Effect of Biological Pre-Treatment with a Cellulolytic Bacterial Consortium On Biogas Production from Crop Residues

Reckson Kamusoko[1], and Patrick Mukumba[2]

Abstract**—**Biological pre-treatment is a promising and sustainable strategy to improve biogas production from crop residues (CRs). In the present study, a novel hot spring cellulolytic microbial consortium (HSCMC) was applied to pre-treat CRs, such as maize stover, wheat straw and soybean straw to enhance biogas yield via anaerobic digestion (AD). Results showed that the ash and volatile solids content of maize stover was significantly reduced, with maximum reductions of 21.3% and 69.2% respectively, after 7 d of pre-treatment compared to the untreated control. The highest reduction in total solid content (83.9%) was observed in wheat straw. Total reducing sugar content increased significantly, with maximum yields rising by 60.9%, 96.3% and 84.7% in maize stover, wheat straw and soybean straw, respectively. Furthermore, the HSCMC pretreatment significantly increased the cumulative methane (CH_4) yield of wheat straw, maize stover and soybean straw by 50.6%, 50.2% and 56.6%, respectively. The substantial increase in CH_4 production from pre-treated CRs suggests that this method is a promising and economically viable strategy for large-scale AD.

Keywords**—**anaerobic digestion, biological pre-treatment, methane yield, renewable energy, lignocellulosic biomass.

I. Introduction

Rapidexhaustion of fossil fuels accompanied by continuous population growth has led to ever-increasing energy crisis in the world [1],[2]. The utilisation 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 yr, putting the global energy security at risk [1]. In addition, concerns about the global climate change have motivated us to search for affordable, clean and sustainable sources of energy [3]. Crop residues (CRs), 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 CRs are generated each year in the world, but their valorisation into biofuels and other value-added products is still undervalued. The annual CRs production is estimated at around 5 billion tonnes the world over. Asia is the leading CRs producer in the world. It accounts for about 47% of the total CRs production. Americas, Europe, Africa and Oceania contribute about 29%, 16%, 7% and 1% to the global CRs production, respectively [6].

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Zimbabwe is an agro-based economy, which produces about 7.8 million tonnes yr^{-1} of residues from harvesting and processing of crops. This corresponds to an energy potential of 8.5 PJ yr^{-1} [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]. CRs 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 CRs will pose severe environmental challenges. For example, burning of CRs releases greenhouse gases that may contribute to global warming and climate change. More so, burying of CRs into the soil can affect crop yields through resurgence of diseases and dilapidated soil conditions [5]. This calls for scientist intervention to develop sustainable strategies that can effectively manage and recycle CRs.

Anaerobic digestion (AD) is a well-established and efficient technology that amalgamates waste management and biogas production [8]. The process intricately involves four interrelated steps, such as hydrolysis, acidogenesis, acetogenesis and methanogenesis. It is facilitated by consortia of bacteria. Biogas is the main resultant product of the methanogenic step [6]-[8]. It is a renewable energy carrier with multiple applications. Biogas is a source of heat and electricity, and a fuel for automobile engines. The solid waste by-product of AD can be used as a fertilizer or as a substrate for edible mushroom cultivation [8]-[11]. However, CRs 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 CRs. About 80% of the cellulose found in CRs can be converted into biogas [9],[13]. Lignin is a hard-to-digest material, limiting the use of CRs for biogas production [12],[13]. Hydrolysis is often rate-limiting in AD of CRs [14]. This can be surpassed by deploying suitable pre-treatment methods that can enhance the biomethane potential (BMP) of CRs.

