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Effect of total solids content on giant reed ensilage and subsequent anaerobic digestion

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ABSTRACT

The effect of total solids (TS) content on giant reed ensilage and subsequent methane production by both liquid anaerobic digestion (L-AD) and solid-state anaerobic digestion (SS-AD) was investigated. Minimal loss of TS (about 1%), cellulose (1.2–2.4%), and hemicellulose (2.9–4.7%) was observed after 30 days of giant reed ensilage at TS contents from 25% to 40%. Ensilage with a TS content of 25% showed higher consumption of water soluble carbohydrates and extractives than those with TS contents of 30–40%, which was consistent with its higher production of organic acids and lower pH. Compared to non-ensiled giant reed, ensiled giant reed achieved up to 15% higher methane yield during AD. Ensiling giant reed at a TS content of 25% resulted in a lower glucose yield during enzymatic hydrolysis and lower methane yield during the subsequent AD, compared with ensiling it at TS contents of 30–40%. Compared to L-AD, SS-AD of non-ensiled or ensiled giant reed showed 12–18% lower methane yields, but about 2 times higher volumetric methane productivities.

1. Introduction

Giant reed is a perennial rhizomatous grass that has been traditionally cultivated in Southern Europe, North Africa, Asia, and the Middle East and has been recently introduced in the USA [1,2]. Due to its attractive features such as high growth rate, suitability for multiple harvests per year, and tolerance to dry environments, giant reed is considered a promising energy crop for production of bioenergy, such as bioethanol, heat, and power [1–5]. Recently, methane production via anaerobic digestion (AD) has been suggested for harnessing energy from giant reed biomass due to its simple process, reliable performance, and low greenhouse gas emissions [2,3,5,6].

Storage of biomass feedstock is a crucial step for a sustainable bioenergy production. High amounts of easily degradable carbohydrates in crops are prone to being lost during suboptimal storage conditions. Ensilage is a traditional storage technology that has been used since the 1800s for storing fodder crops. The ensilage process generally relies on naturally occurring microorganisms, mainly lactic acid bacteria, which convert soluble carbohydrates in the biomass to organic acids, such as lactic acid, acetic acid, propionic acid, and butyric acid, and thus inhibit the growth of detrimental microorganisms by reducing the pH to 3–4 [7]. Ensilage is also known as “wet storage” in contrast to “dry storage”. Compared to dry storage, ensilage has several advantages, such as lower energy input, less loss of total solids (TS) during outdoor storage, increased product uniformity, and reduced risk of fire [8,9]. Furthermore, organic compounds produced during ensilage can readily be utilized for biogas production during the subsequent AD process. Currently, studies on ensilage of giant reed and subsequent methane production by AD are still limited with research gaps, such as performance of the ensilage and AD processes at different solid contents [10].

TS content is one of the major factors that can affect silage quality and the subsequent bioenergy production from the ensiled biomass. TS content can affect the growth of lactic acid bacteria, formation of organic compounds, and pH during the ensilage process [11]. Low TS contents can lead to poor silage and large amounts of leachate [7], while high TS contents favor the growth of fungi [12]. Thus, TS contents between 25% and 35% are suggested for ensilage of herbaceous plants [7]. Previous research studied the effect of different pre-wilting times on the final TS content and subsequent ensilage of different biomass, and found that ensilage at high TS contents can cause reduced levels of organic acids and a pH of 5–6

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Table 1
Properties of initial materials.

Parameters	Giant reed	Inoculum
TS, %	50.02 ± 1.29	6.52 ± 0.02
VS, %TS	93.54 ± 0.20	63.47 ± 0.03
Nitrogen (N), %TS	0.44 ± 0.01	3.88 ± 0.02
Carbon (C), %TS	46.57 ± 0.15	37.14 ± 0.19
C/N	106.90 ± 2.72	10.08 ± 0.10
pH	4.29 ± 0.03	7.94 ± 0.01
NH_3-N, %TS	0.03 ± 0.02	5.80 ± 0.20
Extractives, %TS	17.90 ± 0.10	12.15 ± 1.34
WSC, %TS	4.80 ± 0.18	0.49 ± 0.10
Cellobiose, %TS	1.02 ± 0.06	ND
Glucose, %TS	0.85 ± 0.10	ND
Cellulose, %TS	31.40 ± 0.28	1.22 ± 0.09
Hemicellulose, %TS	16.38 ± 0.25	ND
Lignin, %TS	17.82 ± 0.32	NA
Crude protein, %TS	2.32 ± 0.15	18.11 ± 0.55
Ash, %TS	6.46 ± 0.20	36.53 ± 0.03

TS: total solids; VS: volatile solids; WSC: water soluble carbohydrates; ND: not detectable; NA: not applicable.

[13]. However, to our knowledge, no study has reported the optimal TS content for ensilage of giant reed.

AD processes can be classified according to their TS content into liquid-AD (L-AD; TS content equal to or less than 15%) or solid-state AD (SS-AD; TS content higher than 15%). Compared to L-AD, SS-AD has several advantages such as a smaller reactor volume for the same solid loading, fewer moving parts, lower energy input for heating and mixing, and an end product that is easier to handle [14]. In addition, SS-AD does not have problems such as floating and stratification of fats, fibers, and plastics that commonly occur in L-AD [15]. The major drawback of SS-AD is its lower methane yield and reduced stability compared to L-AD [16,17]. Ensilage at different TS contents may result in different degrees of biomass degradation and organic acid production, which should subsequently affect the AD process using the ensiled biomass as a feedstock. To date, there have been no reports on the effect of ensilage at different TS contents on the organic acid production and its effect on the subsequent SS-AD and L-AD.

