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Circular bioeconomy strategy for livestock waste: Valorizing biogas slurry as a low-nutrient source for *Nannochloropsis sp.* cultivation

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ABSTRACT

The valorization of biogas slurry through microalgal cultivation presents a sustainable approach to waste management and biomass production within the circular bioeconomy. This study investigates the potential of *Nannochloropsis sp*. cultivation in biogas slurry from a continuous digester, evaluating its growth kinetics, phytoremediation efficiency, and protein accumulation. The physicochemical characterization of biogas slurry revealed a dynamic nutrient profile influenced by organic load and microbial activity. Growth modeling using the Gompertz model demonstrated that optimal dilution (P2) with a C/N ratio of 6,751 supported the highest cell production rate (0.2580 day^{-1}) and a shorter lag phase (5.3175 days), attributed to balanced nutrient availability. Phytoremediation analysis indicated significant reductions in chemical oxygen demand (COD) (75.66%), biological oxygen demand (BOD) (68.93%), and ammonium (83.76%), highlighting *Nannochloropsis sp*. as an effective biological treatment agent. Additionally, protein content in P2 (0.1412 µg/mL) closely approached that of the synthetic control medium, demonstrating its potential as an alternative nutrient source for sustainable microalgal cultivation. These findings emphasize the role of biogas slurry in microalgal-based bioremediation and biomass valorization, contributing to waste-to-product innovations aligned with circular bioeconomy and global sustainability goals.

Keywords: biogas slurry, circular bioeconomy, growth kinetic, phytoremediation, *Nannochloropsis sp*.

INTRODUCTION

The global shift towards a circular bioeconomy has gained significant momentum as industries seek to minimize waste, maximize resource efficiency, and reduce environmental impact. In the agricultural sector, livestock waste represents both a challenge and an opportunity for sustainable management (Phiri *et al* 2024). Traditional waste disposal methods contribute to greenhouse gas emissions, soil degradation, and water pollution (Manea *et al* 2024). However, innovative strategies in closed-loop agriculture integrate livestock waste management into bioeconomic frameworks (Wagh *et al* 2024), transforming organic residues into valuable bio-based products such as, organic fertilizers, and biofuels biogas (Panoutsou *et al* 2021). This shift aligns with the principles of circular economy, where waste is repurposed as a resource rather than discarded. A key aspect of this approach is the development of sustainable bioprocessing techniques that ensure waste-derived materials can be safely and effectively integrated into new production cycles. By leveraging anaerobic digestion and biorefinery technologies, livestock waste can be converted into biogas slurry as a nutrient rich by-product that holds promise for further valorization, including its use as a culture medium for microalgae cultivation (Solis *et al* 2020). Despite these advantages, optimizing the reuse of biogas slurry requires a thorough understanding of its physicochemical characteristics and its impact on biological systems.

Microalgae have emerged as a promising biological resource capable of addressing multiple global challenges, including wastewater treatment, CO_2 sequestration, and biofuel production . Their ability to grow rapidly under various conditions, utilizing wastewater as a nutrient source while capturing carbon dioxide, makes them an ideal candidate for sustainable bioremediation and bioeconomy models (Ding *et al* 2020). Compared to conventional remediation technologies, microalgae-based systems offer a cost-effective and environmentally friendly solution for nutrient recovery and pollutant removal. Microalgae biomass holds significant potential in the circular bioeconomy framework, as it can be integrated into diverse applications such as biofertilizers (Pereira *et al* 2023), bioplastics (Ilhami *et al* 2025), nutraceuticals (Parameswari and Lakshmi, 2022), and feed production (Bature *et al* 2022). Furthermore, advancements in biorefinery technologies enable the extraction of multiple valuable compounds from microalgae, enhancing economic feasibility while minimizing environmental impacts (Razzak *et al* 2019). Expanding research on microalgal-based biorefineries can significantly improve the sustainability of waste-to-product strategies, particularly in the development of renewable energy and high-value bioproducts. The implementation of microalgae-based biorefineries aligns with global sustainability goals, offering a viable alternative to conventional waste management and resource recovery strategies (Mahmod *et al* 2025).

Nannochloropsis sp. has been widely recognized for its ability to thrive in nutrient-rich wastewater environments, efficiently assimilating contaminants through its metabolic processes (Santanumurti *et al* 2022). As a photosynthetic microalga (Parsy *et al* 2024), *Nannochloropsis sp.* removes organic pollutants by incorporating them into its cellular metabolism, converting dissolved organic matter into biomass while simultaneously producing valuable bioactive compounds such as polyunsaturated fatty acids, sterols, proteins, and pigments. Due to its high adaptability and fast growth rate, *Nannochloropsis sp*. has been widely studied for its potential applications in aquaculture, biofuel production, and wastewater treatment. Its robust adaptability to varying organic waste conditions makes it a promising candidate for wastewater bioremediation and biomass valorization (Diaz *et al* 2022). In a recent study, *Nannochloropsis sp.* demonstrated a 37.91% reduction in soluble COD in poultry wastewater and a 37.18% reduction in pig manure wastewater, highlighting its significant role in organic matter removal (Sales-Pérez *et al* 2023). These findings indicate that *Nannochloropsis sp*. is not only effective in pollutant reduction but also contributes to sustainable biomass production for further biotechnological applications. Such findings reinforce the feasibility of integrating *Nannochloropsis sp.* into circular bioeconomy strategies, where microalgae cultivation in biogas slurry can provide dual benefits environmental remediation and sustainable biomass production.

