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Applicability of Rice Husk Residue Generated by the Silica Extraction Process to Anaerobic Digestion for Methane Production

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Abstract: Rice husks are a feedstock of biogenic silica because of their high silica content. After silica extraction, a solid residue comprising mostly carbohydrates is present. Solid residue valorization is important for closed-loop systems using rice husk and has minimal negative environmental impacts. In this study, we used solid rice husk that was generated by silica extractionto anaerobic digestion for producing biomethane. The rice husk residue was characterized in terms of total solids, volatile solids, pH, composition, and particle size. Changing the characteristics increased biogas production by 2.48-fold compared to that of raw rice husk. The residue produced 166.4 mL-biogas g^{-1} vs. and 100.4 mL CH 4 g^{-1} VS, much more than previously reported. Microbial community analysis, which was conducted to investigate the biological reasons for increased biogas and methane, found increased Bacteroidetes levels in the rice husk samples. Among archaeal communities, Bathyarchaeota was more abundant in all rice husk samples than in the inoculum. The rice husk residue contained more operational taxonomic units than other samples. These changes in the microbial community significantly influenced the anaerobic digestion of the rice husk residue and improved methane production. Our findings provide a basis for the cleaner utilization of rice husk residue to produce renewable energy.

Keywords: rice husk; anaerobic digestion; biochemical methane potential; methane; microbial community; pyrosequencing

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1. Introduction

Globally, 757 million tons of rice and paddy were produced in 2020, as reported by the Food and Agricultural Organization [1]. Rice byproducts are one of the major crop residues in Asian countries; approximately 90.5% of rice and paddy are produced in Asia [1]. Rice husk is a byproduct of the rice milling process and accounts for 20% of the paddy produced. Since rice husk is already dried and accumulates at rice mills, it is a good candidate for an industrial crop. Rice husks have a high silica content, with an average of 10–20% silica [2].

Silica extracted from rice husks can be utilized in potential value-added applications, such as bio-applications, energy storage, bioremediation, and construction materials [3].

Recently, we developed a two-stage continuous process for the effective extraction of silica from rice husks [4]. This process was operated stably for 80 h with an 89% silica extraction yield. During the extraction process, silica leached into the liquid, and carbohydrates remained in the solid. The liquid part, mainly sodium silicate, has been used to synthesize mesoporous or microsphere silica particles [5–8]. However, approximately 67% of the rice husk components remained in the solid residue. Anaerobic digestion (AD) is a useful tool for producing environmentally friendly and economical energy from crop residues. AD uses the biological processes of many types of bacteria that produce biogas, which is mostly composed of methane and carbon dioxide [9]. The biogas that is produced can be used to generate electricity and heat. Through refining, it can also be used as vehicle fuel (compressed natural gas). Many AD plants have already been built and are in operation, including 92 AD plants in the Republic of Korea [10], approximately 500,000 in Vietnam [11], and more than 40 million household biogas digesters and 26,000 medium and large-scale AD plants in rural areas of China [12]. Anaerobic digesters can be used to produce biofuel using crop residue as a resource, resulting in reduced facility construction costs. Therefore, AD is the most feasible and clean method for producing renewable energy using solid residue. Originally, AD plants using raw rice husks as feedstock often experienced low methane yields because of their complex nature and chemical composition [13], with one study reporting 32.26 mL g^{-1}-VS [14]. To improve the AD of rice husks, pretreatment techniques should be used to overcome the structural obstacles of the substrate and enhance biodegradability. Several studies have previously reported biogas enhancement in the AD of rice husks after enzymatic (lignase) [12,15,16], physical (hammer-milled) [17], and chemical (acidic/alkaline) [14,18] treatments.

The silica extraction process we developed consists of an attrition ball mill and an alkaline hydrothermal treatment. Attrition ball milling has been reported to reduce the size and crystallinity of lignocellulosic biomass, which makes it more suitable for chemical or microbial interactions [19]. Alkaline hydrothermal treatment degrades ester and glycosidic side chains in lignocellulosic biomass, resulting in structural changes in lignin, as well as the swelling and decrystallization of cellulose [20]. It also increases the intraparticle porosity and channel size of lignocellulose, which improves the accessibility to microorganisms and enzymes [21,22]. Therefore, we expected that the rice husk residue, treated by both attrition ball milling and alkaline hydrothermal treatment, would have improved biodegradability.

This study investigated the applicability of rice husk residue generated by silica extraction in AD to produce clean biofuels. The effects of the silica extraction process on the chemical composition, methane production, and microbial community changes were compared with those of raw rice husks. The ideal goal of a circular bioeconomy is to obtain a closed-loop system by minimizing negative impacts on the environment [23]. This study suggests a method for efficiently using rice husk residue generated from silica extraction for clean energy production. We believe that this study will enhance the valorization of rice husk residue, maximize value-added utilization of rice husks, and, ultimately, be a step toward a circular bioeconomy.

2. Materials and Methods

2.1. Substrates and Inoculum

Rice husks were collected from a rice-processing facility in Jinju, Republic of Korea. The rice used in this study was *Oryza sativa*. The sewage sludge used as the inoculum was collected from the Jungnang Sewage Treatment Center in Seoul, Republic of Korea. Sewage sludge was collected directly from sampling values of the sewage treatment plant. The substrates and inoculum were stored at room temperature and at 4 °C, respectively.

