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Sustainable cultivation of microalgae *Euglena* sp. IDN 22 using anaerobic digested manure wastewater: Integrating circular bioeconomy principles in agroindustry

Dhomas Indiwara Prana Jhouhanggir[1], Ambar Pertiwiningrum[1*], Nanung Agus Fitriyanto[1], Eko Agus Suyono[2]

[1] Department of Animal Products Technology, Faculty of Animal Science, Gadjah Mada University, Indonesia
[2] Biotechnology Laboratory, Faculty of Biology, Gadjah Mada University, Indonesia
* Corresponding author's email: artiwi@mail.ugm.ac.id

ABSTRACT

This research was conducted by utilizing anaerobic digested manure wastewater (ADMW) as a growth medium for microalgae *Euglena* sp. IDN 22. This research aimed to determine the effectiveness of using ADMW as a rich nutrient supplement to cultivate microalgae *Euglena* sp. IDN 22. Multiple dilutions of 0%, 25%, 50%, and 75% were applied to ADMW, and the growth of *Euglena* sp. IDN 22 was analyzed using the growth rate of microalgae cell counts. The number of cells was observed with a haemocytometer. The cultivation of *Euglena* sp. IDN 22 in the ADMW medium was conducted in lab-scale (1 L) photobioreactors for 15 days of cultivation. The analysis of 25% ADMW showed C/N and P/N of 7.40 ± 0.17 and 1.27± 0.03, while 50% ADMW analysis yielded 14.99 ± 0.51 and 1.72 ± 0.07. The best growth rate trend of *Euglena* sp. IDN 22 in ADMW was shown by the treatment with 25% ADMW dilution with a maximum growth of 62.92×10^4 cells/ml, compared to a maximum growth of 79.92×10^4 cells/ml with 0% ADMW as a control. Slow growth activity was shown by 50% and 75% ADMW, with a maximum growth of only 10.67×10^4 cells/ml and 4.45×10^4 cells/ml, respectively. The slow growth activity in 50% ADMW, with a maximum growth of only 10.67×10^4 cells/ml, demonstrated a significant difference ($p > 0.01$). The results of this study prove that the integration of 25% ADMW and *Euglena* sp. IDN 22 cultivation can be an effective valorization concept and an opportunity to transform biogas production waste towards a circular economy in agroindustry.

Keywords: anaerobic digested manure wastewater, cell number, cultivation, *Euglena* sp., growth rate.

INTRODUCTION

Sustainable waste management needs to be done to maintain the overall balance of the ecosystem and reduce the environmental burden. The depletion of available natural resources has led to an increased interest in developing the concept of circular economy sustainable production, a process in which valuable by-product resources are kept in use for as long as possible in the production cycle through recovery and reuse (Markou et al., 2019). To address the challenges of waste management, it is essential to integrate the sustainable practices that not only minimize waste generation but also maximize the value extracted from resources throughout their lifecycle (Perdana et al., 2023). The implementation of circular economy principles (Krishnan et al., 2020) allows for the reduction of waste, promotes resource efficiency, and fosters the creation of new economic opportunities by encouraging the reuse, recycling, and upcycling of materials (Sarkar et al., 2022). Therefore, this approach contributes to environmental preservation and economic resilience, creating a pathway toward a more sustainable future.

Biogas production serves as an efficient agro-industrial waste management strategy, significantly reducing organic waste volume while generating renewable energy (Khan et al., 2023). However, despite its energy production benefits, the byproduct of this process, anaerobic digested manure wastewater (ADMW), still poses environmental challenges. These challenges include high transportation requirements, the release of greenhouse gases when stored, and nutrient loss or leaching when applied to land (Baral et al., 2018). To address such challenges, microalgae have been widely integrated with various waste streams, including flue gas, municipal, and agro-industrial wastewater, to utilize their nutrient content for biomass production (Chong et al., 2022). Among the promising microalgal species, *Euglena* sp. has gained attention for its ability to generate biofuels, such as bioethanol and biodiesel, due to its high lipid accumulation (Gissibl et al., 2019). Beyond biofuel production, *Euglena* sp. is also valuable as a raw material for functional foods, feed additives, cosmetics, and pharmaceuticals, enhancing its economic potential (Nurafifah et al., 2023). Importantly, *Euglena* sp. cultivation offers a sustainable approach for ADMW effluent treatment. By utilizing ADMW as a nutrient source, *Euglena* sp. can contribute to wastewater bioremediation while simultaneously reducing the need for external inputs such as fertilizers and freshwater. This dual-purpose application not only mitigates the environmental impact of ADMW, but also enhances the economic feasibility of microalgae-based bioprocesses (Chong et al., 2022).

The integration of microalgae cultivation with ADMW management has been extensively explored in various studies. As photosynthetic organisms, microalgae utilize nutrients from ADMW, carbon dioxide (CO_2), and solar energy to produce biomass through photosynthesis (Chen et al., 2022). Previous research has investigated the use of ADMW for microalgae cultivation with dilution levels of 10%, 20%, 30%, 40%, and 50% in media prepared using centrifuged digestate (CLD) and distilled digestate (DLD). The highest biomass production of *Chlorella vulgaris* and *Arthrospira platensis* was observed at 50% DLD and 10% CLD, respectively. These findings highlight the importance of optimizing dilution levels, which are generally recommended between 30% and 50% for liquid digestate-based microalgae cultivation (Kisielewska et al., 2022).

However, while dilution strategies for certain microalgae species have been studied, there is limited research on the optimal dilution levels for *Euglena* sp. cultivated in ADMW. Given the potential of *Euglena* sp. for biofuel production and wastewater treatment, it is essential to determine the appropriate dilution that maximizes its growth. Therefore, this study aimed to identify the most suitable ADMW dilution for *Euglena* sp. IDN 22 cultivation, bridging this knowledge gap and contributing to the advancement of sustainable microalgae-based bioremediation.

