



Methane production through anaerobic co-digestion of sheep dung and waste paper



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ABSTRACT

A large amount of sheep dung (SD) produced on farms in China is a potential feedstock for production of clean energy in the form of biogas. Similarly, waste paper can be a notable energy source that is worth exploiting. In this study, we assessed the feasibility of co-digesting nitrogen-rich SD with the carbon-rich corrugated board (CB) or waste office paper (OP) in varying volatile solids (VS) ratios, to produce methane. Synergistic effect of co-digesting SD with CB and SD with OP on methane production was found in this study. The highest methane yields of 151.62 and 198.85 mL/g-VS were obtained during the co-digestions of SD with CB at 4:1 ratio (SDCB) and SD with OP at 2:3 ratio (SDOP), respectively. High-throughput 16 S rRNA gene sequencing demonstrated that the microbial diversity and richness in SDCB and SDOP co-digests were higher than in SD and OP mono-digests, respectively. Characteristic bacteria and archaea in the digesters were strongly substrate-related and might contribute to methane production. The validated results indicated that methane production through anaerobic co-digestion of SD and waste paper can be an efficient way that could not only reduce environmental pollution but also contribute to methane production.

1. Introduction

Anaerobic digestion is considered to be a promising technology for disposing of organic wastes and can generate biogas as fuel and effluent as effective fertilizer simultaneously. Biogas, a type of renewable gas fuel composed of mainly methane and carbon dioxide, is becoming increasingly attractive because of the growing concerns over global energy shortage and environmental pollution resulting from the use of fossil fuels [1,2]. Anaerobic digestion of single substrates has some drawbacks, which are correlated with the substrate characteristics. Co-digestion involves mixing two or more types of materials to obtain the complementary characteristics of substrates, which has the advantages, such as equilibrium of macro- and micronutrients, dilution of inhibitory or toxic substances, and augmentation of methane production, compared with the mono-digestions [3–5].

The livestock production in China is mainly focused on pig, sheep, and cattle. In 2015, approximately 162.06 million sheep were produced in China, which is 16.73% more than in 2010 [6]. Sheep dung (SD) is mainly used as compost, which results in the direct release of massive amounts of gases, including N₂O, NH₃, CO₂, and CH₄ produced during

the composting process, into the atmosphere [7,8]. Therefore, the use of SD as compost does not make full use of the resources and might even result in aggravation of the greenhouse effect. Although the anaerobic digestion of SD has been reported to be less than that of manure from cattle, pig or chicken, anaerobic digestion is still a practical technique for utilizing SD [9].

China Paper Association announced in 2016 that the consumption of paper and paperboard reached 10.35 million tons in 2015 in China, which is 2.79% more than in 2014 [10]. Due to quality specifications, there are limitations to increasing the recycled content in paper products and paper can only be recycled through a limited amount of times. Moreover, paper fibers recycling to new paper is very difficult for a waste paper that has been mixed with other wastes such as mud, glue, food, ink, human waste etc. [11,12]. Furthermore, statistical data showed that the paper recycling rate was 48.10% in 2014 in China, suggesting that a substantial quantity of waste paper is disposed of in ways other than recycling and reutilization [13]. Compared to reutilization, other methods for waste paper disposal, such as dumping, incineration, and landfilling, have considerable negative environmental impacts and fewer economic benefits [14–16]. Therefore, it is necessary

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to develop alternative approaches for waste paper disposal. The main limiting factor for the anaerobic digestion of waste paper is the high carbon to nitrogen (C/N) ratio in the substrate. So, the co-digestions of waste paper with the substrates of high nitrogen content such as food wastes and algal sludge have been studied by researchers to improve the digestion performance [3,17].

Compared to SD, corrugated board (CB) and waste office paper (OP) have abundant cellulose, but lack of nitrogen nutrients. Therefore, anaerobic co-digestion of SD with waste paper (CB and OP) was studied both with the substrates separately and with mixtures in various proportions to evaluate the potential benefits of co-digestions. The objectives of this study were (1) to identify the optimal substrate ratio of SD to CB or OP for methane production and provide applicable information for future biogas engineering; (2) to analyze and compare the bacteria and archaea in the mono-digesters and co-digesters. Insights gained from this study would elucidate the complex biochemical interrelationships that encourage digestion process, as well as help to improve the understanding of microorganisms involved in the anaerobic co-digestion.

