

Synergistic Effects of Biochar and Potassium on Enhancing Methane Production from Carbohydrate-Rich Waste in Mesophilic Anaerobic Digestion

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Abstract

This study investigates the synergistic effects of biochar and potassium additives on methane (CH₄) production from carbohydrate-rich waste during two-phase anaerobic digestion. The research aims to determine the optimal concentrations of biochar and potassium that enhance CH₄ yields from white bread waste. A factorial experimental design with 16 treatments, each replicated three times, was used to evaluate different concentrations of biochar (0, 5, 15, 25 g/L) and potassium (0, 1, 2, 3 g/L). Biogas production was measured daily using the volume displacement method, and the concentration of CH₄ was analyzed to calculate both the cumulative CH₄ yield and the daily CH₄ production rate. Additionally, culture samples were collected for the analysis of the microbial community. Experimental results showed that the supplementation of biochar at a concentration of 5 g/L increased CH₄ yield and CH₄ production rate by 29% (from 3804.32 to 4915.78 mL/L) and 22% (from 507.24 to 616.92 mL/L/d) compared to control, respectively. Similarly, potassium supplementation at 3 g/L further boosted CH₄ yield and CH₄ production rate by 14% (from 4833.72 to 5510.04 mL/L) and 34% (from 586.81 to 785.94 mL/L/d) compared to the control. The combination of 5g/L biochar and 3g/L potassium yielded 5899.17 mL/L CH₄, higher than that of adding the individual contribution from the biochar (4915.78 mL/L) and the potassium (5510.04 mL/L), indicating synergistic benefits. Microbial community analysis showed a 53.8% increase in *Methanosaetaceae* under the combined treatment, promoting more efficient CH₄ generation pathways. This combination utilizes biochar's ability to create a microhabitat for methanogenic microbes and potassium's function as a metabolic cofactor. The novelty of this study lies in the synergistic use of biochar and potassium, which significantly accelerates CH₄ production by enhancing microbial activity and stabilising the digestion process.

Keywords: Biochar, Methane optimisation, Microbial community, Potassium, Waste-to-energy.

63 **1. Introduction**

64 The growing demand for sustainable energy has led to an increasing interest in renewable energy
65 sources, with biogas production via the anaerobic digestion (AD) process as a promising alternative.
66 AD of organic waste generates biogas that is rich in methane (CH₄), offering dual benefits of
67 renewable energy production and effective waste management (1,2). However, achieving high CH₄
68 yields from anaerobic digestion remains challenging due to suboptimal microbial activity, slow
69 organic degradation rates, and process instability (3–5). This has led to research into additives like
70 biochar and potassium to improve process efficiency and enhance CH₄ production.

71 Biochar, a carbonaceous material produced via pyrolysis of biomass, has gained attention for its
72 ability to improve anaerobic digestion. Its large surface area, high porosity, and electrochemical
73 properties provide a conducive environment for microbial colonisation, especially for methanogenic
74 archaea(6,7). Biochar's buffering capacity also helps maintain stable pH levels during digestion,
75 which is critical for sustaining microbial activity (8,9). These properties make biochar an attractive
76 additive to stabilise AD processes and promote CH₄ production by facilitating electron transfer
77 between microorganisms and substrates(10,11). Additionally, research has highlighted that biochar
78 can influence enzymatic pathways and microbial community structures, optimizing digestion
79 efficiency(12). Although biochar's role in anaerobic digestion has been extensively researched, its
80 potential synergistic interactions with other additives, such as potassium, remain underexplored.

81 Potassium is an essential nutrient for microbial metabolism and plays a critical role as a cofactor for
82 enzymes in the methanogenesis pathway, especially in converting acetate and hydrogen (H₂) into CH₄
83 (13). Potassium ions stabilize enzymes and help regulate osmotic pressure within microbial cells,
84 which is crucial for the optimal functioning of methanogenic archaea (14). Previous studies have
85 shown that potassium supplementation can increase CH₄ yields by enhancing the metabolic efficiency
86 of methanogens(15–18). However, the combined effects of biochar and potassium on CH₄ production,
87 as well as their potential synergistic effects, have not been thoroughly investigated.

88 Recent studies suggest that the combination of biochar and potassium may exhibit synergistic effects
89 in AD systems. Biochar provides structural support for microbial attachment, while potassium
90 optimizes enzymatic activity, thereby enhancing methanogenesis efficiency. Research by De Vrieze
91 et al. (2016) indicated that potassium-enriched biochar improved CH₄ yields by facilitating microbial
92 interactions and stabilizing pH levels (15). Similarly, studies by Zhang et al. (2024) and Yin et al.
93 (2022) demonstrated that potassium acts as an activator for key methanogenic enzymes, reinforcing
94 the role of biochar in AD(19,20). Moreover, recent investigations into engineered nanoparticles
95 (ENPs) have demonstrated their potential interactions with microbial metabolism in AD, which could
96 provide further insights into the effects of biochar-mineral interactions (21). Additionally, Gao et al.
97 (2022) found that magnetic biochar modified with potassium promoted acetoclastic methanogenesis
98 by stimulating microbial metabolic pathways and enhancing CH₄ production efficiency (22). Despite
99 these promising findings, the specific mechanisms underlying the biochar-potassium synergy in AD
100 remain poorly characterized.

