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Agro-Industrial Waste Revalorization for Bioenergy Purposes in the Developing World

DISSERTATION THESIS

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Declaration

I hereby affirm that I have done this thesis entitled **"Agro-industrial waste revalorization for bioenergy purposes in the developing world"**, except for jointly authored publications that are included. In the case of such publications, my specific contributions to each chapter have been clearly stated at their respective beginnings. Furthermore, I affirm that proper acknowledgment has been provided within this thesis for any references made to the works of others. I also ensure that this work has not been and is not being submitted for any other degree from this or any other university. All sources have been properly quoted and acknowledged through complete references, in accordance with the Citation rules of the FTA.

In Praha, September 25th, 2025

Lydia Mawar Ningsih

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Abstract

Revalorization of agro-industrial waste is a key step towards sustainability, positively impacting both the environment and society. The utilization of agro-industrial waste as a renewable energy source, such as biogas, biopellets, and plant nutrients, using simple technology, is beneficial both environmentally and economically for industry and farmers. Agro-industrial waste management must be considered because the amount is very abundant; however, there are some issues in its management, such as a lack of technology, finance, land, and knowledge in waste management among industries and farmers. In the case of tofu production, 1 kg of soybeans consumes 25.25 L/kg of water and produces wastewater of 14.45 L/kg. The use of tofu wastewater as biogas feedstock produces 310.49 L/day at an OLR of 2 $kgCOD/m^3.day$. The utilization of tofu wastewater can substitute for approximately 2.82% of firewood, 11.86% LPG, and 33.39% biopellets, making it very beneficial in both economic and environmental aspects. Therefore, based on the national capacity for the tofu and tempeh industry reaching 400,000 tons, this results in a reduction of approximately 448,000 tons of CO_2e emissions. Moreover, the high acetic acid content in tofu wastewater (92.40%) and tempeh wastewater (77.32%) can serve as a valuable source of acetic acid, which has significant economic potential and diverse applications in industries such as food production,

pharmaceuticals, and volatile fatty acid (VFA). The utilization of tofu wastewater into biogas using biofilter in ABR enhances the nutrient content in biogas effluent by capturing and biodegrading pollutants. This effluent, already rich in essential nutrients due to the anaerobic condition and compartmentalized design of the ABR, contains the following concentrations in the biogas effluent mix with commercial nutrient (AB MIX): total N (262.5 mg/L), P-available (0.399 mg/L), Ca (4.08 mg/L), Mg (25.24 mg/L), Cu (0.032 mg/L), and Fe (13.09 mg/L). These values comply with the organic fertilizer standard set by the Ministry of Agriculture of Indonesia.
This thesis provided an overview of the performance of agro-industrial waste treatments, including wastewater and solid waste in the developing world, through anaerobic digestion (AD). The results of this study show that the use of simple technology in the utilisation of agro-industrial waste is very useful as a renewable energy source and a source of nutrients for plants. Moreover, it provides information on audit energy as a consideration for micro, small, and medium enterprises (MSMEs) levels and stakeholders in making decisions and investing to utilise the waste, which impacts sustainability to reach net zero and recover energy demand at the industry and global levels.

Keywords: wastewater; renewable energy; sustainability; anaerobic digestion; resource recovery.

Abstrak

Penanggulangan limbah adalah kunci keberlanjutan yang berdampak positif terhadap lingkungan dan masyarakat. Pemanfaatan limbah agroindustri dengan menggunakan teknologi sederhana sebagai sumber energi terbarukan seperti biogas, biopelet, dan nutrisi untuk tanaman dapat memberikan keuntungan secara lingkungan dan ekonomi pada petani dan industri. Manajemen limbah agroindustri harus sangat dipertimbangkan karena jumlahnya yang sangat melimpah, namun disisi lain, terdapat beberapa problematika yang dihadapi, yaitu kurangnya teknologi yang memadai, finansial, lahan dan kurangnya pengetahuan manajemen limbah ditingkat industri dan petani. Sebagai contoh, produksi 1 kg tahu dari kedelai membutuhkan 25,25 L air dan menghasilkan limbah cair sebanyak 14,45 L/kg. Pemanfaatan limbah cair tahu sebagai bahan baku biogas dapat menghasilkan biogas 310.49 L/hari dengan OLR 2 kgCOD/m^3.day. Berdasarkan kapasitas produksi tahu dan tempe nasional sebesar 400.000 ton, potensi penurunan emisi yang dihasilkan mencapai sekitar 448.000-ton CO_2e.

Pemanfaatan limbah cair tahu menjadi biogas memberikan benefit pada aspek lingkungan dan ekonomi, karena dapat mensubtitusi energi tidak terbarukan berkisar 2.82% kayu bakar, 11.86% LPG, dan 33.39% biopelet. Selain itu, kandungan asam asetat yang tinggi dalam air limbah tahu (92,40%) dan air limbah tempe (77,32%) dapat berfungsi sebagai sumber asam

asetat yang berharga, yang memiliki potensi ekonomi yang signifikan dan aplikasi yang beragam dalam industri seperti produksi makanan, farmasi, dan asam lemak volatil (VFA).

Pemanfaatan limbah cair tahu menjadi biogas menggunakan biofilter di ABR meningkatkan kandungan nutrisi pada biogas effluent dengan menangkap dan mengurai polutan secara biologis. Tingginya kandugan nutrisi pada biogas effluent dikarenakan kondisi anaerobik dan desain ABR yang terkompartementalisasi, Kandungan nutrisi biogas effluent mix dengan nutrisi komersil (AB MIX) adalah: N total (262,5 mg/L), P-tersedia (0,399 mg/L), Ca (4,08 mg/L), Mg (25,24 mg/L), Cu (0,032 mg/L), dan Fe (13,09 mg/L). Kandungan nutrisi tersebut mematuhi standar pupuk organik yang ditetapkan oleh Kementerian Pertanian Indonesia.

Disertasi ini memberikan gambaran umum mengenai performa pengolahan limbah agroindustri yakni limbah cair dan limbah padat di negara berkembang khususnya di Indonesia, melalui anaerobik digester atau biodigester. Hasil menunjukkan bahwa penggunaan teknologi sederhana pada pemanfaatan limbah agroindustri sangat efektif sebagai energi terbarukan dan sumber nutrisi untuk pertumbuhan tanaman. Selanjutnya, disertasi ini menyediakan informasi audit energi sebagai bahan pertimbangan untuk membuat Keputusan dalam memanfaatkan limbah pada usaha skala mikro, kecil, dan menengah (UMKM) yang berdampak pada sustainabilitas untuk mencapai net zero dan memulihkan permintaan energi pada level industri dan global.

Kata kunci; limbah cair; energi terbarukan; keberlanjutan; anaerobik digester, pemulihan sumber daya.

Abstrakt

Revalorizace agroprůmyslového odpadu představuje klíčový krok k udržitelnosti, s významnými přínosy pro životní prostředí i společnost. Jeho využití jako obnovitelného zdroje energie – například ve formě bioplynu, biopelet či živin pro rostliny za pomoci jednoduchých technologií – přináší environmentální i ekonomické výhody jak průmyslovým podnikům, tak zemědělcům. Efektivní nakládání s agroprůmyslovým odpadem je nezbytné vzhledem k jeho značnému objemu, avšak čelí řadě překážek, jako je nedostatek vhodných technologií, finančních prostředků, dostupné půdy a odborných znalostí v oblasti odpadového hospodářství, a to zejména mezi podniky a zemědělskými producenty.

V případě výroby tofu platí, že na 1 kg sójových bobů se spotřebuje 25,25 l vody a vzniká 14,45 l odpadní vody. Použitím odpadní vody z tofu jako substrátu pro výrobu bioplynu lze při organickém zatížení 2 kgCOD/m³.den vyrobit 310,49 l bioplynu denně. Využití odpadní vody z tofu tak může nahradit přibližně 2,82 % energie z palivového dřeva, 11,86 % energie z LPG a 33,39 % z biopelet, což je velmi výhodné z hlediska ekonomiky i životního prostředí. Na základě národní kapacity výroby tofu a tempehu ve výši 400 000 tun by toto využití mohlo vést ke snížení emisí přibližně o 448 000 tun CO_2e.

Vysoký obsah kyseliny octové v odpadní vodě z tofu

(92,40 %) a tempehu (77,32 %) z ní činí cenný zdroj kyseliny octové, která má významný ekonomický potenciál a široké využití v potravinářství, farmacii a výrobě těkavých mastných kyselin.

Využití odpadní vody z výroby tofu k produkci bioplynu prostřednictvím biofiltračního anaerobního reaktorového systému přispívá ke zvýšení obsahu živin ve výsledném výstupu, a to díky zachycování a biologickému rozkladu znečišťujících látek. Výstup z tohoto procesu, který je již přirozeně obohacen o esenciální živiny díky anaerobním podmínkám a členitému uspořádání reaktoru, vykazuje následující koncentrace: celkový dusík – 262,5 mg/l, dostupný fosfor – 0,399 mg/l, vápník – 4,08 mg/l, hořčík – 25,24 mg/l, měď – 0,032 mg/l a železo – 13,09 mg/l. Tyto hodnoty splňují standardy pro organická hnojiva stanovené Ministerstvem zemědělství Indonésie.

Tato práce poskytuje přehled o účinnosti metod zpracování agroprůmyslového odpadu, včetně kapalného i pevného odpadu, v rozvojových zemích prostřednictvím anaerobní digesce. Výsledky studie ukazují, že využití jednoduchých technologií při zpracování agroprůmyslového odpadu je velmi přínosné – jak pro výrobu obnovitelné energie a živin pro rostliny. Studie zároveň nabízí cenné informace o energetickém auditu jako nástroji pro rozhodování a investice na úrovni mikro, malých a středních podniků i dalších zainteresovaných subjektů. Využití těchto poznatků přispívá k podpoře udržitelnosti, dosažení uhlíkové neutrality a zajištění energetických potřeb v průmyslovém i globálním měřítku.

Klíčová slova: odpadní voda; obnovitelná energie; udržitelnost; anaerobní digesce; obnova zdrojů.

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ABBREVIATIONS

ABR	Anaerobic Baffled Reactor
AD	Anaerobic digestion
BOD	Biological oxygen demand
CAGR	Compound annual growth rate
CHP	Combined Heat and Power
CO_2	Carbon Dioxide
CH_3COOH	Acetic Acid
$CaSO_4$	Potassium Sulphate
COD	Chemical Oxygen Demand
COP	Climate Change Conference
$_{S}$COD	Soluble Chemical Oxygen Demand
EU	European Union
GC	Gas Chromatography
GNEP	General National Energy Plan
GHG	Greenhouse Gas
HRT	Hydraulic Retention Time
KJ/kg	kilojoule / kilogram
L/kg	Litre/kilogram
LCA	Lice Cycle Assessment
LPG	Liquid Petroleum Gas
MJ/kg	Megajoule/kilogram
MSMEs	Micro, Small, and Medium Enterprises
mt/y	Million tons/year
MWe	Megawatt Electrical
NEP	National Energy Policy
OLR	Organic Loading Rate
SDGs	Sustainable Development Goals

SOP	Standard Operating Procedure
UNDP	United Nations Development Program
TS	Total solid
TSS	Total suspended solids
UAE	United Arab Emirates
USD	United States dollar
VFA	Volatile Fatty Acid
VFA/ALK	VFA/alkalinity
WWTP	Wastewater treatment plant

1. Introduction

The issue of waste management and its consequences is a pressing concern affecting ecosystems, human health, and economic progress. The main types of waste include organic waste (food, yard trimming, and other biodegradable materials), inorganic waste (plastic, metals, glass, and other non-biodegradable materials), and hazardous waste (toxic substances, chemicals, and pollutants) (Adetunji et al., 2023). The emergence of waste is increasing rapidly, predicted up to 46 billion tons by 2050, which is related to the high demand for population growth it does not seem any sign of decreasing (Maalouf & Mavropoulos, 2023). Waste management is a significant challenge worldwide, with the global population generating over 2 billion tons of municipal solid waste in 2023 and expected to grow up to 3.8 billion tons by 2050 (UNEP, 2024). The countries with the largest amount of municipal solid waste per capita are the United States and Denmark, with an average of citizens producing more than 800 kilograms of waste per year (Statista, 2024). Moreover, agriculture and industry are linked to agro-industry sectors, which produce abundant waste each year. This is because the demand for agro-industrial waste for a sustainable resource significantly increases. The agro-industrial activities generate waste of approximately 1.3 billion tons per year, besides that the size market of global agriculture waste is 17.58 billion USD in 2023 and is predicted will reach 28.60 billion USD by 2030, at a Compound annual growth rate (CAGR) of 7.20% by the forecast period 2024 – 2030 (MMR, 2023; Prado-Acebo et al., 2024). However, wastewater also has enormous potential that requires special consideration in its utilization and management. The global potential of wastewater is expected will increase by 24% in 2030 and 51% by 2050, based on the current situation, the potential of wastewater produced annually is 380 trillion L (Qadir et al., 2020).

Based on global waste potential data, a solution is needed to utilize waste so that it is sustainable, has high economic value, and has a positive impact on environmental and socio-economic aspects. The improper waste disposal leads to environmental pollution, climate change, and health risks (Rodríguez Gamboa et al., 2023). The report from UNEP (2024) found that the global cost of waste management was around 252 billion USD in 2020 and will rise to 361 billion USD when considering poor health, hidden costs, population, and climate change due to improper disposal and waste management. If there is no action on waste management, the cost will increase almost twofold to 640.3 billion USD by 2050. The utilization of wastewater could be a solution to provide the alternative energy demand for a half billion people, supplying over 10 times the volume of water currently provided by global desalination capacity and offsetting around 10% of global fertilizer use (Nairobi, 2023). Thus, a simple technology approach is required to utilize agro-industrial waste to minimize the hazard in the ecosystem, which impacts not only the environment but also public health.

This dissertation provides information on the study results on the utilization of agro-industrial wastewater through AD in developing countries, particularly in Indonesia. This dissertation is one of the supporting materials to the targets of the National Energy Policy (NEP) in Indonesia, which expands measures to improve energy security and encourage the energy transition from fossil fuels to renewable energy by 2035 and to reach net zero by 2060 (Setyawati & Setiawan, 2024). Government Regulation No. 79/2014 regarding the NEP expressed the ambition to carry out the transformation by 2025 and 2050, the main energy supply mix, namely: a) new and renewable energy achieve around 31% in 2050, b) for the oil less than 20% in 2050, c) coal less at least 25% in 2050; d) gas less at 24% in 2050. However, in the current situation, the contribution of renewable energy in Indonesia is only about 9% of the total energy mix (Rianawati et al., 2021). Based on the report of IESR, the current energy sector policies are far from sufficient to reduce emissions, as they are projected to only cut 20% of projected emissions by 2030 and maintain an increasing trend until 2060 (IESR, 2024). Renewable energy in Indonesia is usually used for cooking at the household level in rural areas and remote islands. Around 24.5 million households, or 40% of households, still use firewood for cooking in daily life (IRENA, 2017). Currently, the main energy source to meet 10% of Indonesia’s energy needs is coal. Thus, Indonesia is critical to the renewable energy transition. One step to achieve the target of using renewable energy is to utilize industrial waste as biogas. Household biogas generation has been adopted in Indonesia in response to the country's concerns about energy security in rural areas; the total number of biogas plants is 48,038 (Situmeang et al., 2022).

In Indonesia, based on the scale of biogas categories in two plants, namely households or communal scale, and industrial scale. Currently, the capacity of biogas is 28.93 million m^3/year is only 5.8% of the target of 489.8 million m^3/year in 2029 consisting of the capacity of biogas on a small household scale is 26.72 million m^3/year, and on an industrial scale there are 78 biogas plants with 161.6 MWe of capacity (Setiawan et al., 2020). The government will continue to increase the number of biogas installations as a concrete manifestation of the government's ideals for equal distribution of energy throughout the region, including in rural areas. Domestic biogas is greatly useful for small farmers in sustainably utilizing livestock manure to reduce dependence on fossil fuels and firewood for cooking around 28,557 biogas plants were installed, and

9,043 households utilized bio-slurry as fertilizer in 2023 (Marketplace, 2023). Thus, it can improve the quality of living conditions and help reduce the negative impacts of climate change.

The government of Indonesia is aggressively promoting decarbonization to meet the NEP target of generating 17 – 19% energy from renewable energy by 2025. Based on the 5-year analysis report, Indonesia's biogas market value is 149 megawatts (Research, 2024). The current Indonesian government is to increase the demand for renewable energy through efficient waste management and the application of biogas as a sustainable energy source, which focuses on waste-to-energy and the advancement of technology. Biogas feedstock sources are usually used in various types of organic waste such as biomass, animal manure, or kitchen waste (food waste), however, the biogas industry in Indonesia is driven by big-capacity agro-industries like tapioca and palm oil factories, and agricultural waste (Purnomo et al. 2023; Setiawan et al. 2020).

Furthermore, small-scale industries producing organic waste, notably tofu and tempeh manufacturing, offer considerable potential for biogas conversion. This dissertation investigates the revalorization of agro-industrial wastewater derived from Indonesia's micro, small, and medium enterprises (MSMEs) engaged in tofu and tempeh production.

1.1. The potential of tofu and tempeh wastewater in Indonesia

Tofu is a favorite food in Indonesia because it is rich in protein and affordable, with high per capita consumption. The average tofu consumption per capita was 0.158 grams per week in 2021, an increase of 3.27% from the previous year (2021), which was 0.153 kg per week (Hulu, 2023). Tofu consumption is growing rapidly worldwide, not just in Indonesia, driven by its popularity as a meat alternative in the food and beverage industry due to its nutritional benefits and wide availability (Maximize Market Research, 2025). In 2023, the tofu market size reached USD 1.5 billion. Projections indicate steady growth, with the market estimated to rise from USD 1.6 billion in 2024 to USD 2.7 billion by 2032, reflecting a CAGR of 9.01% from 2024 to 2032 (Singh, 2025). The high demand for tofu consumption has an impact on the tofu industry, not only to meet the demand but also on the waste produced and its management. Generally, almost all tofu production processes are carried out conventionally at the home industry level. There are 84,000 tofu industries in Indonesia, and they produce tofu wastewater is 1.024 mt/y (Faisal et al., 2016; Sumiyati et al., 2023).

However, from the processing production of tofu generates 40% solid waste is generated from the total capacity production, commonly in micro, small, and medium enterprises (MSMEs) of the tofu industry in Indonesia, solid waste can be used directly as food ingredients and animal feed (Hartini et al., 2023). That means tofu solid waste does not have a high potential for environmental pollution because it can be used directly, even economically profitable, in contrast to wastewater, which requires special attention in its management and utilization.

Tofu wastewater is formed from the production process step, including washing and rinsing, boiling, pressing, and molding (Maulana & Marsono, 2021). However, the production capacity and the method used in tofu process production will make the difference in tofu wastewater quality. the industry has a high capacity will generate a high amount of wastewater with low concentration, and the industry with low capacity will generate a low concentration and amount of wastewater (Agriculture, 2009). As a case, the daily activities of a small-scale tofu industry in an area of 50 m^2 produce ± 400 Kg of soybeans and consume water approximately ± 8000L (Gaol & Rizky Franchitika, 2024). Furthermore, based on the result of the study, producing 1 kg of soybeans into tofu consumes 25.2 L/kg of water and produces 14.5 L/kg of wastewater (Ningsih, Mazancová, et al., 2024).

The big issue of the tofu and tempeh industry in Indonesia is a lack of wastewater management due to most of the tofu industry is adjacent to the house residence, thus there is no space for a wastewater treatment plant (WWTP) in other words is a narrow place. There are several obstacles to why tofu wastewater cannot be processed optimally in Indonesia (Maharso et al. 2014; Rahmat et al. 2023), namely:

a. narrow space

b. lack of ability in financial and technology

c. lack of knowledge in waste management

d. lack of support from the government and stakeholders

e. lack of entrepreneurial awareness of the dangers of waste in the environment.

Tofu wastewater from washing and rinsing is sufficient with the standard for direct discharge, usually separated from highly concentrated wastewater such as the results of boiling, pressing, and printing. Thus, most washing and rinsing water can be directly discharged into the body of water as municipal sewage (Feng et al., 2024). Tofu wastewater from all steps of the process production is discharged directly without any treatment. Disposal of tofu wastewater directly into the environment causes pollution that is linked to public health. The air pollution (odor) caused by tofu wastewater is felt by the community who stay around the industry, especially in the dry season; thus, the community protests to the industry owner to consider how to treat the wastewater before discharged into the river (Wardani et al., 2020). Tofu industry wastewater is detrimental and can harm the surrounding environment because tofu wastewater is acidic and rich in organic matter; thus, in a short time, the environment that receives the wastewater will become dirty and smelly (Lasmini & Kurniawan, 2022). However, tofu wastewater still contains high nutrients such as COD, VFA, and TS. Moreover, tofu wastewater also contains essential compounds, namely lipid 0.04%, carbohydrate 0.21%, and protein 0.28% (Asiandu et al., 2023). Detailed information of tofu characteristics is provided in Table 1.

Table 1. Tofu and tempeh wastewater characteristics

Parameter	Unit	Tempeh [4]	Tofu	Quality standard [3]
COD	mg/L	24715	4,583.33 [2]	300
BOD_5	mg/L	11333.33	1,788.8 [1]	150
TSS	mg/L	601.67	444 [2]	100
pH	-	4.14	4.9 [1]	6 – 9
PO_4	mg/L	-	76 [1]	-
Total N	mg/L	-	181[1]	-

Source : [1](Hendrasarie et al. 2022), [2](Hardyanti et al., 2023), [3](Lampung, 2010). [4] (Nurhayati et al., 2024).

With great potential, it would be a shame if tofu wastewater were simply thrown away without being utilized. Currently, in Indonesia, many studies have begun to pay attention to the use of tofu wastewater, such as its use as a renewable energy source, liquid organic fertilizer, and food ingredient. This study focused on the revalorization of tofu wastewater through anaerobic digestion with simple technology to convert it into renewable energy using a simple technology approach.

Another wastewater used in this study is tempeh wastewater. Tempeh is a fermented soy product originating from Java Island, particularly Central Java, Indonesia. unlike other traditional soy-based foods, it is unique in that it did not emerge from China or Japan (Romulo & Surya, 2021). Tempeh is a protein-rich fermented soybean product, offers an affordable, nutritious, and sustainable food source that has achieved global popularity (Ahnan-Winarno et al., 2021). Like tofu, tempeh is a staple source of plant-based protein in Indonesia, widely consumed as part of the traditional diet. Based on Statistics Indonesia indicates that Indonesians consumed an average of 0.140 kg of tempeh per week per capita in 2022, reflecting its significance in daily nutrition (Cundari et al., 2023). According to Linear Regression, Indonesia's national soybean consumption in 2024 is forecasted to reach 1,846,288.000 kg. This demand is projected to generate approximately 153,586,868.34 m^3 of wastewater from tofu and tempeh production (85,021,562.400 L of tofu wastewater, and 68,565,305,936.84 L of tempeh wastewater) (Mujayyanah et al., 2025). Moreover, the tempeh global market has shown strong growth, expanding from $5.71 billion in 2024 to an estimated $6.15 billion in 2025 at a

7.8% CAGR. Projection indicates further robust growth, reaching $8.71 billion by 2029 at 9.1% CAGR, driven by the plant-based movement, global cuisine integration, health trends, product innovation, and retail expansion (The Business Research Company, 2024).

Generally, tempeh is similar to tofu in that both are commonly produced from soybeans at home-industry scale. The key difference lies in the production process: tempeh undergoes fungal fermentation, typically with *Rhizopus* molds, while tofu is produced through the coagulation of soy milk (Fransiscus & Simangunsong, 2021). The tempeh industry produces waste, both solid and wastewater, from the production process. A significant amount of water is used for soaking, boiling, washing, and peeling of soybeans, and fermentation (Pakpahan et al., 2021). However, most solid waste, like soybean hulls, is repurposed as animal feed, and wastewater remains an environmental concern. The tempeh production process generates a high amount of wastewater. Producing 1 kg of soybeans consumes 13.3 L of water and generates 12.2 L of wastewater (Pakpahan et al., 2021). On a large scale, processing 100 kg of soybeans results in approximately 2 m^3 of wastewater (Riadi et al., 2021). The wastewater from tempeh processing contains impurities that pollute the environment. The characteristic of tempeh wastewater is provided in Table 1. Similar to the tofu industry, the tempeh industry struggles with wastewater management, as untreated effluent is frequently discharged directly into water bodies. According to Destri et al. (2025), 81% of surveyed tempeh producers (18 out of 22) dispose of untreated wastewater directly into drainage systems, while only 15% (4 producers) route their wastewater to communal wastewater treatment plants (WWTP). The findings suggest that many tofu and tempeh producers remain unaware of proper wastewater management practices and fail to recognize the environmental and public health risks posed by untreated effluent. The tofu and tempeh industry holds significant potential for renewable energy generation, particularly through methane (CH_4) recovery from wastewater. With an estimated methane potential of 19,030,484,523, 225.50 m^3, this approach not only addresses a critical environmental challenge but also presents a substantial opportunity for energy recovery (Mujayyanah et al., 2025). Moreover, Industry tofu Asri can generate approximately 13.65 kWh per day from 7 m^3 of wastewater, equivalent to 410 kWh per month. Additionally, the tofu wastewater is utilized as an energy source for the tofu production process, particularly for cooking (Nurkholis et al., 2025).

The utilization of wastewater from tempeh and tofu agro-industry as a renewable energy source has great potential to address environmental and social issues. However, appropriate technology is required to achieve optimal and targeted results.

The utilization of agro-industrial waste carried out in this research can achieve the SDGs goals proposed by UNDP, namely SDGs 6 (ensure availability and sustainable management of water and sanitation for all), 7 (affordable and clean energy), 11 (sustainable cities and communities), 12 (responsible consumption and production), 13 (climate action) and the top goals from this study is can meet SDGs 17 (partnership and goals), to make the integration of energy system in utilizing wastewater into bioenergy. It is also, as a consideration for industry owners, local government, and stakeholders in the management and utilization of industrial wastewater, especially tofu and tempeh wastewater.

2 Objectives of the study

2.1 Main objective

Indonesia possesses significant potential for agro-industrial waste as a renewable energy source, yet technological challenges hinder its utilization. Converting this waste into energy aligns with Indonesia's National Energy Policy (NEP), supporting energy security, decarbonization by 2035, and net-zero emissions by 2060.

The primary objective of this study is to revalorize agro-industrial wastewater through anaerobic digestion (AD) as a sustainable energy source, employing simple, scalable technology to advance circular economy principles and environmental sustainability.

2.2 Specific objectives

- Chapter 3: To identify inefficiencies and propose strategies for transitioning toward a green and sustainable tofu industry.

- Chapter 4: To evaluate the efficacy of the biofilter integrated in ABR in increasing methane concentration in biogas and producing effluent suitable for use as liquid organic fertilizer.

- Chapter 5: To investigate the acclimatization process of microbial communities in tofu wastewater treatment and to optimize biogas production and stability using a biofilter equipped with ABR.

- Chapter 6: To analyze the impact of varying OLR concentration on biogas production efficiency and methane content.

- Chapter 7: To assess the effect of pretreatment methods on volatile fatty acid (VFA) yield during anaerobic digestion and batch-process efficiency for scalable biogas production.

2.3 Relevance Study

This study is aligned with the sustainable SDGs 7 (clean and affordable energy), SDGs 12 (Responsible consumption and production), and SDGs 13 (climate action), the Paris Agreement to limit the global temperature rise even further to 1.5 ^{0}C which the peak of GHG must before 2025 and at least 43% by 2060, and Indonesia's Government Regulation No. 79/2014 regarding the NEP expressed the ambition to carry out the transformation of energy from non-renewable energy to renewable energy by 2025 and 2050. By providing data from energy audit studies and utilization of agro-industrial wastewater with simple technology as a bioenergy feedstock, this study contributes to the academic literature and serves as a consideration in policy making in utilizing agro-industrial wastewater at the MSMEs in Indonesia. Insights from this study are expected to provide information regarding policy interventions aimed at promoting the Utilization of agro-industrial wastewater into bioenergy using simple technology that can be applied to MSMEs in Indonesia to achieve sustainability, clean energy, and a circular economy.

2.4 Structure of The Thesis

The thesis is structured into six main chapters. Chapter 3.

Provide the information energy audit in the tofu industry; an evaluation of energy consumption towards a green and sustainable industry. Chapter 4. examines the use of biofilter in the anaerobic baffled reactor to improve the quality of methane concentration and effluent as a liquid organic fertiliser. Chapter 5. Examine the acclimatisation process of biogas production from tofu industrial wastewater using a biofilter in the anaerobic baffled reactor (ABR). Chapter 6. Continuation of examination of the effect of organic loading rate (OLR) concentration in anaerobic baffled reactor (ABR) with biofilter to enhance the quality of biogas. Chapter 7. is the examination of the effect of pretreatment on VFA production from tofu and tempeh wastewater through an anaerobic digestion batch.

2.5 Methodological Approach

This dissertation uses primary data with a mixed method approachement combines both qualitative and quantitative methods used in each chapter, namely;

- Chapter 3 used primary data with Semi-structured interviews and questionnaire surveys conducted in 40 tofu industries in Gunung Sulah District, Bandar Lampung City, Lampung Province, Indonesia. The semi-structured interview and questionnaire approach is an interview in which the researcher collects open data (Dejonckheere & Vaughn, 2019).

- Chapter 4 is an examination of tofu wastewater through AD with a simple technology approachement using a biofilter, which impacts to the quality of biogas effluent as liquid organic fertilizer.
- Chapter 5 is the continuation of the examination using a simple technology approachement of a biofilter for the acclimatisation of the AD process from tofu wastewater, which will affect the quality and quantity of biogas as a startup for further study (Chapter 6).
- Chapter 6 is further studies on the use of biofilters in various OLRs to obtain the best production results and quality of biogas composition. Chapters 4 - 6 were conducted on a pilot scale with continuous loading.
- Chapter 7 is the novelty of using tofu and tempeh wastewater for VFA production through an AD batch, which is conducted on a laboratory scale.

3. Energy Audit in The Tofu Industry: An Evaluation of Energy Consumption Towards A Green and Sustainable Industry

Adopted from Ningsih, Lydia Mawar, Jana Mazancová, Udin Hasanudin, and Hynek Roubík. 2024. "Energy Audit in The Tofu Industry: An Evaluation of Energy Consumption Towards A Green and Sustainable Industry." *Journal of Environment, Development, and Sustainability*. https://doi.org/10.1007/s10668-024-05109-z.

Abstract

The tofu industry consumes a lot of energy and water for its production process, and then produces wastewater in large quantities that has a negative effect on the environment. It is because tofu craftsmen still use conventional technology in the production process, and there is no standard operational procedure (SOP) for the use of raw materials and energy sources, and there is also a lack of capability in waste management. This study aims to investigate the audit energy in the tofu industry, including evaluating the consumption energy in different energy sources, determining the production of wastewater in the tofu industry and determining the energy sources available in the tofu industry. The data collection method involved semi-structured interviews and questionnaire surveys carried out in 40 tofu industries in Gunung Sulah district, Bandar Lampung City, Lampung Province, Indonesia. The results showed that the highest consumption in process production in the tofu industry is for cooking, which depends on the type of energy sources, namely firewood 71.1 MJ/kg, LPG 16.9 MJ/kg, and wood pellets 6.0 MJ/kg. However, the consumption of water for the production of the process is 25.2 L/kg and it produces 14.5 L/kg wastewater. The tofu industry is still not efficient in consuming energy because it does not use wastewater to produce a bioenergy product that can be beneficial in economic and environmental aspects. To make energy consumption more efficient in the tofu industry, it is very useful to use wastewater to produce bioenergy material that can replace non-renewable energy as the main energy for process production. Based on the prediction that the potential for methane from tofu wastewater is approximately 0.056 m^3/kg soybeans, the use of tofu wastewater as biogas feedstock can replace 2.82% of firewood, 11.86% LPG, and 33.39% biopellets.

Keywords: Energy audit; tofu industry, sustainability.

3.1. Introduction

The tofu industry is one of the most numerous and dynamic processed food industries in Indonesia. It is because the tofu industry is the main source of income and employment for the local community. The demand for tofu is high about 3.5 million tons per year, this is because the Indonesians always consume tofu in their daily diet as a source of protein (Yuliarti, 2020). Generally, the tofu industry in Indonesia consists of micro, small, and medium-scale enterprises (MSMEs); the micro and small-scale industries are known as "home industry". The tofu industry in Indonesia is a type of heritage business that will be passed down from generation to generation, the number of tofu factories has reached 84,000 business units, including a large and small-scale industry spread across all regions (Putri et al., 2022).

Tofu industry that was observed in this study is located in Gunung Sulah District, Bandar Lampung City. The tofu industry is growing rapidly in Bandar Lampung City with a total of 238 tofu industries, most of them are in Gunung Sulah District with around 115 industries (Primkopti, 2016). Usually, tofu industries concentrated in one location make a cluster in the middle of residential areas. This is because tofu production is very easy with simple technology that can be produced on a home scale, therefore neighbours and relatives who live nearby also produce tofu in their homes. Therefore, the problem arises such as the lack of space to process the wastewater produced by tofu that has an impact on the environment, health, and other social aspects. Hence, the management of tofu industry is very important due to the relationship with the business actor that can give them the benefit from waste management and create a sustainable industry with high environmental quality (Simanjuntak et al., 2021).

The tofu industry faces several challenges, including lack of outreach from relevant stakeholders to obtain a home industry business permit certificate from the government. There are no standard operating procedures (SOP) in the production process, waste management methods, and energy consumption efficiency, so that the impact not only on environment but also on economy aspect (Lisanty et al., 2021). Tofu production consumes a lot of energy, particularly when grinding the soybeans and boiling the soybean porridge. Approximately 90% of micro and small-scale tofu industry uses firewood as fuel for all production processes. The use of non-renewable energy in industry will increase the exploitation of fossil fuels and have significant effects on climate change, such as environmental pollution, especially greenhouse gas (GHG) emissions. However, the use of non-renewable energy in the tofu production process will cause an increase in CO_2 emissions, which is a big issue that caused by industrial activities. Concerns about pollution caused by the burning of fossil fuels at the industry level are increasing globally, not only in developing countries, but also in developed countries (Thakur et al., 2020). Hence, in the middle of an energy transition, considering environmental aspects is a priority by developing research that is beneficial to the environment, especially emphasizing on greenhouse gas (GHG) pollutions linked to conventional energy sources (Adebayo & Ozkan, 2024; Adebayo et al., 2024).