Several methods, including chemical, physical, biological and hybrid technologies are widely reported in literature to improve the BMP of CRs [13],[15]. Biological pre-treatment has been proposed to be more advantageous over the other pre-treatment methods. It is a simple, low capital and energy venture, with limited environmental pollution [13]. Authors

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have extensively reported on the use of single strain systems in pre-treatment of organic matter. Nevertheless, this strategy is not in compliance with the natural degradation criterion, in which consortia of microorganisms work synergistically to deconstruct the substrate [16]. Utilisation of complex microbial systems has been posited to be a very competent strategy for pre-treatment of organic matter. It is considered as a suitable option to overcome drawbacks of feedback regulation and metabolic suppression. More so, microbial consortia are highly resistant to changes in environmental conditions, including temperature, nutrient concentration and the presence of inhibitors [16]. 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 [14], cassava residues [16], cotton stalks [17], and wheat straw [2],[18] that were pre-treated using microbial consortia produced more methane (CH_4) yields compared to control conditions.

Despite, most researchers reporting on the use of single strain pre-treatment, microbial consortia have been proposed to be more effective for degrading cellulosic material. However, there is limited information in literature with respect to the viability of microbial consortia for pre-treatment of CRs to improve biogas production. In this study, a microbial consortium with high cellulolytic activity was constructed using bacteria isolated from local hot springs. These bacteria strains were previously identified using morphological, biochemical and molecular methods [19]. The present study is a continuation of research work on screening novel microbial strains for accelerating the decomposition of cellulosic matter. The aim was to investigate the potential of the constructed microbial consortium to improve biogas production from three lignocellulosic feedstocks, including wheat straw, maize stover and soybean straw.

II. 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 CRs during biological pre-treatment: (a) treatment with a bacteria consortium; and (b) control (without inoculation). Three batch setups were designed for the AD of CRs. Two treatments were allotted to each experimental set up: (a) pre-treated with a bacteria consortium; and (b) non-treated CRs. 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 Makonde District of Zimbabwe. The CRs were air-dried at room temperature and 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 kept in airtight containers until further use. The characteristics of untreated CRs used in this study were adapted from literature [3]. pH, total alkalinity, volatile matter, total nitrogen and total suspended solids of CRs 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 CRs 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 the inoculum for the study. The inoculum was in a semi-solid form. Ash, volatile solids (VS) and total solids (TS) content of the inoculum were 3.49%, 6.18% and 7.19%, respectively. The inoculum was kept in sealed containers and acclimatised at 30 °C for one week prior to AD.

2.3 Construction of microbial consortium

Pure bacteria strains isolated from Lubimbi hot springs in Binga, Zimbabwe, were used to construct a hot spring cellulolytic microbial consortium (HSCMC). Morphological and biochemical features, and 16S rRNA sequence analysis of the strains were formerly performed by Kamusoko et al. [19]. All the strains were rod-shaped, Gram positive and belonged to 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 [19]. HSCMC consortium was produced by mixing equal portions (50 mL) of overnight grown cultures of the three bacteria strains in Luria-Bertani broth + 1% carboxymethylcellulose 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% glycerol at -20 °C until further use.

2.4 Pre-treatment of crop residues

Microbial consortium pre-treatment of CRs was conducted according to the method adapted from Zhang et al. [16]. A mineral salt medium was prepared by dissolving 0.5 g NH_4Cl, 0.5 g NaCl, 0.5 g K_2HPO_4, 0.4 g KH_2PO_4, 0.1 g $MgCl_2.6H_2O$, 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.

A negative control without inoculation of the HSCMC 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 d. All the experiments were performed in triplicate. The digested powder-containing media were designated as the hydrolysates of CRs. Hydrolysates were preserved at -20 °C awaiting the AD process.

2.5 Characterisation of pre-treated crop residues

Pre-treated CRs were characterised 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 CRs. 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 analysed using the standard methods of American Public Health Association (APHA) [20]. 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 the HSCMC on biogas production from CRs. Experiments were performed based on the modified principles of Zhang et al. [16]. Batch reactions were conducted in 500 mL plastic digesters with a working volume of 350 mL. A total of 50 g of the start inoculum was seeded into each digester containing the substrate. Nitrogen gas (N_2) was flushed into the digesters for 5 min to eliminate aerobic conditions. Lastly, $NaHCO_3$ was added to maintain digester pH at 7.0. Digesters were linked via silicon tubing to plastic bottles filled with 2% NaOH solution to dissolve carbon dioxide (CO_2) and hydrogen sulphide (H_2S). Digesters were manually shaken once per day to ensure adequate mixing.