101 The aim of this study is to examine the synergistic interaction between biochar and potassium to
102 enhance methane production from carbohydrate-rich waste, specifically bread waste. While biochar
103 offers a microhabitat for microorganisms and supports stable process conditions, potassium enhances
104 microbial metabolism by acting as a biochemical cofactor. The combination of these two additives
105 could create an optimised environment where biochar supports microbial communities structurally,
106 and potassium facilitates their metabolic functions, leading to accelerated CH₄ production. This
107 synergistic effect is hypothesised to maximise CH₄ yields by enhancing both microbial activity and
108 process stability. By evaluating different concentrations of biochar and potassium in combination,
109 this research aims to determine the optimal conditions for maximising CH₄ yields and advancing the
110 efficiency of biogas production.

111

112 **2. Materials and methods**

113 ***2.1 Materials***

114 The food waste utilised in this study served as the primary feedstock for AD. The bread used in the
 115 experiments was sourced from a local bakery in Malang, East Java, Indonesia. To prepare the waste
 116 for digestion, the bread was grated into smaller, finer particles, averaging approximately 1 to 2 mm
 117 in diameter. A thorough characterisation of the food waste was conducted to evaluate its composition.
 118 This involved several key analyses, including proximate analysis, which measured total solids (TS),
 119 volatile solids (VS), moisture content (MC), and ash content. Bread waste contains a moisture content
 120 of 36.32%, ash content of 8.12%, protein content of 11.03%, fat content of 4.96%, carbohydrate
 121 content of 35.94%, carbon (C) content of 42.7%, hydrogen (H) content of 9.1%, oxygen (O) content
 122 of 45.6%, nitrogen (N) content of 2.3%, a C/N ratio of 18.56%, total solids of 63.68%, and volatile
 123 solids of 91.88% (Table 1). Biochar was produced from rice husks by pyrolysis at a temperature of
 124 600°C. Similarly, biochar also shows varied results for each parameter. These parameters include a
 125 moisture content of 1.64%, ash content of 79.17%, protein content of 3.32%, fat content of 0.27%,
 126 carbohydrate content of 0%, carbon (C) content of 84.14%, hydrogen (H) content of 1.57%, oxygen
 127 (O) content of 13.7%, nitrogen (N) content of 0.58%, a C/N ratio of 145.06%, total solids of 98.36%,
 128 and volatile solids of 20.83%. For the inoculum, sludge was sourced from an anaerobic digester at a
 129 local industrial facility in Malang, Indonesia. The sludge was characterised to ensure its suitability
 130 for the study. Sludge contains a moisture content of 98.9%, ash content of 0.54%, protein content of
 131 2.70%, fat content of 22.80%, carbohydrate content of 24.54%, total solids of 1.09%, volatile solids
 132 of 99.45%. It was found to have a total solids (TS) content of 1.09%, meaning that 1.09% of the
 133 sludge was made up of solid particles, while the remaining 98.91% was water.

134 **Table 1** Content of Bread Waste, Biochar, and Sludge

Content	Unit	Bread Waste	<i>Biochar</i>	<i>Sludge</i>
Moisture Content	%	36.32	1.64	98.9
Ash Content	%	8.12	79.17	0.54
Protein Content	%	11.03	3.32	2.70
Fat Content	%	4.96	0.27	22.80
Carbohydrates	%	35.94	0	24.54
C	%	42.7	84.14	-
H	%	9.1	1.57	-
O	%	45.6	13.7	-
N	%	2.3	0.58	-

Content	Unit	Bread Waste	Biochar	Sludge
C/N Ratio	%	18.56	145.06	-
Total Solids	%	63.68	98.36	1.09
Volatile Solids	%	91.88	20.83	99.45

2.2 Anaerobic digestion experimentation

The research used food waste effluent from the first phase of TPAD as the substrate. The food waste contains 42.7% carbon, 2.3% nitrogen, 9.1% hydrogen, and 0.3% sulfur, with a C/N ratio of 18.7 and a pH of 4.9. It has a total solid content of 61.2% and volatile solids of 59.5%. The preparation of the substrate in the first phase followed the procedure from Sugiarto et al. (2021). The sludge was heated to 95 °C and stirred at 250 rpm for 30 minutes to remove methanogens. A glass bottle bioreactor contained 8 gVS/L of bread, 10 mL of heated sludge, and 50 mL of distilled water, totalling 60 mL. The initial pH was set to 6.4. Bioreactors were incubated at 32°C for six days [25]. A mix of 50 mL of this effluent and 10 mL of seed sludge was placed in a 100 mL serum bottle. The pH was initially adjusted to 7.8 using either hydrochloric acid (HCl) or sodium hydroxide (NaOH). To create an anaerobic environment, the bioreactors were flushed with high-purity nitrogen gas (>99.99%) at a flow rate of 20 L/min for 30 seconds. The bioreactors were then sealed with rubber stoppers and capped with aluminium lids before being placed in an incubator at 37°C for 45 days(1). Experiments on CH₄ yields were split into three sets. The first set aimed to assess the effect of biochar with different biochar concentrations of 0 g/L, 5 g/L, 15 g/L, and 25 g/L. The second set aimed to determine the effect of potassium with various concentrations of 0 g/L, 1 g/L, 2 g/L, and 3 g/L. After identifying the concentration yielding the highest gas production, a parallel study was conducted to examine the synergetic impact of biochar and potassium addition, following the same procedure of biochar and potassium experiments. The third experimental set focused on evaluating the influence of biochar and potassium on CH₄ yields. Each experiment was conducted in triplicate under consistent conditions.

