2-6-2024

Impacts of Harvest Date and Concurrent Alkali Pretreatment and Ensiling on Anaerobic Digestion of Pennycress Biomass

Liangcheng Yang
Illinois State University

Tuba Yasmin Lubna
Illinois State University

Michael A. Moklak
Illinois State University

Barsanti Gautum
Illinois State University

Nicholas J. Heller
Illinois State University

See next page for additional authors

fermentation

MDPI

Article

Impacts of Harvest Date and Concurrent Alkali Pretreatment and Ensiling on Anaerobic Digestion of Pennycress Biomass

Liangcheng Yang [1,2], Tuba Yasmin Lubna [2], Michael A. Moklak [2], Barsanti Gautam [3], Nicholas J. Heller [2], Robert L. Rhykerd [2], David E. Kopsell [2] and John C. Sedbrook [3,*]

[1] Department of Health Sciences, Illinois State University, Normal, IL 61790, USA; lyang@ilstu.edu
[2] Department of Agriculture, Illinois State University, Normal, IL 61790, USA; tylubna@ilstu.edu (T.Y.L.); mamokla@ilstu.edu (M.A.M.); njhelle@ilstu.edu (N.J.H.); rrhyker@ilstu.edu (R.L.R.); dkopsel@ilstu.edu (D.E.K.)
[3] School of Biological Sciences, Illinois State University, Normal, IL 61790, USA
* Correspondence: jcsedbr@ilstu.edu; Tel.: +1-(309)-438-3374

Abstract: Pennycress (*Thlaspi arvense* L.) is an annual cover crop known for its exceptional cold tolerance and high oil and protein yields. Pennycress can be integrated into a corn–soybean rotation in the U.S. However, the utilization of pennycress biomass remains largely unexplored, including assessing compositional changes through its growth and organic matter digestibility. This study harvested pennycress at three growth stages, characterized the biomass for anaerobic digestion (AD), and tested the effects of concurrent alkali pretreatment and ensiling on the biomass methane yield. Results showed that the biomass harvested when the plants were undergoing senescence ("third-harvest") had higher contents of acid detergent fiber, neutral detergent fiber, and lignin, while the biomass harvested when 80–90% of the pods were fully-sized ("second-harvest") had the highest protein content. The AD experiments showed that the first-harvest biomass (90% of flowers opened) failed to produce biogas due to a drop in the pH and alkalinity, the second-harvest biomass was inhibited for methane production (45.74 ± 0.20 L/kg-VS), and the third-harvest biomass had a methane yield of 171.80 ± 4.82 L/kg-VS. After the alkali pretreatment and ensiling, a methane yield of 270.4 ± 3.10 L/kg-VS was obtained from the second-harvest biomass, representing a significant 4.5-fold increase (adjusted for the organic matter loss) relative to the untreated second-harvest biomass.

Keywords: anaerobic digestion; biogas; compositional change; *Thlaspi arvense* L.; pennycress; biomass; silage; cover crop

Citation: Yang, L.; Lubna, T.Y.; Moklak, M.A.; Gautam, B.; Heller, N.J.; Rhykerd, R.L.; Kopsell, D.E.; Sedbrook, J.C. Impacts of Harvest Date and Concurrent Alkali Pretreatment and Ensiling on Anaerobic Digestion of Pennycress Biomass. *Fermentation* **2024**, *10*, 96. https://doi.org/10.3390/fermentation10020096

Academic Editor: Yutuo Wei

Received: 14 December 2023
Revised: 2 February 2024
Accepted: 3 February 2024
Published: 6 February 2024

1. Introduction

Cover crops have been traditionally used to provide ecosystem services, such as reducing soil erosion, retaining nutrients in farm fields, holding soil moisture, and improving biodiversity and soil health [1–3]. Recently, cover crops have been evaluated for their effects on soil organic carbon, nitrous oxide emissions from soil, and improving soil microbial communities [4]. Cover crops can bring additional benefits to farming operations by suppressing weeds, improving nutrient cycling, providing a habitat for beneficial insects and pollinators, and acting as a climate-smart practice by sequestering carbon and reducing the adverse effects of droughts and floods caused by climate change [4–13]. The adoption of cover crops on a large scale could reduce agriculture greenhouse gas emissions by 10%, with a 116 g CO_2 e/m^2-year reduction from nonlegumes and a 135 g CO_2 e/m^2-year reduction from legumes, respectively [12,14]. According to the USDA survey report released in 2021, to prepare for the planting of summer cash crops, most winter cover crops are terminated with herbicide or tillage, but also can be grazed or harvested as hay, silage, or for other applications [1,12,15–18]. Cover crop biomass can contribute to energy production by producing biogas, syngas, bio-oil, and sustainable aviation fuel (SAF) [19]. Several cover

crops, such as cereal rye, carinata, camelina, rapeseed, and pennycress, have been tested for bioenergy production potential [19–21].

Pennycress (*Thlaspi arvense* L.), also referred to as field pennycress, stinkweed, and frenchweed, is a winter annual plant belonging to the Brassicaceae family. Pennycress is widely distributed in temperate regions of all continents except Antarctica, including throughout North America, having remarkable adaptability to many environmental conditions [2,22]. Pennycress natural populations are inherently exceptionally cold tolerant, withstanding temperatures below −20 °C, which is significant for the intended use of domesticated varieties as an overwintering crop [2]. Pennycress can be integrated into a two-year corn–soybean rotation, widely practiced among producers in the U.S. [23,24]. Domesticated pennycress [25–27], commercially released as CoverCress^{TM}, produces oilseeds during the off-season in the lower U.S. Midwest Corn Belt, thereby resulting in farm field revenue from three crops in two years [28,29]. The biomass production of pennycress ranges from 5000 to 8500 kg/ha (harvest index of about 0.3), while the output of its seed oil is 400–800 L/ha [30–32].

Pennycress seeds contain 20–25% crude protein and 30–36% oil content by dry weight [33]. Because of the high protein content, the whole seed or seed press cake after oil extraction can be used as an ingredient in feed for poultry, swine, cattle, fish, and pets [34,35]. The high oil content in pennycress grain makes it a valued feedstock for renewable diesel, biodiesel, and SAF [29,36]. It was estimated that pennycress can provide the aviation fuel industry with 800 million gallons per year [37]. Growing domesticated pennycress in the Midwest Corn Belt has the potential to replace approximately 5% of the present petroleum supplies in the U.S. [28,30].

The use of the pennycress biomass, either as senesced stems and leaves after grain harvest or in its entirety before full maturity, has been little studied despite the biomass yield being significant, ranging from 5000 to 8500 kg/ha [30,31]. Pennycress plant tissues naturally contain a high concentration of glucosinolate, making it a potential source for use in biofumigation for weed control and an effective biocide against several pathogenic organisms, which could be beneficial to organic growers [38,39]. Pennycress stems and leaves have a similar composition to other lignocellulosic biomass; therefore, they can be used in anaerobic digestion (AD) to produce biogas. While domesticated pennycress grain has the highest commercial value, harvesting the biomass in its entirety before full maturity could be an option in years when prolonged cold spring weather prevents full maturation in time to plant the summer crop. Harvesting before full maturity could also be an attractive option, e.g., in the northern U.S. Midwest, where growing days are fewer and pennycress grain production does not fit within the corn–soybean rotation.