METHODS

Culture conditions

The microalgae *Euglena* sp. IDN 22 used in these experiments were obtained from the Biotechnology Laboratory, Faculty of Biology, Gadjah Mada University, Indonesia. The ADMW used as the microalgae growth medium was sourced from the biogas plant at the Faculty of Animal Science, Gadjah Mada University. This biogas plant operates under anaerobic conditions, using feeding substrates primarily composed of crop residues and animal manure, with a substrate-to-water ratio of 1:2. These substrates were chosen due to their abundance in agroindustry and complementary characteristics. Crop residues are rich in carbon (Fu et al., 2021), while manure provides nitrogen (Dalias and Christou, 2020), resulting in a balanced C/N ratio ideal for anaerobic digestion. Compared to ADMW from single substrates, this mixture produces effluent with moderate nutrient levels and lower ammonia toxicity, which is more suitable for microalgae cultivation. The 1:2 ratio has been proven to optimize biogas production (Kouakou et al., 2025). It also maximizes organic matter utilization and produces bioslurry with more stable conditions. The biogas retention time for the ADMW experiment was 40 days. Cultures of *Euglena* sp. IDN 22 were grown in a closed, 1-liter transparent glass photobioreactor under continuous light, with the pH maintained at 7.00. The culture temperature was kept at 24.0°C, and aeration was provided using an air pump system to ensure proper mixing of the culture medium, maintain homogeneity throughout the reactor volume, and supply CO_2 to the culture. Sterility was maintained by spraying 70% alcohol every 24 hours in the *Euglena* sp. IDN 22 cultivation area and photobioreactor to prevent microbial contamination during the cultivation period, as routine daily disinfection helps minimize the risk of airborne or surface-borne contaminants.

ADMW pretreatment

ADMW produced during the biogas production process must be stored for several days before use. In this study, ADMW was stored for 7 days in an open tank to allow residual microbial activity and gas release to stabilize. This period ensures that active methanogenesis subsides, reducing the risk of continued anaerobic activity that may interfere with subsequent microalgal cultivation (Duan et al., 2020). Prior to inoculation, the digested manure was diluted. Dilution is a method to reduce the turbidity of the biogas slurry and provide a suitable medium for growing microalgae (Kisielewska et al., 2022). The composition of the culture medium was adjusted to include 0% ADMW (synthetic medium Cramer Myers) as a control, 25% ADMW, 50% ADMW and 75% ADMW. Table 1 displays the Cramer Myers (CM) nutrient content. Deionized water was used to dilute ADMW. The diluted ADMW sample was filtered twice: first, to remove large particles from the raw ADMW and second to eliminate smaller particles after the initial filtration. Filtration was performed using a 10 µm pore size filter, followed by sterilization in an autoclave to remove both large particles and indigenous bacteria. No additional nutrients were added for the scale-up of the cultivation. The experimental flow of *Euglena* sp. IDN 22 cultivations in ADMW is illustrated in Figure 1.

Nutrient content analysis

The ADMW samples were analyzed to determine the nutrient content at each dilution. The concentrations of total nitrogen (T-N), total phosphorus (T-P), and organic carbon (C) were measured following standard protocols. Total nitrogen (T-N) was determined using the Kjeldahl Method according to SNI 2803:2010. Total phosphorus (T-P) was measured by spectrophotometric analysis using a UV-VIS spectrophotometer at 882 nm (ISRIC, 2002). Organic carbon content was measured using a UV-VIS spectrophotometric method at 561nm (Sari et al., 2023). The C/N and P/N ratios were calculated by comparing the concentrations of organic carbon and total phosphorus to total nitrogen, respectively. Each analysis was performed in triplicate to ensure data accuracy.

Table 1. Cramer Myers (CM) composition used for *Euglena* sp. IDN 22 cultivation

Component	Unit	Volume
$(NH4)_2SO_4$	mg/L	1000
KH_2PO_4	mg/L	1000
$MgSO_4 \cdot 7H_2O$	mg/L	200
$CaCl_2 \cdot 2H_2O$	mg/L	20
Trace metal 10.000x	µL/L	100
$Na2MoO_4 \cdot 2H_2O$ 10.000x	µL/L	100
Vitamin B1 50.000x	µL/L	20
Vitamin B12 40.000x	µL/L	25

Euglena sp. IDN 22 cell density

Cell number is an indicator of the growth rate of microalgae cells. The number of microalgae cell was counted daily using a Neubauer Improved Haemocytometer (Zhang et al., 2020). A 900 µL (microliter) sample of microalgae in ADMW was transferred to a 1 mL microtube, followed by the addition of 100 µL of 70 percent alcohol to the microtube. The sample was then inserted into a 1 mm (millimeter) Neubauer Improved Hemocytometer for observation under a microscope. Thus, this microalgae cell number measurement was taken every day for the entire 15-day observation period.

