ACS OMEGA

http://pubs.acs.org/journal/acsodf Article

Effects of Mechanical Refining on Anaerobic Digestion of Dairy Manure

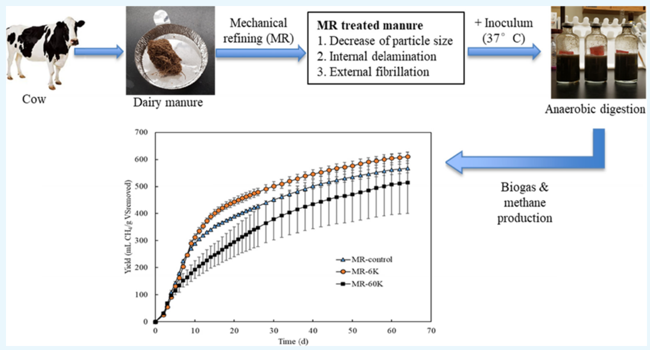
Shengquan Zeng, Hyun Min Jang, Seonghyun Park, Sunkyu Park, and Eunsung Kan*

Cite This: ACS Omega 2021, 6, 16934–16942

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Mechanical refining (MR) is a cost-effective pretreatment in biochemical conversion processes that is employed to overcome biomass recalcitrance. This work studied the effects of MR on biogas and methane produced by the anaerobic digestion (AD) of dairy manure. The cumulative gas volume and yield from the AD of manure refined at 6k revolutions increased by 33.7 and 7.7% for methane and by 32.0 and 6.4% for biogas, respectively, compared to the unrefined manure. This enhancement was reached by increasing manure solubilization, reducing particle size, and achieving external fibrillation and internal delamination of fibers in manure. However, the highly refined manure (subjected to 60k revolutions) exhibited methane and biogas yields that were reduced by 9.5 and 1.5%, respectively. This decrease was observed because the pore structure was ruptured, and finely ground manure particles were aggregated together at high revolutions (60k), thereby inhibiting the release of organic matter from the manure. Therefore, this study indicates that the MR for pretreatment of dairy manure could have great potential for significantly enhancing AD of dairy manure. Further studies will include optimization of conditions of mechanical refining (i.e., mechanical intensity, process time), a continuous AD of dairy manure pretreated by the MR, and scale-up with cost evaluation.

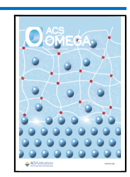


1. INTRODUCTION

A large amount of dairy manure (e.g., 0.92 billion tons per year in the United States) is generated annually worldwide.[1] However, the widespread application of dairy manure to agriculture can cause the contamination of soil, water and air with excessive nutrients, antibiotics, and microbial pathogens.[2−4] Anaerobic digestion (AD) is one of the most reliable techniques to dispose of dairy manure by converting it to biogas and digestate. AD can also lower emissions of greenhouse gas and mitigate the odor nuisance of manure.[2,5] However, current AD of dairy manure has several shortcomings, such as low digestion and biogas production, fluctuating performance, and generation of significant amounts of undigested sludge.[5,6] In particular, approximately 40−50% of dairy manure is composed of biofibers, mainly consisting of lignin tightly associated with cellulose and hemicellulose, which is difficult to effectively degrade and hampers AD performance.[7,8]

For overcoming current limitations of AD process, various pretreatment methods (physical, chemical, and biological methods) have been carried out to enhance digestibility of AD substrates. Physical pretreatment methods, including mechanical, thermal, ultrasonic, and microwave pretreatments, can increase a surface area of AD substrates and enhance their accessibility to microorganisms and enzymes, resulting in increasing biogas and methane yields.[9] The physical pretreatment benefits from short process time and no generation of inhibitor compounds[9,10] while requiring high energy consumption.[9] For chemical pretreatments, various chemicals (acid, alkali, and oxidant) are applied to break down and transform biorecalcitrant substrates (i.e., lignin in biomass) to more easily degradable compounds.[11] The chemical pretreatment shows low energy demand.[12] Nevertheless, the major drawbacks of chemical pretreatment are possible deterioration of substrate structure and generation of undesirable byproducts as additional pollutants despite the low energy demand for chemical pretreatment.[12] Biological pretreatments via microorganisms and enzymes can break down and transform the complex and biorecalcitrant structures of substrates for enhancing AD.[11] Although the biological pretreatment requires a low energy consumption with negligible generation of inhibitory compounds,[12] continuous use of expensive enzyme, and slow and fluctuating microbial reactions limit practical applications of biological pretreatment.[12] To enhance the AD

Received: April 1, 2021
Accepted: June 11, 2021
Published: June 22, 2021



performance while effectively degrading biofibers in dairy manure, various pretreatments have been investigated. For instance, alkali (10% NaOH, 100 °C) and acid (2% HCl, 37 °C) pretreatments improved the methane production potential by 23.6 and 20.6% through the breakdown of fibers in manure.[13] Moreover, Yang et al. also reported that the pretreatment with 7% NaOH and 2% polyethylene glycol at 23 °C resulted in the improvement of methane yield by 33% during the AD of dairy manure, which was mainly due to the reduction of lignin in fiber after pretreatment.[14] However, alkali or acid pretreatments often causes additional pollution from intermediates and byproducts generated by these pretreatments, and corrosion of AD reactors.[12] Ultrasonic treatment with the amplitude of 160 μm_{pp} and duration of 30 s disintegrated the manure and enhanced the methane yield by 62% during the AD process.[15] However, high energy input and difficult scale-up limited practical application of the ultrasonic pretreatment.[12,16] In addition, the combination of ozone and ammonia pretreatment for the AD of dairy manure improved the biogas production by 55−105% compared to the ammonia pretreatment alone, despite high capital costs and high numbers of ozone-associated health problems.[17] Furthermore, Bruni et al. used laccase, cellulase, and hemicellulase to pretreat the biofibers from the digested cow manure and found that enzymatic pretreatments showed no effect on the methane yield in the AD of biofibers.[18]