Daily CH_4 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 CH_4. 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. BMP reactions were done at ambient temperature conditions for 30 d.

A. Data analysis

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

III. RESULTS AND DISCUSSION

3.1 *Effect of pre-treatment on chemical characteristics of crop residues*

The objective of biological pre-treatment was to promote the degradation of CRs and improve the accessibility of holocellulose to microflora. Holocellulose is the main fermentable substrate that acts as a carbon source CRs during AD [5]. Ash, TS, VS and total reducing sugar content are determined as components of proximate analysis for CRs and other types of biomasses. Proximate composition of pre-treated CRs utilised in this study is shown in Table 1. Findings demonstrate a significant variability ($p \leq 0.05$) in different chemical characteristics of pre-treated CRs compared to corresponding control conditions. Ash, VS and TS reductions, and the total reducing sugar increment of CRs was computed and presented in Fig. 1. Ashing is based on the fact that minerals cannot be degraded by heat due to their low volatility. Instead, they are transformed into compounds, such as oxides, sulphates, phosphates, chlorides and silicates while organic matter is burnt under aerobic conditions [21]. However, the reported reduction in ash content in the study was probably due to washouts in the hydrolysates that were removed from the plant biomass to liquid phase. 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 utilisation 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 (i.e. TS and VS) of the feedstock. 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. [14], Wen et al. [22], and Zhang et al. [23].

Compared to control experiments, the total reducing sugar content of pre-treated hydrolysates of maize stover, wheat straw and soybean straw were increased by 60.9%, 96.3% and 84.7%, respectively. This confirmed the efficient degradation of complex carbohydrates to simple sugars by the HSCMC. Similarly, Ali et al. [1] recorded an increased dissolved carbohydrate content by 70.6% after pre-treatment of sawdust with a novel microbial consortium. In another study, Wen et al. [22] reported the highest reducing sugar content of 3 254 mg L^{-1} after 48 h 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 [24,25].

This study implied that an artificial microbial consortium system is more effective than a single bacterial system in solubilising complex sugars in CRs to more readily fermentable intermediates. 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 [26],[27].

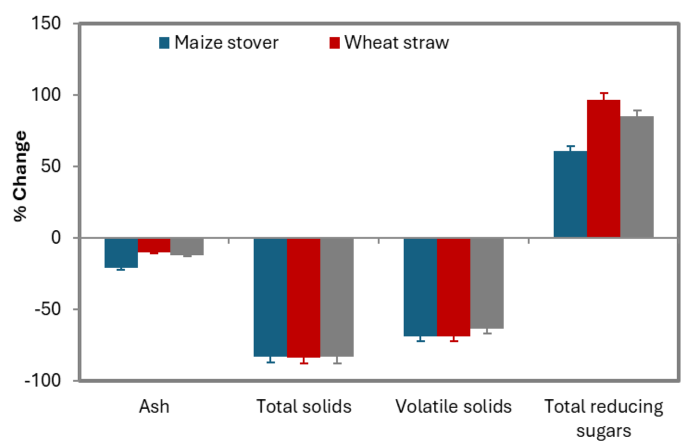
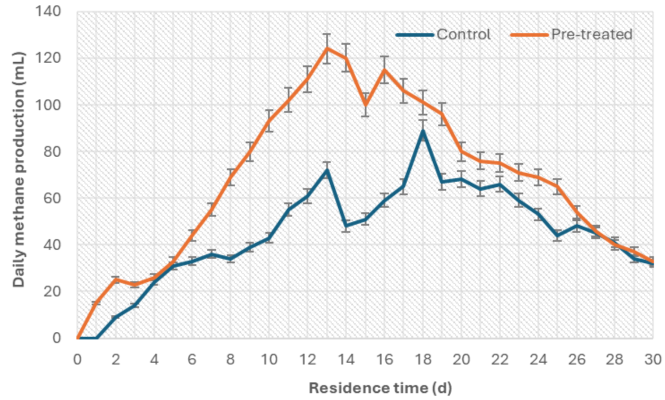