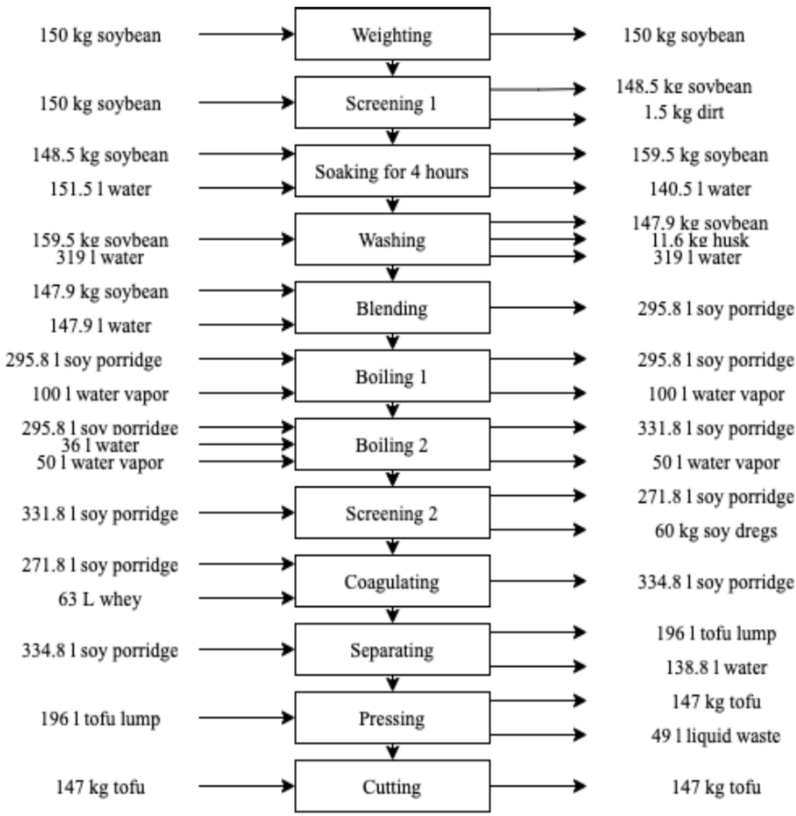

Fig. 1. Flow chart mass balance of the tofu production process (Septifani et al., 2021)

Pollution that occurs from the type of fuel used in industry is one of the roles related to policy makers. This issue arises when there is ambiguity regarding the scope of government organizations at various levels (local, national, and global) that will adopt and implement plan initiatives related to climate change (Liu, 2023). The use of non-renewable energy not only has an impact on the environment and the industrial economy but also has a huge impact on the national and global scale. Improving energy efficiency or using less energy to achieve the same level of production that can meet a variety of government priorities, from economic growth to the reduction of greenhouse gases in energy and food security (Dimitris, 2017).

Furthermore, the tofu industry also consumes a lot of water during the production process, thus producing large amounts of wastewater with high organic content (Fig. 1), which has a negative impact on body water (Aurora et al., 2021). The tofu industry produces two types of waste including; solid waste (tofu dregs) and wastewater. There is no negative impact of tofu dregs due to their ability to be used directly as additional material for human food and animal feed, co-substrate for biogas, and material to make paper (Annisa, 2014; Saputra & Purnomoadi, 2018). In fact, most of the tofu industries have a contract with third parties like industries or farmers to sell their tofu dregs (Lubis et al., 2022). The use of tofu dregs as additional ingredient food for humans and livestock is because it still has a high nutrition content and is rich in protein approximately 18–25%, fat 4.5%, and crude fibre 18.21% (Farabi et al., 2016).

According to the issues faced in the tofu industry, policy makers need a special strategy to handle these issues, including; (1) investment in renewable energy, (2) focus on the main issues of ecological degradation that arise from urbanization and economic expansion, (3) implement policies and strategies related to trade openness to improve environmental quality, (4) policy makers must encourage economic development by supporting a sustainable economic environment (Adebayo et al., 2023). The current situation about global warming linked to climate change has drawn the attention of CO_2 emissions stakeholders. There are some factors that limited attention in the field of energy and environmental literature related to environmental degradation such as the combined impact of uncertainties, climate policy, and economic policy uncertainties, including geopolitical (Adebayo, 2024).

One of the solutions to control the efficiency of energy consumption at the industry level to help the policy maker and the business actor is conducting an energy audit. Conducting an energy audit is one of the efforts to achieve net zero in the tofu industry, as stated in the UN Climate Change Conference in Glasgow (COP26) to accelerate action in controlling climate change and replace non-renewable energy such as fossil fuels (UN, 2023). Like the COP28 output held in Dubai, the United Arab Emirates with 150 heads of state and government were intensely discussed and negotiated to reach an agreement that has managed to reach an innovative agreement focused on phasing out fossil fuels, tripling the capacity of renewable energy, and increasing climate finance for the most vulnerable communities. These factors are to reach the main goals of the Paris Agreement that restrict the global average near-surface temperature increase to 1.5 ºC in preindustrial levels for the long term (UNFCC, 2024; Xu et al., 2024). It is aligned with the definition of EU energy audit standard (EN 16247–1) that energy auditing is a systematic inspection of energy analysis and energy consumption of building, site, system, or organization, the aim being to identify energy flows and potential energy efficiency improvements and report them (Thollander et al., 2020).

Energy audit is a method to estimate the amount of energy consumed in industrial activities, which has an impact on the efficiency of energy consumption. Energy audit as an approach to identify opportunities for energy conservation as the first step in energy management, specifically for industrial energy efficiency (Kaur & Thakur, 2014). Energy management and conversion are the most significant variables for energy consumption since they have a direct impact on the environment and economic issues (Qandil et al., 2021). According to the results of the LCA analysis in the tofu industry, the greatest contribution to the environment in the production of the tofu process is grinding and boiling, including acidification 27.92294 kg of SO_2, eutrophication 1.987027 kg of PO_4, global warming 4026.078 kg of CO_2, human toxicity 436.9892 kg of 1.4-DB eq and photochemical oxidation 0.085625 kg of C_2H_4 eq (Lolo et al., 2021). In addition to that, the energy audit in two different tofu industries (modern and traditional methods) shows that the energy consumption in the modern tofu industry that uses a steam boiler is 0.09 MJ/kg for human energy, 0.15 MJ/kg for electricity, and 0.61 MJ/kg for biomass (firewood). However, the energy consumption in the traditional tofu industry is lower than in the modern industry, namely 0.13 MJ/kg of human energy, 0.71 MJ/kg of biofuel, and 0.82 MJ/kg of biomass (rice husk) (Yanti et al., 2022). The results of energy in PT. Sandria et al. not only show the energy efficiency that is used for production per year, but can also save the cost of energy around 8740.79 USD per year (Wardhana & Damarwan, 2023).

The result of study that eco-innovation and renewable energy is negative on CO_2 across all quantiles and periods; however, there are some factors that have a positive impact on the contribution to CO_2, including: socio-economic condition, political risk, and financial risk (Adebayo & Ozkan, 2024). Therefore, the energy audit in the tofu industry as a method of achieving the

SDGs 7 (affordable and clean energy), 11 (sustainable cities and communities), and 12 (responsible consumption and production) (United Nations, 2024). The energy audit in this study is a toll to distinguish and examine energy administration programme, it could be useful for industry to save the fundamental energy cost and provides some advantages such as increasing profits, better quality, and the most essential fulfilment of leading towards contributing to the global energy saving (Sharma et al., 2021).

To complete the literature gap, this study aims to investigate audit energy in the tofu industry as preliminary data, to evaluate consumption energy in different energy sources, to determine the production of wastewater in the tofu industry, and to find the energy sources available in the tofu industry. The energy audit reveals how much energy is needed for industrial activities through the tofu production process, which allowed us to figure out how to reduce energy consumption and more effectively (Sharma et al., 2021). Thus, it can become a reference for industry to improve industrial activities toward sustainability for environmental protection, such as the development of environmental technology, because it restricts the discharge of waste into the environment. Another benefit of technological innovation is that it can improve environmental quality through improving the energy transition, enhancing the production of renewable energy that is expected to have A good impact on environmental health (Adebayo, 2023).

3.2 Materials and Methods

3.2.1. Target area

Energy audits were carried out in the micro- and small-scale tofu industries in Gunung Sulah District, Bandar Lampung City, Lampung Province, Indonesia. The report of the Central Bureau of Statistics on the classification of scale industry based on the number of laborers is divided into four scales: micro (1- 4 laborers), small (5 - 19 laborers), medium (20 - 99 laborers), and big (more than 100 laborers) (Central Bureau of Statistics, 2019). Gunung Sulah District is the centre of the micro- and small-scale agro-industry in Bandar Lampung city, focusing on the soybean food processing industry, such as tofu, tempeh, and oncom (traditional staple food of West Java).

3.2.2. Technic sampling

In this study, the tofu industry was selected as a sample using simple random sampling. In total sampling, the selected sample can represent the research area. For the homogeneous population, simple random sampling is used. The sampling procedure can be performed at random; the samples and locations were chosen at random to represent the population in the study area. The tofu industry is concentrated in four regions in Gunung Sulah District. To represent each region, ten tofu industries were chosen at random, for a total sample of 40 industries.

3.2.3. Data collection

Semi-structured interviews and questionnaire surveys were conducted in 40 tofu industries in Gunung Sulah District, Bandar Lampung City, Lampung Province, Indonesia. The semi-structured interviews and questionnaire approach is an interview in which the researcher collects open-ended data (Dejonckheere & Vaughn, 2019). This study focuses on the energy consumption for tofu production. Using a semi-structured interview and questionnaire is more relevant and flexible to the cases in this study because it allows the researcher to have new questions during the interview for the participant to explore more details about the information (Aung et al., 2021).

The following steps were taken for data collection: a) visiting each of the tofu industries, b) sample analysis of the caloric value of firewood, biopellets, and LPG in the laboratory (Laboratory of Agro-industrial Technology, Faculty of Agriculture, University of Lampung, Indonesia), c) data analysis. The consumption data were calculated using the following equation:

- Human energy consumption equation (Kasumov et al., 2017) :

$$He = JK \; x \; T \; x \; Hc \text{ ....(1)}$$

He = Human energy (MJ)
JK = Number of labors
T = the length of time worked (hours)
Hc = Human caloric value 0.53 MJ/hour

- Electricity consumption is calculated with the equation:

$$Ec = P \; x \; t \text{ ....(2)}$$

Ec = Electricity consumption (MJ)
P = Electrical power used (Watt)
t = Time used during the process (seconds)

There are two types of fuel oil consumed for soybean grinding machines in the tofu industry, namely gasoline and diesel.

- Gasoline consumption is calculated with the equation:

$$Gc = V \; x \; \rho \; x \; cvg \text{ ....(3)}$$

Gc = gasoline consumed (kJ)
V = volume gasoline used (m^3)

ρ = density of gasoline 0.745 Kg/l (Badarudin & Hardiansyah, 2015)
cvg = caloric value gasoline 43.999 (MJ/Kg) (Irzon, 2012).

- Diesel fuel consumption is calculated using the following equation:

$$Dc = V\ x\ \rho\ x\ cs \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(4)$$

Dc = diesel consumed (MJ)
V = volume of diesel used (liters)
ρ = density of diesel 0.832 kg/l (Badarudin & Hardiansyah, 2015)
cs = diesel caloric value 42.595 (MJ/Kg) (Yohana & Askhabulyamin, 2012)

Currently, three common fuels are used for cooking in the tofu industry namely: firewood, biopellets, and LPG. The energy consumption for cooking can be calculated with the following formula:

- Firewood consumption is calculated with the following equation:

$$Fec = Mb\ x\ cb \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(5)$$

Fec = Firewood energy consumption (MJ)
Mb = mass of firewood (kg)
cb = heat value of firewood 20.13 (MJ/kg)

- The consumption of biopellets is calculated using the equation:

$$Bc = Mb \ x \ cb \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(6)$$

Bc = biopellets fuel energy used (MJ)
Mb = mass of biopellets (kg)
cb = heat value of biopellets 17.187 (MJ/kg)

- The LPG fuel consumption is calculated with the following equation:

$$Gc = Mg \ x \ Cg \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(7)$$

Gc = LPG energy used (MJ)
Mg = Mass of LPG used (Kg)
Cg = caloric value of LPG = 47.081 MJ/Kg

Water consumption is the most important part in the tofu industry in the process of producing wastewater. The volume of water tanks used in the production process is measured manually to ensure the amount of water consumed. In addition, the mass balance is applied to determine the amount of wastewater produced. To calculate the energy from tofu wastewater is use the following equation:

- The amount of methane (CH_4) (Eleutheria et al., 2016) can be calculated with the equation :

$$M = P\ x\ Lw\ x\ 0.9\ x\ COD\ x\ (0.35\ /1000000)\ \ldots\ldots (8)$$

M = Methane production (m^3/day)
Lw = Wastewater production (L/kg soybeans)
P = Production capacity of tofu factory (kg soybeans/day)
0.9 = 90% COD removal
COD = COD concentration (mg/l)
0.35 = methane production potential (m^3/kg COD removal)
1000000 = Conversion factor

3.3. Results and Discussion

3.3.1. Energy consumption for cooking

In Indonesia, approximately 99.99% or 56.54 million MSMEs units play an important role in economic aspects due to their significant impact on the economy, society, and the environment (Indonesia, 2015). The energy sources used in industry and households in Indonesia still rely on non-renewable energy, especially fossil fuels (petroleum and coal) (Haryana, 2018). Currently, there are three types of fuels that are commonly used as the main energy for cooking in the tofu industry, including LPG, firewood, and biopellet. In Fig. 2. the highest energy consumption of fuel for cooking in the tofu industry is firewood 71.1 MJ/kg, followed by LPG 16.9 MJ/kg, and wood pellets 6.0 MJ/kg. Most of the tofu industries that was observed in this study (38 of 40 tofu industries) used firewood as their main energy resource for their production process, particularly for cooking.

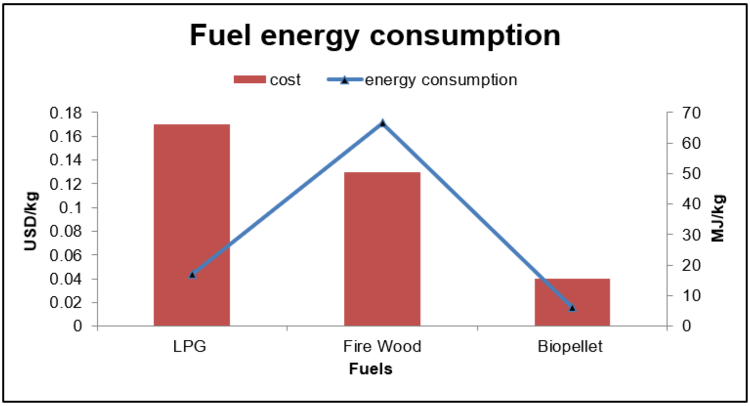

Fig.2 . Fuel consumption for cooking

Generally, the micro- and small-scale tofu industry uses firewood as the main energy source for its production process, with a low capacity of around 30–150 kg of soybeans/day. Compared to other fuels (LPG and biopellet). The use of firewood in the micro and small-scale food industry is quite common because of its long-lasting heat and high calorific value, so it is very usable for the production of tofu. Firewood types have high calorific value that are preferred as the main energy sources in the MSMES tofu industry, usually hardwoods such as rubber, acacia, mahogany, and fruit trees (Insusanty et al., 2016).

The type of firewood used in the tofu industry is old and unproductive rubber wood purchased from firewood sellers around the tofu industry (Rizwan & Miswar, 2012). The use of firewood is essential for rural communities and MSMEs in Indonesia to meet daily demands, not only for industries but also for households (Sylviani et al., 2013). Although the economy in the Southeast Asian region has increased rapidly, the consumption of firewood at the community level has also increased significantly (Dwiprabowo, 2010). The result of the observation in the field is that there is only one micro-scale tofu industry that uses LPG as the main energy source for their process production.

Based on Fig. 2. the consumption of LPG is less than that of firewood, but the cost consumption of LPG is highest among the energy resources (firewood and biopellet). Indonesia has several years of experience in energy transition, from firewood to kerosene and recently in LPG. One of the government's strategies to switch from firewood to LPG is to provide LPG subsidies for MSMEs. However, the owner of the micro- and small-scale tofu industry must meet certain criteria to receive the LPG 45 subsidised. Although LPG is subsidised by the government for MSMEs, in fact, the price is very expensive, so the owners of the tofu industry prefer to use firewood. Another reason why the owner of tofu industries is not interested in using LPG is because of the lack of knowledge of how to use it safely, as there is no training to fulfill the requirements that must be met to use the LPG. Therefore, most of the owners industry consider firewood to be more practical to use and economically profitable for producers; thus, it is still difficult to change the habits in energy consumption (Purnomo, 2022). In addition to that, the competition between industries to get LPG is high, and the leakage of LPG cylinders results in explosions and huge losses for consumers, causing people to be reluctant to switch from firewood to LPG. The explosion of the gas stove caused by a leak in the LPG cylinder and increased the temperature of the gas stove frame, requiring the installation of technology that includes an early warning system and a mitigation mechanism (Kurniawan & Tjahjadi, 2016).

The results from laboratory analysis show that the calorific values of firewood, biopellets, and LPG, respectively, are 20.1, 17.2, and 47.1 MJ/kg. A good quality of fuel has a high calorific value; therefore, the higher the calorific value, the better the quality of the fuel (Gioda et al., 2019). According to Fig. 2. the consumption of fuel used in the tofu industry is very different, because the habits in fuel consumption and the types of fireplaces used are closely related to the energy consumption efficiency. Furthermore, some factors impact energy consumption, such as the production capacity and the length of the production process, and there is no SOP in the tofu industry for its production activities, especially in the use of fuel for the process of cooking soybean porridge.

The common issue of firewood fireplaces is incomplete combustion of firewood in a simple furnace since there is no separation between fuel and ash (Hananto & Fahriannur, 2018). In the case in the rice industry, the lack of awareness of workers is a common event in the industry during processing production, causing energy losses or increasing energy consumption by approximately 0.00016 MJ/kg of rice (Hasrizal & Diswandi Nurba, 2019). Biopellet is renewable energy produced from biomass or agricultural residues. However, to use biopellets requires a large investment and a large space to build a safe boiler, this is why home industries cannot switch from firewood to biopellets. However, biopellets have a high calorific value and a high bound carbon content that makes them more beneficial for industries that use biopellets as energy resources (Prasetyo et al., 2022). The small-scale tofu industry observed in this study requires 350 kg of biopellets per day with a production capacity of approximately 1 ton per day. To meet consumer demand, they are using biopellets as the main energy source and the appropriate type of boiling stove to optimise the efficiency of the soybean cooking process. During the burning process of biopellets, soot and ash are produced, but the quantities are very small compared to firewood. The use of biopellets is very beneficial because it is more efficient, environmentally friendly, and economically friendly, and can save up to 25–41% of the fuel cost (Ari Supriyanti Rikin, 2019). There are several reasons why tofu industry owners still use firewood: (1) Because they do not know about other fuel options, tofu industry owners only know three types of fuel: firewood, fuel oil (gasoline, diesel and kerosene), and biopellets. (2) Preserving the unique taste of tofu that cooked with the firewood (tofu has a distinct flavour when cooked to other fuels, such as bio-pellet or biogas, they must modify the fireplace for cooking to the new design, which required a lot of money, labour, and space (Tambunan & Studi, 2013).

There are many types of fireplaces used in the tofu industry for process production; the common fireplace used is a single furnace and steam boiler. The type of fireplace used is related to the energy consumption; the highest energy consumption in the tofu industry is the operation of a steam boiler of 19.005 MJ/kg, and the lowest is 6.026 MJ/kg for a single furnace (Markumningsih & Purwantana, 2013). The energy consumption is linked to the production cost consumption. Based on Fig. 2. the highest cost energy consumption per kg of production capacity is 0.17 USD for LPG, then 0.13 USD for firewood, and 0.04 USD for biopellets. Several factors impact the use of fuel and energy consumption cost, including financial issues related to consumer purchasing power, and the limited of knowledge of tofu craftsmen in waste management that can be beneficial to the industry in saving energy cost (Dwiprabowo, 2010). The cost of energy consumption in the small-scale industry is around 42.4% of operating costs. However, the percentage of costs is not dominantly high for all production activities but remains a major consideration for industry owners when choosing the types of fuel used (Tambunan & Studi, 2013). Furthermore, the small-scale tofu industry still does not use biopellets in processing production because the target market for biopellets is not intended for small industries. Commonly, biopellets are used by medium- and large-scale industries with high-capacity production. The main market for biopellets is the international market (export activity), such as Asian countries (Japan, South Korea, China, etc.), the European Union (EU), the UAE, and the United States, because the consumers of these countries is much higher than the domestic consumer; hence the potential for the trade of biopellets is enormous (Sidabutar, 2018).

3.3.2 Energy consumption for grinding

The use of fuel oil depends on the type of soybean grinding machine used in the tofu industry. In Indonesia, most of the micro- and small-scale of tofu industries use two types of fuel oil: diesel and gasoline, diesel fuel known as "solar" (Martin et al., 2020). However, in medium- and large-scale tofu industries, they use dynamo machines that consume electricity. In Fig. 3, the total energy of the fuel oil consumption of the grinding machines for diesel and gasoline, respectively, is 3.3 MJ/kg and 1.9 MJ/kg; the fuel consumption is directly proportional to the energy produced. If the consumption of fuel is low, the energy produced will also be low, and vice versa (Sartono, 2016). On the basis of the observation in the tofu industry, the consumption of diesel fuel is higher than that of gasoline because the grinding machines are old and require maintenance; this makes the grinding machines difficult to operate and consumes a lot of fuel during operation. In Indonesia, the grinding machines that consume diesel fuel are limited because the price of diesel fuel is very expensive and in short supply.

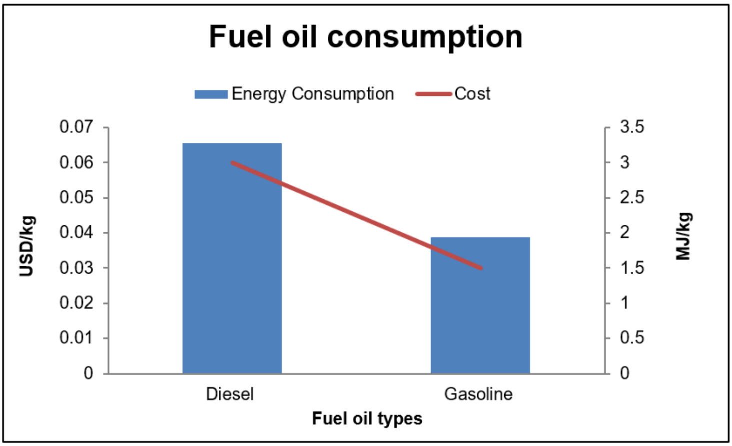

Fig.3. Total fuel oil consumption

The results of interviews with industry owners and tofu craftsmen revealed that there is no SOP in using the grinding machine; they only followed instincts based on their experience. Furthermore, the owner rarely performs maintenance on the grinding machine; thus, it will be very costly to repair and maintain the machine. There are some factors that influence the consumption of fuel oil for the grinding machine, namely: production capacity, the age of the machine, the duration of machine operation, and maintenance (Isbandi, 2021).

Several factors cause downtime or decreased machine performance, such as; (1) no kanban card causes 20%-time loss; it is because the function of the kanban card is to provide information about the strict stock and to start the operation at the right time. (2) 18% of the time lost by diamond grinding (sharpening grinding wheels for grinding rolls) is related to machine performance. (3) there is no operator or skilled worker who causes a loss of around 17% (Kardas, 2017). There are commonly used methods to maintain the quality of engine performance, like having a proactive maintenance schedule, and thus, the engine performance remains good when operating. Not only basic maintenance to prevent corrosion, but also required daily maintenance such as checking all parts of the machine before and after operation due to the machine maintenance linked to the production activities, and minimise productivity disruptions to produce a good quality product (Singh et al., 2022). In general, there are two types of maintenance for grinding machines in the tofu industry, including corrective and preventive maintenance. Most of the micro- and small-scale tofu industry only performs corrective maintenance due to financial issues and the lack of knowledge about machine maintenance and how to use it properly for the workers in the industry. In fact, corrective maintenance is more costly than preventive maintenance due to the repair and replacement of damaged machine parts, so preventive maintenance is highly recommended to keep the grinding machine in good condition for a longer time (Pau Asngadi, 2021). The operators machine in industry need to know how to operate the machine with sufficient experience, because they always work with the machine for a long time during the production process. To support existing resources in the industry, especially machine operators, the industry is obliged to provide machine operation training and its maintenance to improve worker performance to maintain the quality of the product.

Currently, the Indonesian government is in a hurry to replace all diesel engines with new biodiesel engines, including vehicles that use palm oil biodiesel (Martin et al., 2020). The consumption of fuel oil for the soybean grinding machine is related to the cost of fuel consumption. In Fig. 3 shows that the total cost consumption of diesel fuel and gasoline is 0.06 USD/kg and 0.03 USD/kg with the consumption of diesel fuel and gasoline per day, respectively, being 2.5 L/day and 1.52

L/day. Because the grinding machines that use diesel fuel are usually old machines that require maintenance, this has an impact on the cost of fuel consumption and is wasteful in the use of diesel fuel. However, the grinding machine that uses gasoline is a new machine, the price of gasoline is cheaper than diesel fuel, as well as the consumption of gasoline is lower than diesel fuel. Although using an old machine is difficult, tofu craftsmen have another way to grind soybeans, they will ask the nearest tofu industry to grind the soybeans, then pay for the grinding service. Payment for soybean grinding services can be in cash or by purchasing fuel oil that has been used to grind soybeans.

The grinding stage is a very important process in the production of tofu. if this process fails, it will affect the further process because the quality of the tofu is not good in the moulding process, then the industry will suffer losses. Good quality tofu is produced from a perfect grinding process, resulting in a very soft soybean porridge due to the fact that it contains high protein (Wulandari, 2012). Old machines are used with a special treatment to maintain the quality of soybeans as the main ingredient in tofu production. The soybean grinding machine will rest for about 5 to 15 min after grinding, waiting for the machine to cool down so that the quality of the soybean porridge remains good. The working duration of the machine for the production capacity of 30 kg/day is 1.5 h, and the number of breaks during the grinding process is four times. Furthermore, to maintain the quality of soybean porridge, it is necessary to add water during the grinding process. The more water used, the faster the grinding process and the more efficient the energy consumption (Widiyarta et al., 2016). The amount of water consumed during the production process is quite high for grinding soybeans and boiling soybean porridge. The purpose of adding water is to speed up the grinding process and soften the ground soybeans to produce good soybean porridge. So, the addition of water in the boiling process is to obtain as much soluble protein as that contained in soybean porridge as a component of tofu (Mulyani & Hartono, 2013).

3.3.3. Electricity consumption

Electricity consumption in the tofu industry has two functions, including utilities and grinding. The energy consumption for the utilities is not as high as for grinding, because the tofu production process starts early in the morning until noon (5 a.m.–15 p.m.), so the use of electrical energy for lighting does not consume a lot of energy. The source of electrical energy in the micro and small-scale tofu industry is from the State Electricity Company, known as 'PLN', which is used based on each industry capacity (Biantoro & Permana, 2017). In Fig. 4. it is shown that the total electricity consumed for utilities is 0.019 MJ/kg and for grinding is 0.03 MJ/kg. Generally, small, medium, and large-scale of tofu industries use a dynamo machine to grind the soybeans, which uses electricity as the main source of energy. Like grinding machines that use fuel oil as their energy source, grinding machines with a dynamo are also related to the production capacity and the length of work. In the small-scale tofu industry, the production capacity is 1 ton per day, to meet consumer demand, the working duration of the dynamo machine is 12 h continuously non-stop, the duration of duration for the production process is 24 h (with a two-shift system), the electricity consumption for grinding is higher than utilities because the working duration of dynamo machines is longer compared to utilities such as lightning, water pumps, and blowers.

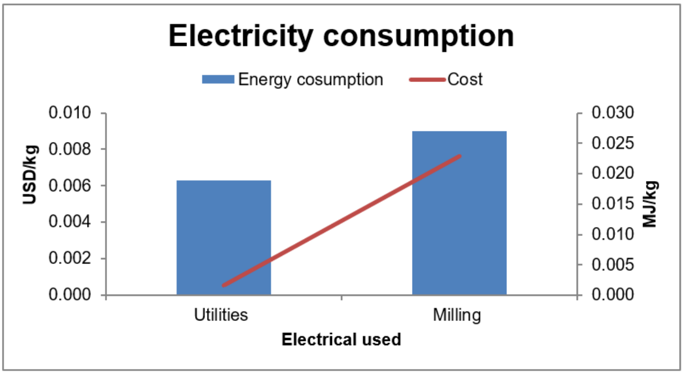

Fig. 4. Electricity consumption

The consumption of electricity for utilities and grinding has an impact on the cost of consumption. According to Fig. 4, the cost of consumption for utilities is 0.001

USD/kg and for grinding is 0.0076 USD/kg. The use of electricity for tofu production in micro and small-scale industries is combined with household electricity, so the electricity bill for industry is combined with the household electricity bill (Aulia, 2015).

In this study, data collection focused on electricity consumption for utilities in tofu production processes such as water pumps, lighting, blowers, and grinding. The highest electricity consumption for utilities in the micro-scale tofu industry is the water pump to fill the water tank, which is used for the tofu production process. In addition, the consumption of electricity in the production process is for lighting and a blower; the blower is very important to burn firewood during the production process, thus the wood always burns. The working times of the water pumps and blowers are different in each tofu industry because each industry has different methods of water consumption and production capacities. According to the LCA analysis, the main problem of energy consumption for tofu processing production is boiling soybean porridge using firewood, and the use of electricity in water pumps and soybean grinding machines (Lolo et al., 2021). The use of electricity for processing production in the tofu industry required a solution to increase efficiency in energy consumption, especially the use of electricity in the management of waste into a bioenergy product to replace non-renewable energy. Utilisation of tofu waste is a solution to energy efficiency in the tofu industry and optimal waste management.

3.3.4. Water consumption

Water consumption is an important aspect that influences industrial sustainability. To make the activities of the tofu industry more sustainable and environmentally friendly, water consumption management is required (Lubis, 2021). In Fig. 5. It is shown that the water consumption in tofu industry is different in each industry due to the production capacity and the production process methods are not the same. From the results of calculations and predictions, water consumption in the micro- and small-scale tofu industries is 25.2 L/kg of soybeans, and the wastewater produced from the production process is 14.5 L/kg of soybeans, which is approximately 53.4% of the water consumed. From the production of the tofu process, around 15–20 L/kg of wastewater will be produced with a high nutritional content (Sadzali, 2010). Because tofu wastewater still has a high nutritional content and acetic acid, tofu craftsmen usually keep some of the wastewater in a container as an acid solution resource for coagulation in the next day's production. The use of wastewater as an acid solution for coagulation will be mixed with CH_3COOH (acetate acid) or $CaSO_4$ (potassium sulphate), the addition of these acids causing different characteristics of wastewater (Yudhistira et al., 2018). Unfortunately, there is no SOP for the use of water in small and medium-scale tofu industries; hence, tofu craftsmen consume unlimited water and rely only on their instinct without a specific amount.

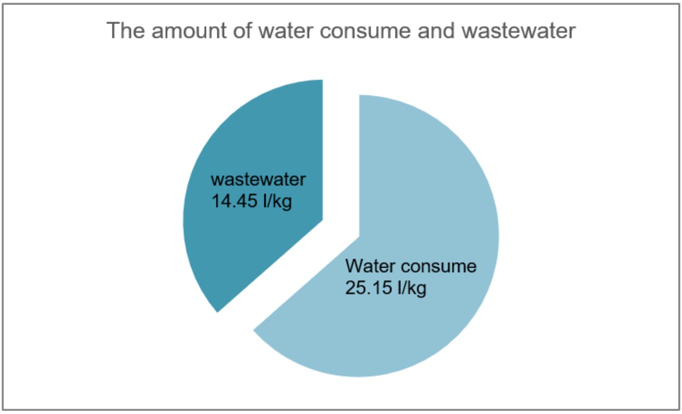

Fig. 5. Water consumption for processing production in the tofu industry

According to Table 1, the characteristic tofu wastewater in the Gunung Sulah district has a high COD content and a low pH value of 5.17. Tofu wastewater characteristics do not meet the Regulation of the Governor of Lampung Province 2006, so wastewater must be treated first before being discharged into water bodies or used as renewable energy, such as biogas. The utilisation of wastewater in biogas is a good solution not only from an environmental point of view, but also from an economic and social point of view to achieve net zero to become a sustainable green industry. The prediction of tofu wastewater from 10 tofu industries with production capacities of 150–500 kg/ day is around 45,900 kg/day with a COD content ranging from 5000–8500 mg/L, which has the potential to produce approximately 128.52 m^3/ day of methane gas (Faisal et al., 2014).

Table 1. Tofu wastewater characteristics

Parameter	Unit	Lab. Analysis	Government regulation (Lampung, 2010)
COD	mg/L	12400	300
BOD_5 *	Mg/L	8852	150
SCOD	mg/L	7150	-
TS	mg/L	3800	-
TSS	mg/L	1188.3	100
pH	-	5.17	6-9
Alkalinity	mg/L	280	-
VFA	mg/L	1500	-

Source ; * (Novan Bagas Sayoga, 2014)

The use of tofu wastewater as biogas feedstock is very profitable for the industry and farmers because the main product of AD is biogas that can be used directly for the tofu industry as the main energy for their process production, by-product is digested which also has economic value as an organic liquid fertiliser that farmers can use to apply to their plantations in field (Budiyono & Syaichurrozi, 2020). Waste management in the tofu industry is very important due to the relationship in the issue of sustainable urbanisation that is essential for emerging nations. The tofu industry is one of the most significant contributors to greenhouse gas pollution, which confirms the theory of the ’life effect’ of urbanisation as an inevitable consequence of urbanisation (Adebayo et al., 2023).

3.3.5. Human energy consumption

Human energy consumption is related to the production capacity and the number of workers. Generally, the micro-scale tofu industry in Indonesia does not have labour because the production capacity is too low. Based on production capacity, there are 3 types of micro- and small-scale tofu industries, namely; (1) low production capacity (20–50 kg soybean/day) with 1–2 labourers, (2) medium production capacity (> 50–75 kg soybean/day) with 2–3 labourers, and (3) high production capacity (100–150 kg soybean/day) with 3 – 4 labourers (Nurhayati, 2012). Therefore, based on its scale, industry is divided into three types including small-scale (1–19 employees), medium-scale (20–99 employees), and large-scale (big industry) (more than 100 employees) (Nurhayati, 2012).