2.2. Rice Husk Sample Preparations

Rice husk residue was prepared using a previously reported silica extraction method [4] as illustrated in Figure 1. The silica extraction process consisted of ball milling and an alkaline hydrothermal treatment. First, the rice husks were treated in a ball mill using attrition pulverizer mill equipment (Hankookmc Co., Incheon, Republic of Korea) with a 2.4 L working volume inner jar, where steel balls of 10 mm in diameter crushed the rice husks. An alkaline solvent, sodium hydroxide (NaOH) (98%, Daejung Chemical & Metals Co., Ltd., Siheung, Republic of Korea), was added to the grinding jar to obtain a concentration of 0.2 M. The steel balls were moved randomly inside the jar by rotating the impeller. The rotation speed and milling time were set at 300 rpm and 30 min, respectively. Following ball milling, the samples were incubated at 80 °C for 3 h without stirring for alkaline leaching. After the alkaline reaction, the liquid was discharged by vacuum filtration. The solid residue was resuspended in water, and its pH was adjusted to neutral using 1 M HCl (Sigma-Aldrich, St. Louis, MO, USA).

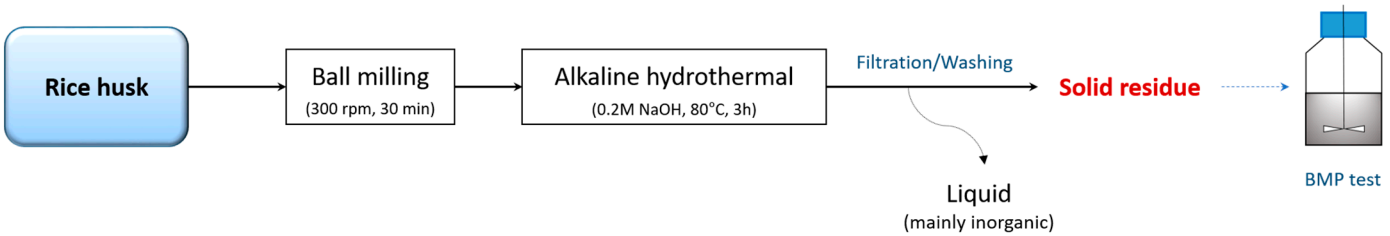


Figure 1. Illustrative scheme of the preparation of rice husk residue samples.

To prepare the ball mill treated sample, fine particles produced by ball milling of the rice husk were transferred to a vertical sieving machine (Analysette3, Fritsch GmbH, Idar-Oberstein, Germany) and shaken for 1 min with a 3 mm amplitude to separate the solid from the liquid, which was used as the substrate. Alkaline treated samples were prepared in the same manner without ball milling using the silica extraction method.

2.3. Biochemical Methane Potential (BMP) Testing

The methane production potential of the samples was assessed using an automatic methane potential test system (AMPTS II) (Bioprocess Control AB, Lund, Sweden) under mesophilic (37 °C) and stirring conditions. The biogas produced during the BMP test was automatically measured using a gas volume meter that was integrated into the test system. For all experimental setups, the initial volatile solids (VS) ratio of the substrate to inoculum was maintained at 1:2. The rice husk substrate (2.5 g) was mixed with 313 mL of inoculum and placed in a 500 mL bottle. The pH values of the bottles were not adjusted. All reactors were purged with N_2 gas (99.999% (*v*:*v*)) for 10 min before sealing. The average results of biogas production from the three tests are shown as values at 0 °C and 1 atm. The blank contained only sewage sludge and no rice husks. The quantity of biogas produced by the samples was subtracted with that produced by the blank to determine the actual biogas production of the samples. The experiment was conducted in triplicate. ANOVA was performed to determine the significance of differences between raw rice husk and rice husk residues.

2.4. Microbial Community Analysis

To analyze the microbial community, deoxyribonucleic acid (DNA) extraction samples (5 mL each) were collected from the BMP bottle and stored at −20 °C until analysis. A PowerMax ®soil DNA isolation kit (Qiagen, Hilden, Germany) was used to extract DNA from each sample. The extracted DNA was used as the template, and the primer set (341F and 805R) [24] was used to amplify the V3–V4 region of the bacterial 16S rRNA gene. The specific primer set Arch519F (5′-CAGCCGCCGCGGTAA-3′) and Arch934R (5′-GTGCTCCCCCGCCAATTC-3′) was used to detect methanogen species [24]. A QI-

Aquick PCR Purification Kit (Qiagen) was used to purify the amplified products. Equal concentrations of purified products were pooled, and short fragments (non-target products) were removed using the AMPure bead kit (Agencourt Bioscience Co., Beverly, MA, USA). The quality and product size were assessed using a Bioanalyzer 2100 (Agilent Technologies Co., Santa Clara, CA, USA) and a DNA 7500 chip. Emulsion PCR was used to assess the mixed amplicons, which were then deposited on Picotiter plates (ChunLab Inc., Seoul, Republic of Korea), using the Illumina MiSeq Sequencing system to perform sequencing according to the manufacturer's instructions. The reads obtained from the different samples were sorted using the unique barcodes of each PCR product. Then, barcodes, linkers, and primer sequences were removed from the original sequencing reads. Any reads with two or more ambiguous nucleotides, low quality scores (average score < 25), or reads shorter than 300 bp were discarded. The Bellerophon method, which compares the BLASTN search results between the forward and reverse half sequences, was used to detect potential chimeric sequences [25]. After removing these, each reader's taxonomic classification was assigned to the EzBioCloud server (www.ezbiocloud.net) to achieve species-level identification (97% cutoff). Typical taxonomic suffixes are _s (for species), _g (genus), _f (family), _o (order), _c (class), and _p (phylum). Unclassified taxa are indicated by _uc [26]. The pyrosequencing reads generated in this study can be found in the European Molecular Biology Laboratory Sequence Read Archive (EMBL SRA) database under the accession numbers SUB9552054 and PRJNA726362. Samples were collected on the 1st and 7th days. The 7th-day samples were analyzed in-depth because biogas production showed a stable value and the microbial community changed significantly compared to the 1st day. The microbial community of the inoculum was analyzed before rice husks were added.

