was achieved at 26 × 10^4 ± 6.75 cells/mL over an optimal period of 8 days. These findings establish a reference point for determining the optimal phase for *Euglena* sp. cultivation using biogas bioslurry, indicating when maximum biomass production can be achieved and maintained sustainably.

Biomass production of *Euglena* sp. in diluted bioslurry

The biomass production of *Euglena* sp. varied significantly across the different bioslurry treatments during the 16-day cultivation period, highlighting the influence of nutrient composition on cell growth and biomass accumulation. The highest biomass was recorded in the synthetic CM medium as a control, achieving a maximum biomass concentration of 22.67 ± 6.81 mg/L. This result underscores the optimized nutrient balance in CM medium, which supports optimal cell proliferation and biomass yield. In the 10% bioslurry treatment, *Euglena* sp. demonstrated a considerable biomass production, reaching a maximum concentration of 8 ±

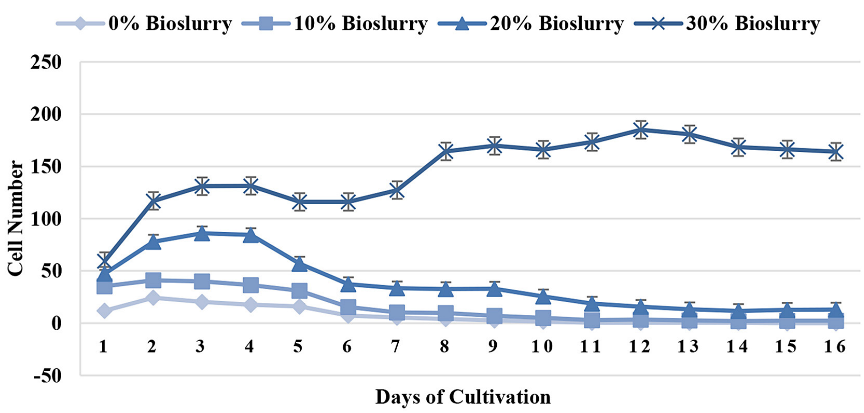

Figure 2. Growth rate of *Euglena* sp. at different bioslurry dilution levels

1.73 mg/L. This indicates that the 10% bioslurry dilution provides sufficient nutrients to support moderate biomass accumulation. The relatively high biomass yield in this treatment suggests that E0% bioslurry dilution is a viable alternative medium for sustainable microalgae cultivation. Conversely, biomass production in the 20% and 30% bioslurry treatments was lower, with maximum concentrations of 6.33 ± 1.53 mg/L and 6.67 ± 1.53 mg/L, respectively. The reduced biomass in these treatments could be attributed to nutrient imbalances or the presence of inhibitory substances at higher bioslurry concentrations, such as excess ammonia or heavy metals, which may negatively affect cell metabolism and biomass accumulation (Figure 3).

The percentage of bioslurry is known to influence biomass production in microalgae cultures. In addition to generating biomass, microalgae cultivation also contributes to CO_2 reduction. Microalgae biomass is rich in lipids and does not overlap with the demand for food production. Microalgae exhibit relatively fast growth rates, short life cycles, and high productivity per hectare. Furthermore, cultivation processes can be practically implemented using wastewater in community applications. The utilization of microalgal biomass in the production of bioactive metabolites has been applied in various industries, including pharmaceuticals, nutrition, and medicine (Bajhaiya et al., 2017). According to previous studies (Geremia et al., 2021), algae biomass accumulated in wastewater can serve as raw material for producing industrially valuable metabolites. The potential of bioslurry in various fields such as agriculture, energy, environment, and human health remains largely unexplored (Gupta, 2016).

Lipid content of *Euglena* sp. in diluted bioslurry

The maximum lipid content of *Euglena* sp. was observed in the control treatment (0% bioslurry), which was higher compared to other bioslurry treatments. This indicates that while bioslurry can support *Euglena* sp. growth, the CM remains the most favorable environment for lipid accumulation. The highest lipid content was recorded in the CM treatment, reaching a maximum of 22.67 ± 2.52 mg/L. Despite this, *Euglena* sp. cultivated in bioslurry dilutions of 10%, 20%, and 30% also exhibited lipid accumulation, with maximum lipid contents of 8.00 ± 1.00 mg/L, 7.33 ± 1.53 mg/L, and 7.67 ± 1.15 mg/L, respectively (Figure 4).

