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OPTIMIZATION OF METHANE PRODUCTION FROM MIXED SUBSTRATES OF COW FAECES AND CARICA SEEDS USING RESPOND SURFACE METHODOLOGY

Optimasi Produksi Metana pada Substrat Campuran Feses Sapi dan Biji Carica Menggunakan *Respond Surface Methodology*

Setio Susanto[1,2], Sutaryo Sutaryo[1]*, Agung Purnomoadi[1], Doni Abeng[1], Rita Purwasih[1,3]
[1]Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, 50275, Indonesia.
[2]Feed Quality Testing and Certification Center, Bekasi, Jawa Barat, 17320, Indonesia.
[3]Department of Agroindustry, Subang State Polytechnic, Subang, 41285, Indonesia.
*Email: soeta@lecturer.undip.ac.id

ABSTRACT

Anaerobic digestion (AD) of dairy cow feces (DCF) has low methane production per ton of waste. A strategy to overcome this drawback is to co-digest DCF and carica seed (CS). Currently, CS is still a waste from the candied carica in the syrup industry and is often just thrown away into the environment. This research aims to evaluate the optimal level of combination for methane production from DCF with co-substrates of germinated (CGM) and non-germinated (CNG) CS meal using Respond Surface Methodology (RSM). This research uses a completely randomized design with a factorial pattern consisting of the first factor being CGM and CNG, and the second factor being the combined level of DCF and CS (CGM or CNG. The result showed that utilization of CS can increase significantly ($p<0.05$) methane production of the final substrate compared to the control (digester treating DCF only). Utilization of CGM as co-substrate with DCF can also increase methane production ($p<0.05$) compared to CNG. All parameters in the liquid phase were in the normal range for AD. Based on the research results, the optimum point with a desirability value close to 1 was achieved at a ratio of CGM10 and DCF90. CS was proven can be used as a co-substrate with DCF to increase methane production of the final substrate and germination can be used as a method to increase the methane yield of CS.

Keywords: *Biogas, Carica Seeds, Dairy Cow Faeces, Germination, Substrate*

ABSTRAK

Penanganan limbah secara anaerob dari feses sapi perah (FSP) memiliki produksi metana yang rendah per ton limbah yang ditangani. Salah satu strategi untuk mengatasi kelemahan ini adalah dengan melakukan co-substrate antara FSP dan biji carica (BC). Saat ini, BC masih menjadi limbah dari industri manisan carica dan sering kali dibuang begitu saja ke lingkungan. Penelitian ini bertujuan untuk mengevaluasi tingkat kombinasi optimal untuk produksi metana dari FSP dengan co-substrat tepung BC baik yang dikecambahkan (CGM) dan maupun yang tidak dikecambahkan (CNG) menggunakan *Respond Surface Methodology* (RSM). Penelitian ini menggunakan rancangan acak lengkap dengan pola faktorial yang terdiri dari faktor pertama yaitu CGM dan CNG, dan faktor kedua yaitu gabungan level FSP dan BC (CGM atau CNG. Hasil penelitian menunjukkan bahwa pemanfaatan BC dapat meningkatkan produksi metana

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dari substrat akhir secara signifikan ($p<0,05$) dibandingkan dengan kontrol (digester yang hanya mengolah FSP). Pemanfaatan CGM sebagai co-substrat dengan FSP juga dapat meningkatkan produksi metana ($p<0,05$) dibandingkan dengan CNG. Semua parameter pada fase cair berada pada kisaran normal untuk AD. Berdasarkan hasil penelitian, titik optimum dengan nilai desirability mendekati 1 dicapai pada rasio CGM10 dan DCF90. CS terbukti dapat digunakan sebagai co-substrat dengan FSP untuk meningkatkan produksi metana substrat akhir dan perkecambahan dapat digunakan sebagai metode untuk meningkatkan produksi metana dari BC.

Kata kunci: Biji Carica, Biogas, Feses Sapi Perah, Germinasi, Substrat

INTRODUCTION

The rapid growth of the world's population has caused serious challenges to the global environment. This phenomenon causes various problems, especially the reduction in non-renewable natural resources and the increase in waste production which can damage the ecosystem (Abdesalam et al. 2017; Harirchi et al. 2022). Therefore, various alternative clean energy sources have been used as renewable energy to replace fossil fuels to overcome environmental and economic problems (Ma et al. 2018; Suanon et al. 2016; Wandera et al. 2018).

The use of energy resulting from biomass through biological processes is identified as a potential solution because biomass is a significant renewable energy source. Biomass-based energy can result from various organic wastes, including animal waste, food waste, industrial waste sludge, and agricultural waste (Abdesalam et al. 2015; Chew et al. 2021; Ganzoury and Allam 2015; Sekoai et al. 2021). Waste processing technology with the anaerobic digestion (AD) process is one of the most interesting, efficient and promising processing techniques. In the AD process, there is a series of complex, multi-stage, interrelated biochemical processes, namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Through these processes, organic waste is degraded into biogas, a mixture of gases including methane, with the help of anaerobic microorganisms (Jin et al. 2018; Keskin et al. 2019; Yilmaz and Sahan 2020). The environment in the AD process is ecologically balanced for the growth of bacterial and archaic microorganisms to degrade organic macromolecules (carbohydrates, proteins and lipids) into biogas under conditions without oxygen.

Research on anaerobic digestion processes involving various types of biomass as a single substrate has been carried out by several researchers (Babaee et al. 2013; Khalid et al. 2011; Sutaryo et al. 2012; Sutaryo et al. 2014). The research results show that there is a main problem in this process, namely nutritional imbalance, especially in the Carbon/Nitrogen (C/N) ratio. These findings are in line with previous studies (Møller et al. 2014; Triolo et al. 2013) reporting that the use of a single substrate from livestock faeces tends to have low nutritional quality so the biogas production is low. Therefore, current research proposes a solution by applying co-substrate to increase biogas production.