Compared to those chemical and biological pretreatment techniques, mechanical pretreatments have lower sensitivity of substrate specificities and can be easily scaled up for industrial applications.[19,20] In addition, mechanical pretreatment does not generate any toxic or inhibitory byproducts such as furfural and hydroxymethylfurfural, which are often found during chemical pretreatment.[19−21] In general, the main objectives of mechanical pretreatment are to lower the particle size and crystallinity and improve the surface area of biorecalcitrant substrates (i.e., lignocellulose), which makes them easily degraded by microorganisms during AD.[12] To date, considerable research has been conducted to apply the mechanical pretreatments of biomass for the AD process.[20,22−25] For instance, Rodriguez et al. reported that the Hollander beater pretreatment of microalgae (*P. canaliculata*) enhanced the methane yield by 45% (283 mL/g volatile solids (VS)).[23] Kang et al. used a grinder to evaluate the effect of particle size of substrate on the AD of grass (*Hybrid Pennisetum*), and achieved a maximum methane yield of 292 mL/g VS in the particle size of 0.25−0.38 mm.[22] However, the application of mechanical pretreatment for the AD of dairy manure is notably limited. Angelidaki and Ahring reported that mechanical maceration using a mechanical blender reduced the size of biofibers in cattle manure and improved the methane yield by 20% for sizes less than 0.35 mm.[8] In addition, Bruni et al. and Tsapekos et al. used kitchen blenders and commercially available heavy plates to pretreat the undigested manure biofibers and found that the mechanical pretreatment had a slightly positive impact on the biodegradability of fibers.[7,18] Despite positive impacts of mechanical pretreatment on AD of dairy manure, high energy input and maintenance costs are still the main bottleneck facing the application of mechanical pretreatments.[12,16]

Among the mechanical pretreatment methods, mechanical refining (MR) is the process developed and widely used in pulp and paper industry, where wood fibers are fibrillated to increase internal and external surface area. Although it is inevitable to have particle size reduction, the design of refiner has been evolved to minimize particle size reduction and reduce overall energy consumption. This is because the strength of paper products will decrease with the particle size reduction. Therefore, the wood fibers are subject to compression and shear force (not a direct cutting action) between refiner plates for a short retention time. In addition, MR can open up the biomass structure via external fibrillation and internal delamination.[26,27] More specifically, during the MR, fibrils can be peeled from the fiber surface, resulting in the fracture of fiber structure, which is known as external fibrillation.[26,28] Moreover, the crosslinking of interfibrillar matrices can be broken by mechanical stress, causing the loosened internal structure, which is referred to as internal delamination.[26,29] The energy consumption for mechanical pretreatment processes is varied upon structure and operating principles of mechanical equipment. In general, the energy input for most of mechanical pretreatment processes is higher than 100 kWh/t,[22,30] while the energy consumption during MR is typically 50−100 kWh/t. It is noted that the refining is performed at low solid content (3−15% solid), which will greatly reduce energy consumption. As a comparison, nanocellulose production requires 1000−2000 kWh/t energy to break cellulose fiber structure. Therefore, compared to other mechanical pretreatment methods, MR is cost-effective and requires reasonable energy consumption.[26] Recently, MR has been adopted in the biochemical conversion of biomass to overcome biomass recalcitrance by external fibrillation and internal delamination of biofibers; thus, the accessibility of biofibers was significantly enhanced.[26,28] Nevertheless, to the best of our knowledge, the application of MR to any AD process has not been reported to date.

The major objective of the present work was to employ MR pretreatment to improve the production of biogas and methane from AD of dairy manure. To the best of our knowledge, the current study is the first to investigate the effects of MR on the AD performance of dairy manure. Moreover, the dairy manure containing biofibers was characterized to identify the change in manure morphologies before and after the MR pretreatment. Finally, the potential roles of MR in the AD process were elucidated.

2. RESULTS AND DISCUSSION

2.1. Characterization of Dairy Manure.

Both the arithmetic mean particle size and the length weighted mean particle size decreased with the increase of refining intensity (Table 1). Compared to the unrefined manure, the length weighted mean particle size of manure markedly decreased from 0.952 to 0.423 mm and 0.367 mm by MR at 6000 (6k) and 60,000 (60k) revolutions. Moreover, considering the distribution of length weighted mean particle size, the length weighted mean particle size of manure refined at 60k revolutions was notably and evenly shortened compared to that refined at 6k revolutions (Figure S1). Similarly, previous

Table 1. Mean Particle Size of Unrefined and Mechanically Refined Dairy Manure

revolution	arithmetic (mm)	length weighted (mm)
unrefined	0.312	0.952
6k	0.201	0.423
60k	0.181	0.367