The increased lipid content in bioslurry treatments suggests that specific nutrient compositions in bioslurry, such as nitrogen and carbon sources, can stimulate lipid biosynthesis in *Euglena* sp. However, excessive nutrient concentrations, as seen in higher bioslurry dilutions, may lead to nutrient imbalances that can limit lipid accumulation. These findings align with prior studies indicating that controlled nutrient availability is critical for optimizing lipid production in microalgae. The data also highlight the potential of diluted bioslurry as an alternative growth medium for *Euglena* sp., especially for sustainable lipid production in biotechnological applications, though it remains less effective than synthetic media. Further optimization of bioslurry concentrations could enhance its efficacy for large-scale applications in the production of biofuels, nutraceuticals, and other lipid-based products. Nutrients such as carbon (C) are key components of microalgae. Carbon (C) functions as a building block for the structure and metabolism of

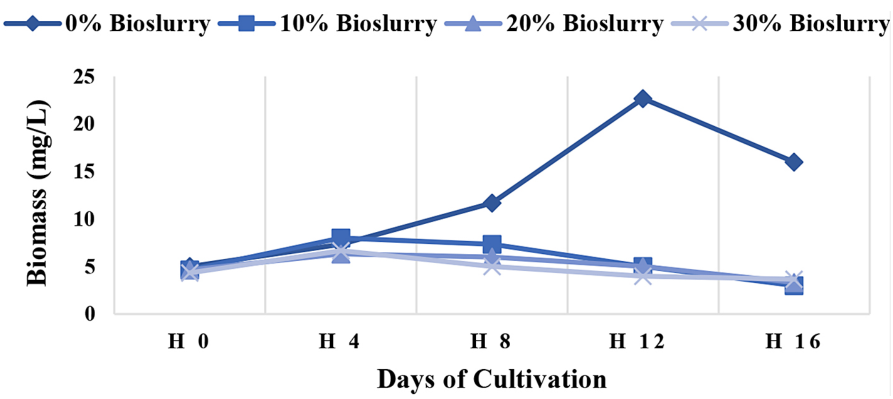

Figure 3. Biomass of *Euglena* sp. at different bioslurry dilution levels

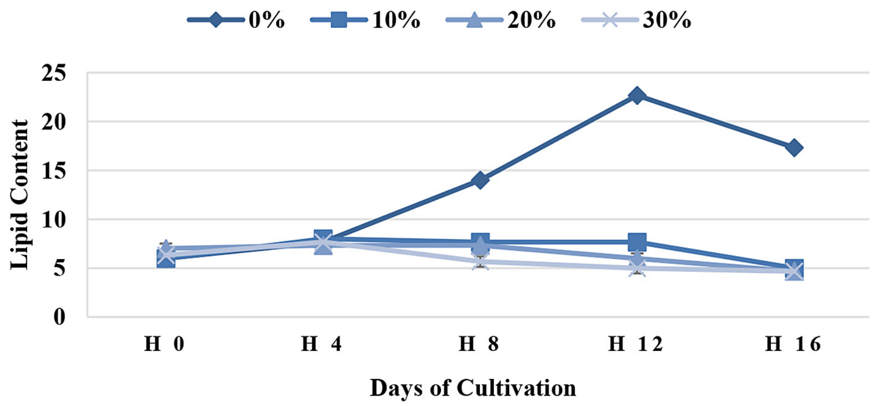

Figure 4. Lipid content of *Euglena* sp. at different bioslurry dilution levels

proteins, carbohydrates, lipids, and nucleic acids, making its availability crucial for microalgal growth. Meanwhile, nitrogen (N) plays a role in controlling protein biosynthesis within cells (Lari et al., 2016). The C/N ratio has been reported as a major factor in microalgal metabolism, with an increase in the C:N ratio shown to enhance the lipid content of microalgae (Lari et al., 2016).