The utilization of biogas slurry as a cultivation medium for microalgae remains an underexplored yet highly promising approach for advancing the circular bioeconomy in agro-industrial systems (Wang *et al* 2017). While anaerobic digestion has been widely adopted for biogas production, the potential of its liquid byproduct biogas slurry for microalgal biomass valorization is still not fully understood (Yang *et al* 2022). Existing studies have primarily focused on the use of biogas slurry as a cultivation substrate without considering the influence of digester type on its composition and suitability for microalgal growth. Since different digester types influence slurry composition, their impact on microalgae growth and nutrient assimilation must be carefully examined. Specifically, the dynamic nutrient profile of continuous digester biogas slurry, which is influenced by steady organic load input and microbial activity (Ajay *et al* 2021), necessitates a deeper understanding of its potential to support microalgal cultivation, particularly in terms of growth kinetics, phytoremediation efficiency, and protein content (Markou *et al* 2018). Given the nutrient-rich composition of slurry from continuous digesters, further research is necessary to determine its optimal utilization for biomass production and pollutant removal (Wang *et al* 2019). Understanding these interactions will enable the development of integrated waste-to-value strategies, ensuring both environmental benefits and economic feasibility in agro-industrial waste management. A better understanding of these interactions will not only optimize cultivation conditions but also enhance the economic feasibility of microalgae-based bioremediation strategies. Additionally, recognizing biogas slurry as a low-nutrient source for microalgae cultivation can further support sustainable resource utilization and circular bioeconomy approaches. This study aims to address these gaps by assessing the effluent characteristic, microalgae growth dynamics, phytoremediation efficiency, and protein accumulation of *Nannochloropsis sp.* cultivated in biogas slurry from continuous digester, offering new perspectives on sustainable resource recovery.

METHODS

Microalgae source and culture conditions

This study was carried out from March to December 2024 at the Leather, Waste, and By-Products Technology Laboratory, Faculty of Animal Science and Biotechnology Laboratory, Faculty of Biology, Gadjah Mada University. The *Nannochloropsis sp.* microalga used in this experiment was obtained from the Brackish Water Aquaculture Development Center in Situbondo, East Java, Indonesia. Cultivation was performed in a modified one-liter photobioreactor within a closed system, ensuring controlled lighting conditions and a neutral pH (Penloglou *et al* 2024). Continuous aeration was applied, and the culture temperature was consistently maintained at 24 °C. This system facilitated even dispersion of the culture medium while supplying adequate CO_2 for photosynthetic activity at a flow rate of 3 liters per minute (Sathyamoorthy *et al* 2021). To uphold sterility throughout cultivation, 96% alcohol was sprayed in the cultivation area at 24-hour intervals.

Biogas slurry preparation

The biogas slurry used in this study was obtained from a continuous digester at Samesta Dairy Cooperative, Sleman, Yogyakarta, Indonesia. The biogas production process utilizes dairy farm waste as the primary substrate, consisting of manure and other organic residues. The biogas slurry is a by-product of a continuous anaerobic digestion process with a retention time of 40 days. This slurry was aerobically conditioned for several days before use to allow any remaining methanogenic bacteria to complete gas production, even though their activity was minimal (Duan *et al* 2020). To eliminate large particulates, the collected biogas slurry was filtered using an unbleached 74-micron nylon filter cloth. The slurry was then diluted into different concentrations: P1, P2, P3, and P0 (Walne's Fertilizer) as the control medium, using deionized water. To ensure a sterile cultivation environment, the diluted slurry samples were autoclaved, eliminating potential contaminants that might interfere with microalgal growth. The experimental flow of *Nannochloropsis sp*. cultivations in biogas slurry is ilustrated in Figure 1. For scaling up the cultivation, no additional nutrients were introduced. The dilution formula (Vn) and dilution concentration (Pn) applied in this study followed the equation.

$$V_n = 500 \text{ mL } (n\text{-}1) + 500 \text{ mL } (n) \quad (1)$$

$$Pn = 0{,}50^n \quad (2)$$

Nutrient content analysis

The nutrient composition of the diluted biogas slurry was examined to evaluate its potential as a nutrient source for microalgae cultivation. The parameters analyzed included total organic carbon (TOC), total nitrogen (TN), C/N ratio, total phosphorus (TP), and total potassium (TK). Organic carbon content was measured using a UV-VIS spectrophotometric method at 561 nm (Sari *et al* 2023). The Kjeldahl method was applied to determine total nitrogen content (SNI: 2803, 2010), while phosphorus and potassium concentration was assessed using

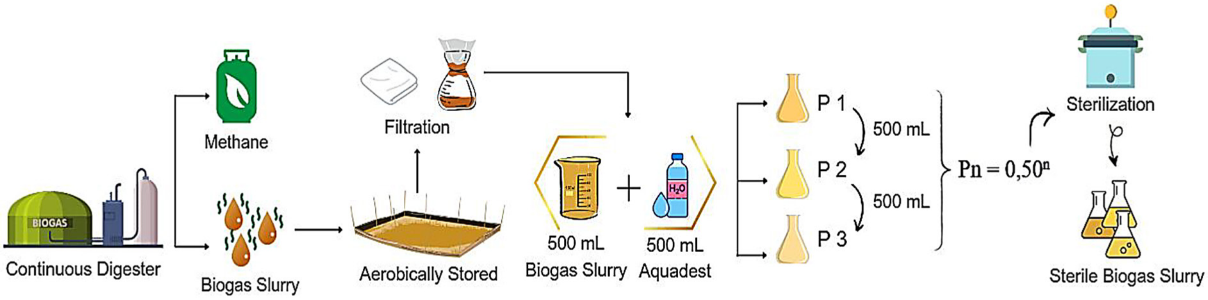

Figure 1. Pretreatment process of biogas slurry

UV-VIS spectrophotometer at 882 nm and 766.5 nm. These analyses provide essential insights into the nutrient availability in biogas slurry, ensuring its suitability for sustainable microalgal growth and biomass production.