Fig. 1. Comparison of ash, volatile solids and total solids reductions, and total reducing sugar increments in non-treated and pre-treated crop residues

3.2 Effect of pre-treatment on biomethane production

Pre-treatment of CRs with HSCMC consortium significantly improved CH_4 yield compared to control conditions. The disparity could be due to positive synergistic effect of the HSCMC that promoted efficient solubilisation of cellulosic compounds in CRs to make more fermentable sugars available to AD. All experimental treatments in this study showed an analogous trend in terms of daily CH_4 production. Several peaks were observed with maximum CH_4 production appearing from day 13 to day 16 of digestion depending on the nature of pre-treated feedstock. The highest



(b)

TABLE 1
PROXIMATE CHARACTERISTICS OF CROP RESIDUES AFTER MICROBIAL CONSORTIUM PRE-TREATMENT

Parameter	Maize stover		Wheat straw		Soybean straw	
	Control	Treated	Control	Treated	Control	Treated
Ash (%)	5.34 ± 0.49	4.20 ± 0.25*	6.67 ± 0.58	5.99 ± 0.28**	4.55 ± 0.70	3.99 ± 0.21**
Volatile solids (%)	85.53 ± 3.31	26.33 ± 0.51*	89.60 ± 0.40	28.00 ± 0.87*	87.26 ± 2.09	31.73 ± 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 ($p \leq 0.05$).

CH_4 production for unpre-treated CRs appeared within day 16 to day 18 of AD. .Further increase in digestion time caused a gradual decline in CH_4 production conforming loss of organic material and carbon for microbial growth [16].

Profiles for daily CH_4 production and cumulative CH_4 yields of untreated and pre-treated wheat straw are shown in Fig. 2. In the first 5 d of AD, CH_4 production rate was low, probably due to hydrolysis and acidification of complex organic matter into simple molecules by bacteria. Thereafter, there was a sharp rise in CH_4 production owing to increased microbial activity in the digester. Maximum CH_4 production of 89 mL d^{-1} for non-treated wheat straw was achieved after 18 d of AD. However, pre-treatment of wheat straw increased daily CH_4 production to a maximum volume of 124 mL after 13 d of digestion. Maximum cumulative CH_4 yield of pre-treated wheat straw increased by 50.6% over the corresponding control. This implied that microbial consortium pre-treatment can enhance the disintegration of holocellulose and the overall structure of wheat straw. Besides increased biogas production, the reported enhanced CH_4 yield after pre-treatment was also due to increased CH_4 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. [18] significantly increased the total CH_4 yield of wheat straw by 80.3% through biological pre-treatment using a functional microbial consortium comprised of bacteria and fungi.

(a)