Microalgae approach to various applications

Microalgal biomass can be converted into a variety of valuable bioproducts, including food, feed, pharmaceuticals, biopolymers, bioplastics, and bulk chemicals. Euglena exhibits highly complex metabolic potential as a unicellular organism, offering significant opportunities for the production of value-added biomolecules (O'Neil, 2015). The commercial potential of Euglena was recognized after the discovery of the high protein content and amino acid profile of E. gracilis (Kott, 1964). Additionally, Euglena cells contain nutritionally important fatty acids, such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and vitamins, underscoring their industrial potential as a valuable resource (Hayashi, 1993) (Figure 5).

Euglena sp. microalgae have been studied for their potential in sustainable biofuel production, with lipids, proteins, and carbohydrates serving as precursors for biofuels in microalgae. Lipids in *Euglena* exhibit a dominance of C16 methyl esters (42%) and C18 methyl esters (50%), emphasizing their biofuel properties, which are similar to vegetable oils (O'Neil, 2015). Lipids are biomolecules that serve as energy and carbon reserves within microalgal cells (Vitova, 2015). They ensure the survival of microalgal cells, particularly during nighttime, and provide energy for essential biological processes during cell division, including DNA replication, nuclear division, and cytokinesis. Several studies have reported significant lipid content in *Euglena*, comprising up to 13 different types of fatty acids, of which approximately 50% are unsaturated fatty acids (Mahapatra, 2013). Enhanced lipid biosynthesis efficiency under photosynthetic, heterotrophic, and anaerobic conditions has further advanced *Euglena's* biotechnological potential as a source for biofuel production.

This study highlights the potential of bioslurry as a sustainable medium for cultivating *Euglena* sp., demonstrating its dual role in reducing waste and supporting microalgal growth. The findings indicate that a 10% bioslurry dilution offers an optimal nutrient balance, promoting effective growth and moderate lipid accumulation in *Euglena* sp. while mitigating issues associated with higher concentrations, such as turbidity and nutrient imbalances. These results underscore the feasibility of integrating bioslurry into circular agricultural practices, contributing to nutrient recycling and CO_2 reduction. Future research should focus on optimizing bioslurry preparation methods and exploring its large-scale application for industrially valuable bioproducts, such as biofuels and nutraceuticals.

The cultivation of *Euglena* sp. utilizing a biogas bioslurry medium can be adapted for biogas purification. In its metabolism, microalgae inherently require carbon dioxide (CO_2) to perform photosynthesis. Increased fixation directly correlates with enhanced microalgal growth, consequently leading to an increase in microalgal biomass (Hadiyanto and Widayat, 2014). During the photosynthetic process, (CO_2) is assimilated

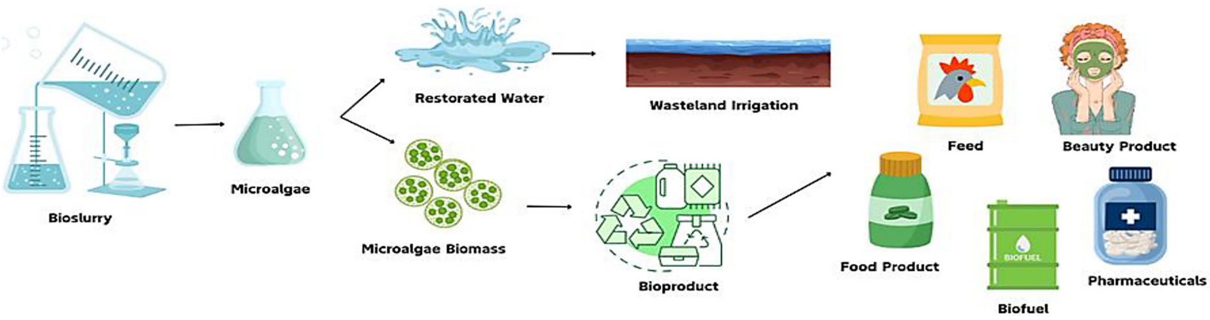

Figure 5. Microalgae applications across various industries and sectors

by the microalgae as a primary nutrient source. This (CO_2) fixation process effectively reduces the (CO_2) content within the biogas stream while simultaneously supporting the proliferation of the microalgae (Anwar, 2023).