2.3 Analytical methods

158 The pH of the samples was measured using a digital pH meter. Before each use, the pH meter was
159 carefully calibrated using standard buffer solutions with known pH values of 7.0 and 9.2 to ensure
160 accuracy. Total solids (TS) were determined to quantify the overall solid material present in the
161 sample. In contrast, volatile solids (VS) were analysed to identify the portion of these solids that are
162 organic and can be decomposed by microorganisms during the digestion process. Moisture content
163 (MC) was measured to determine the water content in the samples, and ash content was assessed to
164 evaluate the inorganic residue remaining after the complete combustion of the sample. All these
165 analyses were conducted in accordance with Standard Method 2540 G, a widely recognised protocol
166 for evaluating such parameters in environmental samples. The daily volume of CH₄ produced was
167 recorded using the water displacement method. The recorded gas volumes were then converted to
168 standard temperature and pressure (STP) conditions (273K and 1 atm) using the ideal gas law, which
169 allows for consistent comparison of CH₄ production data[12]. To estimate the theoretical CH₄ yield
170 from the organic biomass, the Buswell equation was applied. This equation assumes a complete
171 breakdown of the organic material into CH₄ and other byproducts. The necessary inputs for this
172 calculation—namely, the levels of carbon (C), hydrogen (H), nitrogen (N), oxygen (O), and sulphur
173 (S) in the substrate samples—were obtained through detailed elemental analysis. The elemental
174 composition of the food waste was determined using a ThermoFisher Scientific FlashSmart™
175 CHNS/O elemental analyser (EA). This instrument provides precise measurements of carbon,
176 hydrogen, nitrogen, and sulphur content in the samples. Additionally, the elemental composition in
177 the leachate (liquid byproduct) was analysed using Inductively Coupled Plasma Optical Emission
178 Spectrometry (ICP-OES), specifically with a Thermo Scientific iCAP 7400 Duo ICP-OES device.
179 This technique allows for the accurate determination of various elemental concentrations within the
180 samples. Finally, the potential CH₄ yield in the anaerobic digestion process was theoretically
181 calculated using the modified Buswell-Boyle equation(23). This equation models the biochemical
182 conversion of organic matter into CH₄, providing a theoretical estimate based on the elemental
183 composition of the substrate.

$$C_c H_h O_o N_n S_s + \left(c - \frac{h}{4} - \frac{o}{2} + \frac{3n}{4} + \frac{s}{2} \right) H_2O$$

$$\rightarrow \left(\frac{c}{4} + \frac{h}{8} - \frac{o}{4} - \frac{3n}{8} - \frac{s}{4} \right) CH_4 + \left(\frac{c}{2} - \frac{h}{8} + \frac{o}{4} + \frac{3n}{8} + \frac{s}{4} \right) CO_2 + nNH_3 + sH_2S$$

Where: c,h,o,n,s represent the moles of each mass fraction of the elements carbon (C), hydrogen (H), oxygen (O), nitrogen (N), and sulphur (S).

2.4 Microbial analysis

To study the effect of biochar addition on microbial communities, 1 mL of the liquid sample was taken from each experiment at both the initial and final stages of CH₄ production, respectively. Samples were stored at -20°C prior to microbial community analysis. Microbial communities were determined at the Genetika Science Indonesia (GSI) by DNA extraction and diversity profiling. Sediment total DNA was extracted using a ZymoBIOMICS DNA Miniprep Kit (Zymo Research. D4300). Primer pairs were used to amplify the regions of the 16S rRNA genes for archaea (V4-V5 regions). Specifically, Arch519f CAGCCGCCGCGGTAA and Arch915r GTGCTCCCCGCCAATTCCT were chosen to amplify the archaeal gene regions (7). High-throughput DNA sequencing was performed on an Illumina MiSeq. The raw sequences were clustered into operational taxonomic units (OTU) with a similarity threshold of 97%, and taxonomy assignment was performed by QIIME using the Greengenes database.