Effluent quality parameters analysis

The diluted biogas slurry was examined for effluent quality parameters to assess its suitability and environmental impact as a cultivation medium for microalgae. The analyzed parameters included turbidity, chemical oxygen demand (COD), biological oxygen demand (BOD), and ammonium (NH_4^+-N). Turbidity was measured following SNI 06-6989.25-2005, while COD analysis adhered to SNI 6989.2:2019, and BOD was evaluated using SNI 69 89.72-2009. Ammonium (NH_4^+-N) concentrations were assessed in accordance with SNI 06-6989.30-2005.

Optical density analysis

The growth rate of microalgae was assessed using the optical density (OD) method, which involved measuring absorbance at 680 nm with a spectrophotometer (Suzuki, 2017). The OD values obtained were subsequently analyzed to determine microalgal growth kinetics using the logistic and Gompertz models. These models provide a deeper understanding of growth patterns and biomass accumulation, which are essential for optimizing cultivation conditions.

Growth kinetic modelling of *Nannochloropsis sp.*

The Gompertz model was utilized to characterize cell population dynamics during the exponential growth phase. Unlike simpler models, it accounts for additional parameters, including maximum cell production (r_m) and lag time (tL). This model was implemented using Equations 3 and 4, where SSR represents the sum of squares residual, and SST refers to the total sum of squares (Hanief *et al* 2020).

$$x = Xo + [Xmax.exp \left[-exp\left(\left(\frac{rm.exp(1)}{Xmax}\right)(tL-t)+1\right)\right] \quad (3)$$

$$R^2 = 1 - \left(\frac{SSR}{SST}\right) \quad (4)$$

Protein contents analysis

The protein content of *Nannochloropsis sp*. was quantified using the Bradford assay. The microalgae culture was first centrifuged, and the resulting supernatant was combined with an SDS solution. The mixture was then heated at 95 °C for 5 minutes, followed by rapid cooling at 4 °C for another 5 minutes. After incubation, Bradford reagent was added to the samples, and the absorbance was measured at 595 nm using an ELISA reader. Protein concentration was determined by constructing a standard curve based on bovine serum albumin (BSA) standards at concentrations of 25, 50, 75, and 100 µg/mL, employing linear regression analysis for quantification.

Statistical analysis

The data were analyzed using analysis of variance (ANOVA) to assess differences among treatment groups. Duncan's multiple range test (DMRT) was subsequently performed to identify specific group differences. Statistical significance was established at a 95% confidence level. This analytical approach allowed for a comprehensive comparison of results across the experimental conditions, ensuring robust and reliable conclusions.

RESULTS AND DISCUSSION

Physicocemical characteristics of biogas slurry

The physicochemical characteristics of biogas slurry provide essential insights into its suitability as a microalgal cultivation medium (Huang *et al* 2022), as shown in Table 1. The total nitrogen (TN) content in the slurry exhibited a decreasing trend with dilution, ranging from 0.179 ± 0.016% (P0) to 0.058 ± 0.004% (P3), indicating a progressive reduction in nutrient concentration (Malhotra *et al* 2022). Similarly, total organic carbon (TOC) declined significantly from 3.075 ± 0.327% to 0.160 ± 0.016%, which reflects the impact of dilution on organic matter availability (Akkaya and Can-Guven, 2022). The carbon-to-nitrogen (C/N) ratio followed a similar pattern, with the highest value of 17,184 ± 0,401 at P0 and the lowest at 2.762 ± 0.218 in P3, suggesting that dilution influences the balance between carbon and nitrogen sources (Chong *et al* 2022).

Table 1. Physicochemical characteristics of biogas slurry from continuous digesters at different dilution levels

Characteristics of biogas slurry	Dilution treatments of biogas slurry			
	P0 (Initial BS)	BS P1	BS P2	BS P3
TN (%)	0.179 ± 0.016	0.112 ± 0.015	0.082 ± 0.006	0.058 ± 0.004
TOC (%)	3.075 ± 0.327	1.038 ± 0.165	0.553 ± 0.051	0.160 ± 0.016
C/N Rasio	17.184 ± 0.401	9.354 ± 1.848	6.751 ± 0.179	2.762 ± 0.218
TK (%)	0.065 ± 0.006	0.048 ± 0.002	0.031 ± 0.002	0.018 ± 0.002
TP (%)	0.021 ± 0.012	0.010 ± 0.002	0.008 ± 0.001	0.005 ± 0.001
NH_4+-N (mg/L)	81.677 ± 3.522	60.851 ± 3.076	49.419 ± 2.112	29.511 ± 1.516
COD (mg/L)	608.573 ± 30.901	455.507 ± 46.965	287.192 ± 13.842	192.973 ± 5.628
BOD (mg/L)	381.027 ± 27.141	282.700 ± 17.803	187.671 ± 6.414	102.393 ± 3.772

Note: BS (biogas slurry); TN (total nitrogen); TOC (total organic carbon); TK (total potassium); TP (total phosphorus); NH4+ (ammonium); COD (chemical oxygen demand); BOD (biologycal oxygen demand).

Additionally, key macronutrients such as potassium (K) and phosphorus (P) play essential roles in microalgal growth and metabolism. Potassium is vital for enzyme activation, osmotic regulation, and photosynthetic efficiency (Shah *et al* 2024), while phosphorus is a fundamental component of nucleic acids, ATP, and phospholipids, facilitating cell division and energy transfer (Khan *et al* 2023). In this study, their concentrations ranged from 0.065 ± 0.006% to 0.018 ± 0.002% for K and 0.021 ± 0.012% to 0.005 ± 0.001% for P, indicating their availability to support microalgal growth and biochemical functions. The ammonium (NH_4^+) concentration also exhibited a decreasing pattern, from 81.677 ± 3.522 mg/L at P0 to 29.511 ± 1.516 mg/L at P3, indicating potential implications for ammonia toxicity management in microalgal cultivation. Ammonium is the most preferred nitrogen source for microalgae due to its direct assimilation into cellular metabolism (Salbitani and Carfargna, 2021). However, excessive concentrations can be toxic, disrupting enzymatic activity and inhibiting growth (Chuka-ogwude *et al* 2020). To mitigate the inhibitory effects of elevated nutrient concentrations in algal cultures, researchers often employ dilution strategies to adjust the ammonia-N levels within the optimal range of 50–100mg/L, ensuring a favorable growth environment while preventing toxicity-related stress (Torres Franco *et al* 2018).

