Enhanced anaerobic digestion of prairie biomass through hydroxycinnamic acid extraction

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Abstract:

This study explores the hydroxycinnamic acid extraction from prairie biomass as a potential value-added pretreatment for enhancing the performance of anaerobic digestion. Pretreatment increased the biomethane potential of prairie biomass by 33%; when the extraction residue was left on the biomass, the biomethane potential increased by 100%. When the treated biomass was co-digested with manure, a 134% and 25% increase in methane productivity and methane content was obtained, respectively, relative to raw biomass co-digested with manure. Hydroxycinnamic acid extraction also improved anaerobic digestion performance under biochar supplementation and liquid digestate recirculation conditions. Lastly, the extraction process was optimized for hydroxycinnamic acid yield. It was found that increases in treatment temperature and time could further increase yield by 5%. Collectively, the results show hydroxycinnamic acid extraction can be used as a highly effective pretreatment for improving the anaerobic digestion of prairie biomass.

Keywords

Anaerobic digestion, pretreatment, hydroxycinnamic acid, prairie biomass, biorefinery

1. Introduction

Despite years of soil conservation efforts, modern agricultural practices continue to degrade soil and reduce biodiversity in farmland (Grudens-Schuck et al., 2017). Prairie strips are an effective conservation practice that offers numerous soil, environmental, and ecological benefits. For example, Schulte et al. (2017) reported significant improvements in the abundance or biodiversity of different birds, insects, and pollinators, as well as a 20 times improvement in soil retention by replacing only 10% of cropland with prairie.

However, farmers must also receive economic benefit to incentivize them to implement prairie strips into their existing farmland. As a lignocellulosic feedstock, prairie biomass has a large potential to produce bio-based chemicals and fuels. Unlike traditional lignocellulosic biomass, such as corn stover or wheat straw, the cultivation of prairie biomass can result in numerous environmental and ecological benefits (Schulte et al., 2017), making its valorization essential. Given the surge of anaerobic digestion (AD) in the United States in recent years, prairie biomass may be a suitable candidate for biogas production.

Anaerobic digestion (AD) is an established technology to produce methane for heat, electricity, and renewable natural gas. Due to its low substrate specificity, AD is viable for a wide range of organic substrates. Although full scale AD has been primarily implemented with food wastes, manure, and biosolids in wastewater treatment facilities, co-digestion of lignocellulosic biomass with other feedstocks has several advantages over mono-substrate digestion, such as easier control of carbon to nitrogen (C:N) ratio, a balanced nutrient composition of the feedstock, and enhanced gas production (Lima et al., 2016; Rabii et al., 2021). In fact, co-digestion is increasingly popular for farm-based digesters, as the high carbon content in the lignocellulosic biomass can balance the C:N ratio in nitrogen-rich manures and thus, generate significantly more biogas (Ward et al., 2008). However, due to the recalcitrant nature of lignocellulose, a pretreatment step may be needed for an efficient biomass degradation (Sawatdeenarunat et al., 2015).

Various methods have been explored for pretreating lignocellulosic biomass in an AD process. For example, Chandra et al. (2012) evaluated the effect of an alkaline and a hydrothermal pretreatment on digestibility of wheat straw for AD. The authors reported a 112% increase in specific methane yield of wheat straw treated with 4% sodium hydroxide, and a 20% increase when treated with sodium hydroxide and hydrothermal processing. Jackowiak et al. (2011) reported a 28% increase in methane yield from wheat straw after performing a microwave pretreatment. Mustafa et al. (2016) reported a fungal pretreatment on rice straw using *Pleurotus ostreatus*, with a 120% increase in methane yield relative to untreated rice straw.

Although these methods showed enhanced AD performance, biomass pretreatment also incurs large costs and energy investments (Hartmann et al., 2000, Tao et al., 2011, Vasco-Correa and Shah, 2019). Developing an efficient pretreatment method while producing value-added products can improve the economics of AD processes. Recently, researchers at Iowa State University reported the extraction of hydroxycinnamic acids (HCA) from corn stover through a mild alkaline treatment process (Johnston et al., 2020). This delignification process improved the accessibility of cellulose to enzymatic hydrolysis. The HCA extraction offers a potentially economically viable method for utilizing lignocellulosic biomass by enhancing its conversion efficiency and producing marketable coproducts to offset costs.

The extracted HCAs are widely known for their benefits in the pharmaceutical and cosmetic industry (Taofig et al., 2017). Two HCAs are extracted from the HCA extraction process: ferulic acid and p-coumaric acid. Ferulic acid is effective in tyrosinase and collagenase inhibition and has been used as an ingredient for anti-aging skin serums (Taofig et al., 2017). P-coumaric acid can be used as an antioxidant and antimicrobial compound for skin serums (Taofig et al., 2017); this compound has garnered attention as a platform chemical for high-value products such as polyhydroxyalkanoates (Linger et al., 2014). In addition to the improvement in ethanol yield, HCA extraction from corn stover showed a drastic improvement in the economics of a cellulosic ethanol plant when compared to traditional acid pretreatment (Johnston et al., 2020). The aim of this study is to investigate the potential of using HCA extraction on prairie biomass as a pretreatment for AD.