RESULTS AND DISCUSSION

Nutrient removal of ADMW

The characterization results of the ADMW culture medium utilized in the experimental variants are presented in Table 2, while Figure 2 illustrates the nutrient removal efficiency across multiple dilutions of ADMW. In microalgae cultivation, the composition of the medium is crucial, with a particular focus on maintaining a well-balanced nutrient profile (Chakraborty et al., 2023). The carbon-to-nitrogen (C/N) ratio and phosphorus-to-nitrogen (P/N) ratio must be optimized to support the physiological demands of microalgae, ensuring efficient growth and biomass production (Razzak et al., 2024). An optimal C/N ratio is crucial, as deviations from this balance can impede the microalgal growth. Specifically, a high C/N ratio may lead to an excess of carbon, resulting in nutrient limitation that restricts the development of microalgae. Conversely, a low C/N ratio can cause nitrogen

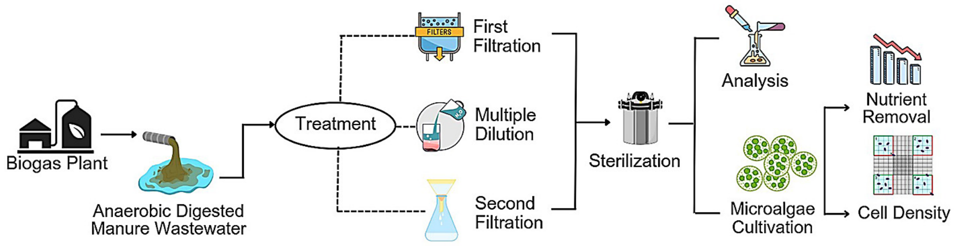

Figure 1. Experimental flow of *Euglena* sp. IDN 22 cultivations in ADMW

imbalance, leading to ammonia toxicity, which can inhibit growth (Malolan et al., 2020).

In this study, the 75% diluted ADMW exhibited higher concentrations of nitrogen, organic carbon, phosphorus, potassium, and a more favourable C/N ratio compared to the 50% and 25% dilutions. The variations in nutrient concentrations observed are directly attributed to the dilution levels of ADMW, which modulate the nutrient availability in the culture medium. This finding underscores the importance of optimizing dilution factors to achieve a balanced nutrient supply that promotes microalgal growth and enhances the efficiency of nutrient removal. The results suggest that higher ADMW concentrations may offer a more conducive environment for microalgae, though further investigation is necessary to identify the specific dilution thresholds that maximize both nutrient removal and biomass production. Such insights are critical for scaling up microalgae cultivation systems, as they provide a foundation for fine-tuning nutrient profiles to improve the overall system performance.

The retention of essential nutrients in ADMW following anaerobic digestion of biogas makes it a promising supplement for microalgae cultivation (Jiminez et al., 2020). However, while all diluted ADMW concentrations provided the nutrients necessary for microalgae growth, the nutrient content in the 25% ADMW dilution was found to be the most suitable for promoting optimal development of microalgae. This dilution demonstrated the highest nutrient absorption efficiency. In contrast, the 50% and 75% ADMW dilutions exhibited elevated C/N ratios, which can be detrimental to microalgae growth. The optimal C/N ratio for microalgae is generally within the range of 4 to 8 (Kisielewska et al., 2022), and deviations from this range can result in unfavorable growth conditions. An excessive carbon supply, which often accompanies a high C/N ratio, may inhibit the production of photosynthetic pigments in microalgae, thereby reducing photosynthetic efficiency and biomass accumulation.

Critical limitation of ADMW as a microalgae growth medium is its low phosphorus-to-nitrogen (P/N) ratio, which fails to meet the stoichiometric phosphorus requirements for optimal microalgal growth (Cheng et al., 2021). Both the 50% and 75% ADMW dilutions exhibited similarly low P/N ratios, which suggest a deficiency in phosphorus relative to nitrogen. While the ideal P/N ratio varies by microalgae species, a range of 7–16 has been proposed as optimal for ensuring balanced nutrient availability (Chowdury et al., 2020). A P/N ratio outside this range with excess nitrogen often indicates nutrient imbalance. The surplus nitrogen is usually in the form of ammonia, which can be

Table 2. Nutrient characteristics of ADMW at different dilution levels

Parameters (mg/L)	ADMW concentration					
	ADMW 25%		ADMW 50%		ADMW 75%	
	Before	After	Before	After	Before	After
TN	1.59 ± 0.06	0.43 ± 0.004	2.23 ± 0.06	1.39 ± 0.06	3.82 ± 0.10	3.04 ± 0.10
TOC	11.77 ± 0.47	2.77 ± 0.22	33.41 ± 0.29	22.40 ± 0.30	69.21 ± 0.83	64.58 ± 0.75
TP	2.02 ± 0.08	0.22 ± 0.03	3.84 ± 0.06	0.89 ± 0.02	7.70 ± 0.20	7.10 ± 0.17
C/N	7.40 ± 0.17	6.50 ± 0.29	14.99 ± 0.51	16.16 ± 0.45	18.11 ± 0.62	21.23 ± 0.43
P/N	1.27 ± 0.03	0.51 ± 0.04	1.72 ± 0.07	1.74 ± 0.05	2.01 ± 0.10	2.33 ± 0.02

Note: ADMW (anaerobic digested manure waste water); TN (total nitrogen); TOC (total organic carbon); TP (total phosphorus).

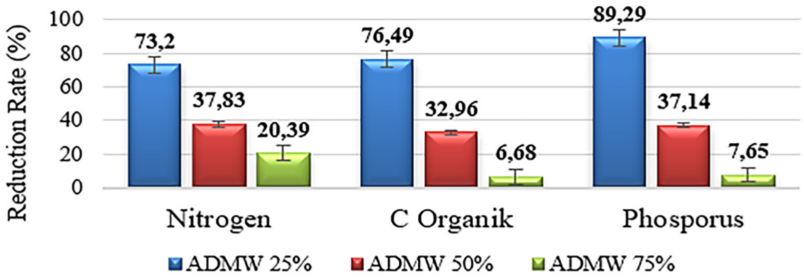

Figure 2. Nutrient removal analysis of ADMW following the cultivation of *Euglena* sp. IDN 22

toxic to microalgae under certain conditions (Salbitani and Carfagna, 2021).