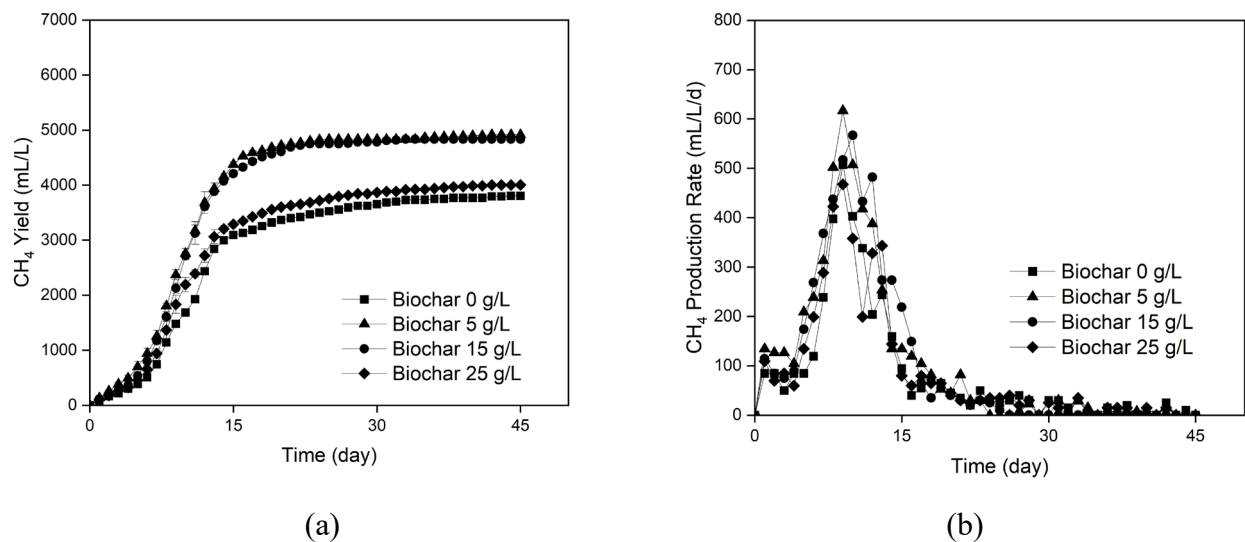
3. Results and Discussion

3.1 The Effect of Biochar Concentration Variations on Biogas Production

The results of CH₄ yield and production rates during anaerobic digestion are presented in **Figure 1**. The control culture (0 g/L biochar) started producing CH₄ immediately and reached its peak production rate on day 9, with a maximum rate of 507.24 mL/L/d. However, production rates dropped sharply after this peak and had nearly ceased by day 45. The cumulative yield for the control was 3804.32 mL/L by the end of the experiment. Cultures with 5 g/L biochar addition showed the highest cumulative yield, reaching 4915.78 mL/L, and achieved a peak production rate slightly earlier on day

210 9 (616.92 mL/L/d). In contrast, cultures with higher biochar concentrations (15 and 25 g/L) exhibited
 211 lower cumulative yields and peak production rates, with 15 g/L reaching 4833.72 mL/L and 25 g/L
 212 reaching 4003.24 mL/L in cumulative yield. The production rate for these cultures was also lower,
 213 with 15 g/L and 25 g/L peaking at 566.81 mL/L/d on day 10 and 467.46 mL/L/d on day 9,
 214 respectively. The decrease in both CH₄ yield and production rates with increasing biochar
 215 concentration may suggest an inhibitory effect at higher dosages. This is consistent with findings by
 216 Sunyoto et al. (2016), which indicate that excessive biochar can adsorb volatile fatty acids, thereby
 217 reducing their availability for CH₄ production (24). Additionally, the peak production rates in the
 218 early stages of digestion are likely due to the rapid degradation of easily metabolizable substrates by
 219 methanogens, a phenomenon observed in previous studies like Pan et al. (2019). In the later stages,
 220 biogas production decreased, possibly due to changes in environmental conditions within the digester,
 221 such as pH shifts or the accumulation of inhibitory by-products, which can impact methanogenic
 222 activity [18](25). The results from production rate data also indicate fluctuations due to environmental
 223 factors within the reactor, such as pH changes or accumulation of by-products. As a result, in the later
 224 days of the degradation process within the digester, the activity of methanogenic microorganisms
 225 may be inhibited(25).

226



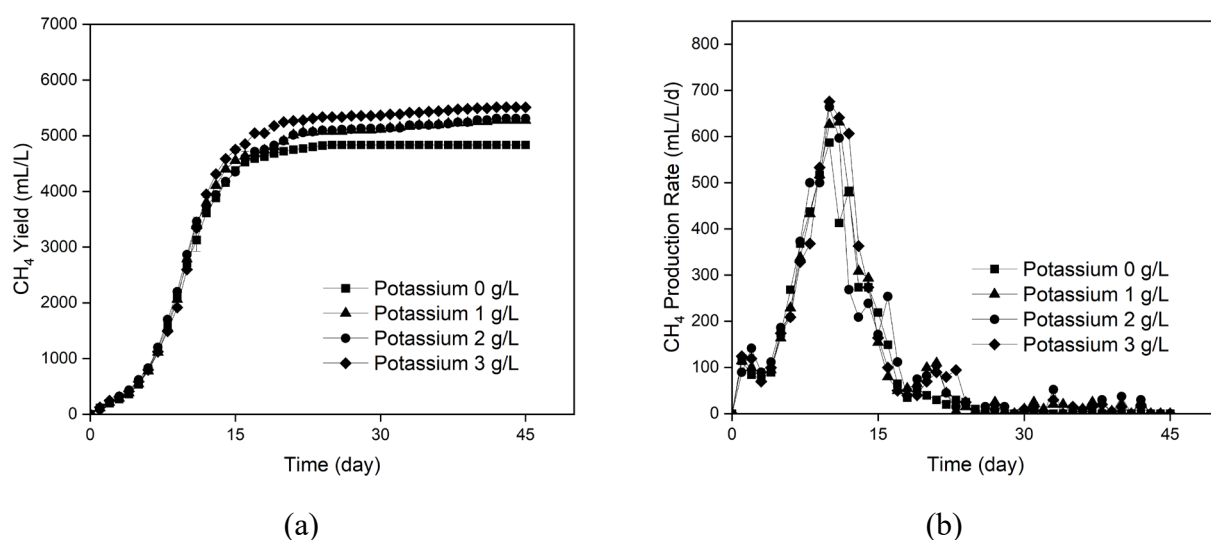
227 **Figure 1** Effect of biochar on (a) CH₄ yield and (b) Production Rate of Biogas

228

229 ***3.2 The Effect of Potassium Concentration Variations on Biogas Production***