2. Materials and Methods

2.1 Raw materials and inoculum for anaerobic digestion

The prairie biomass used in this study was a mixture of ~59 species that were harvested, dried, and milled to ¼ inch particle size. Detailed information on the cultivation and speciation of the prairie biomass was reported previously (Kordbacheh et al. 2018). The cow manure was obtained from a local beef farm in Kingsley, Iowa. The manure was chilled upon collection, delivered to the laboratory, aliquoted into air-tight 250-mL HDPE bottles and stored at -20°C. The biochar was produced from an autothermal fast pyrolysis of corn stover (Polin et al. 2019).

The seed for AD experiments was obtained from the Water Pollution Control Plant in Ames, Iowa. The facility uses anaerobic digesters treating biosolids in a two-stage system. The slurry from the second-stage digester was stored in a sealed, 5-gallon bucket at 4°C. Table 1 summarizes the chemical composition of the materials used in this study.

2.2 Extraction of hydroxycinnamic acids from prairie biomass

Extraction of HCA from prairie biomass was based on the protocol reported by Johnston et al. (2020), with the modification of using potassium hydroxide, instead of sodium hydroxide, as the treatment reagent. The choice of potassium hydroxide was to improve the fertilizer properties of the digestate, whereas less expensive sodium hydroxide would potentially introduce the detrimental effects of sodium on plant health (Pawlett and Tibbett, 2015, Castro et al., 2017). Figure 1 shows the overall schematics of the HCA extraction procedure and the resultant biomass used for AD experiments. In short, a solution containing 50% ethanol, 30% deionized water and 20% potassium hydroxide solution (4N) was refluxed with prairie biomass at 80°C for two hours. The slurry was filtered through a 0.45 μm glass microfiber filter. The filtrate was stored at -20°C for HCA analysis. The solids were either directly collected (designated as "treated biomass"), or thoroughly washed with DI water to remove any attached organic residues and then dried at 70°C for 72 hours (designated as "treated and cleaned biomass"). Both the treated biomass and treated and cleaned biomass were stored in an air-tight jar at 4°C for no more than 48 hours prior to use in AD.

2.3 Batch anaerobic digestion experiment

Batch AD was conducted in 160 mL serum bottles with 100 mL working volume. Each bottle was inoculated with 80 mL of seed culture, and sparged with nitrogen gas for 5 minutes prior to being sealed and crimped with a butyl septa and aluminum cap to create an anaerobic environment. Serum bottles were held in an incubator at 37°C and 160 rpm for 12 days to degas. Then, prairie biomass and DI water were added to the bottles to achieve an organic load of 5 gVS/L. Three types of prairie biomass were used: 1) raw biomass, 2) treated biomass, and 3) treated and cleaned biomass (Figure 1). Positive controls containing all content but without biomass were also tested. Methane production from each bottle was corrected with the methane produced from the positive control.

2.4 Continuous anaerobic digestion setup and experimental conditions

Continuous AD was conducted in 1-L digesters with 500 mL working volume to implement co-digestion of prairie biomass and manure. To initiate the culture, 500 mL of inoculum was added to each digester; the digesters were flushed with nitrogen gas for 5 minutes to ensure an anaerobic environment. Feeding was conducted by removing a specific volume of digestate and replenishing with an equal volume of fresh feed. For the first 14 days, each digester was fed raw prairie biomass and manure at a 2:1 ratio (g VS/g VS). On day 14, the feed was switched to a feedstock containing a specified type of prairie biomass mixed with manure based on the experimental design. DI water was added to the feed to adjust solids content to 10%. The feeding rate was based on a 30-day hydraulic retention time. After the feeding operation, nitrogen gas was used to flush the digester to expel any encroaching oxygen for two minutes. It should be noted that this flushing process were performed daily to ensure consistent anaerobic conditions as lab-scale digesters are more susceptible to oxygen encroachment. For large scale anaerobic digesters, however, this daily flushing process may not be necessary. The digesters were incubated in a shaker at 35°C with continuous agitation (120 rpm).

Continuous AD was conducted to assess the impact of HCA extraction at two prairie biomass to manure ratios (1:2 and 2:1 gVS: gVS). Then, co-digestion was performed with biochar supplementation and liquid digestate recirculation (LR), respectively. Biochar-supplemented AD was performed by adding biochar into the digesters at 5 g/L on day 14, followed by daily feeding of biochar to maintain this concentration for the remaining of experimental period. To implement LR operation, on a daily basis, the digestate was centrifuged for 5 minutes at 5,000 g to collect the supernatant, which was used to partially replace the makeup DI water used in the feed. Two levels of water volume replacement by the liquid digestate were used, 25% and 50%.

Data for the continuous digesters were collected on a daily basis. Digesters reached steady-state after 90 days with stable biogas production. The digestates were also sampled on a daily basis for pH measurement, and then stored at -20°C for further analysis.

2.5 Optimization of temperature and time for Hydroxycinnamic acid extraction

The extraction time and temperature on the HCA yield were optimized using a central composite design (Table 2). The HCA yield (HCA) as a response was correlated with temperature (T) and time (t) through a second order polynomial equation:

$$\mathrm{HCA} = \beta_0 + \beta_i \times T + \beta_j \times t + \beta_{ii} \times T^2 + \beta_{jj} \times t^2 + \beta_j \times T \times t \quad (1)$$

where β were coefficients estimated. F-test was used to evaluate significance. JMP16 and Rstudio software were used for design matrix creation and statistical analysis.