The nutrient composition of ADMW, including the C/N and P/N ratios, is largely influenced by the source of the biogas feedstock and the operational conditions during anaerobic digestion. These findings highlight the necessity of modifying the biogas feedstock to achieve a more balanced nutrient profile. Combining ADMW with feedstocks that have lower or higher C/N ratios such as animal manure or sewage (low C/N) or organic solid waste (high C/N) could help optimize the C/N ratio and rectify the nutrient deficiencies observed, thereby enhancing microalgal growth and improving the overall efficiency of the cultivation process (Zheng et al., 2017).

In conclusion, while ADMW demonstrates potential as a nutrient source for microalgae, its effectiveness is contingent upon careful management of its nutrient composition, particularly in relation to the C/N and P/N ratios. Further optimization of the feedstock and operational parameters in biogas production could enhance the utility of ADMW in large-scale microalgal cultivation systems.

Euglena sp. IDN 22 growth rates

Euglena sp. IDN 22 was able to survive in all diluted ADMW samples, as shown in Figure 3.

Table 3. Maximum cell density of *Euglena* sp. IDN 22 at each ADMW concentration

ADMW concentration	Growth rate (n × 10^4 cells/ml)
0%	79.92 ± 1.50[a]
25%	62.92 ± 1.01[b]
50%	10.67 ± 1.42[c]
75%	4.55 ± 0.44[d]

Note: ADMW (anaerobic digested manure waste water); [abcd] (different subsets indicate significant differences at a significance level of $p < 0.01$)

Euglena sp. IDN 22 showed differences in growth rate among ADMW treatments during 15 days of cultivation, indicating that different nutrient content can affect the cell growth and development of microalgae (Yaakob et al., 2021). The data analysis results based on Table 3 showed significant differences ($p < 0.05$) among all ADMW concentration treatments, indicating that each dilution level had a distinct effect. The best growth rate was recorded in CM as a synthetic medium, reaching a maximum growth rate of $79.92 \times 10^4 \pm 1.50$ cells/ml. However, *Euglena* sp. IDN 22 can also grow well and survive in 25% ADMW with the same death phase as a synthetic medium with a maximum growth rate of $62.92 \times 10^4 \pm 1.01$ cells/ml. *Euglena* sp. IDN 22 at both 0% and 25% ADMW showed the same death phase after day 13 of cultivation due to nutrient depletion. In contrast, the 50% and 75% ADMW experiments showed slower growth rate, entering the death phase after day 5 of *Euglena* sp. IDN 22 cultivations with maximum growth rates of only $10.67 \times 10^4 \pm 1.42$ and $4.45 \times 10^4 \pm 0.44$ cells/ml, respectively. The growth performance of the synthetic medium served as an experimental control in *Euglena* sp. IDN 22 cultivations, yielding similar results to those observed in 25% ADMW when compared to 50% and 75% ADMW. The death phases of 50% and 75% ADMW occurred faster than other treatments due to the incompatibility of nutrient content for *Euglena* sp. IDN 22.

The morphology of *Euglena* sp. IDN 22 cultivated in ADMW is illustrated in Figure 4. This unicellular flagellate possesses a prominent flagellum, facilitating locomotion through aquatic environments. Additionally, it features a red-pigmented eyespot, or stigma, functioning as a photoreceptive organelle that enables the organism to detect light direction and intensity, thereby guiding phototactic movement toward optimal light conditions for photosynthesis

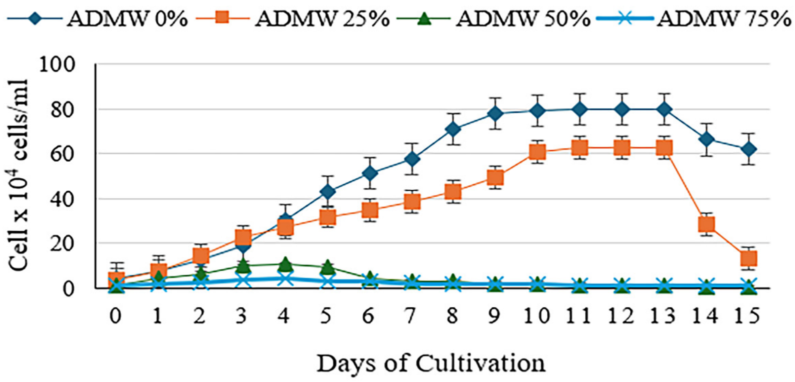

Figure 3. Growth curves of *Euglena* sp. IDN 22 in ADMW at different dilution levels

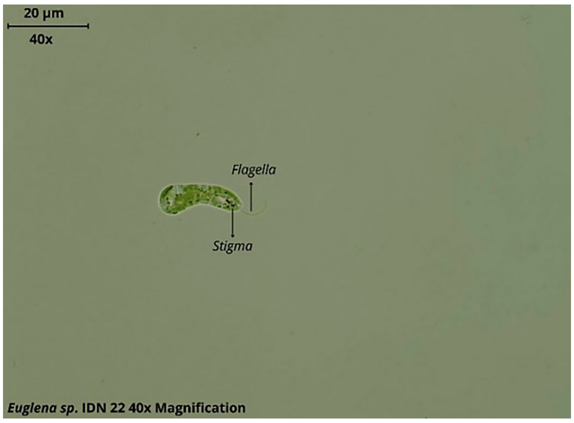

Figure 4. Microalgae *Euglena* sp. IDN 22 observed under a microscope at 40x magnification

(Duchene and Drouard, 2021). The performance of *Euglena* sp. IDN 22 in ADMW is significantly influenced by the availability of a balanced nutrient profile.