Various research methods have been tested in the use of co-substrates for biogas production, namely corn silage, sunflower, and Sudanese grass (Amon et al. 2007), tapioca industrial waste (Hasanudin et al. 2023), tofu industrial waste (Purwanti et al. 2022), pineapple skin (Arifan et al. 2022), and cogon grass (Mustikasari et al. 2023). The research results showed that the use of co-substrates is effective in overcoming nutritional imbalances, improving substrate quality, and ultimately increasing biogas productivity. Further research is needed to explore other potential biomass as co-substrates of dairy cow faeces (DCF).

Carica fruit (*Carica pubescens* (CP)) is a typical highland plant in Indonesia, which is often found on the Dieng plateau, Central Java, Indonesia. Data from BPS (2022) records that carica production in 2021 is 3,286 tons. Solid waste from carica fruit consists of 20% peel and 50% seeds (Arija et al., 2022), which are often thrown into landfills by the candied carica in the syrup industry. In 2021, therefore the CS production in Dieng was 1,643 tons. Utilization of carica seeds (CS), is very important in supporting the concept of clean production. This approach aims to increase the efficiency of natural resource use and prevent environmental pollution. The mixture of CS with DJF as a co-substrate is expected can increase methane production of the final substrate since CS is a nutritious biomass (Table 1).

Table 1. Nutritional content of CP seeds with germination and without germination

Nutritional contents	CNG	CGM	Sig (2-tailed)	Significant
TS (%)	94.24±0.12	91.34±0.00	0.001	Yes
VS (%)	89.99±0.19	87.12±0.04	0.002	Yes
Crude Protein (%)	33.09±0.80	33.93±0.01	0.273	No
Crude Fat (%)	37.50±0.86	33.08±0.22	0.019	Yes
Crude Fibre (%)	22.24±0.39	24.72±0.40	0.025	Yes
NDF (%)	30.17±0.03	32.24±0.12	0.002	Yes
ADF (%)	22.45±1.05	25.33±0.62	0.000	Yes
Lignin (%)	16.67±0.67	18.74±0.46	0.012	Yes
Hemicellulose (%)	8.06±0.57	7.51±0.31	0.352	No
Cellulose (%)	5.12±0.13	6.55±0.00	0.004	Yes
Lysine (%)	2.67	3.06	Not Test	-
Methionine (%)	0.22	0.26	Not Test	-
TS (%)	94.24±0.12	91.34±0.00	0.001	Yes

The nutritional content of CP seeds can be increased through the germination process. Previous research by (Medugu et al. 2012; Sugiharto et al. 2022) stated that the germination process can cause several changes in the seed, such as the decomposition of certain components and the transport of minerals from one part of the seed to another. It is hoped that the use of CP seeds with the germination process will not only improve the nutritional value of the seed waste but also support sustainable biogas production.

Optimizing parameters in biogas installations is the key to increasing biogas production from organic waste. Conventional optimization methods are limited because they require many trials and a long time (Safari et al. 2018). To overcome this, one of the statistical programs namely Response Surface Methodology (RSM) is used as an optimization approach. Current research aims to examine the effect of the combination of Holstein Friesian cow faeces and CP seeds as mixed substrates on biogas production and then find the optimal combination for obtaining maximum biogas production using the RSM approach.

MATERIALS AND METHODS

Materials

The inoculum was the slurry output from the active reactor treating cow faeces at the Faculty of Animal Husbandry and Agriculture, Diponegoro University, Indonesia. Before use, the slurry was filtered with a filter cloth. The characteristics of inoculum were pH of 7.53, Total Solid (TS) of 0.810 %, and Volatile Solids (VS) of 0.423 %. Dairy cow faeces were obtained from the Faculty of Animal Husbandry and Agriculture, Diponegoro University, Indonesia. Carica (CP) seeds were obtained from processing waste from the Carica fruit candy factory in Wonosobo, Central Java Province, Indonesia. Before use, CP seeds were washed to remove their epidermis. To make germinated CP seeds, the cleaned CPD seeds were germinated for 21-30 days. Hence, there were two types of CP seeds, namely germinated CP seeds (CGM) and non-germinated CP seeds (CNG). The two types of CP seeds were dried directly under the hot sun for 2 days. After drying, they were ground with a grinder (Retsch-ZM 200, Germany) for further processing.

Methods

In this research, 114 glass bottles with a capacity of 500 mL were prepared as batch digesters. The inoculum as much as 200 g was filled in each digester. Then, substrates were added with various of mixed substrates dairy cow faeces (DCF) and CGM or CNG flour with percentage combinations of 98:2, 96:4, 94:6, 92:8, 90:10 (w/w) with 4 replications. Thus, the substrate combination included CGM2 (98% DCF and 2% CGM), CNG2 (96% DCF and 2% CNG), CGM4 (96% DCF and 4% CGM), CNG4 (96% DCF and 4% CNG), CGM6 (94% DCF and 6% CGM), CNG6 (94% DCF and 6% CNG), CGM8 (92% DCF and 8% CGM), CNG8 (92% DCF and 8% CNG), CGM10 (90% DCF and 10% CGM), CNG10 (90% DCF and 10% CNG) and 100% DCF as a control. The filled digester was sealed, flushed with nitrogen for two minutes, and stored for 90 days in an incubator at 37°C. As many as 48 main digesters were used to measure methane volume production which was measured on days 3, 7, 12, 20, 30, 45, 60, 75, and 90. For the analysis of pH and TAN, as many as 22 digesters were operated and disassembled for each period of 0, 30, and 60 days. Meanwhile, for analysis of pH and TAN on day 90, samples were taken from the main digesters. These were conducted because it was not possible to dismantle the main digesters to take samples to observe the pH, TAN and VFA during the AD process. The resulting pH and TAN data were averaged and then would be discussed descriptively in the Results and Discussion. Meanwhile, the VFA analysis was carried out for samples on day 60. VS reduction analysis was carried out by observing VS reduction at the beginning and the end of the AD process. The VS reduction was analysed to obtain information about the process performance of the microorganisms in the digesters.

