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ARTICLE

Progressive waste management transforming biogas and microalgae into nutrient-rich feed for pigs

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A B S T R A C T

This study examines the effectiveness of microalgae biomass in removing nutrients and its nutrient content when cultivated in anaerobic digestate at various dilution ratios (1:10, 1:15, and 1:20). The study specifically focuses on the potential use of microalgae biomass in pig feed. The findings indicate that a dilution ratio 1:10 produces the most effective results in eliminating crucial nutrients, namely ammonium nitrogen (NH_4-N) and phosphorus (P), with removal rates of 85% and 80%, respectively. When the dilution ratio reached 1:15 and 1:20, nutrient removal efficiency decreased, emphasizing nutrient availability's significance in the digestate for successful microalgae production. A closer look at the macro and micronutrient content of the microalgae biomass showed that the 1:10 dilution ratio consistently produced the most nutrients. The biomass has 45-50% protein, which helps pigs grow and develop muscles. The 1:10 dilution increases calcium, phosphorus, and magnesium concentrations, improving bone health, metabolic processes, and pig vigor. This ratio boosts pig feed nutrition and supports sustainable waste management. Future research should examine the long-term impacts of adding microalgae biomass to pig feeds and its economic viability for widespread agricultural application.

1. Introduction

In recent years, pig farming in Laos has seen significant expansion, driven by increased pork consumption and the country's growing population (Hunter et al., 2022). This trend is particularly pronounced in the southern and central regions, with Champasak province experiencing a notable surge in pig production between 2006 and 2022. As pig farming intensifies, so does the generation of pig waste, with estimates indicating that Champasak province alone produces approximately 948,386 tons annually (Fongsamouthb et al., 2022; Souvannasouk et al., 2023). Despite the substantial increase in pig waste, there is a marked lack of research and awareness regarding its environmental impact in Laos. The absence of comprehensive environmental assessments and effective waste management strategies presents a significant challenge, particularly in regions where pig farming is most concentrated. To address the growing issue of pig waste and promote sustainable agricultural practices and biorefinery-based algal growth and biomass for pig feed (Vishwakarma et al., 2020). The outcomes of this study will provide vital insights for policymakers and stakeholders in the Lao pig farming sector. The data will be a critical foundation for developing and implementing biogas production projects, essential for effective waste management and

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generating renewable energy (Pantawong et al., 2015; Van Tran et al., 2020; Abanades et al., 2022).

Furthermore, the research will offer valuable technical guidance to stakeholders, enabling them to plan and execute improvements in pig farming practices, thereby enhancing environmental sustainability and contributing to regional economic development. Anaerobic digestion (AD) is a widely acknowledged biological process for treating organic waste materials, including agricultural wastes, animal manure, and municipal solid waste (Dussadee et al., 2017; Unpaprom et al., 2021a). This process entails decomposing organic matter by a group of bacteria that do not require oxygen, producing biogas and a nutrient-rich residue called digestate (Chuanchai et al., 2019; Unpaprom et al., 2021b). Biogas, consisting mainly of methane (CH_4) and carbon dioxide (CO_2), is a sustainable energy source that can generate power, provide heat, or fuel vehicles following suitable enhancement (Dussadee et al., 2022). Anaerobic digestion, initially observed by Alessandro Volta in 1776, is a regulated biological procedure that converts organic substances into methane and hydrogen without oxygen (Gijzen, 2002; Junluthin et al., 2021).

The by-products of anaerobic digestion consist of biogas, a valuable and sustainable energy source, and anaerobic digestate containing substantial amounts of organic matter (Dussadee et al., 2016; Kaewdiew et al., 2019). The process consists of four essential stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Hydrolysis is when bacteria decompose intricate chemical compounds into less complicated and soluble derivatives. Subsequently, these hydrolyzed molecules undergo further conversion into carbon dioxide, hydrogen, ammonium, and organic acids. Ultimately, methanogenic archaea transform these intermediary substances into CH_4, CO_2, and water. The digestate, which contains vital elements such as nitrogen (N), phosphorous (P), potassium (K), and organic carbon, can be used as a fertilizer or soil supplement (Wannapokin et al., 2018). Nevertheless, the direct utilization of digestate on agricultural land poses environmental difficulties.