AD is a highly tolerant bioconversion technology and has been used to produce biogas from various organic wastes, such as animal waste, municipal solids, food waste, and lignocellulosic biomass [40–43]. Biogas mainly contains methane and CO_2, and can be used to generate heat, electricity, or transportation fuels [44,45]. While pennycress grain production has been intensively studied, to our knowledge, no publications exist on the utilization of pennycress biomass for AD. Analyzing the digestibility and potential methane yield of pennycress biomass can be one important step in determining the value and economic feasibility of harvesting and processing pennycress at different growth stages suited for different crop rotations and agricultural regions.

The primary objective of this research was to investigate the AD of pennycress biomass at different growth stages. Domesticated pennycress integrated within a corn–soybean rotation is usually planted in mid-September to mid-October and harvested in mid-May to early June in the U.S. Midwest. Verhoff et al. recently introduced a method for the phenological staging of pennycress, which started with stage 0—germination and ended with stage 9—maturity [46]. We were particularly interested in researching the biomass characteristics and biogas yields from pennycress biomass at the later growth stages, including stage 7—seed fill, stage 8—ripening, and stage 9—maturity (see Materials and Methods, Section 2.2, for additional phenological staging details). It is common to see biomass com-

positional and weight changes at different growth stages from other crops [47,48], but how that affects the pennycress biogas yield has not been studied. Also, the storage of biomass, especially green biomass, is necessary in the biomass feedstock logistics supply chain, but can be challenging, as organic matter can be lost and acids can be generated [49]. Therefore, we intended to evaluate the effects of a commonly used storage/pretreatment method on the pennycress biogas yield. The specific objectives of this study were to: (1) characterize the pennycress biomass at three growth stages/harvest dates; (2) evaluate the biogas yields from pennycress biomass and optimize the AD conditions to improve the methane yields; (3) test the effects of a concurrent alkali pretreatment and ensiling of pennycress biomass on the methane yield.

2. Materials and Methods

2.1. Overall Experimental Design

The overall experimental design is shown in Figure 1. Pennycress biomass was harvested at three growth stages and used as feedstock in AD. The effects of the total solids (TSs), inoculation rate, and particle size on the methane yield were evaluated. In addition, the harvested biomass was pretreated with alkali and ensiling, and then used as AD feedstock for the methane yield.

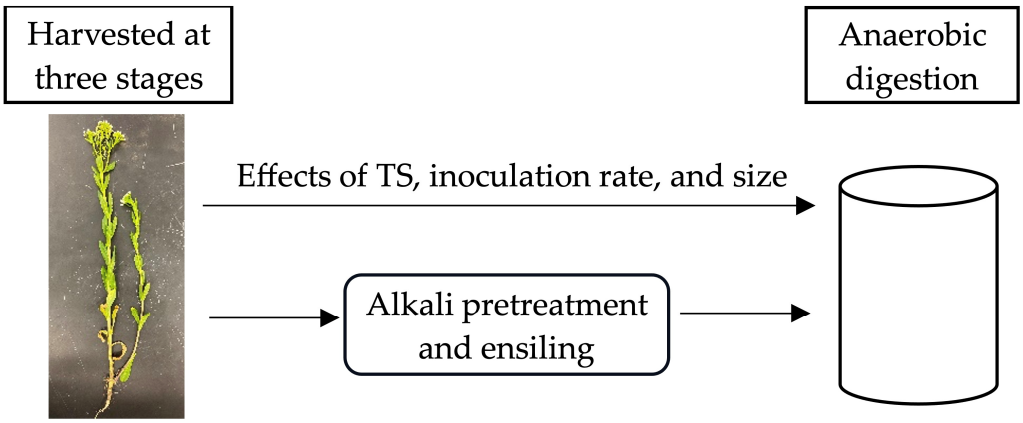

Figure 1. Overall experimental design.

2.2. AD Feedstocks, Inoculum, and Micronutrients

Cover crop biomass. Pennycress breeding line B36, which did not contain domestication traits, including the reduced seed coat fiber "yellow-seed" trait, was planted on the Illinois State University (ISU) farm near Lexington, IL (lat. 40°30′ N), USA, and harvested three times in 2023. The first harvest was on 27 April 2023. The plants were at the growth stages of 6.9–7.1, close to their final total height, and 90% of flowers opened. The biomass was green and had a high moisture content. The second harvest was taken on 12 May 2023 at the growth stage of 7.8–7.9, with 80–90% of pods fully sized. Biomass had started to turn yellow. The third harvest was on 26 May 2023 at the growth stage of 9; the plants were mature and uniformly yellow, but the pod shatter was not yet occurring. More information about the pennycress crop growth stages can be found in [46]. The length and weight of the plant biomass were measured using 10 randomly selected stalks with the root tissue removed. Biomass (without the root) was chopped to 2.54 cm (1 inch) or 1.27 cm (0.5 inch) pieces, and then stored at −10 °C prior to use.

Biomass concurrent alkali pretreatment and ensiling. Pennycress biomass was chopped into 2.54 cm pieces and then loaded into plastic silage bags. NaOH (97+%, Certified ACS, Fisher Chemical, Norristown, PA, USA) powders were added at a dosage of 5% of the biomass dry matter, similar to the dosages (4–10%) used in other studies [50,51]. The alkali powders were mixed with the biomass by shaking. The bags were pressed to push out air, then sealed and put inside of an airtight plastic container for one month in a dark

environment at room temperature (approximately 20 °C). Both the fresh and pretreated biomass were used as AD feedstocks.

Effluent and micronutrients. The inoculum utilized in this study was digestion effluent obtained from a mesophilic anaerobic digester operated by BNWRD (Bloomington Normal Water Reclamation District in Bloomington, IL, USA). Municipal sewage sludge was fed into this digester. Digestion effluent contains nutrients and microbes adapted to the anaerobic digestion process. To preserve the quality of the inoculum, it was kept in a 37 °C incubator before use. Because most lignocellulosic biomass, including the pennycress biomass, lack adequate micronutrients for AD, commercial BioGas1 AD micronutrients (purchased from Aquafic, Inc., Madison, WI, USA) containing cobalt, nickel, and iron were added to help stabilize the AD process.

2.3. AD Experimental Setup

Batch AD experiments were carried out in 0.5-L glass reactors. Each condition was triplicated. Reactors were kept inside of incubators with temperature controlled at 37 °C. To test the effects of solids content on the methane yield, digestion effluent and deionized (DI) water were added to achieve overall total solid (TS) contents of 4%, 6%, and 8% (wet basis). The inoculation rate (feedstock/inoculum, also known as F/I ratio, based on volatile solid contents) was controlled at 2.0. A total of 54 reactors (3 growth stages × 2 biomass sizes × 3 solids contents × 3 reactors for each condition) were created as treatments and two reactors without feedstock were created as controls. In addition, 6 reactors were created to test the effects of inoculation rate (adjusted the F/I ratio from 2.0 to 1.0) and another 6 reactors were created to test the effects of the concurrent alkali pretreatment and ensiling on biogas yield, respectively. Each reactor received 1 mL of BioGas1 micronutrient based on the manufacturer's recommendations. A 5 L gas bag (purchased from the CEL Scientific, Santa Fe Springs, CA, USA) was connected to each reactor to collect biogas every 2–7 days, depending on the volume of biogas produced during the experimental period.