2.5. Analytical Methods

Total solids (TS) and vs. were measured using standard methods [27]. TS was weighed by placing the sample in a drying oven (Daihan Scientific Co. Ltd., Seoul, Republic of Korea) at 105 °C for 24 h. vs. was measured by burning the sample at 550 °C for 1 h using a muffle furnace (Intec Systems Inc., Seongnam, Republic of Korea). After burning, the weights of the samples were measured three times, and the average values were used. The dry matter of the substrate was then analyzed. The total carbon and nitrogen contents of the samples were measured using an elemental analyzer (CS744, LECO Co., St. Joseph, MI, USA) compliant with ISO standards [28]. The biogas composition was determined using a gas chromatography (GC)-thermal conductivity detector (YL6500 GC system, Young In Chromass Co., Anyang, Republic of Korea) with a Carbonxen®-1006 PLOT capillary GC column (Supelco Inc., Bellefonte, PA, USA). The oven temperature of the gas chromatograph was set to 65 °C. The temperatures of the injector and detector of the gas chromatograph were set to 230 °C. The carbohydrate, lignin, and ash contents were measured according to the standard procedure specified by the National Renewable Energy Laboratory [29]. In this method, two-step acid hydrolysis is performed using concentrated and diluted sulfuric acid to separate sugars from cellulose and hemicellulose. A high-performance liquid chromatograph equipped with a refractive index detector (Waters 2414, Waters Co., Milford, MA, USA) was used to examine the sugar composition. Sugars were eluted with degassed distilled water at a flow rate of 0.5 mL min^{-1} using a Sugar-Pak column (Waters Co., Milford, MA, USA), which was maintained at 70 °C. The acid-soluble lignin content was measured using ultraviolet spectroscopy (Jasco V-550 UV/VIS spectrophotometer, Jasco, Hachioji, Japan) at 320 nm. The acid-insoluble lignin content was determined by burning the samples at 575 °C [30]. All measurements were conducted in triplicate for error analysis. The standard deviations for the results are shown as error bars within the graphs.

3. Results and Discussion

3.1. Composition and Physical Property Changes after Silica Extraction

The characteristics of rice husks are important factors in AD. The characteristics of the samples are listed in Table 1.

Table 1. Characteristics of raw rice husk, solid residue, and inoculum used in this study.

	TS (wt %)	VS [a] (wt %)	pH	C (wt %)	N (wt %)	C/N	Carbohydrate (wt %)	Lignin (wt %)	Ash (wt %)
Raw rice husk	94.3 ± 0.13	86.8 ± 0.20	6.51	44.2	0.87	50.8	51.8	28.9	13.7
Solid residue	96.3 ± 0.14	97.2 ± 0.27	7.22	50.9	1.22	41.7	62.4	30.8	3.0
Inoculum	22.6 ± 0.02	70.7 ± 1.04	8.23	34.2	4.13	8.28	6.2	12.8	42.7

[a] Dry basis.

The carbohydrates in rice husks are the main energy source for anaerobic microbes. The raw rice husk used in this study was composed of 51.8 wt % carbohydrates, 28.9 wt % lignin, and 13.7 wt % ash. The high ash content of rice husk is a notable characteristic. After silica extraction, the rice husk residue was composed of 62.4 wt % carbohydrate, 30.84 wt % lignin, and 3.02 wt % ash (Table 1). The silica extraction process removed mainly ash. Rice husk ash contains mainly amorphous silica and other metallic compounds. Ash content interferes with the accessibility of carbohydrates to anaerobic microbes. Removing ash content would improve the accessibility of carbohydrates to microbes. Alkaline hydrothermal treatment has been reported to cause redeposition/relocalization of lignin [31], resulting in a reduced recalcitrance of lignocellulosic biomass. Therefore, the alkaline treatment used in this study is expected to improve accessibility to microbes.

The rice husks had a diameter of approximately 6–7 mm before silica extraction. Because ball milling was applied during silica extraction, the solid residue was reduced in size. Specifically, 25.4 wt % of the residue particles were collected by a sieve of <300 µm and 62.1 wt % by 300–1000 µm. This indicates that the silica extraction method used in this study is also effective in reducing biomass particle sizes.

The silica extraction process did not significantly change the TS, but the vs. increased because of the significant loss of ash (Table 1). The pH of the rice husk residue increased slightly, which could be due to NaOH remaining after washing. The carbon-to-nitrogen (C/N) ratio in the raw rice husk was 50.8. Ideally, the C/N ratio should be between 10 and 30 [32]. After silica extraction, the C/N ratio of the solid residue was 41.7. Therefore, the solid residue had a C/N ratio closer to the optimum range.

3.2. Improved Biogas and Methane Production

The biogas yield from the solid residue was approximately 2.48-times higher than that from the raw rice husk sample (Figure 2). The methane production in the solid residue was 2.18-times higher than that in the raw rice husk (Table 2). The solid residue sample showed a biogas production of 166.4 mL g^{-1} vs. and methane yield of 100.4 mL CH_4 g^{-1} vs. (Table 2). These values are significantly higher than the previously reported values of 18–75 mL g^{-1} TS even though the previous studies pretreated the rice husk with enzymatic (lignase), physical (hammer-milled), and chemical (acidic/alkaline) treatments, respectively [14,16–18,33]. To investigate the effects of a single factor, two samples were prepared separately either by ball milling or alkaline hydrothermal treatment. The final biogas yield from ball milling and alkaline hydrothermal treatment was 1.86- and 1.80-times higher, respectively, than that of the raw rice husks (Figure 2 and Table 2). The increase in biogas from the single treatments was lower than that from the combinational treatment of the solid residue sample. This indicates that the combinational treatment for extracting silica has a synergistic effect from both single treatments. The single treated samples showed different patterns in biogas production. The ball mill treated sample showed a much higher increase in biogas on the first day compared to the alkaline hydrothermal

treated sample (Figure 2). Ball milling reduced the size of the rice husk particles, which increased the surface area and improved the accessibility of microbes to the rice husk particles. In contrast, the alkaline hydrothermal treated sample showed a constant increase in biogas during the initial three days. Alkaline hydrothermal treatment removed most of the inorganics from rice husks and broke down the lignin, allowing unrestricted access to carbohydrates. Through the synergistic effect of these single treatments, a large increase in final biogas yield was obtained in the solid residue.