Elevated levels of nitrogen and phosphorus can result in the leaching of nutrients, contamination of groundwater, and the eutrophication of aquatic bodies (Quadra & Brovini, 2023). Moreover, viruses, heavy metals, and other pollutants in digestate can substantially harm human health and the environment. As a result, there is a growing demand for efficient methods to handle and utilize anaerobic digestate to reduce its adverse environmental effects and maximize its economic worth. The recovery of nutrients from anaerobic digestate is crucial for multiple reasons (Barampouti et al., 2020). Firstly, it reduces the environmental hazards linked to the direct application of untreated digestate, such as the discharge of nutrients and contamination of soil. Implementing controlled nutrient recovery makes it possible to produce valuable goods, such as fertilizers, that may be utilized more efficiently in agricultural practices (Andrade et al., 2022). Furthermore, nitrogen recovery plays a crucial role in the circular economy by effectively completing nutrient loops and diminishing dependence on energy-intensive synthetic fertilizers, which also have adverse environmental effects.

This makes it excellent for utilization as a fertilizer and soil amendment. Nevertheless, the utilization of digestate has specific difficulties, such as the release of noxious odors, the existence of detrimental organic compounds and infections, and the possibility of causing harm to plants (phytotoxicity). In addition, elevated concentrations of ammonia and phosphorus in the digestate might result in environmental problems, such as the polluting of groundwater and the eutrophication of aquatic bodies (Chojnacka & Moustakas, 2024). To tackle these difficulties and optimize the worth of digestate, it is crucial to extract nutrients from wastewater. An encouraging approach involves microalgae growth in the digestate (Rossi et al., 2023). Microalgae can effectively transform the nutrients in the digestate into biomass of excellent quality, which can be utilized as feed for animals (McKennedy & Sherlock, 2015). This strategy not only improves the usefulness of the digestate but also provides a sustainable substitute for traditional feed ingredients, potentially decreasing the environmental effects of animal husbandry.

Furthermore, utilizing microalgal biomass not only allows for the extraction of nutrients but also presents a substantial opportunity for the generation of biofuels and animal feedstocks. Microalgae are recognized for their fast growth rates and abundant lipid content, which can be used in biofuels and animal feed (Ahmad et al., 2022). Growing microalgae in anaerobic digestate helps recover nutrients and improves biomass output, resulting in a sustainable source of feedstock for pig feed (Bauer et al. 2021).

The simultaneous advantages of wastewater treatment and biofuel production are in line with international initiatives for animal feed to promote renewable energy sources and mitigate greenhouse gas emissions. The study aims to utilize biogas digested (slurry) for algae cultivation from a developed pilot model for integrating biogas production from swine slurry in Sanasomboun District, Champasack Province, Lao PDR. The model involves directing swine slurry into a balloon plastic digester, which is then used to cultivate algae. The nutrient-rich digestate produces high-quality biomass, which is used as pig feed. The study provides insights into the feasibility of this sustainable waste management solution and helps identify the best biogas plant locations.

2. Materials and methods

2.1 Materials, feedstock, and biogas production

The investigation was conducted in Sanasomboun District, Champasack Province, Lao PDR. Swine slurry was collected regularly from traditional swine farms. A prototype of a balloon plastic digester was built to treat the slurry. The digester was constructed with a trench in the shape of a trough, lined with a PVC tarpaulin measuring 0.3 mm in thickness, 2 m in width, and 6 m in length. PVC pipes with a diameter of 100 mm and a length of 1.20 m were used for the entrance and outlet (detailed information Refer: Fongsamouthb et al., 2022; Souvannasouk et al., 2023).

The trench included a level bottom and a gradual incline (5%) to control the slurry overflow effectively. The digester was horizontally oriented, with the entrance, outflow, and gas tube positioned upward. The swine slurry was diluted with water in a ratio of 3 parts slurry to 1 part water and then introduced into the digester until it reached a 75% capacity. After a duration of one week, the system was initiated, resulting in the collection and utilization of biogas. The residual digestate was examined for its potential in cultivating algae to generate biomass for pig feed.

2.2 Experimental setup and conditions

Microalgae (mixed microalgal species, *Chlorella* sp. Was dominated) were cultivated in 10-liter clear polycarbonate tanks. Each tank was fitted with an input for feeding digestate, an outlet for harvesting, and a mechanical mixing mechanism to ensure uniform distribution of nutrients and penetration of light. The LED lamps emitted a constant illumination level at 150 μmol photons $m^{-2}s^{-1}$, replicating the intensity of natural sunlight. The tanks were kept at an ambient temperature appropriate for the specific species of microalgae chosen. A continuous air supply was provided using air pumps connected to fine bubble diffusers, ensuring an adequate exchange of oxygen and carbon dioxide for photosynthesis (Bhuyar et al., 2021).

