

Article

Not peer-reviewed version

Effect of Biological Pre-treatment with Cellulolytic Bacteria Consortium on Biogas Production from Crop Residues

[Reckson Kamusoko](#) * and [Patrick Mukumba](#)

Posted Date: 3 September 2024

doi: 10.20944/preprints202409.0278.v1

Keywords: anaerobic digestion; biogas; cellulose; crop residues; microbial consortium; pre-treatment



Preprints.org is a free multidiscipline platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This is an open access article distributed under the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Article

Effect of Biological Pre-Treatment with Cellulolytic Bacteria Consortium on Biogas Production from Crop Residues

Reckson Kamusoko * and Patrick Mukumba

Faculty of Science and Agriculture, Department of Physics, University of Fort Hare, Ring Road, Alice, 5700, South Africa

* Correspondence: rkamsoko.kamusoko@gmail.com

Abstract: Biological pre-treatment holds much promise as an innovative and sustainable strategy to improve biogas production from crop residues. In the present study, a novel hot spring cellulolytic microbial consortium (HSCMC) was used to pre-treat crop residues, such as maize stover, wheat straw and soybean straw to improve biogas production by anaerobic digestion (AD). Results indicated that the ash and volatile solids in maize stover were significantly reduced by maximum values of 21.3 % and 69.2 % respectively, after 7 days of pre-treatment over non-treated residues. The maximum decline of 83.9 % in total solid content was reported in wheat straw. The total reducing sugar content was increased by maximum yields of 60.9 %, 96.3 % and 84.7 % in maize stover, wheat straw and soybean straw, respectively. Furthermore, the HSCMC consortium enhanced the cumulative methane yield of wheat straw, maize stover and soybean straw by 50.6 %, 50.2 % and 56.6 %, respectively. Increased methane yield from pre-treated crop residues implies that this method could be an economically viable option for large-scale valorization of the AD process.

Keywords: anaerobic digestion; biogas; cellulose; crop residues; microbial consortium; pre-treatment

1. Introduction

Rapid exhaustion of fossil fuels accompanied with continuous population growth has led to ever-increasing energy crisis in the world [1,2]. The utilization of fossil fuels is estimated to increase by 105-fold by 2050, outweighing the supply from natural fossil reserves. At this rate, the natural oil reserves are likely to be depleted in less than 30 years, putting the global energy security at risk [1]. In addition, concerns about global climate change have motivated to search for affordable, clean and sustainable sources of energy [3]. Crop residues, such as straws, husks, stover, stalks, baggase, hulls and cobs are potential sources of renewable energy that can replace fossil fuels [4,5]. Vast amounts of crop residues are generated each year in the world, but their valorization into biofuels and other value-added products in a biorefinery is still undervalued. The annual crop residues production is estimated at above 5 billion tons the world over. Asia is the leading crop residues producer in the world. It accounts for about 47 % of the total annual crop residues production. Americas, Europe, Africa and Oceania contribute around 29 %, 16 %, 7 % and 1 % to the global crop residues production, respectively [6].

Zimbabwe is an agro-based economy, which produces about 7.8 million tons per year of residues from harvesting and processing of crops. This corresponds to an energy potential of 8.5 PJ per year [4,7]. Sugarcane and cereals are the major producers of agricultural residues in Zimbabwe. They account for about 34 % and 25 % of the total annual residues production, respectively. Approximately 2 % of the residues come from legumes, and in particular soybean and groundnuts [4]. Crop residues are mostly exploited via traditional practices, such as use for animal feed, burying into the soil for nutrient recycling and burning [5,7]. Improper discharge of crop residues will pose severe environmental degradation challenges. For example, burning of crop residues releases greenhouse gases that may contribute to global warming and climate change. More so, burying of crop residues into the soil can affect crop yields through resurgence of diseases and dilapidated soil conditions [5].

This calls for scientist intervention to develop suitable strategies that can effectively manage and recycle crop residues in an eco-friendly and sustainable way.

The anaerobic digestion (AD) of crop residues is considered a well-established and efficient technology that amalgamates waste management and biofuel production [8]. AD is an intricate reaction, which involves four interrelated steps, such as hydrolysis, acidogenesis, acetogenesis and methanogenesis. The process is facilitated by consortia of bacteria. Biogas is the main resultant product of the methanogenic step in AD [6–8]. It is a renewable energy carrier with multiple applications. Biogas can be used as a source of heat and electricity, or as a fuel for automobile engines. In addition, a solid digestate substance that can be used as a fertilizer in agriculture or as a substrate for edible mushroom cultivation is produced [7,9–11]. However, crop residues are highly lignocellulosic due to the structural heterogeneity, complexity and rigidity of the cell wall, which is resistant to microbial degradation [1,12]. Holocellulose (i.e. cellulose and hemicelluloses) is the most fermentable substrate in crop residues. About 80 % of the cellulose found in crop residues can be converted into biogas [9,13,14]. Lignin is the hard-to-digest material that is resistant to hydrolysis during AD [12–14]. The recalcitrant nature of lignin limits the use of crop residues for biogas production. The hydrolysis step is often rate-limiting in AD of crop residues [15]. This restriction can be surpassed by deploying appropriate pre-treatment methods to enhance the biomethane potential (BMP) of crop residues.