https://doi.org/10.1021/acsomega.1c01760
ACS Omega 2021, 6, 16934−16942

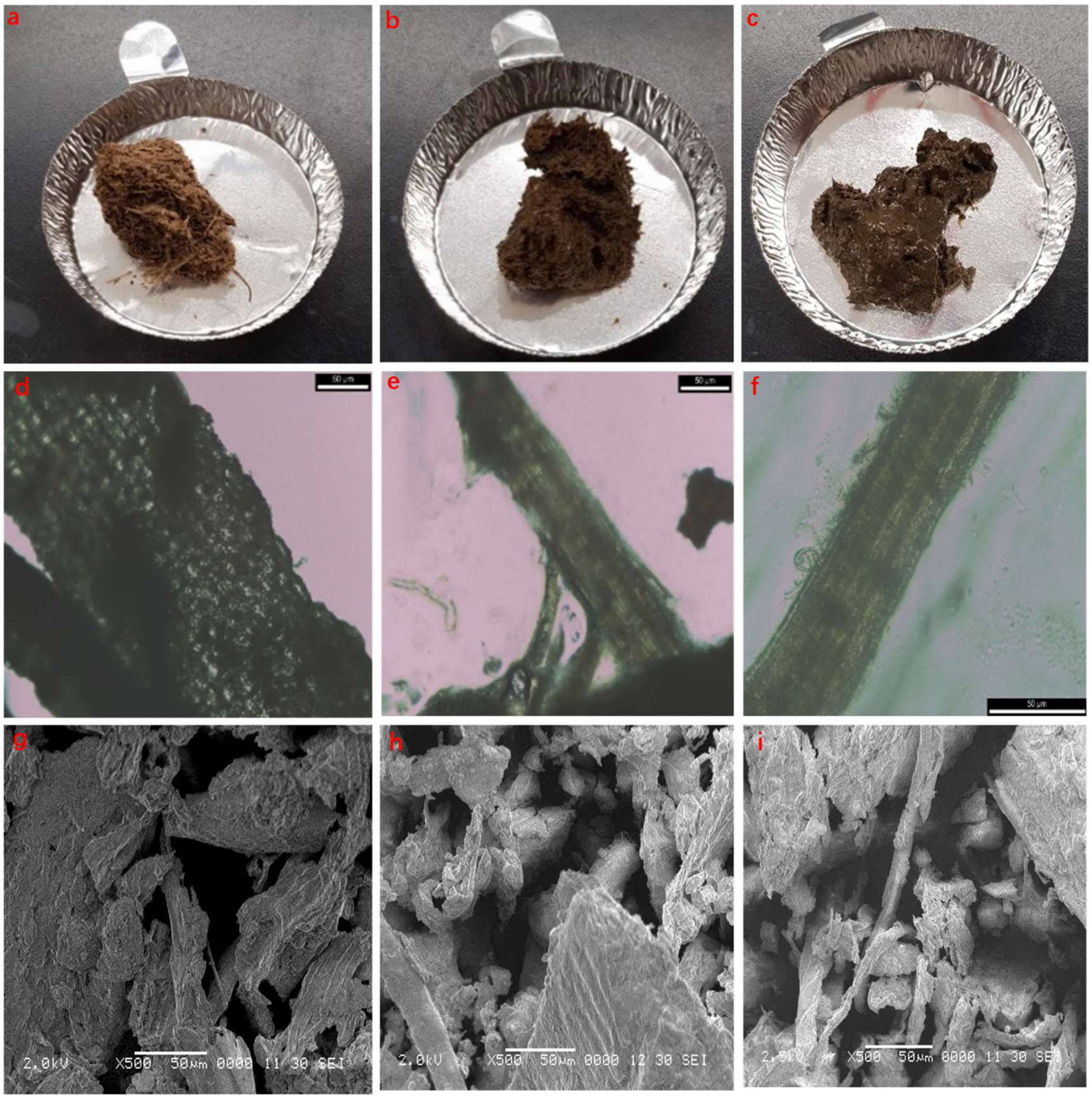


Figure 1. Photograph (a-c), optical microscope (d-f) and SEM (g-i) images of unrefined (a, d, g) and refined manure with 6k revolutions (b, e, h) and 60k revolutions (c, f, i).

studies have also found that size reduction of biomass was one of the primary consequences of MR, and particle size decreased with improvement in the refining intensity.[28,29]

The surface morphologies and texture of manure particles generated by MR at different revolutions were investigated using photographs, optical microscopy and SEM images (Figure 1). The morphology of manure became a slurry as the intensity of refining increased (Figure 1a−c). Some fibers were observed in the unrefined manure and refined manure at 6k revolutions, but few were observed in the refined manure at 60k revolutions. When fibers are subjected to MR, external fibrillation can occur on the surface of fibers and result in the production of small fibers.[26] Hairy features, resulting from small fibers, were observed in the images of refined manure at 6 and 60k revolutions (Figure 1d−f). Due to the production of small fibers, the surface area can increase after MR.[31] Moreover, Figure 1d−f also shows that the degree of fibrillation increased with increased refining intensity. From the SEM images (Figure 1g−i), the unrefined manure appeared as largely intact clusters that were aggregated; however, the MR produced smaller particles and increased the surface area, which may result from the disruption of manure structure and the delamination of the cell walls of fibers.[28] Similar observations were also mentioned by Chen et al. and De Assis et al., which found the same change in biomass structure after MR.[28,29] Chen et al. reported that MR (Szego milling at 1160 rpm) caused the surface disruption and severe delamination of corn stover.[29] De Assis et al. also found that the pretreatment of bagasse by MR at 2k−6k revolutions resulted in the cell−cell separation at middle lamella and increase of single fibers.[28]

The effects of MR on the internal structure of manure were evaluated by measuring the water retention value (WRV). Figure 2 shows that the refined manure samples had higher WRVs than the unrefined samples. Compared to unrefined manure, the WRV values of refined manure at 6 and 60k revolutions increased from 0.84 to 2.28 g/g and 1.68 g/g, respectively. In fact, the refining process induces internal delamination, which results from breakage of the crosslinking of interfibrillar matrices.[26] Thus, the cavities generated by internal delamination provide more opportunities for water molecules to stay within the internal structure of fibers.

https://doi.org/10.1021/acsomega.1c01760
ACS Omega 2021, 6, 16934−16942

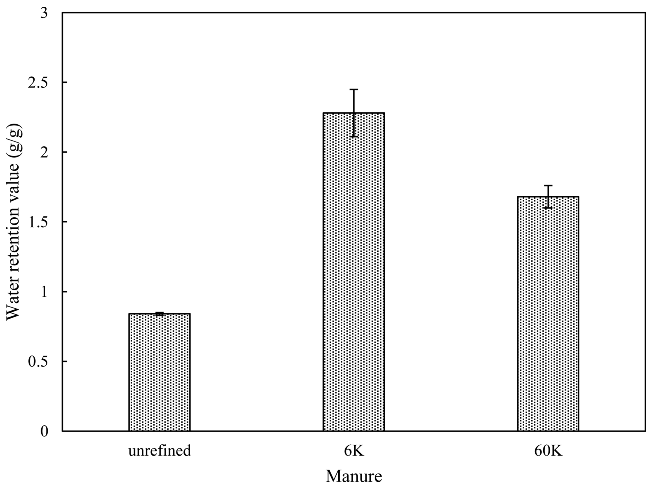


Figure 2. Water retention value (WRV) of unrefined and refined manure at 6 and 60 K revolutions.

However, the WRV of refined manure at 60k revolutions was lower than that at 6k revolutions. This decrease may result from the collapsed pore structure, which decreased the water molecule accessibility to the internal structure of fibers. Chen et al. and Hui et al. also concluded that MR at high revolutions could destroy the pores and cause the loss of macropores.[27,32] Moreover, finely ground manure particles might aggregate and block the newly formed surface area of fibers.

The effect of MR on manure solubility is shown in Table 2. Compared to the unrefined manure, the refined manure at 6k revolutions showed a drastic enhancement of water quality parameters and metabolites (i.e., sCOD concentration by 47.83%, soluble total N by 122%, soluble total P by 102%, and total volatile fatty acids (TVFAs) by 90.67%). This finding indicated that after MR at 6k revolutions, more organic and inorganic compounds from the manure were released to the liquid phase, due to the disintegration of the manure matrix and the disruption of fibers in the dairy manure. Low proportions of soluble phosphate in total P (5.50−7.53%) and soluble ammonia in total N (0.56−1.06%) revealed that the majority of soluble total N and P were bonded to organic compounds of manure, in which anaerobic fermentative bacteria (acetogenic and acidogenic) would access and convert organic matters into biogas and small organic acid (i.e., acetic and butyric acids) as metabolites. Nah et al. also reported that after the mechanical pretreatment (via a collision-plate at 30 bar) of waste sludge for AD, the sCOD, TP and soluble protein concentrations were significantly increased.[33] However, compared to the unrefined manure, the manure with MR at 60k revolutions showed a reduction in most of the water quality parameters (i.e., sCOD concentration by 47.83%, TP concentration by 52.12%, total nitrogen (TN) concentration by 38.89%, PO_4^{3-} concentration by 45.16%, and ammonium concentration by 45.61%) (Table 2). This would come from the aggregation of finely ground manure particles resulting from strong compression and shear stress from MR at high revolutions (60k), which could inhibit the release of organic and inorganic substances from the manure to the aqueous phase. In addition, the pore structure of fibers might be destroyed with MR at high revolutions, resulting in the blocking of pores, which would also show negative effects on the release of organic and inorganic compounds from pores into the aqueous phase.