2.4. Sampling and Analytical Methods

The concentrations of CH_4 in the collected biogas samples were measured using a biogas analyzer (Landtec Biogas 5000, Dexter, MI, USA). The analyzer was calibrated by the manufacturer before the test and was checked monthly using the calibration gas (mixture 3: 60% CH_4 and 40% CO_2) and 100 ppm H_2S, both purchased from Landtec. To reduce the interference of water vapor on the biogas measurement, an in-line membrane filter was installed to remove water vapor prior to the analysis. The filter was replaced every month. The volume of the biogas produced from the bench tests was measured at a normal lab condition (approximately 20 °C) using the built-in flowrate meter with a flowrate of 0.55 lpm (liter per minute).

Liquid and solid samples were collected at the beginning and the end of the experiments. The digested feedstock samples were stored in a −20 °C freezer before analysis. The TSs, volatile solids (VSs), pH, alkalinity, and ammonium-N of the samples were measured based on a slightly revised Standard Methods Examination of Water and Wastewater [52]. Specifically, the samples were dried in an oven (Fisher Scientific Isotemp) at 105 °C for 24 h to calculate the TS content based on the weight difference. The dried samples were then put into a 450 °C oven (Fish-Scientific Isotemp Muffle Furnace, West Sacramento, CA, USA) for 4 h to measure the volatile compounds. The pH value and alkalinity were found by diluting a 10 g sample with DI water for a total of 100 g and then measured using a pH titrator (Hach AT1000 Potentiometric Titrator, Hach Company, Loveland, CO, USA). Ammonium-N was analyzed using a Hach DR1900 Spectrophotometer, following the Hach method 10031. Solid samples were analyzed by a commercial lab (Rock River Laboratory, Watertown, WI, USA) for the crude protein, ADF (acid detergent fiber), NDF (neutral detergent fiber), lignin, total C, and total N using AOAC and Ankom Technologies methods.

2.5. Data Analysis

Measured biogas and methane production data were fitted into a modified Gompertz model (Equation (1)) to determine the theoretical cumulative methane potential (P), maximum daily methane yield (R_m), and lag phase (λ) during the AD process, where H is the amount of methane that is calculated at a given time *t*.

$$H(t) = P \times \left\{ -\exp\left[\frac{R_m \times e}{P} (\lambda - t) + 1 \right] \right\} \quad (1)$$

Details about this model have been provided in a previous study [53]. The three parameters and their standard errors were calculated using an online tool (https://mt.procycla.es/bmp/app, accessed on 30 November 2023). ANOVA (analysis of variance) and *t* tests were conducted to compare the digester performance, such as the methane yields, using software R Studio (Version 2023.09.1 + 494, Posit Software, Boston, MA, USA, Statistical analysis). For the figures and tables, the mean averages and standard errors were reported. Outliers were removed prior to the mean average and standard error calculations. A significance level of 0.05 was used. Figures were created using the software SigmaPlot (Version 13, SPSS Inc., Chicago, IL, USA).

3. Results and Discussions

3.1. Effects of Harvest Date on Biomass Characteristics

Pennycress plants from the first harvest (see Materials and Methods) were relatively shorter than the plants from the other two harvests, while the biomass from the third harvest had a significantly ($p < 0.05$) lower wet weight per stem (Figure 2 and Table 1). The measured dry matter per stem was almost the same from each of the three harvests. The contents of ADF, NDF, lignin, and C increased significantly as the plants grew and matured. The C content in the third-harvest biomass (49.0%) was much higher than its content in the first-harvest biomass (40.7%). The crude protein and N contents increased from the first harvest to the second harvest, but then slightly decreased in the third harvest as the plant reached full maturity and the yellowing stage. Similar compositional changes were observed by Meserszmit et al. [48], who reported higher contents of lignin and NDF in *Molinia* meadows biomass collected on later harvest dates and lower N content at maturity. Yield and changes in pennycress biomass composition can be important factors for farmers to determine harvest timing.

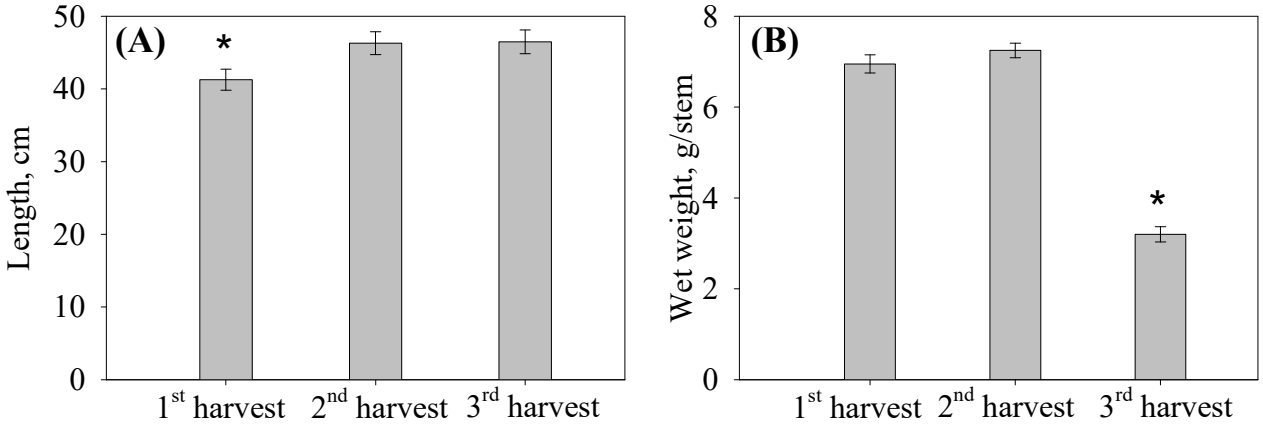

Figure 2. Measured pennycress plant height ("length") at the time of harvest (**A**) and corresponding wet weight (**B**). The * indicates a significant difference (see Materials and Methods).

Table 1. Composition of the pennycress biomass.

Composition	1st Harvest	2nd Harvest	3rd Harvest
Length, cm	41.28 ± 1.46	46.29 ± 1.59	46.48 ± 1.64
Wet wt, g/stem	6.95 ± 0.20	7.25 ± 0.16	3.20 ± 0.17
Dry matter, g/stem	1.65 ± 0.04	1.67 ± 0.04	1.62 ± 0.09
Total solids, %	23.81 ± 0.35	23.03 ± 0.13	50.55 ±1.13
Volatile solids, %	21.62 ± 0.31	21.34 ± 0.11	45.97 ± 1.02
Protein, %	13.56 ± 0.91	14.44 ± 0.01	13.19 ± 0.28
ADF, %	32.69 ± 1.40	37.84 ± 0.58	41.47 ± 1.50
NDF, %	36.59 ± 0.01	43.85 ± 1.29	44.95 ± 0.01
Lignin, %	6.81 ± 0.17	8.71 ± 0.01	8.95 ± 0.02
C, %	40.70 ± 1.10	43.65 ± 0.25	49.00 ± 0.10
N, %	2.09 ± 0.07	2.31 ± 0.01	2.12 ± 0.05

Note: Composition was measured from plant biomass (stems and leaves) cut into 2.54 cm lengths. Wet weight, total solids, and volatile solids are the wet basis. All others are the dry basis.