2.6 Analyses

Biogas volume was measured using a water displacement setup for both batch and continuous AD experiments, i.e., the biogas was continuously vented into a water displacement column containing H_2SO_4 solution (0.5N). Daily biogas production was measured by the volume of the displaced liquid. Biogas composition was measured using gas chromatography with thermal conductivity detector operated at 100°C and HP-PLOT/Q column (30 m × 0.32 mm ×

0.02 mm) 50°C. Helium was used as the carrier gas with a flow rate of 2 mL/min (Zhou et al., 2019).

Lignin content of prairie biomass was analyzed using the acetyl bromide soluble lignin (ABSL) assay (Asgher et al., 2017, Barnes and Anderson, 2017). Cellulose and hemicellulose content was determined by Celignis Biomass Analysis Laboratory (Limerick, Ireland) using the NREL Laboratory Analytical Procedure (LAP) TP-510-42618 (Sluiter et al., 2012). HCA concentration was measured using high performance liquid chromatography with a ThermoFisher Scientific/Dionex Ultimate 3000 system (Sunnyvale, CA, USA) as described previously (Johnston et al., 2020). Digestate phenolic content was measured using the Folin-Ciocalteu method (Blainski et al., 2013). Ammonia concentration was measured using Hach TNT 832 Ammonia test kit.

3. Results and Discussion

3.1 Methane potential of hydroxycinnamic acid-extracted prairie biomass

The effects of HCA extraction on the methane production of prairie biomass were evaluated through a biochemical methane potential test. Figure 2 shows the cumulative methane production of prairie biomass under different treatment methods. Raw biomass produced 235 mL/gVS of methane, while the treated and cleaned biomass improved methane production to 312 mL/gVS. This improvement was attributed to the delignification of the prairie biomass during HCA extraction. The treated biomass (without cleaning) produced 470 mL/gVS of methane, a two-fold increase over raw prairie biomass, and a 51% increase over the treated and cleaned biomass. The high methane yield from this type of biomass may result from the organic residuals attached to the treated biomass, contributing to extra methane production.

HCA extraction has been reported as an effective delignification process for cellulosic ethanol production (Johnston et al., 2020). However, this method has not been investigated for its potential to improve AD performance. The results reported in Figure 2 clearly demonstrate the beneficial effects of HCA extraction on the AD of prairie biomass. In general, chemical-based pretreatments of lignocellulosic biomass release toxic byproducts which may inhibit microorganisms (Kumar et al., 2017). This is generally not desirable for industrial purposes due to extra detoxification processes and higher operation costs. In this work, however, the KOH-based HCA extraction of prairie biomass actually benefitted AD, with the treated and uncleaned biomass (containing organic residue on the biomass) producing a higher methane yield than the treated and cleaned biomass (with organic residues removed) (Figure 2). This unique feature represents an advantage of prairie pretreatment. The reasons for this observation may be due to (i) the leftover ethanol presented in the HCA-extracted biomass which is eventually converted into methane through the DIET pathway (Feng et al., 2021), and (ii) acetic acid extracted from solubilization of acetyl groups of hemicellulose during alkaline extraction (Chen et al., 2013). Considering the high performance achieved from the treated biomass (without cleaning), this type of biomass was used as the substrate in co-digestion with manure in the following studies.

3.2 Co-digestion of hydroxycinnamic acid-extracted prairie biomass and manure

3.2.1 Effects of manure loading

The effects of the HCA extraction of prairie biomass were evaluated in co-digestion with cow manure. Figure 3 shows the daily methane production of the co-digestion systems with two prairie biomass to manure ratios. Under both ratios, the treated prairie biomass enhanced methane production over raw prairie biomass. Compared to 1:2 biomass to manure ratio (Figure

3a), a larger difference in methane yield between the treated and raw biomass was observed at 2:1 biomass to manure ratio (Figure 3b). Table 3 summarizes the steady-state biogas production results. At a 1:2 biomass to manure ratio, treated biomass resulted in an average methane productivity of 190.9 mL/gVS/day, a 47% increase relative to raw prairie biomass. At a 2:1 ratio, treated biomass increased the methane productivity from 148.2 mL/gVS/day to 346.1 mL/gVS/day, a 134% increase compared to raw biomass. Table 3 shows that HCA extraction also increased the methane content in the biogas, particularly at the higher prairie to manure ratio. Overall, Figure 3 and Table 3 show that HCA-extracted biomass resulted in higher methane productivity and methane content in co-digestion system than the raw biomass. Furthermore, the treated biomass can be fed at high prairie to manure ratios without inhibition of biogas production. In fact, a superior biogas production performance achieved with HCA-extracted biomass at a 2:1 biomass to manure ratio. Therefore, this ratio was further used in the following AD experiments.

3.2.2 Co-digestion of prairie biomass and manure with biochar supplementation

In recent years, biochar has drawn increasing attention as an additive in AD systems with enhanced digestion performance such enhanced biogas production, increasing alkalinity, and mitigating toxicity of inhibitory compounds (Torri and Fabbri et al., 2014). In this work, the co-digestion systems were further evaluated under biochar-supplemented conditions in order to evaluate the beneficial effect of HCA extraction under a variety of scenarios. As shown in Table 4, in digesters supplemented with biochar at 5 g/L, the enhancement in AD performance from the treated biomass relative to the raw biomass was even greater. For the treated biomass, the average methane productivity increased by 150% compared to raw biomass, while the methane content increased by 24%. When compared to Table 3 where no biochar was used, Table 4 shows that digestors fed with biochar performed significantly better. For example, treated biomass in biochar supplemented digesters resulted in a methane productivity of 400.4 mL/gVS/day (Table 4), a 16% increase compared to the same feedstock without biochar supplementation (Table 3).