Several methods, including chemical, physical, biological and hybrid pre-treatment technologies are widely reported in literature to improve the BMP of crop residues [13,14,16]. Biological pre-treatment has been proposed to be more advantageous over the other pre-treatment methods. It is often considered to be a simple, low capital and energy venture, with limited pollution to the natural ecosystem. Furthermore, it does not produce toxic by-products that may restrain the AD pathway [13,14]. Authors have extensively reported on the use of single strain systems for biological pre-treatment of lignocellulosic matter. Nevertheless, this strategy is not in compliance with the natural degradation criterion, in which consortia of microorganisms work synergistically to deconstruct the substrate [17]. Utilization of complex microbial systems has been posited to be a very competent strategy for pre-treatment of organic matter. It is regarded as a suitable option to overcome drawbacks of feedback regulation and metabolic suppression associated with single strain pre-treatment. More so, microbial consortia are highly resistant to changes in environmental conditions, including temperature, nutrient concentration and the presence of inhibitory compounds [17]. Many authors have designed mixed microbial consortia or co-cultures that have revealed promising results. As an exemplar, the AD of sawdust waste [1], corn straw [5], waste paper, cardboard [15], cassava residues [17], cotton stalks [18] and wheat straw [2,19] that were pre-treated using microbial consortia produced more methane yields compared to control conditions.

Despite, most researchers reporting on the use of single strain as a pre-treatment strategy, microbial consortium systems have been proposed to be more effective for degradation of cellulosic material. However, there is limited information in literature with respect to the viability of microbial consortium for pre-treatment of crop residues to improve biogas production. In this study, a microbial consortium with high cellulolytic activity was constructed using cellulolytic bacteria isolated from local hot springs. The bacteria strains were previously identified using morphological, biochemical and molecular methods [20]. The present study is a continuation of research work on screening novel microbial strains for accelerating the decomposition of cellulosic matter for AD. The aim was to investigate the potential of the constructed microbial consortium to improve biogas production from three lignocellulosic feedstocks i.e wheat straw, maize stover and soybean straw.

2. Materials and Methods

2.1. Experimental Design

A completely randomized design with three replications was used in this study. Two treatments were assigned to each of the three crop residues during biological pre-treatment: (a) treatment with a bacteria consortium; and (b) control (without inoculation). Three batch setups were designed for the AD of crop residues. Two treatments were allotted to each experimental set up: (a) pre-treated with a bacteria consortium; and (b) non-treated crop residues. A batch experimental set up containing inoculum only was included in the study.

2.2. Raw Materials

Maize stover, wheat straw and soybean straw were collected in clean polythene bags from crop fields located in Chinhoyi, Makonde District, Zimbabwe. The crop residues were air-dried at room temperature and utilized as substrates for pre-treatment using a constructed microbial consortium. The residues were mechanically ground into small particle sizes using a hammer mill. The final powder was obtained by passing through a 1 mm pore size standard testing sieve. The powder samples were then kept in air tight containers until further use. The comprehensive characteristics of untreated crop residues used in this study were adapted from literature [3]. The pH, total alkalinity, volatile matter, total nitrogen and total suspended solids values of crop residues were found to vary from 5.3-5.5, 232.7-448.3 mg L⁻¹, 74.1-78.1 %, 3.1-8.2 % and 699.95-2 039.5 mg L⁻¹, respectively. Cellulose, hemicelluloses, lignin and extractives content of raw crop residues ranged from 34.6-37.8 %, 19.7-28.2 %, 16.2-23.5 % and 12.0-23.7 %, respectively [3]. Rumen waste of cattle obtained from a local abattoir was used as inoculum for the study. The inoculum was in a semi-solid form. The ash, volatile solids (VS) and total solids (TS) content of the inoculum were 3.49 %, 6.18 % and 7.19 %, respectively.

2.3. Construction of Microbial Consortium

Pure bacteria strains belonging to *B. subtilis*, *Bacillus* sp., and *B. licheniformis* were used to construct a hot spring cellulolytic microbial consortium called HSCMC consortium. The cellulolytic bacteria strains were isolated from Lubimbi hot springs in Binga, Zimbabwe. The morphological and biochemical features, and 16S rRNA sequence analysis of the bacteria strains were obtained from the previous study by Kamusoko et al. [20]. All the strains were rod-shaped, Gram positive and found to belong to a group of motile *Bacillus*. Homology analysis showed the bacteria strains to be 99.13 %, 98.26 % and 98.91 % analogous to *B. subtilis*, *Bacillus* sp., and *B. licheniformis*, respectively [20]. The HSCMC consortium was produced by mixing equal portions (50 mL) of overnight grown cultures of the three bacteria strains in Luria-Bertani (LB) broth + 1 % carboxymethylcellulose (CMC) medium. The co-culture was sub-cultured numerous times to acquire a steady microbial community that can efficiently degrade lignocellulosic biomass. The final mixed microbial culture was stored in 60 % sterile glycerol at -20 °C until further use.