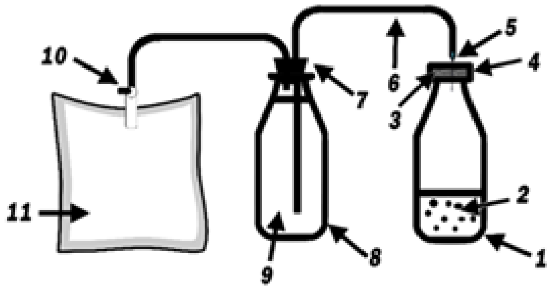
Analysis and Observation

Analysis of the TS content was conducted by heating the samples at 105°C for 12 hours. Then, the dried samples were heated in a furnace at 550°C for 6 hours to obtain the ash content data. VS content was calculated by subtracting the ash content from TS content. Analysis of fibre contents (Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL)) was carried out by following the standard method by Van Soest et al. (1991). According to Møller et al. (2014), ADL was assumed to be lignin. Cellulose was the difference value between ADL and ADF. Hemicellulose was the difference value between ADF and NDF. Crude Protein analysis was determined using the Kjeldahl method.

Methane production from the main digester was collected using a 500 mL glass bottle containing a 4% (w/w) NaOH solution (Merck®, cat no: 1064981000) for capturing CO_2 (Sutaryo et al. 2022a) so that only methane was accommodated in a Tedlar gasbag (Hedetech-Dupont, China) with a capacity of 1 L (Figure 1). Then, the water displacement method was used to measure the methane volume (Sutaryo et al. 2020) following Figure 2.

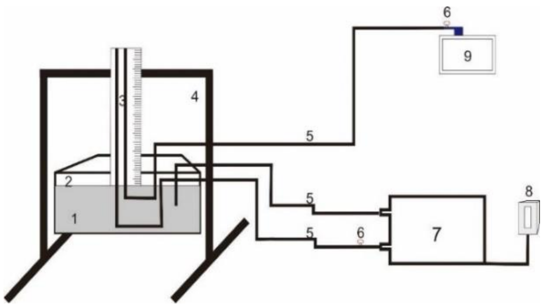
The pH level was measured using a digital pH meter (OHAUST ® ST 300). The total ammonia nitrogen (TAN) concentration was analysed through the Photometric method using a Spectroquant Nova 60A instrument and the Spectroquant® Ammonium Test Method reagent set (paint no: 1006830001). Meanwhile, the total volatile fatty acid (VFA) was measured using the Perkin Elmer Clarus® 680 Gas Chromatograph instrument.

The methane production data were analyzed statistically using a two-factor analysis of variance with a confidence level of 95%, followed by the Duncan test. Then, an optimization process was conducted using Response Surface Methodology (RSM).



1. Batch digester
2. Inoculum and substrate
3. Rubber stoper
4. Aluminium crimp
5. Hypodermic syringe
6. Teflon tube
7. Black rubber stopper
8. Infusion bottle
9. NaOH solution
10. Valve
11. Tedlar gas bag

Figure 1. Method for collecting methane from a batch digester (Sutaryo et al. 2022b)



1. Tab Water
2. Water Container
3. Measuring glass
4. Measuring glass Holder
5. Teflon tube
6. Valve to pump and valve to tedlar gas bag
7. Water pump
8. Swictch
9. Tedlar gas bag

Figure 2. Apparatus for measuring methane volume (Sutaryo et al. 2020)

RESULTS AND DISCUSSION

Characteristics of Co-substrates

The substrate used in this research was cow faeces as the main substrate and CP seeds (non-germinated and germinated) as the co-substrates. Cow faeces have high crude fibre but low crude protein, while CP seeds have low crude fibre but high crude protein. The different forms of CP seeds (germinated (CGM) and non-germinated (CNG)) can influence biogas production. Hence, it is important to carry out characterization to determine the nutritional content of the CP seeds. The characterization results are presented in Table 2.

Table 2. Volatile solid proportion, pH, concentrations of TAN, VFA Total, and VS Reduction

Treatment	VS Proportion of CNG/CGM to the mixed substrate (%)	Parameters			
		TAN (mg/L)	Total VFA (mg/L)	Volatile Solid Reduction (%)	pH
DCF 100% (Control)	0	304.63±85.46	15.73	33.86±2.89	7.45±0.02
CGM2	52.83	282.50±74.28	8.21	33.16±5.79	7.46±0.15
CGM4	55.35	280.00±114.38	6.82	35.66±9.33	7.45±0.14
CGM6	57.62	251.62±78.25	6.77	38.72±6.01	7.44±0.12
CGM8	59.67	264.12±104.87	5.00	40.87±1.13	7.43±0.11
CGM10	61.52	269.87±114.13	8.01	42.19±1.43	7.39±0.10
CNG2	52.94	273.37±65.55	13.77	33.24±8.99	7.47±0.14
CNG4	55.56	251.00±102.53	3.08	32.61±12.34	7.46±0.17
CNG6	57.90	210.12±68.39	7.11	37.53±10.70	7.44±0.15
CNG8	60.00	256.37±63.92	2.31	36.07±1.37	7.45±0.16
CNG10	61.92	249.37±90.38	8.18	37.41±6.76	7.45±0.14

Based on the proximate test (Table 1), the germination process decreased TS and VS in the CP seeds, indicating an increase in plant cellular structure during the germination process (Nkhata et al. 2015). Crude fibre, which consists of cellulose and lignin, increased significantly during the germination process but not hemicellulose. This increase in crude fibre is in accordance with research by Laxmi et al. (2015). Germination also increased the crude protein but not significantly. Then, based on the amino acid test, the germination increased lysine and methionine, showing a significant increase in the biological value of protein.

The concentration of crude fat in germinated CS in this experiment was decreased. This phenomenon can be caused by increased lipase enzyme activity and changes in cellular metabolism. During the germination process, the lipase enzyme breaks down fat into fatty acids and glycerol, which are used as energy sources and structural materials to support the growth and development of plant embryos. As a result, the crude fat content in seeds was decreased (Permana et al. 2013). The increasing activity of various enzymes during the germination process will break down some various complex compounds into their monomers so that these monomers will be easier to digest while the germinated CS is processed anaerobically. Table 1 shows the nutritional contents of CP seeds, which mostly increase due to the germination process. This finding is similar to research conducted by Medugu et al. (2012) reporting that there is the breakdown of certain components in the grain during germination, causing the degradation of complex compounds to become simple compounds. It is hoped that the germinated substrate is easily digested by anaerobic microorganisms in the biogas digesters.

pH

The level of pH effectively influences the digester's performance and biogas production. According to (Lahbab et al. 2021; Harirchi et al. 2022) the optimum pH range in the AD process is 6.5 - 7.5. The optimum pH level can induce the growth of methane-producing microorganisms to increase their population (Boone et al. 2011). In this research, the average pH value in digesters ranged from 7.39 to 7.47 (Table 2). This means that the pH values were in the optimum pH range for the AD process.