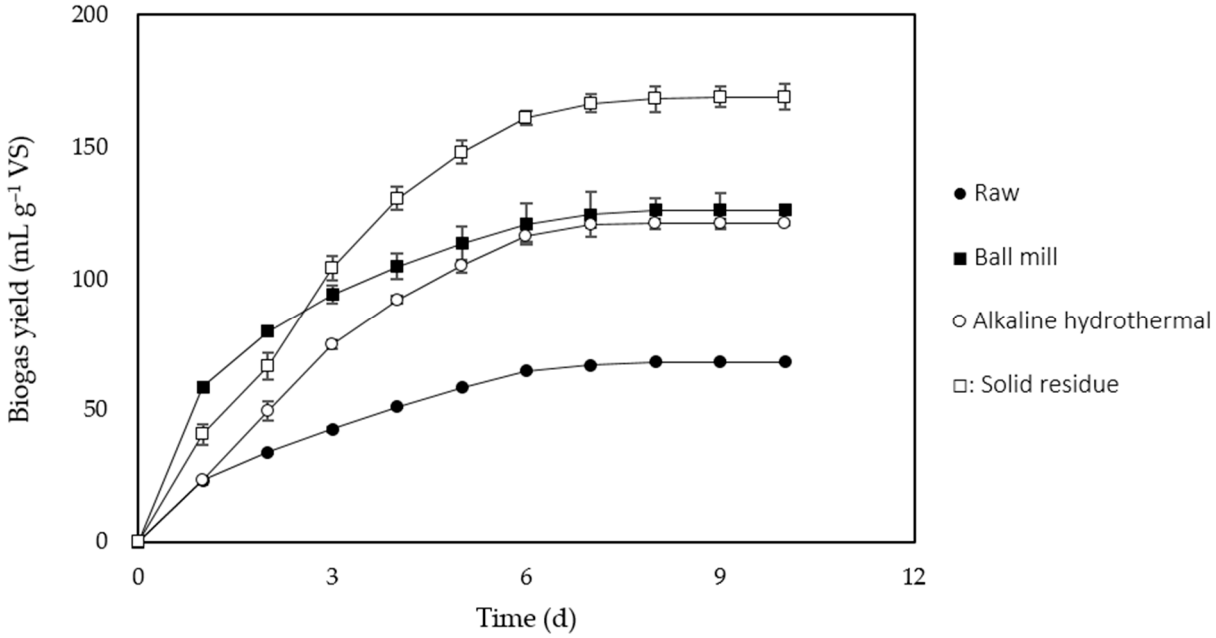


Figure 2. Time course biogas yields from AD of rice husk samples.

Table 2. Biogas and methane yields.

	Raw	Solid Residue	Ball Mill	Alkaline Hydrothermal
Biogas (mL g^{-1} VS)	67.1	166.4	124.5	120.7
Methane (mL CH_4 g^{-1} VS)	46.1	100.4	83.9	80.3

Although the pH of the mixed liquid in the BMP bottles was not adjusted in this study, it was maintained at 7.3–7.7 during AD. Considering that the optimal pH range for methanogenesis is between 6.2 and 8.0 [34], we assumed that the pH should not be an issue in our AD tests.

3.3. Microbial Community Analysis

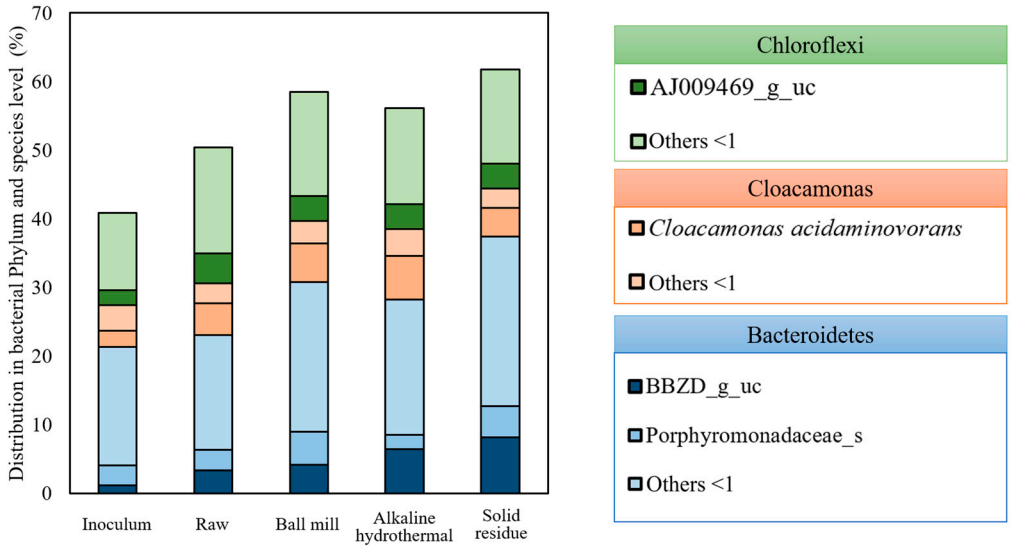
After AD, microbial communities were analyzed in both the rice husk samples and inoculum. Regarding the bacterial community, three dominant bacterial phyla were found in the inoculum: Bacteroidetes, Choloroflexi, and Cloacamonas (Figure 3a). In the rice husk samples, slight increases in the microbiome levels were found in the three bacterial phyla. However, Bacteroidetes levels significantly increased to 38% in the solid residue (Figure 3a) compared to the inoculum. The Bacteroidetes level increase was also significant in the single treated samples, but not as much as in the solid residue. Bacteroidetes produce short-chain carboxylic acids, hydrogen, and carbon dioxide as the end products of cellulolytic fermentation processes [35–38]. The short-chain carboxylic acids generated by Bacteroidetes are composed of C2-C3 carbon chain carboxylic acids, which can be converted into biological methane via a collaborative process of degradation and methanogenesis [39]. Therefore, the increase in