3.2.3 Co-digestion of prairie biomass and manure with liquid recirculation

The co-digestion of prairie biomass and manure was also investigated with liquid recirculation (LR) operation. As seen in Table 5, HCA extraction significantly increased biogas productivity and methane content at both LR ratios. Compared to raw biomass, treated biomass increased methane productivity by 69.6% and 62.4% for LR ratios of 25% and 50%, respectively. Between the two LR ratios, however, the biogas and methane productivities for the same type of biomass were similar. Compared to co-digestion without LR operation (Table 3, 2:1 ratio of biomass to manure), the biogas and methane productivities with LR operation were biomass dependent; LR significantly increased biogas productivity when raw biomass was used, while LR slightly decreased biogas productivity for the treated biomass (Tables 3 & 5).

3.3 Digestate characteristics as a result of hydroxycinnamic acid extraction

In addition to the biogas production, HCA extraction also influenced the digestate characteristics in AD systems. As shown in Figure 4a, digesters fed with treated biomass held a higher pH relative to raw biomass. When treated biomass was used as AD feedstock, the digestate pH from different operational conditions were very similar. For the raw biomass, biochar and LR operations slightly reduced digestate pH. The higher digestate pH from treated biomass was probably due to the residual KOH in the treated biomass, which in turn increased the methane content in the biogas, as CO_2 solubilizes readily under higher pH conditions. Figure 4b shows the ammonia concentration in the digestates of different AD systems. Treated biomass significantly decreased the ammonia concentration compared to the raw biomass with the same conditions. For both types of biomass (raw vs treated), biochar slightly reduced ammonia concentration, while LR significantly increased the ammonia concentration.

Total phenolic content (TPC) of the digestates were also determined as HCA extraction may generate lignin-derived byproducts, particularly phenols. As shown in Figure 5, The TPC varied significantly based on the biomass type and AD condition. Compared to the raw prairie biomass, the treated biomass in the control and LR operation conditions led to significant increases in TPC, likely due to the presence of phenolic byproducts in the residue of the treated biomass, such as the HCAs themselves. However, biochar-supplemented AD systems maintained a low TPC content regardless of the biomass type, indicating biochar's ability to adsorb phenolics (Torri and Fabbri et al., 2014).

3.4 Optimizing hydroxycinnamic acid extraction of prairie biomass

The works reported above focused on the beneficial effects of HCA extraction on prairie grass anaerobic digestion process, based on HCA extraction process adapted from Johnston et al., (2020). However, developing an optimal HCA extraction condition also has its own merit considering the value of HCA and its economic and environmental implications to the AD processes. As such, two important operational parameters during HCA extraction process, temperature and time, were optimized to maximize HCA yield.

Based on the experimental design matrix in Table 2, the HCA yield (HCA) was correlated as a function of temperature (T) and reaction time (t) as follows:

$$HCA = -145.1 + 3.3\times T + 0.58\times t - 0.02\times T^2 - 0.0024\times t^2 + 0.0013\times T\times t \quad (2)$$

Table 6 displays the parameter estimates and their corresponding F-test and p-value. Both F-test ($p < 0.0001$) and R^2 (0.97) suggest the model is significant and reliable. Both time and temperature showed linear and quadratic effects on the HCA yield, but the interactive effect of these two parameters was not significant. Figure 6a shows the response surface plot of the HCA yield as a function of temperature and time. It shows the maximum predicted HCA yield was achieved at 87°C for 144 minutes with a yield of 40.5 g HCA/kg biomass being achieved.

To validate the above optimization process, HCA extraction was conducted experimentally under the optimized condition. Figure 6b shows the cumulative HCA yields and the two individual HCA species obtained at the optimal condition. A cumulative HCA yield of 38.2 g/kg was obtained, of which 22.6 g/kg was p-coumaric acid and 15.5 g/kg was ferulic acid. The HCA yield obtained experimentally was 5.6% less than the predicted value (40.5 g HCA/kg). In the earlier AD experiments, an HCA yield of 36.4 g/kg was obtained, which consisted of 21.3 g/kg p-coumaric acid and 15.1 g/kg ferulic acid. The optimized condition resulted in a 5% increase in cumulative HCA yield ($p < 0.05$). The optimized condition resulted in an increased delignification of prairie biomass, suggesting that AD performance can be even further enhanced under the optimized condition.

In addition to extracting HCA, the alkali treatment also removes lignin from prairie biomass. Figure 6c shows the acetyl bromide soluble lignin (ABSL) content of the raw and treated prairie biomass. Under the optimized condition, HCA extraction removed 28.3% of the ABSL in prairie biomass. Meanwhile, under the conditions used for AD experiments, HCA extraction removed 23.4% of ABSL from the prairie biomass. The difference in total ABSL content between the two treated groups were not statistically significant ($p > 0.05$).

4. Conclusion

This study demonstrated the beneficial effects of HCA extraction of prairie biomass for AD. HCA-extraction improved the biomethane potential of prairie biomass by two-fold. When co-digested with manure, HCA-extracted biomass resulted in a 134% improvement in methane productivity over raw biomass. The HCA-extracted biomass also outperformed raw biomass in biochar-supplemented and LR-operated co-digestion systems, with a 150% and 62-70% increase in methane production for these two conditions, respectively. Optimization of the HCA extraction process increased HCA yields and delignification at higher treatment temperature and time, which indicate potential further enhancements in AD performance.