2.2. Effect of Mechanical Refining on Biogas and Methane Production. The cumulative biogas and methane volumes and yields from the unrefined and refined diary manure are listed in Figure 3. Figure 3 indicates that AD of the manure with MR at 6k revolutions (MR-6k) achieved higher cumulative biogas and higher methane volume and yield than AD of the unrefined manure (MR-control). The cumulative gas volume and yield of MR-6k were 2342 mL and 1110.74 mL biogas/g $VS_{removed}$ for biogas, and 1289.29 mL and 611.47 mL CH_4/g $VS_{removed}$ for methane, respectively. Compared to the MR-control, the cumulative gas volume and yield of MR-6k were improved by 32.02 and 6.35% for biogas and 33.65 and 7.66% for methane, respectively. The results strongly correspond with the beneficial effects of mechanical pretreatment of substrates on biogas and methane production during AD, as indicated by other researchers.[24,25] Rodriguez et al. reported that the mechanical pretreatment of substrates using a Hollander beater improved the methane yield by 21% during mesophilic AD of waste paper.[24] Tsapekos et al. also applied six mechanical pretreatment methods to meadow grass and evaluated their effects on biomass biodegradability.[25] Compared with the untreated meadow grass, all the pretreatment methods resulted in increasing methane production by 8−25%.

Interestingly, MR-60k (AD of the manure with MR at 60k revolutions) led to the decrease in biogas yield by 1.51% (from 1044.45 mL biogas/g $VS_{removed}$ to 1028.68 mL biogas/g $VS_{removed}$) and methane yield by 9.45% (from 567.98 mL CH_4/g $VS_{removed}$ to 514.21 mL CH_4/g $VS_{removed}$) compared to the MR-control (Figure 3). Tsapekos et al. also reported that biomass biodegradability increased by 20% through mechanical pretreatment under relatively gentle operation conditions (600 rpm); however, a higher methane yield was not achieved by more intense operation (1200 rpm).[20] Izumi et al. also used bead milling to decrease the particle size of the substrates of AD for improving the methane yield.[34] The researchers reported that excessive reduction of the particle size (from 0.718 to 0.393 mm) of the substrates led to a decrease in methane production (from 322 to 254 mL g-total COD^{-1}) during the AD process.

In this study, the modified Gompertz model was fitted to the experimental data to evaluate the effects of MR on microbial kinetics during AD. Table 3 shows that MR-6k achieved significantly higher R_{max} (27.32 mL CH_4/g $VS_{removed}$·d) and P (565.76 mL CH_4/g $VS_{removed}$) than the MR-control. Compared to the MR-control, both R_{max} and P were increased by 13.31 and 8.69%, respectively, in MR-6k. These results are highly consistent with previous studies that demonstrated the positive

Table 2. Solubility of Dairy Manure before and after Mechanical Refining

revolution	sCOD (mg/kg TS)	TP-PO_4^{3-} (mg/kg TS)	TN-N (mg/kg TS)	PO_4^{3-} (mg/kg TS)	NH_3-N (mg/kg TS)	TVFAs (mg COD/kg TS)	acetic acid (mg COD/kg TS)	propionic acid (mg COD/kg TS)
unrefined	2300	3300	1800	217	11.4	1019	475	133
6k	3400	6670	4000	367	42.4	1943	744	134
60k	1200	1580	1100	119	6.2	1399	470	65

https://doi.org/10.1021/acsomega.1c01760
ACS Omega 2021, 6, 16934−16942

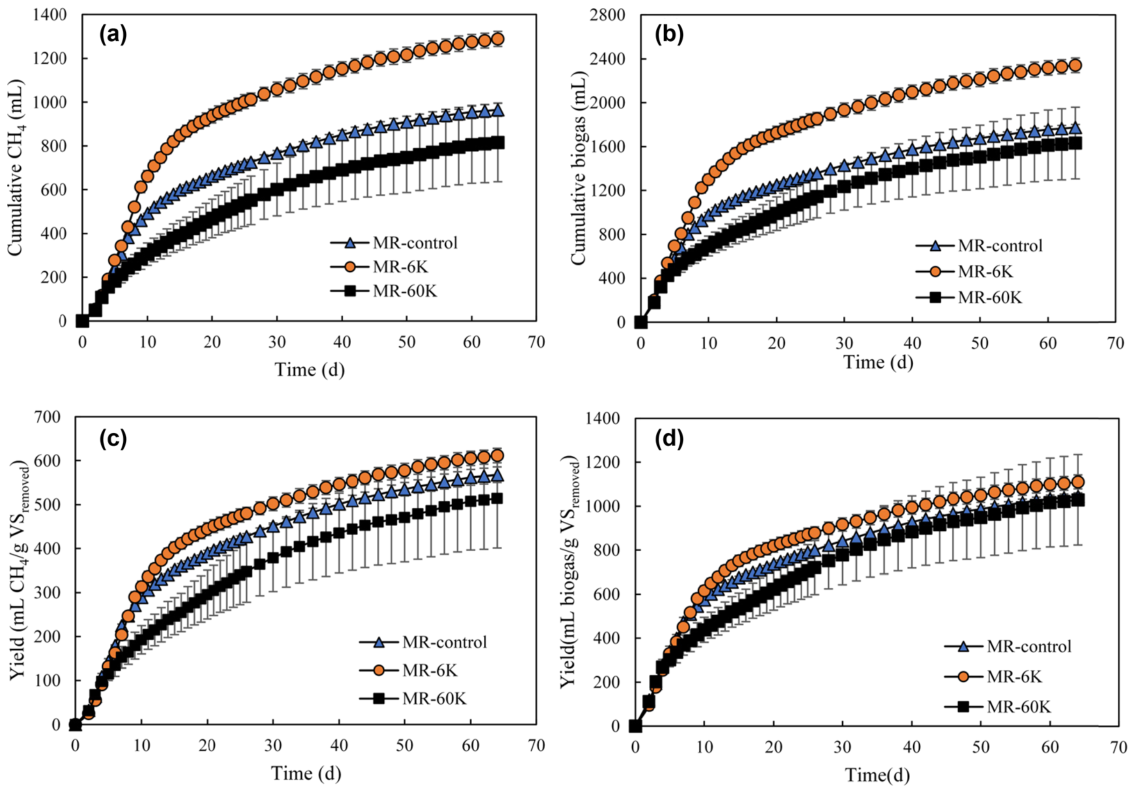


Figure 3. Cumulative methane and biogas volume (a, b) and yield (c, d) from AD of dairy manure.