2.4. Biological Pre-Treatment of Crop Residues by Microbial Consortium

Crop residues were pre-treated using the HSCMC consortium to improve the hydrolysis rate prior to AD. A mineral salt medium was prepared by dissolving 0.5 g NH₄Cl, 0.5 g NaCl, 0.5 g K₂HPO₄, 0.4 g KH₂PO₄, 0.1 g MgCl₂.6H₂O, 1.5 g yeast extract and 1 g peptone in 1 000 mL of distilled water. The pH of the medium was adjusted to 7.0 using 1 M NaOH and 1 M HCl solution. Each ground powder of maize stover, wheat straw and soybean straw, with TS concentration of 20 % dry weight was mixed with 73 mL of mineral salt medium in 250 mL conical flasks. Distilled water was added to make a total working volume of 100 mL. The flasks were autoclaved at 121 °C for 20 min and inoculated with 5 mL of overnight culture (5 % v/v) of the HSCMC consortium.

A negative control without inoculation of the HSCMC consortium was prepared for each experimental setup. The flasks were sealed with cotton plugs and foil paper, and incubated in a water bath at 37 °C and 160 rpm for 7 days. All the experiments were performed in triplicate. The digested powder-containing media were designated as the hydrolysates of crop residues. The hydrolysates were preserved at -20 °C awaiting the AD process.

2.5. Characterization of Pre-treated Crop Residues

Pre-treated crop residues were characterized for total reducing sugar, ash, TS and VS content. The 3,5-dinitrosalicylic acid (DNS) assay was used to determine the total reducing sugar content in pre-treated crop residues. The reaction mixture consisted of 2 mL sample and 2 mL DNS reagent heated in a water bath at 90 °C for 15 min and cooled on ice. Absorbance was measured at 540 nm using a spectrophotometer (UV-1900i, Shimadzu, Germany) and the sugar concentration was estimated by extrapolating from the standard calibration curve of glucose. Ash, VS and TS content were analyzed using the standard methods of American Public Health Association (APHA) [21]. Each

experiment was conducted in triplicate. All values were expressed on % dry matter basis of the means for triplicate measurements.

2.6. Anaerobic Digestion of Crop Residues

Bench-scale AD in a batch fermentation mode was conducted to determine the effect of pre-treatment with HSCMC consortium on biogas production from crop residues. Batch reactions were conducted in 500 mL plastic digesters with a working volume of 350 mL. A total of 50 g of start inoculum was seeded into each digester containing the substrate. Nitrogen gas (N₂) was flushed into the digesters for 5 min to eliminate aerobic conditions. Lastly, NaHCO₃ was added to maintain digester pH at 7.0. The digesters were linked via silicon tubing to plastic bottles filled with 2 % NaOH solution to dissolve carbon dioxide (CO₂) and hydrogen sulfide (H₂S). The digesters were manually shaken once per day to ensure adequate mixing.

Daily methane production was measured using the liquid displacement method, in which the amount of NaOH solution discharged was assumed to be equal to the volume of methane. Control conditions containing only inoculum were run in parallel under similar conditions to evaluate the yield of biogas in the background of the trial digesters. The BMP reactions were performed at ambient temperature conditions for a period of 30 days.

2.7. Data Analysis

Statistical analysis was computed using OriginPro Version 8.5 software package. All the experiments were replicated three times. Therefore, each analytical result was presented as the mean of the three measurements \pm standard deviation. The standard deviations and statistical differences were analyzed using one-way ANOVA at $p \leq 0.05$. Comparison among means was performed using Tukey's Honest Significant Difference (HSD) test.

3. Results and Discussion

3.1. Effect of Biological Pre-Treatment on Chemical Characteristics of Crop Residues

The objective of biological pre-treatment was to promote the degradation of crop residues and improve the accessibility of holocellulose to microflora. Holocellulose is the main fermentable substrate that acts as a carbon source in crop residues during AD [5]. Ash, TS, VS and total reducing sugar content are usually determined as components of proximate analysis for crop residues and other types of biomass. The proximate composition of pre-treated crop residues utilized in this study is shown in Table 1. Findings demonstrate a significant variability ($p \leq 0.05$) in different chemical characteristics of pre-treated crop residues compared to corresponding control conditions. Ash, VS and TS reductions, and the total reducing sugar increment of crop residues was computed and presented in Figure 1. Ashing is based on the fact that minerals cannot be degraded by heat due to their low volatility. Instead, they are transformed to compounds, such as oxides, sulfates, phosphates, chlorides and silicates while organic matter is burnt under aerobic conditions [22]. However, the reported reduction in ash content in the study was probably due to vaporization of water and volatiles in the hydrolysates that were later removed from the plant biomass to liquid phase. The maximum reduction in ash and VS content of 21.3 % and 69.2 %, respectively, were reported from maize stover. Wheat straw recorded the highest decline of 83.9 % in TS content. This dry weight loss is directly linked to robust utilization of biomass by complex bacteria population in a synergistic way. According to Zhong et al. [5], biomass transformation may be shown by a decrease in the quantity of dry matter (TS and VS) of the feedstock. The decomposition of organic matter is a critical aspect for enhancing biogas production. Results obtained in this study are consistent with those found in scientific reports by Ali et al. [1], Yuan et al. [15], Wen et al. [23] and Zhang et al. [24].