The audit of human energy consumption in industry is linked to physiological factors of the worker as the key in determining the size of physical work for heavy or light workloads. The workload category in the tofu industry consists of medium and heavy workloads, the medium workload is for the grinding section, and the heavy workload is for the boiling and moulding section. Human energy consumption in tofu industry consists of the washing and soaking process, milling, boiling, filtering, clumping and acidification, and moulding (Fathimahhayati et al., 2019; Yanti et al., 2022). The detailed information on human energy consumption in the MSMEs tofu industry is provided in Table 2.

Table 2. Human energy consumption in MSMEs tofu industry.

Types of human energy consumption	Amount	Reference
Milling	2.59 kcal/minute	(Fathimahhayati et al., 2019)
Boiling	8.54 kcal/minute	
Filter section	7.06 kcal/minute	
Washing and soaking	44.208 Kj/kg	(Trilaksono, 2022)
Milling	20.945 Kj/kg	
Boiling/cooking	57.6 Kj/kg	
Filtering	11.52 Kj/kg	
Clumping and acidification	11.304 Kj/kg	
Molding		
Cutting	14.76 Kj/kg	
Total of human energy consumption	7.253 Kj/kg 167.59 Kj/kg	
Human energy consumption in tofu	0.13 MJ/kg	(Yanti. et al., 2022)

industry, Sampang Village (conventional method)	
Human energy consumption in the tofu industry, Brani Village (modern method using a steam boiler)	0.09 MJ/kg

Based on the information provided in Table 2. human energy consumption in each industry is different due to the methods, including the section of process production and capacity production. In Fig. 6, it is shown that the average human energy consumption in the tofu industry is 0.09 MJ/kg with two labourers in each industry. The highest human energy consumption is in industry 38, it is because of the imbalance between production capacity and the number of workers. Industry 38 has 5 labourers with a production capacity of 75 kg/day, which means that the consumption of human energy is higher than necessary. Low production capacity means low human energy consumption; conversely, high production capacity in large-scale industry means the consumption of human energy is also high (Soleh et al., 2016). Human energy is linked not only to labour, but also to wages as one of the main problems for MSMEs in the tofu industry (Soleh et al., 2016).

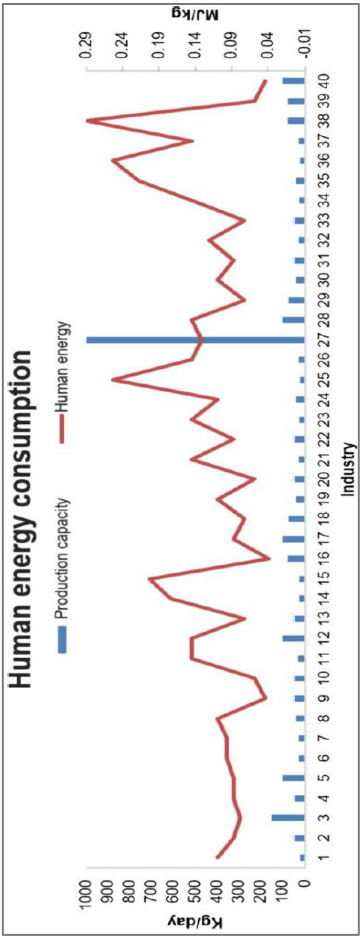

Fig.6. Human energy in the tofu industries.

Based on the results of interviews with various tofu artisans and the owner of the tofu industry, there are some issues related to labour and wage: (1) the owner cannot afford the labourer's compensation, (2) the price of fuel is expensive due to the limited availability of firewood, which has created competition in the industry, (3) People's purchasing power is low due to the pandemic situation and several activities such as schools, universities and offices are not fully active (work from home) which has an impact on low industrial income, (4) The price of soybeans as the main material is high due to imports from the United States (US), so the price will change depending on the situation and is very fluctuating, (5) human energy consumption serves to control the gap between environmental and economic accounting.

Generally, the analysis of environmental aspects ignores the aspect of labour (human energy consumption). Even though from the economic perspective that labour cost is costly, the audit of human energy consumption is useful to reduce the disparities between environmental and economic aspects. Thus, it is easy to take a wise policy on which parts that prioritize for these aspects (Zhang & Dornfeld, 2007). In the economic aspect, the goals of audits of human energy consumption are to optimise labour productivity based on skills and knowledge in the field to obtain efficiency that relates to the ratio of production to labour absorption according to industry or production per capita. Therefore, it can be inferred that wages depend on the skill and education levels of labour, the increase in wages and salaries has a positive effect on labour productivity (Kebede & Heshmati, 2020). This situation reveals that the employee in the countries of Mexico, Indonesia, Nigeria, and Turkey (MINT) comes from the rural to urban region to get better employment, lifestyle, healthcare and education that put some strain on the resources of the city and environment (Adebayo et al., 2023).

The types of workers in the agro-industry are typically sort workers, including casual and temporary employees, depending on how much the industry can afford to pay them based on the agreement between the two parties. Generally (International Labour Organization, 2007). The labour in the MSMEs tofu industry is a typical part-time worker who has other jobs outside of production activities in the tofu industry, such as construction laborers, online taxi drivers, seasonal factory workers (sugar cane factories, pineapple can factories, etc.), they will continue to work in their respective fields after finishing working in the tofu industry. There are two types of work carried out by laborers in the tofu industry, namely: (1) men labouring in tofu production processes that focus on washing, grinding, and moulding. (2) women workers usually work on tofu wrapping, frying, selling tofu in traditional markets, and managing sales funds. Generally, the women workers who helped in the tofu industry are the wives or daughters of the industry owner. They sell tofu in the traditional market from the morning until the afternoon, and they also manage the finances for the industry and profit. According to the division of labour, the energy consumption of the men workers is focused on processing, while the energy consumption of the women workers is focused on product sales and financial management (Soleh et al., 2016). Because most of the tofu industries in Indonesia are on a home industry scale, usually the owner works alone or assisted by their wives or relatives, thus the division of labour tasks is not obligated like in the medium and large-scale industry, so it will be more economically efficient (Rosita et al., 2019). However, when the owner of the industry gets sick and customer demand increases, they will hire freelance workers to help them in the production process activities, then the owner of the industry lets the workers adjust their preferred allocation time that suits them better (Golden, 2012).

3.3.6. Total energy consumption

The tofu industry consumes a lot of energy during the production process. The energy sources used in the production process of the tofu are mainly biomass (firewood) and fuel oil (gasoline and diesel). One thing that is closely related to energy consumption in tofu industry is production capacity as a standard to determine the amount of energy consumed in an industry. Each stage of the tofu production process requires energy, consisting of washing and soaking, cooking, filtering, clumping and wrapping. (Wahyuni, 2006). The total energy consumption in the tofu industry consists of human energy, energy consumption for cooking and grinding, and electrical consumption (for utilities and grinding).

In Fig. 7, it is shown that the average total of energy consumption is 70.68 MJ/kg with a cost energy consumption of 0.15 USD/kg. The lowest energy and cost consumption based on Fig. 7 is in industry 27, namely 6.1 MJ/kg with 0.041 USD. Industry 27 is a small-scale industry that uses the dynamo machine to grind soybeans and biopellets as the main energy sources for cooking, so that energy consumption is low and cheaper than other fuels which used in tofu industry, such as firewood, LPG, and fuel oil (diesel and gasoline for grinding machines). The production capacity in industry 27 is high around 1 ton per day, the duration of process production activities is 24 h; they have a shift system for workers that makes human energy consumption not so high. According to the audit, the energy and the calculation of the water consumption for 1 kg of soybean is 70.68 MJ/kg and 25.25 L/kg of water. Grinding and cooking are the steps that require a lot of energy, in this stage the fuel used is mainly firewood and human energy. From preparation to extinguishing firewood, a long process is required, especially if the wood is in wet condition (usually in the rainy season) and produces GHG in high amounts. The owner of the industry only knows the price of firewood per day that is consumed, without knowing how many sticks of firewood are used,

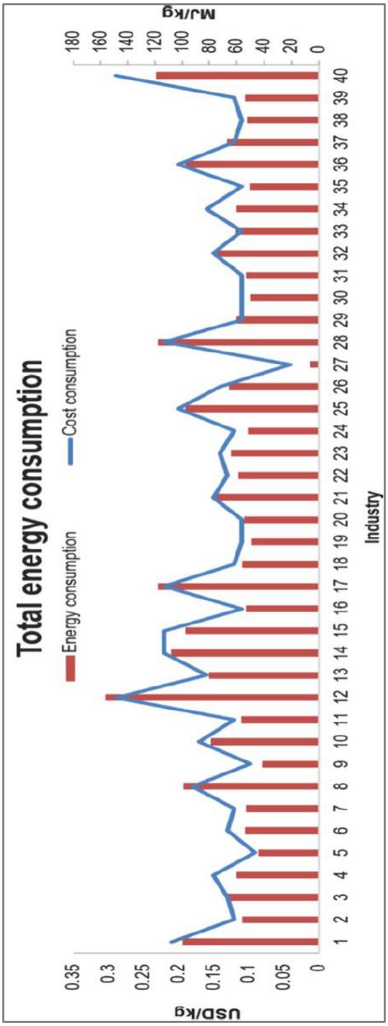

Fig. 7. Total energy consumption in the tofu industry

which is why the energy consumption in the tofu industry is high and unpredictable. Usually, the remaining wood charcoal from the tofu production process is excessive, and it is then used to meet the household needs of the owner's industry. However, the energy consumption in the tofu industry is high; nevertheless, it is still more efficient in life cycle assessment than other food industries that have protein sources, such as chicken and beef (Sahirman, 2014). Based on the LCA calculations, the resource input required to produce and package tofu, for 1 kg of packaged tofu, is 16% of CO_2e (1699.52 g/kg of tofu CO_2e) from soybean production, 52% from the tofu production process, 23% from packaging, and 9% from transportation (Mejia et al., 2017). The result of the calculation using equation no. 8 is that the utilisation of tofu wastewater into biogas will decrease the emission of methane by approximately 0.056 m^3/kg soybeans, or equivalent to approximately 1.12 kg CO_2e/kg soybeans. Therefore, with the production capacity of the tofu and tempeh industry in Lampung Province in 2022 at 54,000 tons (Fakhruddin, 2022), the use of wastewater in biogas production will reduce CO2e emissions by approximately 60,480 tons. Therefore, the national production capacity for the tofu and tempeh industry in 2022 is 400,000 tons 88, and the reduction in carbon emissions is around 448,000 tons of CO_2e.

The use of tofu wastewater in biogas is very useful not only to replace current non-renewable energy sources used for processing production but also to reduce the emission of GHG. To make energy consumption in the tofu industry more efficient, wastewater can be used as a renewable energy material, such as biogas feedstock, which will be more environmentally and economically friendly. The main energy sources for cooking (firewood, LPG, and biopellets) and electricity for utilities can be replaced with biogas so that the tofu industry can be more sustainable and become a green industry.

3.4. Conclusion and Recommendation

3.4.1. Conclusion

The energy consumption in the tofu industry is not efficient as there is no SOP in its production process, and most of them still use non-renewable energy sources. The total energy consumption in the tofu industry for the production process is 70.68 MJ/kg, with an energy consumption cost of 0.15 USD/kg that consists of human energy, energy for cooking and grinding, and electricity. The water consumption in the production process is 25.25 L/kg of soybeans and produces 14.45 L/kg of wastewater. From 14.45 L/kg of tofu wastewater, it will produce 0.056 m^3/kg of methane, which has a great potential to use as biogas feedstock.

3.4.2. Recommendation

To improve the quality of industrial activities to become sustainable and efficient in energy consumption, the use of wastewater into biogas is highly recommended. The utilization of tofu wastewater into biogas can substitute the non-renewable energy of approximately 2.82% of firewood, 11.86% LPG, and 33.39% biopellets. It is very beneficial in economic and environmental aspects.

Author contributions

LMN: Writing–Original draft, investigation, formal analysis, visualization, writing– review and editing; JM, UH: Writing–review and editing, validation; HR: Conceptualization, validation, writing–review and editing, funding acquisition, supervision.

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Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest: The authors declare no conflict of interest.

Ethics approval

Not applicable.

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4. The Use of Biofilter in Anaerobic Baffled Reactor to Improve Quality of Methane Concentration and Effluent as Liquid Organic Fertiliser

Adapted from: Ningsih, Lydia Mawar, Udin Hasanudin, and Hynek Roubík. 2024. "The Use of Biofilter in Anaerobic Baffled Reactor to Improve Quality of Methane Concentration and Effluent as Liquid Organic Fertiliser." *Journal of Ecological Engineering* 25 (9): 226–34. https://doi.org/10.12911/22998993/191261.

Abstract

The biofilter used is a simple technology in anaerobic digestion to remove pollutants from the substrate to enhance biogas production and nutrient effluent, which can be used as liquid organic fertiliser. This study aims to determine the effect of using a biofilter to improve biogas production and biogas effluent as an organic fertiliser material. The results show that the highest methane concentration is 60.64% at a dosage of 200 L·day^{-1}. The total solid (TS) content of biogas effluent exhibits a decrease of approximately 44% across all substrate doses, with respective percentages of TS of 0.16%, 0.03%, 0.025%, and 0.034% for 50 L·day^{-1}, 100 L·day^{-1}, 150 L·day^{-1}, and 200 L·day^{-1}, respectively. The use of

biofilters in an ABR can significantly enhance the quality of biogas effluent, rendering it suitable for use as a liquid organic fertiliser. By capturing and biodegrading pollutants, the biofilter component can further enrich the nutrient content of the effluent, which already contains essential nutrients due to the anaerobic conditions and compartmentalised design of the ABR. The nutrient content in the biogas effluent mix with nutrition (AB mix) namely; N-total 262.5 $mg \cdot L^{-1}$, P-available 0.399 $mg \cdot L^{-1}$, Ca 4.08 $mg \cdot L^{-1}$, Mg 25.24 $mg \cdot L^{-1}$, Cu 0.032 $mg \cdot L^{-1}$, and Fe 13.09 $mg \cdot L^{-1}$ follows the standard organic fertiliser of the Minister of Agriculture of Indonesia.

Keywords: tofu industry; wastewater; renewable energy; anaerobic digestion; organic waste; fertiliser; nutrient content.

4.1 Introduction

Biogas is a renewable energy produced by anaerobic digestion and is considered one of the low-carbon fuel sources to meet the demand for energy in any way (Sawyerr et al., 2019). Many methods are used to obtain high-quality biogas production, such as a biofilter, to effectively in- increase the quality of methane and remove pollutants to improve the quality of biogas effluent (Dumont, 2015). The removal of substrate pollutants not only improves biogas production but also biogas effluent because it can decrease the smell, including pollutant gases such as H_2S, CO_2, nitrogen, and heavy metal content (Hooton et al., 2019; Mielcarek et al., 2021). One of the suitable technologies that can face these challenges is the biofilter because it effectively removes volatile organic compounds and heavy metals in the substrate (Pachaiappan et al., 2022). Biofilter and biotrickling have the same principle: a gas stream is passed through a solid layer, then pollutant-degrading organisms are immobilised as a biofilm. H_2S is absorbed into sulphur (S) by the metabolic activity of microorganisms in biofilms that depend on the available oxygen (Garcia-Peña et al., 2012).

The combination of biofilter and activated carbon effectively removes 98.13% hexamethylcyclotrisiloxane, 96.61% Octamethylcyclotetrasiloxane, and 78.85% Decamethylcyclopentasiloxane (Yang and Corsolini, 2019). The use of a biofilter on a large scale with a mixed culture with specific bacteria strains in anaerobic digestion successfully removes 99% of 1,058 ppmv of H_2S (Kang et al., 2010). Furthermore, a biofilter in an anaerobic digester in semi-continuous mode achieves the removal of COD 95% and sCOD 81.73%, with a methane concentration of 55.089% (Kang et al., 2010; Mawaddah et al., 2019). Biogas produces a by-product known as digestate for solids or effluent for liquids; the effluent can be utilised as an organic fertiliser due to its still high nutrient content (Devarenjan et al., 2019; Koszel and Lorencowicz, 2017). Several countries in the European Union have practiced the application of biogas effluent as an organic fertiliser (EU), such as Germany, Denmark, Austria, Sweden, and Switzerland, have taken a step toward concern and have been further implemented for the biogas sector development (Al Seadi et al., 2013). Organic fertiliser from biogas effluent has been applied in many crops with significant results that can improve soil fertility and increase yield and productivity (Chang et al., 2022; Kefalew and Lami, 2021; Sürmen and Emre, 2022). The application of organic fertiliser from biogas effluent that is mixed with other amendments results in the equivalent yield of corn and mungbean, which was applied with 100% recommended NPK fertiliser, and can also reduce 25% of the NPK fertiliser (Nghia et al., 2022). This study aims to determine the effect of using a biofilter to improve biogas production and biogas effluent as an organic fertiliser material.

4.2 Materials and Methods

The research was conducted at the Laboratory of Management of Waste at the Faculty of Agriculture, University of Lampung, Indonesia. Tofu wastewater was collected from the tofu industry in Gunung Sulah district, Bandar Lampung City, Lampung Province, Indonesia. The inoculum was collected from the second biogas effluent pond in the Tapioca industry, Central Lampung Province, Indonesia.

4.2.1. Experimental set-up

The anaerobic digester used in this study is the anaerobic baffled reactor (ABR), the total volume is 0.927 m^3 (Fig.1). The ABR was inoculated with the 50% inoculum and the 50% substrate (1:1). The ABR was recirculated for 7 days to make the situation in all parts of the chamber stable with a pH of 7.16. The dosage of substrate is 50 kg COD·m^{-3} per day, 100 kg COD·$m^{3\ -1}$ per day, 150 kg COD·$m^{3\ -1}$ per day, and 200 kg COD·$m^{3\ -1}$ per day. The total biofilter used in the chamber of the ABR is 700 balls with a total weight of 3.56 kg.

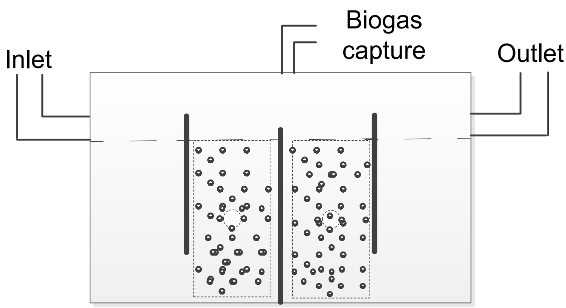


Figure 1. Design of an anaerobic baffled reactor combined with biofilter

4.3. Data analysis

4.3.1. Tofu wastewater and inoculum

Tofu wastewater was analyzed to determine the characterization before treatment through ABR with biofilter as initial data. COD and CODs were measured using a spectrophotometer, total solid (TS) was conducted using oven dry at 105 ^{0}C, and TSS with a muffle furnace based on weighing the dry mass after processing. The information about tofu wastewater and inoculum is provided in Table 1.

Table 1. Characteristics of tofu wastewater before treatment and inoculum

Parameter	Unit	Tofu wastewater	Inoculum
COD	mg. L^{-1}	12400	-
CODs	mg. L^{-1}	7150	-
N-Total	mg. L^{-1}	-	-
TSS	mg. L^{-1}	1188.25	-
TS	%	0.38	3.99
pH	-	5.17	7.95
Alkalinity	mg. L^{-1}	280	800
VFA	mg. L^{-1}	1500	1224
C/N ratio	%	-	4.66
VS	mg. L^{-1}	-	1188.25

4.3.2. Biogas composition

Biogas composition was analysed two times namely in the first week and at the end of digestion. The composition of biogas and the quality of methane were observed to obtain the presentation of the gas composition (CH_4, CO2, and N_2) using the Shimadzu

Shincorbon ST 50-80 D-375 gas chromatography (GC) model. However, the removal TS, TSS, and volatile solids (VS) was observed thrice in a week.

4.3.3. Biogas effluent

The biogas effluent was analysed to determine the nutrient content, which can be used as a liquid organic fertiliser. The nutrient analysed in this study is Nitrogen (N-total). P-available, Calcium (Ca), Magnesium (Mg), Copper (Cu), Iron (Fe), Potassium (K), and C-organic. There are three treatments for biogas effluent, namely: 100% biogas effluent, a mixture of biogas effluent and nutrition (AB mix), and 100% nutrition (AB mix)

4.4. Results and Discussion

4.4.1. The effect of biofilter on biogas composition

The quality of biogas composition, especially CH_4, depends on some factors such as pH, temperature, types of substrates, organic loading rate (OLR), HRT, and performance reactor (digester design) (Mawaddah et al., 2019; Li et al., 2020). Based on Table 2, the concentration of methane is increased gradually, but CO_2 and H_2S are still high. Hence, it can be indicated that the biofilter in ABR does not significantly affect in removal of the gas pollutant, but can increase methane concentration. The enhancement of methane is related to the nutritional content of tofu wastewater as the substrate. Tofu wastewater is rich in protein, which has great potential to provide nitrogen as the main nutrient for anaerobic microorganism activities, and the neutralising effect of VFA through the formation of ammonia (Wresta et al., 2021).

Based on Table 2, the highest concentration of CH_4 is 60.64% at a dosage substrate of 200 L·day$^{-1,}$ and the lowest is 49.78% at 50 L·day^{-1}. This is because of the effect of the dosage of the substrate that is loaded into anaerobic digestion. One of the factors that affects methane yield is the ratio of inoculum and substrate; however, during biodegradation process produces the production rate and synergetic effect (Corsino et al., 2021).

Table 2. Biogas composition

Dosage of substrate	Biogas Composition			
	CH_4 (%)	CO_2 (%)	N_2 (%)	H_2S (ppm)
50 L. day^{-1}	49.78	36.105	14.215	980
100 L. day^{-1}	56.93	37.64	5.416	410
150 L. day^{-1}	55.26	42.749	1.905	610
200 L. day^{-1}	60.64	37.775	1.573	630

Changing the dosage of substrate, same as in nu- nutrient content, will affect the overall methane and biogas production process (Gokul Prasad, 2022). The effect of increasing cattle feed supplement from 543 $L \cdot kg^{-1}$ to 894 $L \cdot kg^{-1}$ VS is not affected in enhancing methane quality; however, it significantly affects to biogas yield and cumulative biogas production rate, because of the absence of a relationship between supplement addition and the methane content in biogas (Zieli et al., 2019). Macro and micronutrient content in the substrate affects reactor performance; however, the content of nitrogen and phosphorus in industrial wastewater is inadequate (Ravichandran and Balaji, 2020).

Generally, biogas produced from dairy manure as feedstock has lower trace chemical concentrations, but the toxicity response of combustion is higher compared to other feedstocks (Li et al., 2020). The high con-concentration of methane is the key to the quality of biogas because it has a high calorific value for combustion (Muntaha et al., 2022). However, based on the result in Table 2. the use of biofilters is not so effective in reducing the concentration of greenhouse gases, namely H_2S and CO_2. In this study, the biogas composition ratio is still within reasonable limits, which do not exceed the concentration of each gas. Generally, the range amount of H_2S in biogas is from 100 ppm to 10,000 ppm, as well as a CO_2 concentration of approximately 20–30%, depending on the type of substrate used; excessive concentration will affect the caloric value and corrosiveness during combustion

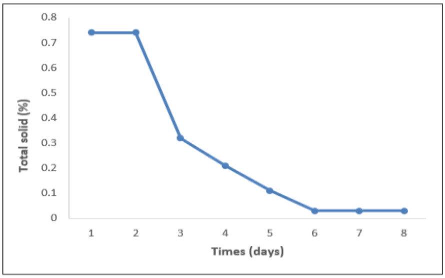
(Silva and Mezzari, 2022). To remove the gas pollutant in biogas, a special treatment is needed cause the characteristics of the gas, a combination of monoethanolamine (MEA) as adsorbent, and gas flow rate is 0.1963 cm^2 and 0.3 $L \cdot min^{-1,}$ which can remove up to 0 ppm H_2S and 0.20%. CO_2 (Kalsum et al., 2022).

4.4.2. The effect of biofilter in the removal of pollutants

Total solids is one parameter to measure the quantity and quality of substrate solid waste and wastewater, which impact anaerobic digestion performance through microorganism activities. It is because of the behaviour of microbial community in the reactor related to TS in feedstock that influences the efficiency fermentation process (Shrestha et al., 2020; Yi et al., 2014). The high content of TS results in low biogas production but increases biogas yield, because TS is linked to the substrate availability in the reactor, which can increase biogas yield (Jeppu et al., 2022).

In Figure 2. the removal of TS in each dose of substrate is provided. The highest TS removal is 95.95% in dosage 50 $L \cdot kg^{-1}$ per day, from 0.74% to 0.03. TS removal in dosage substrate 100 $L \cdot kg^{-1}$ per day, 150 $L \cdot kg^{-1}$ per day, and 200 $L \cdot kg^{-1}$ per day, respectively, are 42.86%, 100%, and 8.57%. The reduction in TS removal was caused by an increase in substrate dosage from 100 L·kg^{-1} per day, up to 200 L·kg^{-1} per day in the reactor, because the dosage of substrate is excessive and may cause the performance of the anaerobic digestion process not optimal in removing.

a



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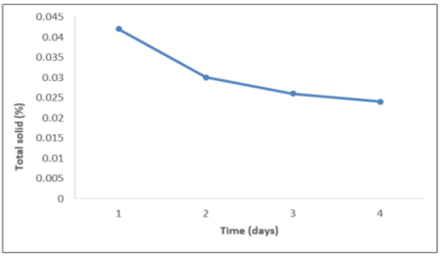

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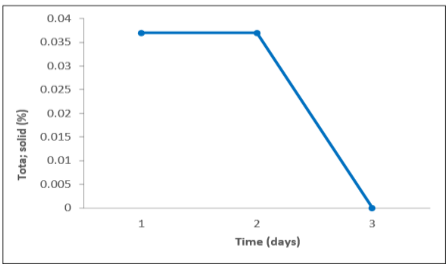

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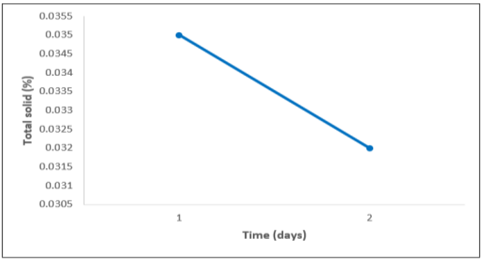
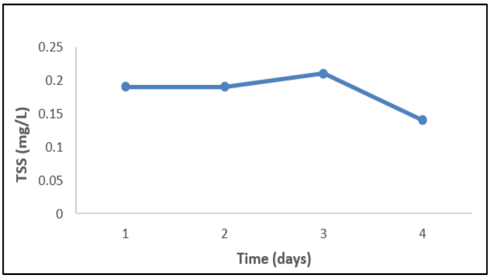


Fig 2. Total solid (TS) in biogas effluent; a) dosage substrate 50 L. Kg^{-1} per day, b) dosage substrate 100 L. Kg^{-1} per day, c) dosage substrate 150 L. Kg^{-1} per day, and d) dosage substrate 200 L. Kg per day.

The substrate dosage is important to determine the performance removal pollutants; the particle stability in any coagulant depends on the substrate dosage. If a small amount of substrate is added, it will not affect the stability of the particles; however, the excessive dose added will have an effect like restabilisation and production of excessive sludge (Igwegbe and Okechukwu Dominic Onukwuli, 2019).In addition, the use of bio-filters also affects the removal of pollutants in the anaerobic digestion process, such as greenhouse gas (GHG), COD, and solid content in biogas effluent. The use of biofilter in ABR is because the biofilter has a high surface area, a high void ratio, and low density that can preserve more biomass (Ravichandran and Balaji, 2020). The use of quarts of sand biofilter reduces 91.9% TSS, 84.1% turbidity, 86.1% colour, 77.7% organic matter, and 81.9%, the effect in lowering TSS and turbidity is decreasing the consumption of coagulants in subsequent raw water treatment (Suprihatin et al., 2017). The result of TSS removal in each substrate dosage is shown in Figure 3. The efficiency of TSS removal for all dosages is 99.98%. The TSS content for each dosage at 50 $L \cdot kg^{-1}$ per day, 100 $L \cdot kg^{-1}$ per day, 150 $L \cdot kg^{-1}$ per day, and 200 $L \cdot kg^{-1}$ per day, respectively are 0.18 $mg \cdot L^{-1}$, 0.16 $mg \cdot L^{-1}$, 0.20 $mg \cdot L^{-1}$, and 0.25 $mg \cdot L^{-1}$.

a



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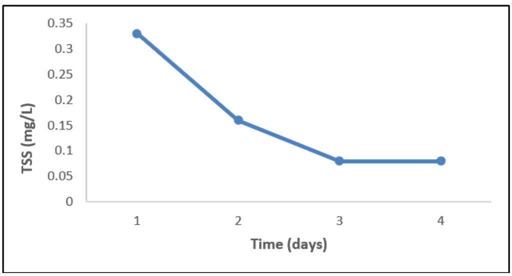

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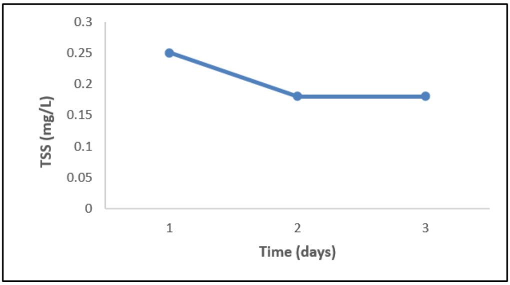

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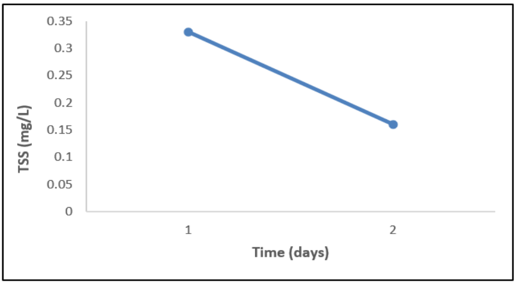
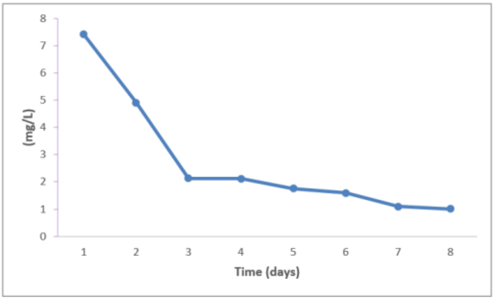


Fig 3. Total solid suspended (TSS) in biogas effluent; a) dosage substrate 50 L. Kg^{-1} per day, b) dosage substrate 100 L. Kg^{-1} per day, c) dosage substrate 150 L. Kg^{-1} per day, and d) dosage substrate 200 L. Kg per day.

The removal efficiency of TS, TSS, and VS in this study is due to the substrate was filtered in the first step before loading into ABR, it is to reduces the hydraulic retention time (HRT) of organic matter in the anaerobic digestion process and increases the production of biogas and the methane quality. The HRT and the dosage of the substrate affected the performance of the reactor and the removal of pollutants. The TS and VS content also influences reactor performance in the substrate due to the microbial activity involved in the efficiency of anaerobic digestion (Orhorhoro et al., 2017). Increasing HRT from 17 to 34 hours affects all parameters, including reducing the concentration of TSS and VSS, resulting in 75% and 90% of COD removal (Hassan et al., 2022).

Moreover, Figure 4 shows that VS removal for all the dosages of the substrate, the highest VS removal is 66.80% at dosage 150 $L \cdot kg^{-1}$ per day from 0.89 $mg \cdot L^{-1}$ to 0.74 $mg \cdot L^{-1}$. VS removal at dosages of 50 $L \cdot Kg^{-1}$ per day, 100 $L \cdot Kg^{-1}$ per day, and 200 $L \cdot Kg^{-1}$ per day, respectively, are 48.18%, 58.69%, and 41.16%. Based on the result in Fig 4. the decrease of VS removal can be initiated because of the excessively high substrate load into ABR. The effect of increasing the OLR in the reactor is re-reduce the efficiency of VS removal (Blasius et al., 2020). The highest VS removal is 75% at OLR 1 g $VS \cdot L^{-1}$ per day at 55 °C, but when the OLR increased in the maximum dosage at 7 g $VS \cdot L^{-1}$ per day, the VS removal gradually decreased to 44% (Gou et al., 2014). The combination of temperature and OLR also influenced the removal of VS in mesophilic treatment is more efficient in waste treatment than thermophilic for the removal of COD and TS, methane yield, and biochemical methane potential value, TVS removal at OLR 0.15 and 0.30 g $TVS \cdot L \cdot d^{-1}$ is 79.5% and 80.1%. However, TVS removal at OLR 0.45, 0.60, and 0.90 g TVS·L·d^{-1}, respectively, is 54.4%, 44.4%, and 32.7% (Blasius et al., 2020).

a



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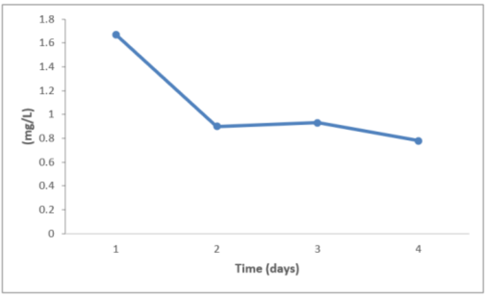

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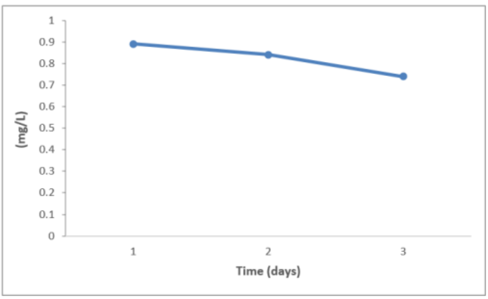

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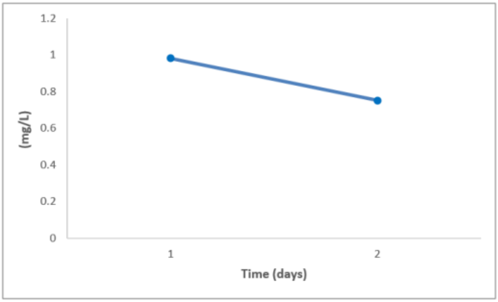

Fig 4. Volatile solid (VS) in biogas effluent; a) dosage substrate 50 L. Kg^{-1} per day, b) dosage substrate 100 L. Kg^{-1} per day, c) dosage substrate 150 L. Kg^{-1} per day, and d) dosage substrate 200 L. Kg^{1} per day.

The use of biofilters in the anaerobic digestion process is a promising and economically friendly solution for the physical and chemical disinfection of wastewater (Maurya et al., 2020). In a biofilter system, acidogenic and methanogenic microorganisms adhere to and colonise the surface of the biofilter, forming a biofilm layer that facilitates the conversion of organic matter to methane (Damayanti et al., 2020). Consequently, the biofilter system not only excels in removing pollutants from wastewater but also enriches the quality of the biogas effluent, which possesses significant potential as a valuable organic fertiliser.