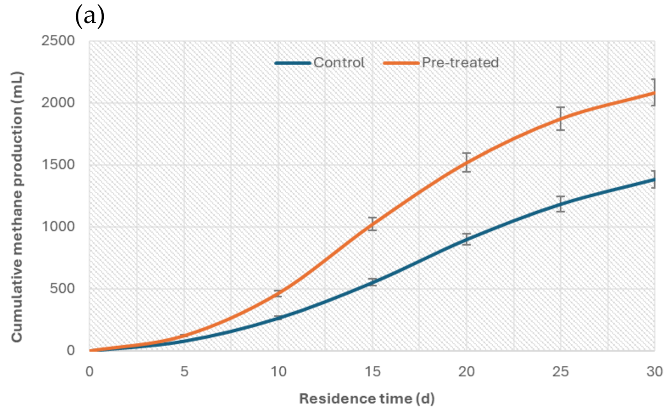


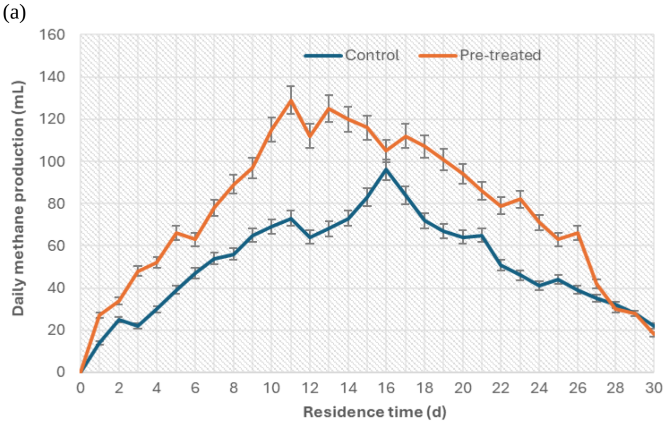
Fig. 2. Comparison of daily methane production (a) and cumulative methane yield (b) for pre-treated and untreated wheat straw

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. Using maize stover for biogas production is restricted by large amounts lignin of about 16% [3]. This can lead to a low recovery of monomeric units from its lignocellulose. Microbial pre-treatment is a potent strategy to improve the BMP of maize stover.

Fig. 3 is a comparison of daily CH_4 production and cumulative CH_4 yields of untreated and pre-treated maize stover. Variability in terms of daily CH_4 production and cumulative CH_4 yield between pre-treated maize stover and control conditions was reported. This can be attributed to sustained efforts of the HSCMC to degrade the structural components of maize stover. Total daily CH_4 yield of 96 mL for untreated maize stover was observed after 16 d of bioreactor operation. This value was lower than the maximum CH_4 yield of 129 mL d^{-1} obtained after 11 d of digesting maize stover pre-treated with the HSCMC. The second peak of pre-treated maize stover was reported after 13 d, yielding 125 mL of CH_4. Thereafter, a steady decrease in CH_4 production was observed with some noticeable peaks. Findings demonstrated that pre-treatment of maize stover with constructed microbial consortium was more favourable to enhance CH_4 production.

Total cumulative CH_4 yield of pre-treated maize stover was found to increase by 50.2% compared to untreated samples. This showed that the HSCMC could be a suitable alternative for saccharification of maize stover. A slight rise in total CH_4 yield of 6.9% obtained from pre-treated maize straw using aerobic microbial consortium was lower than the findings of this study [28]. However, Zhong et al. [5] reported maximum CH_4 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 [29]. Whereas in a study by Li et al. [30], maize stalk pre-treated with composite microbes produced 131.6% higher total CH_4 than the control counterpart. Pre-treatment with mixed microbes could be an effective method to improve the rate of CH_4 production from maize residues.

(a)



(b)

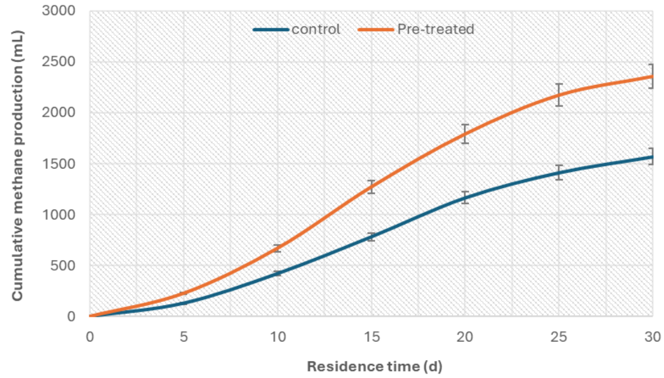


Fig. 3. Comparison of daily methane production rate (a) and cumulative methane yield (b) for pre-treated and untreated maize stover

High lignin content in a feedstock restrains its use for biogas production, hence an effective pre-treatment strategy is a pre-requisite [3]. This can be ascribed 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 CH_4. Daily CH_4 production and cumulative CH_4 yield of untreated and pre-treated soybean straw are shown in Fig. 4. Maximum daily CH_4 production of 84 mL and 121 mL were observed for unpre-treated and pre-treated soybean straw after 18 d and 15 d of digestion, respectively. This disparity suggested that pre-treatment with microbial consortium is an alternative way to reduce the fermentation period of soybean straw.