3.2. Effects of Harvest Date, Solids Content, and Particle Size on Methane Yield

Methane yields from the biomass harvested at three different stages varied dramatically. Digesters with the first-harvest biomass failed to produce biogas from all solids and particle size conditions (Figure 3), likely due to the low pH (below 6.5) and alkalinity conditions (Table 2) [54]. Digesters usually need to be kept at a pH of 7.5–8.5 and an alkalinity of 3000–5000 mg/L $CaCO_3$ for normal operation [54]. It is likely that the organic compounds in the immature first-harvest biomass were quickly converted into short-chain fatty acids (SCFAs), which caused the pH drop and digestion failure. Reducing the biomass size to 1.27 cm could have enhanced the transportation and formation of SCFAs, which lowered the pH values even more (Table 2). Inhibition due to a low pH also occurred in the digesters with the second-harvest biomass. The methane yields were below 50 L/kg-VS. Digesters with the third-harvest biomass had normal pH conditions and produced 171.80 ± 4.82 L of methane per kg-VS at a TS of 4%, as shown in Figure 2A and Table S1, which is still lower than reported methane yields from the other lignocellulosic biomass. For example, previous studies have reported methane yields of 187, 182–285, and 179–274 L/kg-VS from cereal rye [55], corn stover [56,57], and wheat straw [58,59], respectively.

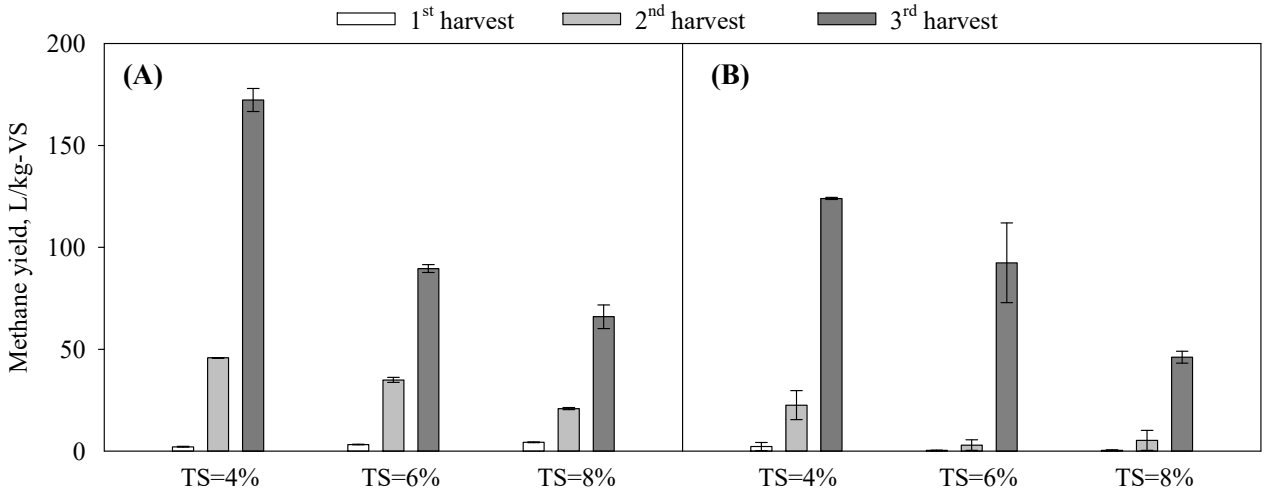

Figure 3. Measured methane yields at a F/I ratio of 2, from biomass cut to 2.54 cm lengths (**A**) or to 1.27 cm lengths (**B**). Legend for the biomass type assayed is located above the graphs. Numerical data are available in Table S1.

Table 2. Characteristics of feedstocks after AD.

	2.54 cm Biomass			1.27 cm Biomass		
TS	**1st Harvest**	**2nd Harvest**	**3rd Harvest**	**1st Harvest**	**2nd Harvest**	**3rd Harvest**
		pH			pH	
4%	5.94 ± 0.07	7.24 ± 0.61	8.13 ± 0.14	5.02 ± 0.04	7.27 ± 0.90	7.91 ± 0.20
6%	6.22 ± 0.02	6.47 ± 0.30	8.12 ± 0.06	5.41 ± 0.02	6.46 ± 1.17	8.31 ± 0.13
8%	6.48 ± 0.14	7.35 ± 0.59	8.27 ± 0.27	5.49 ± 0.02	6.39 ± 0.98	7.95 ± 0.10
		Alkalinity, mg/L $CaCO_3$			Alkalinity, mg/L $CaCO_3$	
4%	1854 ± 0	2414 ± 177	3058 ± 243	1052 ± 219	2679 ± 612	2710 ± 254
6%	3507 ± 157	3240 ± 197	3616 ± 143	3244 ± 79	2995 ± 658	3461 ± 56
8%	4665 ± 91	4457 ± 379	4331 ± 34	3469 ± 5	3713 ± 425	4293 ± 76
		NH_4-N, mg/L			NH_4-N, mg/L	
4%	787 ± 34	692 ± 34	617 ± 29	663 ± 3	675 ± 25	608 ± 18
6%	1067 ± 44	1035 ± 51	842 ± 25	1220 ± 0	953 ± 28	903 ± 43
8%	1475 ± 13	1353 ± 34	1190 ± 30	1220 ± 35	1228 ± 18	1163 ± 23

The effects of the solids content on the biogas yield were also significant. At a TS of 6% and 8%, the methane yields were much lower than at a TS of 4%. A high solids content could have accumulated inhibitors, such as NH_4-N (Table 2), and reduced the accessibility of biodegradable organic compounds to methanogens, resulting in the inhibition in the biogas production [60]. In most conditions, the reduced particulate size can increase the biogas yield as it improves the lignocellulosic degradability [61]. However, for feedstocks that contain or can generate high levels of fatty acids, such as food waste, an excessive reduction in the particle size of the feedstock can result in fatty acid accumulation, which reduces the methane production and digester stability [62]. For this study, lower pH values were noted in the digesters with the 1.27 cm biomass, suggesting an overaccumulation of fatty acids which caused the lower methane yields with a higher variance than the digesters with the 2.54 cm biomass [63].

3.3. Effects of Inoculation Rate and Concurrent Alkali Pretreatment and Ensiling on Methane Yield

Adding the extra inoculum, i.e., adjusting the F/I ratio from 2 to 1, did not save the digesters with the first-harvest biomass or improve the performance of digesters with the second-harvest biomass (Figure 4A vs. Figure 3A). An even higher inoculation rate likely will be needed to buffer the acids generated from this biomass. The methane yield from the third-harvest biomass increased 17.50% from 171.80 ± 4.82 to 202.00 ± 2.17 L/kg-VS (Figure 4A and Table S2). The average pH value (8.65), alkalinity (5435 mg/L $CaCO_3$), and NH_4–N concentration (1252 mg/L) were all slightly higher than at F/I of 2 and were within normal ranges [60,64].

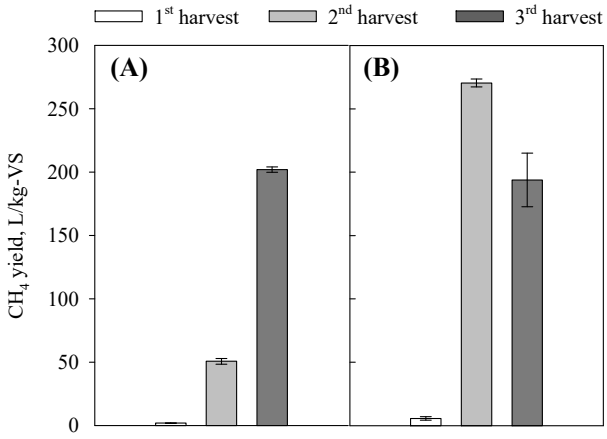

Figure 4. Measured methane yields from biomass cut into 2.54 cm lengths. (**A**) Biomass with an increased inoculation rate of F/I = 1; (**B**) Biomass after concurrent alkali pretreatment and ensiling. Numerical data are available in Table S2.