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Fig. 1. Diagram of the HCA extraction of prairie biomass and the subsequent treated biomass used as a feedstock for AD experiments.

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Table 1. Chemical composition of the inoculum, manure and prairie biomass used in anaerobic digestion experiments.

Parameters	Unit	Inoculum	Manure	Prairie Biomass
Total Solids	% wt	2.15	17.2	93.9
Volatile Solids	% wt	1.57	14.5	86
pH		7.6	8.2	/
C	% wt		41.1	77.99
H	% wt		4.63	8.64
N	% wt		2.11	1.01
S	% wt		0.015	0.1
O (by difference)	% wt		52.1	12.26
Cellulose	% wt			31.27%
Hemicellulose	% wt			24.86%
Lignin	% wt			16.73%

Table 2. Central composite design matrix to optimize HCA yield as a function of temperature and time during HCA extraction.

Run#	Temperature (T)		Time (t)		HCA yield (g/kg)
	Coded	Actual (oC)	Coded	Actual (min)	
1	-1.414	41.7	0	105	0
2	-1	50	-1	60	0.4
3	-1	50	1	150	8.3
4	0	70	-1.414	41.4	9.5
5	0	70	0	105	31.4
6	0	70	0	105	30.7
7	0	70	0	105	31.4
8	0	70	0	105	32
9	0	70	0	105	33.6
10	0	70	1.414	168.6	37.3
11	1	90	-1	60	24.5
12	1	90	1	150	37.1
13	1.414	98.3	0	105	34.3

Table 3. Biogas production from the co-digestion of prairie biomass and cow manure [a].

Ratio of prairie biomass to manure	Prairie Biomass	Biogas Productivity (mL/gVS/day)	Methane content (% CH4)	Methane Productivity (mL/gVS/day)
1:2	Raw	212.1 ± 6.7	61.3 ± 1.9	130.0 ± 4.1
	Treated	298.3 ± 10.1	64 ± 2.1	190.9 ± 6.4
2:1	Raw	283.9 ± 7.7	52.2 ± 1.1	148.2 ± 2.5
	Treated	532.5 ± 16.4	65 ± 0.9	346.1 ± 11.1

a. Data are presented as means ± standard deviations of 14 consecutive samples at steady state in continuous AD experiments.

Table 4. Biogas production in the co-digestion of prairie biomass and manure under biochar-supplemented conditions [a,b].

Prairie Biomass	Biogas Productivity (mL/gVS/day)	Methane content (% CH4)	Methane Productivity (mL/gVS/day)
Raw	308.0 ± 10.1	52.0 ± 0.9	160.0 ± 7.1
Treated	620.8 ± 28.8	64.5 ± 0.7	400.4 ± 15.0

a. Data are presented as means ± standard deviations of 14 consecutive samples at steady state in continuous AD experiments.
b. Biochar was fed and maintained at a concentration of 5 g/L.

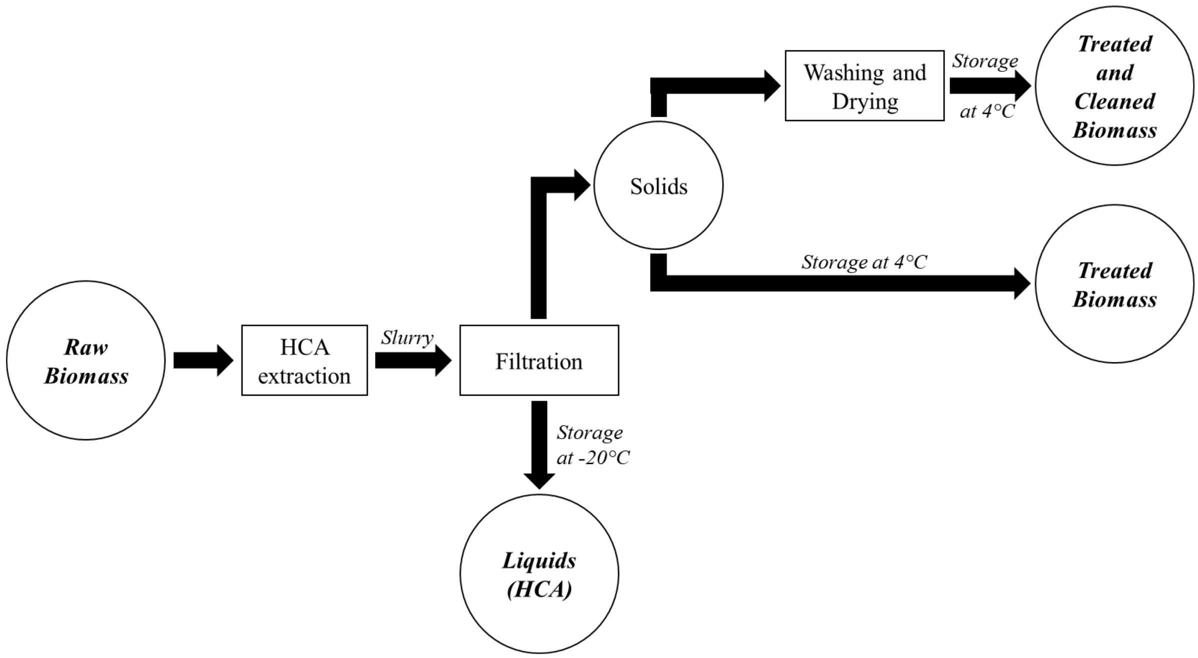
Table 5. Biogas production of co-digestion of prairie biomass and manure under digestate recirculation conditions [a].