Table 1. Proximate characteristics of crop residues after microbial consortium pre-treatment (% dry basis, apart from reducing sugars).

Parameter	Maize stover		Wheat straw		Soybean straw	
	Control	Treated	Control	Treated	Control	Treated
Ash	5.34 \pm 0.49	4.20 \pm 0.25*	6.67 \pm 0.58	5.99 \pm 0.28**	4.55 \pm 0.70	3.99 \pm 0.21**
Volatile solids	85.53 \pm 3.31	26.33 \pm 0.51*	89.60 \pm 0.40	28.00 \pm 0.87*	87.26 \pm 2.09	31.73 \pm 0.98*

Total solids	94.63 ± 0.78	15.90 ± 0.76*	92.70 ± 0.80	14.93 ± 0.24*	91.23 ± 2.04	15.17 ± 0.44*
Total reducing sugars (mg L ⁻¹)	65.13 ± 0.71	126.03 ± 2.47*	52.60 ± 1.31	103.27 ± 2.58*	48.80 ± 1.71	90.17 ± 2.74*

Values are means of the three replicates ± standard deviation. *Showed significant difference in comparison to corresponding control conditions using Tukeys test ($p \leq 0.05$). **Showed no significant difference compared to corresponding control conditions using Tukeys test ($p \leq 0.05$).

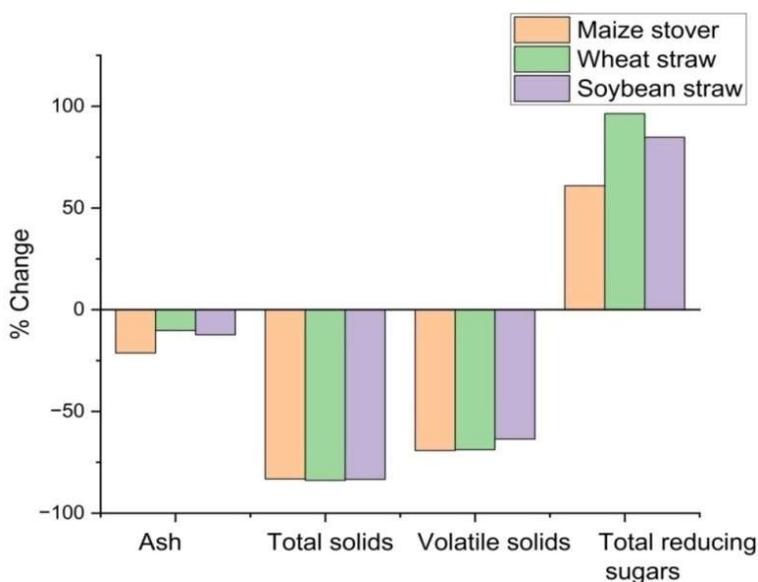


Figure 1. Comparison of ash, volatile solids and total solids reductions, and total reducing sugar increments in non-treated and pre-treated crop residues.

Compared to control experiments, the total reducing sugar content of the pre-treated hydrolysates of maize stover, wheat straw and soybean straw were increased by 60.9 %, 96.3 % and 84.7 %, respectively. This confirms the efficient degradation of complex carbohydrates to simple sugars by HSCMC consortium. Similarly, Ali et al. [1] recorded increased dissolved carbohydrate content by 70.6 % after pre-treatment of sawdust with a novel microbial consortium. In yet another study, Wen et al. [23] reported the highest reducing sugar content of 3 254 mg L⁻¹ after 48 hr of wheat straw saccharification by microbial consortium WSD-5. However, the rate of accumulation of reducing sugars in this study was higher compared to single strain microbial pre-treatment [25,26].

This study implies that an artificial microbial consortium system is more effective than a single bacterial system in solubilizing complex sugars in crop residues to more readily fermentable intermediates. A microbial consortium is associated with cross-feeding which facilitates removal of by-products, thereby suppressing feedback inhibition during pre-treatment. Moreover, it has better adaptability and stability to harsh conditions over a single microorganism [27,28].