4.4.3. The effect of biofilter on biogas effluent

The anaerobic digestion process yields biogas as its primary product and a nutrient-rich liquid digestate as a by-product. This liquid by-product, characterized by its high nutrient content, can be effectively utilized as a liquid organic fertilizer or a nutrient source for hydroponic plant cultivation. The elemental composition of the biogas effluent reveals a significant presence of carbon (37.92 wt%), hydrogen (4.113 wt%), nitrogen (46.287 wt%), oxygen (1.56 wt%), and sulfur (0.047 wt%). This distinctive elemental profile renders the biogas effluent a valuable resource with considerable economic potential for further utilization and product development (Qian et al., 2022). Biogas effluent is a valuable organic material that can be utilized as a high-quality fertilizer, rich in essential nutrients like nitrogen, phosphorus, and potassium, which are vital for plant growth (Chang et al., 2022). The characterisation of the biogas effluent is shown in Table 3.

Based on Table 3. the macronutrients in the biogas effluent are still high and are not very different from the commercial AB mix of nutrients for hydroponic plants. Therefore, it can be indicated that it is worthy of being used as an organic fertiliser or nutrients for hydroponic plants. The use of biogas effluent as fertiliser is a wise solution for both environmental and economic aspects because the product is useful to improve soil fertility, including microorganisms in the soil, and can replace synthetic fertiliser to improve biodiversity (Farghali et al., 2022).

Table 3. Macronutrient content of biogas effluent treated with commercial nutrition

Parameter	100% nutrition	100% effluent	Mix effluent and nutrition (AB mix)	Unit
Nitrogen (N-total)	202.75	175.63	262.5	mg. L^{-1}
P-available (P)	1.22	0.675	0.399	mg. L^{-1}
Calcium (Ca)	1.02	1.83	4.08	mg. L^{-1}
Magnesium (Mg)	20.83	24.25	25.24	mg. L^{-1}
Copper (Cu)	<0.007	<0.007	0.032	mg. L^{-1}
Iron (Fe)	8.7	9.53	13.09	mg. L^{-1}
Potassium (K)	830	140	440	mg. L^{-1}
C-Organic	0.088	0.39	0.49	%

The use of biogas effluent is needed to obtain additional material that can increase the nutrient content for the growth of plants and meet the Regulation of the Minister of Agriculture of the Republic of Indonesia. The standard of liquid organic fertiliser based on PERMENTAN No. 70/ permentan/SR.140/10/ 2011 is provided in Table 4. According to the standard organic fertiliser in Table 4. That biogas effluent can be combined with various materials to meet the standards as an organic fertilizer. These materials include fish emulsion, coal, slag, sugarcane husk charcoal, and organic garbage.

Table 4. Standard organic fertiliser (The regulation of Minister of Agriculture of Indonesia, 2011)

Parameter	Unit	Standard organic fertilizer
C-organic	%	Min 15
pH	-	4-9
Macronutrient		
N	%	Min 4
P_2O_5	%	Min 4
K_2O	%	Min 4
Micronutrient		
Total Fe	Ppm	Max 9000
Available Fe	Ppm	Max 500
Mn	Ppm	Max 5000
Zn	Ppm	Max 5000
Cu	Ppm	Max 5000
Mo	Ppm	Max 20
Na	Ppm	Max 2000
Cl	Ppm	Max 5000

Additionally, other organic materials such as manure, food waste, and shale can also be used. (Nghia et al., 2022; Nurweni et al., 2019). The combination of these materials with biogas effluent can enhance its nutrient content and make it suitable for use as an organic fertilizer. However, information is missing on the specific proportions of these materials that should be added to the biogas effluent to meet the standards. The utilization of biogas effluent as an organic fertilizer can positively impact farmers' ability to achieve the highest net profit by reducing the dependence on synthetic fertilizers (Hooton et al., 2019). Biogas effluent can be an effective substitute for approximately 25% of the chemical fertilizer NPK 1 ton, ha^{-1} as the recommended application dose, as it provides essential nutrients for plant growth (Nghia et al., 2022). This substitution can lead to cost savings for farmers, as biogas effluent is a readily available and renewable resource. Additionally, the use of biogas effluent as an organic fertilizer can contribute to a more circular economy and reduce the environmental impact of synthetic fertilizer usage (Chojnacka & Moustakas, 2024).

Mixing biogas effluent with 100% nutrients (AB mix) increases the nutrient content so that it meets the liquid organic fertiliser standards required by PERMENTAN No. 70/ permentan/SR.140/10/ 2011, which can be applied to plants using soil or hydroponic growing media, however, several things must be considered in its application apart from nutrition, pH and heavy metal content are also taken into consideration (Bergstrand et al., 2020). However, information on the specific economic benefits of using biogas effluent as a substitute for chemical fertilisers is lacking in terms of net profit. Further research is needed to quantify the economic advantages of this practice under different farming conditions.

4.5. Conclusions

The use of biofilters with variations of the substrate dosage in anaerobic digestion is very efficient in removing the pollutant and increasing the nutrient of the biogas effluent that can be used as organic fertiliser. The highest removal of TS is 95.95% at a dosage 50 L. Kg^{-1} per day, the removal of TSS is 99.98% for the entire dosage of substrate, and the removal of VS is 66.80% in the dosage of substrate 150 L. Kg^{-1} per day. The treatment of biogas effluent with a nutrition mix (AB mix) enhances the nutrient content by increasing the concentration of phosphorus, nitrogen, and other essential micronutrients, making it compliant with the standard of organic fertilizer as specified in the Regulation of the Ministry of Agriculture of Indonesia, PERMENTAN No. 70/Permentan/SR.140/10/2011.

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5. Acclimatisation Process of Biogas Production from Tofu Industrial Wastewater Using Biofilter in Anaerobic Baffled Reactor (ABR)

Adapted from: Lydia Mawar Ningsih, Udin Hasanudin, Hynek Roubik. Acclimatisation Process of Biogas Production from Tofu Industrial Wastewater Using Biofilter in Anaerobic Baffled Reactor (ABR). Journal of Renewable Energy.
https://doi.org/10.1016/j.renene.2024.121519

Abstract

A biofilter is a simple technology used in an anaerobic baffled reactor (ABR) to keep biological solids (inoculum) from being easily carried by the inlet substrate and to shorten the hydraulic retention time (HRT). The principle of a biofilter is to form a biofilm on the packed bed of the biofilter in ABR so that it contains immobilised microorganisms. This study aims to know the performance of the biofilter reactor on biogas production during the acclimatisation process. The results show biofilters shorten the HRT and effectively remove pollutants, increasing biogas production and methane quality. The total solid content decreases by around 44%, from 0.38% to 0.17%. The biogas production during acclimatisation was 1806.41 L and COD removal was 95 %. The biogas composition of CH_4 was 58.05 %, CO2 38.23 %, and N2 3.2 %. This study provides preliminary findings for further studies on the use of tofu wastewater as a biogas feedstock with different concentrate substrates, which is very useful for sustainability activities and improving the industry to become green.

Keywords: tofu industry, wastewater, biogas, biofilter

5.1 Introduction

Tofu is a product made with soybean, a legume with great nutritional value. It is a favourite food in Indonesia and other Asian populations; it also has an increasing demand in Europe and is gaining popularity worldwide due to its health benefits and price. Its production begins with soymilk obtained from fresh soybeans [1,2]. In Indonesia, most of the tofu production is carried out by small industries called home industries [3]. During the processing of tofu, a large amount of wastewater is produced which has a bad effect on the environment [null]. The tofu industry generated two types of waste, namely solid waste (tofu dregs) and wastewater [4]. From 150 kg of soybean processing to tofu, 210 kg of tofu dregs, and wastewater of approximately 2115.51 kg are produced [5]. Tofu dregs or okara is a by-product that harms the environment and economy. It comes from the soybean production process, which is the residue component of the filtering of the water-soluble fraction in the form of ground soybean during the production of soymilk [6].

However, in Indonesia tofu dregs do not affect the environment as they can be used directly as the main material for human food or animal feed or other materials such as bioethanol, a nitrogen source in fermentation, and to produce other soy-based foods [7,8]. The main problem of industrial tofu wastewater is its direct disposal in bodies of water without any treatment which is harmful to the environment [5,9]. The owners of the tofu processing plants generally do not have enough capacity to manage their wastewater due to the complexity of the wastewater treatment process [10]. On the other hand, tofu wastewater still has a high nutritional content that can be used as the main material for new products such as biogas as it has a COD range of approximately 1400–11000 mg/L, BOD5 431 mg/L, TSS 244 mg/L, and a pH of about 3.47 [11–13]. Furthermore, the high content of BOD and COD in tofu wastewater causes unpleasant odours in the air and surrounding environment and various types of pollution in water bodies (ground, surface, and river) [null]. The small and medium-scale tofu industry commonly does not have space to treat wastewater, such as wastewater treatment plants (WWTP), as the industry presence is clustered and in the middle of the settlement [11,14]. Therefore, the utilisation of tofu wastewater for biogas is a good solution to prevent environmental pollution and is economically friendly to the industry to replace fossil energy [15]. The substitute for biogas energy is not only for industry activities; it can also be supplied to the community that lives in the surrounding area that does not have access to a natural gas supply for its household energy, such as cooking and lightning [16]. Acclimatisation is required as an initial test to obtain optimal biogas production and quality. Acclimatisation is very important as a pretreatment for microorganism activities in the anaerobic digestion process. Acclimatisation refers to the process of adapting an anaerobic microorganism to a specific environment or operating conditions, such as temperature, pH, or substrate composition, to optimise its performance and stability in the breakdown of organic matter [17–19]. This process is crucial for efficient anaerobic digestion, enabling microorganisms to thrive and produce biogas effectively.

Acclimatisation can be achieved through gradual changes in operating conditions, continuous feeding, and adaptation of the inoculum. This process contributes to stable anaerobic digestion, even under extreme conditions, such as high levels of ammonia [20,21]. Moreover, acclimatisation is essential for the efficient breakdown of organic matter, biogas production, and sustainable energy sources. By adapting microorganisms to specific conditions, acclimatisation improves the overall efficiency and stability of the anaerobic digester [null].

The biofilter is a technology that can be useful for tofu wastewater during the acclimatisation in the anaerobic digestion process [22]. Biofilter technology for tofu wastewater treatment has some advantages, such as effectively removing organic matter, reducing odour, and producing high-quality effluent [23]. The use of a biofilter in an anaerobic digester is to keep the inoculum so that the inoculum is not easily carried away by wastewater and to shorten the residence time of organic matter (HRT) during the digestion process [24]. The use of biofilters has various advantages helpful for water and wastewater treatment; not only can some of the pollutants be removed, but also greenhouse gases (GHG) such as hydrogen sulphate (H_2S), carbon dioxide (CO_2) and methane (CH_4) can be removed. A study by Nguyen et al. [25] found that a biofilter is effective in removing 30 % of total organic carbon (TOC), 50 % of non-methane volatile organic carbon (NMVOC), and 51 % of NH_3.

The effect of methane by biofilter is very slight in decreasing methane (CH_4) by around 6%. A study by Li et al. (2016) also found that the biofilter also has a significant effect in reducing the content of COD and total phenol in the effluent of coal gasification wastewater (CGW), respectively, 234 mg/L and 14.2 mg/L, they also found that the methane concentration reached 169 ml CH_4/L/day. The present study focuses on the effect of biofilter technology on the anaerobic biofilter reactor (ABR) on the production and composition of biogas, especially the quality of methane during acclimatisation as one of the solutions for wastewater treatment for the transition of tofu industry fuels from fossil fuels to zero carbon emissions.

5.2. Material and Methods

5.2.1. Location

This study was conducted on a pilot scale reactor using ABR, and the sample was analysed at the Agro-industrial waste management laboratory, Faculty of Agriculture, University of Lampung, Indonesia.

5.2.2. Experimental setup

5.2.2.1 Biofilter setup

The types of biofilter used in the ABR are dense plastic bio balls. The biofilter was placed in an unused fruit box and then weighed to ensure that the weight of each box was the same. The total number of biofilters used in the ABR chamber was 700 balls and 10 boxes of biofilter with a total weight of 3.56 kg (Fig.1.).

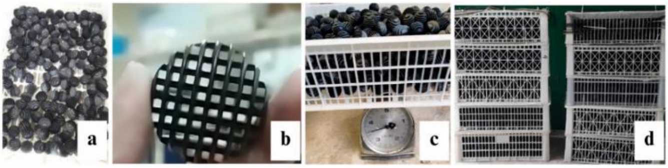

Fig.1. Biofilter used in the ABR; a) plastic biofilter, b) detail of biofilter, c) biofilter measurements, d) total biofilter used in the ABR

5.2.2.2 Anaerobic digestion system

The anaerobic digester used in this study was an anaerobic baffled reactor (ABR) with a total volume of 0.927 m^3 (Fig.2.). The ABR was inoculated with 50% inoculum and 50% substrate/tofu wastewater (a 1:1 ratio). The ABR was recirculated for 7 days to stabilise the chamber pH to 7.16. Then, to avoid microorganism metabolism inhibition, the loading rate concentration was 30 kg COD/m^3 per day and carried out periodically for 26 days. The ABR design is provide in Fig.2.

5.2.3 Sample collection

5.2.3.1 Tofu wastewater and inoculum

The tofu wastewater was collected from micro, small, and medium-scale (MSMEs) of tofu industry in Gunung Sulah District, Bandar Lampung City, Lampung Province, Indonesia. The inoculum was collected from the biogas plant in the tapioca industry in Central Lampung Province, Indonesia. The location is provided in Fig.3.

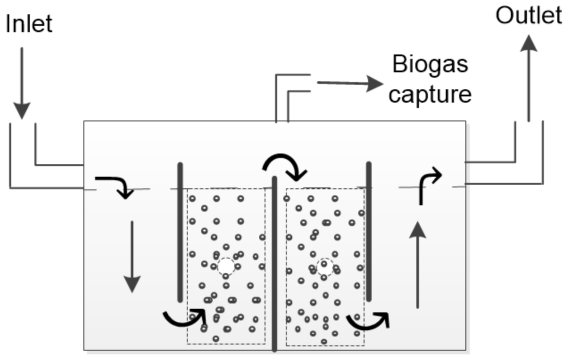

Fig.2. Design of ABR combined with biofilter

5.2.4 Data collection

5.2.4.1 Initial data

The initial data consisted of the characteristics of tofu wastewater and inoculum;

a. Inoculum

The data was analysed to obtain data on the C/N ratio, TS, VS, Alkalinity, VFA, and pH.

b. Tofu wastewater characteristics

Tofu wastewater was analysed to obtain CODs, COD, TS, TSS, pH, alkalinity, and VFA.

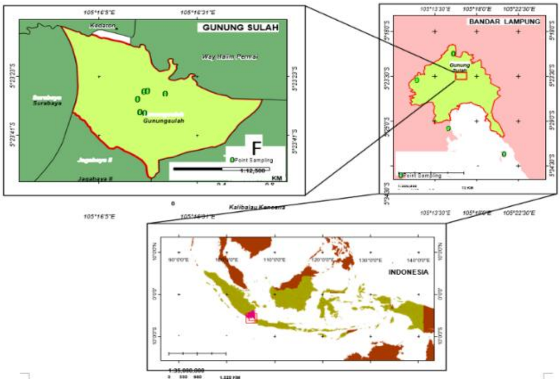

Fig.3. Tofu wastewater collection location in MSMEs of the tofu industry

5.2.4.2 Data analysis

Data for anaerobic digestion were collected to obtain the quality of biogas production, which consists of:

a. Biogas production

Biogas production was observed once every 24 hours using a gas flow meter (wet gas flow meter) model W-NK-10 A SINAGAWA.

b. Biogas composition and methane quality

The composition of biogas and the quality of methane were observed to obtain the presentation of the gas composition (CH_4, CO_2, and N_2) using a gas chromatography (GC) model Simadzu GC-2014 with a thermal conductivity detector at a temperature of 200 °C, injection pressure 100 kPa, injection time 1 min, and injection temperature 100 °C. The GC was equipped with a shin-carbon column of 4.0 m length and 3 mm inner diameter. Helium was used as a carrier gas with a 40 ml/min flow rate.

5.3 Results and Discussion

5.3.1 Characteristics of tofu wastewater and inoculum

The characteristics of the nutritional content of tofu wastewater and inoculum is very important due to the effectiveness of the feedstock in the anaerobic digestion process to produce biogas and improve the quality of biogas. Generally, the inoculum is a microbe or strain species selected to improve the transformation of microbes. The inoculum used in this study was taken from the second pond of a biogas outlet in the tapioca industry, which produces a very good sludge as a seed for the anaerobic reaction, as shown in Table 1.

Table 1. Characteristics of the inoculum

Parameter	Amount	Unit
pH	7.95	-
C/N ratio	4.66	%
TS	39900	Mg/L
VS	1188.25	mg/L
Alkalinity	800	mg/L
VFA	1224	mg/L

Inoculums are known as starter cultures to produce fermented products in the agri-food industry, such as cheese, yogurt, etc. In anaerobic digestion, the main sources of inoculum are anaerobic sewage sludge and manure, and digested from agricultural biogas plants [26]. The inoculum is a crucial factor in the initiation of biogas production and quality [27]. The best inoculum for the anaerobic digester method is the outlet of an anaerobic reaction, such as digested sludge [28].

Inoculums from agro-industry, such as the tapioca industry wastewater, are rich in carbohydrates and sugar, as well as sugar mill wastewater, which has a very good inoculum for anaerobic digestion and also has a high content of organic matter that can potentially be used for fertigation [29,30]. One of the key factors that affects the performance of anaerobic reactors is the content of macronutrients and trace elements in the inoculum and substrate [29]. Furthermore, the result of the analysis of tofu wastewater before anaerobic digestion shows that it has a high COD content and does not comply with the standard of Indonesian government No.15. 2014 for direct discharge into the environment, the results of this analysis are supported by several previous studies, which are shown in Table 2. Not only has high COD content, tofu wastewater also has a low pH, which can harm the environment.

However, tofu wastewater still has high organic compounds and nutrients such as total sugar, N-total, nitrate, and total Phosphorus, which can be reused and are profitable [33, 34].

Table. 2. Characteristics of tofu wastewater (control)

Parameter	Unit	Laboratory analysis	Indonesian Government Standard No.5. 2014	Reference		
				1)	2)	3)
BOD	mg/L	-	150	924.97	2369	580
COD	mg/L	12400	300	9900	9350	5759
CODs	mg/L	7150	-	-	-	-
N-Total	mg/L	-	25	673.01	676	-
TSS	mg/L	1188.25	200	-	19888	552
TS	%	0.38	-	-	-	-
pH	-	5.17	6.00 – 9.00	3.76	6.77	3.9
Alkalinity	mg/L	280	-	-	-	-
VFA	mg/L	1500	-	-	-	-

Sources: 1) [31], 2) [32], 3) [13].

The utilisation of tofu wastewater in biogas is a good solution that prevents water pollution and replaces fossils as the main energy source in the industry for the tofu production process. In addition to that, biogas effluent can be used as organic fertiliser, which has high economic value, so it can be an additional income for the tofu industry [35].

5.3.2 Production and quality of biogas during acclimatisation

Acclimatisation in anaerobic digestion is a process of adaptation of bacteria that have been transported to tofu wastewater to be processed into biogas. The acclimatisation process is also known as the "seeding process", adding the substrate concentration starts with a small dosage of loading rate up to the actual loading rate to be processed [36]. The concentration of the initial loading rate should start low so that the growth of microorganisms will not be overloaded, the reason being to make the up-flow velocity of gas and liquid become low, then the growth of granular and flocculent is encouraged [37].

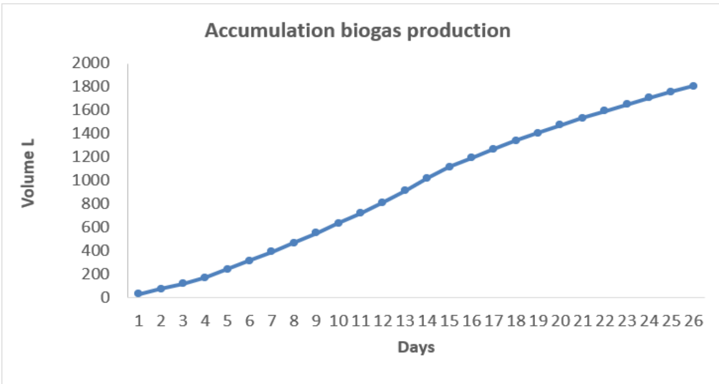

Fig. 4. Accumulation of biogas production during acclimatisation

The working system of the biofilter in ABR is the same as that of the biofilter system in general. The gas is passed through a stack of biofilters where the pollutant-decomposing microorganism is immobilised as a biofilm [38]. The accumulation of biogas production during the acclimatisation term is a complex process that involves the adaptation of microorganisms to the substrate and environmental conditions.

The anaerobic digester used in this study is ABR combined with a biofilter, also known as an attached growth digester. The HRT in the acclimatisation period is 26 days, and the substrate dosage is 30 L/day with an organic loading rate (OLR) concentration of 0.40 g COD/L.day^{-1}, resulting in the optimal accumulation of biogas production during the acclimatisation period of 1806.41 L (Fig.4.). The ability of the digester to adapt efficiently to new substrates, including the types of substrates and the concentration, is affected by the rate of acclimatisation impacts [39]. Biogas production strongly depends on growth supplements and certain compounds that can enhance biogas production [40].

The effect of biofilters on the accumulation of biogas production during the acclimatisation term is a complex process as it can influence the balance between biomass accumulation and removal [41]. The three phases of the biofilter are: the start-up (days 0–25), stable operation (days 26–80), and clogging (days 81–105), with the performance of removal and biomass accumulation, respectively, 2.3 kg, 1.1, and 0.5 kg biomass/m^3 filter bed/day [42]. Biofilters in biogas can facilitate the removal of gas such as H_2S from biogas, which is essential for its use as an energy source; however, the concentration of H_2S can be increased with increasing biogas production. During acclimatisation on day 6, the concentration of H_2S reaches 45.24 ± 0.01 mL, higher than 20.50 ± 0.70 ml in the non-acclimatization treatment [43]. During the acclimatisation period, waste-activated sludge can increase the CH_4 production rate by approximately 0.45 m^3/day kgCOD removal at an OLR ~20 kgCOD/m^3/day on the 6th day of HRT. This indicates that the accumulation of biogas production during acclimatisation can be significant, with a substantial increase in the methane content. As shown in Fig. 4 the increase in the accumulation of biogas production starts on day 8 and continues to day 26, increasing from 466.80 to 1806.41 L/day.

From day 1–7, there is reduced biogas production due to the lag phase; in this phase, the microorganism must adapt to the new condition; this phase is based on the presence of nutrients in the substrate that are ready to use. Additionally, cumulative biogas production is influenced by several factors such as pH, temperature, type of substrate, and C/N ratio; these factors will affect the microorganisms in the anaerobic digestion process [44]. Three phases of cumulative biogas production are described, known as the ’sigmoidal curve’, namely the lag phase, growth, and asymptotic phases [45].

Biogas is a renewable energy whose main products are methane (CH_4) and carbon dioxide (CO_2), as well as hydrogen sulfur (H_2S) and nitrogen (N_2), which are formed through an anaerobic digestion process [46]. The main gas produced from biogas is CH_4, which has an economic advantage that can be used as an energy source and to operate a treatment plant [47]. The composition of the biogas consists of 60–70 % methane, 30–40 % CO_2, 1–2% nitrogen, and 1000–3000 ppm H_2S [38]. Our findings showed that the use of biofilters has not only affected the quantity but also the quality of biogas (Table 3). Based on Table 3, the concentration of CH_4 in the acclimatisation term is 58.05 % and the production of CH_4 is 0.147 L/g-CODremoval.

Table 3. Biogas composition during the acclimatisation

Parameter	Amount
CH_4	58.05%
CO_2	38.23%
N_2	3.2%

However, the production of CH_4 in this study indicated that it was lower than the theoretical production of methane (0.35 L/g-COD removal). This is because several factors affect the production of biogas and CH_4 during acclimatisation: the dissolution of CH_4 in the wastewater during the anaerobic digestion process and the influx of oxygen due to a leaky reactor. The critical factor is the types of substrates that are used in anaerobic digestion as different methane potential of carbohydrates and proteins as the main sources of metabolism of acetogenic bacteria will result in different methane production (0.373 vs. 0.417 L/g) [48, 49].

Temperature and OLR concentration also affect methane production; the highest temperature is 33.4 ^{o}C and OLR is 3.072 g COD total/L.day results in a methane yield of 0.167 L/g COD total [48]. Although methane production does not reach or approach the standard COD stoichiometry that converts to methane, overall, biogas production and methane concentration results are enhanced during the acclimatisation period in ABR with a biofilter. The conversion of tofu wastewater into biogas can reduce CO_2 by approximately 1.12 kg CO_2e/kg of soybeans and produce CH_4 0.056 m3/kg of soybeans. It can also substitute 2.82 % of firewood, 11.86 % of LPG, and 33.39 % of biopellets, and can save fuel costs [50].

The result of this study shows that CH_4 concentration is high enough for ignition. However, there is no specific standard for CH_4 concentration for the ignition due to the variation depending on the specific application and the conditions used, generally, at least a CH_4 concentration of 30 % is needed for combustion [51,52]. Moreover, catalytic combustion gas requires a minimum CH_4 concentration of 25 % [53]. Furthermore, the concentration of CH_4 under 5 % gas mixture is too low and not enough for ignition, a gas mixture greater than 15

% is too high and lacks oxygen, however, a concentration between 5 and 15 % is sufficient for combustion [54]. It can be indicated that the concentration of CH_4 in this study is high and can be used for combustion.

The use of biofilter in ABR as a simple technology has merits and demerits in its application. Its merits include the resistance to organic and hydraulic shock, effective degradation of the organic compound and biogas production, a fixed-bed biological reactor with one or more filtration chambers in series, and can be used for wastewater treatment [55–57]. The demerits of biofilters in ABR must also be taken into account, these include, the inability to reduce pathogens and nutrients to an acceptable discharge level, and a long start-up process in the absence of adapted seed sludge [18,58,59]. Overall, based on the study results, ABR with a biofilter is very meritorious because it produces high CH_4 concentration and high biogas production, thus it can be considered for the tofu industry owners and related stakeholders in utilising tofu wastewater as a biogas feedstock. However, both the merits and demerits of the ABR technology must be duly considered and reviewed when deciding to implement this method, as they may affect the outcome.

5.3.3. The effect of gas and liquid temperature in digester

The general principle of a biofilter is to form a biofilm on the packed bed so that it contains immobilized microorganisms [47]. The use of biofilters in anaerobic digestion is common in reducing some of the composition of biogas, such as H_2S and CO_2; reducing these compounds will improve the quality of biogas [38]. Biofilters affect the processing of biogas production by shortening HRT as the microorganisms grow and attach to the bio ball [28], and the wastewater flows up and downward through the column of the biofilter. Therefore, the contact between the microorganism and the substrate becomes more effective. Afrizal et al. [60] reported that the effect of using a biofilter with the addition of FeCl3 in the anaerobic digestion of tofu wastewater can reduce the concentration of pollutants, namely, COD removal 94.09 %, TSS removal 94.02 %, and the biogas produced is 8.190 ml.

The transition from using non-renewable energy to renewable energy in industry, transportation, and agriculture will be supported by the European Green Deal Investment Plan, the new flagship initiative of the. Transition activities are under the "Green Deal", countries that are expected to reduce overall emissions by around 50 – 55 % by 2030 and achieve the 10-year term target [61]. Factors such as HRT, pH, and temperatures influence biogas production. These have an effect not only on the activities of microorganisms but also on the productivity and quality of biogas. The anaerobic microorganism is very sensitive to the pH and temperatures of the substrates. The range temperature of liquid and gas in this study was 29.15 ◦C - 29.70 ◦C. The temperature interval will be different depending on the types of wastewaters as the production of the process in each industry is different [62]. The temperature of liquid and gas in ABR is not much different because the weather temperature in Indonesia is relatively constant year-round, both at night and during the day, about 26.8 – 27 ºC (Fig. 5.).

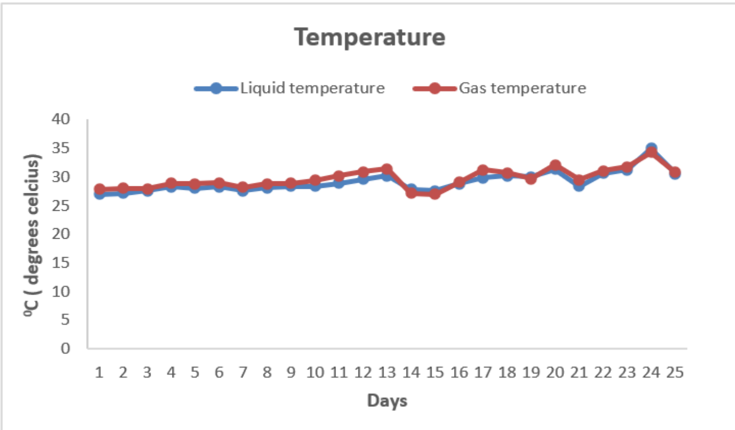

Fig. 5. Temperature of liquid and gas in ABR

The relationship between digester temperature and biogas is very closely related to the anaerobic digestion process, which will affect the quality of biogas; an increase in the temperature in the digestion process will increase the quality of biogas [63]. Temperature is the most important parameter for the biological model that can be affected by the metabolism of microorganisms in the digester; the higher the temperature, the faster the development of biogas [64]. The effect of temperature on the microbial substrate is the absorption kinetics rate at a stable gas formation rate [65].

The low temperature affects the anaerobic digestion process. Low temperatures harm anaerobic digestion. With a decrease in the temperature of the anaerobic process, the result is an increase in the viscosity and solubility of the liquid of gas compounds while reducing the diffusivity of the dissolved substrate [66]. There are advantages and disadvantages of the effective temperature effect in the anaerobic digestion process, such as the activities of microorganisms to form and consume acidity, and the fermentation process is not balanced [67]. The mesophilic temperature is very ideal for the anaerobic digestion process because it has a significant effect on quality, especially methane and productivity biogas, in kinetics and conversion [63]. Temperature significantly affects the anaerobic digestion process, and higher temperature will enhance the dynamic of microbial growth to allow waste to be more optimally converted into biogas [68]

5.3.4. The efficiency of TS and VS removal

The advantage of using a biofilter for an anaerobic digester is that the inlet substrate does not easily carry biological solids, and the HRT is shorter. The high concentration of biological solids allows for treating the high-strength of soluble wastes [69]. The recommended TS content for food waste treatment is 15–20 % due to its suitability for hydrolysis and methane production [70]. The use of biofilters for anaerobic digestion was reported by Nguyen et al. [25]. Combining a biofilter and a scrubber significantly reduces greenhouse gas emissions, but does not significantly reduce methane concentration in waste gas. The efficiency of the cleaning process through the anaerobic filter is 80–95 %, which can be an option for wastewater treatment plants (WWTP) [71].

The use of biofilters in ABR efficiently removes the solid content of biogas effluents such as TS and VS. The TS/VS ratio is a crucial parameter in the anaerobic digestion process, serving as an indicator of the organic content in the substrate (C. [72]). As shown in Fig. 6 the average of the TS biogas effluent is 0.17 %, and the efficiency of (TS removal) is 50 %. Fig. 6 also shows that the TS/VS ratio of biogas effluent gradually increased from 0.06 in the first week up to 0.08 % in the third week, but in the 4th week, the ratio slightly decreased to 0.12. A higher TS/VS ratio indicates a higher organic content, which can be affected by the anaerobic digester process, whether wet or dry. The TS/VS ratio determines the ability of the substrate to produce biomethane under anaerobic conditions, with a higher ratio related to the COD/VS ratio indicating a higher potential for biomethane production [73,74]. The TS/VS ratio also affects the stability of the effluent from the digester; the VS/TS ratio of 0.54 is in HRT on day 7, reducing organic content <45 % [75]. Based on Fig. 6 the highest TS/VS ratio is 13 % in the third week with TS and VS removal, respectively 0.2 and 1.5 %.

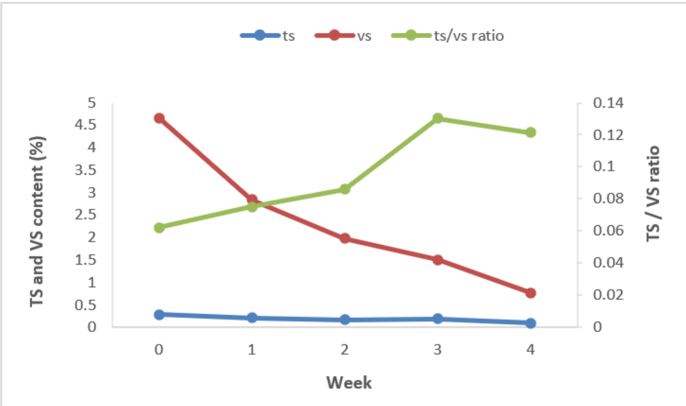

Fig. 6. TS/VS ratio of biogas effluent in acclimatisation term

However, the results of this study show that the ratio of TS/VS is small as the substrate used is tofu wastewater with almost no solid content. Biogas production depends on the types of feedstocks that are being fermented. Generally, the concentration of solid sewage sludge digestion is approximately 8 and 10 % [71]. Therefore, based on the results of the total solid in Fig. 6 it can be concluded that there is no solid content in the biogas effluent due to the application of the biofilter in the digester. The best combination of technology for tapioca wastewater treatment in COD removal is the Anaerobic-Aerobic Fixed Bio-filter (2F2B) with bee-nest shaped biofilter which contains the indigenous bacterial consortium, and the efficiency of TS removal is 86.2 % [76].

Some factors in this study affected the TS of biogas effluent, namely: I) the types of substrates, and tofu wastewater that contains a small amount of TS that is 0.38 %. II) Filtration before loading substrates into the ABR. The first step before loading the substrate into the ABR is filtering to make retention time shorter and anaerobic digestion faster. III) The solid is stuck in the biofilter and forms a biofilm. Controlling and maintaining biomass on the filter surface is the key point in the use of biofilters. There are three phases of the pollutant removal mechanism by biofiltration, namely solid, liquid, and gas. Attached biomass that grows around the filter medium is an important factor in the efficiency of the biofiltration system [77]. The operation of biofilters depends on microbial activity; for consistent and effective operation, a constant source of substrate is required [23]. The biofilter system uses the activities of microorganisms to improve the quality of water or air by biological oxidation of various organic substances. There are three factors of biological process in biofilters for wastewater treatment; the attachment of microorganisms in the biofilter, growth, decay, and detachment of microorganisms of microorganisms [78].