Table 3. Parameters Values of Modified Gompertz Model Fitted with the Experimental Data

mechanical refining	lag phase, λ (d)	R_{max} (mL CH_4/g $VS_{removed}$·d)	P (mL CH_4/ g $VS_{removed}$)	R^2
MR-control	0	24.11	520.53	0.9535
MR-6k	0	27.32	565.76	0.9719
MR-60k	0	15.05	490.90	0.9765

impact of mechanical pretreatment on R_{max} and P in AD.[20,22] However, both R_{max} and P of MR-60k decreased by 37.58 and 5.69% compared to the MR-control, and 44.91 and 13.23% compared to MR-6k, respectively. Similarly, Tsapekos et al. also found that compared to mechanical pretreatment operated at moderate rotating conditions (600 rpm) during AD of meadow grass, the P value significantly decreased with the highest rotating speeds (900 and 1200 rpm).[20] In the previous study, it was found that the full scale mechanical pretreatment caused significant damage to the surface of lignocellulosic biomass and augmented access to the organic matter, resulting in a decreased lag phase (λ) during AD.[35] However, in this study, λ values in all AD experiments were zero because the inocula for all AD tests were highly activated and already fully adapted to mesophilic conditions before the AD experiments began. If the inoculum used in this study would not be adapted in advance, the lag phase would take longer. However, it is expected that MR can reduce the lag phase in the AD process since MR can overcome the recalcitrance of biomass, which increases enzyme and microorganism accessibility to substrates. In addition to pretreatment techniques, the addition of additives (such as nano-structured metal materials and biochar) into the AD process can also decrease the lag phase, because they can improve the microorganism growth, activity, and metabolism during AD.[5,36,37]

Table 4. Operation Parameters for AD Process with Different Dairy Manure

operation parameters	MR-control		MR-6k		MR-60k	
	initial	final	initial	final	initial	final
sCOD (g/L)	6.94	2.89	9.18	3.48	4.16	2.09
TP-PO_4^{3-}(mg/L)	62.6	22.6	77.1	31.7	44.4	39.3
TN-N(mg/L)	810	1120	1020	1150	720	730
NH_3-N(mg/L)	504	767	500	761	508	561
PO_4^{3-}(mg/L)	8.78	10.15	8.52	14.45	8.83	19.6
TA (mg $CaCO_3$/L)	3900	4940	4090	5060	3100	3890
TVFA (mg COD/L)	38.4	70.1	131.2	64.0	107.4	35.5
Acetic acid (mg COD/L)	32.3	59.2	96.9	48.5	85.3	31.8
propionic acid (mg COD/L)	6.1	10.9	24.1	9.8	14.0	3.7
pH	7.44	7.19	7.86	7.27	7.96	6.97
VS (g/L)	60.77	43.78	69.17	48.09	57.75	41.89

https://doi.org/10.1021/acsomega.1c01760
ACS Omega 2021, 6, 16934−16942

2.3. Potential Roles of Mechanical Refining in AD of Dairy Manure. Mechanical stress on lignocellulosic biomass in the refining process can cause various alternations to biomass structure.[26] There are three mechanisms associated with the MR of biomass: cutting, external fibrillation, and internal delamination.[26,28] Through cutting, refined manure at 6k revolutions had smaller particles, higher surface area, and easier access to bacteria and enzymes associated with AD, resulting in a higher production of biogas and methane during AD than unrefined manure.[26] Similar to cutting, MR also led to higher surface area and anaerobic biodegradability through external fibrillation of biofibers in the manure.[29] In addition, the internal delamination induced by MR caused the rupture of crosslinking of interfibrillar matrices and the generation of cavities inside the cell wall structure.[26,27] Thus, the approachability of cellulose in biofibers to enzyme and bacteria was significantly improved after MR, thereby enhancing the hydrolysis efficiency and methane production. However, MR at high revolutions (60k) caused the breakage of pore structure of biofibers in manure, which decreased the manure digestibility due to the reduced enzyme attack during the AD process.

From the AD parameters listed in Table 4, the initial concentrations of sCOD, TP and TN at MR-6k were higher than those of the MR-control. Moreover, as discussed above, the MR enhanced the release of organic P and N from manure to the liquid phase. Thus, it can be concluded that the MR resulted in higher soluble organic substances in the manure sample, which can be easily utilized by the microorganisms associated with AD for increasing methane production. However, MR-60k had lower sCOD, TP and TN compared to the MR-control and MR-6k, which might result from aggregation of finely ground manure particles, which would prevent the release of organic substances from the manure. In addition, MR-6k had a higher consumption of sCOD (62%) than the MR-control (58%) and MR-60k (50%). Furthermore, the final NH_3-N concentration from the MR-60k was lower than that from the MR-control and MR-6k, indicating lower degradation and conversion of refined manure at 60k revolutions to soluble ammonia. This also explains the lower production of methane from MR-60k than the MR-control and MR-6k.

As listed in Table 4, the initial TVFAs concentration of MR-6k was higher than those of the MR-control and MR-60k. In addition, the inoculum used in this study was highly activated and could directly consume and convert VFAs to methane. Thus, a higher initial concentration of TVFAs in MR-6k would be beneficial for biogas and methane production in this study. However, similar to sCOD, TP and TN, MR-60k reduced the release of VFAs from the manure due to aggregation of finely ground manure particles. It is reported that acetic acid is the primary precursor for methane production, while propionic acid can be converted to acetic acid in the absence of acetic acid.[34,38] Thus, higher initial concentrations of acetic and propionic acid at MR-6k led to higher methane production than for the MR-control and MR-60k. Table 4 also shows the total alkalinity (TA) concentration of MR-6k before and after AD was higher than that of the MR-control and MR-60k. Having higher TA in MR-6k could provide higher buffering capacity during the AD of manure, which can improve the AD process stability by alleviating the pH drop from the accumulation of VFAs.[5] This could also enhance the methane production in MR-6k compared to the MR-control and MR-60k. Overall, the appropriate mechanical pretreatment of dairy manure containing biofiber (6k revolutions in this study) increased the release of organic substances and AD metabolites from the manure to enhance biogas and methane production.

Therefore, the MR pretreatment can be considered as a cost-effective method to enhance microbial metabolisms and overcome current limitations in AD of dairy manure. Due to the existence of fibers and biorecalcitrant substrates in various AD processes, the MR can also be applied to pretreat various substrates to enhance AD performance. Since the MR has been used for pulp and paper industry at large scale, the MR could be applied to farm- and industrial-scale AD. Moreover, the combination of the AD and other pretreatment methods in the AD process could be a promising approach. Possible pretreatment of AD substrates by combination of MR and chemical or biological pretreatment would significantly increase interactions between substrates and microorganisms, accelerate microbial reactions and enhance biogas production during AD processes. Future studies will focus on detailed optimization of MR (i.e., mechanical intensity, process time), achieving a continuous AD process with mechanically pretreated manure, application in pretreatment of other substrates, and scale-up studies.