230 The results of CH₄ yield and production rates during the anaerobic digestion are presented in **Figure**
231 **2**. The control culture (0 g/L potassium) began CH₄ production immediately and reached its peak
232 production rate on day 10, with a maximum rate of 586.81 mL/L/d. However, the rate of production
233 rapidly declined after this peak and had essentially ceased by day 45. The cumulative CH₄ yield of
234 the control was 4833.72 mL/L by the end of the experiment. The data shows that the addition of
235 potassium increased CH₄ yield and production rates during the anaerobic digestion. The cultures with
236 the highest potassium by 3 g/L potassium exhibited the highest cumulative yield and maximum rate
237 of production, achieving a peak rate of 785.94 mL/L/d on day 10 and a cumulative yield of 5510.04
238 mL/L. In contrast, cultures with lower potassium concentrations (1 and 2 g/L) demonstrated lower
239 cumulative yields and peak production rates, with 1 g/L reaching a cumulative yield of 5276.31 mL/L
240 and 2 g/L achieving 5311.13 mL/L. The CH₄ production rate values for these cultures were also lower,
241 with 1 g/L peaking at 631.57 mL/L/d on day 11 and 2 g/L peaking at 663.89 mL/L/d on day 10. The
242 data from the graph shows that the addition of potassium has a positive effect on biogas production.
243 In this study, during anaerobic digestion, treatment with the supplementation of potassium by 3 g/L
244 demonstrated the most suitable conditions for methanogenic microorganisms, as potassium helps
245 these microorganisms grow and function optimally. Research by Zhang et al. (2024) explains that
246 potassium serves as an activator for various enzymes involved in energy storage and is crucial for
247 cell structure and function(19). Potassium supports the enzymes required by methanogenic
248 microorganisms to convert acetic acid and H₂ into CH₄. This is supported by the fact that potassium
249 acts as a cofactor for enzymes, assisting various enzymes within cells to function more effectively
250 and ensuring efficient metabolic processes(26). Meanwhile, the production rate data shows that
251 treatment with the supplementation of potassium by 3 g/L exhibited the peak biogas production rate
252 due to the optimal growth and enzymatic activity of methanogenic microorganisms supported by the
253 potassium dosage. Research by Liu et al. (2023) explains that potassium addition can significantly
254 influence CH₄ production(27). Furthermore, treatments with appropriate potassium additions indicate

255 that enzymatic activity peaks, supported by nutrient availability and substrate, leading to maximum
 256 biogas production rates. This is supported by studies conducted by Yin et al. (2022), which highlight
 257 potassium's role in optimising enzyme function(26). Towards the later days, small fluctuations in
 258 production rates were observed, indicating the presence of remaining substrates that are difficult to
 259 degrade. Subsequently, production rates declined to zero across all treatments. This aligns with
 260 research by Aye et al. (2019), which states that when substrates used in biogas production no longer
 261 generate biogas, they have been fully degraded(28).



263 **Figure 2** Effect of potassium on (a) CH₄ Yield and (b) Production Rate of Biogas

265 3.3 The Effect of A Mixture of Biochar and Potassium on Biogas Production

266 The results of biogas accumulation and production rates during the anaerobic digestion are presented
 267 in **Figure 3**. Treatments with the supplementation of biochar by 5 g/L and various potassium
 268 concentrations by 0 g/L, 1 g/L, 2 g/L, and 3g/L produced cumulative biogas volumes of 4946.19 mL/L,
 269 6267.32 mL/L, 6069.30 mL/L, and 5899.17 mL/L, respectively. The production rates for treatments
 270 with the supplementation of biochar by 5 g/L and various potassium concentrations by 0 g/L, 1 g/L,
 271 2 g/L, and 3 g/L were 616.24 mL/L/d (peak on day 10), 691.57 mL/L/d (peak on day 11), 606.92
 272 mL/L/d (peak on day 11), and 636.76 mL/L/d (peak on day 10). Treatment 5 g/L of biochar and 1 g/L
 273 of potassium exhibited the highest biogas production volume, while treatment 5 g/L of biochar and 2

g/L of potassium showed the lowest biogas production volume. The addition of a mixture of biochar and potassium during anaerobic digestion can enhance biogas production. However, excessive amounts of potassium may not always correlate linearly with increased biogas production, as observed in fluctuating and non-linear trends on the graph corresponding to the potassium additions. Therefore, it is crucial to balance the amount of potassium added to avoid excess and potential negative effects. Optimising the levels of biochar and potassium can maximise biogas production efficiently without compromising the stability of the anaerobic process. The biogas production results from the anaerobic digestion with varying mixtures of biochar and potassium concentrations typically show the highest production rates from day 8 to day 11. During this period, the digester conditions are usually more stable, leading to increased biogas production. In the days following this period, biogas production may fluctuate due to changes in substrate composition and concentrations of additional materials. This occurs because microorganisms start depleting the nutrients present in the substrate, resulting in decreasing pressure each day until depletion (24).