Moreover, the chemical oxygen demand (COD) and biological oxygen demand (BOD) levels demonstrated a dilution-dependent reduction, with COD ranging from 608.573 ± 30.901 mg/L to 192.973 ± 5.628 mg/L and BOD from 381.027 ± 27.141 mg/L to 102.393 ± 3.772 mg/L, highlighting the effect of organic matter degradation in the system. The reduction in COD and BOD is highly beneficial for media adjustment, as it minimizes the risk of oxygen depletion and microbial competition, creating a more favorable environment for microalgal growth (Baihaqi and Pratama, 2023). These findings underscore the potential of biogas slurry as a nutrient source for microalgal cultivation, while also emphasizing the need for optimization in terms of dilution to maintain an optimal nutrient balance without reaching inhibitory concentrations.

Biogas slurry exhibits nutrient characteristics comparable to other organic waste-derived media used in microalgal cultivation (Singh *et al* 2023). Compared to livestock wastewater and municipal sludge, biogas slurry offers a more balanced carbon-to-nitrogen (C/N) ratio, which is crucial for nitrogen assimilation and organic carbon availability (Markou *et al* 2020). While untreated livestock wastewater often contains high ammonia concentrations that can be toxic to microalgae (Kisielewska *et al* 2022), biogas slurry especially at higher dilution levels provides a more controlled nitrogen profile suitable for biomass production (Wang *et al* 2019). Additionally, continuous digester slurry demonstrates greater nutrient stability than batch digestate due to steady-state organic load input (Ajay *et al* 2021). This dilution process not only achieves the targeted COD concentration but also reduces turbidity and coloration in the wastewater. These improvements enhance light penetration, which is crucial for establishing optimal conditions for microalgal growth and photosynthetic efficiency (Tan *et al* 2022). These findings suggest that biogas slurry from continuous digesters can be an effective alternative to conventional wastewater-based culture media, especially when proper dilution strategies are applied (Malhotra *et al* 2022).

The composition of biogas slurry is influenced by feedstock type, digester operation, and post-treatment processes (Yadav *et al* 2023). In this study, dairy cattle manure served as the primary substrate, contributing to high total nitrogen (TN) and total organic carbon (TOC) levels. The 40-day retention time in a continuous anaerobic digester allowed for progressive organic matter degradation, reducing COD and BOD levels. Dilution significantly impacted nutrient concentrations, with TN, TOC, and ammonium (NH_4^+) decreasing across different treatments. While ammonium is an essential nitrogen source, excessive levels can be toxic to microalgae, necessitating optimal dilution for growth optimization (Gutiérrez-Casiano *et al* 2022). These findings emphasize the importance of proper nutrient management when utilizing biogas slurry as a microalgal cultivation medium.

Growth kinetic modeling of *Nannochloropsis sp.*

The application of the Gompertz model for growth kinetics provides a robust analytical framework for understanding the proliferation (Wang and Guo, 2024) of *Nannochloropsis sp.* cultivated in biogas slurry with different dilution treatments. This model effectively captures the sigmoidal growth pattern of microalgae by estimating key parameters such as the cell production rate (r_m) and lag phase (tL). One key advantage of the Gompertz model is its accuracy in describing microalgal growth under varying conditions, aiding cultivation optimization in wastewater-based media (Hanief *et al* 2020).

The growth kinetics of *Nannochloropsis sp.* cultivated in biogas slurry were successfully modeled using the Gompertz model (Figure 3), which was derived based on the optical density measurements of the microalgae shown in Figure 2. The results (Table 2) indicate that P2 exhibited the highest cell production rate (0.2580 day^{-1}) and a shorter lag phase (5.3175 days) compared to other treatments, suggesting an optimal balance of macronutrients and micronutrients. In contrast, P1 displayed the lowest cell production rate (0.0914 day^{-1}) and the longest lag phase (7.0685 days), likely due to nutrient imbalances or inhibitory effects from residual organic compounds. The control group (P0), cultivated in a standard medium, showed the highest cell production rate (0.2911 day^{-1}), demonstrating that *Nannochloropsis sp.* achieves near-optimal growth in well-balanced nutrient conditions. The high R^2 values (>0,96) across treatments confirm the suitability of the Gompertz model in describing microalgal growth dynamics under varying wastewater compositions.

Microalgal growth is largely influenced by the availability of essential nutrients, including nitrogen, phosphorus, and trace metals, which facilitate cellular metabolism and biomass accumulation (Razzak *et al* 2024). The superior performance of P2 suggests that the biogas slurry at this dilution provided sufficient nutrient availability while mitigating inhibitory effects