2.4. Energy Balance. In this study, the energy balance was made based on the experimental results from the AD of dairy manure pretreated by MR at 6k revolutions. The AD of dairy manure pretreated by the MR at 6k revolutions resulted in an increase of 43.50 mL CH_4/g VS of methane production compared with the AD of untreated manure (Figure 3). Thus, the increased energy resulting from MR pretreatment was 435 kWh/t VS (with the energy content of methane of 10 Wh/L CH_4[22]). According to the typical energy demand of MR process, the energy input of MR for dairy manure in this study was estimated to be 40 kWh/t VS.[26] Thus, the application of MR for AD of dairy manure led to a positive net energy output of 395 kWh/t VS, which was 9.9 times the energy input. Kang et al. showed that the net energy output was 1.7 times the energy input under the application of mechanical pretreatment (Grinder) in the process of AD of *Hybrid Pennisetum*.[22] Lindmark et al. also found that mechanical pretreatment (Disperser) led to the energy production which was 2−3 times higher than the energy input during the AD of ley crop silage.[39] Therefore, MR can be considered as a cost-effective method for the improvement of performance of AD of dairy manure.

3. CONCLUSIONS

The effects of MR on anaerobic digestion of dairy manure were investigated in this study. MR-6k showed a significant enhancement of biogas and methane production due to the increase in manure solubilization, reduction in particle size, and external fibrillation and internal delamination of fibers in manure compared to the MR-control. The cumulative gas volume and yield from MR-6k increased to 33.7 and 7.7% for methane and to 32.0 and 6.4% for biogas, respectively. However, MR-60k exhibited even lower biogas and methane production than the MR-control and MR-6k due to the rupture of pore structure and aggregation of finely ground manure particles, which could prevent access and digestibility of microorganisms and enzymes associated with AD. Therefore, an appropriate refining intensity of MR need to be figured out for the pretreatment of AD substrates. Otherwise, it will cause negative effects on AD performance. Future studies will focus on detailed optimization of MR (i.e., mechanical intensity, process time) and scale-up/cost evaluation studies.

https://doi.org/10.1021/acsomega.1c01760
ACS Omega 2021, 6, 16934−16942

4. MATERIALS AND METHODS

4.1. Preparation of Substrate and Anaerobic Inoculum. The dairy manure used as the substrate for AD experiments in this study was obtained from the manure pit at the Southwest Dairy Center at Tarleton State University (Stephenville, TX, USA). In order to keep consistent quality and quantity of manure in every AD reactor, the dairy manure was oven-dried at 70 °C for 24 h and was subsequently crushed. The dry dairy manure consisted of C (16.6%), H (2.3%), O (31.0%), N (1.1%), S (0.2%), and ash (48.8%).[5]

The inoculum was obtained as reported in our previous work.[5] Briefly, the inoculum was acquired from the bottom of a lagoon at the Tarleton dairy farm and incubated with the dry manure under anaerobic conditions at 37 °C for one month. Then, the activated inoculum was used for further AD experiments. The main characteristic parameters of the inoculum were pH: 8.28 ± 0.08, total solids (TS): 137.65 ± 10.11 g/L, VS: 27.17 ± 0.17 g/L, soluble COD (sCOD): 2.09 ± 0.01 g/L, TA: 2.87 ± 0.11 g $CaCO_3$/L, and TVFAs: 9.4 ± 0.9 mg COD/L.

4.2. Mechanical Refining of Dairy Manure. MR was used to pretreat the dry dairy manure by a PFI refiner (Figure S2), which is usually used for the treatment of pulp fibers. Before the refining, deionized water was mixed with the manure to obtain homogenous samples. The mixture was placed evenly on the wall of a rotating disk of a PFI mill. After that step, samples were refined at two different refining intensities (6000 and 60,000 revolutions) for 5 min. During the PFI refining, the dairy manure slurry was thrown against the wall of milling housing by the rotation of the rotor. Then, the impacts of rotor bars resulted in the generation of shearing and compression forces, which led to intrafiber bond breakage, external fibrillation, and fiber cutting.[27]

4.3. AD of Unrefined and Mechanically Refined Dairy Manure. BMP (Biochemical Methane Potential) tests were implemented to evaluate the effects of MR on the AD of dairy manure. First, 100 mL of inoculum and dairy manure (that is, the unrefined and mechanically refined dairy manure) were added to 280-mL serum bottles. The ratio of inoculum to dairy manure in this study was set at 1 (based on TS). After the inoculation, rubber plugs and aluminum crimps were used to seal the serum bottles. Then, the bottles were filled with nitrogen gas for 1 min to ensure anaerobic conditions. The incubation temperature in this study was fixed at mesophilic temperature (37 °C). The experiment sets were referred as MR-control, MR-6k, and MR-60k, which represent the AD of unrefined, 6k (6,000 revolutions) and 60k (60,000 revolutions) refined dairy manure. The AD tests were performed in duplicate, and all bottles were mixed well once a day.

4.4. Analytical Methods. TS, VS, pH, sCOD, NH_3−N, TN, PO_4^{3-}, TP (total phosphorus), TA, and TVFAs were measured on the basis of the standard methods for the evaluation of water and wastewater.[40] The manure solubility for soluble COD, TP, TN, ammonium, phosphate, and VFAs were conducted as follows: 1 g of dry manure was added to 100 mL of deionized water in a 250-mL conical flask and shaken for 24 h at 150 rpm. Then, the manure leachate was separated by centrifugation and used for measurement.

The biogas volume was determined using a 60-mL syringe, and the methane concentration of biogas was measured by a gas chromatograph (GC) (GC-2014, Shimadzu Corp., Japan) fitted with a packed column and a thermal conductivity detector (TCD) and a flame ionization detector (FID). The temperatures for the oven, TCD, and FID were set to 80, 110, and 250 °C, respectively. VFAs analysis was also preformed using the gas chromatograph as previously described.[5] For the measurement of VFA, helium was the carrier gas, and the temperature of FID was fixed at 250 °C.

To measure the particle size distribution, 1 g of dry manure sample was disintegrated in 1 L of distilled water to measure the manure particle length. Then, 200 mL of each disintegrated sample was added to the beaker and diluted to 600 mL to obtain manure particle frequency values under 15. Average manure particle length was measured by utilizing a Fiber Quality Analyzer (FQA). Optical microscopy images of each sample were obtained by a Nikon E200. The images of surface morphology of the manure were acquired using a Hitachi HT7700 field emission scanning electron microscope (SEM).