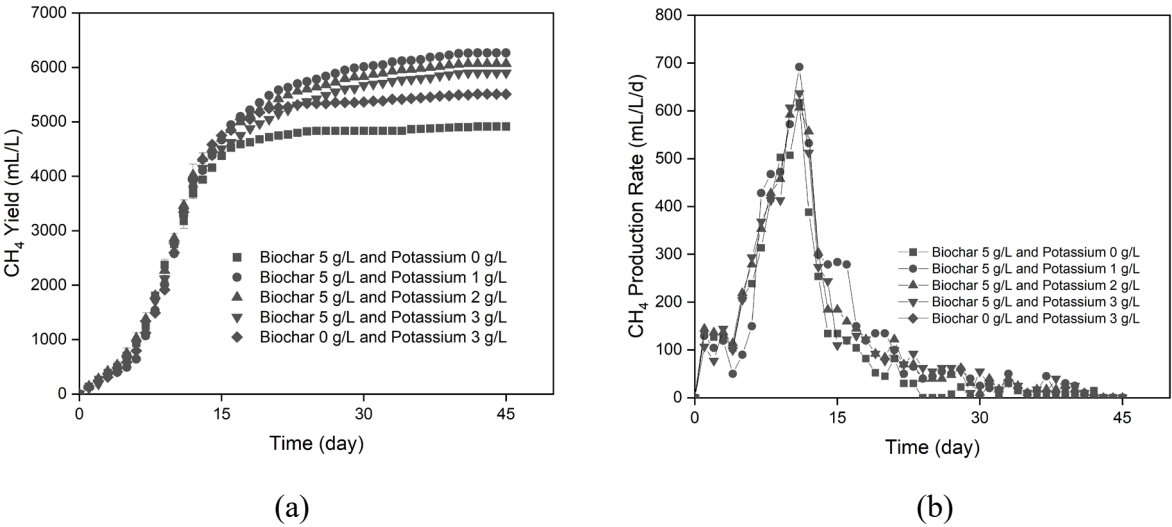


Figure 3 Effect of biochar-potassium on (a) Accumulated Production and (b) Production Rate

To further investigate the interactions between biochar and potassium in anaerobic digestion, a theoretical calculation was performed assuming an additive effect without synergy. Based on individual CH₄ yield increases from biochar (5 g/L) and potassium (3 g/L), the expected CH₄ yield, assuming no synergistic interaction, was calculated as 6621.5 mL/L. However, the actual

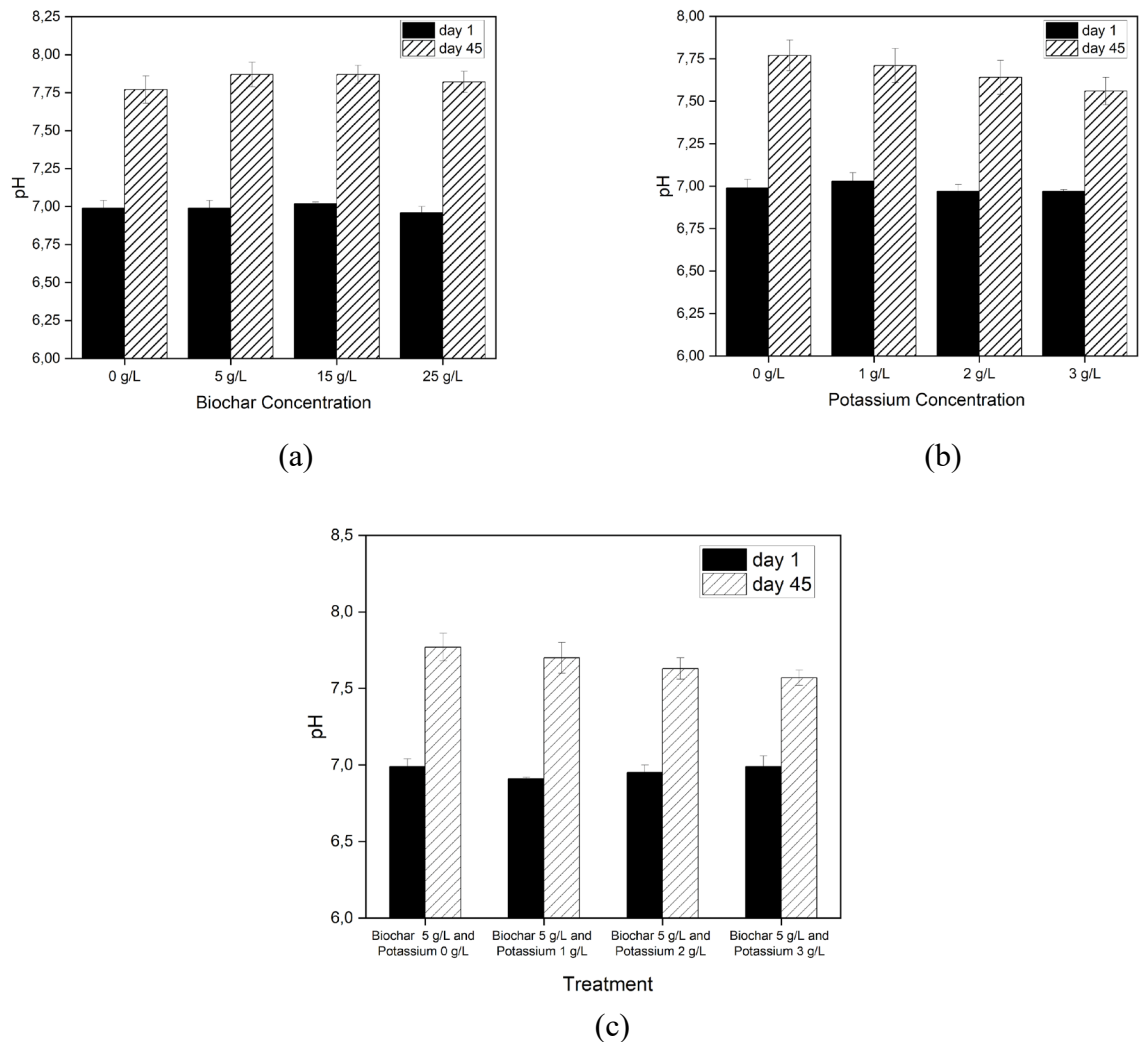
experimental CH₄ yield from the combined biochar (5 g/L) and potassium (3 g/L) treatment was 5899.17 mL/L, which is 722.33 mL/L lower than the expected theoretical value. Despite this difference, the observed methane production still indicates a synergistic interaction, as the combined treatment resulted in an enhanced CH₄ yield compared to the individual effects of biochar and potassium alone. The deviation from the theoretical value may be attributed to variations in microbial activity, substrate utilisation efficiency, or environmental factors within the digester. Biochar and potassium contribute to methanogenesis through different mechanisms—biochar provides a conducive microhabitat for microbial colonisation. It facilitates electron transfer, while potassium enhances enzymatic activity and regulates osmotic balance, both of which promote methane production (14). The combined presence of biochar and potassium likely led to an optimised anaerobic digestion environment, reinforcing the synergistic effect observed in this study.