The nutrient composition of ADMW, including total nitrogen, total phosphorus, and organic carbon, plays a critical role in the metabolism and growth of microalgae cultures (Chong et al., 2022). Nitrogen is essential for the synthesis of proteins, nucleic acids, and other cellular components, typically absorbed from nitrate, nitrite, or ammonium. Phosphorus, a key element in ATP, nucleic acids, and phospholipids, plays a central role in energy transfer and cell membrane structure (Shah et al., 2024). An imbalance in these nutrients, whether an excess or deficiency, can severely affect the microalgal metabolism, leading to reduced growth, impaired photosynthetic activity, and diminished biomass production (Al-Mallahi and Ishii, 2022). Therefore, maintaining an optimal C/N/P ratio is crucial for maximizing the productivity of microalgae, as it ensures the availability of these essential nutrients in the correct proportions for efficient metabolic function.

Optimal ADMW dilution is essential for achieving the ideal conditions necessary for microalgal cultivation (Gao et al., 2021). Nutrients in ADMW can support microalgal growth but its composition may also be toxic depending on the waste source. This is especially true if the microalgae strain has not adapted to such conditions (Praveen et al., 2018). Additionally, the slower growth observed in microalgae cultures grown in 50% and 75% ADMW can be correlated with increased turbidity. Turbidity is an important factor in assessing the suitability of wastewater for microalgae cultivation, as it is caused by suspended particles that reduce light transmission through the medium (Franco et al., 2018). Since light is a key driver of photosynthesis, the scattering and absorption of light by suspended solids – such as clay, sludge, finely divided inorganic and organic materials, and other microscopic organisms negatively impact photosynthetic efficiency (Chuka-ogwude, 2020).

Integrated ADMW-microalgae as a valorization approach

Combining anaerobic digestion with microalgae cultivation can offer many environmental benefits, particularly through the production of abundant algal biomass to serve as biofuel feedstock, fertilizer, or animal feed, and thus provide a valuable solution for managing manure wastes (Razzak et al., 2017). As shown in Figure 5, a futuristic industrialization and scaling of the process proposed in Figure 1 can facilitate waste valorization to maximize microalgal biomass production, thereby potentially reducing negative effects on the surrounding environment and increasing overall economic benefits (Chandrasekhar et al., 2021). The use of anaerobic digestate as a substrate for microalgae cultivation media is gaining significant attention as a bioremediation technology for livestock wastewater, while simultaneously producing sustainable microalgae biomass feedstock (Yang et al., 2022). Microalgae, including *Euglena* sp. IDN 22, can efficiently utilize nutrients from the anaerobic digestate fraction to reduce both inorganic and organic carbon content while producing microalgal biomass that can be utilized for bioenergy applications (Fabris et al., 2020).

The integration of microalgae cultivation technology with ADMW treatment represents a strategic approach for achieving a cyclic utilization of ADMW. This system not only facilitates microalgal growth but also contributes to the removal of excess nutrients in ADMW, thus mitigating the risk of nutrient runoff and preventing the eutrophication of water bodies, a critical environmental issue (Yang et al., 2022). Furthermore, microalgal biomass can be converted into a wide range of valuable bioproducts, such as food, feed, pharmaceuticals, biopolymers, bioplastics, and bulk chemicals. For instance, high-protein microalgae offer an alternative feed source for livestock and aquaculture industries, reducing the reliance on traditional animal feed sources and contributing to more sustainable food production systems (Khanra et al., 2018). The integrated system with microalgae cultivation technology exemplifies a circular economy approach, effectively addressing the challenges of organic waste management by transforming agricultural and organic waste into valuable resources. This process plays a crucial role in minimizing the environmental impacts associated with agriculture, particularly in terms of nutrient pollution, greenhouse gas emissions, and the land-use changes required for conventional animal feed production (Markou et al., 2019). By closing the loop of organic waste through the production of microalgae, the system supports sustainable agricultural practices while contributing to the reduction of environmental degradation.

The relevance of this approach to the sustainable development goals (SDGs) is substantial. First, it directly addresses SDGs 2 (Zero Hunger) by providing a sustainable alternative for food and feed production. The use of microalgae as a protein-rich source for both livestock and aquaculture can help increase food security and reduce reliance on conventional feed crops. Additionally, this system supports SDGs 6 (Clean Water and Sanitation) by mitigating nutrient pollution and preventing eutrophication, which is a significant threat to water quality. SDGs 7 (Affordable and Clean Energy) is also impacted through the potential for microalgae biomass to be converted into biofuels and other renewable energy sources. Moreover, the system contributes to SDGs 8 (Decent Work and Economic Growth) by promoting

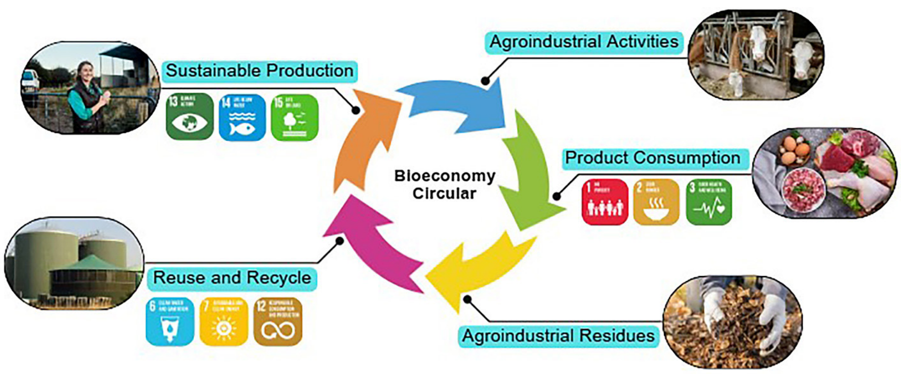

Figure 5. Schematic representation of ADMW-microalgae integration with various sustainable development goals (SDGs)

the growth of green technologies as well as industries related to bioproducts and sustainable agriculture. It is closely aligned with SDGs 9 (Industry, Innovation, and Infrastructure), fostering innovation in waste management and resource recovery. Furthermore, SDGs 13 (Climate Action) is addressed through the reduction of greenhouse gas emissions and the promotion of low-carbon technologies. Finally, this system supports SDGs 14 (Life Below Water) and SDGs 15 (Life on Land) by reducing the environmental impacts of agricultural runoff, contributing to the protection of aquatic ecosystems and biodiversity.