LR ratio[b]	Prairie Biomass	Biogas Productivity (mL/gVS/day)	Methane content (%CH4)	Methane Productivity (mL/gVS/day)
25%	Raw	347.0 ± 6.0	51.8 ± 0.5	179.9 ± 2.6
	Treated	490.8 ± 31.6	62.2 ± 0.5	305.1 ± 19.3
50%	Raw	335.2 ± 20.1	53.6 ± 0.4	179.6 ± 12.2
	Treated	482.2 ± 25.7	60.5 ± 0.7	291.6 ± 17.3

a. Data are presented as means ± standard deviations of 14 consecutive samples at steady state in continuous AD experiments.
b. LR ratio represents the portion of DI water replaced with liquid digestate during digester feeding.

Table 6. Parameter estimates and F-test statistics for the central composite design optimizing the HCA yield as a function of time and temperature.

Coefficient	**Variable**	**Estimate**	**F-value**	**P-value**
β_0	Intercept	-145.1	536.39	<0.0001
β_j	Temperature (T)	3.3	136.23	<0.0001
β_i	Time (t)	0.58	47.4	0.0002
β_{jj}	Temperature^2 (T^2)	-0.02	47.3	0.0002
β_{ii}	Time^2 (t^2)	-0.0024	17.59	0.0041
β_{ij}	Temperature·Time (T·t)	0.0013	0.5853	0.4693



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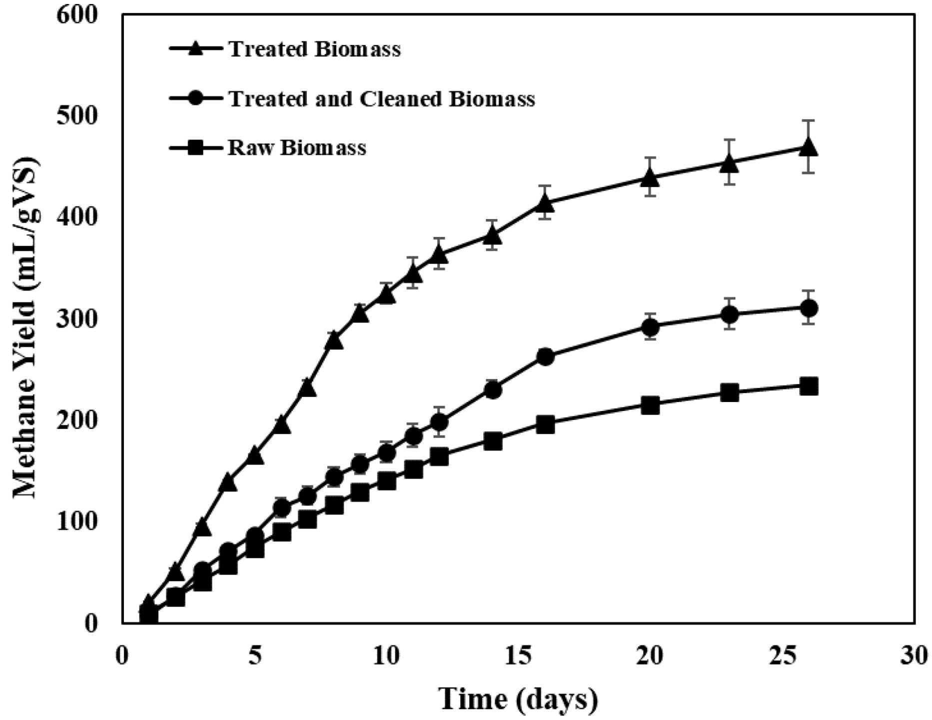