After the concurrent alkali pretreatment and ensiling, the average methane yield from the third-harvest biomass was 193.9 ± 21.2 L/kg-VS (Figure 4B and Table S2), which was higher, but not significantly different (p = 0.416), from the same biomass without the alkali pretreatment (171.80 ± 4.82 L/kg-VS, Figure 3A). The third-harvest biomass was dry, so the distribution of alkali and its reaction with fatty acids during the ensiling process could be uneven, which could have caused the high variance of methane yields (Figure 4B). The pH and alkalinity of the digested feedstock are included in Supplementary Materials Table S3.

The methane yields from the second-harvest biomass (270.4 ± 3.10 L/kg-VS, Figure 4B and Table S2) were 5.9-fold and significantly higher than the biomass without the alkali pretreatment (45.7 ± 0.21 L/kg-VS, Figure 3A). It is well established that organics can be converted to acids during wet ensiling [65]. These acids can be neutralized by the added alkali, resulting in the organics being suitable for methanogens to produce methane gas. The pH value and alkalinity were 7.96 and 4,676 mg/L $CaCO_3$, respectively, which are within the normal operational ranges. The 5.9-fold increase in the methane production shows the potential of using concurrent alkali pretreatment and ensiling as an effective method to buffer acids and improve biogas production for wet pennycress biomass. Similar synergistic effects were reported in previous studies. For example, a 5–15% methane yield increase was observed from coensiling cover crops and barley straw [66], and a significant methane yield increase was obtained from two cover crops after long-term alkaline pretreatment and storage [67,68].

The reactors with the first-harvest biomass, however, still failed to produce methane gas after the pretreatment due to the low pH condition (5.47 ± 0.09, Table S3). Likely an even higher alkali dosage, i.e., 8–10% of biomass dry matter, will be needed to produce methane gas from early harvested pennycress [50,51]. However, this will increase the cost of digestion.

The biomass characteristics and AD conditions also impacted the methane production dynamics. As shown in Figure 5, different cumulative methane yield curves were obtained from the four experiments. The third-harvest biomass that was alkali pretreated showed the greatest initial rate of methane production and cumulative yield. These data support the hypothesis that the concurrent alkali pretreatment and ensiling prepared the biomass for AD by converting organic compounds into digestible acids for methane production. The extremely short lag phase (λ), 0.28 days based on modeling results (Table 3), also suggested that the compounds were immediately available for methane production [69]. Similarly, a short lag phase (1.35 days) and a high maximum daily yield (R_m = 21.2 L/kg-VS.d) were obtained from the pretreated second-harvest biomass (Table 3). The higher inoculation rate (F/I of 1) also shortened the lag phase (5.92 days) compared to the experiment using F/I of 2 (20.6 days; Table 3).

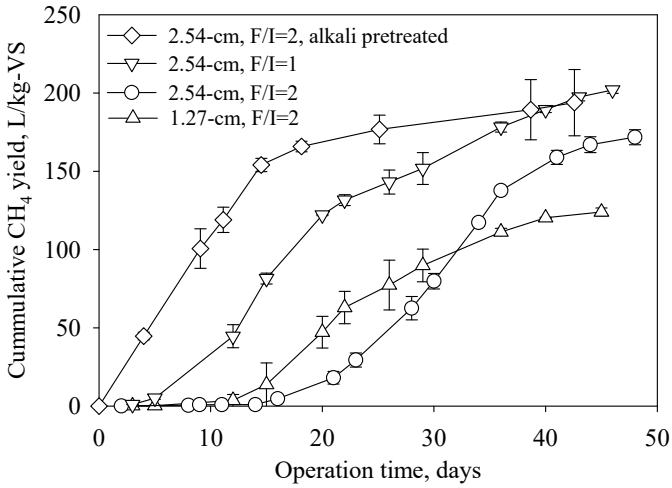

Figure 5. Cumulative methane yields from the third-harvest biomass at the four conditions.

Table 3. Modeling results based on the modified Gompertz equation.

Biomass, AD Conditions	P (L/kg-VS)	R_m (L/kg-VS.d)	λ (d)
1.27 cm, 3rd harvest, F/I = 2, TS = 4%	130 ± 6.09	6.24 ± 0.49	12.8 ± 0.73
2.54 cm 3rd-harvest biomass, F/I = 2, TS = 4%	181 ± 4.5	9.15 ± 0.50	20.6 ± 0.51
2.54 cm 3rd-harvest biomass, F/I = 1, TS = 4%	200 ± 8.2	8.22 ± 0.78	5.92 ± 1.13
2.54 cm, 3rd harvest, F/I = 2, TS = 4%, pretreated	191 ± 3.31	11.7 ± 0.81	0.28 ± 0.63
2.54 cm, 2nd harvest, F/I = 2, TS = 4%, pretreated	266 ± 3.01	21.2 ± 1.08	1.35 ± 0.37

Organic matter loss and associated energy loss are other important factors to consider for biomass storage. Organics can be vaporized, contaminated, used by microbes for reproduction, or converted to CO_2, and losses may reach up to 60% [70]. There are several important factors affecting the organic matter and energy loss during storage, such as the moisture content and compactness of the biomass, the amount of oxygen available to the microbes, the pH and temperature conditions, and the storage duration. Inappropriate storage can cause methane yield reduction. For example, Vlierberghe et al. reported that the wet storage of cover crops led to a high butyric acid accumulation and a 13% reduction in the biomethane potential [67]. For this study, a decrease in the VS content (wet basis) was observed in all samples. On average, reductions of 23.9%, 9.9%, and 3.3% in the VS content were measured from the first-, second-, and third-harvest biomass, respectively, after the concurrent alkali pretreatment and ensiling. The wet samples lost more volatile solids than the dry samples, which is similar to findings in other studies [67,68].

With the VS reduction considered, the concurrent alkali pretreatment and ensiling still increased the overall methane yield by 450% (4.5-fold), and 6% for the second- and third-harvest biomass, respectively. The 6% increase from the third-harvest biomass was not significant, but the AD retention time likely can be shortened for large-scale applications. The 4.5-fold increase for the second-harvest biomass was significant and may be further improved if the alkali dosage and ensiling conditions are optimized. A longer-term (4–8 months) concurrent alkali pretreatment and ensiling test will be needed to evaluate its effects on organic matter loss and methane yield for year-round biomass storage and anaerobic digestion. Based on the data obtained from this experiment (methane yield = 270.4 L/kg-VS, VS/TS = 0.93, VS loss during ensiling = 9.88%, biogas temperature = 20 °C), the estimated pennycress dry matter yield of 2 MT/ha [71], and the 70% of biogas energy available for output, the potential energy output from the pretreated second-harvest biomass was estimated to be 11,496 MJ/ha, equal to 95 GGE/ha (gallon gasoline equivalent).