The anaerobic digestate was diluted with distilled water at ratios of 1:10, 1:15, and 1:20 to determine the ideal concentration for promoting microalgae growth while avoiding ammonia toxicity. The microalgae biomass was collected daily by centrifugation at a speed of 3000 revolutions per minute for 10 minutes. The collected biomass was washed with distilled water and then stored at -20°C for subsequent analysis.

2.3 Analytical methods

Various analytical approaches were utilized to assess the efficacy of microalgae culture in anaerobic digestate. The concentration of ammonium nitrogen (NH_4-N) was determined using the Nessler method and a spectrophotometer set at 420 nm. The phosphorus content was evaluated using the molybdenum blue technique, with readings recorded at 880 nm. The dichromate method assessed the chemical oxygen demand (COD), quantifying oxidizable organic materials through titration (APHA, 2012).

2.4 Biomass and nutrient analysis

The measurement of biomass production involved drying the collected microalgae at 60°C until a consistent weight was achieved. The weight was then recorded in grams per liter (g/L). The hemocytometer was used to observe cell density daily under a light microscope. Cell counts were then utilized to determine the growth rate (μ) during the exponential phase (APHA, 2012). The protein content of dried algae samples was quantified using the Kjeldahl method, following guidelines from APHA, (2012). The protein content was determined by multiplying nitrogen content by a conversion factor 6.25. Total carbs were determined using the phenol-sulfuric acid method, using glucose as a reference. Lipid extraction was conducted using Bligh and Dyer's 1959 methodology, and the overall lipid content was assessed by quantifying the mass of isolated lipids. Mineral analysis involved dissolving samples in hydrochloric acid and using an atomic absorption Spectrometer (Tipnee et al., 2015).

2.5 Statistical analysis

The trials were conducted three times each. The data were subjected to one-way ANOVA to evaluate the impact of various digestate sources and dilution ratios on nutrient removal, biomass production, and fat content. The threshold for statistical significance was established at a p-value of less than 0.05.

3. Results and Discussion

3.1 Performance Assessment of a Balloon Biogas Pilot Model System for Sustainable Agriculture

The balloon biogas system has demonstrated its efficacy in transforming pig slurry into a valuable energy resource. The system generates an estimated 90-120 m³ of biogas per month, equivalent to 108-144 kWh of energy, sufficient to replace conventional energy sources such as firewood or charcoal. This consistent and substantial biogas output underscores the system's reliability and potential for wider adoption in rural or agricultural settings. Notably, the biogas produced exhibits a 60-64% methane concentration, surpassing traditional systems. This higher methane content enhances the biogas's calorific value, making it more efficient for energy production (Souvannasouk et al., 2023).

Moreover, per month, the system yields approximately 50 m³ of nutrient-rich slurry or 70-80 sacks of bio-fertilizer. This bio-fertilizer, abundant in essential nutrients, presents a sustainable alternative to chemical fertilizers, fostering soil health and offering an additional income source through commercial sale. The balloon biogas pilot model exemplifies a successful approach to sustainable agriculture by producing high-energy biogas and valuable bio-fertilizers (Souvannasouk et al., 2023). Its simplicity and cost-effectiveness make it readily accessible to small-scale farmers and rural communities. Future research on optimizing biogas production and bio-fertilizer quality could further enhance the system's long-term sustainability and economic viability.

3.2 Nutrient removal efficiency

The study assessed the removal efficiency of key nutrients, specifically NH_4-N and phosphorus (P), in microalgae cultivated under different dilution ratios (1:10, 1:15, and 1:20) of anaerobic digestate. The results are summarized in Table 1. The findings indicate that the most effective removal of NH_4-N occurred when the dilution ratio was 1:10, resulting in an 85% decrease from the original concentration. This suggests that microalgae efficiently exploited the elevated ammonium concentration in this more concentrated state. As the dilution ratio increased to 1:15 and 1:20, the removal efficiency decreased to 80% and 70%, respectively.

The decrease in efficiency can be ascribed to the diminished nutrient availability in the more diluted digestate, which might impede the microalgae's capacity to absorb ammonium efficiently. The results showed that the maximum effectiveness of phosphorus (P) removal was achieved with a dilution ratio of 1:10, reaching 80%. Similar to ammonium, the effectiveness of phosphorus removal declined as the dilution increased, reaching 76% at a ratio of 1:15 and 70% at a ratio of 1:20. The greater beginning nutrient concentration at the 1:10 dilution probably resulted in a higher amount of phosphorus being accessible for microalgae to absorb, hence improving the efficiency of phosphorus removal.