The unrefined, 6 and 60k refined samples were utilized to measure WRV, which was described in the previous study.[28] Each sample slurry was placed in a WRV vial, and samples were centrifuged for 30 min at 24 °C to remove free water from the samples. The speed of centrifugation was 900 G. Samples were weighed immediately after centrifugation to minimize evaporation loss. After that step, samples were dried at 105 °C overnight to calculate the amount of bound water in the sample. Then, the WRV was calculated by using the equation:

$$\mathrm{WRV} = \frac{W_{\mathrm{wet}} - W_{\mathrm{dry}}}{W_{\mathrm{dry}}} \quad (1)$$

where W_{wet} is the wet sample weight and W_{dry} is the oven-dried sample weight.

4.5. Kinetic Study. For evaluating the effects of MR on lag phase, maximum production potential and production rate during the AD experiments, the experimental data from BMP tests were fitted with the modified Gompertz model described by the following equation (eq 2):[5,13]

$$M(t) = P \times \exp\left\{-\exp\left[\frac{R_{\max} \times e}{P}(\lambda - t) + 1\right]\right\} \quad (2)$$

where $M(t)$ represents the methane yield at a time t (mL/g $VS_{removed}$), P represents the maximum methane potential (mL/g $VS_{removed}$), R_{max} represents the maximum methane production rate (mL/g $VS_{removed} \cdot d$), λ represents the lag phase (d) and e is Euler's constant (2.7183). In this study, the determination coefficient (R^2) was calculated for evaluating the accuracy of prediction of the model (Text S1).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c01760.

Methods for data analysis; length weighted manure length distribution; PFI mill (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Eunsung Kan − *Department of Biological and Agricultural Engineering & Texas A&M AgriLife Research Center, Texas A&M University, College Station, Texas 77843, United States; Department of Wildlife, Sustainability, and Ecosystem Sciences, Tarleton State University, Stephenville, Texas*

https://doi.org/10.1021/acsomega.1c01760
ACS Omega 2021, 6, 16934−16942

76401, United States; orcid.org/0000-0001-6298-6096; Phone: +1-254-968-4144; Email: eunsung.kan@ag.tamu.edu; Fax: +1-254-968-3759

Authors

Shengquan Zeng − *Department of Biological and Agricultural Engineering & Texas A&M AgriLife Research Center, Texas A&M University, College Station, Texas 77843, United States*

Hyun Min Jang − *Department of Environmental Engineering and Soil Environment Research Center, Jeonbuk National University, Jeonju, Jeollabukdo 54896, Republic of Korea*

Seonghyun Park − *Department of Forest Biomaterials, North Carolina State University, Raleigh, North Carolina 27607, United States*

Sunkyu Park − *Department of Forest Biomaterials, North Carolina State University, Raleigh, North Carolina 27607, United States;* orcid.org/0000-0002-9332-9061

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.1c01760

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by Texas A&M University Chancellor Research Initiative Fund.

■ REFERENCES

(1) Zhang, H.; Schroder, J. *Animal manure production and utilization in the US. In Applied manure and nutrient chemistry for sustainable agriculture and environment*; He, Z.; Zhang, H., Eds.; Springer: Dordrecht, 2014; 1−21.

(2) Atandi, E.; Rahman, S. Prospect of anaerobic co-digestion of dairy manure: a review. *Environ. Technol. Rev.* **2012**, *1*, 127−135.

(3) Nasir, I. M.; Mohd Ghazi, T. I.; Omar, R. Anaerobic digestion technology in livestock manure treatment for biogas production: A review. *Eng. Life Sci.* **2012**, *12*, 258−269.

(4) Aguirre-Villegas, H. A.; Larson, R. A.; Sharara, M. A. Anaerobic digestion, solid-liquid separation, and drying of dairy manure: Measuring constituents and modeling emission. *Sci. Total Environ.* **2019**, *696*, No. 134059.

(5) Jang, H. M.; Choi, Y. K.; Kan, E. Effects of dairy manure-derived biochar on psychrophilic, mesophilic and thermophilic anaerobic digestions of dairy manure. *Bioresour. Technol.* **2018**, *250*, 927−931.

(6) Li, K.; Liu, R.; Sun, C. Comparison of anaerobic digestion characteristics and kinetics of four livestock manures with different substrate concentrations. *Bioresour. Technol.* **2015**, *198*, 133−140.

(7) Tsapekos, P.; Kougias, P. G.; Frison, A.; Raga, R.; Angelidaki, I. Improving methane production from digested manure biofibers by mechanical and thermal alkaline pretreatment. *Bioresour. Technol.* **2016**, *216*, 545−552.

(8) Angelidaki, I.; Ahring, B. K. Methods for increasing the biogas potential from the recalcitrant organic matter contained in manure. *Water Sci. Technol.* **2000**, *41*, 189−194.

(9) Atelge, M. R.; Atabani, A. E.; Banu, J. R.; Krisa, D.; Kaya, M.; Eskicioglu, C.; Kumar, G.; Lee, C.; Yildiz, Y. Ş.; Unalan, S.; Mohanasundaram, R.; Duman, F. A critical review of pretreatment technologies to enhance anaerobic digestion and energy recovery. *Fuel* **2020**, *270*, No. 117494.

(10) Volschan Junior, I.; de Almeida, R.; Cammarota, M. C. A review of sludge pretreatment methods and co-digestion to boost biogas production and energy self-sufficiency in wastewater treatment plants. *J. Water Process Eng.* **2021**, *40*, No. 101857.

(11) Abraham, A.; Mathew, A. K.; Park, H.; Choi, O.; Sindhu, R.; Parameswaran, B.; Pandey, A.; Park, J. H.; Sang, B. I. Pretreatment strategies for enhanced biogas production from lignocellulosic biomass. *Bioresour. Technol.* **2020**, *301*, No. 122725.

(12) Rodriguez, C.; Alaswad, A.; Mooney, J.; Prescott, T.; Olabi, A. G. Pre-treatment techniques used for anaerobic digestion of algae. *Fuel Process. Technol.* **2015**, *138*, 765−779.

(13) Passos, F.; Ortega, V.; Donoso-Bravo, A. Thermochemical pretreatment and anaerobic digestion of dairy cow manure: Experimental and economic evaluation. *Bioresour. Technol.* **2017**, *227*, 239−246.

(14) Yang, Q.; Wang, H.; Larson, R.; Runge, T. M. Comparative study of chemical pretreatments of dairy manure for enhanced biomethane production. *BioResources* **2017**, *12*, 7363−7375.

(15) Wu-Haan, W.; Burns, R. T.; Moody, L. B.; Grewell, D.; Raman, R. Evaluation of ultrasonic pretreatment on anaerobic digestion of different animal manures. *Trans. ASABE* **2010**, *53*, 577−583.