Limiting factors of integrated ADMW-microalgae

ADMW is the residual organic matter from biogas production process and highly dependent on the characteristics of the feedstock (Chong et al., 2022) also the operating conditions of the digester (Baral et al., 2018). ADMW is rich in several macronutrients (N, P, K, S, Mg, Ca, Fe, and Na) and may also contain trace elements (Co, Fe, Se, Mo, and Ni) that are derived from the feedstock used (Jiminez et al., 2020). Overall, nutrients in anaerobic digestion, such as nitrogen, phosphorus, and potassium are conserved and converted into more organic forms during anaerobic digestion (Yang et al., 2022). As photosynthetic organisms, microalgae utilize phosphorus (P) and nitrogen (N) in ADMW for their growth (Chong et al., 2022). Therefore, the C/N ratio of ADMW can serve as an indicator of nutrient availability, which can affect both microalgal growth and product quality. The C/N ratio has been known to affect biomass production in microalgae culture. A higher C/N ratio results in higher biomass. Research indicates that the C/N ratio of ADMW is unsuitable for microalgae growth. At the same time, a C/N ratio of 4 to 8 (Chong et al., 2022) is optimal for microalgae growth, whereas the P/N ratio of 7–16 (Chowdury et al., 2020) has been recommended to provide adequate balanced nutrients for microalgae.

To scale up microalgae cultivation from laboratory to commercial levels, several treatments must be implemented to address the inherent challenges associated with using anaerobic digestate as a growth medium (Razzak et al., 2024). One of the primary obstacles is the high turbidity of the digestate, which can impede light penetration essential for photosynthetic activity. This turbidity, often caused by suspended solids, can reduce the efficiency of light transmission, thus limiting the growth potential of microalgae (Kisielewska et al., 2022). Another significant issue is the excessive and unbalanced nutrient concentrations in the digestate. While nutrients are vital for microalgae growth, an overabundance or imbalance – particularly in the C/N/P ratios – can lead to nutrient toxicity, restricting microalgal metabolism and growth rates (Zheng et al., 2017). Additionally, the presence of competing microorganisms in anaerobic digestate can further complicate the cultivation process. These microorganisms may outcompete microalgae for available nutrients, thus reducing the overall productivity of the system (Chong et al., 2022). Furthermore, the potential toxicity of certain organic compounds and high ammonia concentrations in the digestate presents a major hurdle. Ammonia, in particular, is known to inhibit microalgal growth at elevated concentrations, further limiting the efficiency of cultivation (Al-Mallahi and Ishii, 2022).

CONCLUSIONS

ADMW is gaining significant attention as a sustainable microalgae biomass feedstock and a bioremediation technique for livestock effluent. Microalgae, such as *Euglena* sp. IDN 22, can efficiently use nutrients from the anaerobic digestate fraction, thereby lowering the amount of both inorganic and organic carbon in optimal nutrient composition. This study experimented with growing *Euglena* sp. IDN 22 in 0% ADMW, 25% ADMW, 50% ADMW and 75% ADMW. The results showed that 25% ADMW provided the most optimal nutrient content for the growth of *Euglena* sp. IDN 22, despite 50% and 75% ADMW having higher nutrient levels. The C/N and P/N ratios in 25% ADMW were 7.40 ± 0.17 and 1.27 ± 0.03, respectively, whereas 50% ADMW exhibited ratios of 14.99 ± 0.51 and 1.72 ± 0.07, and 75% ADMW showed ratios of 18.11 ± 0.62 and 2.01 ± 0.10. These results were evident in the cultivation of *Euglena* sp. IDN 22 in 0% ADMW (control), 25% ADMW, 50% ADMW, and 75% ADMW in the study, which had maximum growth rates of $79.92 \times 10^4 \pm 1.5$ cells/ml, $62.92 \times 10^4 \pm 1.01$ cells/ml, $10.67 \times 10^4 \pm 1.42$ cells/ml, and $4.45 \times 10^4 \pm 0.44$ cells/ml, respectively. The integration of anaerobic digestate management with microalgae cultivation presents a win-win strategy for both sustainable waste management and sustainable microalgae production. This strategy can positively impact the environment, provide an effective solution for agricultural waste, and produce a large amount of microalgae biomass, which can be used as a raw material for a variety of medicines, fertilizers, or animal feed. Future research should address several challenges, such as high turbidity, inappropriate nutrient content, competing biological contaminants, and metal toxicity in ADMW medium.