CONCLUSIONS

This study demonstrates that biogas-derived bioslurry has a significant impact on the growth performance of *Euglena sp.*, primarily due to its content of essential nutrients such as carbon (C), nitrogen (N), and phosphor (P), which are critical for cellular metabolism and growth. While an increase in bioslurry concentration was associated with a decline in specific growth rate and biomass accumulation, the levels of primary metabolites remained relatively comparable to those observed in the control group (Cramer-Myers medium). This research critically explores the application of biogas slurry (bioslurry) as a culture medium for *Euglena* sp. to assess its viability in microalgae cultivation. The 10% bioslurry dilution exhibited favorable nutrient ratios, with C/N and P/N values of 2.73 ± 0.17 and 0.58 ± 0.03, respectively, while the 20% dilution showed increased C/N and reduced P/N ratios of 5.30 ± 0.51 and 0.37 ± 0.07. Among the tested conditions, the 10% dilution achieved the highest growth performance, with a peak cell density of $26 \times 10^4 \pm 6.75$ cells/mL, though it remained lower than the control (0% bioslurry) at $169 \times 10^4 \pm 5.14$ cells/mL. Conversely, the 20% and 30% dilutions significantly hindered growth, with peak densities of $20 \times 10^4 \pm 2.08$ cells/mL and $24 \times 10^4 \pm 7.02$ cells/mL, respectively ($p>0.05$). The highest lipid content was observed in the control medium (0% bioslurry) at 22.67 ± 2.52 mg/L, followed by 10%, 20%, and 30% dilutions with lipid concentrations of 8.00 ± 1.00 mg/L, 7.33 ± 1.53 mg/L, and 7.67 ± 1.15 mg/L, respectively. These results underscore the potential of a 10% bioslurry dilution as a practical strategy for enhancing *Euglena* sp. cultivation, offering a sustainable pathway to valorize biogas production residues within a circular agricultural economy framework. These findings suggest that, despite certain limitations at higher concentrations, bioslurry holds considerable potential as an alternative nutrient source for microalgal cultivation, offering a sustainable and cost-effective approach for biomass production in biotechnological applications.

Acknowledgements

The authors would like to sincerely acknowledge the financial support provided by Postgraduate Grant from Gadjah Mada University, Indonesia.

REFERENCES

1. Anwar, D. 2023. Reduction of carbon dioxide (CO_2) levels in biogas by biofixation of *Chlorella Vulgaris* And *Scenedesmus obliquus* microalgae. *CHEMTAG Journal of Chemical Engineering, 4*(2). https://doi.org/10.56444/cjce.v4i2.4432
2. Budiyono, Syaichurrozi, I., Sumardiono, S., Sasongko, S. B. (2014). Production of *Spirulina platenis* biomass using digested vinasse as cultivation medium. *Academix Journals*, *9*(2), 88–100. https://doi.org/10.3923/tasr.2014.93.102
3. Chotimah, S. N., (2010). *Biogas production from food waste with variations and substrate temperatures in an anaerobic biodigester*. Thesis. Sebelas Maret University, Surakarta.
4. Chynoweth, D. P., (2002). *Review of biomethane from marine biomass.* Gainsvville: Department of Agriculture and Biological Engineering,