(16) Cesaro, A.; Belgiorno, V. Pretreatment methods to improve anaerobic biodegradability of organic municipal solid waste fractions. *Chem. Eng. J.* **2014**, *240*, 24−37.

(17) Ai, P.; Zhang, X.; Dinamarca, C.; Elsayed, M.; Yu, L.; Xi, J.; Mei, Z. Different effects of ozone and aqueous ammonia in a combined pretreatment method on rice straw and dairy manure fiber for enhancing biomethane production. *Bioresour. Technol.* **2019**, *282*, 275−284.

(18) Bruni, E.; Jensen, A. P.; Angelidaki, I. Comparative study of mechanical, hydrothermal, chemical and enzymatic treatments of digested biofibers to improve biogas production. *Bioresour. Technol.* **2010**, *101*, 8713−8717.

(19) Carrere, H.; Antonopoulou, G.; Affes, R.; Passos, F.; Battimelli, A.; Lyberatos, G.; Ferrer, I. Review of feedstock pretreatment strategies for improved anaerobic digestion: From lab-scale research to full-scale application. *Bioresour. Technol.* **2016**, *199*, 386−397.

(20) Tsapekos, P.; Kougias, P. G.; Angelidaki, I. Mechanical pretreatment for increased biogas production from lignocellulosic biomass; predicting the methane yield from structural plant components. *Waste Manag.* **2018**, *78*, 903−910.

(21) Ariunbaatar, J.; Panico, A.; Esposito, G.; Pirozzi, F.; Lens, P. N. L. Pretreatment methods to enhance anaerobic digestion of organic solid waste. *Appl. Energy* **2014**, *123*, 143−156.

(22) Kang, X.; Zhang, Y.; Song, B.; Sun, Y.; Li, L.; He, Y.; Kong, X.; Luo, X.; Yuan, Z. The effect of mechanical pretreatment on the anaerobic digestion of *Hybrid Pennisetum*. *Fuel* **2019**, *252*, 469−474.

(23) Rodriguez, C.; Alaswad, A.; El-Hassan, Z.; Olabi, A. G. Improvement of methane production from *P. canaliculata* through mechanical pretreatment. *Renew. Energy* **2018**, *119*, 73−78.

(24) Rodriguez, C.; Alaswad, A.; El-Hassan, Z.; Olabi, A. G. Mechanical pretreatment of waste paper for biogas production. *Waste Manag.* **2017**, *68*, 157−164.

(25) Tsapekos, P.; Kougias, P. G.; Angelidaki, I. Biogas production from ensiled meadow grass; effect of mechanical pretreatments and rapid determination of substrate biodegradability via physicochemical methods. *Bioresour. Technol.* **2015**, *182*, 329−335.

(26) Park, J.; Jones, B.; Koo, B.; Chen, X.; Tucker, M.; Yu, J. H.; Pschorn, T.; Venditti, R.; Park, S. Use of mechanical refining to improve the production of low-cost sugars from lignocellulosic biomass. *Bioresour. Technol.* **2016**, *199*, 59−67.

(27) Chen, X.; Kuhn, E.; Wang, W.; Park, S.; Flanegan, K.; Trass, O.; Tenlep, L.; Tao, L.; Tucker, M. Comparison of different mechanical refining technologies on the enzymatic digestibility of low severity acid pretreated corn stover. *Bioresour. Technol.* **2013**, *147*, 401−408.

(28) de Assis, T.; Huang, S.; Driemeier, C. E.; Donohoe, B. S.; Kim, C.; Kim, S. H.; Gonzalez, R.; Jameel, H.; Park, S. Toward an understanding of the increase in enzymatic hydrolysis by mechanical refining. *Biotechnol. Biofuels* **2018**, *11*, 289.

(29) Chen, X.; Wang, W.; Ciesielski, P.; Trass, O.; Park, S.; Tao, L.; Tucker, M. P. Improving sugar yields and reducing enzyme loadings in the deacetylation and mechanical refining (DMR) process through multistage disk and szego refining and corresponding techno-economic analysis. *ACS Sustainable Chem. Eng.* **2016**, *4*, 324−333.

(30) Barakat, A.; de Vries, H.; Rouau, X. Dry fractionation process as an important step in current and future lignocellulose biorefineries: a review. *Bioresour. Technol.* **2013**, *134*, 362−373.
(31) Henn, A. R.; Fraundorf, P. B. A quantitative measure of the degree of fibrillation of short reinforcing fibres. *J. Mater. Sci.* **1990**, *25*, 3659−3663.
(32) Hui, L.; Liu, Z.; Ni, Y. Characterization of high-yield pulp (HYP) by the solute exclusion technique. *Bioresour. Technol.* **2009**, *100*, 6630−6634.
(33) Nah, I. W.; Kang, Y. W.; Hwang, K.-Y.; Song, W.-K. Mechanical pretreatment of waste activated sludge for anaerobic digestion process. *Water Res.* **2000**, *34*, 2362−2368.
(34) Izumi, K.; Okishio, Y.-K.; Nagao, N.; Niwa, C.; Yamamoto, S.; Toda, T. Effects of particle size on anaerobic digestion of food waste. *Int. Biodeter. Biodegr.* **2010**, *64*, 601−608.
(35) Tsapekos, P.; Kougias, P. G.; Egelund, H.; Larsen, U.; Pedersen, J.; Trénel, P.; Angelidaki, I. Mechanical pretreatment at harvesting increases the bioenergy output from marginal land grasses. *Renew. Energy* **2017**, *111*, 914−921.
(36) Pan, J.; Ma, J.; Liu, X.; Zhai, L.; Ouyang, X.; Liu, H. Effects of different types of biochar on the anaerobic digestion of chicken manure. *Bioresour. Technol.* **2019**, *275*, 258−265.
(37) Shen, R.; Jing, Y.; Feng, J.; Luo, J.; Yu, J.; Zhao, L. Performance of enhanced anaerobic digestion with different pyrolysis biochars and microbial communities. *Bioresour. Technol.* **2020**, *296*, No. 122354.
(38) Zheng, Z.; Liu, J.; Yuan, X.; Wang, X.; Zhu, W.; Yang, F.; Cui, Z. Effect of dairy manure to switchgrass co-digestion ratio on methane production and the bacterial community in batch anaerobic digestion. *Appl. Energy* **2015**, *151*, 249−257.
(39) Lindmark, J.; Leksell, N.; Schnürer, A.; Thorin, E. Effects of mechanical pre-treatment on the biogas yield from ley crop silage. *Appl. Energy* **2012**, *97*, 498−502.
(40) Eaton, D. A. *Standard methods for the examination of water and wastewater*. American Public Health Association (APHA): Washington, DC, USA 2005.

https://doi.org/10.1021/acsomega.1c01760
ACS Omega 2021, 6, 16934−16942