Bacteroidetes levels in the solid residue can explain the increased methane production in the solid residue sample.

Subsequently, the bacterial community was analyzed at the genus level to determine the dominant bacterial species (Figure 3b). Significantly, an uncultured genus, BBZD_g_uc, was the most prevalent genus of Bacteroidetes in the rice husk residue. In the raw rice husk sample, AJ009469_g, which belongs to Anaerolinaceae (Chloroflexi), was the most prevalent (12.99%) (Figure 3b). AJ009469_g has rarely been reported in microbiome samples, but it has been found in biomethanation processes that use a chemically defined medium rather than a complex carbon source [40–42]. In contrast, BBZD_g_uc was detected in the anaerobic co-digestion of cassava pulp with pig manure [43], AD sludge [44], and fish intestines [45]. BBZD_g_uc appears to be directly related to the digestion of lignocellulosic biomass. The species *Cloacamonas acidaminovorans* was observed as one of the main species, accounting for 4.1–6.3% of the bacterial communities in all samples (Figure 3a). Recent studies have demonstrated that *C. acidaminovorans* is involved in the AD of cellulose [46]. *C. acidaminovorans* are protein and polysaccharide degraders in dry fermentation processes [47]. Among archaeal communities, Bathyarchaeota was more abundant in all rice husk samples than in the inoculum.

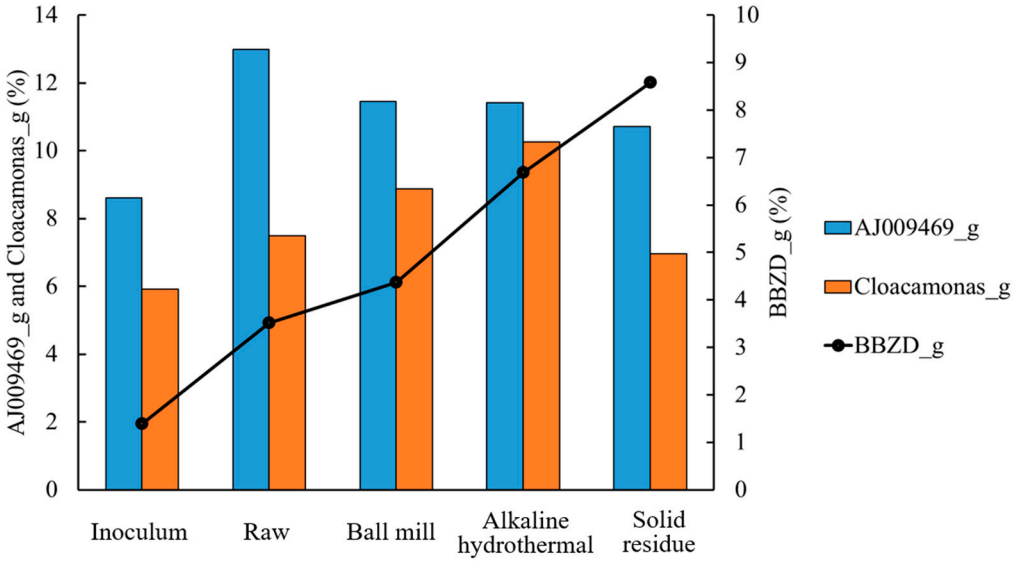
Similar to the bacterial communities, the archaeal communities were analyzed using pyrosequencing. Interestingly, Bathyarchaeota was more abundant in all rice husk samples than in the inoculum (Figure 3c). Although Bathyarchaeota has yet to be successfully cultured [48], a microbiome containing this species positively contributes to methanogenesis of lignocellulosic biomass via AD [49]. Additionally, Bathyarchaeota may be involved in the fermentation of lignocellulosic biomass and cellulose utilization [50]. The distribution of Bathyarchaeota was not correlated with methane production, but the solid residue and alkaline hydrothermal treated samples showed more operational taxonomic units than other samples in the rarefaction curve using alpha diversity analysis (data not shown). This means that alkaline treatment increased the overall archaeal diversity and affected methane production.

The solid residue sample tested in this study induced changes in the microbial community of anaerobic digesters, which eventually led to increased biogas and methane production.