Total Ammonia Nitrogen (TAN), Total Volatile Fatty Acid (VFA), and Volatile Solid Reduction

Ammonia has an important role in the AD process because it is an essential nutrient for anaerobic microorganism growth. However, it should be noted that TAN concentrations > 1,700 mg/L can inhibit methane formation (Cristou et al. 2021). The analysis results in Table 2 show that the TAN concentration value was in the range of 210.10 to 282.50 mg/L. A previous study (Czatzowska et al. 2020) stated that a TAN concentration of around 200 mg/L is beneficial for the anaerobic digestion process because the methanogenic microorganisms can grow well in this condition.

In anaerobic systems, VFAs are generated during the AD process, but too high concentrations of VFAs can disrupt the balance of the system (Alavi-Borazjani et al. 2020). Accumulation of VFAs can lower pH levels, increase acid production, and ultimately lead to AD process failure. VFA concentrations can vary based on feedstocks, inoculums, operational conditions, and digester configurations.

The final total VFA after the AD process for substrates of cow manure and kitchen waste was <1,000 mg/L (Li et al. 2014), cow manure and Barley was <20,000 mg acetic acid /L (Akyol et al. 2016), and fish waste and cow dung was 816 mg/L (Solli et al. 2014). In this study, the total VFA was in the range of 2.31 to 13.77 mg/L (Table 2) indicating that the difference in substrate combination did not affect the pH level significantly.

The VS reduction in this study was in the range 33.16 to 42.19% (Table 4). The VS reduction was increased with the increasing proportion of CS in the final substrate, which caused an increase in methane production. This indicates that the anaerobic microorganism's can digest organic matter in the CS properly. Utilization of CS as co-substrate with FSP, can increase anaerobic microorganism activity at least by improving nutrient quality and C/N ratio of the final substrate (Sutaryo et al. 2023).

Production of Methane

The analysis results showed that the use of mixed substrates significantly increased cumulative methane production (mL/g VS) ($p<0.05$) up to day 90 (Table 3, 4, and 5). Treatment of CGM10 resulted in the highest methane production (514.01 mL/g VS) and treatment of CNG2 resulted in the lowest methane production (425.12 mL/g VS). Compared to the control variable, the AD of cow feces with co-substrates of CP seeds (either in the form of CGM or CNG) at various compositions resulted in higher biogas production with a significant difference ($p<0.05$). Furthermore, there was no significant difference ($p>0.05$) between CNG2 and CNG4.

Table 3. Cumulative Methane Production on day 30

Factor	Treatment					Average
	B1	**B2**	**B3**	**B4**	**B5**	
			--------------------(ml/g VS)-----------------			
A1	395.19±8.05	402.67±12.99	424.62±9.81	431.14±12.19	453.78±14.58	421.48±29.89^{y}
A2	367.28±3.50	382.57±15.61	392.71±13.52	408.10±7.81	430.72±16.17	396.27±24.87^{x}
Average	**381.23±15.98^{a}**	**392.62±17.09^{a}**	**408.66±20.26^{b}**	**419.62±15.54^{b}**	**442.25±18.85^{c}**	

^{abc} : values in each row in the same parameter followed by the same letters not significantly different ($p>0.05$)

^{x,y} : values in each column in the same parameter followed by the same letters not significantly different ($p>0.05$)

Table 4. Cumulative Methane Production on day 60

Factor	Treatment					Average
	B1	**B2**	**B3**	**B4**	**B5**	
			--------------------(ml/g VS)-----------------			
A1	425.98±6.68	447.81±10.76	458.61±11.00	466.34±7.47	488.78±10.83	457.50±22.88^{y}
A2	408.35±13.29	412.76±21.86	423.46±12.59	442.79±9.85	459.14±15.87	429.29±23.80^{x}
Average	**417.17±13.53^{a}**	**430.28±24.61^{b}**	**441.03±21.74^{b}**	**454.56±14.96^{c}**	**473.95±20.23^{d}**	

^{abc} : values in each row in the same parameter followed by the same letters not significantly different ($p>0.05$)

^{x,y} : values in each column in the same parameter followed by the same letters not significantly different ($p>0.05$)

Table 5. Cumulative Methane Production on day 90

Factor	Treatment					Average
	B1	**B2**	**B3**	**B4**	**B5**	
			--------------------(ml/g VS)-----------------			
A1	463.76±12.08	470.91±11.93	488.29±4.69	492.29±7.92	514.01±4.68	485.85±19.72^{y}
A2	425.12±18.25	427.85±23.09	451.38±9.83	455.36±10.83	469.55±15.87	445.85±22.64^{x}
Average	**444.44±25.14^{a}**	**449.38±28.62^{a}**	**469.83±20.98^{b}**	**473.83±21.61^{b}**	**491.78±26.12^{c}**	

^{abc} : values in each row in the same parameter followed by the same letters not significantly different ($p>0.05$)

^{x,y} : values in each column in the same parameter followed by the same letters not significantly different ($p>0.05$)

Comparison between CGM (A1) and CNG (A2) factors shows that the CGM factor generated a higher methane production. It indicates that the germination pretreatment had a positive impact on the AD process. The germination process significantly changed the grain composition and improved the nutritional quality of grains.

Research conducted by Diaz-Batalia (2023) showed that germination can reduce anti-nutrient content and increase amino acids. Winarti et al. (2024) reported that CS contains anti-nutrient compounds such as flavonoids and polyphenols. These anti-nutrient compounds will be degraded during the germination process, resulting in a decrease in anti-nutrient levels. These anti-nutrient compounds have the potential to inhibit the activity of methane-forming bacteria (Hütter et al. 2023).