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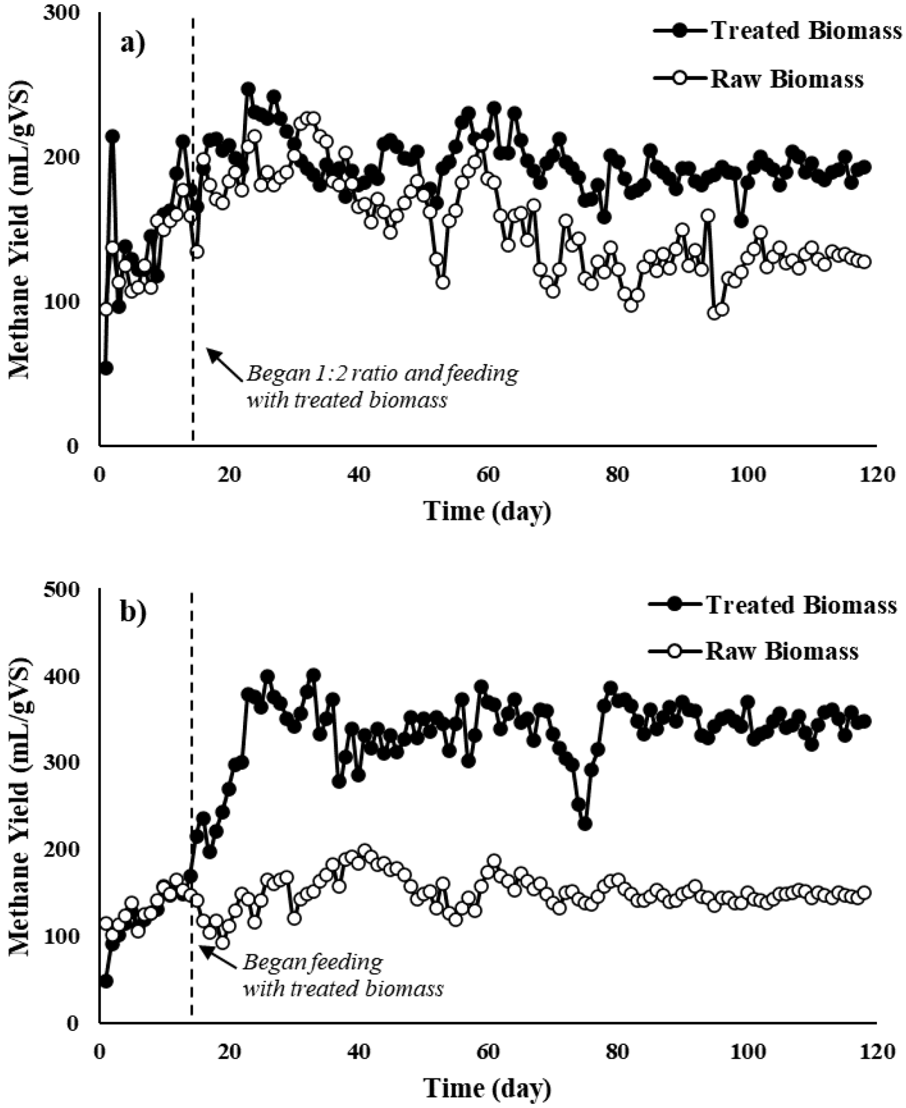

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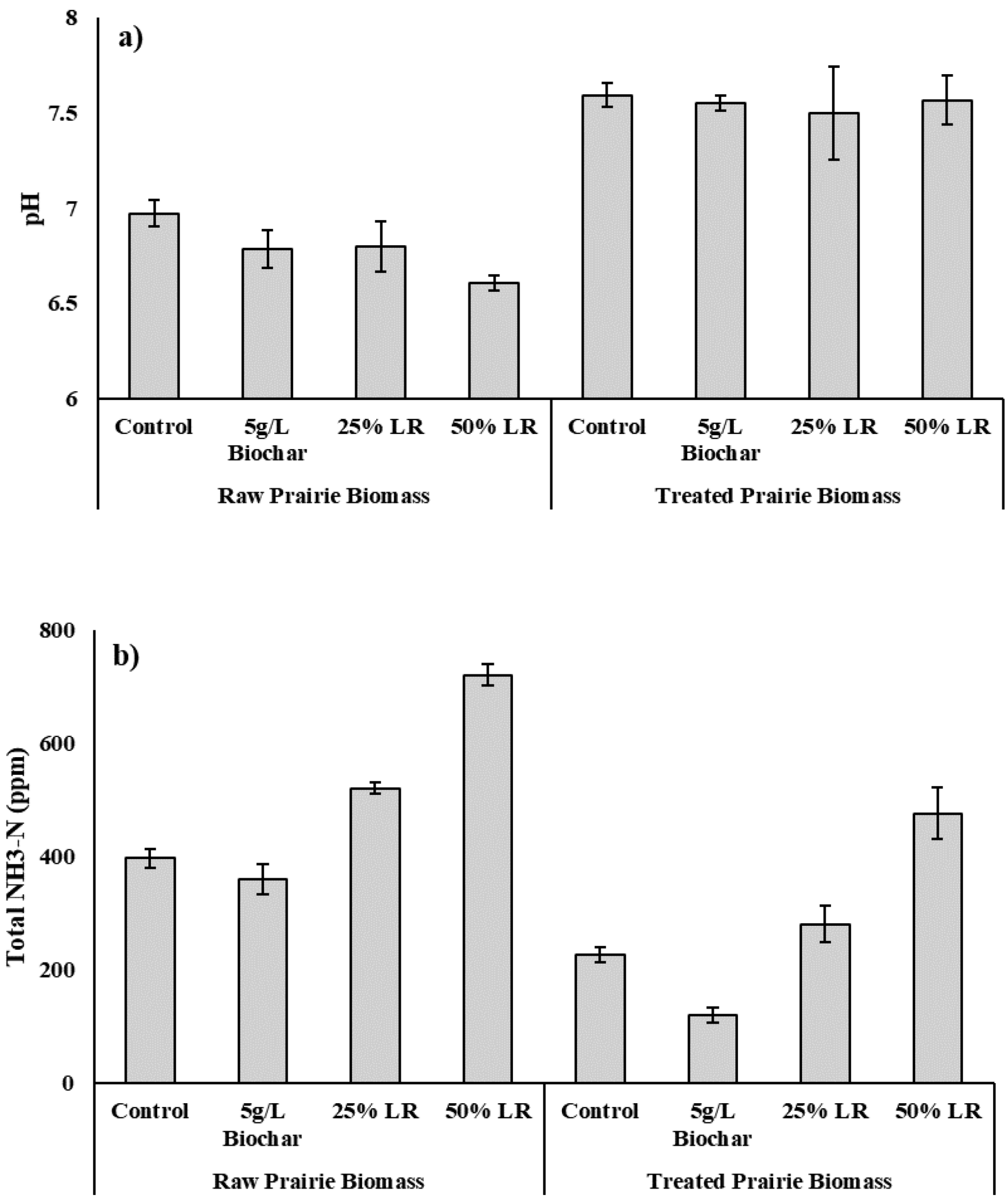