Amongst the three CRs, soybean straw reported the highest increase in cumulative CH_4 yield. Maximum CH_4 yield of soybean straw was enhanced by 56.6% as a result biological pre-treatment using the HSCMC. This could be probably due to high volatile matter content of 78.1% exhibited by soybean straw [3]. Many researchers have successfully developed a wide range of microbial consortia that have shown strong degradation capability on CRs [5],[14],[16]-[18]. But these microbial consortia have never been immensely explored for their ability to increase the hydrolysis of soybean straw for an efficient AD. 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 showed more profound effect on pre-treatment of soybean straw for CH_4 production than the MCI consortium. MC1 consortium increased CH_4 yield of pre-treated sterilized and unsterilized soybean straw by 36.9% and 34.3%, respectively [31].

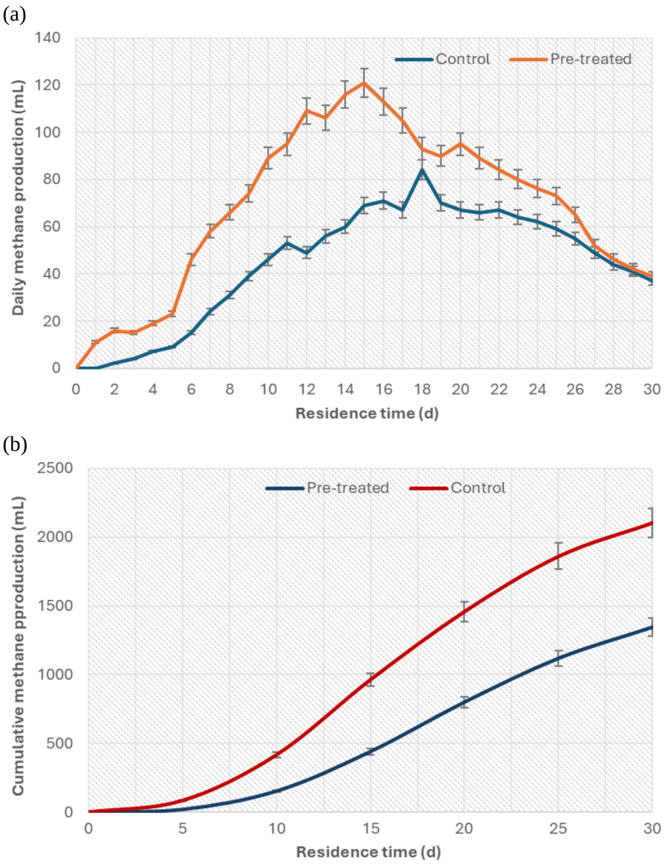


Fig. 4. Comparison of daily methane production rate (a) and cumulative methane yield (b) for pretreated and untreated soybean straw

IV. Conclusion

Development of microbial consortia is an innovative platform for improving the hydrolysis rate and CH_4 potential of CRs. Pre-treatment of CRs using the HSCMC consortium enriched from local hot springs achieved more daily CH_4 and cumulative CH_4 yield from wheat straw, maize stover and soybean straw than non-treated conditions. This method could be a suitable futuristic option that can be upgraded towards large-scale operation. However, further research is still needed to optimise the process and assess the environmental and techno-economic impacts on scalability of microbial consortium HSCMC pre-treatment of CRs for use in biogas production.

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