Previous studies (Medugu et al. 2012; Sugiharto et al. 2022) showed that there is a breakdown of certain components in the seeds and the transport of minerals from one part of the seed to another during the germination process. In addition, previous studies (Fouad dan Rehab 2015; Lien et al. 2017; Sugiharto 2021; Betran-Orocho et al. 2020) also stated an increase in the nutritional quality of grain after the germination process. Current research also found the same findings that the germination process increased the nutritional quality of the CP seeds as shown in Table 1. Research conducted by Purwasih et al. (2024) demonstrates that germination can increase significantly ($p<0.05$) methane production of papaya seeds.

The use of a pretreatment process with a grinder to reduce the substrate size has been proven to be effective in aiding digestion by microorganisms. These findings provided valuable information in the context of this research, indicating that the combination of germination and grinding pretreatments can positively improve the nutritional quality of the seeds and then influence methane production.

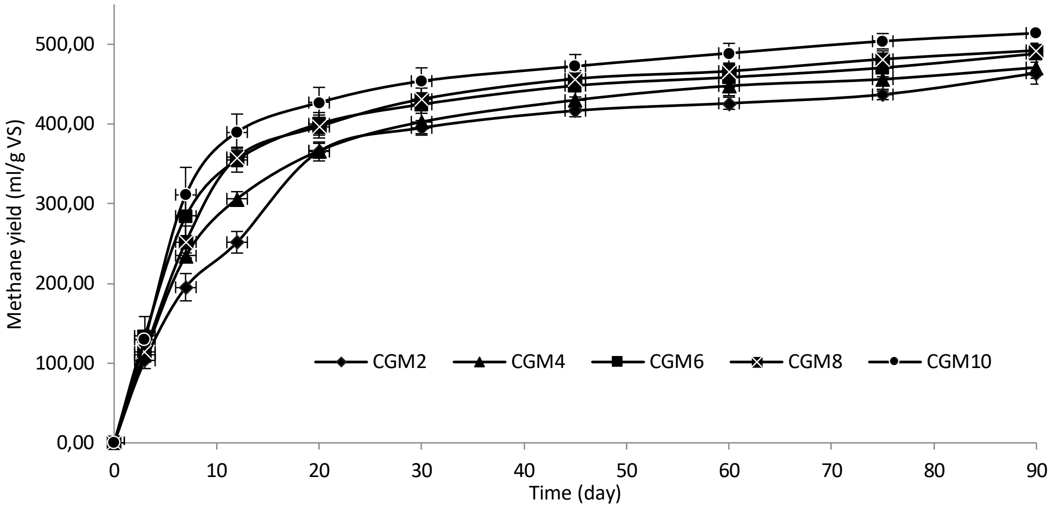


Figure 3. Profile of methane production using CGM as a co-substrate until 90 days

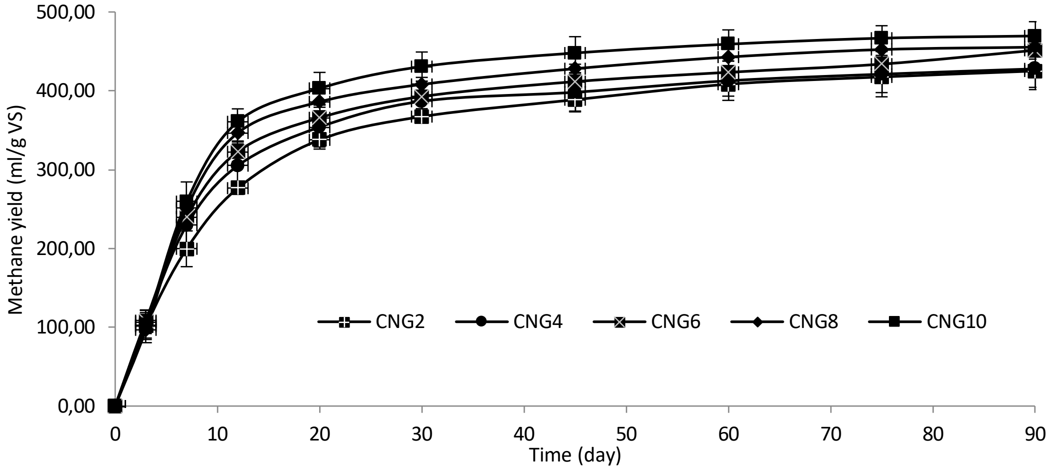
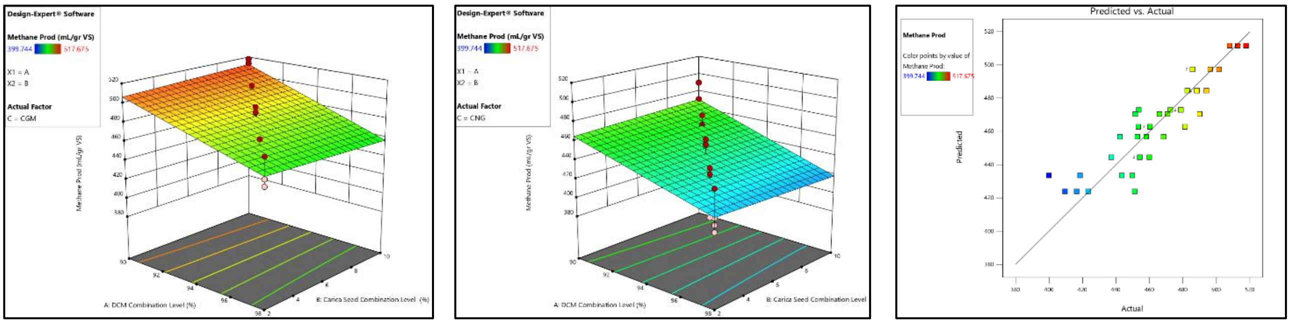


Figure 4. Profile of methane production using CNM as a co-substrate until 90 days

Figures 3 and 4 show the methane production rate. The increase in the addition of CP seeds either with or without germination pretreatment significantly increased methane production (p>0.05). The increase in CP seed composition was directly proportional to the increase in methane production. Hence, the higher the composition of CP seeds was in mixed substrates, the higher the methane production was generated. These results indicated the potential for increasing methane production through the addition of CP seeds. Based on Figures 3 and 4, the methane production rate on day 3 reached its highest value until day 12 day, and then it decreased until day 90. This indicates that the optimal methane production began on day 3. This shows that the co-substrate that has been ground with a grinder (physical pretreatment) can be hydrolyzed quickly by microorganisms to produce methane.