3.2. Effect of Microbial Pre-Treatment on Biomethane Production

Pre-treatment of crop residues with HSCMC consortium significantly improved methane yield compared to control conditions. The disparity could be due to positive synergistic effect of HSCMC consortium that promoted efficient solubilization of cellulosic compounds in crop residues to make more fermentable sugars available to AD [29]. All experimental treatments in this study showed an analogous trend in terms of daily methane production. Several peaks were observed with maximum methane production appearing from day 13 to day 16 of digestion depending on the nature of pre-treated feedstock. The highest methane production for unpre-treated crop residues appeared within day 16 to day 18 of AD. Further increase in digestion time caused a gradual decline in methane production conforming loss of organic material and carbon for microbial growth [17].

The profiles for daily methane production and cumulative methane yields of untreated and pre-treated wheat straw are shown in Figure 2. The maximum methane production of 89 mL day⁻¹ for non-treated wheat straw was achieved after 18 days of AD. However, pre-treatment of wheat straw

increased daily methane production to a maximum volume of 124 mL after 13 days of digestion. The maximum cumulative methane yield of pre-treated wheat straw was increased by 50.6 % over the corresponding control. Besides increased biogas production, the reported enhanced methane yield after pre-treatment was also due to increased methane ratio in accumulated biogas [1,2]. The findings are higher than the values of 22.2 % and 36.6 % obtained after pre-treatment of wheat straw using microbial consortium TC-5 under mesophilic and thermophilic conditions, respectively [2]. In contrast, Zhong et al. [19] significantly increased the total methane yield of wheat straw by 80.3 % through biological pre-treatment using a functional microbial consortium comprised of bacteria and fungi.

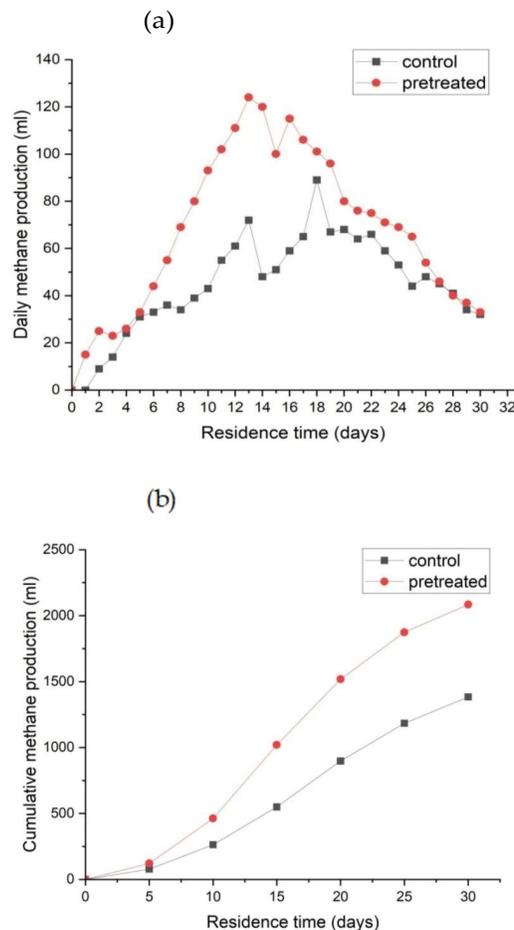


Figure 2. Comparison of daily methane production (a) and cumulative methane yield (b) for pre-treated and untreated wheat straw.

Maize stover consists of leaves, cobs, stalks and husks that are left in maize production fields. This agricultural waste is highly rich in carbohydrates, and thus makes it an ideal feedstock for biogas production. The use of maize stover for biogas production is restricted by large amounts lignin of about 16 % [3,29]. This can lead to a low recovery of monomeric units from lignocellulose. Microbial pre-treatment is a potent strategy to improve the methane potential of maize stover [29].

Figure 3 is a comparison of daily methane production and cumulative methane yields of untreated and pre-treated maize stover. Variability in terms of daily methane production and cumulative methane yield between pre-treated maize stover and control conditions was reported. The total daily methane yield of 96 mL for untreated maize stover was observed after 16 days of bioreactor operation. This value was lower than the maximum methane yield of 129 mL day⁻¹ obtained after pre-treatment of maize stover using HSCMC consortium.