303

304 ***3.4 pH Analysis on Biogas Production***

The pH value during the anaerobic digestion process is also a crucial parameter for biogas production, as pH can affect the activity of microorganisms when digesting organic material into biogas. In the anaerobic digestion, the initial pH of each treatment was set at 7 ± 0.1 , in accordance with the research by Goswami et al. (2016), which states that the optimal pH value for anaerobic digestion ranges from 6.5 to 8.2. pH measurements were taken after the end of the first phase of anaerobic digestion, specifically on days 1 and 45. These results are depicted in Figure 4, which shows an increase in pH values for each treatment. This rise in pH is due to the activity of methanogenic bacteria consuming volatile fatty acids and converting them into CH₄ gas (29). According to Sugiarto et al. (2021), the increase in pH during anaerobic digestion occurs because volatile fatty acids such as acetic acid, butyric acid, propionic acid, and valeric acid formed in the first phase are decomposed by methanogenic microorganisms(30). These organic acids initially lower the pH due to their acidic nature, so when microorganisms degrade VFAs, these acids are removed from the digester, resulting in a stabilisation and increase in pH(31). The use of biochar can stabilise the pH in the digester from

318 fluctuations. The role of biochar as a buffer in anaerobic digestion can absorb excess bases, preventing
 319 a drastic increase in pH that could affect degradation by methanogenic bacteria(32). The addition of
 320 potassium concentrations to each digester did not significantly affect the final pH conditions in the
 321 anaerobic digestion. However, treatment with 3 g/L of potassium showed a moderate increase in pH,
 322 as the 3 g/L potassium dose still worked optimally in the anaerobic digestion process. In comparison,
 323 the inhibitory effects of potassium addition were only observed at doses of 4 g/L and more(33). On
 324 the last day of the anaerobic digestion, the pH values of all treatments ranged from 7.50 to 7.87.
 325

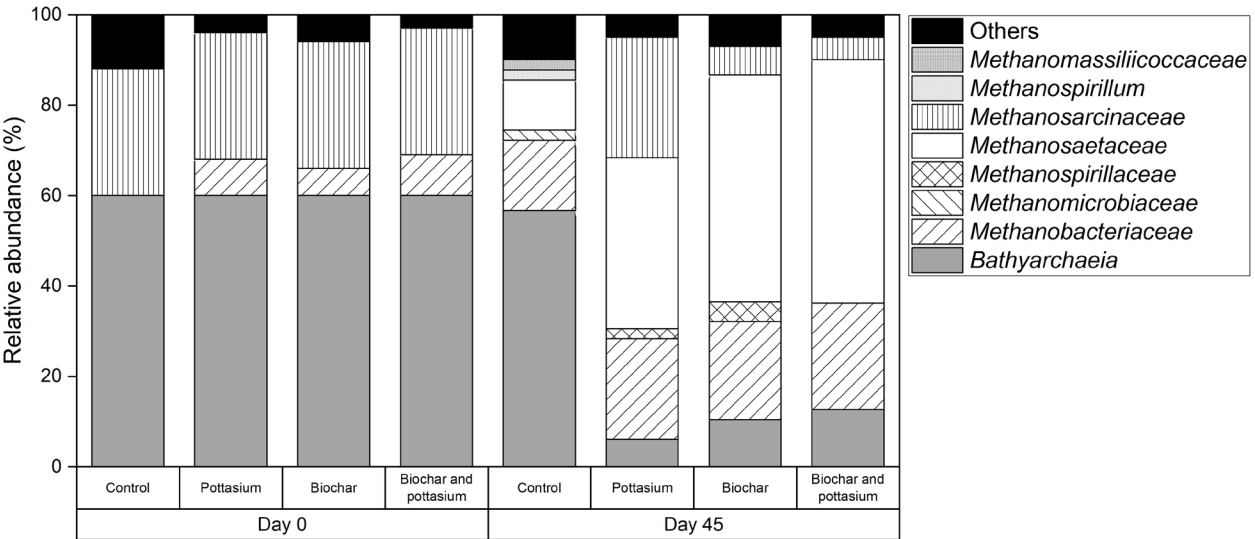


326 **Figure 4** Effect of pH on Biogas Production in Anaerobic Digestion with Various Treatments; (a)
 327 Addition of Biochar, (b) Addition of Potassium, (c) Addition of Biochar and Potassium.

328

329 Microbial Analysis

330 The study reveals distinct changes in the abundance of methanogenic families between Day 0 and Day 45
 331 under different treatment conditions, including potassium, biochar, and their combination. These shifts
 332 highlight how biochar and potassium promote the development of key methanogens, improve metabolic
 333 pathways, and enhance CH₄ production efficiency over time.