Studies on the effects of germination on methane production are still minimal. One of them is a study by Purwasih et al. (2024) which examined the effects of gemination of papaya seeds (PS) on methane production as a single substrate, where the treatment was able to increase methane production (p<0.05) by 180%, 57%, and 81% for fresh PS, fresh PS meal, and dry PS, respectively. In this study, when germinated CS was used as a co-substrate with FSP, it significantly increased methane production by 6.31% compared to non-germination.



(A) (B) (C)

Figure 5. Response of variations of combination level of DCF and CP seeds (CGM) on methane production (A). Response of variations of combination level of DCF and CP seeds (CNG) on methane production (B). Comparison between experimental data and predicted data (C).

This experiment used 44 combinations of factors to find the optimum condition using the RSM method with central composite design (CCD). The CCD is conducted to explore and optimize the response of a system that is influenced by several input variables. This design involves a combination of centre points, factorial points, and axial points to create a surface response model that approaches the optimal condition. The RSM analysis (Figures 5A and 5B) was conducted in this research to show the relationship between CP seed combination level (%), DCF combination level (%), and type of CP seeds (CNG and CGM). The validity of the model was evaluated through the coefficient of determination (R^2). The R^2 shows the extent to which the experimental variables and their interactions can explain variations in the observed response values. The use of R^2 aims to assess the quality of the model, with a high correlation between the predicted value and the observed value. The R^2 value of 0.8273 indicates that the model can explain 82.73 of the observed response variability.

The predicted data obtained from the model are close to the experimental data obtained from the experiment (Figure 5(c)). The graphic plot depicts a satisfactory correlation between the experimental data and the predicted data. It shows the closeness of both values to a linear line. According to Table 6, the recommended value for the optimum desirability condition is close to 1. Therefore the use of CGM as a co-substrate at a combination level of 10% (DCF combination level 90%) is recommended. This optimization uses a model to find the best factors to increase methane production byconsidering other factors. In RSM, the desirability value describes the range of responses, reflecting the desired range in each response (Myers and Montgomery 2002). In simultaneous optimization, each response must have a maximum value according to the target to be achieved. Thus, the goal of optimization is to find an optimal solution that meets ideal conditions.

Table 6. Recommended Optimization Value at Maximum Desirability

No	DCF Combination Level	Carica Seed Combination Level	Type of Carica Seed	Methane Production	Desirability	No
1	**90.00**	**10.00**	**CGM**	**511.60**	**0.948**	**Selected**
2	90.00	9.93	CGM	511.55	0.948	
3	90.00	9.87	CGM	511.51	0.948	
4	90.04	10.00	CGM	511.31	0.946	
5	90.00	9.40	CGM	511.19	0.945	
6	90.15	10.00	CGM	510.58	0.94	
7	90.00	7.72	CGM	510.04	0.935	
8	90.00	7.31	CGM	509.76	0.933	
9	90.00	6.38	CGM	509.12	0.927	
10	90.00	2.80	CGM	506.68	0.907	

CONCLUSION

The results showed that the addition of CS (CGM and CNG), as a co-substrate of DCF up to a percentage of 10% co-substrate addition can increase methane production compared to the control. The highest methane production was 514 mL/g VS in the CGM10 treatment and the lowest methane production was 425 mL/g VS (CNG2). However, for commercial applications, additional costs required for the increase in biogas production obtained caused the germination process have to be recalculated. The results of optimization using RSM, the optimum point with a desirability value approaching 1 was achieved in the CGM10 treatment (90% DCF and 10% CGM). The concentration of VFA and TAN was sufficient to meet the needs of microorganisms, and the pH value was at the optimum level for anaerobic digestion. The use of RSM in this study not only found the optimum conditions for the use of CS, but also showed a methodological approach that could be adopted in similar studies in the future.

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REFERENCES

Abdelsalam E, Samer M, Abdel-Hadi MA, Hassan HE, Badr Y (2015) Effect of $CoCl_2$, $NiCl_2$ and $FeCl_3$ on biogas and methane production. Misr J. Agric. Eng 32:843-862. doi:10.21608/mjae.2015.98656

Abdelsalam E, Samer M, Attia YA, Abdel-Hadi MA, Hassan HE, Badr Y (2017) Effects of Co and Ni nanoparticles on biogas and methane production from anaerobic digestion of slurry. Energy Conv. Man 141:108-119. doi:10.1016/j.enconman.2016.05.051

Akyol C, Ozbayram EG, Ince O, Kleinsteuber S, Ince B (2016) Anaerobic co-digestion of cow manure and barley: effect of cow manure to barley ratio on methane production and digestion stability. Environ. Prog. Sustain. Energy 35:589–595. https:doi.org/10.1002/ep.12250

Alavi-Borazjani SA, Capela I, Tarelho LAC (2020) Over-acidification control strategies for enhanced biogas production from anaerobic digestion: a review. Biomass Bioenerg 143:105833. doi:10.1016/j.biombioe.2020.105833

Amon T, Amon B, Kryvoruchko V, Zollitsch W, Mayer K, Gruber L (2007) Biogas production from maize and dairy cattle manure-Influence of biomass composition on the methane yield. Agric. Ecosyst. Environ 118:173-182. doi:10.1016/j.agee.2006.05.007

Arifan F, Broto RTDW, Sumardiono S, Sutaryo, Dewi AL, Yudanto, YA, Sapatra, EF (2022) Effect of thermal pretreatment of pineapple peel waste in biogas production using response surface methodology. Int. J. Technol 13:619-632. doi:10.14716/ijtech.v13i3.4747