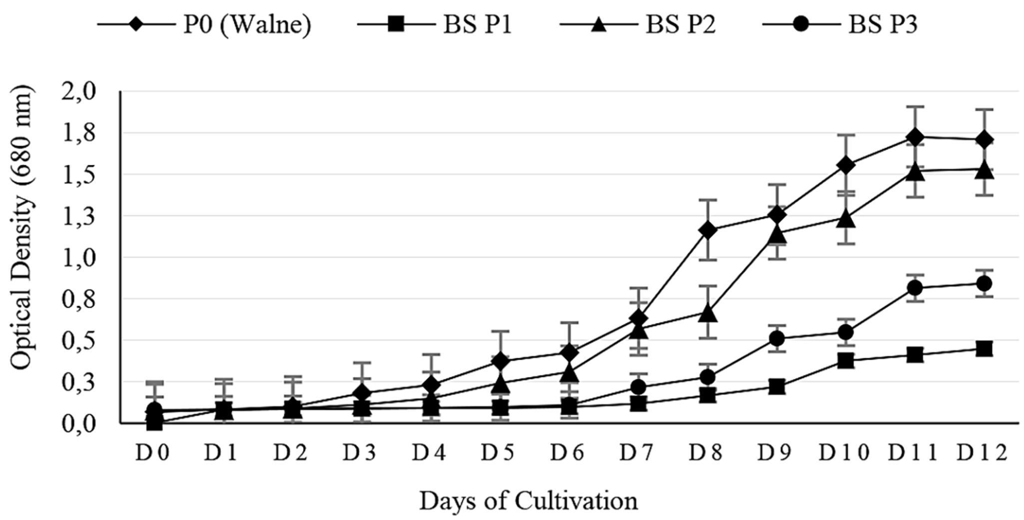

Figure 2. Optical density of *Nannochloropsis sp*. at different dilution levels of biogas slurry

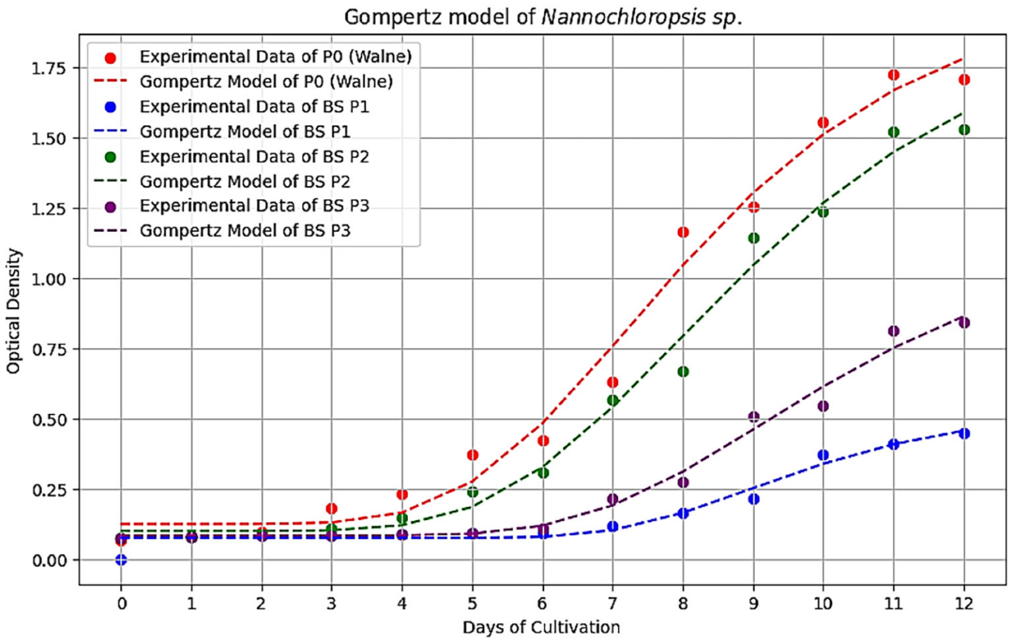

Figure 3. Simulated growth model of *Nannochloropsis sp*. using gompertz model analysis

associated with undiluted or excessively diluted conditions (Torres Franco *et al* 2018). This aligns with findings by Chong *et al* (2022), which indicate that optimal C/N ratios for microalgal growth range between 4 and 8, with P2 exhibiting a C/N ratio of 6.751, supporting efficient nutrient assimilation. The moderate performance of P3 (cell production rate: 0.1557 day^{-1}, lag time: 6.5842 days) indicates that excessive dilution may reduce nutrient concentrations below the optimal threshold, thereby limiting growth potential (Malhotra *et al* 2022). *Nannochloropsis sp.* employs photosynthetic activity, nutrient uptake mechanisms, and extracellular enzymatic processes to assimilate dissolved organic and inorganic compounds, contributing to phytoremediation and biomass valorization. These findings underscore the potential of biogas slurry as an alternative growth medium, supporting sustainable microalgal biotechnolo gy within circular bioeconomy frameworks.

Protein content of *Nannochloropsis sp.*

The protein content of *Nannochloropsis sp.* cultivated in biogas slurry with different dilution treatments (P1, P2, and P3) and Walne's fertilizer as the control (P0) revealed variations in protein accumulation and productivity, as shown in Table 3. The highest protein content was observed in P0 (0.1751 ± 0.0055 µg/mL), which served as the control using Walne's fertilizer, a well-balanced synthetic medium (Astriandari *et al* 2023). Among the biogas slurry dilutions, P2 exhibited the highest protein accumulation (0.1412 ± 0.0005 µg/mL), followed by P3 (0.0971 ± 0.0013 µg/mL) and P1 (0.0862 ± 0.0007 µg/mL). This trend suggests that nutrient dilution plays a critical role in protein biosynthesis (Fernandes *et al* 2022), with P2 providing an optimal balance of nitrogen and carbon sources. The protein productivity followed a similar pattern, with P2 yielding 2.0217 ± 0.0001 µg/mL/day, whereas P1 and P3 had lower values (1.0102 ± 0.0004 µg/mL/day

Table 2. Growth kinetic parameters of *Nannochloropsis sp.* cultivated in biogas slurry

Treatment	Gompertz model analysis		
	Cell production rate (r_m)	Lag time (tL)	R^2
P0 (C/N	0.2911	4.8322	0.9867
P1	0.0914	7.0685	0.9631
P2	0.2580	5.3175	0.9894
P3	0.1557	6.5842	0.9864