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REFERENCES

1. Abomohra, A., Elsayed E. F., Esakkimuthu M., S., El-Sheekh M., Hanelt D. (2020). Potential of fat, oil and grease (FOG) for biodiesel production: a critical review on the recent progress and future perspectives. *Progress in Energy and Combustion Science 81*, 1–32. https://doi.org/10.1016/j.pecs.2020.100868
2. Al-Mallahi, J. and Ishii, K. (2022). Attempts to alleviate inhibitory factors of anaerobic digestate for enhanced microalgae cultivation and nutrients removal: A review. *Journal of Environmental Management. 304*, 114266. https://doi.org/10.1016/j.jenvman.2021.114266
3. Baral K. R., Jego G., Amon B., Bol R., Chantigny M. H., Olesen J. E., Petersen S. O. (2018). Greenhouse gas emissions during storage of manure and digestates: key role of methane for prediction and mitigation. *Agricultural Systems 166*, 26–35. https://doi.org/10.1016/j.agsy.2018.07.009
4. Chandrasekhar K., Raj T., Ramanaiah S., Kumar G., Banu J. R., Varjani S., Sharma P., Pandey A., Kumar S., and Kim S. H. 2021. Algae biorefinery: a promising approach to promote microalgae industry and waste utilization. *Journal of Biotechnology 10*, 1–16. https://doi.org/10.1016/j.jbiotec.2021.12.008
5. Chowdury, K. H., Nahar, N. and Deb, K. U. (2020). The growth factors involved in microalgae cultivation for biofuel production: A review. *Computational Water, Energy, and Environmental Engineering. 9*(4). https://doi.org/10.4236/cweee.2020.94012
6. Chen, J., Dai L., Mataya D., Cobb K., Chen P., Ruan R. (2022). Enhanced sustainable integration of CO_2 utilization and wastewater treatment using microalgae in circular economy concept. *Bioresource Technology 366*, 128188. https://doi.org/10.1016/j.biortech.2022.128188
7. Cheng Y. W., Chong C. C., Lam M. K., Leong W. H., Chuah L. F., Yusup S., Setiabudi H. D., Tang Y., Lim J. W. 2021. Identification of microbial inhibitions and mitigation strategies towards cleaner bioconversions of palm oil mill effluent (POME): A review. *Journal of Clean Production 280*, 124346. https://doi.org/10.1016/j.jclepro.2020.124346
8. Chong C. C., Cheng Y. W., Ishak S., Lam M. K., J. Lim W., Tan I. S., Show P. L., Lee K. T. (2022). Anaerobic digestate as a low-cost nutrient source for sustainable microalgae cultivation: A way forward through waste valorization approach. *Science of the Total Environment. 803*, 1–16. https://doi.org/10.1016/j.scitotenv.2021.150070
9. Chuka-ogwude, D., J. Ogbonna and N. R. Moheimani. (2020). A review on microalgal culture to treat anaerobic digestate food waste effluent. *Algal Research. 47*, 101841. https://doi.org/10.1016/j.algal.2020.101841
10. Dalias, P. and Christou, A. (2025). Nitrogen supplying capacity of animal manures to the soil in relation to the length of their storage. *Nitrogen. 1*(1), 52–66. https://doi.org/10.3390/nitrogen1010006
11. Duan N., Khoshnevisan, Lin B., Liu C., Liu. H. (2020). Life cycle assessment of anaerobic digestion of pig manure coupled with different digestate treatment technologies. *Environmental International 137*, 1–16. https://doi.org/10.1016/j.envint.2020.105522
12. Duchene, A-M. and Drouard, L. (2021). Chapter nine - Last news on plant mitochondria—A follow-up of ABR volume 63: Mitochondrial genome evolution. *Advances in Botanical Research. 100*, 275–299. https://doi.org/10.1016/bs.abr.2021.01.004
13. Fabris M., Abbriano R. M., Pernice M., Sutherland D. L., Commault A. S, Hall C.C., Labeew L., McCauley J. I., Kuzhiuparambil U., Ray P., Kahlke T., Ralph P. J. 2020. Emerging technologies in algal biotechnology: toward the establishment of a sustainable, algae-based bioeconomy. *Frontiers in Plant Science 11*, 279. https://doi.org/10.3389/fpls.2020.00279
14. Franco T. A. F., da Encarnação, Araújo S., Passos F., de L. Chernicharo C. A., Mota Filho C. R., and Figueredo C. C. 2018. Treatment of food waste digestate using microalgae-based systems with low-intensity light-emitting diodes. *Water Science Technology 78*, 225–234. https://doi.org/10.2166/wst.2018.198
15. Fu, B., Chen, L., Huang, H., Qu, P., and Wei, Z. (2025). Impacts of crop residues on soil health: a

review. *Environmental Pollutants and Bioavailability. 33*(1), 164–173. https://doi.org/10.1080/26395940.2021.1948354