5.3.5. The efficiency of CODremoval in acclimatisation term

The use of biofilter in ABR during the acclimatisation terms affects not only biogas production and quality, removal of the TS and TS/VS ratio of biogas effluent but also COD removal. COD removal is a crucial process in wastewater treatment, and acclimatisation plays a significant role in optimising this process. Acclimatisation involves adapting microorganisms to specific conditions to enhance their ability to remove pollutants, including COD [79]. Based on Fig. 7. COD removal efficiency is 95%, a decrease from 12400 to 100 mg/L. At first, COD removal is not much, around 61.67%, as the microorganism still does not show much growth on the surface of the biofilter. However, over time, the efficiency of COD removal increases by up to 95%. The acclimatisation process also refers to the adaptation of microorganisms and growth in the media filter to attach and form the biofilm; usually, stable conditions can be achieved after an acclimatisation process of approximately 30 days from the perspective of COD removal [80]

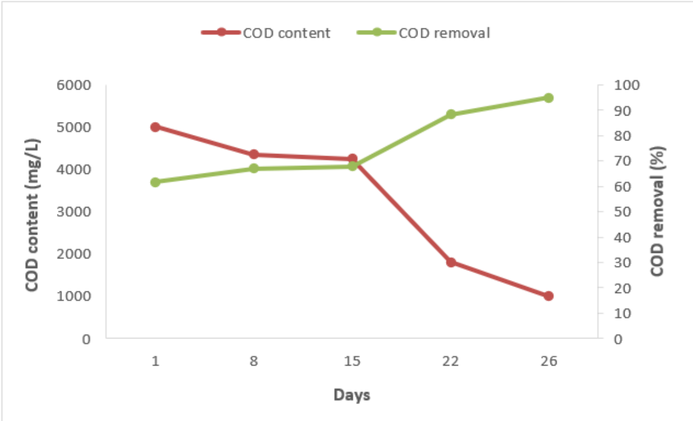

Fig.7. COD removal during the acclimatisation term

The efficiency of COD removal during acclimatisation term varies depending on the specific conditions and system used. The efficiency of COD removal in the bio-electrochemical system (BES-UASB) is 82%, with biogas production yields of approximately 0.33 m^3/kg-CODremoved, and biomethane production greater than 60% [81]. To achieve high efficiency of COD removal during the acclimatisation period, several operating conditions have been identified as optimal, such as influent COD content, biomass concentration, and (food/microorganism) F/M ratio, and hydraulic retention time (HRT) [82].

Acclimatisation can also affect the initial phase of COD removal; for instance, wastewater treatment with the optimal glycogen accumulation organisms (GAO) at 21.34 % results in a COD removal rate of 90.2 % [83]. Thus, the evaluation of the capacity to remove COD is essential. According to Ya'acob et al. [84], the treatment of municipal wastewater using an acclimatised mixed culture reaches the optimum of COD and N removal, respectively, 70.41 % and 64.29 % achieved at 150 rpm with HRT for 5 days. Another important key in COD removal of this study is the use of biofilter, which means biofilters are effective in removing COD from wastewater. According to various studies, the efficiency of COD removal by biofilter is influenced by factors such as HRT, temperature, and OLR. The use of biofilters filled with fly ashes from sewage sludge thermal treatment (FASST LWA) as an effective method for airport de-icing wastewater, obtained the highest N removal reached at the temperature of 00 C (34.93 ± 4.54 %) and hydraulic at 5.0 L m^2 d^{-1}, however, the most efficient in COD removal is 82.35 % ± 1.53 % at temperature 25 and hydraulic loading of biofilter to 10.0 L m^{-2} d^{-1} [85]. In general, acclimatisation is a critical factor in improving COD removal in wastewater treatment, and understanding its effectiveness in COD is essential to optimise treatment processes.

5.4. Conclusion

The use of biofilters in ABR for tofu wastewater treatment significantly increases the production of biogas and methane quality, respectively, 69.48 L/day and 58.05%, and the efficiency of COD removal is 95%. The operation of the biofilter is based on the microorganism that sticks to the surface formed by the biofilm to convert it into bioenergy that very effectively removes the total solid content of effluent biogas of approximately 44% (from 0.38% to 0.17%) with TS/VS ratio 0.13 which can be useful for utilising the effluent as a liquid organic fertiliser material. The advantage of using a biofilter in anaerobic digestion is that it makes the HRT shorter and more economical due to the density of the inoculum and the substrate attached to it, so it can be used in the long term. This is highly recommended for industrial-scale wastewater treatment that produces large amounts of wastewater in the production process.

5.5. Recommendation

The use of a biofilter in ABR is a simple and economically friendly technology that can be used for the long term to prevent the inoculum from being easily carried during substrate loading. Furthermore, biofilter technology is highly efficient in removing pollutants through an anaerobic process and increasing biogas quality. Continuous AD method wastewater treatment with high discharge and shorter HRT is highly recommended.

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Ethics approval and consent to participate

Not applicable.

Consent to publication

All authors have read and agreed to publish this version of the manuscript.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Credit authorship contribution statement

Lydia Mawar Ningsih: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis. **Udin Hasanudin:** Writing – review & editing, Validation, Resources, Methodology, Investigation. **Hynek Roubík:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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6. The Effect of Organic Loading Rate (OLR) Concentration in Anaerobic Baffled Reactor (ABR) With Biofilter to Enhance The Quality of Biogas

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Abstract

The utilization of tofu industry wastewater into biogas has a great advantage that can towards the industry to become sustainable because it can prevent pollution in the environment, including social economy aspects. The use of simple technology biofilter it can shorten the hydraulic retention time (HRT) and improve the quality of production and composition of biogas and effluent. This study aims to obtain the effectiveness of biofilters in anaerobic baffled reactors (ABRs) and optimize organic loading rate (OLR) variants to improve the quality of biogas produced in enhancing the efficiency of pollutant removal. The loading method is continuous with variant OLR concentration at 0.66, 1.33, 2, and 2.67 KgCOD/m^3.day. The result shows that the highest CH_4 concentration is 60.64 % achieved on an OLR of 2.67

KgCOD/m^3.day, however, the highest biogas production is 310.49 L/day at an OLR of 2 KgCOD/m^3.day. The use of biofilters is very effective in removing COD and has a positive impact on the VFA/alkalinity ratio for all OLR concentrations. The efficiency of COD removal is 92.87%, from 5220 to 240 mg/L at an OLR of 0.66 KgCOD/m^3.day. However, the concentration of H_2S at an OLR of 2.67 L/day KgCOD/m^3.day is still high, 630 ppm, but smaller compared to 980 ppm at an OLR of 0.66 KgCOD/m^3.day. Thus, it can be indicated that the combination of biofilter in ABR is significantly effective in increasing biogas production and methane concentration, also in removing COD, and the VFA/alkalinity ratio.

Keywords: wastewater; biogas; biofilter; renewable energy; methane concentration

6.1. Introduction

The current trend of using food manufacturing waste for energy conversion is driven by the increasing demand for energy because of climate change. Industrial sustainability is driven by regulations that incentivize the integration of waste utilization into industrial processes (Musa et al., 2018). Converting tofu industry waste into biogas is a viable solution that not only helps mitigate environmental impacts but also offers a sustainable alternative to non-renewable energy sources currently used in tofu production, thereby reducing the industry's reliance on fossil fuels. (Herdiana et al., 2022). The process of anaerobic digestion (AD) is related to the activities of microorganisms that can convert biomass into biogas with methane (CH_4) as the main product that can be used for combustion (Nagao et al., 2012). The production of high-quality biogas is significantly influenced by two critical factors: the optimal dosage of the substrate and the selection of suitable substrate types, both of which play a crucial role in the anaerobic digestion process (Nwokolo et al., 2020). As the primary energy source for microorganisms, the composition of the substrate has a profound impact on biogas production, with the carbon-to-nitrogen (C/N) ratio being a critical parameter that influences the delicate balance of the anaerobic digestion process, thereby affecting the overall efficiency and quality of biogas generation (Cioabla et al., 2012; Uddin & Wright, 2023). The type of substrate used also influences biogas production, with different substrates producing varying amounts of methane and carbon dioxide (Almomani & Bhosale, 2020). Furthermore, the dosage of substrate affects the efficiency of the anaerobic digestion process, with a high water content decreasing the efficiency of methane per ton of fresh matter (Czekała et al., 2023).

In general, careful selection and dosing of the substrate are essential to optimize biogas production. Raw materials for biogas production are classified into two primary categories: monosubstrates and cosubstrates. Monosubstrates, comprising slurry, manure, and ruminant animal stomach contents, possess inherent fermentation capabilities due to the presence of methane-producing bacteria and provide a broad spectrum of macro- and micronutrients essential for microorganism growth. On the contrary, cosubstrates, including agricultural by-products, energy crop biomass, and greenhouse waste, are added to the digester to optimize process efficiency, ensure adequate hydration, and prevent inhibition (Akande et al., 2023; Ignatowicz et al., 2021, 2023).

A direct correlation was observed between the mass of added corn silage and the fermentation mass index, as measured by the VFA/TIC (total inorganic carbon) ratio. Following an adjustment of the corn silage dose from 31 t/d to 27 t/d on the 70th day of operation of the biogas plant, the VFA/TIC ratio stabilized at a consistent level of approximately 0.3 - 5, indicating a balanced fermentation process (Ignatowicz et al., 2021). Furthermore, the inoculum/substrate ratio was found to have a significant impact on maximum specific biogas production. In particular, an increase in the inoculum/substrate ratio to 1, as observed in Digester D-1, resulted in a substantial 32.4% increase in biogas production, from 0.25 to 0.37 L/g.Vs/day. Furthermore, improvements in biogas production were also achieved at ratios of 2 and 4, compared to the control experiment

(Owamah et al., 2021). Similarly to the impact of increasing the OLR in the reactor, the quality of biogas produced through anaerobic digestion is significantly influenced by the organic loading rate (OLR), which is, in turn, dependent on the characteristics of the substrate fed into the reactor, including its chemical oxygen demand (COD), biochemical oxygen demand (BOD) and total solids (TS) content (Jiang et al., 2020; Moguel-castañeda et al., 2020).

OLR is a measurement of the quantity of the inlet substrate that loads into the digester in the unit (g/L^{-d}), OLR can be increased by the influent flow rate and inlet substrate concentration, there are no constraints due to excess substrate, as OLR increases, biogas production also increases (Labatut & Pronto, 2018). OLR plays a role in the wastewater treatment process; optimum OLR depends on the types of substrate used, including the characteristics of the organic substrate (Jayanta Bhattacharya et al., 2018). Increasing the OLR means that the concentration of substrate in AD also increases the results of biogas yield. The amount of OLR 1.37, 2.74, and 6.85 kg VS substrate/(m^3.d) produces biogas yields in 438.9, 477.3, 480.1, and 188.7 mL/(g VSsubstrate.d), respectively (M. Sun et al., 2017). Increasing OLR from 2.05 to 3.15 kg VS/m^3.day in mesophilic temperature improves biogas production by approximately 73% and methane quality by approximately 10.5% (Huang, 2012). When OLR increases at 4.33 $kg/m^3.d$ in a fixed-bed anaerobic reactor at 4 °C, biogas production is 5.33 L, 69.3% methane, and the COD removal rate is 59.8%, also, the pH value range is stable from 7.2 to 6.8 (H. Zhao et al., 2020).

Another important factor that influences the quality of biogas production is the type of substrate. The type of substrate used in AD significantly influences biogas yield, quality, and process stability (Liu et al., 2022). It is because the characteristics of the substrate, such as C/N ratio, pH value, and chemical composition, play a pivotal role in determining efficiency and stability in the AD process, which involves the degradation of kinetic biomass (Nwokolo et al., 2020). This study uses tofu wastewater as a substrate for the AD process. Tofu wastewater is rich in protein, which can provide Nitrogen as the main nutrient required by anaerobic microorganisms, and neutralization of the VFA effect by ammonia generation (Wresta et al., 2021). Tofu wastewater has a high COD content of around 7796.07 mg/L, TS 1.07%, pH 5.12, and VS 68.99 mg/L (Darwin et al., 2019). Moreover, tofu wastewater has a high organic content, namely, carbs, lipids, and protein, these components can be used for renewable energy like biogas. The ratio of 50% of tofu wastewater : 50% cow manure with HRT 168 hrs resulted in the highest biogas pressure of 2.5 mm H_2O (Purwanti et al., 2023). The use of tofu wastewater as biogas feedstock with biofilter in ABR resulted in biogas accumulation of 1806.4 L during the acclimatization and methane concentration of 58.05% CH_4, with the efficiency of COD removal 95% (Ningsih, Hasanudin, et al., 2024). Moreover, it is necessary to use technology that can improve the quality and production of biogas, one of which is a biofilter that can be combined with a variant of OLR.

The biofilter is a technology that is used to improve the quality of biogas, very effectively removing the content of BOD, COD, and pollutants such as H_2S and NH_3 (Montebello, 2013; Su et al., 2014). The configuration of the aerobic-anoxic trickling filter (AATF) is efficient for nitrification and denitrification at hydraulic load (5.6 m^3 m^{-2} d^{-1}) and organic load (0.26 kg COD m-3 d-1) N removal ranging from 60 to 74% with effluent ammonia-N less than 13 mg/L, with COD removal 90% (Victoria & Foresti, 2011). The biofilter removed 30% TOC, 50% non-methane volatile organic carbon (NMVOC), 51% NH3, and 6% CH_4, however, the concentration of N_2O increased by 26% (Nguyen et al., 2014). In addition to that, the biofilter also has the function of controlling the emission of waste gases from processed anaerobic digestion, the ORGUS® biofilter successfully removes the pollutant 92% of volatile organic compounds, NH_3 <0.1 ppm, and 1 ppb H_2S, a decrease in the average value of 373 $ou_E.m^{-3}$ (Sempere et al., 2015). It can be concluded from the previous study that the biofilter is not only good for biogas quality but also increases the biogas effluent (Victoria & Foresti, 2011). This paper is a further study on the utilization of tofu wastewater as a raw material for biogas using simple biofilter technology on ABR. This study aims to investigate the effectiveness of integrating biofilters in anaerobic baffled reactors (ABRs) and optimizing OLR concentration variants to improve the quality of biogas produced and the efficiency of pollutant removal.

6.2. Materials and methods

6.2.1. Location

This study was carried out on a pilot scale, and all the laboratory analyses were conducted in the Agro-industrial Waste Management Laboratory, Agro-industrial Technology Faculty, University of Lampung, Indonesia.

6.2.2. Organic loading rate (OLR)

The organic loading rate (OLR) refers to the daily amount of waste or organic material loaded into the reactor per unit reactor volume. OLR is a crucial parameter in anaerobic digestion processes, as it determines the flow of organic matter to be degraded and ultimately converted into biogas (Dabestani-Rahmatabad et al., 2024). The calculation of OLR in this study is in equation (1).

$$\text{OLR} = \frac{S}{HRT \; x \; 1000} \qquad \text{(Equation 1)}$$

where S is the COD substrate concentration, HRT is the hydraulic retention time, and OLR is the organic loading rate (KgCOD/m3 ·day). Table 1 provides the OLR for each treatment.

Table 1. The OLR for each dosage substrate loaded into the ABR

Dosage substrate (L/d)	OLR (KgCOD/m3 ·day)
50	0.66
100	1.33
150	2
200	2.67

6.2.3. Experimental setup

6.2.3.1. Anaerobic digestion system

The anaerobic digester used in this study is an anaerobic baffled reactor (ABR), the total volume is 0.927 m^3. The Bio ball plastic or biofilter is put inside the ABR the total of biofilters used in the chamber of the ABR is 700 balls with a total weight of 3.560 kg. The anaerobic baffled reactor (ABR) was inoculated with a 1:1 ratio of inoculum to substrate (50% : 50%) and recirculated for 7 days to establish a stable environment throughout the chamber, with a pH of 7.16. This step was crucial in preventing the inhibition of microbial metabolism and ensuring optimal conditions for biogas production. The substrate dosage for start-up is 30 L/day with continuous loading.

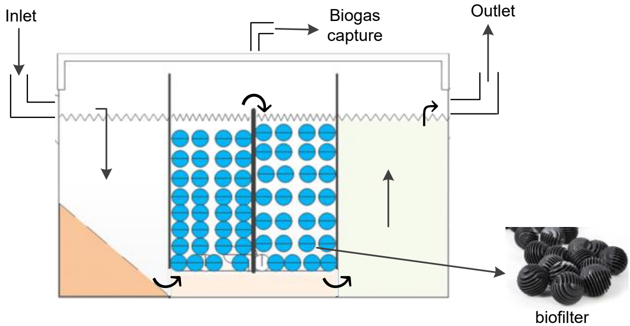


Fig 1. Design of an anaerobic baffled reactor combined with a biofilter

The anaerobic baffled reactor (ABR) was operated at a hydraulic retention time (HRT) of 30 days for acclimatization, with its performance monitored through tracking of COD removal and biogas production to ensure a stable environment. Upon achieving stability, the reactor was deemed ready for operation, and the OLR was incrementally increased from 0.66, 1.33, 2, to 2.67 KgCOD/m3·day, enabling the assessment of the ABR's performance under varying conditions.

6.2.4. Data collection

6.2.4.1. Initial data

The initial data consists of the characteristics of tofu wastewater and inoculum:

a. Inoculum:
   Analyzed to obtain the data about C/N ratio, TS, VS, Alkalinity, VFA, and pH.

b. Tofu wastewater characteristics
   Tofu wastewater was analyzed to obtain about CODs, COD, TS, TSS, pH, alkalinity, and VFA

6.2.4.2. Anaerobic digestion results

The data of anaerobic digestion is used to obtain the quality of biogas production, which consists of biogas effluent and biogas production:

a. Effluent biogas
   The biogas effluent was analyzed every 3 days (twice in a week) to obtain COD, VFA, alkalinity, and pH.

b. Biogas production
   Biogas production is observed every 1 x 24 hours using a gas flow meter (wet gas flow meter) model W-NK-10 A SINAGAWA.

c. Biogas composition
   The composition of biogas and the quality of methane were observed to obtain the presentation of the gas composition (CH_4, CO_2, and N_2) using the Shimadzu

Shincorbon ST 50-80 D-375 gas chromatography (GC) model.

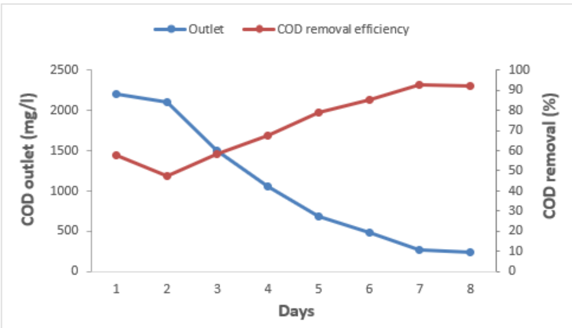
6.3. Results and Discussion

6.3.1. COD removal

COD is a key parameter for measuring pollutants and the quality of water, wastewater, and aqueous hazardous waste (Hu & D Grasso, 2005). COD is the amount of oxygen that is consumed in the chemical oxidation of organic matter by a strong oxidant. Based on Fig. 2. COD removal rates exhibited a non-linear response to increasing OLR. Specifically, COD removal at OLR of 1.33 and 2 $KgCOD/m^3 \cdot day$ is 77.34% and 81.49%, respectively. However, a subsequent increase in OLR to 2.67 $KgCOD/m^3 \cdot day$ resulted in a rebound effect, with COD removal rates of 65.88%, although it remained significantly lower than optimal removal rates of 92.87% (corresponding decrease from 5220 to 240 mg/L) achieved at an OLR of 0.66 $KgCOD/m^3 \cdot day$. Increasing the OLR concentration in anaerobic digestion can have positive and negative effects on the COD removal; a higher substrate dosage can lead to an increase in biomethane production and COD removal efficiency, as more organic matter is available for microbial degradation (Thakur et al., 2023).

COD removal is an important indicator of the reactor performance, COD removal is more efficient as the OLR increases, which accumulates in the digester when the concentration of OLR is high (Hassan et al., 2015; H. Zhao et al., 2020). Reducing COD removal also affects the reactor reduction sequence, with a sudden increase in the organic loading rate (OLR) from 10.08 to 18.52 g COD/L in the anaerobic membrane Bioreactor (AMBR) and from 10.17 to 23.33 g COD/L in the anaerobic sequencing batch reactor (ASBR), leading to a decrease in COD removal efficiency.

(a)



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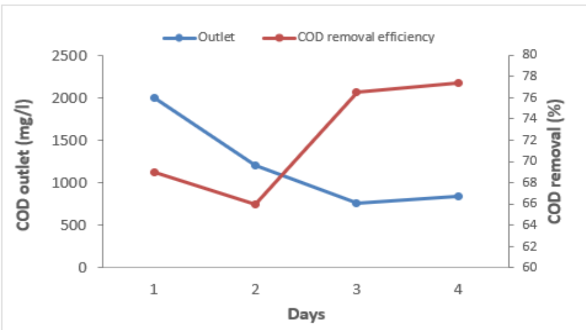

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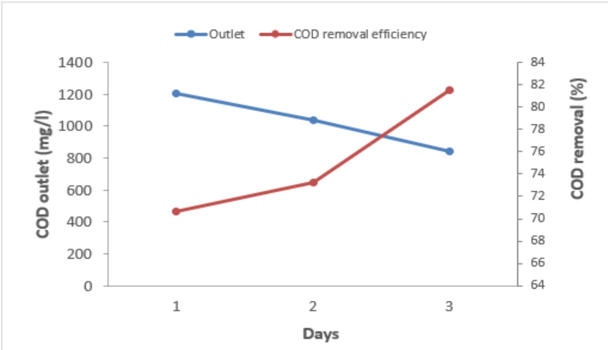

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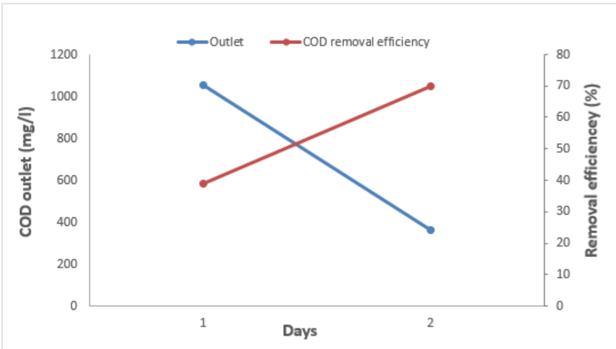


Fig **2.** COD removal at an OLR of (a) 0.66 KgCOD/m^3·day, (b) 1.33 KgCOD/m^3·day, (c) 2 KgCOD/m^3·day, and (d) 2.67 KgCOD/m^3·day.

On the contrary, optimal COD and BOD removal rates of 82.49% and 90.65%, respectively, were achieved at a relatively low OLR of 3.79 g COD/L (Eslami et al., 2018). Excess dosage substrate load can result in anaerobic conditions, leading to a decrease in COD removal efficiency and possibly even inhibiting microbial activity; also, it can lead to mass transfer limitations, which negatively impact COD removal (Kassongo et al., 2022; Kawai et al., 2016).

Additionally, OLR affected the performance of the anaerobic digestion process in terms of organic removal efficiency, VFA yield, methane production rate, and system stability (Musa et al., 2018). Increasing the OLR concentration will affect the COD removal; however, an increase in the OLR at an over-range will reduce the COD removal and cause instability in the reactor performance (Krishnan et al., 2016). Generally, OLR and substrate concentration significantly affect the anaerobic digestion process in degradation performance, metabolic activities, and biogas production (Ahmad et al., 2021). Removal of the chemical oxygen demand (COD) is a complex process influenced by multiple factors, including the concentration and types of substrate, the OLR, the hydraulic retention time (HRT), and the reactor performance. The high removal efficiency for the HRT and dosage substrate variants is influenced by the relatively high concentration of biomass in the reactor (Rinquest et al., 2019). Although the dosage of substrate and OLR plays a significant role, it is not the sole determinant of COD removal efficiency. In particular, HRT also has a profound impact, with shorter HRTs typically resulting in poor COD removal and longer

HRTs leading to improved COD removal rates (Yanqoritha et al., 2018). The COD removal in the UASB at OLR 2.63 kg/m^3 d. was only 30.9%, however, when the OLR decreased to 2.29 kg/m^3 d., the COD removal efficiency improved to 49.8%. The increase in COD removal can be attributed to the alleviation of toxicity caused by high concentrations of ammonia nitrogen, which previously hindered the stability and performance of the UASB reactor (B. Zhao et al., 2015).

Longer HRT has a significant effect on COD removal because it can result in higher COD removal efficiency. For example, a study found that the highest efficiency COD removal is 70.39% achieved for 24 h HRT with OLR 0.895 g COD/L/d at 15 L min^{-1} (Abdulsalam et al., 2020). In this study, the longest HRT is 26 days at an OLR of 0.66 KgCOD/m^3·day, which is very efficient in COD removal among other treatments. The HRT at OLR 1.33, 2, and 2.67 KgCOD/m^3·day, respectively, is 18 days, 9 days, and 6 days. The longer HRT in anaerobic digestion will make the pH and COD peaks the same, as well as in COD flow to the methane reactors (Asplund, 2005). The inverse relationship between COD and hydraulic retention time (HRT) has been substantiated by Herlina et al. (2020), who observed a significant decrease in COD from 1346.4 mg/L to 448.8 mg/L over 6 days, corresponding to a COD removal efficiency of 66.66%. This finding suggests that a longer HRT in anaerobic reactors is conducive to higher COD removal efficiencies, as microorganisms are provided with sufficient time to break down organic matter and reduce COD levels.

Moreover, the combination of biofilters in ABR may influence the shorter HRT with high COD removal efficiency, because biofilter has a large surface area for attached growth, thereby accelerating the AD process due to immobilization of microorganisms (Dorji et al., 2021). The use of biofilter in refinery wastewater treatment reaches the maximum of COD 52 to 56% and TOC removal (43 to 51%) after the circulation 8 - 12 times at a flow rate of 1 ml/min (Sinha & Mukherji, 2024). The use of biofilter in ABR during the acclimatization term results in shorter HRT and significantly removes pollutants; the efficiency of COD removal is 95%, and TS removal is 44% from 0.38 to 0.17 % (Ningsih, Hasanudin, et al., 2024). Moreover, the biofilter mixes with the peat-perlite with HRT 1.1 days, achieving the highest COD removal of 91% and 92% for the color removal (direct blue 2 dye) (Angélica Guillén et al., 2022).

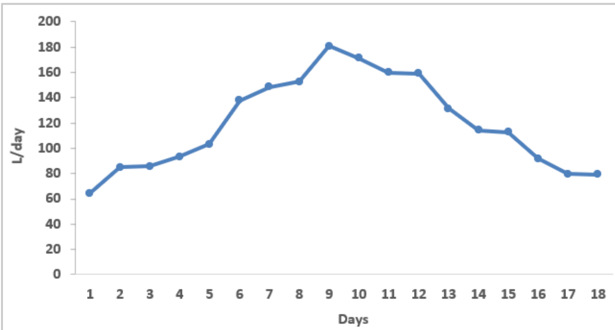
6.3.2. The effect of OLR in the production and composition of biogas

One of the main factors affecting biogas production in the anaerobic digestion process is OLR, especially in the continuous flow mode (Ramanathan et al., 2022). The concentration of OLR loaded into the ABR is significantly related to enhancing biogas production and composition. Based on Fig. 3, the average biogas production at an OLR of 0.66 $KgCOD/m^3 \cdot day$ is 199.50 L/day. Biogas production at OLR 1.33 and 2 $KgCOD/m^3 \cdot day$ is 148.69 and 310.49 L/day, respectively. However, when the OLR increased up to 2.67 $KgCOD/m^3 \cdot day$, the biogas production decreased extremely to 137.11 L/day. The low biogas production at high OLR is because the substrate concentration load into the reactor is too high, resulting in the accumulation of VFA, which can inhibit microbial growth and production (Dabestani-Rahmatabad et al., 2024).

The dosage substrate, also known as the inoculum-to-substrate ratio, an optimal ratio is crucial, as it affects biogas production rates and accumulation (Corsino et al., 2021). The dosage substrate of 33 kg amaranth and 250 L control manure mixture results in 0.542 $Nm^3.m^3\ d^{-1}$, which is 3 times higher than slurry: manure (80:20), namely 0.160 $Nm^3.m^3\ d^{-1}$ (Kristof & Gaduš, 2018). Furthermore, the small biogas production is also influenced by the reactor performance. At an OLR of 10 $g\ L^{-1}d^{-1}$ in two reactors, different biogas production in R1 is 8 L/day, while in R2, with the same OLR, it has the highest result of 27 L/day. Low biogas production due to the shock load received by the reactor, as well as the accumulation of volatile fatty acids (VFA) at a higher OLR, resulted in inadequate growth of microbial biomass

(*Methanosarcina bacteria*) (Musa et al., 2020). Small biogas production in high OLR is because the loading of new substrates in excessive volumes daily will change the environment in the reactor and reduce the performance of microorganisms during acclimatization (initial period), causing the activity of hydrolysis bacteria to be higher than the activity of methanogenesis bacteria (Odey et al., 2018; Tsegaye & Leta, 2022).

(a)



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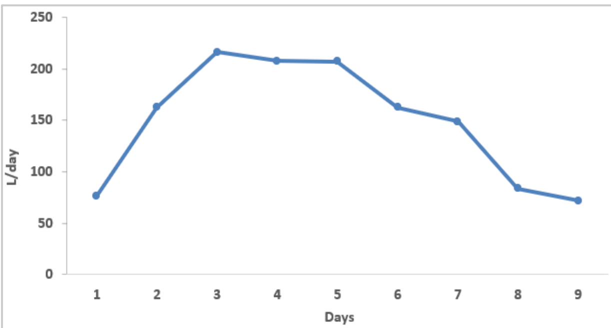

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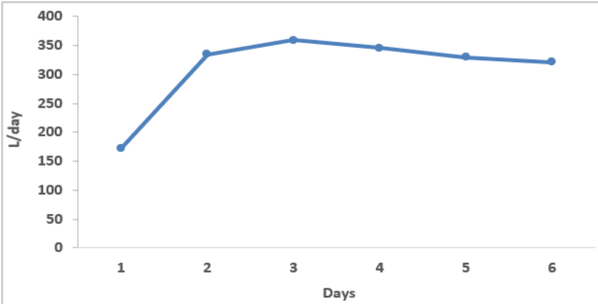

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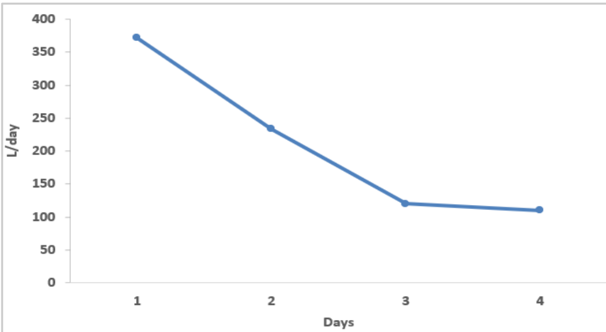


Fig 3. biogas production at an OLR of (a) 0.66 KgCOD/m^3·day, (b) 1.33 KgCOD/m^3·day, (c) 2 KgCOD/m^3·day, and (d) 2.67 KgCOD/m^3·day.

On the other hand, the optimal conditions for biogas production are achieved with a longer hydraulic retention time (HRT) and a smaller OLR, whereas high OLR and shorter HRTs result in reduced biogas production. The longer hydraulic retention time (HRT) allows for a more thorough breakdown of organic compounds, enabling the degradation of non-biodegradable matter and subsequent conversion into biogas. On the contrary, a shorter HRT may not provide sufficient time for complete degradation, resulting in residual organic matter that cannot be converted to biogas (Odey et al., 2018).

However, according to Fig.3. the highest biogas production is 310.49 L/day achieved at an OLR of 2 KgCOD/m^3.day with HRT 6 days, and the lowest is 137.11 L/day at an OLR of 2.67 KgCOD/m^3.day with HRT 4 days. A high concentration of OLR load into the reactor may positively and negatively affect biogas production and microbial biomass development. A high-OLR can lead to increased biogas production, as it provides more substrate for microorganisms to break down and convert to biogas (Moestedt et al., 2013). The highest hydrogen consumption rate of 68 mg COD/L/h was observed at an OLR of 3.25 g VS/L/d, which was the maximum value tested. In particular, this increase in hydrogen production did not result in a significant accumulation of organic acids, despite elevated partial hydrogen pressures (Dabestani-Rahmatabad et al., 2024). The concentration of substrate has a dual impact on the anaerobic digestion process. Not only improve biogas production, but it also affects the quality of the biogas production, specifically the methane content (Aili Hamzah et al., 2023). The biogas composition is provided in Table 2.

Table 2. Biogas composition in all OLR

OLR concentration ($KgCOD/m^3.day$)	Biogas composition			
	CH_4 (%)	CO_2 (%)	N_2 (%)	H_2S (ppm)
0.66	49.78	36.105	14.215	980
1.33	56.93	37.64	5.416	410
2	55.26	42.749	1.905	610
2.67	60.64	37.775	1.573	630

The use of biofilter in ABR effectively increases methane concentration, based on Table 2. the highest is 60.64% achieved at OLR 2.67 $KgCOD/m^3.day$, the lowest is 49.78% at an OLR 0.66 $KgCOD/m^3.day$; however, the methane concentration at OLR 1.33 and 2 $KgCOD/m^3.day$ is in the same range, 56.93% and 55.26% respectively. Biofilters influence the composition of biogas because they can remove impurities and contaminants from the biogas stream, thus improving the overall quality and efficiency of biogas production (Nguyen et al., 2014). Based on Table 2. It is indicated that the methane concentration is gradually increased with increasing OLR, it is because the OLR significantly affects microbial community activities related to the quality of biogas composition and production that resulting in a low level of syntrophic methanogenesis (Sihlangu et al., 2024).

The increased OLR to 4.0 ± 0.3 g/L/d at R1's top sludge makes archaea dominant, consisting of Methanosaeta 41.6%, Methanobacterium 34.4%, and Methanolinea

13.7%, which cause the breakdown of complex organic matter and release a large amount of hydrogen (Mou et al., 2024). At High OLR, microbial profiling revealed enriched diversity among acidogenic and acetogenic bacteria, facilitating efficient substrate breakdown, which makes an imbalance of acetoclastic and hydrogenotrophic methanogens (Methanosarcina and Methanothermobacter) in the process of biomethane (Yellezuome et al., 2024).