Table 3. Protein content and productivity of *Nannochloropsis sp*. cultivated in biogas slurry with different dilution treatments

Treatments	Protein accumulation ± SD (µg/mL)	Protein productivity ± SD (µg/mL/day)
P0	0.1751 ± 0.0055[c]	2.8673 ± 0.0007[b]
P1	0.0862 ± 0.0007[a]	1.0102 ± 0.0004[a]
P2	0.1412 ± 0.0005[b]	2.0217 ± 0.0001[b]
P3	0.0971 ± 0.0013[a]	1.2213 ± 0.0001[a]

Note: SD (standard deviation); abc – different subsets indicate significant differences at a significance level of $p<0.01$)

and 1.2213 ± 0.0001 µg/mL/day). The lower protein content in P1 and P3 could be attributed to nutrient limitations or potential inhibitory effects from residual compounds in the biogas slurry (Al-Mallahi and Ishii, 2022). These findings indicate that moderate dilution enhances protein accumulation, whereas excessive or insufficient dilution may lead to suboptimal growth and metabolic activity.

These differences highlight the influence of nutrient availability on microalgal metabolism and biochemical composition. The protein content of *Nannochloropsis sp.* cultivated in biogas slurry varied across different dilution treatments, indicating that nutrient availability plays a crucial role in protein accumulation (Kusmayadi *et al* 2023). The highest protein content was observed in the control group, which utilized Walne's fertilizer as the culture medium (Ramli *et al* 2023), suggesting that the optimized composition of synthetic nutrients facilitates protein synthesis. Among the biogas slurry treatments, the variation in protein accumulation can be attributed to differences in nitrogen availability, ammonium concentration, and overall nutrient balance (Truong *et al* 2024). The dilution levels influenced the C/N ratio, which is a key determinant in nitrogen assimilation for protein biosynthesis (Cai *et al* 2022). Excessive ammonium concentrations in undiluted or minimally diluted biogas slurry may have exerted inhibitory effects on cellular metabolism (Metin and Altinbas, 2024), leading to lower protein accumulation in certain treatments. Conversely, moderate dilution improved nutrient accessibility while reducing potential toxicity, resulting in a more favorable environment for protein production. Among the biogas slurry dilutions, P2 exhibited the closest protein content to P0, indicating that this dilution level provides an optimal balance of nutrients for *Nannochloropsis sp.* growth and protein biosynthesis. This suggests that P2 can serve as a promising alternative culture medium, reducing the reliance on synthetic fertilizers while maintaining efficient protein production.

The protein content and productivity of *Nannochloropsis sp*. cultivated in biogas slurry have significant implications for industrial applications, particularly in the food, cosmetics, feed, biofuels, nutraceutical, and pharmaceutical industries (Gamal and Shreadah, 2024). *Nannochloropsis sp.* is well known for its high-value biochemical composition, including essential amino acids, omega-3 fatty acids, and bioactive peptides (Paterson *et al* 2024), making it an attractive candidate for sustainable feed. The findings of this study suggest that biogas slurry-based cultivation can provide a cost-effective alternative to synthetic media, supporting the development of low cost microalgal biomass for feed and bioenergy applications. However, further optimization is required to enhance protein accumulation and productivity, particularly through nutrient supplementation and metabolic engineering approaches. Additionally, the integration of biogas slurry valorization into circular bioeconomy frameworks aligns with sustainable waste management strategies, reducing environmental impact while generating high-value bioproducts.

Phytoremediation capability

The ability of *Nannochloropsis sp*. to remove pollutants from biogas slurry was evaluated based on the reduction of chemical oxygen demand (COD), biological oxygen demand (BOD_5), and ammonium (NH_4^+) under different dilution treatments (P1, P2, and P3). The results (Table 4) indicate that dilution level plays a crucial role in determining phytoremediation efficiency (Santos *et al* 2021), with P2 demonstrating the highest pollutant removal rates across all parameters. This highlights the importance of maintaining a well-balanced nutrient composition in wastewater, as

Table 4. Phytoremediation capability *Nannochloropsis sp.* cultivated in biogas slurry with different dilution treatments

Parameter	Reduction levels (%)		
	P1	P2	P3
COD	31.08 ± 2.30[a]	75.66 ± 2.59[b]	45.76 ± 2.97[c]
BOD_5	22.17 ± 2.05[a]	68.93 ± 2.63[b]	38.42 ± 1.90[c]
NH_4^+	20.59 ± 1.36[a]	83.76 ± 1.87[b]	23.25 ± 1.23[a]

Note: COD (chemical oxygen demand); BOD (biologycal oxygen demand); $NH4^+$ (ammonium); [abc] (different subsets indicate significant differences at a significance level of $p<0.01$).

an optimal nutrient balance supports microalgal growth and enhances its capacity for effective phytoremediation (Gupta *et al* 2024).

The highest COD reduction was observed in P2 (75.66 ± 2.59%), followed by P3 (45.76 ± 2.97%) and P1 (31.08 ± 2.30%) as shown in Figure 4. This trend suggests that moderate dilution optimizes organic matter degradation, likely by reducing inhibitory effects from excess organic compounds while maintaining sufficient nutrient levels for microalgal metabolism (Sun *et al* 2018). The BOD_5 removal efficiency exhibited a similar pattern, with P2 achieving 68.93 ± 2.63%, whereas P3 and P1 resulted in 38.42 ± 1.90% and 22.17 ± 2.05%, respectively. Since BOD represents the biodegradable fraction of organic matter, the lower removal rates in P1 may be due to oxygen limitation or high organic load, which could have hindered *Nannochloropsis sp.*'s capacity to fully assimilate available nutrients. The most notable finding was the significant reduction in ammonium (NH_4^+), where P2 achieved the highest removal rate of 83.76 ± 1.87%, compared to 23.25 ± 1.23% in P3 and 20.59 ± 1.36% in P1.