4. Conclusions

The harvest date affected the pennycress biomass length, wet weight, composition, and biogas yield. Higher contents of ADF, NDF, lignin, and C were found in biomass collected on later harvest dates. The digesters with the first-harvest biomass failed due to the pH drop, and the performance did not improve with a higher inoculation rate. Without the alkali pretreatment, the highest methane yield of 171.80 ± 4.82 L/kg-VS was obtained from the 2.54 cm, third-harvest biomass at a solid content of 4% and a feedstock/inoculum ratio of 2. After the one-month concurrent alkali pretreatment and ensiling, the volatile solid content was reduced by 23.9%, 9.9%, and 3.3% in the first-, second-, and third-harvest biomass, respectively. However, this pretreatment conditioned the biomass to reduce the inhibition, and the overall methane yield from the pretreated second-harvest biomass was significantly increased to 270.4 ± 3.10 L/kg-VS, a 4.5-fold increase from the untreated second-harvest biomass with the organic matter loss adjusted. Modeling results suggested that the treatment also shortened the anaerobic digestion lag phase. We conclude that, with proper pennycress biomass pretreatment, it could be an attractive feedstock for biomethane production. Pilot-scale tests, technoeconomic analysis, and environmental impact studies can be the next steps.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation10020096/s1, Table S1: Measured methane yields (L/kg-VS) at an F/I ratio of 2 from biomass cut to 2.54 cm or 1.27 cm lengths. Table S2: Measured methane yields (L/kg-VS) from biomass cut into 2.54 cm lengths. Table S3: Characteristics of the AD digestate after alkali pretreatment and ensiling.

Author Contributions: Conceptualization, J.C.S. and L.Y.; Methodology, T.Y.L., M.A.M. and L.Y.; Validation, J.C.S. and L.Y.; Formal Analysis, T.Y.L. and M.A.M.; Investigation, T.Y.L., M.A.M. and B.G.; Resources, J.C.S., L.Y., N.J.H., R.L.R. and D.E.K.; Data Curation, T.Y.L. and L.Y.; Writing—Original Draft Preparation, L.Y., T.Y.L. and B.G.; Writing—Review and Editing, L.Y., N.J.H., R.L.R., D.E.K. and J.C.S.; Visualization, L.Y.; Supervision, L.Y., J.C.S. and R.L.R.; Project Administration, J.C.S. and L.Y.; Funding Acquisition, J.C.S., L.Y. and R.L.R. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the U.S. Department of Agriculture, Agriculture and Food Research Initiative Competitive, grant no. 2019–69012-29851, to J.C.S. and N.J.H., and the Iowa Soybean Association (no grant no.) to L.Y. and R.L.R.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data are within the manuscript and Supplementary Materials.

Acknowledgments: This study was supported by ISU Farm manager Jason Lindbom and his crews. Pennycress B36 seeds were kindly provided by CoverCress, Inc.

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

References

1. Wallander, S.; Smith, D.; Bowman, M.; Classeen, R. *Cover Crop Trends, Programs, and Practices in the United States*; EIB 222; U.S. Department of Agriculture, Economic Research Service: Washington, DC, USA, 2021.
2. Warwick, S.I.; Francis, A.; Susko, D.J. The Biology of Canadian Weeds. 9. *Thlaspi arvense L. (Updated). Can. J. Plant Sci.* **2002**, *82*, 803–823. [CrossRef]
3. Eerd, L.L.V.; Chahal, I.; Peng, Y.; Awrey, J.C. Influence of Cover Crops at the Four Spheres: A Review of Ecosystem Services, Potential Barriers, and Future Directions for North America. *Sci. Total Environ.* **2023**, *858*, 159990. [CrossRef] [PubMed]
4. Park, B.; Rejesus, R.M.; Aglasan, S.; Che, Y.; Hagen, S.C. Payments from Agricultural Conservation Programs and Cover Crop Adoption. *Appl. Econ. Perspect. Policy* **2023**, *45*, 984–1007. [CrossRef]
5. Wittwer, R.; Dorn, B.; Jossi, W.; Heijden, M.V. Cover Crops Support Ecological Intensification of Arable Cropping Systems. *Sci. Rep.* **2017**, *7*, 41911. [CrossRef]
6. Bergtold, J.S.; Duffy, P.A.; Hite, D.; Raper, R.L. Demographic and Management Factors Affecting the Adoption and Perceived Yield Benefit of Winter Cover Crops in the Southeast. *J. Agric. Appl. Econ.* **2012**, *44*, 99–116. [CrossRef]
7. Castellano, M.J.; Helmers, M.J.; Sawyer, J.E.; Barker, D.W.; Christianson, L. Nitrogen, Carbon, and Phosphorus Bal-ances in Iowa Cropping Systems: Sustaining the Soil Resource. In Proceedings of the 2012 Integrated Crop Management Conference, Iowa State University, Ames, IA, USA, 28–29 November 2012; pp. 145–156.
8. Kladivko, E.J.; Kaspar, T.C.; Jaynes, D.B.; Malone, R.W.; Singer, J.; Morin, X.K.; Searchinger, T. Cover Crops in the Upper Midwestern United States: Potential Adoption and Reduction of Nitrate Leaching in the Mississippi River Basin. *J. Soil Water Conserv.* **2014**, *69*, 279–291. [CrossRef]
9. Snapp, S.; Swinton, S.; Labarta, R.; Mutch, D.; Black, R.; Leep, R.; O'Neil, K. Evaluating Cover Crops for Benefits, Costs and Performance within Cropping System Niches. *Agron. J.* **2015**, *97*, 322–332. [CrossRef]
10. Poeplau, C.; Don, A. Carbon Sequestration in Agricultural Soils via Cultivation of Cover Crops—A Meta-Analysis. *Agric. Ecosyst. Environ.* **2015**, *200*, 33–41. [CrossRef]
11. Laloy, E.; Bielders, C. Effect of Intercropping Period Management on Runoff and Erosion in a Maize Cropping System. *J. Environ. Qual.* **2010**, *39*, 1001–1008. [CrossRef]
12. Kaye, J.P.; Quemada, M. Using Cover Crops to Mitigate and Adapt to Climate Change. A Review. *Agron. Sustain. Dev.* **2017**, *37*, 4. [CrossRef]
13. Myers, R.; Watts, C. Progress and Perspectives with Cover Crops: Interpreting Three Years of Farmer Surveys on Cover Crops. *J. Soil Water Conserv.* **2015**, *70*, 125A–129A. [CrossRef]
14. USEPA. Sources of Greenhouse Gas Emissions. Available online: https://www.epa.gov/ghgemissions/sources-greenhouse-gas-emissions (accessed on 27 November 2023).