Arija F, Purwanto P, Hadiyanto H (2022) The opportunities of cleaner production in carica (*Carica pubescens*) industry to reduce hazardous waste. J. Bioresour. Environ. Sci 1:20-26. doi:10.14710/jbes.2022.14235

Babaee A, Shayegan J, Roshani A (2013) Anaerobic slurry co-digestion of poultry manure and straw: effect of organic loading and temperature. J. Environ. Health Sci. Eng 11:15. doi:10.1186/2052-336X-11-15

Badan Pusat Statistik (BPS) (2022) Kecamatan Kejajar dalam angka 2022. Badan Pusat Statistik, Jakarta

Beltrán-Orozco M del C, Martínez-Olguín A, Robles-Ramírez M del C (2020) Changes in the nutritional composition and antioxidant capacity of chia seeds (*Salvia hispanica* L.) during germination process. Food Sci. Biotechnol 29:751-757. doi:10.1007/s10068-019-00726-1

Boone DR, Garrity G, Castenholz RW (2011) Bergey's manual of systematic bacteriology: volume one: the archaea and the deeply branching and phototrophic bacteria. Springer, New York

Chew KR, Leong HY, Khoo KS, Vo DVN, Anjum H, Chang CK, Show PL (2021) Effects of anaerobic digestion of food waste on biogas production and environmental impacts: a review. Environ. Chem. Lett 19:2921-2939. doi:10.1007/s10311-021-01220-z

Christou ML, Vasileiadis S, Kalamaras SD, Karpouzas DG, Angelidaki I, Kotsopoulos TA (2021) Ammonia-induced inhibition of manure-based continuous biomethanation process under different organic loading rates and associated microbial community dynamics. Bioresour. Technol 320. doi:10.1016/j.biortech.2020.124323

Czatzkowska M, Harnisz M, Korzeniewska E, Koniuszewska I (2020) Inhibitors of the methane fermentation process with particular emphasis on the microbiological aspect: A review. Energy Sci Eng 8:1880-1897. doi:10.1002/ese3.609

Díaz-Batalla L, Aguilar-Arteaga K, Castro-Rosas J, Nallely Falfán-Cortés R, Navarro-Cortez RO, Gómez-Aldapa CA (2023) Common bean (Phaseolus vulgaris L.) seed germination improves the essential amino acid profile, flavonoid content and expansion index. Czech J. Food Sci 41:73–77. doi:10.17221/5/2022-CJFS

Fouad AA, Rehab FMA (2015) Effect of germination time on proximate analysis, bioactive compounds and antioxidant activity of lentil (*Lens culinaris* Medik.) sprouts. Acta Sci. Pol. Technol 14:233-246. doi:10.17306/J.AFS.2015.3.25

Ganzoury MA, Allam NK (2015) Impact of nanotechnology on biogas production: A mini-review. Renew. Sustain. Energy Reviews 50:1392-1404. doi:10.1016/j.rser.2015.05.073

Harirchi S, Wainaina S, Sar T, Nojoumi SA, Parchami M, Parchami M, Varjani S, Khanal SK, Wong J, Awasthi MK, Taherzadeh, MJ (2022) Microbiological insights into anaerobic digestion for biogas, hydrogen or volatile fatty acids (VFAs): a review. Bioengineered 13:6521-6557. doi:10.1080/21655979.2022.2035986

Hasanudin U, Safira ND, Nurainy F, Utomo TP, Haryanto A (2023) Improving biogas production in tapioca industry by using onggok as co-substrate. Int. J. Renew Energy Res 13:741-749. doi:10.20508/ijrer.v13i2.13814.g8748

Hütter M, Sailer G, Hülsemann B, Müller J, Poetsch J (2023) Impact of Thermo-Mechanical Pretreatment of Sargassum muticum on Anaerobic Co-Digestion with Wheat Straw. Fermentation. doi.org/10.3390/fermentation9090820

Jin W, Xu X, Yang F, Li C, Zhou M (2018) Performance enhancement by rumen cultures in anaerobic co-digestion of corn straw with pig manure. Biomass Bioenerg 115:120-129. doi:10.1016/j.biombioe.2018.05.001

Keskin T, Arslan K, Karaalp D, Azbar N (2019) The Determination of the trace element effects on basal medium by using the statistical optimization approach for biogas production from chicken manure. Waste Biomass Valori 10:2497-2506. doi:10.1007/s12649-018-0273-2

Khalid A, Arshad M, Anjum M, Mahmood T, Dawson L (2011) The anaerobic digestion of solid organic waste. Waste Manag 31:1737-1744. doi:10.1016/j.wasman.2011.03.021

Lahbab A, Djaafri M, Kalloum S, Benatiallah A, Atelge MR, Atabani AE (2021) Co-digestion of vegetable peel with cow dung without external inoculum for biogas production: Experimental and a new modelling test in a batch mode. Fuel 306. doi:10.1016/j.fuel.2021.121627

Laxmi G, Chaturvedi N, Richa S (2015) The impact of malting on nutritional composition of foxtail millet, wheat and chickpea. J. Nutrition Food Sci 05. doi:10.4172/2155-9600.1000407

Li H, Guo XL, Cao FF, Wang Y (2014) Process evolution of dry anaerobic Co-digestion of cattle manure with kitchen waste. Chem. Biochem. Eng. Q 28:161-166. https://www.researchgate.net/publication/279903174

Lien DTP, Tram PTB, Toan HT (2017) Effect of germination on antioxidant capacity and nutritional quality of soybean seeds (*Glycinemax* (L.) Merr.). Can Tho University J. Sci 06:93-101. doi:10.22144/ctu.jen.2017.032

Ma J, Bashir MA, Pan J, Qiu L, Liu H, Zhai L, Rehim A (2018) Enhancing performance and stability of anaerobic digestion of chicken manure using thermally modified bentonite. J. Cleaner Prod 183:11-19. doi:10.1016/j.jclepro.2018.02.121

Medugu CI, Saleh B, Igwebuike JU, Ndirmbita RL (2012) Strategies to Improve the Utilization of Tannin-Rich Feed Materials by Poultry. Int. J. Poultry Sci 11:417-423. doi:10.3923/ijps.2012.417.423