334

335 **Figure 5.** Effect of potassium and biochar addition on microbial community dynamics

336 Figure 5 shows how different treatments (potassium, biochar, and their combination) affected the
 337 microbial community dynamics. At the beginning of the experiment, *Bathyarchaeia* and
 338 *Methanobacteriaceae* dominated the methanogenic communities, each contributing 56.66 % of the
 339 total population. Families associated with acetoclastic methanogenesis, such as *Methanosaetaceae*
 340 and *Methanosarcinaceae*, were either absent or present in very low numbers. This suggests that the
 341 microbial community was still developing and had not yet shifted toward optimised CH₄ production
 342 pathways. This condition typically occurs in anaerobic digestion systems during an initial lag phase,
 343 as methanogenic archaea require time to establish and thrive within these anaerobic environments.
 344 By Day 45, significant changes were observed, especially in the groups treated with biochar,
 345 potassium, or their combination. One of the most significant trends was the increase in the abundance
 346 of *Methanosaetaceae*, an acetoclastic methanogen responsible for converting acetate to CH₄. In the
 347 control group, there was only a minor increase in *Methanosaetaceae*, indicating that the natural
 348 progression of microbial communities during anaerobic digestion leads to the slow growth of

349 acetoclastic methanogens. This suggests that external interventions like biochar or potassium are
350 necessary to speed up microbial shifts.

351 The samples treated with potassium showed a 37.78% increase in *Methanosaetaceae*. Potassium
352 plays a critical role in microbial metabolism by stabilising pH levels, which helps maintain optimal
353 conditions for methanogens. Studies have shown that potassium enhances microbial stability,
354 preventing sudden pH drops or toxic conditions that can disrupt methanogenic activity. In
355 comparison, samples treated with biochar showed an even more significant increase, with
356 *Methanosaetaceae* rising to 50.17 % of the total community by Day 45. Biochar offers numerous
357 benefits, such as providing more surfaces for microbial colonisation and enhancing electron transfer.
358 This is consistent with earlier research that discovered biochar enhances acetoclastic methanogenesis
359 by creating a better environment for microbial communities[6].

360 The combination of biochar and potassium had a significant effect, causing *Methanosaetaceae* to
361 increase to 53.80%. This shows that biochar and potassium work together to stabilise the anaerobic
362 digestion process and promote acetoclastic methanogenesis. Biochar provides a favourable
363 microhabitat for methanogenic archaea by enhancing microbial attachment, facilitating direct
364 interspecies electron transfer (DIET), and reducing the inhibitory effects of toxic intermediates [19].
365 Potassium, on the other hand, plays a more intricate role in methanogenesis beyond serving as a
366 general cofactor. It supports intracellular ionic balance and activates essential enzymes, including
367 methyl-coenzyme M reductase, which catalyses the final step in methane production. The availability
368 of potassium may enhance the metabolic activity of methanogenic archaea by improving enzyme
369 kinetics and ATP synthesis pathways, thereby increasing methane yield [11]. As acetoclastic
370 methanogens like *Methanosaetaceae* increased, the abundance of hydrogenotrophic methanogens,
371 such as *Methanomicrobiaceae* and *Methanospirillaceae*, declined across treatments. This suggests a
372 shift from hydrogenotrophic to acetoclastic pathways as the dominant CH₄ production route. Such a
373 shift is beneficial, as acetoclastic methanogenesis is generally more efficient in stable anaerobic
374 systems with sufficient acetate concentrations(22). The presence of biochar further supports this

375 transition by promoting acetate utilisation and limiting hydrogen accumulation, which favours the
376 growth of acetoclastic methanogens over hydrogenotrophic ones(7). Together, these treatments
377 enhance CH₄ production by promoting the dominance of acetoclastic methanogenesis, leading to
378 higher biogas yields.

379

380 **4. Conclusions**

381 This study demonstrates that the combined use of biochar and potassium significantly enhances CH₄
382 production and process stability during mesophilic anaerobic digestion (AD) of carbohydrate-rich
383 bread waste. The CH₄ yield and production rate of the substrate with the addition of a combination
384 of biochar and potassium is higher than the sum of the individual contributions from biochar and
385 potassium, showcasing the synergistic effects between biochar and potassium. Biochar facilitates
386 microbial aggregation and serves as an electron shuttle, enhancing direct interspecies electron transfer
387 (DIET), while potassium functions as an essential cofactor for methanogenic enzymes, promoting
388 more efficient metabolic pathways. The interaction between biochar and potassium creates a stable
389 and enriched microbial ecosystem, leading to enhanced acetoclastic methanogenesis and improved
390 methane yield. Microbial analysis revealed a 53.8% increase in acetoclastic methanogens
391 (*Methanosaetaceae*), indicating a shift toward more efficient CH₄ pathways. Additionally, the
392 buffering capacity of biochar helps maintain pH stability, reducing potential inhibitory effects of
393 volatile fatty acids, while potassium contributes to ion balance, ensuring optimal enzymatic function.
394 Future research should explore scaling these findings to full-scale biogas plants and further
395 investigate the long-term stability and optimisation of biochar-potassium interactions in AD systems.

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401 **Conflict of Interest**

402 The authors declare no conflict of interest.

403 **Author Contribution**

404 Yusron Sugiarto took the lead in designing and supervising the study. Ary Mustofa Ahmad and Inggit
405 Kresna Maharsih conducted the experiments and performed data analysis. Nimas Mayang Sabrina
406 Sunyoto assisted with data collection, while Hendrix Yulis Setyawan managed the statistical analyses.
407 Muhamad Hafizhur Rofiq contributed to the preparation of materials, and Mingming Zhu provided
408 resources and reviewed the manuscript. All authors reviewed and approved the final version of the
409 manuscript.

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