These findings demonstrate the potential of *Nannochloropsis sp.* in effectively performing phytoremediation when provided with an optimal nutrient composition from biogas slurry. The presence of essential macronutrients, particularly nitrogen and carbon sources, supports microalgal metabolism, enabling the assimilation and breakdown of pollutants (Emparan *et al* 2020). The ability of *Nannochloropsis sp*. to reduce COD, BOD, and ammonium concentrations is primarily driven by its photosynthetic activity, nutrient uptake mechanisms, and extracellular enzymatic processes. Through photosynthetic activity, *Nannochloropsis sp*. converts light energy into chemical energy (Shah *et al* 2024), releasing oxygen that enhances aerobic degradation of organic matter, further aiding in COD and BOD reduction (Sales-Pérez *et al* 2023). Additionally, its nutrient uptake mechanisms enable the assimilation of nitrogen, including ammonium (NH_4^+), and phosphorus from biogas slurry, which supports biomass production while lowering nutrient pollution levels. Furthermore, *Nannochloropsis sp*. employs extracellular enzymatic processes, secreting enzymes that break

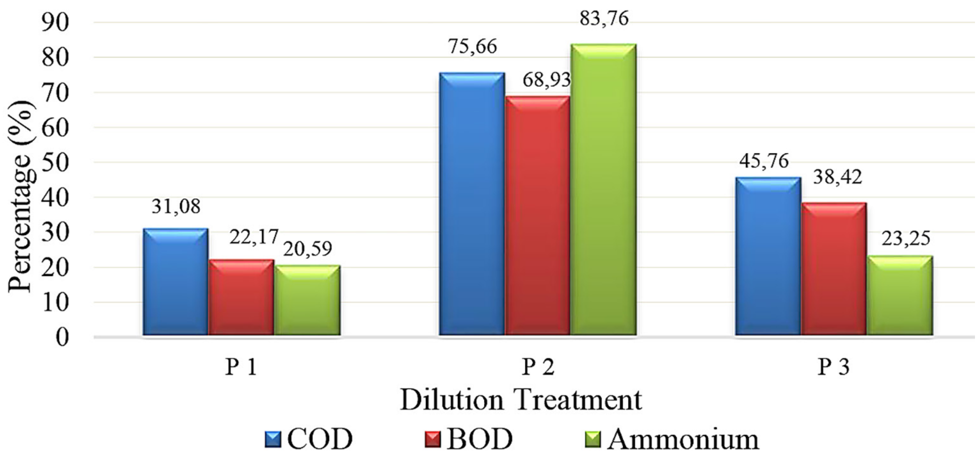

Figure 4. Reduction levels of BOD, COD, TSS, and ammonium in biogas slurry by microalgae *Nannochloropsis sp.*

down complex organic compounds, facilitating the biodegradation of pollutants in wastewater (Muthukumaran *et al* 2024).

The integration of *Nannochloropsis sp*. cultivation into biogas slurry treatment presents an innovative phytoremediation approach, leveraging the microalga's ability to assimilate nutrients and organic pollutants from wastewater streams. Compared to other phytoremediation systems such as constructed wetlands, macrophyte-based treatments, and bacterial bioreactors, microalgal-based remediation offers advantages in nutrient uptake efficiency, biomass valorization, and industrial scalability (Diaz *et al* 2022). While constructed wetlands and bacterial bioreactors are widely used for wastewater treatment, they require large land areas, extended retention times (Xu *et al* 2024), costly operational inputs (Al-Asheh *et al* 2024) and pH adjustments. In contrast, microalgal phytoremediation systems, particularly those utilizing high-biomass-producing species like *Nannochloropsis sp*., offer a compact, scalable, and cost-effective alternative that integrates well into closed-loop biotechnological processes. Unlike conventional methods focused solely on pollutant degradation, microalgal systems facilitate efficient nutrient removal while generating high-value biomass for biofuels, aquaculture feeds, and bioproducts, aligning with circular bioeconomy principles.

Circular bioeconomy and SDGs implications

The valorization of biogas slurry within a circular bioeconomy framework represents a sustainable approach to addressing both waste management challenges and renewable resource utilization (Elalami *et al* 2021). The integration of biogas slurry into microalgal cultivation systems not only mitigates environmental pollution but also contributes to high-value biomass production, supporting a zero-waste concept in agro-industrial processes (Catenacci *et al* 2022). Unlike conventional waste disposal practices, which often lead to nutrient losses, greenhouse gas emissions, and water contamination (Manea *et al* 2024), the repurposing of biogas slurry as a nutrient-rich medium for *Nannochloropsis sp*. cultivation aligns with the principles of a circular economy, where waste streams are efficiently transformed into valuable bioresources (Rubert *et al* 2024). The application of microalgae in waste-derived cultivation systems enhances bioremediation efficiency (Blanco-Vieites *et al* 2024), reduces dependency on synthetic fertilizers (Zhang *et al* 2024), and fosters the development of sustainable aquaculture and biofuel industries (Wang *et al* 2023). This approach (Figure 5) underscores the necessity of advancing biotechnological innovations that optimize waste-to-product pathways while minimizing environmental footprints.