Møller HB, Moset V, Brask M, Weisbjerg MR, Lund P (2014) Feces composition and manure derived methane yield from dairy cows: Influence of diet with focus on fat supplement and roughage type. Atmos. Environ 94:36-43. doi:10.1016/j.atmosenv.2014.05.009

Mustikasari AR, Sutaryo S, Ufidiyati N, Purnomoadi A (2023) The Effect of Using Acidified Imperata cylindrica as a Co-substrate with Dairy Cow Manure on the Digesters Performance. Trop Anim Sci J 46:361–366. doi:10.5398/tasj.2023.46.3.361

Myers RH, Montgomery DC (2002) Response Surface Methodology: Product and Process Optimization Using Designed Experiments, 2nd Edition, John Wiley & Sons, New York

Nkhata SG, Ayua E, Kamau EH, Shingiro JB (2018) Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. Food Sci. Nutr 6(8):2446-2458. doi:10.1002/fsn3.846

Permana IDGM, Idrati R, Hastuti P, Suparmo (2013) Indogenous lipase activities during cocoa bean (*Theobroma cacao* L.) germination. Agritech 33(2): 176-181.

Purwanti A, Setyo Arbintarso E, Setyowati Rahayu S, Rahayu Gusmarwani S, Puri Dwi Pangestu M, Prayogo W (2022) Optimization of biogas production from tofu wastewater. J. Environ. Eng. Sustain. Technol 9:24-29. doi:10.21776/ub.jeest.2022.009.01.4

Purwasih R, Sutaryo S, Purbowati E, Purnomoadi A (2024) Evaluation of germination as pretreatment method to increase methane production: A case study in papaya seed. Case Stud Chem Environ Eng 10. doi:10.1016/j.cscee.2024.100788

Safari M, Abdi R, Adl M, Kafashan J (2018) Optimization of biogas productivity in lab-scale by response surface methodology. Renew Energ. 118:368-375. doi:10.1016/j.renene.2017.11.025

Sekoai PT, Ghimire A, Ezeokoli OT, Rao S, Ngan WY, Habimana O, Yao Y, Yang P, Fung HY, Yoro KO, Daramola MO, Hung CH (2021) Valorization of volatile fatty acids from the dark fermentation waste Streams-A promising pathway for a biorefinery concept. Renew. Sustain. Energy Rev 143. doi:10.1016/j.rser.2021.110971

Solli L, Bergersen O, Sørheim R, Briseid T (2014) Effects of a gradually increased load of fish waste silage in co-digestion with cow manure on methane production. Waste Manag 34(8):1553-1559. doi:10.1016/j.wasman.2014.04.011

Suanon F, Sun Q, Mama D, Li J, Dimon B, Yu CP (2016) Effect of nanoscale zero-valent iron and magnetite (Fe_3O_4) on the fate of metals during anaerobic digestion of sludge. Water Res 88:897-903. doi:10.1016/j.watres.2015.11.014

Sugiharto S (2021) The use of sprouted grains as dietary feed ingredients for broilers-a brief overview. Livestock Res. Rural Dev 33:38.

Sugiharto S, Agusetyaningsih I, Widiastuti E, Wahyuni HI, Yudiarti T, Sartono TA (2022) Germinated papaya seed alone or in combination with chitosan on growth, health and meat quality of broilers during grower period. Vet. Anim. Sci 18:1-7. doi:10.1016/j.vas.2022.100273

Sutaryo S, Ward AJ, Møller HB (2012) Thermophilic anaerobic co-digestion of separated solids from acidified dairy cow manure. Bioresour. Technol 114:195-200. doi:10.1016/j.biortech.2012.03.041

Sutaryo S, Ward AJ, Møller HB (2014) The effect of low-temperature thermal pre-treatment on methane yield of pig manure fractions. Animal Prod. 16:55-62.

Sutaryo S, Sempana AN, Lestari CMS, Ward AJ (2020) Performance comparison of single and two-phase biogas digesters treating dairy cattle manure at tropical ambient temperature. Trop. Anim. Sci. J 43:354-359. doi:10.5398/tasj.2020.43.4.354

Sutaryo S, Sempana AN, Mulya RM, Sulistyaningrum D, Ali MS, Damarjati RI, Purbowati E, Adiwinarti R, Purnomoadi A (2022^{a}) Methane Production of pistia stratiotes as a single substrate and as a co-substrate with dairy cow manure. Fermentation 8:1-9. doi:10.3390/fermentation8120736

Sutaryo S, Sempana AN, Prayoga I, Chaniaji FG, Dwitama SD, Sugandi NF, Purnomoadi A, Ward AJ (2022^{b}) Increased methane yield from dairy cow manure by co-substrate with *Salvinia molesta*. Asia Pac. J. Sci. Technol 32:2147-2160. doi:10.14456/apst.2023.39

Sutaryo S, Huda S, Toba GA, Izza AS, Rianto E (2023) Anaerobic co-digestion of tempe wastewater and dairy cow dung. Livestock Res. Rural Dev 35:1-5.

Triolo JM, Ward AJ, Pedersen L, Sommer SG (2013) Characteristics of animal slurry as a key biomass for biogas production in Denmark. In: Biomass Now - Sustainable Growth and Use. InTech. doi:10.5772/54424

Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 74:3583-3597. doi:10.3168/jds.S0022-0302(91)78551-2

Wandera SM, Qiao W, Algapani DE, Bi S, Yin D, Qi X, Liu Y, Dach J, Dong R (2018) Searching for possibilities to improve the performance of full scale agricultural biogas plants. Renew. Energy 116:720-727. doi:10.1016/j.renene.2017.09.087

Winarti W, Yudiarti T, Widiastuti E, Wahyuni HI, Sartono TA, Sugiharto S (2024) Nutritional value and antioxidant activity of sprouts from seeds of Carica papaya – their benefits for broiler nutrition. Bulg J Agric Sci 30:107–114

Yılmaz Ş, Şahan T (2020) Utilization of pumice for improving biogas production from poultry manure by anaerobic digestion: A modeling and process optimization study using response surface methodology. Biomass Bioenerg 138. doi:10.1016/j.biombioe.2020.105601