(**a**)

Figure 3. *Cont.*



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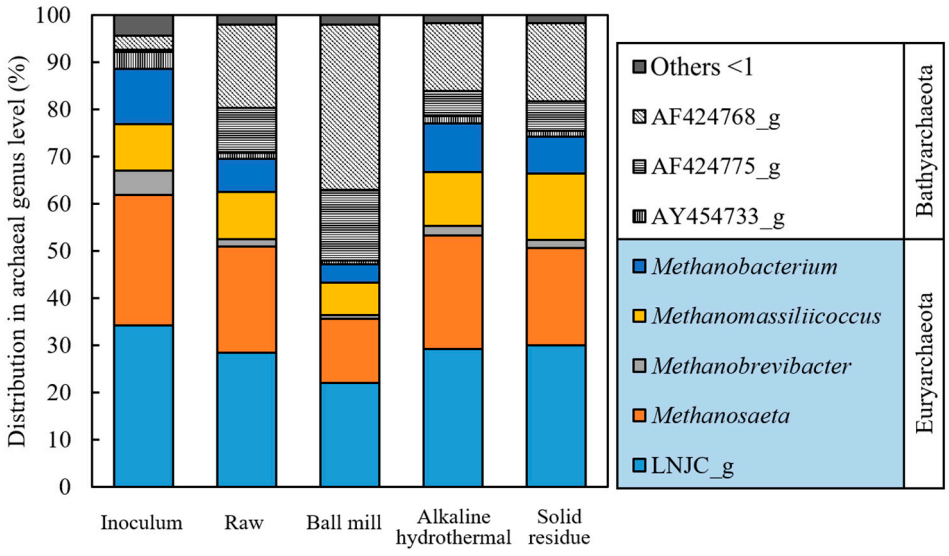

(**c**)

Figure 3. Distribution at the level of (**a**) bacterial phylum, (**b**) bacterial genus, and (**c**) archaeal genus of rice husk samples and inoculum. (**a**) Green boxes represent uncultured Chloroflexi, orange boxes represent *Cloacamonas*, and blue boxes represent uncultured Bacteroidetes. (**b**) Blue boxes represent uncultured AJ009469, orange boxes show Cloacamonas_g, and the black line represents uncultured BBZD_g. (**c**) Textured boxes represent an uncultured genus belonging to Bathyarchaeota, and colored boxes show a genus belonging to Euryarchaeota.

4. Conclusions

The effects of solid residue produced from the silica extraction process on AD were investigated. Because the silica extraction process, consisting of ball milling and alkaline hydrothermal processes, enhanced accessibility of the solid residue, this led to improved biogas production. The combinational treatment for silica extraction had the synergistic effects of both ball milling and alkaline hydrothermal treatments, which outperformed the effects of the individual treatments on their own. The microbial community differed significantly depending on the rice husk samples, according to metagenome analysis. The solid residue

sample with the highest abundance of Bacteroidetes exhibited the highest methane production. It was concluded that the solid residue generated from silica extraction produced higher methane in an anaerobic digester than raw rice husk and is applicable in AD.

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References

1. FAOSTAT. Crops and Livestock Products. 2022. Available online: http://www.fao.org/faostat/en/#data/QC. (accessed on 3 August 2022).
2. Ding, T.P.; Ma, G.R.; Shui, M.X.; Wan, D.F.; Li, R.H. Silicon isotope study on rice plants from the Zhejiang province, China. *Chem. Geol.* **2005**, *218*, 41–50. [CrossRef]
3. Chun, J.; Lee, J.H. Recent Progress on the Development of Engineered Silica Particles Derived from Rice Husk. *Sustainability* **2020**, *12*, 10683. [CrossRef]
4. Park, J.Y.; Gu, Y.M.; Park, S.Y.; Hwang, E.T.; Sang, B.-I.; Chun, J.; Lee, J.H. Two-Stage Continuous Process for the Extraction of Silica from Rice Husk Using Attrition Ball Milling and Alkaline Leaching Methods. *Sustainability* **2021**, *13*, 7350. [CrossRef]
5. Chun, J.; Gu, Y.M.; Hwang, J.; Oh, K.K.; Lee, J.H. Synthesis of ordered mesoporous silica with various pore structures using high-purity silica extracted from rice husk. *J. Ind. Eng. Chem.* **2020**, *81*, 135–143. [CrossRef]
6. Jang, I.-S.; Park, J.Y.; Park, H.; Gu, Y.M.; Lee, J.H.; Chun, J. Simplified Synthesis of Spherical Silica Microparticles from Rice Husk. *Chem. Eng. Technol.* **2022**, *45*, 381–384. [CrossRef]
7. Kim, S.; Park, J.Y.; Gu, Y.M.; Jang, I.-S.; Park, H.; Oh, K.K.; Lee, J.H.; Chun, J. Eco-friendly and facile synthesis of size-controlled spherical silica particles from rice husk. *Nanoscale Adv.* **2021**, *3*, 6965–6973. [CrossRef] [PubMed]
8. Park, J.Y.; Mun, W.; Chun, J.; Sang, B.-I.; Mitchell, R.J.; Lee, J.H. Alkali Extraction to Detoxify Rice Husk-Derived Silica and Increase Its Biocompatibility. *ACS Sustain. Chem. Eng.* **2022**, *10*, 7811–7817. [CrossRef]
9. Izumi, K.; Okishio, Y.-K.; Nagao, N.; Niwa, C.; Yamamoto, S.; Toda, T. Effects of particle size on anaerobic digestion of food waste. *Int. Biodeterior. Biodegrad.* **2010**, *64*, 601–608. [CrossRef]
10. Sung, T.; Kim, S.; Kim, K.C. Thermoeconomic analysis of a biogas-fueled micro-gas turbine with a bottoming organic Rankine cycle for a sewage sludge and food waste treatment plant in the Republic of Korea. *Appl. Therm. Eng.* **2017**, *127*, 963–974. [CrossRef]
11. Roubík, H.; Barrera, S.; Van Dung, D.; Phung, L.D.; Mazancová, J. Emission reduction potential of household biogas plants in developing countries: The case of central Vietnam. *J. Clean. Prod.* **2020**, *270*, 122257. [CrossRef]
12. Song, Z.; Zhang, C.; Yang, G.; Feng, Y.; Ren, G.; Han, X. Comparison of biogas development from households and medium and large-scale biogas plants in rural China. *Renew. Sustain. Energy Rev.* **2014**, *33*, 204–213. [CrossRef]
13. Jabeen, M.; Zeshan; Yousaf, S.; Haider, M.R.; Malik, R.N. High-solids anaerobic co-digestion of food waste and rice husk at different organic loading rates. *Int. Biodeterior. Biodegrad.* **2015**, *102*, 149–153. [CrossRef]
14. Olugbemide, A.D.; Lajide, L.; Adebayo, A.; Owolabi, B.J. Enhanced Biogas Production from Rice Husk Through Solid-State Chemical Pretreatments. *Waste Biomass-Valorization* **2020**, *11*, 2397–2407. [CrossRef]
15. Díaz, A.B.; Blandino, A.; Belleli, C.; Caro, I. An Effective Process for Pretreating Rice Husk To Enhance Enzyme Hydrolysis. *Ind. Eng. Chem. Res.* **2014**, *53*, 10870–10875. [CrossRef]
16. Matin, H.H.A.; Hadiyanto, H. Optimization of biogas production from rice husk waste by solid state anaerobic digestion (SSAD) using response surface methodology. *J. Environ. Sci. Technol.* **2018**, *11*, 147–156.