University of Florida, 2002. https//doi.org/102478/v10145-010-0030-7

5. Effendi, H., Kawaroe, M., Lestari, D. F., Mursalin, Permadi, T. (2016). Distribution of phytoplankton diversity andabundance in Mahakam Delta, East Kalimantan. *Procedia Environmental Sciences, 33*, 496–504. https://doi.org/10.1016/j.proenv.2016.03.102
6. Elystia, S., Dian L., Sri R. M. (2020). Lipid production from microalgae scenedesmus sp. in tofu liquid waste media with variations in waste concentration and photoperiod. *Al-Ard Journal of Environmental Engineering*. University of Riau. Pekanbaru. https://doi.org/10.29080/alard.v5i2.519
7. Geremia, E., Ripa, M., Catone, C. M., Ulgiati, S. A. (2021). Review about microalgae wastewater treatment for bioremediation and biomass production – a new challenge for Europe. *Environments 8*(12), 136. https://doi.org/10.3390/environments8120136
8. Gupta, P. L., Lee, S. M., Choi, H. J. (2015). A mini review: Photobioreactors for large scale algal cultivation. *World J Microbiol Biotechnol 31*, 1409–1417. https://doi.org/10.1007/s11274-015-1892-4
9. Hadiyanto and Widayat. (2014). CO_2 biofixation by microalgae *Chlamydomonas* sp. in tubular photobioreactor. *Reaktor, 15*(1), April 2014, Hal. 37–42. https://doi.org/10.14710/reaktor.15.1.37-42
10. Hayashi, M., Toda, K., Misawa, Y., Kitaoka, S. (1993). Preparation of *Euglena gracilis* enriched with eicosapentaenoic and docosahexaenoic acids. *Aquaculture Science, 41*(2), 169–176. https://doi.org/10.29080/alard.v5i2.519
11. Heifetz, P. B., Forster, B., Osmond, C. B., Giles, L. J. et al. (2000). Effects of acetate on facultative autotrophy in *Chlamydomonas reinhardtii* assessed by photosynthetic measurements and stable isotope analyses. *Plant. Physiol., 122*, 1439–1445. https://doi.org/10.1104/pp.122.4.1439
12. Hidayatullah, Gunawan, Mudikdjo, K., Erliza, N. (2005). Melalui Penerapan Konsep Produksi Bersih. 8(1), 124–136. https://doi.org/10.21082/jpptp.v8n1.2005.p%p
13. Judd, S. J., Al Momani, F. A. O., Znad, H., Al Ketife, A. M. D. (2017). The cost benefit of algal technology for combined CO_2 mitigation and nutrient abatement. *Renewable and Sustainable Energy Reviews, 71*(September 2016), 379–387. https://doi.org/10.1016/j.rser.2016.12.068
14. Kings, A. J., Raj, R. E., Miriam, L. M., Visvanathan, M. A. (2017). Growth studies on microalgae Euglena sanguinea in various natural eco-friendly composite media to optimize the lipid productivity. *Bioresource technology, 244*, 1349–1357. https://doi.org/10.1104/pp.122.4.1439
15. Kotoula D., Iliopoulou A., Irakleous-Palaiologou E., Gatidou G., Aloupi M., Antonopoulou P., Fountoulakis M.S., Stasinakis A.S. (2020) Municipal wastewater treatment by combining in series microalgae Chlorella sorokiniana and macrophyte Lemna minor: preliminary results. *J. Clean. Prod. 271*, 122704. https://doi.org/10.1016/j.jclepro.2020.122704
16. Kott, Y., Wachs, A. M. (1964). Amino acid composition of bulk protein of Euglena grown in waste water. *Applied Microbiology, 12*(4), 292–294. https://doi.org/10.1128/am.12.4.292-294.1964
17. Kottuparambil, S., Thankamony, R. L., Agusti, S. (2019). Euglena as a potential natural source of value-added metabolites. A review. *Algal Research, 37*(August 2018), 154–159. https://doi.org/10.1016/j.algal.2018.11.024
18. Kuroda, M., Horino, T., Inoue, D., Morikawa, M., Ike, M. (2018). Biomass production and nutrient removal through cultivation of *Euglena gracilis* in domestic wastewater. *Japanese Journal of Water Treatment Biology, 54*(4), 105–113. https://doi.org/10.2521/jswtb.54.105
19. Lari, Z., Moradi-Kheibari, N., danAhmadzadeh, H., Abrishamchi, P.,Moheimani, N.R., Murry, M.A. (2016). Bioprocess engineering of microalgae to optimize lipid production through nutrient management. *Journal of Applied Phycology, 26*(6), 3235–3250. https://doi.org/10.1007/s10811-016-0884-6
20. Lumina, P., P, P. M. (2018). Research India Publications. http://www.ripublication.comThe Format of the IJOPCM, first submission. *International Journal of Applied Engineering Research, 13*(7), 249–252. http://www.ripublication.com
21. Mahapatra, D. M., Chanakya, H. N., Ramachandra, T. V. (2013). Euglena sp. as a suitable source of lipids for potential use as biofuel and sustainable wastewater treatment. *Journal of Applied Phycology*, *25*(3), 855–865. https://doi.org/10.1007/s10811-013-9979-5
22. Malolan, R., Jayaraman R. S., Adithya S., Arun J., Gopinath k. P., Sundar Rajan P. Nasig O., Kim W., Govarthanan m. (2020). Anaerobic digestate water for *Chorella pyrenoidosa* cultivation and employed as co-substrate with cow dung and chicken manure for methane and hydrogen production: a closed loop approach. *Chemosphere 266*, 128963. http://doi.org/10.1016/j.chemosphere.2020.128963
23. O'Neill, E. C., Trick, M., Henrissat, B., Field, R. A. (2015). Euglena in time: Evolution, control of central metabolic processes and multi-domain proteins in carbohydrate and natural product biochemistry. *Perspectives in Science, 6*, 84–93. https://doi.org/10.1016/j.pisc.2015.07.002
24. Puja Asiandu, A., Puspito Nugroho, A., Saifun Naser, A., Ryan Sadewo, B., Donny Koerniawan, M., Budiman, A., Juniarti Siregar, U., Tri Suwanti, L., Agus Suyono, E. (2022). The effect of tofu wastewater and pH on the growth kinetics and biomass composition of *Euglena* sp. *Current Applied Science*