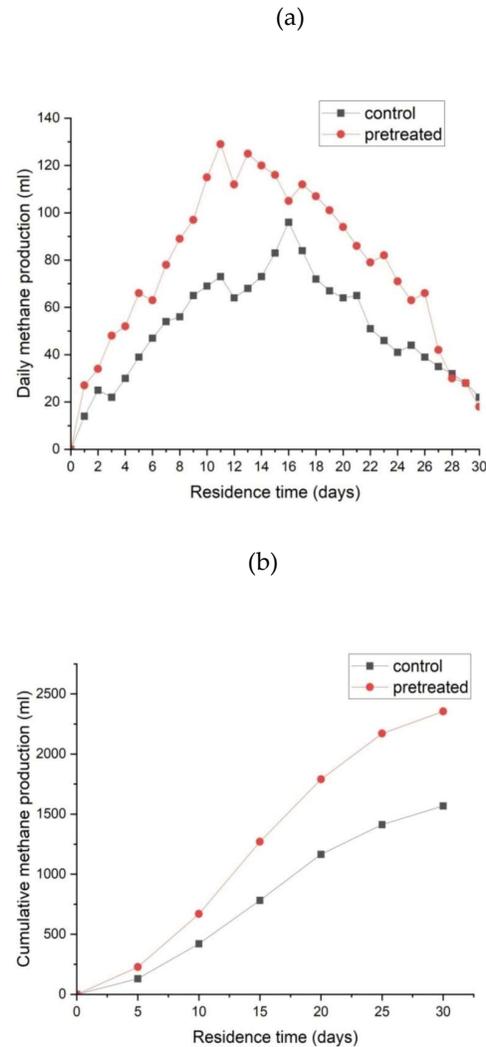


Figure 3. Comparison of daily methane production rate (a) and cumulative methane yield (b) for pre-treated and untreated maize stover.

The total cumulative methane yield of pre-treated maize stover was found to increase by 50.2 % compared to untreated samples. This study demonstrates that microbial consortium HSCMC could be a suitable alternative for saccharification of maize stover. A slight rise in total methane yield of 6.9 % obtained from pre-treated maize straw using aerobic microbial consortium was lower than the findings of this study [30]. However, Zhong et al. [5] reported maximum methane yield from microbially pre-treated maize straw, which was higher by 75.6 % than control. Similarly, pre-treatment of maize stover using a microbial consortium (BYND-9) improved biogas production by 62.85 % compared to non-treated residues [31].

High lignin content in a feedstock restrains its use for biogas production, hence an effective pre-treatment strategy is a pre-requisite [3,29]. This can be attributed to the amorphous and complex nature of lignin, which is resistant to microbial degradation. Generally, the lignin content of 23.5 % of the studied soybean straw was very high [3]. This could have caused a weak impact on its ability to produce methane on a daily basis compared to wheat straw and maize stover. The daily methane production and cumulative methane yield of untreated and pre-treated soybean straw are shown in Figure 4. The maximum daily methane production of 84 mL and 121 mL were observed for untreated and pre-treated soybean straw after 18 days and 15 days of digestion, respectively.

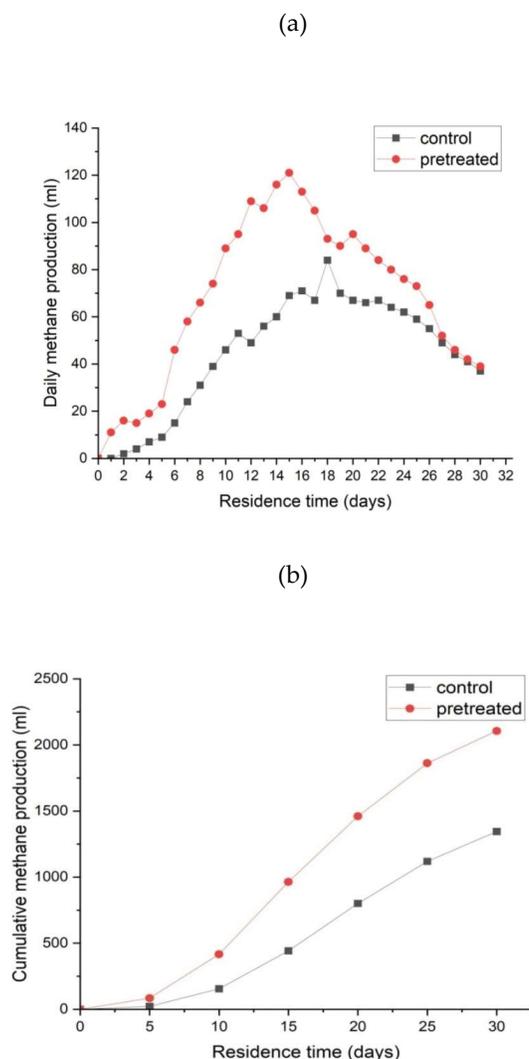


Figure 4. Comparison of daily methane production rate (a) and cumulative methane yield (b) for pretreated and untreated soybean straw.

Amongst the three crop residues, soybean straw reported the highest increase in cumulative methane yield. The maximum methane yield of soybean straw was enhanced by 56.6 % as a result biological pre-treatment using microbial consortium HSCMC. This could be probably due to high volatile matter content of 78.1 % exhibited by soybean straw [3,29]. Many researchers have successfully developed a wide range of microbial consortia that have shown strong degradation capability on crop residues [5,15,17–19]. But these microbial consortia have never been immensely explored for their ability to increase the hydrolysis of soybean straw for an efficient AD process. This points to limited published data on the utility of microbial consortia for pre-treatment of soybean straw for enhanced biogas production. Nonetheless, the HSCMC consortium showed more profound effect on pre-treatment of soybean straw for methane production than the MCI consortium. The MCI consortium increased methane yield of pre-treated sterilized and unsterilized soybean straw by 36.9 % and 34.3 %, respectively [32].