The quantity and quality of biogas are not only influenced by the OLR concentration and the HRT, but also by the types of biofilter used in ABR. There are many types of biofilters used in anaerobic digestion. Biofilters are made from different materials such as sand, stone, activated carbon, plastic, and reticulated foam polymers (Chelliapan et al., 2020). Those materials have an advantage in enhancing biogas production due to their low void volume, but a risk in accumulating non-biodegradable solids gradually (Periyasamy et al., 2021). This study uses a biofilter made from plastic material, which improves biogas production but is still not effective in removing gas pollutants, especially H_2S.

The result in Table 2. shows that the highest H_2S content is 980 ppm at an OLR of 0.66 $KgCOD/m^3$.day, and the lowest is 410 ppm at an OLR of 1.33 $KgCOD/m^3$.day, however, the content of H_2S at an OLR of 2 and 2.67 $KgCOD/m^3$.day is not different. The removal of gases such as methane (CH_4), nitrous oxide (N_2), hydrogen sulfide (H_2S), and ammonia (NH_3) from biofilters in anaerobic digestion plants can improve the composition of biogas. However, several factors affect biofilter performance, including temperature, moisture content, pH, nutrients, oxygen level, gas velocity, and pressure drops (Dumont, 2015).

The H_2S is a significant component of biogas, and its presence has both positive and negative impacts on biogas production, combustion, and overall system operation. The heat produced from burning biogas with an H_2S concentration of up to 200-500 ppmv is acceptable, but it is preferable to a zero content, because more than 500 ppm causes corrosiveness (corrodes metal), lowering engine oil in the cogeneration unit where biogas is burned to produce electrical power, resulting in a malfunction (Rodriguez et al., 2014; Valdebenito-Rolack et al., 2021). The most efficient method for removing H_2S in biogas is biological methods, including biofilters, biotrickling filters, and bioscrubbers. The use of a desulfurization biofilter, the efficiency of H_2S removal ranged from 26.10 to 75.80% (Becker et al., 2024).

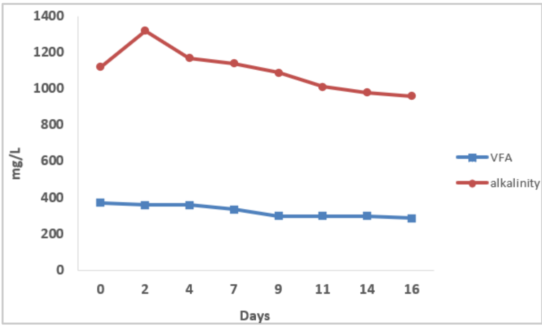
Although using the biofilter, the H_2S content for all doses in this study is still high, more than 400 ppm, which can damage combustion equipment and negatively impact human health. Moreover, based on Table 1. the highest of N_2 in biogas composition is 14.21% achieved at an OLR of 0.66 KgCOD/m^3.day, followed by an OLR of 1.33 KgCOD/m^3.day with 5.41%, which means there is the presence of oxygen in the biofilter that can affect the composition of the biogas during the anaerobic digestion process. It can be concluded that the use of biofilters in this study is inefficient in reducing greenhouse gases, including CO_2 and H_2S, but effectively increases biogas production. Hence, optimizing biofilters for H2S removal warrants further investigation to enhance process efficiency and long-term operational stability in the AD system.

6.3.3. The effect of OLR in VFA and alkalinity ratio

VFA is another parameter related to biogas production in the anaerobic digestion process. The fluctuation in VFA concentration is due to the availability of acetic acid produced by acetogen bacteria to form biogas (Pampang et al., 2020). The concentration of OLR has a significant impact on VFA and alkalinity in anaerobic digestion. Increasing the proportion of organic waste content in the substrate results in increased VFA production. Based on Fig 4. the average VFA content in biogas effluent at an OLR of 0.66, 1.33, 2, and 2.67 KgCOD/m^3.day, respectively, is 327, 270, 352, and 402 mg/L.

The result of this study indicated that the higher dosage substrate (optimum OLR at 2.67 $KgCOD/m^3$.day) results in a high content of VFA, due to the synergistic effects of co-degradation, leading to a higher production of VFA at a higher organic waste fraction (Owusu-Agyeman et al., 2022). However, high substrate loading can decrease pH value, indicating higher VFA production than alkalinity (Owusu-Agyeman et al., 2020a). The relationship between VFA and alkalinity is a critical balance in the anaerobic digester.

(a)



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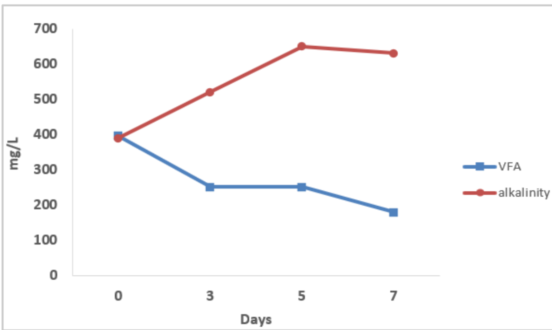

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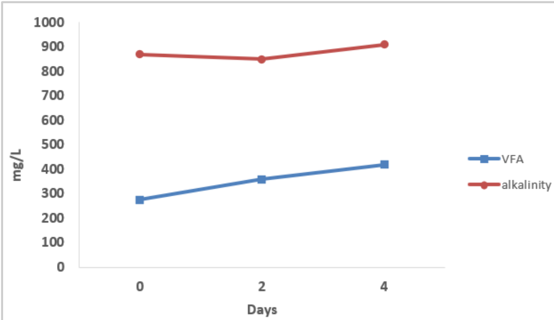

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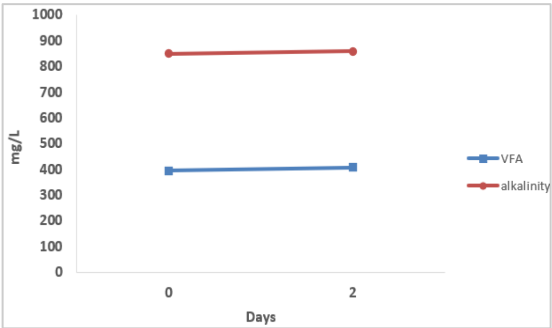

Fig 4. Ratio VFA/ALK at OLR of (a) 0.66 KgCOD/m^3.day, (b) 1.33 KgCOD/m^3.day, (c) 2 KgCOD/m^3.day, and (d) 2.67 KgCOD/m^3.day.

A change in this balance may occur days before a pH change, and maintaining a specific ratio between VFA and alkalinity is essential (Hamawand & Baillie, 2015; Palacios-Ruiz et al., 2008). Alkalinity plays a crucial role in anaerobic characterization, as it represents the buffering capacity of water to neutralize acids formed during the digestion process (Bernie Sheff, 2019). This buffering capacity is essential to maintain a stable pH, which is critical for the optimal growth and activity of microorganisms, particularly methanogens involved in anaerobic digestion. Moreover, alkalinity helps to neutralize the VFA produced during anaerobic digestion, preventing a decrease in pH that could inhibit the digestion process (S. Chen et al., 2015; Gopikumar et al., 2016). A healthy balance between VFA and alkalinity can be achieved through careful operational control of feed rates, mixing, and heating treatments (Inizan et al., 2019; Waqas et al., 2018). The ratio of VFA/ALK in this study at OLR of 1.33, 2, and 2.67 KgCOD/m^3.day is not much different, respectively 0.49, 0.40, and 0.47. However, the VFA/ALK ratio at an OLR 0.66 KgCOD/m^3.day is 0.3. According to the reference that the range of VFA/ALK ratio is 0.1 to 0.35, and 0.1 to 0.25 is ideal; however, if the ratio is 0.5, the digester situation is sour (Council, 2017).

Based on Bioenergie (2016), the ratio of VFA/ALK is should not be higher than 0.8. The VFA/ALK ratio is often used for process evaluation because the analysis results of different processes are not comparable due to the empirical nature of the formula. A ratio above 0.8 may be a sign of process instability and impact the low biogas production and failure (Aramrueang et al., 2022; Calabrò et al., 2021). The ratio of VFA/ALK for all OLR concentrations in this study is ideal, due to under 0.5 and 0.8. The VFA/alkalinity ratio, along with the percentage VS, has a significant impact on the digestion process. The performance of the digester is linked to the ratio VFA/alkalinity (VFA/ALK), ideally around 0.3 $gCH_3COOH/gCaCO_3$, to ensure digester stability and optimal biogas production. The treatment of raw compost in completely stirred tank reactors reaches the tolerated VFA/ALK values of 0.5 $gCH_3COOH/gCaCO_3$, however, this value is higher than the technical literature assumed (Siciliano et al., 2019).

A good VFA/ALK ratio is indicative of healthy microorganisms and a well-functioning biological system. The use of biofilters can help maintain an optimal VFA/ALK ratio by providing a suitable environment for microorganisms to grow and thrive. In an acidic hydrolysis environment, biofilters utilize naturally occurring bacteria to oxidize the odor-causing compounds, including VFA (Pressley et al., 2023). The use biofilter can decrease VFA content and odor of effluent. In this study, the use of a biofilter is very efficient in the removal of VFA in variants of OLR concentration at 0.66, 1.33, 2, and 2.67 KgCOD/m^3.day, respectively, is 83.44, 89.20, 67.88, and 73.84%. The use of biofilter for long-term operation is highly suggested in the removal of VFA, with relatively low and steady pressure drop, the efficiency of removal of VFA is 95% at substrate concentration up to 22.4 g/m^3 (Tsang et al., 2008). The removal of VFAs by biotrickling filters is a biological process that involves the degradation of these compounds by microorganisms. It can be optimized by controlling factors such as temperature, pH, and nutrient availability (Ding et al., 2011; Eregowda, 2019). Overall, the use of biofilter has shown promise as a stable and effective technology for the removal of VFA and increasing the quality of biogas in long-term operation.

6.4. Conclusion

The use of biofilters in ABR is effective in enhancing biogas production, the highest is 310.49 L/day at an OLR of 2 $KgCOD/m^3$.day. However, the highest methane concentration is 60.64% achieved at OLR 2.67 $KgCOD/m^3$.day. Not only effectively enhance the quality and quantity of biogas, but also significantly in removing of pollutants, with the highest COD removal is 92.87% at OLR 0.66 $KgCOD/m^3$.day. Furthermore, the lowest of the VFA/ALK ratio is 0.3, achieved at an OLR of 0.66 kg COD/m^3·day. This value is considered optimal as it remains below the threshold of 0.5, beyond which process instability, souring, and potential operational risk may arise in the ABR. However, biofilters are still not effective in removing H_2S because the levels are still high. The highest of H_2S concentration is 980 ppm at an OLR of 0.66 $KgCOD/m^3$.day. Overall, biofilters demonstrate promise in the AD system. Thus, future research should focus on optimization to enhance both treatment efficiency and biogas quality, particularly through improvements in biofilter design and operational parameters.

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Authors' Contributions Statement

Lydia Mawar Ningsih: Writing - Original Draft, Methodology, Investigation, Visualisation, Formal Analysis. Udin Hasanudin: Methodology, Resources, Writing - Review & Editing, Investigation, Validation. Hynek Roubík: Conceptualisation, Methodology, Validation, Writing - Review & Editing, Funding acquisition, Supervision.

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7. The Effect of Pretreatment on VFA Production from Tofu and Tempeh Wastewater Through Anaerobic Digestion

Adopted from: Ningsih, Lydia Mawar, Mohammad Taherzadeh, Steven Wainaina, and Hynek Roubík. 2024. "The effect of pretreatment on VFA production from tofu and tempeh wastewater through anaerobic digestion." Under review Journal.

Abstract

Tofu and tempeh, derived from soybeans, are widely consumed for their nutritional value and high protein content. However, the production of these foods generates nutrient-rich wastewater that poses environmental challenges while offering opportunities for valorization. This study investigates the production of volatile fatty acids (VFAs) and acetic acid from tofu and tempeh wastewater via batch anaerobic digestion, utilizing various pretreatment methods. The pretreatments included adjustment of the pH to 6 and inoculum treatments with and without heat shock under mesophilic and thermophilic conditions. Results demonstrated that the highest average total VFA concentrations of 10.08 g/L and 9.79 g/L were achieved for tempeh at T3 (tempeh wastewater + pH6 +

thermophilic + heat shock) and tofu wastewater at TF3 (tofu wastewater + pH6 + thermophilic + heat shock), respectively. The highest acetic acid concentrations were observed under mesophilic conditions, reaching 77.32% for tempeh wastewater at T7 (tempeh wastewater + unadjusted pH + mesophilic + heat shock) and 92.40% for tofu wastewater at TF10 (Tempeh wastewater + pH6 + mesophilic + non-heat shock). Notably, increased VFA production was associated with reduced cumulative methane yields, such as 3.65 mL/g-VS for tempeh at T3 and 25.23 mL/g-VS for tofu wastewater at TF3. These findings indicate the effectiveness of the pretreatment strategies in enhancing VFA and acetic acid production, suggesting significant potential for industrial applications. Further research is recommended to optimize production processes and explore the broader utilization of VFAs and acetic acid in the bioeconomy, promoting sustainability.

Keywords: volatile fatty acid; wastewater; sustainability; methane; anaerobic digestion; waste management.

7.1. Introduction

Soy products are the most prevalent protein sources in the human diet. However, they produce substantial amounts of wastewater, approximately 10 liters per liter of raw material. The treatment costs associated with soy and dairy are also high, amounting to 130 US$ per cubic meter of effluent-treated (Wang & Serventi, 2019). The by-products of tofu and tempeh production are solid waste and wastewater. The solid wastes from the tofu and tempeh industries are distinct due to the varying production methods. Solid waste from the tofu industry is commonly referred to as 'tofu dregs or okara', which is more abundant than solid waste from the tempeh industry (banana leaves, plastic and soybean skins). For every kilogram of soybeans processed into tofu, approximately 1.2 kilograms of soybean residue are produced (Szulc et al., 2023). Solid waste from tamari and tofu does not pose environmental harm, as most of it is sold to other industries or farmers for direct use in human food production and animal feed (Azhari, 2016).

Tofu wastewater is formed from the processing production (soaking, washing soybeans, washing the equipment used for the production process, filtering, and pressing tofu in the molding process) (Hajar et al., 2021). However, tempeh wastewater in the process of production is from washing, boiling, soaking, and mixing (Pramaningsih et al., 2022). The quantity of wastewater from tofu process production is tremendous, in the case of the small-scale industry with a capacity production of 150 kg of soybeans per day produced 147 kg of tofu, and the by-product generated is 71.6 kg of solid waste consists of 60 kg pulp and 11.6 kg soybeans skin, and

637.3 L wastewater (Septifani et al., 2021). Tofu and tempeh wastewater still have a high protein and nutrition that can be utilized as other materials, such as bioenergy, and as acid solution sources due to the effect of acid solution used in the coagulation process, such as acetic acid (CH_3OOH) and calcium sulfate ($CaSO_4$) (Yudhistira et al., 2016). The types of acid solutions used for coagulation and the method used in the production process of tofu impact the characteristics and quality of wastewater like BOD, COD, TSS, and pH value (Sayow et al., 2020). The characteristic of tofu and tempeh wastewater is provided in Table 1.

Table 1. Characteristic of tempeh and tofu wastewater

Parameter	Tofu wastewater *	Tempeh wastewater **	Unit
BOD	7904	4146.50	mg/L
COD	2290	32297.71	mg/L
TSS	64	0.75	mg/L
pH	2.65	4.8 ***	

Sources: * (Amalia et al., 2022), ** (Pakpahan et al., 2021), *** (Nurhayati et al., 2011).

There are some obstacles for industry owners, especially in micro, small and medium enterprises (MSMEs) in managing wastewater, namely lack of knowledge in waste management, the narrow space to manage their wastewater, financial and technical issues, including lack of training and campaign from stakeholders linked to industrial activities. Moreover, the production of tofu and tempeh in Indonesia is often scattered throughout urban areas, making it difficult to implement efficient whey utilization practices (Crops, 2016; Ningsih, Mazancová, et al., 2024). Hence, to prevent the risk of causing harm to the environment and social aspects, it is vital for the soy processing industry to manage and recycle the wastewater by considering environmental, social, and economic factors, even though it will impact the production cost (Li et al., 2021; Puspawati & Soesilo, 2018). Those challenges can be achieved by implementing alternative waste management strategies that address the chemical and physical characteristics and pollution load in each step of the wastewater generation process (Pramaningsih et al., 2022).

A highly promising solution is to utilize soy wastewater to produce VFA as the acetic acid source through anaerobic digestion. Usually, micro and small-scale tofu industries in Indonesia use tofu whey for coagulation due to its economic friendly and easy-to-use (Yuwono, Sudarminto S. Waziiroh, 2020). Whey can serve as a coagulant in the food industry, similar to the tofu production process, offering an alternative to conventional coagulants (Corzo-Martínez et al., 2016). Utilization of soy wastewater, especially tofu wastewater is extensively used in the recovery of compounds or nutrition for microbial or enzymatic treatment to produce new beverages like nata de soya (Chua & Liu, 2019;

Puspawati et al., 2019). The addition of 30% *Acetobacter xylinum* bacteria to tofu wastewater in producing nata de soya resulted in the highest organoleptic tests (color, flavor, and elasticity), thickness (2.76 cm), yield (51.4%), fiber (54.025), and water content (85.8%) (Marlinda & Basuki, 2023). Even though research on soy whey coagulation is still in its nascent stages compared to dairy whey coagulation, which shares common objectives, dairy whey has been extensively studied for its potential applications in the production of bioactive peptides, lactose, and milk fractions with distinct properties (Barba, 2021; Rebouillat & Ortega-Requena, 2015).

In contrast, soy whey has been extensively studied for its potential applications in the production of soy protein isolates, soy cheese, and other soy-based products. Conversely, dairy whey research has a more extensive history, with a greater emphasis on its nutritional and functional characteristics. Although soy whey research is still in its nascent stages, it holds the potential to provide valuable insights and knowledge that can be applied to the valorization of dairy whey (Figueroa Pires et al., 2021; Hueso et al., 2022). VFA production through an anaerobic digestion process is a traditional alternative that promises benefits from both economic and environmental aspects (Pinto et al., 2023). VFAs are produced from the intermediate phase of the newly developed anaerobic digestion process (Owusu-

Agyeman et al., 2020b; Patel et al., 2021). The product of anaerobic digestion is biogas with H_2 and VFAs as intermediate products. Despite the challenges associated with VFA production, it has garnered significant attention due to the superior value-added compared to biogas. The biogas market in 2024 is $82.9 billion at a compound annual growth rate (CAGR) of 9%, and the global market of VFAs is $98.2 billion at a CAGR of 9.5% (Business Research Company, 2024). VFA production through an anaerobic digestion process is achieved at a shorter hydraulic retention time (HRT) than for biogas. Although VFAs are composed of 2-6 carbon organic acids, the primary acids are typically acetic acid and butyric acid. These acids can be converted into high-value chemicals such as bioplastics and biofuel, which confer a higher economic value compared to biomethane (Sun et al., 2025).

To optimize VFA production during anaerobic digestion, pretreatment is of paramount importance. The key pretreatment techniques employed to achieve this include pH adjustment, temperature control, heat shock treatment for microorganisms, and methane inhibition (Castro-Fernandez et al., 2024; Strazzera et al., 2018; J. Sun et al., 2021). The pH value is a very essential factor that impacts VFA yield and affects to the competition between acidogenesis and methanogenesis in the process of anaerobic digestion. At pH 5.5 the highest concentration of VFA composition is acetic acid around

50%, however, at pH 7 obtaining a VFA yield of 36.6% g COD-VFA/g COD substrate with 20% of propionic and 30% butyric acid (Castro-Fernandez et al., 2024). Additionally, heat shock or thermal treatment ranges from 140 – 170 °C, and the addition of 0.5–3% HCl and H_2SO_4 increases the solubilization of organic matter and can inhibit methanogens (Strazzera et al., 2018). The effect of pretreatment influences microbial diversity; thermal treatment will reduce it and be more selective. The combination of thermal and acid pretreatments significantly affects to shift of the dominant microbial communities from non-dominant into more prominent such as Cloacimonadota and Spirochaetota (Hidalgo et al., 2023). This study aimed to obtain optimum total VFA production from tofu and tempeh wastewater through batch anaerobic digestion with adjusted substrate pH, heat shock inoculum pretreatment, and temperature treatment in the water bath.

7.2. Materials and methods

7.2.1. Sample preparation (tempeh and tofu wastewater)

The wastewater was generated through the simulated production of tofu and tempeh in a laboratory setting. The preparation of tempeh and tofu wastewater differs due to the distinct production processes involved. In brief, soaking soybeans overnight makes the size of soybeans bigger than before soaked and soft thus easy to peel.

Then, the soybeans were washed to remove all the dirt after peeling, and the soybeans were boiled until the white foam came out. To obtain tempeh wastewater, the boiled soybeans are filtered, and the boiled water is collected. Tempeh wastewater used in this study was collected from the soaking, washing, and boiling steps. The flowchart of tempeh and tofu production is provided in the appendix (Fig.1a).

The preparation of tofu and tempeh wastewater is identical, but the subsequent steps differ after soybeans are boiled. Following boiling, the soybeans are milled into soy porridge. Subsequently, the porridge is filtered to obtain soy milk. Soy milk is boiled until white foam appears. Furthermore, soy milk is poured into a tofu mold and cooled to approximately 50°C. Subsequently, an acid solution is poured into the mold for the coagulation process. The acid solution utilized in this study is lemon juice. The top of the mold is pressed with a heavy weight to compact and separate the water. Tofu wastewater collected from soaking, washing, boiling, and tofu molding is used in this study. The wastewater resulting from boiling and molding is known as 'whey', which can generally be used as an acid solution for subsequent process production in small-scale industries.

7.2.2. Preparation of inoculum

The inoculum used in this study was granular sludge collected from an up-flow anaerobic sludge blanket (UASB) reactor treating municipal sewage wastewater (Hammarby Sjöstad, Stockholm, Sweden). The inoculum was incubated for one week at 37°C (mesophilic temperature) and 57°C (thermophilic temperature) for thermophilic in an incubator. Before use, the inoculum was treated for heat shock (thermal pretreatment) to maximize the production as a result of the inhibited activity and growth of methanogens. The inoculum was added to a 100 mL experimental serum glass bottle, then put in the water bath, and heated at 80 °C for 15 minutes for heat shock treatment (Jomnonkhaow et al., 2021).

Table 2. Characterization of substrate and inoculum before the experiment

	wastewater		
Parameter	Tempeh	Tofu	inoculum
COD (mg/L)	10,200	26,400	-
TS (%)	6.25	2.1	4.2
TSS (mg/L)	9.6	10.8	3.01
VS (mg/L)	5	2	3.33
VSS (mg/L)	0.00065	0.0023	0.0033
pH	5.82	5.61	7.38

Furthermore, the mixture was immediately cooled down in an ice chamber. The heat shock was applied to both inoculum, namely mesophilic and thermophilic. There are 4 types of inoculums used in this study, namely mesophilic + heat shock, mesophilic + non-heat shock, thermophilic + heat shock, and thermophilic + non-heat shock. Initial measurements of substrate and inoculum were carried out as initial data for characterization before experiments on anaerobic digestion batches.

7.2.3. Methods

7.2.3.1. Experimental setup for batch anaerobic digestion

The study was conducted in an anaerobic digestion batch experiment in the water bath. The assay was conducted in a 120 ml serum glass bottle, with an 80 ml working volume. The substrate and inoculum were mixed at a 1:1 ratio (40 mL each) to maintain balanced conditions. The serum glass bottle was tightly sealed, and immediately flushed with Nitrogen gas for 2 minutes to replace the oxygen inside the reactor to obtain an anaerobic condition.

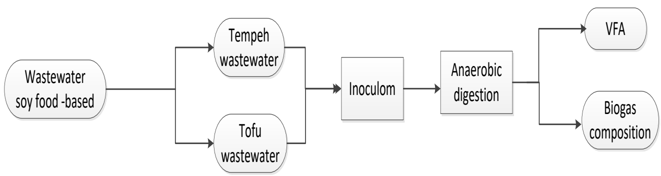


Fig 2. The experimental setup of the anaerobic digestion batch.

Then, the reactor was incubated in a water bath shaker at 37 °C and 57 °C at 100 rpm. Three times a week, 250 µl of biogas was taken using a gas-tight syringe (VICI, Precision Sampling Inc., USA) to analyze biogas composition, and 1 ml of liquid was taken from the reactor using a syringe for VFA (volatile fatty acid) analysis. The experiment was carried out for 33 days with three replicates (Table 3).

Table 3. Variable samples of the anaerobic digestion batch.

Type of wastewater			
Tofu wastewater		Tempeh wastewater	
name of samples	detail of samples	name of samples	detail of samples
TF1	Tofu wastewater + Unjasted pH + thermophilic + heat shock	T1	Tempeh wastewater + Unjasted pH + thermophilic + heat shock
TF2	Tofu wastewater + Unjasted pH + thermophilic + non- heat shock	T2	Tempeh wastewater + Unjasted pH + thermophilic + non- heat shock
TF3	Tofu wastewater + pH6 + thermophilic + heat shock	T3	Tempeh wastewater + pH6 + thermophilic + heat shock
TF4	Tofu wastewater + pH6 + thermophilic + non-heat shock	T4	Tempeh wastewater + pH6 + thermophilic + non-heat shock
TF5	Blank + thermophilic + heat shock	T5	Blank + thermophilic + heat shock
TF6	Blank + thermophilic + non-heat shock	T6	Blank + thermophilic + non-heat shock
TF7	Tofu wastewater + unadjusted pH + mesophilic + heat shock	T7	Tempeh wastewater + unadjusted pH + mesophilic + heat shock
TF8	Tofu wastewater + unadjusted pH + mesophilic + non-heat shock	T8	Tempeh wastewater + unadjusted pH + mesophilic + non-heat shock
TF9	Tofu wastewater + pH6 + mesophilic + heat shock	T9	Tempeh wastewater + pH6 + mesophilic + heat shock
TF10	Tofu wastewater + pH6 + mesophilic + non-heat shock	T10	Tempeh wastewater + pH6 + mesophilic + non-heat shock
TF11	Blank + mesophilic + heat shock	T11	Blank + mesophilic + heat shock
TF12	Blank + mesophilic + non- heat shock	T12	Blank + mesophilic + non- heat shock

Notes : TF; tofu wastewater, T; tempeh wastewater

7.2.3.2. Analytical method

TS, VS, TSS, and VSS were measured using an oven and muffle furnace at 105 °C and 550 °C with the standard method American Public Health Association (APHA-AWWA-WEF-2005). pH value was analyzed by the pH meter (Mettler Toledo F20 FiveEasy, OH, USA). The COD was measured using a CSB 15,000 test kit, and the concentration of COD was analyzed with a Nanocolor 500D Photometer (MACHEREY-NAGEL GmbH & Co. KG, Germany).

The analysis of biogas composition was performed using gas chromatography (GC) (Clarus 550; Perkin-Elmer, Norwalk, CT, USA) with a column (Carboxen™ 1000, 6́ x 1.8́ OD, 60/80 mesh, Supelco, Shelton, CT.USA). Furthermore, the VFA was analysed by GC (Clarus 550; Perkin-Elmer, Norwalk, CT, USA) with a capillary column (Elite-WAX ETR, 30 m x 0.32 mm x 1.00 µm, Perkin-Elmer, Shelton, CT, USA) and a flame ionized detector (FID). Before VFA analysis, the wastewater was mixed with acid mix (25% (v/v) formic acid and 25 % (v/v) ortho-phosphoric acid at a ratio of 1:3), centrifuged at 10,000 rpm for 5 minutes. Then filtered the supernatant was filtered through a 0.2 µm syringe filter to remove undissolved particles, and then added Butanol at a concentration of 1 g/L as an internal standard. Put into the vial and add Milli-Q water; the total volume is 1 ml.

The total production of VFA and the biogas composition from batch tests were compared to evaluate the effects of inoculum pretreatment, adjusted pH, and thermal pretreatment using statistical analysis. An analysis of variance (ANOVA) test, followed by Duncan's multiple range test, was used at the significance level of p-value < 0.05

7.3. Results and discussion

Tofu and tempeh wastewater have a high potential to be used as a source of acetic acid; the research results in this study confirm this fact through an anaerobic digestion process. The result shows the dominant VFA compound to be acetic acid in both wastewaters (tofu and tempeh wastewater). In addition, the effect of heat shock treatment on the inoculum, pH value of the substrate, and temperature in the water bath resulted in variations in methane and VFA concentrations in each treatment, which were discussed in detail in this section.

7.3.1. The effect of pH and Heat-shock treatment in total VFA and VFA distribution of tempeh wastewater

The effect of pH and heat shock treatment on VFA production is crucial to the anaerobic digestion process. The results of the study show that unadjusted pH and heat shock treatment can improve VFA yield. Based on the results provided on the Table. 4 and Table. 5. the production total VFA in the blank samples for both heat shock and non-heat shock treatment at fermentation day 14 was not high, and it only lasted for a few days. Total VFA for T5 and T6 at fermentation days 14, respectively, is 4.08 and 2.02 g/L (Table 4).

Table 4. The results of blank samples T5 and T6 at HRT days 14.

VFA composition	Blank samples	
	T5	T6
Acetic acid (%)	60.31	100
Propionic acid (%)	11.53	0
Isobutyric acid (%)	7.76	0
Butyric acid (%)	6.65	0
Isovaleric acid (%)	13.74	0
Valeric acid (%)	0	0
Caproic acid (%)	0	0
Total VFA (g/L)	4.08	2.02

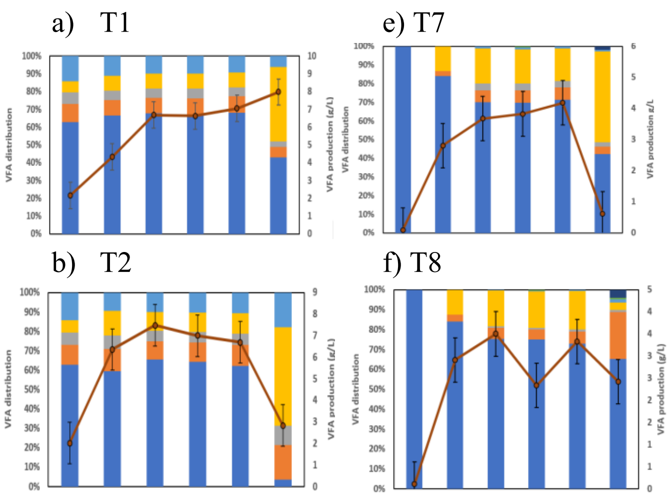
The high VFA production is influenced by several factors such as the types and dosage of substrates, the concentration of organic loading rate (OLR), temperature treatment, and reactor performance. A higher OLR at 9 VS/L-d at a temperature of 40 °C results in the highest VFA (Owusu-Agyeman et al., 2020c). Moreover, the total VFA production in mesophilic conditions at T11 and T12, respectively is 3.73 and 2.36 g/L (Table 5.). In addition, the highest concentration of acetic acid in the blank sample is reached in non-heat shock treatment for both temperature conditions, namely 100% at T6 and T12.

Table. 5. The results of blank samples T11 and T6 at HRT days 12.

VFA composition	Blank samples	
	T11	T12
Acetic acid (%)	56.56	100
Propionic acid (%)	11.52	0
Isobutyric acid (%)	8.31	0
Butyric acid (%)	7.77	0
Isovaleric acid (%)	15.81	0
Valeric acid (%)	0	0
Caproic acid (%)	0	0
Total VFA (g/L)	3.73	2.36

However, the blank sample with heat shock treatment at both temperatures produced low concentrations of acetic acid, namely 60.31% at T6 and 56.56% at T11. Although it produces high levels of acetic acid in the non-heat shock treatment, the total VFA in the heat shock treatment is higher. Hence, it can be concluded that the combination of heat shock and thermophilic treatment significantly affects in VFA production. The production of total VFA in thermophilic conditions is higher than in mesophilic conditions, however, the concentration of acetic acid is not much different and is still in the same range for all treatments in blank samples. Pretreatment of inoculum is one of the effective methods to improve acidification in anaerobic digestion, reaching the optimum pretreatment method depends on the type of feedstock and inoculum (Tian et al., 2024).

Furthermore, the highest total VFA production in thermophilic conditions was 10.08 mg/L, achieved at T3, followed by 2.45 mg/L at T7 for mesophilic conditions (Fig 2.). However, acetic acid concentration from tempeh wastewater was the highest compared to butyric acid, propionic acid, and isobutyric acid for all treatments. Based on Fig. 2, the anaerobic digestion process on tempeh wastewater resulted in a concentration of acetic acid exceeding 50% compared to the other VFA compositions, such as propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, and caproic acid. The concentration of acetic acid for all treatments of tempeh wastewater was high. The high acetic acid content in anaerobic digestion inhibited the process of methanogens producing biogas. However, the acetic acid concentration decreased extremely at fermentation days 32 for all the treatments, with the lowest acetic acid concentration of 5.32% achieved in T1, followed by T4 (19.53%). Furthermore, the highest acetic acid concentration was attained at T7, T8, T10, and (Fig. 2) at the fermentation day 1. The average acetic acid concentration in thermophilic conditions (T1, T2, T3, and T4) was 66.95, 53.79, 69.39, and 56.19%. Additionally, the average acetic acid concentration in mesophilic conditions (T7, T8, and T9) was 75.84%, 77.32%, and 75.53%, which were within the same range. Consequently, the low acetic acid concentration at T9 (69.71%) was observed. The high acetic acid concentration on the first day of fermentation was probably due to the metabolic regulation of the microorganisms. The results from the addition of initial sugar from 320 – 450 g/L increased acetic acid rapidly by 1.06 – 1.62 g/L in the process of the final wine, it is due to the regulation of yeast metabolism, which is driven by hyperosmotic stress (Deng et al., 2023).