and Technology, 23(2). https://doi.org/10.55003/cast.2022.02.23.010

25. Regnault, A., Chervin, D., Chammai, A., Piton, F., Calvayrac, R., Mazliak, P. (1995). Lipid composition of *Euglena gracilis* in relation to carbon-nitrogen balance. *Phytochemistry, 40*(3), 725–733. https://doi.org/10.1016/0031-9422(95)00268-C
26. Rubiyatno, Matsui, T., Mori, K., Toyama, T. (2021). Paramylon production by Euglena gracilis via mixotrophic cultivation using sewage effluent and waste organic compounds. *Bioresource Technology Reports, 15*(June), 100735. https://doi.org/10.1016/j.biteb.2021.100735
27. Tossavainen, M., Katyal Chopra, N., Kostia, S., Valkonen, K., Sharma, A. K., Sharma, S., Ojala, A., Romantschuk, M. (2018). Conversion of biowaste leachate to valuable biomass and lipids in mixed cultures of *Euglena gracilis* and chlorophytes. *Algal Research, 35*(October 2017), 76–84. https://doi.org/10.1016/j.algal.2018.08.007
28. Vidyawati, D. S. and Herlina, F. (2019). The effect of phytoremediation of water hyacinth (Eichornia crassipes) through dilution on the quality of tofu industrial liquid waste. *LenteraBio 8*(2), Mei 2019: 113–119. https://ejournal.unesa.ac.id/index.php/lenterabio/issue/view/1762
29. Virama, H.C.M. (2015). Droop model analysis for microalgae growth under nutrient limitations. final project of mathematics department. *Faculty of Mathematics and Natural Sciences, Sepuluh Nopember Institute of Technology.* Surabaya. Indonesia.
30. Vitova, M., Bisova, K., Kawano, S., Zachleder, V. (2015). Accumulation of energy reserves in alga e: from cell cycles to biotechnological applications. *Biotechnology advances, 33*(6), 1204–1218. https://doi.org/10.1016/j.biotechadv.2015.04.012
31. Wisha, U. J., Yusuf M., Maslukah L. (2016). Kelimpahan Fitoplankton dan Konsentrasi TSS Sebagai Indikator Penentu Kondisi Perairan Muara Sungai Porong. *Jurnal Kelautan, 9*(2), 122–129. https://doi.org/10.21107/jk.v9i2.1298
32. Wu, M, Qin, H, Deng, J, Liu, Y, Lei, A, Zhu, H. Wang, J. (2021). A new pilotscale fermentation mode enhances *Euglena gracilis* biomass and paramylon (β-1, 3-glucan) production. *Journal of Cleaner Production, 321*, 128996. https://doi.org/10.1016/j.jclepro.2021.128996
33. Zhang M., Gu L., Zheng P., Chen Z., Dou X., Qin Q., Cai X. (2020) Improvement of cell counting method for Neubauer Counting Chamber. *Journal of Clinical Laboratory Analysis 34*(1), e23024. https://doi.org/10.1002/jcla.23024