4. Conclusions

Development of a microbial consortium is an innovative platform for improving the hydrolysis and methane potential of crop residues. In this study, the HSCMC consortium was enriched from local hot springs and utilized for pre-treatment of wheat straw, maize stover and soybean straw. The HSCMC consortium significantly reduced ($p \leq 0.05$) the ash, VS and TS content of crop residues. It

also increased the concentration for the total reducing sugar content of pre-treated hydrolysates of maize stover, wheat straw and soybean straw by 60.9 %, 96.3 % and 84.7 %, respectively. Biological pre-treatment using HSCMC consortium enhanced the cumulative methane yield of wheat straw, maize stover and soybean straw by 50.6 %, 50.2 % and 56.6 %, respectively. Microbial consortium pre-treatment could be a suitable futuristic option for improving the bioconversion of crop residues to yield methane at large-scale operation. However, further investigation is still needed to assess the environmental and techno-economic impacts on scalability of microbial consortium HSCMC pre-treatment of crop residues for use in biogas production.

Author Contributions: Conceptualization, R.K. and P.M.; methodology, R.K. and P.M.; formal analysis, R.K.; investigation, R.K.; resources, R.K.; data curation, R.K.; writing-original draft preparation, R.K.; writing-review and editing, R.K. and P.M.; visualization, R.K. and P.M.; supervision, P.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Informed Consent Statement: Not Applicable.

Data Availability Statement: Not Applicable.

Acknowledgments: The authors are grateful to the Department of Research and Innovation (DRI) at the University of Fort Hare, Department of Science and Innovation (DSI), Technology Innovation Agency (TIA), National Research Foundation (NRF), Eskom TESP and Research Niche Area: Renewable Energy-Wind, for their financial support.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Ali, S.S.; Abomohra, A.E.; Sun, J. Effective bio-pretreatment of sawdust waste with a novel microbial consortium for enhanced biomethanation. *Bioresour. Technol.* **2017**, *238*, 425-432.
2. Kong, X.P.; Du, J.; Ye, X.; Xi, Y.; Jin, H.; Zhang, M.; Guo, D. Enhanced methane production from wheat straw with the assistance from lignocellulolytic microbial consortium TC-5. *Bioresour. Technol.* **2018**, *263*, 33-39.
3. Kamusoko, R.; Jingura, R.M.; Parawira, W.; Chikwambi, Z. Characterization of lignocellulosic crop residues for potential biogas production in Zimbabwe. *Biofuels Bioprod. Bioref.* **2022**, *16*, 1165-1171.
4. Jingura, R.M.; Matengaifa, R. The potential for energy production from crop residues in Zimbabwe. *Biomass Bioenergy.* **2008**, *32*, 1287-1292.
5. Zhong, W.; Zhang, Z.; Luo, Y.; Sun, S.; Qiao, W.; Xiao, M. Effect of biological pre-treatments in enhancing corn straw biogas production. *Bioresour. Technol.* **2011**, *102*, 11177-11182.
6. Shinde, R.; Shahi, D.K.; Mahapatra, P.; Singh, C.S.; Naik, S.K.; Thombare, N.; Singh, A.K. Management of crop residues with special reference to the on-farm utilization methods: A review. *Ind. Crops Prod.* **2022**, *181*, Article 114772.
7. Kamusoko, R.; Jingura, R.M.; Chikwambi, Z.; Parawira, W. Strategies for valorization of crop residues into biofuels and other value-added products. *Biofuels Bioprod. Bioref.* **2021**, *15*, 1950-1964.
8. Achinas, S.; Achinas, V.; Euverink, G.J.W. A technological overview of biogas production from biowaste. *Engineering.* **2017**, *3*, 299-307.
9. Sukhesh, M.J.; Rao, P.V. Anaerobic digestion of crop residues: Technological developments and environmental impact in the Indian context. *Biocatal. Agric. Biotechnol.* **2018**, *16*, 513-528.
10. Sindhu, R.; Gnansounou, E.; Rebello, S.; Binod, P.; Varjani, S.; Thakur, I.S.; Thakur, I.S.; Nair, R.; Pandey, A. Conversion of food and kitchen waste to value-added products. *J. Environ. Manag.* **2019**, *241*, 619-630.
11. Dar, R.A.; Parmar, M.; Dar, E.A.; Sani, R.K.; Phutela, U.G. Biomethanation of agricultural residues: Potential, limitations and possible solutions. *Renew. Sustain. Energy Rev.* **2021**, *135*, 110217.
12. Paudel, S.R.; Banjara, S.P.; Choi, O.K.; Park, K.Y.; Kim, Y.M.; Lee, J.W. Pre-treatment of agricultural biomass for anaerobic digestion: Current state and challenges. *Bioresour. Technol.* **2017**, *245*, 1194-1205.
13. Abraham, A.; Mathew, A.K.; Park, H.; Choi, O.; Sindhu, R.; Parameswaran, B.; Pandey, A.; Park, J.H.; Sang, B. Pre-treatment strategies for enhanced biogas production from lignocellulosic biomass. *Bioresour. Technol.* **2020**, *301*, 122725.
14. Awogbemi, O.; Kallon, D.V.V. Pre-treatment techniques for agricultural waste. *Case Stud. Chem. Environ. Eng.* **2022**, *6*, 100229.
15. Yuan, X.; Cao, Y.; Li, J.; Wen, B.; Zhu, W.; Wang, X.; Cui, Z. Effect of pre-treatment by a microbial consortium on methane production of waste paper and cardboard. *Bioresour. Technol.* **2012**, *118*, 281-288.