17. Contreras, L.M.; Schelle, H.; Sebrango, C.R.; Pereda, I. Methane potential and biodegradability of rice straw, rice husk and rice residues from the drying process. *Water Sci. Technol.* **2012**, *65*, 1142–1149. [CrossRef] [PubMed]
18. Syafrudin; Nugraha, W.D.; Matin, H.H.A.; Budiyono. The effect of enzymatic pretreatment and c/n ratio to biogas production from rice husk waste during solid state anaerobic digestion (SS-AD). *MATEC Web Conf.* **2017**, *101*, 02016. [CrossRef]
19. Lee, J.H.; Kwon, J.H.; Kim, T.H.; Choi, W.I. Impact of planetary ball mills on corn stover characteristics and enzymatic digestibility depending on grinding ball properties. *Bioresour. Technol.* **2017**, *241*, 1094–1100. [CrossRef]
20. Reilly, M.; Dinsdale, R.; Guwy, A. Enhanced biomethane potential from wheat straw by low temperature alkaline calcium hydroxide pre-treatment. *Bioresour. Technol.* **2015**, *189*, 258–265. [CrossRef]
21. Dizaji, H.B.; Zeng, T.; Hartmann, I.; Enke, D.; Schliermann, T.; Lenz, V.; Bidabadi, M. Generation of High Quality Biogenic Silica by Combustion of Rice Husk and Rice Straw Combined with Pre- and Post-Treatment Strategies—A Review. *Appl. Sci.* **2019**, *9*, 1083. [CrossRef]
22. Dehghani, M.; Karimi, K.; Sadeghi, M. Pretreatment of Rice Straw for the Improvement of Biogas Production. *Energy Fuels* **2015**, *29*, 3770–3775. [CrossRef]
23. Devi, A.; Bajar, S.; Kour, H.; Kothari, R.; Pant, D.; Singh, A. Lignocellulosic Biomass Valorization for Bioethanol Production: A Circular Bioeconomy Approach. *BioEnergy Res.* **2022**, *15*, 1820–1841. [CrossRef] [PubMed]
24. Fadrosh, D.W.; Ma, B.; Gajer, P.; Sengamalay, N.; Ott, S.; Brotman, R.M.; Ravel, J. An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. *Microbiome* **2014**, *2*, 6. [CrossRef]
25. Huber, T.; Faulkner, G.; Hugenholtz, P. Bellerophon: A program to detect chimeric sequences in multiple sequence alignments. *Bioinformatics* **2004**, *20*, 2317–2319. [CrossRef] [PubMed]
26. Kim, O.-S.; Cho, Y.-J.; Lee, K.; Yoon, S.-H.; Kim, M.; Na, H.; Park, S.-C.; Jeon, Y.S.; Lee, J.-H.; Yi, H.; et al. Introducing EzTaxon-e: A prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int. J. Syst. Evol. Microbiol.* **2012**, *62*, 716–721. [CrossRef] [PubMed]
27. SOLIDS. *Standard Methods for the Examination of Water and Wastewater*; American Public Health Association: Washington, DC, USA, 2018.
28. *ISO 16948:2015*; Solid Biofuels—Determination of Total Content of Carbon, Hydrogen and Nitrogen. International Organization for Standardization: Geneva, Switzerland, 2015. Available online: https://www.iso.org/standard/58004.html (accessed on 3 April 2021).
29. NREL (National Renewable Energy Laboratory). *Determination of Structural Carbohydrates and Lignin in Biomass: Laboratory Analytical Procedure*; Technical Report NREL/TP-510-42618; National Renewable Energy Laboratory: Golden, CO, USA, 2008.
30. Kline, L.M.; Hayes, D.G.; Womac, A.R.; Labbé, N. Simplified determination of lignin content in hard and soft woods via UV-spectrophotometric analysis of biomass dissolved in ionic liquids. *Bioresources* **2010**, *5*, 1366–1383. [CrossRef]
31. Zhang, Y.; Chen, X.; Gu, Y.; Zhou, X. A physicochemical method for increasing methane production from rice straw: Extrusion combined with alkali pretreatment. *Appl. Energy* **2015**, *160*, 39–48. [CrossRef]
32. Wang, X.; Yang, G.; Feng, Y.; Ren, G.; Han, X. Optimizing feeding composition and carbon–nitrogen ratios for improved methane yield during anaerobic co-digestion of dairy, chicken manure and wheat straw. *Bioresour. Technol.* **2012**, *120*, 78–83. [CrossRef]
33. Kalra, M.; Panwar, J. Anaerobic digestion of rice crop residues. *Agric. Wastes* **1986**, *17*, 263–269. [CrossRef]
34. Linville, J.L.; Shen, Y.; Schoene, R.P.; Nguyen, M.; Urgun-Demirtas, M.; Snyder, S.W. Impact of trace element additives on anaerobic digestion of sewage sludge with in-situ carbon dioxide sequestration. *Process Biochem.* **2016**, *51*, 1283–1289. [CrossRef]
35. Betian, H.G.; Linehan, B.A.; Bryant, M.P.; Holdeman, L.V. Isolation of a cellulolytic Bacteroides sp. from human feces. *Appl. Environ. Microbiol.* **1977**, *33*, 1009–1010. [CrossRef] [PubMed]
36. Halliwell, G.; Bryant, M.P. The Cellulolytic Activity of Pure Strains of Bacteria from the Rumen of Cattle. *J. Gen. Microbiol.* **1963**, *32*, 441–448. [CrossRef] [PubMed]
37. Hatamoto, M.; Kaneshige, M.; Nakamura, A.; Yamaguchi, T. Bacteroides luti sp. nov., an anaerobic, cellulolytic and xylanolytic bacterium isolated from methanogenic sludge. *Int. J. Syst. Evol. Microbiol.* **2014**, *64*, 1770–1774. [CrossRef] [PubMed]
38. Robert, C.; Chassard, C.; Lawson, P.A.; Bernalier-Donadille, A. Bacteroides cellulosilyticus sp. nov., a cellulolytic bacterium from the human gut microbial community. *Int. J. Syst. Evol. Microbiol.* **2007**, *57*, 1516–1520. [CrossRef] [PubMed]
39. Fenchel, T.; Blackburn, H.; King, G.M.; Blackburn, T.H. *Bacterial Biogeochemistry: The Ecophysiology of Mineral Cycling*; Academic Press: Cambridge, MA, USA, 2012.
40. Choi, T.-S.; Song, Y.-C.; Joicy, A. Influence of conductive material on the bioelectrochemical removal of organic matter and nitrogen from low strength wastewater. *Bioresour. Technol.* **2018**, *259*, 407–413. [CrossRef]
41. Shin, H.C.; Ju, D.H.; Jeon, B.S.; Choi, O.; Kim, H.W.; Um, Y.; Lee, D.H.; Sang, B.I. Analysis of the microbial community in an acidic hollow-fiber membrane biofilm reactor (Hf-MBfR) used for the biological conversion of carbon dioxide to methane. *PLoS ONE* **2015**, *10*, e0144999. [CrossRef]
42. Song, Y.-C.; Joicy, A.; Jang, S.-H. Direct interspecies electron transfer in bulk solution significantly contributes to bioelectrochemical nitrogen removal. *Int. J. Hydrog. Energy* **2019**, *44*, 2180–2190. [CrossRef]
43. Panichnumsin, P.; Ahring, B.; Nopharatana, A.; Chaiprasert, P. Microbial community structure and performance of an anaerobic reactor digesting cassava pulp and pig manure. *Water Sci. Technol.* **2012**, *66*, 1590–1600. [CrossRef]
44. Rivière, D.; Desvignes, V.; Pelletier, E.; Chaussonnerie, S.; Guermazi, S.; Weissenbach, J.; Li, T.; Camacho, P.; Sghir, A. Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge. *ISME J.* **2009**, *3*, 700–714. [CrossRef]