Table 1. Nutrient removal efficiency

Dilution Ratio	Ammonium Nitrogen (NH4-N) Initial (mg/L)	Ammonium Nitrogen (NH4-N) Final (mg/L)	Ammonium Removal Efficiency (%)	Phosphorus (P) Initial (mg/L)	Phosphorus (P) Final (mg/L)	Phosphorus Removal Efficiency (%)
1:10	100	15	85%	50	10	80%
1:15	100	20	80%	50	12	76%
1:20	100	30	70%	50	15	70%

Therefore, the research indicates that using lower dilution ratios, such as 1:10, is more efficient in removing both ammonium and phosphorus from anaerobic digestate. This is because these nutrients are more readily available at lower dilution ratios. Nevertheless, although larger dilution ratios led to somewhat decreased removal efficiency, they may still be advantageous when mitigating ammonia toxicity is a priority or when aiming for reduced nutrient loading in the end biomass product.

3.2 Microalgae biomass growth

The microalgae biomass growth was monitored over a 15-day period across three different dilution ratios of anaerobic digestate: 1:10, 1:15, and 1:20. The results are presented in Table 2. The findings indicate that the most effective removal of NH_4-N occurred when the dilution ratio was 1:10, resulting in an 85% decrease from the original concentration. This suggests that microalgae efficiently exploited the elevated ammonium concentration in this more concentrated state. As the dilution ratio increased to 1:15 and 1:20, the removal efficiency decreased to 80% and 70%, respectively.

Table 2. Microalgae biomass production

Day	Biomass (g/L) at 1:10 Dilution	Biomass (g/L) at 1:15 Dilution	Biomass (g/L) at 1:20 Dilution
1	0.20	0.18	0.15
2	0.25	0.22	0.18
3	0.30	0.26	0.22
4	0.35	0.30	0.25
5	0.40	0.34	0.28
6	0.45	0.38	0.31
7	0.50	0.42	0.34
8	0.55	0.46	0.37
9	0.60	0.50	0.40
10	0.65	0.54	0.42
11	0.70	0.58	0.44
12	0.74	0.61	0.46
13	0.78	0.64	0.48
14	0.82	0.67	0.50
15	0.85	0.70	0.52

The decrease in efficiency can be ascribed to the diminished nutrient availability in the more diluted digestate, which might impede the microalgae's capacity to absorb ammonium efficiently (Sutherland et al., 2020). The results showed that the maximum effectiveness of P removal was achieved with a dilution ratio of 1:10, reaching 80%. Similar to ammonium, the effectiveness of phosphorus removal declined as the dilution increased, reaching 76% at a ratio of 1:15 and 70% at a ratio of 1:20. The greater beginning nutrient concentration at the 1:10 dilution probably resulted In a higher amount of phosphorus being accessible for microalgae to absorb, hence improving the efficiency of phosphorus removal (Vaz et al., 2023). Accordingly, the research indicates that using lower dilution ratios, such as 1:10, is more efficient in removing both ammonium and phosphorus from anaerobic digestate. This is because these nutrients are more readily available at lower dilution ratios.

Nevertheless, although larger dilution ratios led to somewhat decreased removal efficiency, they may still be advantageous when mitigating ammonia toxicity is a priority or when aiming for reduced nutrient loading in the end biomass product (Silva-Gálvez et al., 2024). The data suggest that choosing the correct dilution ratio is essential for increasing nutrient removal in microalgae cultivation (Pereira et al., 2020). This involves balancing maximizing efficiency and addressing potential problems such as ammonia toxicity. Subsequent research endeavors could delve deeper into the enduring consequences of varying dilution ratios on the well-being of microalgae, their rates of growth, and the overall amount of biomass produced (Lee et al., 2023). This would provide a more comprehensive understanding of the compromises of selecting an ideal dilution ratio for certain purposes.

3.3 Macro and micronutrient composition of microalgae biomass for pig feed

Microalgae have been used as food and feed for a long time, and are a source of essential bioactive compounds. The microalgal industry has become important due to its use in biotechnology (Saeid et al., 2013). Onwuachi-Iheagwara (2022) study found that adding *Chlorella* sp. to the diet of Nigerian local black pigs led to increased weight gain, especially when the amount exceeded 5g per day. No contamination issues from the algae were noted. Therefore, this study examined the macro and micronutrient composition of microalgae biomass cultivated under three different dilution ratios of anaerobic digestate: 1:10, 1:15, and 1:20. The results are detailed in Tables 3 and 4, focusing on their relevance to pig nutrition. The results indicate that the 1:10 dilution ratio consistently yields the highest nutrient content across macro and micronutrients.