16. Catherine, C.; Twizerimana, M. Biogas production from thermo-chemically pre-treated sweet potato root waste. *Heliyon*, **2022**, *8*, e10376.
17. Zhang, Q.; He, J.; Tian, M.; Mao, Z.; Tang, L.; Zhang, J.; Zhang, H. Enhancement of methane production from cassava residues by biological pre-treatment using a constructed microbial consortium. *Bioresour. Technol.* **2011**, *102*, 8899-8906.
18. Bai, Y.; Li, W.; Chen, C.; Liao, P. Biological pre-treatment of cotton stalks and domestication of inocula in biogas fermentation. *Microbiol. China*. **2010**, *37*, 513-519.
19. Zhong, C.; Wang, C.; Wang, F.; Jia, H.; Wei, P.; Zhao, Y. Enhanced biogas production from wheat straw with the application of synergistic microbium consortium pre-treatment. *RSC Adv.* **2016**, *6*, 60187.
20. Kamusoko, R.; Jingura, R.M.; Parawira, W.; Chikwambi, Z. Isolation and characterization of cellulolytic bacteria from Lubimbi hot springs in Binga, Zimbabwe. *J. Biosci. Biotechnol.* **2023**, *12*, 33-39.
21. American Public Health Association (APHA). *Standard Methods for the Examination of Water and Wastewater*, 19th edn. APHA Inc.: New York, United States of America, 1995.
22. Liu, 2019. Effect of sample size, dry ashing, temperature and duration on determination of ash content in algae and other biomass. *Algal Res.* **2019**, *40*, 101486.
23. Wen, B.; Yuan, X.; Cao, Y.; Liu, Y.; Wang, X.; Cui, Z. Optimization of liquid fermentation of microbial consortium WSD-5 followed by saccharification and acidification of wheat straw. *Bioresour. Technol.* **2012**, *118*, 141-149.
24. Zhang, H.; Zhang, P.; Ye, J.; Wu, Y.; Fang, W.; Gou, X.; Zeng, G. Improvement of methane production from rice straw with rumen fluid pre-treatment: A feasibility study. *Inter. Biodeter. Biodegradation.* **2016**, *113*, 9e16.
25. Xu, W.; Fu, S.; Yang, Z.; Lu, J.; Guo, R. Improved methane production from corn straw by micro-aerobic pre-treatment with pure bacteria system. *Bioresour. Technol.* **2018**, *259*, 18-23.
26. Shah, T.A.; Lee, C.C.; Orts, W.J.; Tabassum, R. Biological pre-treatment of rice straw by ligninolytic *Bacillus* sp. strains for enhancing biogas production. *Environ. Prog. Sustain. Energy.* **2019**, *38*, 3.
27. Cao, Z.; Yan, W.; Ding, M.; Yuan, Y. Construction of microbial consortia for microbial degradation of complex compounds. *Front. Bioengin. Biotechnol.* **2012**, *10*, 1051233.
28. Liu, C.; Li, Z.; Guan, X.; Xu, Y.; Huang, N.; Liu, K. Improving pulping performance as well as reducing consumption and increasing efficiency via microbial consortium pre-treating bamboo. *Fermentation*, **2023**, *9*(4), 400.
29. Kamusoko, R. Isolation and characterization of cellulolytic bacteria for improving biogas production from crop residues through biological pre-treatment and co-digestion. PhD Thesis. *Unpublished*.
30. Zhu, Q.; Wang, Y.; Tan, F.; Wu, B.; Dai, L.; He, M.; Hu, G. Promotion of biogasification efficiency by pre-treatment and bioaugmentation of corn straw with microbial consortium. *E3S Web of Conf.* **2019**, *118*, 01032.
31. Zhao, Y.; Xu, C.; Ai, S.; Wang, H.; Gao, Y.; Yan, L.; Mei, Z.; Wang, W. Biological pretreatment enhances the activity of functional microorganisms and the ability of methanogenesis during anaerobic digestion. *Bioresour. Technol.* **2019**, *290*, 121660.
32. Yan-zhuan, C.; Hai-bo, Z.; Wan, S.; Hong-yan, C.; Shuang-dui, Y.; Yu-shan, B.; Zong-jan, C.; Jia-jia, L. Effects of MC1 pre-treatment on hydrolysis characteristics and methane production efficiency of soybean straw. *J. Agro-Environ. Sci.* **2010**, *39*(9), 2074-2080.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.