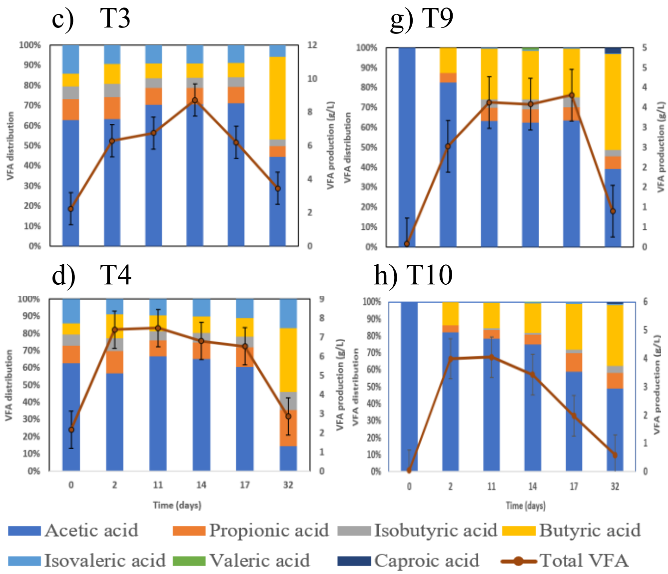


Fig 2. VFA concentration and distribution in tempeh wastewater

However, decreasing the concentration of acetic acid causes an increase in the number of carbons in the VFA molecular structure, particularly butyric acid, propionic acid, isobutyric acid, and isovaleric acid. The highest butyric acid content was 80.58% at T7 treatment, followed by 76.55% at T2 (Fig. 2). This high VFA content is based on the accumulation of acetate that produces high ammonia concentrations which can inhibit acetogenesis and methanogenesis reactions (Fernandes, 2020). The situation during the anaerobic digestion process can lead to high results of acetic acid and glycerol, as a byproduct of fermentation. In general, the concentration of acetic acid in mesophilic conditions was higher than in thermophilic (Fig. 2). However, this is not related to the total VFA produced. Overall, the total VFA was higher in thermophilic conditions and lower in mesophilic. The relation between temperature and VFA accumulation in anaerobic digestion is complex and influenced by several factors. Some studies suggested that thermophilic conditions can lead to increased VFA accumulation, while others indicate that mesophilic conditions may result in higher VFA yield. A study by David Fernández-Domínguez et al. (2020) reported that the highest VFA yield is 0.49–0.59 $gCOD_{VFA}/gVS$ achieved at a temperature of 35°C (mesophilic conditions), but the VFA composition was not influenced by the fermentation temperature. On the other hand, the thermophilic temperature can increase the rate of hydrolysis and acidogenesis, resulting in high concentrations of acetic acid and isovaleric acid and VFA accumulation. Thermophilic conditions can improve the activities and growth of bacteria which can release the α-glucosidase and protease (Hao & Wang, 2015).

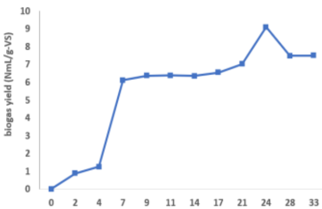
The decrease of acetic acid content at the end of retention time (fermentation) due to certain microorganisms like Acetobacter oxidizing ethanol to acetic acid during the advanced stage of fermentation (Hata et al., 2023). However, it is not always the acetic acid that will decrease at the end of retention time, several factors can decrease acetic acid during the anaerobic digestion process, such as strain yeast and the type of substrate with low acetic production are used (Chidi Boredi Silas, 2016). Additionally, the environmental factors that slow down the anaerobic digestion process, such as low temperatures, can also lead to lower acetic acid production at a temperature of 20 °C, acetic acid decreases, moving slightly to the middle of the exponential growth phase (Shang et al., 2016). The higher the concentration of acetic acid, the higher the inhibition of methanogens to produce methane, which affects the quantity and quality of biogas as the final result of the anaerobic digestion process, the increase of acetic acid up to 45 g/L can inhibit the cell growth and ethanol oxidation (Song et al., 2022).

The types of substrates used in the anaerobic digestion process also affect the production of VFA. The premier sewage sludge is particularly effective in generating a substantial quantity of VFA and acetic acid, owing to its high content of readily biodegradable and soluble monomeric organic matter, including glucose, fructose, and amino acids (Al-Sulaimi et al., 2022). The substrate utilized in this research was tempeh and tofu wastewater, which are high in protein content. Consequently, during the fermentation process associated with their production, a high concentration of acetic acid is produced. This occurs as microorganisms break down the organic compounds present in soybeans, resulting in acetic acid as a byproduct (Chua & Liu, 2019; Sakinah et al., 2019). However, the fermentation process also leads to the production of other acidic compounds, such as propionic acid, which can further contribute to the acidity of the wastewater (Nieto-Veloza et al., 2021).

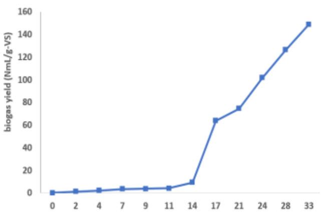
Moreover, based on the result in Fig 3. the lowest cumulative methane is 0.22 mL/g-VS achieved at T9, and the highest is 44.98 mL/g-VS at T2. The relation between VFA production and the quality of biogas composition in this study is influenced by pretreatment heat shock in the inoculum. Heat shock treatment serves to select the microbial communities that can thrive under pressure conditions, including halting methanogenesis, thus the selected microbial communities can produce high VFA (de Almeida et al., 2024). The heat shock treatment had a significantly positive impact on VFA production, contrary to the non-heat shock treatment. This study also employed adjustment pH as a parameter of observation (pH 6 and acidity pH) that influences the performance of reactors and the activities of microorganisms. This is because methanogenesis is highly sensitive to acidic conditions (pH changes) and is active within the range of pH 6.8 to 7.24 (Bahira et al., 2018).

According to Fig 3. in terms of methane production, both treatments show a lazy S-profile with a slow rate until the first day, then an increase in period retention time 14 – 21 days, that which point the rate begins to decrease. The cumulative methane at T2 (44.98 mL/g-VS) is higher than at T10 (24.78 mL/g-VS). This is because the pH substrate has a substantial impact on biogas production. The pH range of 6.4 – 7.6 is considered ideal for the growth of bacteria and optimal for biogas production (A. ali et al., 2021). Both pH and temperature critically influence biogas production.

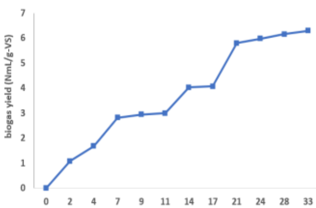
a) T1



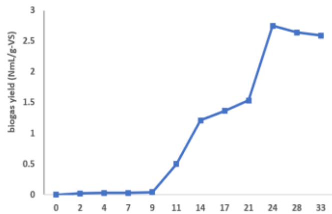
b) T2



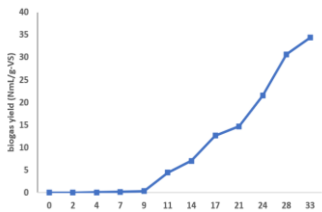
c) T3



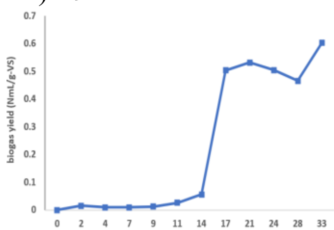
g) T7



h) T8



i) T9



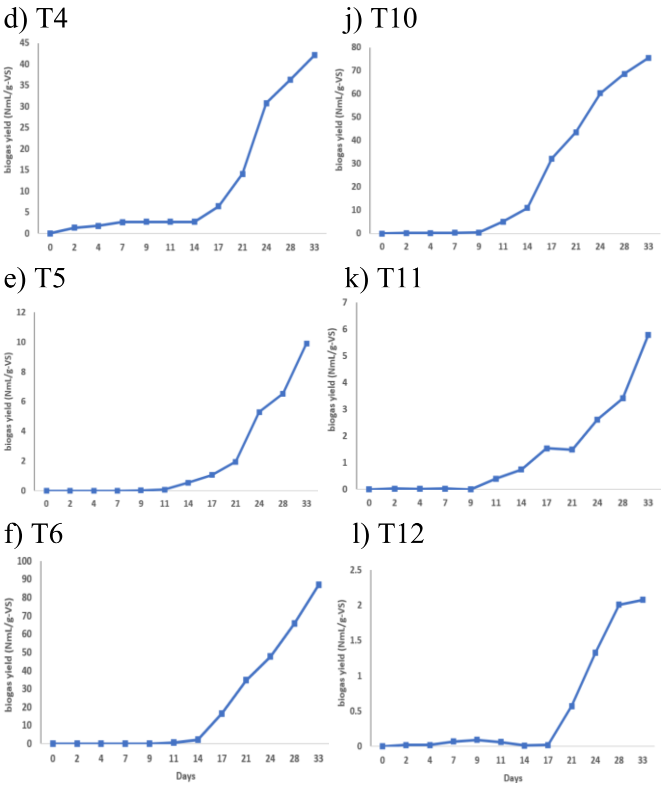


Fig 3. Methane content of tempeh wastewater

The highest specific biogas yield reaches 161.09 mg/L of COD removal at 50 °C, demonstrating that thermophilic conditions (50–60°C) optimize bacterial activity and enhance production efficiency (Deepanraj et al., 2015). The kinetics of biogas production in mesophilic and thermophilic conditions are similar, however, the energy in thermophilic conditions is considerably higher compared to mesophilic conditions, thus, considerably required in optimizing the biogas production (Al-Zoubi et al., 2024).

7.3.2. The effect of pH and Heat-Shock treatment in total VFA and VFA distribution of tofu wastewater

The effect of pH and HS treatment on tofu wastewater differs slightly from tempeh wastewater. The total VFA of tofu wastewater is not significantly different from tempeh wastewater, because both wastewaters form a similar composition of organic matter and contain a high level of protein, carbohydrate, and other compounds that can contribute to VFA production during anaerobic (Hardyanti et al., 2023; Widyarani et al., 2018). Moreover, the high concentration of acetic acid in tofu wastewater is related to its fermentation process, which contains 8 types of amino acids, i.e., Aspartic acid, Glutamic acid, Arginine, Serine, Glycine, Leucine, Lysine, and Histidine, that can be converted to produce VFA (Li et al., 2021).

The relationship between VFA and amino acids serves as the starting material for producing VFA. However, the acetic acid of tofu wastewater tends to stabilize or not much decrease at the end of fermentation, unlike in tempeh wastewater.

Table 6. The results of blank samples at TF5 and TF6 in HRT days 2

VFA composition	Blank samples	
	TF5	TF6
Acetic acid (%)	58.77	62.44
Propionic acid (%)	12.72	13.18
Isobutyric acid (%)	8.78	9.25
Butyric acid (%)	8.1	8.79
Isovaleric acid (%)	15.1	16.67
Valeric acid (%)	0	0
Caproic acid (%)	0	0
Total VFA (g/L)	7.92	7.95

It is because tofu production involves coagulating soy milk with agents like acidic whey, acetic acid, gypsum (calcium sulfate dihydrate), and tofu seed solution (tofu wastewater that is left over one night), which could contribute acetic acid to the higher levels in tofu wastewater (Qiao et al., 2010; Sayow et al., 2020).

Moreover, tempeh production involves fermenting cooked soybeans with microorganisms, which might result in a different acid profile. Based on the results provided in Tables 6. and 7, the total VFA in the blank samples of tofu wastewater in thermophilic and mesophilic conditions with heat and non-heat shock treatment were in the same range. Total VFA in thermophilic conditions with heat shock treatment (TF5) is 7.92 g/L which is not different from non-heat shock treatment (TF6) 7.95 g/L (Table 6).

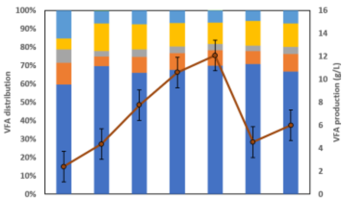
Table 7. The results of blank samples at TF11 and TF12 in HRT days 22

VFA composition	Blank samples	
	TF11	TF12
Acetic acid (%)	59.90	55.62
Propionic acid (%)	11.52	27.5
Isobutyric acid (%)	7.83	0
Butyric acid (%)	6.91	0
Isovaleric acid (%)	13.82	16.8
Valeric acid (%)	0	0
Caproic acid (%)	0	0
Total VFA (g/L)	7.2	7.44

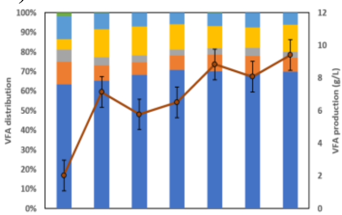
Similarly, total VFA under mesophilic conditions with heat and non-heat treatment was significantly not different 7.2 g/L at TF11 and 7.44 g/L at TF12 (Table 7). The highest acetic acid content in blank samples of tofu wastewater is 62.44% reached at TF6 (Table. 6). However, the acetic acid content in blank samples of tofu wastewater at TF5 (58.77%), TF11 (59.90%), and TF12 (55.62%) is not much different. The main composition of VFA in tofu wastewater consists of acetic acid, butyric acid, propionic acid, isobutyric acid, and isovaleric acid.

The highest average content of acetic acid from all treatments is achieved at TF10 and TF7, respectively, 92.40% and 91.68% (Fig. 4). The fermentation anaerobic digestion process of tofu wastewater was the same as that of tempeh wastewater. The highest acetic acid content based on the fermentation period is 100% achieved at TF7 (at day 1 HRT) (Fig.4). However, the highest total VFA is 9.79 g/L achieved by TF4 at fermentation day 2 HRT.

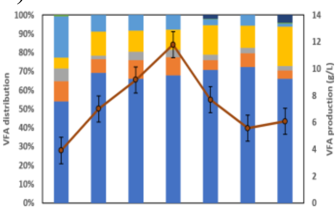
a) TF1



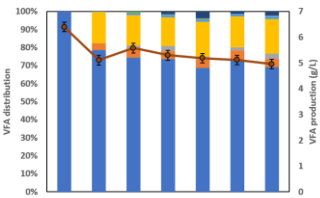
b) TF2



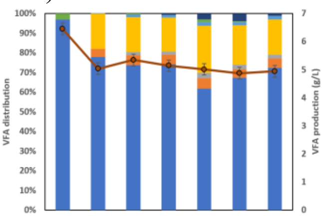
c) TF3



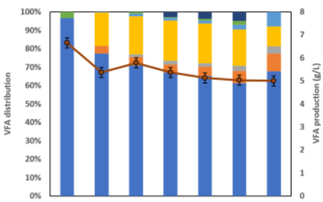
e) TF7



f) TF8



g) TF9



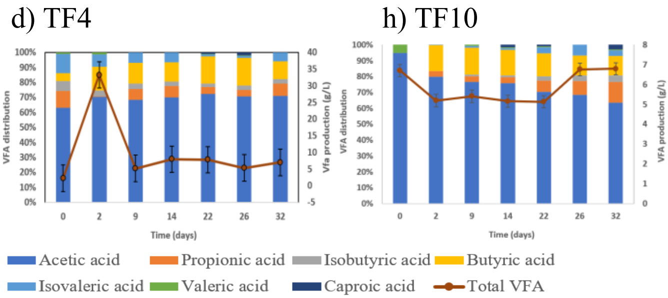


Fig 4. VFA concentration and distribution in tofu wastewater

Hence, fermentation significantly affects the composition and total VFA production as a parameter on an anaerobic digester for performance and stability (Shi et al., 2017).

The short retention time of 1.5 days produces a high VFA accumulation of 0.48 $\pm$ 0.01 g COD_{VFA}/g $TCOD_{fed}$ because the short retention time is inappropriate for methanogens and slow-growing in consumed VFA to biogas production (Law et al., 2023). The short fermentation at 8 h results in the highest main component of VFA, including acetic acid 1.1845 $\pm$ 0.0165, propanoic acid 0.5160 $\pm$ 0.0141, and butyric acid 0.0148 $\pm$ 0.0009 mM/L, with VFA yield 48.20 $\pm$ 1.21% (Khan et al., 2019). It can be indicated that the short fermentation increases the production of the main VFA component, which is more beneficial in the acidogenesis phase because it allows for a more efficient conversion of simple monomers into VFA (Lago et al., 2023; Pramanik et al., 2020). In general, the total VFA production in both wastewater (tempeh and tofu synthetic wastewater) with heat shock pretreatment in thermophilic conditions is higher than non-heat shock pretreatment in mesophilic conditions.

The pretreatment heat shock in the inoculum is significantly effective in enhancing VFA production, which is linked to the dynamics of the bacterial community (Blasco et al., 2020). Heat shock, also known as thermal treatment as a pretreatment for inoculum, it has a positive impact on inoculum to enhance VFA production. The heat shock inoculum treatment is effective in increasing VFA yield 9315 ± 652 mg COD/L at alkaline pH and inhibits nonsporulating bacteria and methanogenic archaea *(Methanobacteriaceae*) (Logan et al., 2023). However, other study reports that a low pH value can improve the VFA and its composition. The highest VFA production yield from fermentation dairy milk was 0.92 gCOD/gVS, including the acid profile of VFA reached at pH 5 (Atasoy & Cetecioglu, 2022). This is because the adaptation of the microbial community in acidic pH increases VFA production, which also impacts in biogas composition.

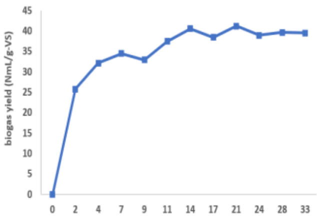
Based on Fig. 5. the highest cumulative methane from synthetic tofu wastewater through an anaerobic digestion process was 33.42 mL/g-VS reached at TF1, followed by TF3 (29.88 mL/g-VS), and TF4 (25.23 mL/g-VS).

Furthermore, the lowest cumulative methane was 0.25 mL/g-VS at TF7 and TF9. Hence, it can be indicated that the cumulative methane in thermophilic is higher than in mesophilic conditions, which is the same as the result from tempeh wastewater. According to Fig. 5. the cumulative methane in tofu wastewater also has a lazy S-profile, same as in tempeh wastewater. Lazy S-profile refers to a slow rate of biogas production at the beginning, followed by a rapid increase, and then a gradual decrease. The slow rate of biogas production indicated unstable operational performance and lower biogas production (Wang et al., 2019). Based on the results of this study, high cumulative methane in tempeh and tofu wastewater was found at thermophilic temperatures with heat shock pretreatment.

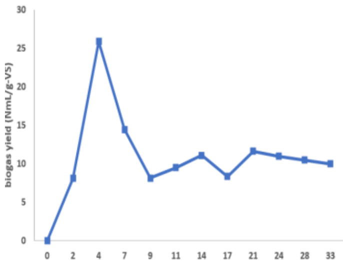
The same applies to the total VFA production in both wastewaters; the total VFA in the thermophilic temperature treatment was higher than at mesophilic. Acetic acid serves as an essential substrate for methanogenesis, directly influencing microbial metabolism, particularly in methane-producing bacteria. When fermentation temperatures decrease, both total VFA and acetic acid concentration progressively decline, negatively impacting methane production (Wang et al., 2019).

There was a relationship between total VFA and cumulative methane; if total VFA is high, then methane content is low. The results show that the highest total VFA in tempeh wastewater was 10.08 g/L at T3 (Fig 2.) but the cumulative methane was low at 3.65 mL/g-VS (Fig 3.).

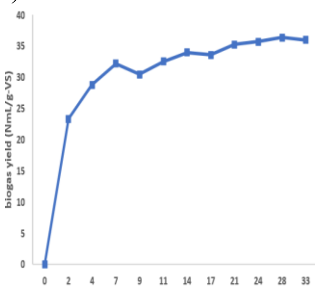
a) TF1



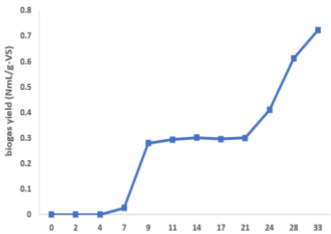
b) TF2



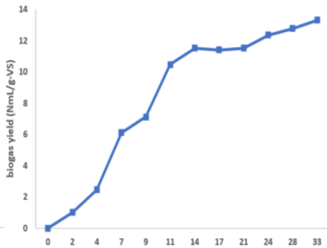
c) TF3



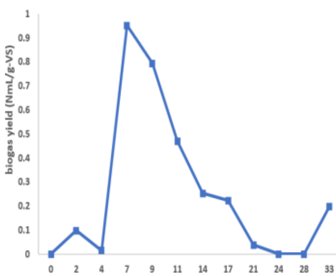
g) TF7



h) TF8



i) TF9



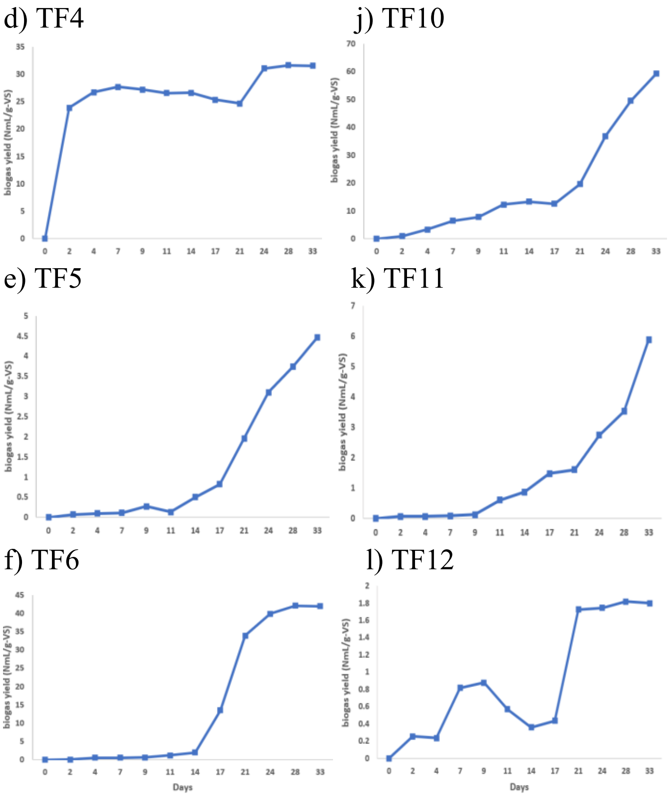


Fig 5. Methane content in tofu wastewater

It can be indicated that methane production is related to VFA production, which is a decreasing methane content along with an increasing VFA (Tampio et al., 2019). However, in tofu wastewater, the highest total VFA was 9.79 g/L reached at TF3 (Fig 4.) with a high cumulative methane of 25.23 mL/g-VS (Fig 5.). This is probably because tofu wastewater had a higher carbohydrate content than tempeh wastewater, thus achieving high total VFA and cumulative methane. The type of substrate with high carbohydrate content and biodegradability reached a high methane yield and VFA yield (Mahmoud et al., 2023). Therefore, further study is needed to optimize the production of VFA from tofu wastewater.

Overall, the findings of this study demonstrate the significant potential of producing high acetic acid content from two distinct types of wastewater for various industrial applications. Acetic acid holds immense value due to its multifaceted utilization in industry, which is driven by its substantial economic worth and substantial global demand. The market size of acetic acid in 2023 is projected to reach USD 23.23 billion. The forecast period 2024 – 2032 of the acetic acid market will grow to a 5.10 % compound annual growth rate (CAGR), and the value will reach USD 36.36 billion by 2032 (Market, 2023). Further research is needed to utilize tofu and tempeh wastewater as a source of acetic acid, which is applied in the food manufacturing process or as other materials.

7.4. Conclusion

Anaerobic digestion of tofu and tempeh wastewater resulted in efficient production of high concentrations of total volatile fatty acids (VFA) and acetic acids. The highest average total VFA in tempeh and tofu wastewater, respectively, was 10.08 and 9.79 g/L, achieved in T3 and TF3. Additionally, the highest concentration of VFA composition is acetic acid from both wastewaters. The highest average acetic acid concentration in tempeh wastewater is 77.32% in T7 and 92.40% in TF10 for tofu wastewater. High VFA production has an impact on the methane concentration. The highest total VFA in tempeh wastewater at T3 has a low cumulative methane of 3.65 mL/g-VS. However, tofu wastewater has a high content of carbohydrates, thus resulting in high VFA and cumulative methane of 25.23 mL/g-VS at TF3. This study concluded that tempeh and tofu wastewater have a high potential to produce VFA as a source of acetic acid. The combination of pretreatment heat shock, pH 6, and thermophilic conditions results in the highest total VFA and low cumulative methane. However, further research is needed to optimize the production of VFA and acetic acid, including their application as additives in the food industry.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations
Conflict of interest the authors declare no conflict of interest.

Ethics approval is not applicable.

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8. Discussion

The global transition toward renewable energy and sustainable waste management has spurred significant interest in anaerobic digestion (AD) technologies for converting waste into biogas. in Indonesia, tofu and tempeh production, a staple industry generating vast quantities of high-organic wastewater, represents a prime opportunity for biogas adoption. Recent studies highlight the efficacy of biofilter-enhanced ABR in addressing two critical challenges: wastewater treatment and renewable energy production. These findings are particularly relevant for Indonesia, the world's leading producer of tofu and tempeh, where small-scale industries contribute substantially to organic wastewater pollution. Globally, this innovation aligns with circular economy principles and decarbonization goals, offering scalable solutions for waste valorization and energy security.

The ABR-biofilter system presents a transformative approach for Indonesia to pioneer sustainable agro-industrial waste management, delivering dual benefits: high biogas yields (up to 60.64% from tofu wastewater at an OLR 2.67 kg COD/$m^3 \cdot$day) and effective pollution control (95% COD removal). Benchmarking against international studies further highlights the system's performance. For example, the combination of CSTR and ABR treating dairy effluents achieved an 82% COD removal, with a biogas yield of 0.26 m^3/kg and an average methane content of 76% ± 2% (Jürgensen et al., 2018). Similarly, anaerobic digestion of olive mill wastewater mixed with agro-industrial substrate produced a methane concentration of 60 ± 4.7% for mixture A (30% v/v OMW with 70% poultry and liquid pig manure) and 61 ± 3.4% for mixture B (40% v/v OMW with 60% poultry manure and cheese whey) (Thanos et al., 2021). Like in another study, anaerobic co-digestion of dairy wastewater with 8% crude glycerol derived from slaughterhouse sludge (CG8) produced a methane concentration of 73.10 ± 24.03% and an accumulated biogas volume of 4383.3 ± 110.2 ml (Chou & Su, 2019). Likewise, co-digestion using cattle manure as inoculum reached a maximum biogas production of 342.22 ml/gVS and methane yield of 369.63 ± 4.05 mL/gVS at 60% fraction whey and a short lag phase of 0.76± 0.17 days (Bella & Venkateswara Rao, 2022). Moreover, a pilot-scale Spiral Symmetric Stream Anaerobic Bioreactor (P-SSSAB) treating soybeans processing wastewater reached a biogas yield of 0.69 m^3/kgCOD, methane yield of 0.48 m^3/kgCOD, methane concentration between 69.98 – 72.22% and COD removal of 91.06% (Chen et al., 2021).

Although substrate composition differs, these comparisons suggest that tofu wastewater exhibits methane yields and treatment efficiencies comparable to those of other high-organic agro-industrial residues, reinforcing the global relevance of this ABR-biofilter model and its potential contribution to low-carbon economies. However, challenges such as H_2S emissions (410 – 980 ppm) require further optimization through hybrid treatment systems.

This discussion synthesizes experimental results, evaluates their implications for Indonesia's energy and environmental policies, and explores scalability for global applications. Based on national production capacity data, Indonesia's tofu and tempeh industry processes 1,864,288,000 kg of soybeans annually, generating tofu wastewater (85,021,562.400 L) and tempeh wastewater (68,565,305,937 L). By utilizing these wastewater streams for renewable energy production through AD, the model predicts annual methane (CH_4) emission reduction of 332.094 m^3 CH_4 from tofu wastewater and 276.816 m^3 CH_4 from tempeh wastewater. The captured annual CH_4 from the tofu and tempeh industry has the potential to generate approximately 5,99,100 kWh of electrical energy per year. Assuming a biogas generator efficiency of 35% for a small-scale CHP system, would result in about 2,099,685 kWh/year of usable electricity. This energy could supply power to roughly 1,750 households annually, based on average household electricity consumption data.

Although not financially feasible, the project is technically viable because it provides benefits by converting wastewater into bioenergy for local community use rather than for commercial purposes (Appendix F). However, strong government support in terms of funding, technical assistance, and monitoring is essential. In addition, the combustion of CH_4 would prevent the emission of approximately 1,650 tons of CO_2-equivalent per year, thereby contributing to national greenhouse gas (GHG) reduction targets and climate mitigation efforts.

Additionally, this dissertation demonstrates that wastewater from tofu and tempeh processing can serve as an effective substrate for volatile fatty acid (VFA) production, with acetic acid as the dominant component. Notably, AD of tempeh wastewater under thermophilic conditions (57 °C) with heat shock pretreatment yielded 10.08 g/L total VFA, of which acetic acid accounted for 77.33%, under mesophilic conditions. Similarly, tofu wastewater adjusted to pH 6 produced 9.79 g/L VFAs, with acetic acid reaching 92.40%. These results highlight the technical feasibility and novelty of utilizing tofu and tempeh wastewater for high-efficiency VFA production. The high acetic acid content in tempeh and tofu wastewater presents significant potential for reuse as an acidic solution in the food industry, particularly for coagulation in the tofu production and as a substrate for nata de tofu fermentation. This practice has long been empirically employed by the majority of small-scale tofu producers within Indonesia's MSME sector. Tofu and tempeh wastewater demonstrate outstanding potential for VFA production. Serving dual purposes as a source of food-grade acetic acid and as a versatile platform for chemical synthesis. As critical intermediates in anaerobic fermentation, VFAs, primarily acetic, propionic, and butyric acid, can be catalytically upgraded to value-added alcohols or serve as a key precursor for flavor compounds, pharmaceutical ingredients, and specialty chemicals (Fan et al., 2021). This pathway represents a highly promising approach for biochemical recovery, offering both economic and environmental benefits through waste valorization.

The utilization of tofu and tempeh wastewater through AD has promising results. From a regulatory standpoint, Indonesia has demonstrated growing support for the development of renewable energy, including bioenergy. Notably, Presidential Regulation No. 112 of 2022 on the Acceleration of Renewable Energy Development highlights biomass and biogas as key components in diversifying the national energy mix. This regulation aligns with the broader targets outlined in the NEP and GNEP, which aim to increase the share of renewable energy in the total energy mix to at least 23% by 2025 and to achieve net-zero emissions by 2060.

The utilization of tofu and tempeh industrial wastewater for dual biogas and VFA production, employing locally available labor and construction materials, offers significant socio-economic advantages. These include rural employment generation, improved waste management practices, and enhanced energy security in decentralized regions. This integrated biogas technology represents more than just a sustainable energy solution for Indonesia's tofu and tempeh industry: it serves as a multi-benefit approach that simultaneously addresses greenhouse gas mitigation while aligning with national energy policies and climate commitments. The existing regulatory support, coupled with positive techno-economic feasibility indicators, demonstrates substantial potential for scaling these initiatives to other agro-industrial sectors nationwide.

9. Conclusion

This dissertation addresses the research gap in utilizing tofu wastewater in Indonesia, demonstrating its strong potential as a raw material for renewable energy. The findings provide a practical solution for wastewater management, particularly relevant to Indonesia's MSME tofu industry, where waste treatment remains suboptimal due to limited awareness among business owners, local governments, and stakeholders.

The study highlights two key applications:

- Biogas and VFA production: agro-industrial wastewater, especially from tofu processing, can be effectively converted into biogas and VFA through AD.

- Acetic acid recovery: The wastewater is also a viable source of acetic acid, which can be reused in tofu production or other industries (food, cosmetics, chemicals, and cleaning products).

A simple biofilter technology in ABR proved efficient for biogas production, with benefits including:

- Low-cost, long-term usability, reducing maintenance expenses for MSMEs.
- Nutrient-rich effluent usable as liquid organic fertilizer, offering additional income streams.

In summary, this research presents simple, high-impact technologies for converting agro-industrial wastewater into renewable energy and valuable byproducts. The results serve as a reference for policymakers, industry owners, and stakeholders to adopt sustainable, circular economy practices in wastewater management.

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APPENDICES

- **Appendix A – Sample questionnaire from an energy audit in the tofu industry, Indonesia.**

Czech University of Life Science at prague
Faculty of Tropical AgriSciences
Sustainable Technology Department

Tanggal wawancara : 23-08-2020
Nama : Ibu Sumarni
Alamat : G. Sulah.

Kuisioner ini digunakan untuk penelitian mengetahui kapasitas penggunaan energi dalam memproduksi tahu, karakteristik limbah cair tahu dan untuk mengetahui aspek sosial-ekonomi dari indsutri tahu di Kelurahan Gunung Sulah, Bandar Lampung, Indonesia. Beban pencemaran dari kegiatan produksi tahu akan disesuaikan dengan baku mutu limbah cair tahu menurut peraturan Kementerian Lingkungan Hidup No. 5. Tahun 2014, untuk menganalisis pemanfaatan limbah cair tahu sebagai bahan bioenergi (biogas) dan dampaknya terhadap penurunan emisi serta aspek sosial-ekonominya.
Studi kasus : Industri tahu di Kelurahan Gunung Sulah, Bandar Lampung oleh Lydia Mawar Ningsih, mahasiswa S3 (Ph.D. Student) Sustainable Technology Department, Faculty of Tropical AgriSciences, Czech University of Life Science at Prague kolaborasi dengan Fakultas Pertanian, Universitas Lampung.
Saya mohon kesediaan Bapak/Ibu/Saudara/i untuk mengisi kuisioner ini dengan teliti dan lengkap sehingga menjadi data yang objektif.
Atas kesediannya saya ucapkan terimakasih.

Instrumen penelitian.