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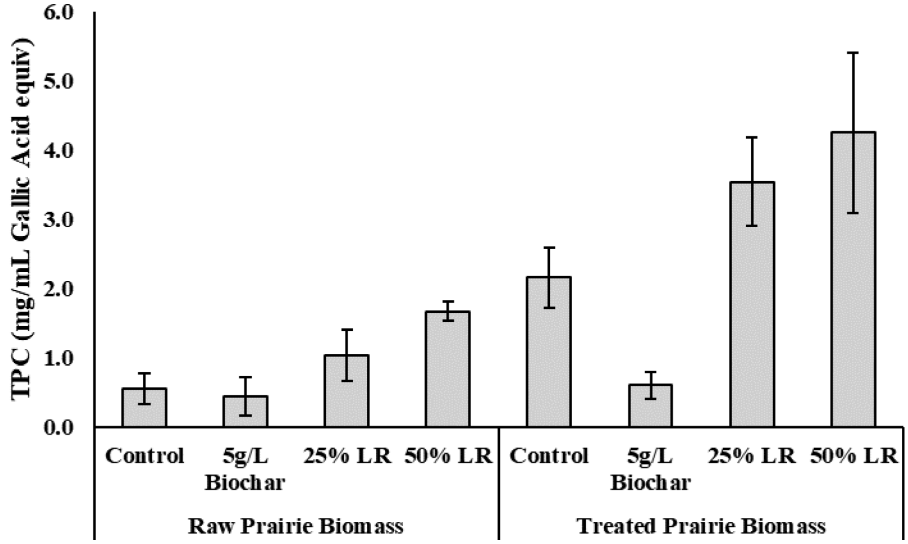

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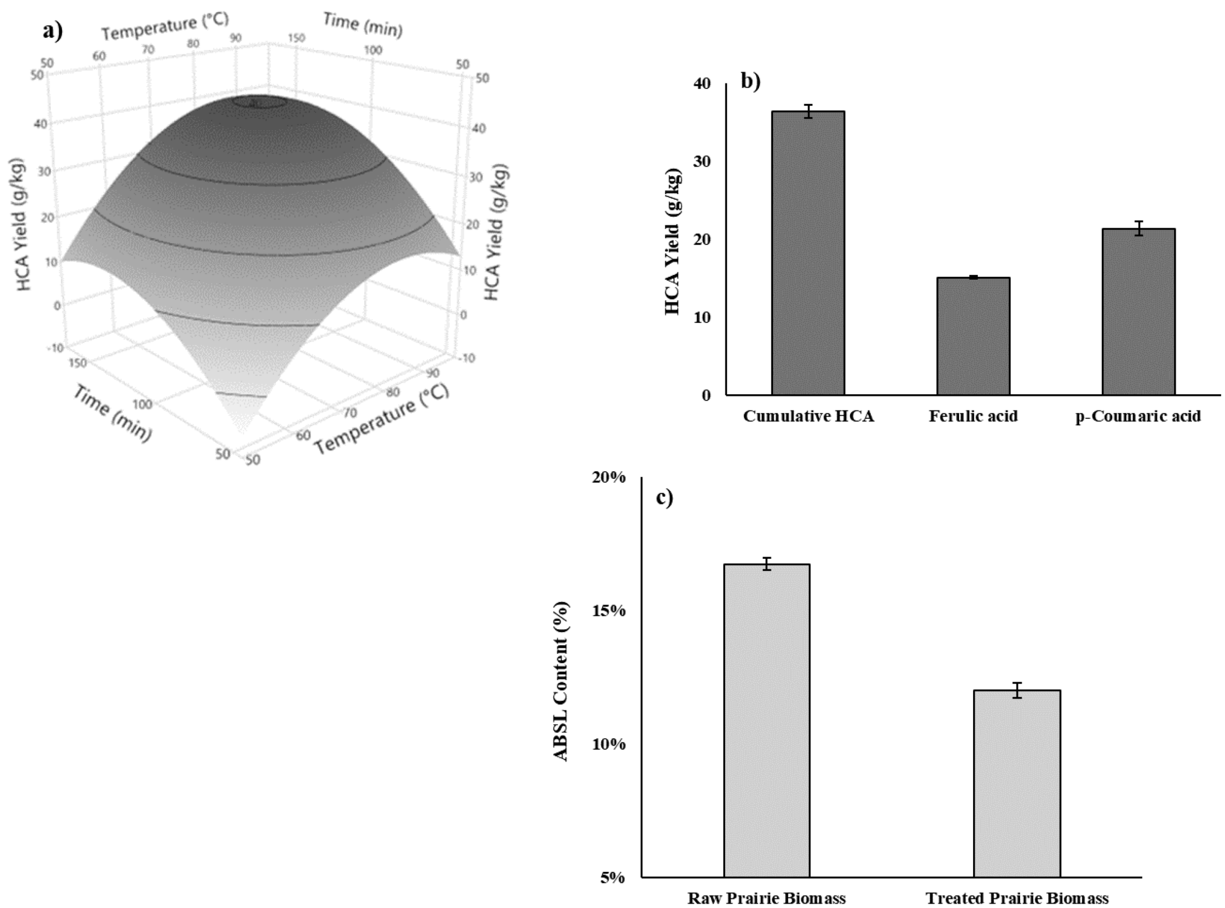

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