In order to address the above mentioned research gaps, the objectives of this study were to: (1) evaluate the effect of TS content on the giant reed ensilage process, including accumulation of organic compounds; loss of TS, cellulose, hemicellulose, water soluble carbohydrates (WSC), and extractives; and glucose yield by enzymatic hydrolysis; and (2) evaluate and compare methane production by SS-AD and L-AD of giant reed after ensilage at different TS contents.

2. Materials and methods

2.1. Feedstock and inoculum

Giant reed was harvested from a research farm near Columbus, OH, USA, on December 10, 2014. On the day it was harvested, the biomass was ground to pass through a 12 mm sieve using a shredder-chipper (Mighty Mac, Mackissic Inc., Parker Ford, PA, USA), and then stored at 4 °C in a walk-in cooler for one week before use. AD effluent obtained from a mesophilic liquid anaerobic digester (KB BioEnergy, Akron, OH, USA) was used as inoculum for AD of giant reed. The inoculum was kept in air-tight buckets at 4 °C, and activated at 37 °C for 1 week prior to use. Three samples were taken from both the processed giant reed and inoculum to characterize their compositions. Characteristics of the giant reed and inoculum for AD are shown in Table 1.

2.2. Ensilage of giant reed

Water was added to giant reed biomass to reach TS contents of 25%, 30%, 35%, and 40% (w/w), and then packed in 1-gallon zippered plastic bags (Ziploc Vacuum Freezer System, SC Johnson Inc., Racine, WI, USA) with 1 kg of biomass in each bag. The bags were vacuumed to minimize the oxygen content inside. Ensilage was then conducted at room temperature (25 ± 3 °C) with triplicates of each TS content level. After 30 days, the biomass was taken out of the bags. The biomass for the triplicates with the same TS content were mixed thoroughly. Three samples of the mixed biomass were taken for compositional analysis and the remaining ensiled biomass was stored at −20 °C until being used for AD tests and enzymatic hydrolysis.

2.3. Enzymatic hydrolysis of giant reed

Enzymatic hydrolysis of fresh or ensiled giant reed was carried out according to the National Renewable Energy Laboratory (NREL) Laboratory Analytical Procedures (LAP) [18]. Cellulase (Cellic CTec 2, Novozymes, Denmark) was used in this study, and its activity was determined to be 137 FPU/ml based on the NREL LAP (NREL/TP-510-42628) [19]. Samples were supplemented with the cellulase at a dosage of 60 FPU/g of cellulose, and incubated at 50 °C with shaking at 180 rpm for 72 h. The hydrolysate was filtered through a 0.2 μm nylon membrane filter before sugar analysis by HPLC. The glucose yield by enzymatic hydrolysis was calculated as follows:

$$\text{Glucoseyield}\,(\%) = \frac{M_s}{f \times M_p} \times 100 \quad (1)$$

where M_s is the amount of glucose released by enzymatic hydrolysis, M_p is the amount of cellulose in the giant reed feedstock determined by acid hydrolysis, and f (180/162) is the conversion factor for cellulose to glucose [20].

2.4. Anaerobic digestion of giant reed

L-AD was set up by mixing fresh or ensiled giant reed, inoculum, and deionized (DI) water to reach a feedstock-to-inoculum (F/I) ratio of 1.0 (based on volatile solids, VS), and a TS content of 8%. SS-AD was set up similarly but at an F/I ratio of 2.0 and a TS content of 20%. AD trials with only inoculum were also set up as the control. Both L-AD and SS-AD were conducted in 1-L reactors at 37 ± 1 °C for 40 days in triplicate. Biogas generated was collected in a 5-L Tedlar gas bag (CEL Scientific, Santa Fe Springs, CA, USA) that was connected to the outlet of each reactor. Biogas composition and volume were measured every 2 to 4 days during the 40-day AD period.

2.5. Analytical methods

TS, volatile solids (VS), total Kjeldahl nitrogen (TKN), total ammonia nitrogen (TAN), and pH of samples were measured based on the Standard Methods Examination of Water and Wastewater [21]. Total carbon and total nitrogen contents in samples were determined using an elemental analyzer (Elementar Vario Max CNS, Elementar Americas, Mt. Laurel, NJ, USA). Crude protein content was calculated by multiplying total organic nitrogen (TKN minus TAN) by a factor of 6.25 [21,22]. Water soluble carbohydrates (WSC), expressed as total reducing sugar, were measured using the 3,5-dinitrosalicylic acid method [23].

Organic acids (lactic acid, acetic acid, propionic acid, and butyric acid) and ethanol were analyzed with high performance liquid chromatograph (HPLC) (Shimadzu, LC-20AB, Columbia, MD, USA) using a Phenomenex Rezex RFQ-Fast Fruit H^+ column (Phenomenex Inc., Torrance, CA, USA) and a micro-guard cartridge (Catalog