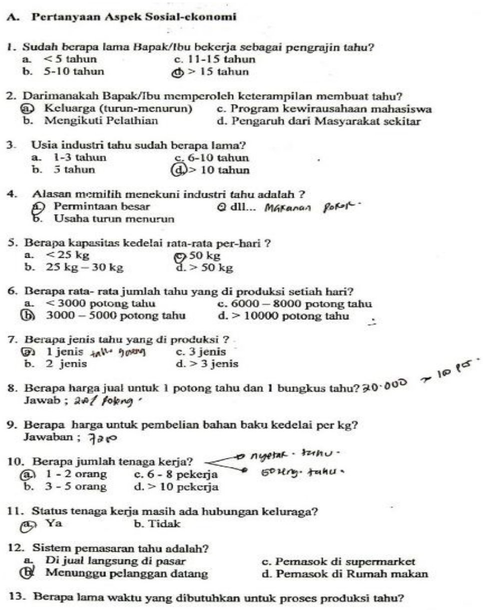
I. Identitas Responden

Nama responden : ibu Sumarni
No. Responden : 2.
Umur : 55 tahun.
Jenis kelamin : perempuan
Pekerjaan : Pengrajin tahu.
Pendidikan terakhir : SD.
Alamat :
Tanggal : 23-08-2020

II. Profil Industri Tahu

Nama industri : Ibu Sumarni
Nama pemilik :
Tahun berdiri : 1989.
Lokasi Industri : Gunung Sulah.
Luas bangunan industri : 4 x 8

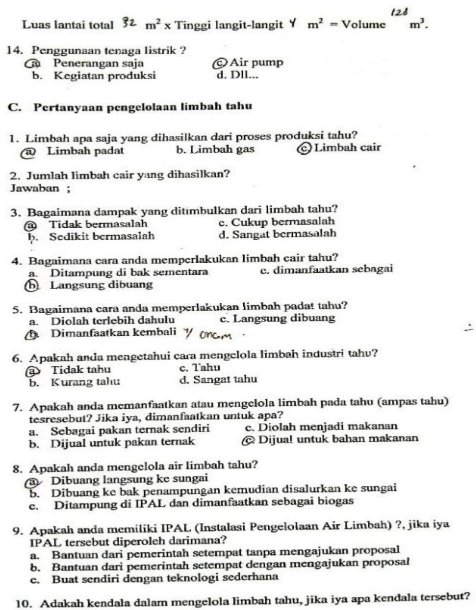
Figure 1. Questionnaire of industry owner information



A. Pertanyaan Aspek Sosial-ekonomi

1. Sudah berapa lama Bapak/Ibu bekerja sebagai pengrajin tahu?
   a. < 5 tahun c. 11-15 tahun
   b. 5-10 tahun d. > 15 tahun
2. Darimanakah Bapak/Ibu memperoleh keterampilan membuat tahu?
   a. Keluarga (turun-menurun) c. Program kewirausahaan mahasiswa
   b. Mengikuti Pelathian d. Pengaruh dari Masyarakat sekitar
3. Usia industri tahu sudah berapa lama?
   a. 1-3 tahun c. 6-10 tahun
   b. 5 tahun d. > 10 tahun
4. Alasan memilih menekuni industri tahu adalah ?
   a. Permintaan besar c. dll... Makanan pokok.
   b. Usaha turun menurun
5. Berapa kapasitas kedelai rata-rata per-hari ?
   a. < 25 kg c. 50 kg
   b. 25 kg – 30 kg d. > 50 kg
6. Berapa rata- rata jumlah tahu yang di produksi setiah hari?
   a. < 3000 potong tahu c. 6000 – 8000 potong tahu
   b. 3000 – 5000 potong tahu d. > 10000 potong tahu
7. Berapa jenis tahu yang di produksi ?
   a. 1 jenis tahu goreng c. 3 jenis
   b. 2 jenis d. > 3 jenis
8. Berapa harga jual untuk 1 potong tahu dan 1 bungkus tahu? 20.000 → 10 pcs.
   Jawab ; 200/ potong.
9. Berapa harga untuk pembelian bahan baku kedelai per kg?
   Jawaban ; 7200
10. Berapa jumlah tenaga kerja? → nyetak tahu. → 50 Kg. tahu.
    a. 1 - 2 orang c. 6 - 8 pekerja
    b. 3 - 5 orang d. > 10 pekerja
11. Status tenaga kerja masih ada hubungan keluraga?
    a. Ya b. Tidak
12. Sistem pemasaran tahu adalah?
    a. Di jual langsung di pasar c. Pemasok di supermarket
    b. Menunggu pelanggan datang d. Pemasok di Rumah makan
13. Berapa lama waktu yang dibutuhkan untuk proses produksi tahu?

Figure 2. Questionnaire of the socio-economic aspect.



Luas lantai total 32 m^2 x Tinggi langit-langit 4 m^2 = Volume 128 m^3.

14. Penggunaan tenaga listrik ?
    a. Penerangan saja c. Air pump
    b. Kegiatan produksi d. Dll...

C. Pertanyaan pengelolaan limbah tahu

1. Limbah apa saja yang dihasilkan dari proses produksi tahu?
   a. Limbah padat b. Limbah gas c. Limbah cair
2. Jumlah limbah cair yang dihasilkan?
   Jawaban ;
3. Bagaimana dampak yang ditimbulkan dari limbah tahu?
   a. Tidak bermasalah c. Cukup bermasalah
   b. Sedikit bermasalah d. Sangat bermasalah
4. Bagaimana cara anda memperlakukan limbah cair tahu?
   a. Ditampung di bak sementara c. dimanfaatkan sebagai
   b. Langsung dibuang
5. Bagaimana cara anda memperlakukan limbah padat tahu?
   a. Diolah terlebih dahulu c. Langsung dibuang
   b. Dimanfaatkan kembali ⁄/ oncom .
6. Apakah anda mengetahui cara mengelola limbah industri tahu?
   a. Tidak tahu c. Tahu
   b. Kurang tahu d. Sangat tahu
7. Apakah anda memanfaatkan atau mengelola limbah pada tahu (ampas tahu) tesresebut? Jika iya, dimanfaatkan untuk apa?
   a. Sebagai pakan ternak sendiri c. Diolah menjadi makanan
   b. Dijual untuk pakan ternak c. Dijual untuk bahan makanan
8. Apakah anda mengelola air limbah tahu?
   a. Dibuang langsung ke sungai
   b. Dibuang ke bak penampungan kemudian disalurkan ke sungai
   c. Ditampung di IPAL dan dimanfaatkan sebagai biogas
9. Apakah anda memiliki IPAL (Instalasi Pengelolaan Air Limbah) ?, jika iya IPAL tersebut diperoleh darimana?
   a. Bantuan dari pemerintah setempat tanpa mengajukan proposal
   b. Bantuan dari pemerintah setempat dengan mengajukan proposal
   c. Buat sendiri dengan teknologi sederhana
10. Adakah kendala dalam mengelola limbah tahu, jika iya apa kendala tersebut?

Figure 3. Questionnaire of waste management.

a. 1-2 jam
b. 3 jam
c. > 3 jam
d. Tergantung keadaan

± 4 jam 4/perendaman

B. Pertanyaan konsumsi energi

1. Dalam 1 kali produksi berapa banyak air yang digunakan L/hari?
   Jawaban ; 2x bak.

2. Jenis cairan penggumpal yang digunakan?
   a. Asam cuka (CH_3COOH)
   b. Asam Sulfat (H_2SO_4)
   c. dll ... tawa.

3. Jenis bahan bakar yang digunakan ?
   a. Kayu bakar
   b. Gas LPG
   c. Listrik
   d. Dll, bensin

4. Alasan memilih jenis bahan bakar tersebut adalah?
   Jawaban ; Awet barangnya.

5. Harga bahan bakar biomas (kayu bakar) yang digunakan per hari?
   Jawaban ; 70 ribu

6. Berapa banyak bahan bakar (kayu bakar) yang dibutuhkan tahu per hari?
   Jawaban ; 1/2

7. Harga bahan bakar minyak yang digunakan per hari?
   Jawaban : 10 ribu

8. Berapa banyak bahan bakar minyak yang digunakan per hari?
   Jawaban ; 1,5 liter

9. Berapa mesin yang digunakan untuk memproduksi tahu?
   a. 1 buah
   b. 2-3 buah
   c. 5 buah
   d. > 5 buah

10. Berapa lama mesin beroperasi?
    a. 2 jam
    b. 3-5 jam
    c. > 5 jam

11. Berapa ruang yang digunakan untuk memproduksi?
    a. 1 ruangan
    b. 2-3 ruangan
    c. > 3 ruangan

12. Waktu operasional
    a. Senin – minggu (/ jam)
    b. Sabtu (/ jam)
    c. Total operasional per tahun (/ jam)

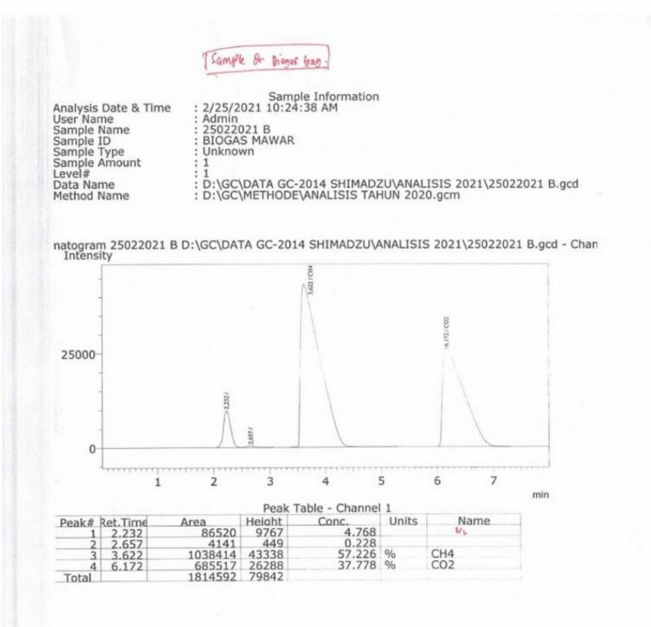
    7 x 4,5 jam → 31,5 jam.

13. Karakteristik bangunan

    4,5 x 3

Figure 4. Questionnaire of energy consumption

- **Appendix B – The results of biogas composition, terms related to acclimatization are provided in Figures 1–3**



[Sample & biogas bag]

Sample Information

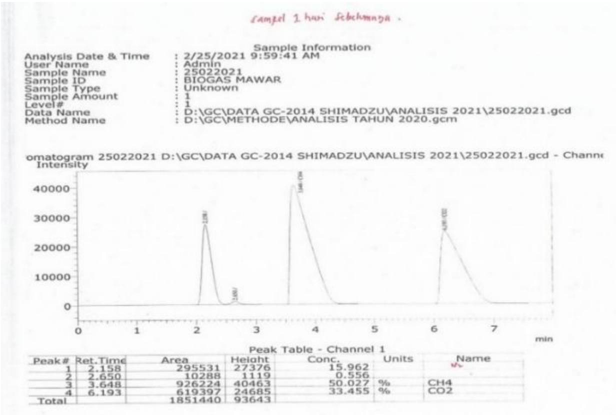
Analysis Date & Time : 2/25/2021 10:24:38 AM
User Name : Admin
Sample Name : 25022021 B
Sample ID : BIOGAS MAWAR
Sample Type : Unknown
Sample Amount : 1
Level# : 1
Data Name : D:\GC\DATA GC-2014 SHIMADZU\ANALISIS 2021\25022021 B.gcd
Method Name : D:\GC\METHODE\ANALISIS TAHUN 2020.gcm

natogram 25022021 B D:\GC\DATA GC-2014 SHIMADZU\ANALISIS 2021\25022021 B.gcd - Chan

Peak Table - Channel 1

Peak#	Ret.Time	Area	Height	Conc.	Units	Name
1	2.232	86520	9767	4.768		N_2
2	2.657	4141	449	0.228		
3	3.622	1038414	43338	57.226	%	CH4
4	6.172	685517	26288	37.778	%	CO2
Total		1814592	79842			

Figure 1. Biogas composition from the biogas bag in the acclimatization term.



sampel 1 hari sebelumnya .

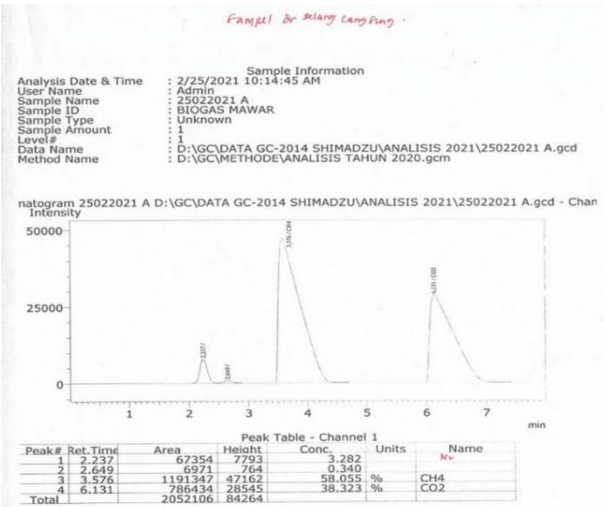
Sample Information

Analysis Date & Time : 2/25/2021 9:59:41 AM
User Name : Admin
Sample Name : 25022021
Sample ID : BIOGAS MAWAR
Sample Type : Unknown
Sample Amount : 1
Level# : 1
Data Name : D:\GC\DATA GC-2014 SHIMADZU\ANALISIS 2021\25022021.gcd
Method Name : D:\GC\METHODE\ANALISIS TAHUN 2020.gcm

Peak Table - Channel 1

Peak#	Ret.Time	Area	Height	Conc.	Units	Name
1	2.158	295531	27376	15.962		N2
2	2.650	10288	1119	0.556		
3	3.648	926224	40463	50.027	%	CH4
4	6.193	619397	24685	33.455	%	CO2
Total		1851440	93643			

Figure 2. Biogas composition analyzed from the biogas bag
One day earlier during the acclimatization term.



Sampel dr selang langsung .

Sample Information

Analysis Date & Time : 2/25/2021 10:14:45 AM
User Name : Admin
Sample Name : 25022021 A
Sample ID : BIOGAS MAWAR
Sample Type : Unknown
Sample Amount : 1
Level# : 1
Data Name : D:\GC\DATA GC-2014 SHIMADZU\ANALISIS 2021\25022021 A.gcd
Method Name : D:\GC\METHODE\ANALISIS TAHUN 2020.gcm

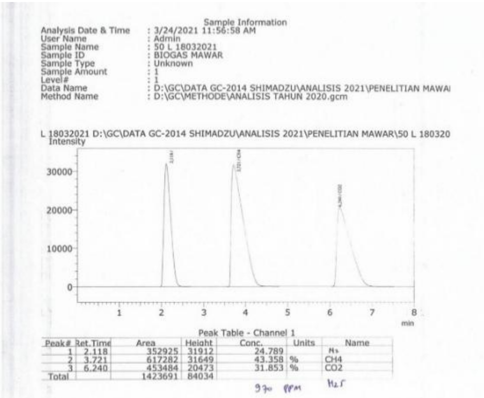
Peak Table - Channel 1

Peak#	Ret.Time	Area	Height	Conc.	Units	Name
1	2.237	67354	7793	3.282		N2
2	2.649	6971	764	0.340		
3	3.576	1191347	47162	58.055	%	CH4
4	6.131	786434	28545	38.323	%	CO2
Total		2052106	84264			

Figure 3. Biogas composition analyzed from the pipe of AD directly

- **Appendix C - The results of biogas composition, in variance of dosage substrate**

a)



Sample Information

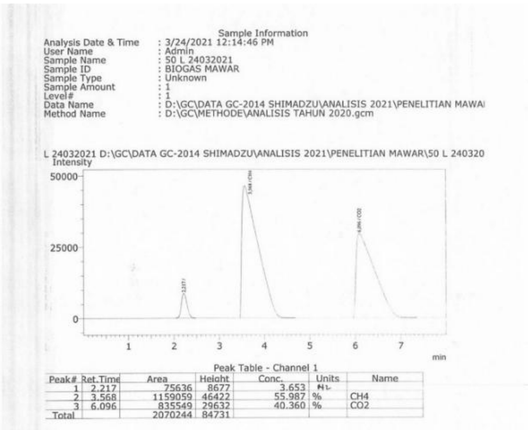
Analysis Date & Time	: 3/24/2021 11:56:58 AM
User Name	: Admin
Sample Name	: 50 L 18032021
Sample ID	: BIOGAS MAWAR
Sample Type	: Unknown
Sample Amount	: 1
Level#	: 1
Data Name	: D:\GC\DATA GC-2014 SHIMADZU\ANALISIS 2021\PENELITIAN MAWAI
Method Name	: D:\GC\METHODE\ANALISIS TAHUN 2020.gcm

Peak Table - Channel 1

Peak#	Ret.Time	Area	Height	Conc.	Units	Name
1	2.118	352925	31912	24.789		H_2
2	3.721	617282	31649	43.358	%	CH4
3	6.240	453484	20473	31.853	%	CO2
Total		1423691	84034			

970 ppm H_2S

b)



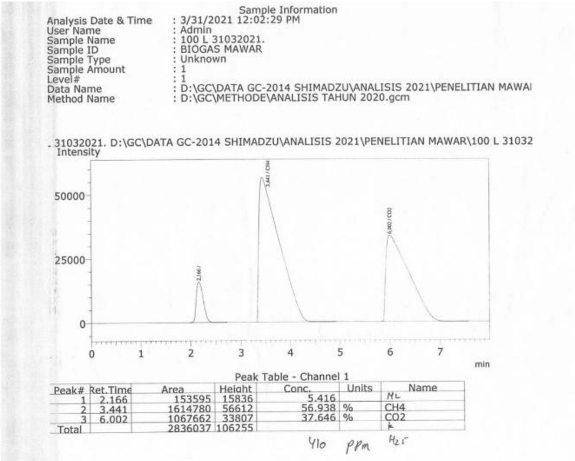
Sample Information

Analysis Date & Time	: 3/24/2021 12:14:46 PM
User Name	: Admin
Sample Name	: 50 L 24032021
Sample ID	: BIOGAS MAWAR
Sample Type	: Unknown
Sample Amount	: 1
Level#	: 1
Data Name	: D:\GC\DATA GC-2014 SHIMADZU\ANALISIS 2021\PENELITIAN MAWAI
Method Name	: D:\GC\METHODE\ANALISIS TAHUN 2020.gcm

Peak Table - Channel 1

Peak#	Ret.Time	Area	Height	Conc.	Units	Name
1	2.217	75636	8677	3.653	H_2	
2	3.568	1159059	46422	55.987	%	CH4
3	6.096	835549	29632	40.360	%	CO2
Total		2070244	84731			

Figure 1. a) biogas composition in the first week and b) the last week for dosage substrate 50 L.



Sample Information

Analysis Date & Time : 3/31/2021 12:02:29 PM
User Name : Admin
Sample Name : 100 L 31032021.
Sample ID : BIOGAS MAWAR
Sample Type : Unknown
Sample Amount : 1
Level# : 1
Data Name : D:\GC\DATA GC-2014 SHIMADZU\ANALISIS 2021\PENELITIAN MAWAI
Method Name : D:\GC\METHODE\ANALISIS TAHUN 2020.gcm

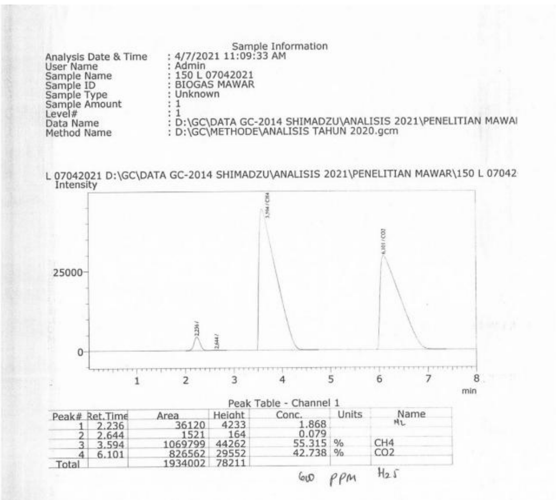
Peak Table - Channel 1

Peak#	Ret.Time	Area	Height	Conc.	Units	Name
1	2.166	153595	15836	5.416		H_2
2	3.441	1614780	56612	56.938	%	CH4
3	6.002	1067662	33807	37.646	%	CO2
Total		2836037	106255			

410 ppm H_2S

Figure 2. Biogas composition in the last week for dosage substrate 100 L

a)



Sample Information

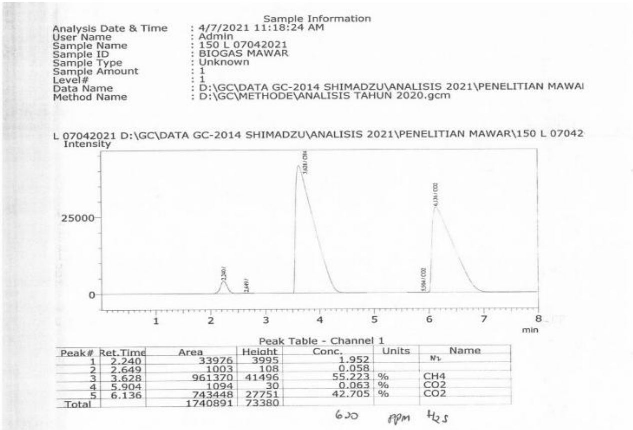
Analysis Date & Time : 4/7/2021 11:09:33 AM
User Name : Admin
Sample Name : 150 L 07042021
Sample ID : BIOGAS MAWAR
Sample Type : Unknown
Sample Amount : 1
Level# : 1
Data Name : D:\GC\DATA GC-2014 SHIMADZU\ANALISIS 2021\PENELITIAN MAWAI
Method Name : D:\GC\METHODE\ANALISIS TAHUN 2020.gcm

Peak Table - Channel 1

Peak#	Ret.Time	Area	Height	Conc.	Units	Name
1	2.236	36120	4233	1.868		H_2
2	2.644	1521	164	0.079		
3	3.594	1069799	44262	55.315	%	CH4
4	6.101	826562	29552	42.738	%	CO2
Total		1934002	78211			

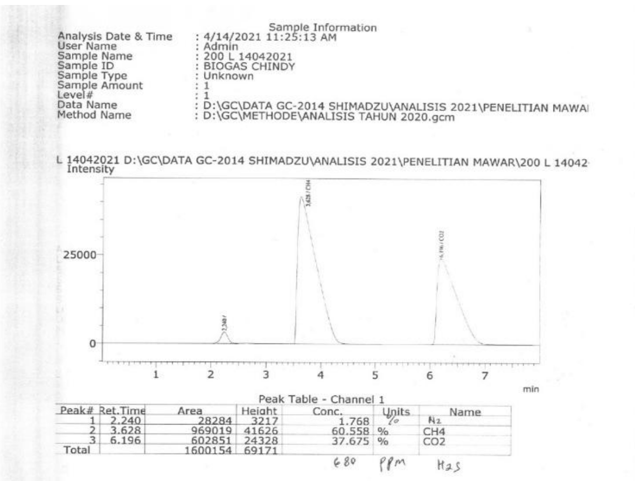
600 ppm H_2S

b)



Peak#	Ret.Time	Area	Height	Conc.	Units	Name
1	2.240	33976	3995	1.952		N2
2	2.649	1003	108	0.058		
3	3.628	961370	41496	55.223	%	CH4
4	5.904	1094	30	0.063	%	CO2
5	6.136	743448	27751	42.705	%	CO2
Total		1740891	73380			

Figure 3. a) biogas composition in the first week and b) the last week for dosage substrate 150 L



Peak#	Ret.Time	Area	Height	Conc.	Units	Name
1	2.240	28284	3217	1.768	%	N2
2	3.628	969019	41626	60.558	%	CH4
3	6.196	602851	24328	37.675	%	CO2
Total		1600154	69171			

Figure 4. Biogas composition in the last week for dosage substrate 200 L

- **Appendix D – Analysis of nutrient content of organic fertilizer from biogas effluent mix with commercial products**

Table S1. Analysis of nutrient content of biogas effluent with AB mix

Kode Dok : F-LAB-5.10.1
Revisi : 0

LABORATORIUM ANALISIS POLINELA

SERTIFIKAT ANALISIS
CERTIFICATE OF ANALYSIS (COA)

No. Sertifikat (*Certificate No*) : 129.b/09/PL15.13.17/INV/2021
Pelanggan (*Customer*) : Widia Rini
Tanggal sampling (*Date of Sampling*) : -
Tanggal diterima (*Date of Received*) : 2 September 2021
Jenis Sampel (*Subject of Sample*) : Limbah Cair
Identitas Sampel (*Customer Sample Id*) : Effluent+AB Mix
Deskripsi Sampel (*Discription of Sample*) : -

No.	Parameter	Unit	Result	Regulation	Method
1	Nitrogen (N-total)	mg/l	262.50	-	Kjedahl-Spektro
2	P-tersedia	mg/l	0.399	-	Spektrophotometri
3	Kalium	%	0.044	-	AAS
4	C-Organik	%	0.049	-	Walkley-Black
5	Kalsium (Ca)	mg/l	4.08	-	AAS
6	Magnesium (Mg)	mg/l	25.54	-	AAS
7	Tembaga (Cu)	ppm	0.032	-	AAS
8	Besi (Fe)	ppm	13.09	-	AAS

Bandar Lampung, 6 Oktober 2021
Manajer Teknik,

Yatim Rahayu Widodo
NIP. 196203271989031002

Table S2. Analysis of nutrient content of 100% biogas effluent

Kode Dok	: F-LAB-5.10.1
Revisi	: 0

LABORATORIUM ANALISIS POLINELA

SERTIFIKAT ANALISIS
CERTIFICATE OF ANALYSIS (COA)

No. Sertifikat (*Certificate No*) : 129.a/09/PL15.13.17/INV/2021
Jenis Sampel (*Subject of Sample*) : Limbah Cair
Pelanggan (*Customer*) : Widia Rini
Identitas Sampel (*Customer Sample Id*) : 100% Effluent
Tanggal sampling (*Date of Sampling*) : -
Deskripsi Sampel (*Discription of Sample*) : -
Tanggal diterima (*Date of Received*) : 2 September 2021

No.	*Parameter*	*Unit*	*Result*	*Regulation*	*Method*
1	Nitrogen (N-total)	mg/l	175.63	-	Kjedahl-Spektro
2	P-tersedia	mg/l	0.675	-	Spektrophotometri
3	Kalium	%	0.014	-	AAS
4	C-Organik	%	0.039	-	Walkley-Black
5	Kalsium (Ca)	mg/l	1.83	-	AAS
6	Magnesium (Mg)	mg/l	24.25	-	AAS
7	Tembaga (Cu)	ppm	<0.007	-	AAS
8	Besi (Fe)	ppm	9.53	-	AAS

Bandar Lampung, 6 Oktober 2021
Manajer Teknik,

Yatim Rahayu Widodo
NIP. 196203271989031002

Certified System
IAS-ANZ
KAN
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ISO 9001
SAI GLOBAL
QEC26269

Table S3. Analysis of nutrient content of 100% AB mix

Kode Dok	: F-LAB-5.10.1
Revisi	: 0

LABORATORIUM ANALISIS POLINELA

SERTIFIKAT ANALISIS
CERTIFICATE OF ANALYSIS (COA)

No. Sertifikat (*Certificate No*) : 129.c/09/PL15.13.17/INV/2021
Jenis Sampel (*Subject of Sample*) : Limbah Cair
Pelanggan (*Customer*) : Widia Rini
Identitas Sampel (*Customer Sample Id*) : 100% AB Mix
Tanggal sampling (*Date of Sampling*) : -
Deskripsi Sampel (*Discription of Sample*) : -
Tanggal diterima (*Date of Received*) : 2 September 2021

No.	*Parameter*	*Unit*	*Result*	*Regulation*	*Method*
1	Nitrogen (N-total)	mg/l	202.75	-	Kjedahl-Spektro
2	P-tersedia	mg/l	1.22	-	Spektrophotometri
3	Kalium	%	0.083	-	AAS
4	C-Organik	%	0.088	-	Walkley-Black
5	Kalsium (Ca)	mg/l	1.02	-	AAS
6	Magnesium (Mg)	mg/l	20.83	-	AAS
7	Tembaga (Cu)	ppm	<0.007	-	AAS
8	Besi (Fe)	ppm	8.70	-	AAS

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Yatim Rahayu Widodo
NIP. 196203271989031002

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Quality ISO 9001
QEC26269

- **Appendix E – Flowchart of tofu and tempeh production**

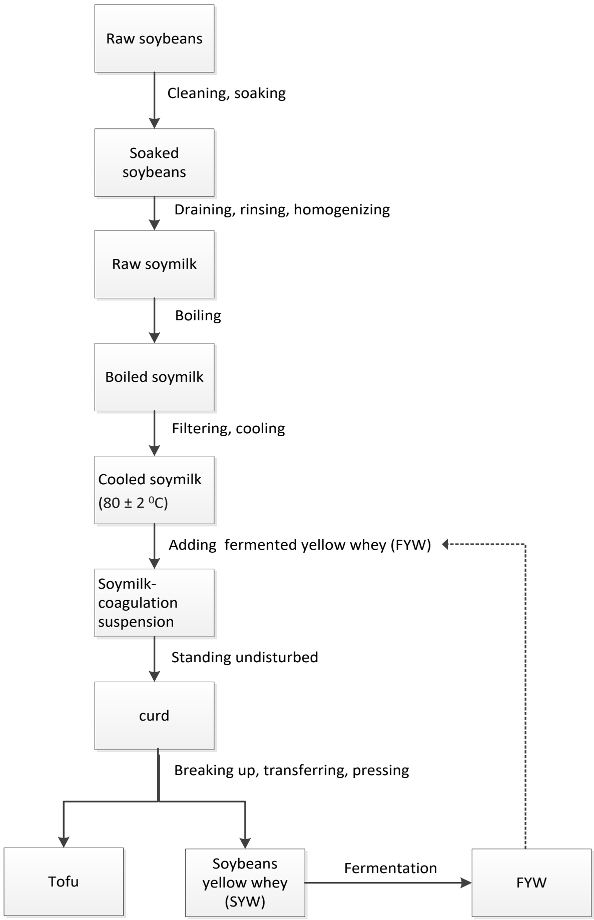

Fig.1. a) Flow chart of tofu process production, (Huang et al., 2021).

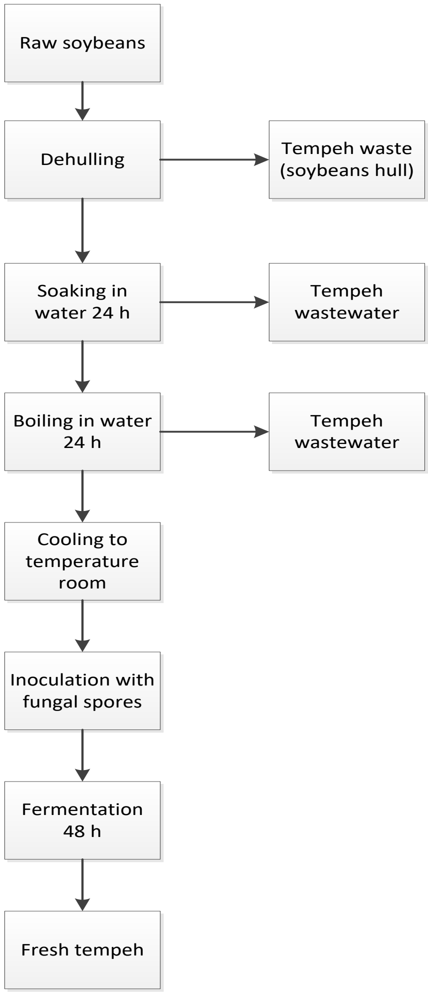

Fig. 1b. Flow chart of tempeh process production, Sources; (Chaerun, 2009)

Appendix F – Calculation of Economic Feasibility

Data project

- COD content = 12400 mg/L = 12.4 g/l
- Flowrate wastewater = 46.24 m^3/day
- COD removal = 95%
- CH_4 = 58.05%
- LHV CH_4 = 35.9 MJ/m^3
- Capex = €52,310
- Opex = €9417.63 / year
- Project life time = 10 years

a. CH_4 production;

12.4 g/l x 46.24 m^3/day x 0.35 l/gCOD x 0.95
= 190.64 CH_4 m^3/day

b. Energy production per day

35.9 MJ/m^3 x 190.64 m^3/day
= 6844.24 MJ/day

c. Saving cost per day

- Firewood;

$$\frac{6844.24 \text{ MJ/day}}{186 \text{ kg} \times 66.55 \text{ MJ/kg}} \times 100 = 55.29\%$$

55.29% x €5.09 = €2.28 per day

€2.28 x 365 = €832 per year

- Biopellet;

$$\frac{6844.24 \text{ MJ/day}}{350 \text{ kg x } 6.02 \text{ MJ/kg}} \text{ X } 100 = 53.95\%$$

53.95% x €30.08 = €13.85 per day

€13.85 x 365 = €5055 per year

- LPG

$$\frac{6844.24 \text{ MJ/day}}{20 \text{ x } 18.83 \text{ MJ/kg}} \text{ X } 100 = 18.17\ \%$$

18.17% x €6.27 = €5.13 per day

€5.13 x 365 = €1872 per year

d. Total saving cost per year :

€832 + €5055 + €1872 = €7760.17

e. Cash flow (CF) :

€7760.17 - €9417.63 = -1657.46 €

f. NPV :

$$NPV = \sum_{t=1}^{n} \frac{CF_t}{(1+r)^t} - Capex$$

r = 10% and n = 10

$$PV = -1657.46 \times \frac{1-(1+0.1)^{-10}}{0.1}$$

$= -10,\ 188.2$

NPV = PV – Capex

= - 10,188.2 - 52,310 = - 62,498.2 €

- **Appendix G – Substitution energy for non-renewable energy in tofu industry**

Table G1. Biogas production and CH_4 concentration in each OLR

OLR kgCOD/m^3·day	Biogas (L/day)	CH_4 (%)
0.66	199.50	49.78
1.33	148.69	56.93
2	310.49	55.26
2.67	137.11	60.64

a. Calculation for OLR at 0.66 kgCOD/m^3·day

Energy = 0.19950 x 0.4978 x 35.9 = 3.56 MJ/day

b. Substitution

$$\text{Firewood} = \frac{3.56}{66.55} \times 100\% = 5.3\%$$

$$\text{LPG} = \frac{3.56}{18.83} \times 100\% = 18.93\%$$

$$\text{Biopellet} = \frac{3.56}{6.02} \times 100\% = 59.2\%$$

c. Calculation for OLR 1.33 kgCOD/m^3·day

Energy = 0.14869 x 0.5693 x 35.9 = 3.03 MJ/day

d. Substitution

$$\text{Firewood} = \frac{3.03}{66.55} \times 100\% = 4.56\%$$

$$\text{LPG} = \frac{3.03}{18.83} \times 100\% = 16.13\%$$

$$\text{Biopellet} = \frac{3.03}{6.02} \times 100\% = 50.48\%$$

e. Calculation for OLR 2 $kgCOD/m^{3}\cdot day$

Energy = 0.3149 x 0.5526 x 35.9 = 6.15 MJ/day

f. Substitution

$$\text{Firewood} = \frac{6.15}{66.55} \times 100\% = 9.2\%$$

$$\text{LPG} = \frac{6.15}{18.83} \times 100\% = 32.71\%$$

$$\text{Biopellet} = \frac{6.15}{6.02} \times 100\% = 102.31\%$$

g. Calculation for OLR 2.67 $kgCOD/m^{3}\cdot day$

Energy = 0.137 x 0.6064 x 35.9 = 2.98 MJ/day

h. Substitution

$$\text{Firewood} = \frac{2.98}{66.55} \times 100\% = 4.48\%$$

$$\text{LPG} = \frac{2.98}{18.83} \times 100\% = 15.85\%$$

$$\text{Biopellet} = \frac{2.98}{6.02} \times 100\% = 49.58\%$$

Table G2. Substitution of non-renewable energy from biogas production in each OLR

OLR kgCOD/m^3·day	Biogas (L/day)	CH_4 (%)	Substitution energy (%)
0.66	199.50	49.78	Firewood = 5.3 LPG = 18.93 Biopellet = 59.2
1.33	148.69	56.93	Firewood = 4.56 LPG = 16.13 Biopellet = 50.48
2	310.49	55.26	Firewood = 9.2 LPG = 32.71 Biopellet = 102.31
2.67	137.11	60.64	Firewood = 4.48 LPG = 15.85 Biopellet = 49.58