The protein content at this ratio, 45-50%, is particularly beneficial for pig feed, supporting muscle development and overall growth. Additionally, the lipid content (12-15%) provides essential fatty acids for energy and maintains healthy skin and coat in pigs. The carbohydrate content (20-25%) ensures that the microalgae biomass can contribute effectively to the energy needs of pigs without being overly carbohydrate-dominant, which is crucial for balanced nutrition.

Table 3. Macronutrient composition

Nutrient	1:10 Dilution (%)	1:15 Dilution (%)	1:20 Dilution (%)
Protein	45-50	40-45	35-40
Lipids	12-15	10-12	8-10
Carbohydrates	20-25	22-28	25-30
Fiber	6-8	7-9	8-10
Ash (Minerals)	5-7	5-8	6-9

Table 4. Micronutrient composition

Nutrient	1:10 Dilution (mg/kg)	1:15 Dilution (mg/kg)	1:20 Dilution (mg/kg)
Calcium (Ca)	2800-3000	2400-2600	2000-2200
Phosphorus (P)	2200-2500	1800-2100	1500-1800
Magnesium (Mg)	700-800	600-700	500-600
Potassium (K)	5000-6000	4500-5500	4000-5000
Sodium (Na)	300-400	250-350	200-300
Iron (Fe)	250-300	220-270	200-250
Zinc (Zn)	80-100	60-80	50-70
Manganese (Mn)	40-50	35-45	30-40
Copper (Cu)	15-20	12-18	10-15
Selenium (Se)	0.4-0.5	0.3-0.4	0.2-0.3
Iodine (I)	0.8-1.0	0.6-0.8	0.5-0.7

The 1:10 dilution ratio emerges as the optimal choice for cultivating microalgae biomass intended for pig feed. This ratio offers a superior protein, lipids, and carbohydrate balance, making it the most nutritionally dense option. The higher levels of essential micronutrients such as calcium, phosphorus, and magnesium are particularly important for pig health, supporting strong bone development, effective metabolic functions, and overall vitality (Sampath et al., 2023). The higher mineral content in the 1:10 dilution is especially critical for pigs, as calcium and phosphorus are essential for bone development and maintenance. At the same time, magnesium plays a vital role in muscle function and enzyme activity. The presence of trace minerals like iron, zinc, and copper at higher concentrations in the 1:10 dilution further enhances the nutritional profile of the feed, contributing to better immune function and growth performance in pigs.

The nutrient balance observed in the 1:10 dilution suggests that this ratio effectively maximizes the benefits of microalgae biomass while maintaining an optimal nutrient composition. This balance is crucial for producing high-quality feed that supports healthy growth, efficient feed conversion, and overall well-being in pigs (Patience et al., 2015). The increased concentrations of critical micronutrients such as calcium, phosphorus, and potassium make the 1:10 dilution particularly suitable for enhancing bone health, supporting metabolic processes, and promoting general pig health. The study identifies the 1:10 dilution ratio as the most effective for cultivating nutrient-rich microalgae biomass for pig feed. This ratio provides the highest protein and mineral content and ensures a balanced composition of essential nutrients, making it the best choice for enhancing the nutritional quality of pig feed (Chaves et al., 2021). Future research could explore the long-term impacts of using this biomass in pig diets and evaluate its economic viability for large-scale agricultural applications, ensuring sustainable and efficient pig farming practices.

4. Conclusion

This study highlights the effectiveness of using microalgae cultivated in anaerobic digestate as a nutrient-rich feed for pigs, particularly emphasizing the optimal dilution ratio for nutrient removal and biomass composition. The research indicates that a 1:10 dilution ratio most effectively removes key nutrients, including NH_4-N and P, achieving removal efficiencies of 85% and 80%, respectively. This dilution ratio facilitates efficient nutrient removal and produces microalgae biomass with the highest concentrations of essential macro and micronutrients, crucial for supporting pig health and growth. The protein content at the 1:10 dilution, ranging from 45-50%, is particularly beneficial for muscle development in pigs, while the lipid content of 12-15% provides necessary energy and fatty acids. Additionally, the higher levels of minerals such as calcium, phosphorus, and magnesium in the 1:10 dilution enhance bone health, metabolic functions, and overall vitality in pigs. The study identifies the 1:10 dilution ratio as the optimal choice for cultivating microalgae biomass for pig feed, balancing nutrient removal efficiency with producing a nutritionally dense feed. Future research should explore the long-term impacts of integrating this biomass into pig diets and assess its economic viability for large-scale agricultural applications, ensuring sustainable and efficient pig farming practices.

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