45. Han, S.; Liu, Y.; Zhou, Z.; He, S.; Cao, Y.; Shi, P.; Yao, B.; Ringø, E. Analysis of bacterial diversity in the intestine of grass carp (Ctenopharyngodon idellus) based on 16S rDNA gene sequences. *Aquac. Res.* **2010**, *42*, 47–56. [CrossRef]
46. Limam, R.D.; Chouari, R.; Mazéas, L.; Wu, T.; Li, T.; Grossin-Debattista, J.; Guerquin-Kern, J.; Saidi, M.; Landoulsi, A.; Sghir, A.; et al. Members of the uncultured bacterial candidate division WWE 1 are implicated in anaerobic digestion of cellulose. *Microbiologyopen* **2014**, *3*, 157–167. [CrossRef] [PubMed]
47. Stolze, Y.; Zakrzewski, M.; Maus, I.; Eikmeyer, F.; Jaenicke, S.; Rottmann, N.; Siebner, C.; Pühler, A.; Schlüter, A. Comparative metagenomics of biogas-producing microbial communities from production-scale biogas plants operating under wet or dry fermentation conditions. *Biotechnol. Biofuels* **2015**, *8*, 14. [CrossRef] [PubMed]
48. Evans, P.; Parks, D.H.; Chadwick, G.; Robbins, S.J.; Orphan, V.; Golding, S.D.; Tyson, G.W. Methane metabolism in the archaeal phylum Bathyarchaeota revealed by genome-centric metagenomics. *Science* **2015**, *350*, 434–438. [CrossRef] [PubMed]
49. Li, Y.; Zhao, J.; Achinas, S.; Zhang, Z.; Krooneman, J.; Euverink, G.J.W. The biomethanation of cow manure in a continuous anaerobic digester can be boosted via a bioaugmentation culture containing Bathyarchaeota. *Sci. Total. Environ.* **2020**, *745*, 141042. [CrossRef] [PubMed]
50. Maus, I.; Rumming, M.; Bergmann, I.; Heeg, K.; Pohl, M.; Nettmann, E.; Jaenicke, S.; Blom, J.; Pühler, A.; Schlüter, A.; et al. Characterization of Bathyarchaeota genomes assembled from metagenomes of biofilms residing in mesophilic and thermophilic biogas reactors. *Biotechnol. Biofuels* **2018**, *11*, 167. [CrossRef] [PubMed]