16. Gao K., Liu Q., Gao Z., Xue C., Qian P., Dong J., Gao Z., Deng X. (2021). A dilution strategy used to enhance nutrient removal and biomass production of *Chlorella sorokiniana* in frigon wastewater. Algal Research 58, 2211–9264. https://doi.org/10.1016/j.algal.2021.102438
17. Gissibl A., Sun A., Care A., Nevalainen H., and Sunna A. (2019). Bioproducts from *Euglena gracilis*: synthesis and applications. *Frontiers in bioengineering and biotechnology 7*, 108. https://doi.org/10.3389/fbioe.2019.00108
18. Jimenez R., Markou G., Saida T., Barakat A., Chapsal C., Monlau, F. (2020). Production of microalgal slow-release fertilizer by valorizing liquid agricultural digestate: growth experiments with tomatoes. *Applied Science 10*(11), 1–19. https://doi.org/10.3390/app10113890
19. Kouakou, A. R., Kouadio, M. C., Oseni, D., Adjoumani, K. E. I., Ehouman, A. D., and Yao, B. (2025). Optimization of the manure/water ratio for maximum biogas production: Case of the FONSTI-CRDI digester in the East of Côte d'Ivoire. *Earthline Journal of Chemical Sciences. 12*(2), 137–153. https://doi.org/10.34198/ejcs.12225.137153
20. Khan, A., Niazi, M.B.K., Ansar, R., et al., (2023). Thermochemical conversion of agricultural waste to hydrogen, methane, and biofuels: a review. *Fuel 351*, 128947. https://doi.org/10.1016/j.fuel.2023.128947
21. Khanra S., Mondal M., Halder G., Tiwari O. N., Gayen K., Bhowmick T. K. (2018). Downstream processing of microalgae for pigments, protein and carbohydrate in industrial application: a review. *Food and Bioproduct Processing 110*, 60–84. http://dx.doi.org/10.1016/j.fbp.2018.02.002
22. Kisielewska M., Debowski M., Zielinski M., Kazimierowicz J., Quattrocelli P., Bordiean A. (2022). Effects of liquid digestate treatment on sustainable microalgae biomass production. *Bioenergy Research 15*, 357–370. https://doi.org/10.1007/s12155-021-10251-x
23. Krishnan, R., Agarwal, R., Bajada, C., et al., (2020). *Redesigning a food supply chain for environmental sustainability – an analysis of resource use and recovery*. J. Clean. Prod. 242 (UTS Business School, University of Technology Sydney, PO Box 123, BroadwayNSW 2007, Australia). https://doi.org/10.1016/j.jclepro.2019.118374
24. 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 Chlorella 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. https://doi.org/10.1016/j.chemosphere.2020.128963
25. Markou, G., Dimiotrios A., Christos E., Athanasios B., Raffaele T., Anthi P., Nikolaos T. 2019. Cultivation and safety aspects of Arthrospira platensis (Spirulina) grown with struvite recovered from anaerobic digestion plant as phosphorus source. *Journal of Algal Research 44*(1), 1–8. https://doi.org/10.1016/j.algal.2019.101716
26. Nurafifah, I., Hardianto M. A., Erfianti T., Amelia R., Maghfiroh K. Q. (2023). The effect of acidic pH on growth kinetics, biomass productivity, and primary metabolite contents of *Euglena sp. Makara Journal of Sciences 27*(2), 97–105. https://doi.org/10.7454/mss.v27i2.1506
27. Perdana, T., Kusnandar, K., Perdana, H. H., and Hermiatin, F. R. (2023). Circular supply chain governance for sustainable fresh agricultural products: Minimizing food loss and utilizing agricultural waste. *Sustainable Production and Consumption. 41*, 391–403. https://doi.org/10.1016/j.spc.2023.09.001
28. Praveen, P., Guo Y., Kang H., Lefebvre C, Loh K. C. (2018). Enhancing microalgae cultivation in anaerobic digestate through nitrification. *Chemical Engineering Journal 354*, 905–912. https://doi.org/10.1016/j.cej.2018.08.099
29. Razzak, S. A., Ali S. A. M., Hossain M. M., deLasa H. (2017). Biological CO_2 fixation with production of microalgae in wastewater – A review. *Renewable and Sustainable Energy Reviews 76*, 379–390. https://doi.org/10.1016/j.rser.2017.02.038
30. Razzak, S. A., Bahar, K., Islam, K. M. O., Haniffa, A. K., Faruque, M. O., Hossain, S. M. Z., and Hossain M. M. (2024). Microalgae cultivation in photobioreactors: sustainable solutions for a greener future. *Green Chemical Engineering. 5*(4), 418–439. https://doi.org/10.1016/j.gce.2023.10.004
31. Salbitani, G. and Carfagna, S. (2021). Ammonium utilization in microalgae: A sustainable method for wastewater treatment. *Sustainability. 13*(2), 956. https://doi.org/10.3390/su13020956
32. Sari, R., Maryam and Yusmah, R. A. (2023). Determination of c-organic in soil to improve crop productivity and sustainability of crop life by UV-VIS spectrophotometric method. *Jurnal Teknologi Pertanian. 12*(1), 11–19. https://doi.org/10.32520/jtp.v12i1.2598
33. Sarkar, B., Debnath, A., Chiu, A. S. F., et al., 2022. Circular economy-driven two-stage supply chain management for nullifying waste. *J. Clean. Prod. 339*, 130513. https://doi.org/10.1016/j.jclepro.2022.130513
34. Shah, I. H., Jinhui, W., Li, X., Hameed, M. K., Manzoor, M. A., Li, P., Zhang, Y., Niu, Q., and Chang

L. (2024). Exploring the role of nitrogen and potassium in photosynthesis implications for sugar: Accumulation and translocation in horticultural crops. *Scientia Horticulturae. 327*, 112832. https://doi.org/10.1016/j.scienta.2023.112832

35. Vats, N., Khan A., Ahmad K. (2020). Options for enhanced anaerobic digestion of waste and biomass - a review. *Biosystem Engineering. 45*: 1–15. https://doi.org/10.1007/s42853-019-00040-y

36. Yaakob, M. A., Mohamed, R. M. S. R., Al-Gheethi, A., Gokare, R. A., and Ambati, R. R. (2021). Influence of nitrogen and phosphorus on microalgal growth, biomass, lipid, and fatty acid production: an overview. *Cells. 10*(2), 393. https://doi.org/10.3390/cells10020393

37. Yang, W., Li S., Qv M., Dai D., Liu D., Wang W., Tang C., Zhu L. (2022). Microalgal cultivation for the upgraded biogas by removing CO_2, coupled with the treatment of slurry from anaerobic digestion: A review. *Bioresource Technology 364*, 128118. https://doi.org/10.1016/j.biortech.2022.128118

38. Zeng, Z., Zheng P., Ding A., Zhang M., Abbas G., Li W. (2017). Source analysis of organic matter in swine wastewater after anaerobic digestion with EEM-PARAFAC. *Environmental Science and Pollution Research 24*(1), 6770–6778. https://doi.org/10.1007/s11356-016-8324-3

39. 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