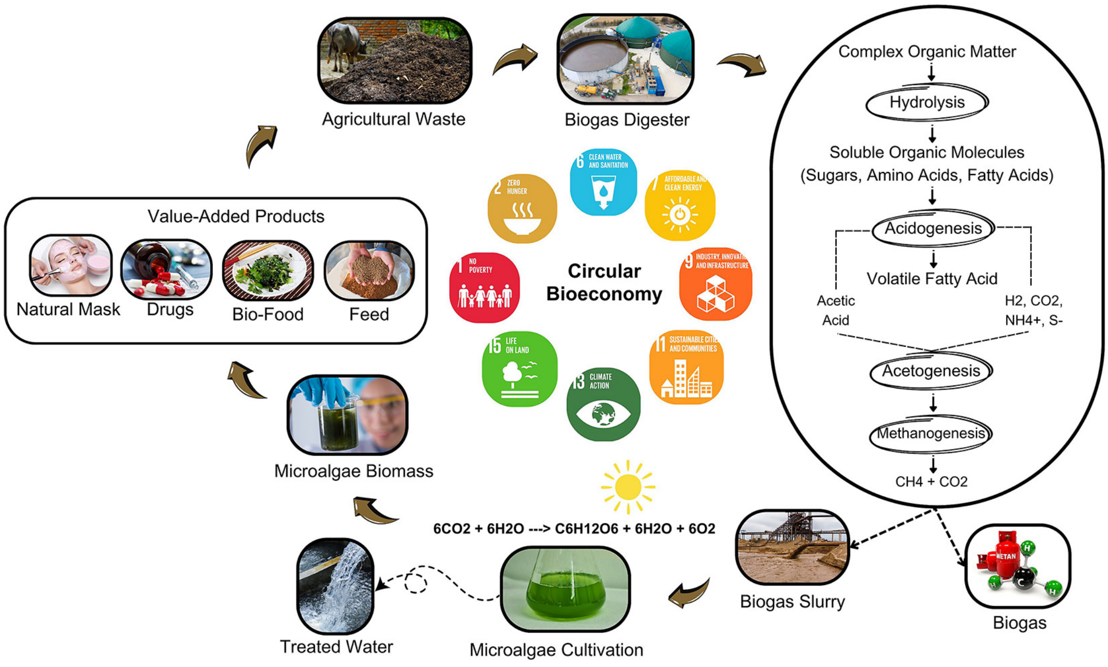

Figure 5. Agricultural waste valorization in a circular bioeconomy framework

The relevance of biogas slurry valorization to the United Nations Sustainable Development Goals (SDGs) is evident in its contribution to multiple global sustainability targets (Olabi *et al* 2023). Specifically, this strategy aligns with SDGs 6 (Clean Water and Sanitation) by reducing organic pollutants and nutrient overload in wastewater, thereby improving water quality (Diaz *et al* 2022). Furthermore, its role in bioenergy generation and resource recovery supports SDGs 7 (Affordable and Clean Energy), as microalgal biomass cultivated in biogas slurry can be further processed into biofuels and bioproducts. Additionally, this approach promotes SDGs 12 (Responsible Consumption and Production) by creating a closed-loop system that enhances resource efficiency, reduces waste, and fosters the development of sustainable bio-based industries (Goswami *et al* 2021). The ability of *Nannochloropsis sp*. to sequester CO_2 during cultivation also contributes to SDGs 13 (Climate Action) by mitigating carbon emissions and advancing climate-smart agricultural practices ((Arora *et al* 2021). These multidimensional benefits highlight the transformative potential of microalgal biorefineries in achieving global sustainability targets.

Despite its potential, the scalability of biogas slurry valorization faces several technical, economic, and policy-related challenges that must be addressed to enable widespread adoption. One of the primary concerns is the variability in biogas slurry composition, which requires standardization and optimization strategies to ensure consistent microalgal growth and biomass yield. Additionally, the economic feasibility of large-scale microalgal cultivation depends on advancements in photobioreactor design, energy-efficient harvesting technologies, and cost-effective nutrient supplementation methods (Diaz *et al* 2022). From a policy perspective, the integration of biogas slurry valorization into waste management regulations and renewable energy policies is crucial to creating market incentives that promote the commercialization of microalgae-based bioproducts (Choo *et al* 2020). The development of public-private partnerships and circular economy policies that support waste valorization initiatives can further drive innovation and investment in this sector. By overcoming these barriers, the large-scale implementation of microalgal cultivation in biogas slurry can play a pivotal role in transitioning toward a more sustainable, resilient, and resource-efficient bioeconomy.

CONCLUSION

This study successfully demonstrates the potential of biogas slurry as a nutrient source for the cultivation of Nannochloropsis sp. The research objectives were achieved by confirming that microalgae can thrive at different biogas slurry dilutions, with optimal growth observed in P2 (C/N ratio of 6,751), which maintained a balanced nutrient composition. This finding aligns with previous studies indicating that an optimal C/N ratio between 4 and 8 enhances microalgal growth and metabolic activity. The results suggest that biogas slurry not only supports sufficient nutrient availability but also facilitates efficient pollutant removal, thereby improving water quality and biomass production. The P2 treatment exhibited the highest cell production rate (0.2580 day^{-1}) and achieved COD, BOD, and ammonium removal efficiencies of 75.66%, 68.93%, and 83.76%, respectively, demonstrating the microalga's strong phytoremediation capability.

These findings highlight biogas slurry valorization as an effective and sustainable approach for integrating microalgae-based bioremediation into wastewater management strategies. The ability of Nannochloropsis sp. to convert biogas slurry into high-protein biomass (0.1412 µg/mL in P2) reinforces its potential as an alternative nutrient source for large-scale microalgal cultivation. Furthermore, this study provides a practical framework for transforming biogas production waste into a valuable bioresource, supporting circular bioeconomy principles. The integration of microalgae cultivation with biogas slurry treatment promotes environmental sustainability and aligns with key Sustainable Development Goals, particularly those related to clean water, responsible production, and climate action. Future studies should focus on optimizing large-scale applications, refining cultivation conditions, and assessing economic feasibility to further advance this waste-to-resource innovation.

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