15. Lemaire, G.; Franzluebbers, A.; Carvalho, P.C.; Dedieu, B. Integrated Crop–Livestock Systems: Strategies to Achieve Synergy between Agricultural Production and Environmental Quality. *Agric. Ecosyst. Environ.* **2014**, *190*, 4–8. [CrossRef]
16. Planisich, A.; Utsumi, S.A.; Larripa, M.; Galli, J.R. Grazing of Cover Crops in Integrated Crop-Livestock Systems. *Animal* **2021**, *15*, 100054. [CrossRef]
17. Finney, D.M.; Creamer, N.G.; Schultheis, J.R.; Wagger, M.G.; Brownie, C. Sorghum Sudangrass as a Summer Cover and Hay Crop for Organic Fall Cabbage Production. *Renew. Agric. Food Syst.* **2009**, *24*, 225–233. [CrossRef]
18. Franzluebbers, A.J.; Stuedemann, J.A. Soil Physical Responses to Cattle Grazing Cover Crops under Conventional and No Tillage in the Southern Piedmont USA. *Soil Tillage Res.* **2008**, *100*, 141–153. [CrossRef]
19. Yang, L.; Lamont, L.D.; Liu, S.; Guo, C.; Stoner, S. A Review on Potential Biofuel Yields from Cover Crops. *Fermentation* **2023**, *9*, 912. [CrossRef]
20. Robertson, K.A. Biomass Potential in Sustainable Aviation Fuel Development: Switchgrass Production Optimization and Carinata Oilseed Enterprise Viability Analysis. Master's Thesis, University of Tennessee, Knoxville, Tennessee, 2020.
21. Rezki, B.; Essamlali, Y.; Aadil, M.; Semlal, N.; Zahouily, M. Biodiesel production from rapeseed oil and low free fatty acid waste cooking oil using a cesium modified natural phosphate catalyst. *RSC Adv.* **2020**, *10*, 41065–41077. [CrossRef]
22. Fan, J.; Shonnard, D.R.; Kalnes, T.N.; Johnsen, P.B.; Rao, S. A Life Cycle Assessment of Pennycress (*Thlaspi arvense* L.)—Derived Jet Fuel and Diesel. *Biomass Bioenergy* **2013**, *55*, 87–100. [CrossRef]
23. Trejo-Pech, C.O.; Larson, J.A.; English, B.C.; Yu, T.E. Cost and Profitability Analysis of a Prospective Pennycress to Sustainable Aviation Fuel Supply Chain in Southern USA. *Energies* **2019**, *12*, 3055. [CrossRef]
24. Phippen, W.B.; Phippen, M.E. Soybean seed yield and quality as a response to field pennycress residue. *Crop Sci.* **2012**, *52*, 2767. [CrossRef]
25. Chopra, R.; Johnson, E.B.; Emenecker, R.; Cahoon, E.B.; Lyons, J.; Kliebenstein, D.J. Identification and Stacking of Crucial Traits Required for the Domestication of Pennycress. *Nat. Food* **2020**, *1*, 84–91. [CrossRef]
26. McGinn, M.; Phippen, W.B.; Chopra, R.; Bansal, S.; Jarvis, B.A.; Phippen, M.E. Molecular Tools Enabling Pennycress (*Thlaspi arvense*) as a Model Plant and Oilseed Cash Cover Crop. *Plant Biotechnol. J.* **2018**, *17*, 776–788. [CrossRef]
27. Marks, M.; Chopra, R.; Sedbrook, J. Technologies enabling rapid crop improvements for sustainable agriculture: Example pennycress (*Thlaspi arvense* L.). *Emerg. Top. Life Sci.* **2021**, *5*, 325–335. [CrossRef]
28. Jarvis, B.A.; Romsdahl, T.B.; McGinn, M.G.; Nazarenus, T.J.; Cahoon, E.B.; Chapman, K.D.; Sedbrook, J.C. CRISPR/Cas9-Induced Fad2 and Rod1 Mutations Stacked With Fae1 Confer High Oleic Acid Seed Oil in Penny-cress (*Thlaspi arvense* L.). *Front. Plant Sci.* **2021**, *12*, 652319. [CrossRef] [PubMed]
29. Phippen, W.B.; Rhykerd, R.; Sedbrook, J.C.; Handel, C.; Csonka, S. From Farm to Flight: CoverCress as a Low Carbon Intensity Cash Cover Crop for Sustainable Aviation Fuel Production. A Review of Progress Towards Commercialization. *Front. Energy Res.* **2022**, *10*, 793776. [CrossRef]
30. Moser, B.R.; Knothe, G.; Vaughn, S.F.; Isbell, T.A. Production and Evaluation of Biodiesel from Field Pennycress (*Thlaspi arvense* L.) Oil. *Energy Fuels* **2009**, *23*, 4149–4155. [CrossRef]
31. Mousavi-Avval, S.H.; Shah, A. Life Cycle Energy and Environmental Impacts of Hydroprocessed Renewable Jet Fuel Production from Pennycress. *Appl. Energy* **2021**, *297*, 117098. [CrossRef]
32. Sedbrook, J.C.; Phippen, W.B.; Marks, M.D. New Approaches to Facilitate Rapid Domestication of a Wild Plant to an Oilseed Crop: Example Pennycress (*Thlaspi arvense* L.). *Plant Sci.* **2014**, *227*, 122–132. [CrossRef]
33. Sun, X.; Boardman, K.; Marks, D.; Wyse, D.L.; Hu, B. Fungal Bioprocessing to Improve Quality of Pennycress Meal as a Potential Feeding Ingredient for Monogastric Animals. *Fermentation* **2023**, *9*, 732. [CrossRef]
34. Alhotan, R.A.; Wang, R.L.; Holser, R.A.; Pesti, G.M. Nutritive Value and the Maximum Inclusion Level of Pennycress Meal for Broiler Chickens. *Poult. Sci.* **2017**, *96*, 2281–2293. [CrossRef]
35. Hartnell, G.F.; Lemke, S.; Moore, D.; Matthews, A.; Nemeth, M.A.; Brister, R.; Liu, S.; Aulbach, C. Performance and health of broiler chickens fed low erucic acid, lower fiber pennycress (CoverCressTM) grain. *Poult. Sci.* **2023**, *102*, 102432. [CrossRef]
36. Mousavi-Avval, S.; Hkanal, S.; Shah, A. Assessment of Potential Pennycress Availability and Suitable Sites for Sustainable Aviation Fuel Refineries in Ohio. *Sustainability* **2023**, *15*, 10589. [CrossRef]
37. Markel, E.; English, B.C.; Hellwinckel, C.; Menard, R.J. Potential for Pennycress to Support a Renewable Jet Fuel Industry. *Ecol. Pollut. Environ. Sci.* **2018**, *4*, 95–102. Available online: https://hdl.handle.net/2376/111635 (accessed on 20 October 2023).
38. Vaughn, S.F.; Isbell, T.A.; Weisleder, D.; Berhow, M.A. Biofumigant compounds released by field pennycress (*Thlaspi arvense*) seedmeal. *J. Chem. Ecol.* **2005**, *31*, 167–177. [CrossRef]
39. Moore, S.A.; Wells, M.S.; Gesch, R.W.; Becker, R.L.; Rosen, C.J.; Wilson, M.L. Pennycress as a Cash Cover-Crop: Improving the Sustainability of Sweet Corn Production Systems. *Agronomy* **2020**, *10*, 614. [CrossRef]
40. Xu, F.; Li, Y.; Ge, X.; Yang, L.; Li, Y. Anaerobic digestion of food waste—Challenges and opportunities. *Bioresour. Technol.* **2018**, *247*, 1047–1058. [CrossRef]
41. Ampese, L.; Sganzerla, W.G.; Ziero, H.D. Research progress, trends, and updates on anaerobic digestion technology: A bibliometric analysis. *J. Clean. Prod.* **2022**, *331*, 130004. [CrossRef]

42. Bhatnagar, N.; Ryan, D.; Murphy, R.; Enright, A.M. A comprehensive review of green policy, anaerobic digestion of animal manure and chicken litter feedstock potential—Global and Irish perspective. *Renew. Sustain. Energy Rev.* **2022**, *154*, 111884. [CrossRef]
43. Ervasti, S.; Kostensalo, J.; Tampio, E. Effects of seasonal and local co-feedstocks on the performance of continuous anaerobic digestion of cattle slurry. *Bioresour. Technol. Rep.* **2022**, *19*, 101207. [CrossRef]
44. Yang, L.; Ge, X.; Wan, C.; Yu, F.; Li, Y. Progress and perspectives in converting biogas to transportation fuels. *Renew. Sustain. Energy Rev.* **2014**, *40*, 1133–1152. [CrossRef]
45. Ge, X.; Yang, L.; Sheet, J.P.; Yu, Z.; Li, Y. Biological conversion of methane to liquid fuels: Status and opportunities. *Biotechnol. Adv.* **2014**, *32*, 1460–1475. [CrossRef]
46. Verhoff, K.; Phippen, W.; Heller, N.; Lindsey, A. Winter-type oilseed pennycress crop staging guide. *Crop Forage Turfgrass Manag.* **2022**, *8*, e20165. [CrossRef]
47. Mast, B.; Lemmer, A.; Oechsner, H.; Reinhardt-Hanisch, A.; Claupein, W.; Graeff, S. Methane Yield Potential of Novel Perennial Biogas Crops Influenced by Harvest Date. *Ind. Crop. Prod.* **2014**, *58*, 194–203. [CrossRef]
48. Meserszmit, M.; Chrabąszcz, M.; Chylińska, M.; Szymańska-Chargot, M.; Trojanowska-Olichwer, A.; Kącki, Z. The Effect of Harvest Date and the Chemical Characteristics of Biomass from Molinia Meadows on Methane Yield. *Biomass Bioenergy* **2019**, *130*, 105391. [CrossRef]
49. Wendt, L.M.; Zhao, H. Review on Bioenergy Storage Systems for Preserving and Improving Feedstock Value. *Front. Bioeng. Biotechnol.* **2020**, *8*, 370. [CrossRef]
50. Kim, J.S.; Lee, Y.Y.; Kim, T.H. A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. *Bioresour. Technol.* **2015**, *199*, 42–48. [CrossRef]
51. Chandra, R.; Takeuchi, H.; Hasegawa, T.; Kumar, R. Improving biodegradability and biogas production of wheat straw substrates using sodium hydroxide and hydrothermal pretreatments. *Energy* **2012**, *43*, 273–282. [CrossRef]
52. Rice, E.W.; Baird, R.B.; Eaton, A.D. *Standard Methods for the Examination of Water and Wastewater*, 23rd ed.; American Public Health Association; American Water Works Association: Washington, DC, USA; Water Environment Federation: Alexandria, VA, USA, 2017.
53. Dechrugsa, S.; Kantachote, D.; Chaiprapat, S. Effects of inoculum to substrate ratio, substrate mix ratio and inoculum source on batch co-digestion of grass and pig manure. *Bioresour. Technol.* **2013**, *146*, 101–108. [CrossRef]
54. Anderson, G.K.; Yang, G. pH Control in Anaerobic Treatment of Industrial Wastewater. *J. Environ. Eng.* **1992**, *118*, 551–567. [CrossRef]
55. Yang, L.; Lamont, L.; Sedbrook, J.; Heller, N.; Kopsell, D. Anaerobic Digestion of Cereal Rye Cover Crop. *Fementation* **2022**, *8*, 617. [CrossRef]
56. Wang, S.; Li, F.; Wu, D.; Zhang, P.; Wang, H.; Tao, X.; Ye, J.; Nabi, M. Enzyme pretreatment enhancing biogas yield from corn stover: Feasibility, optimization, and mechanism analysis. *J. Agric. Food Chem.* **2018**, *66*, 10026–10032. [CrossRef]
57. Lizasoain, J.; Trulea, A.; Gittinger, J.; Kral, I. Corn stover for biogas production: Effect of steam explosion pretreatment on the gas yields and on the biodegradation kinetics of the primary structural compounds. *Bioresour. Technol.* **2017**, *244*, 949–956. [CrossRef]
58. Singh, R.; Behera, S.; Yadav, Y.K.; Kumar, S. *Potential of Wheat Straw for Biogas Production Using Thermophiles*; Sardar Swaran Singh National Institute of Renewable Energy: Kapurthala, India, 2014; Volume 3. [CrossRef]
59. Mancini, G.; Papirio, S.; Lens, P.N.; Esposito, G. Increased biogas production from wheat straw by chemical pretreatments. *Renew. Energy* **2018**, *119*, 608–614. [CrossRef]
60. Wang, Z.; Xu, F.; Li, Y. Effects of total ammonia nitrogen concentration on solid-state anaerobic digestion of corn stover. *Bioresour. Technol.* **2013**, *144*, 281–287. [CrossRef]
61. Luo, L.; Qu, Y.; Gong, W.; Qin, L.; Li, W.; Sun, Y. Effect of Particle Size on the Aerobic and Anaerobic Digestion Characteristics of Whole Rice Straw. *Energies* **2021**, *14*, 3960. [CrossRef]
62. Izumi, K.; Okishio, Y.; Nagao, N.; Niwa, C.; Yamamoto, S.; Toda, T. Effects of particle size on anaerobic digestion of food waste. *Int. Biodeterior. Biodegrad.* **2010**, *64*, 601–608. [CrossRef]
63. Latif, M.A.; Mehta, C.M.; Batstone, D.J. Influence of low pH on continuous anaerobic digestion of waste activated sludge. *Water Res.* **2017**, *113*, 42–49. [CrossRef]
64. Karthikeyan, O.P.; Visvanathan, C. Effect of C/N ratio and ammonia-N accumulation in a pilot-scale thermophilic dry anaerobic digester. *Bioresour. Technol.* **2012**, *113*, 294–302.
65. Essien, D.; Richard, T.L. Ensiled Wet Storage Accelerates Pretreatment for Bioconversion of Corn Stover. *Front. Bioeng. Biotechnol.* **2018**, *6*, 195. [CrossRef] [PubMed]
66. Feng, L.; Perschke, Y.; Fontaine, D.; Ward, A.J.; Eriksen, J.; Sorensen, P.; Moller, H.B. Co-ensiling of cover crops and barley straw for biogas production. *Renew. Energy* **2019**, *142*, 677–683. [CrossRef]
67. Vlierberghe, C.V.; Escudie, R.; Bernet, N.; Santa-Catalina, G.; Frederic, S.; Carrere, H. Conditions for efficient alkaline storage of cover crops for biomethane production. *Bioresour. Technol.* **2022**, *348*, 126722. [CrossRef] [PubMed]
68. Vlierberghe, C.V.; Escudie, R.; Bernet, N.; Frederic, S.; Carrere, H. Long term alkaline storage and pretreatment process of cover crops for anaerobic digestion. *Bioresour. Technol.* **2021**, *330*, 124986. [CrossRef] [PubMed]
69. Mao, C.; Wang, X.; Xi, J.; Feng, Y.; Ren, G. Linkage of kinetic parameters with process parameters and operational conditions during anaerobic digestion. *Energy* **2017**, *135*, 352–360. [CrossRef]

70. Franco, R.T.; Buffiere, P.; Bayard, R. Ensiling for biogas production: Critical parameters. A review. *Biomass Bioenergy* **2016**, *94*, 94–104. [CrossRef]
71. Phippen, W.B.; Gallant, J.V.; Phippen, M.E. Evaluation of planting method and seeding rates with field pennycress (*Thlaspi arvense* L.). In Proceedings of the 22nd AAIC Annual Meeting, Fort Collins, CO, USA, 18–22 September 2010.