2. Materials and methods

2.1. Substrates and inoculum

SD was collected from a sheep farm in Shunyi District, Beijing. Next, a blender was used to mix SD with deionized water to obtain the homogeneous state. Waste paper (CB and OP) was collected from the Beijing University of Chemical Technology campus. The two types of waste paper were cut into pieces and subsequently ground into a fibrous mass with a length of less than 0.5 cm. The inoculum was obtained from Donghuashan Biogas Plant in Beijing and degassed for 20 days to reduce the influence of background methane production.

2.2. Analytical methods

Total solids (TS) and volatile solids (VS) of the substrates and inoculum were determined according to the standard methods [18]. The elemental carbon, hydrogen, nitrogen, and sulfur compositions were quantified by an elemental analyzer (Vario EL cube, Elementar, Germany), and the oxygen content was determined using an oxygen analyzer (2400 II, PerkinElmer, USA). The pH was measured with a pH meter (Mettler Toledo, USA). Cellulose, hemicellulose, and lignin contents were determined by the analyses of neutral detergent fiber, acid detergent fiber, and acid detergent lignin, respectively, using a fiber analyzer (A2000, ANKOM, USA) [19]. Daily biogas production was calculated from the difference in the pressure, measured using a barometer (WAL Mess-und Regelsysteme GmbH, Germany), before and after the release of biogas from each digester. The biogas composition was measured daily by using a gas chromatography (GC) system (7890B, Agilent, USA), equipped with an analytical column and a thermal conductivity detector. The concentration of volatile fatty acids (VFAs) was determined by a GC system (7890A, Agilent, USA), equipped with a DB-wax capillary column and a flame ionization detector [20]. HACH test kits (HACH, USA) were used to measure the concentrations of total ammonia nitrogen (TAN) and total alkalinity (TA).

2.3. Experimental design

Anaerobic digester used in this study was shown in Suppl. Fig. 1. All digesters, each with a 0.5 L working volume, were used for the anaerobic digestion at 37 °C in triplicate. The ratios of SD to CB or SD to OP based on the VS content were 0:5, 1:4, 2:3, 3:2, 4:1 and 5:0, with the initial VS loading of 3 g-VS/L. Each digester was seeded with the inoculum at the substrate to inoculum ratio of 1, which was also calculated on the basis of VS content [21]. The initial pH inside the digesters was adjusted to 7.0 ± 0.1. Subsequently, each bottle was purged with

N₂ for 3 min to eliminate the air, and then the bottles were sealed with rubber stoppers. Three blank digesters (control), containing 3 g-VS/L of inoculum and without the substrate, were used for the validation of methane production. All digesters were shaken at the speed of 150 rpm every day for 1 min. After 43 days, when the anaerobic digestion process was complete, the digesters were collected to analyze the microbial communities.

2.4. Carbon to nitrogen ratios of substrates

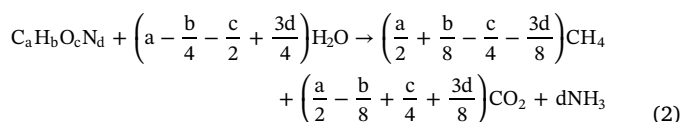
The C/N ratios of the substrates were calculated using Eq. (1).

$$C/N = \frac{C_1X_1 + C_2X_2}{N_1X_1 + N_2X_2} \quad (1)$$

In Eq. (1), C₁ and C₂ (% TS) are the elemental mass fractions of carbon and N₁ and N₂ (% TS) are the elemental mass fractions of nitrogen in SD and waste paper, respectively. X₁ and X₂ are the TS fractions of SD and waste paper in the co-digestions, respectively.

2.5. Theoretical maximum methane yield and biodegradability

Theoretical maximum methane yield (TMY, mL/g-VS) was calculated from the elemental composition, lignin and ash contents of the substrates, using the Buswell's formula [22] and Chen's formula [23], as shown in Eqs. (2) and (3), respectively.



$$TMY = \frac{22.4 \times 1000 \times \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8}\right)}{12a + b + 16c + 14d} \times (1 - \text{lignin}\% - \text{ash}\%) \quad (3)$$

Biodegradability (BD) was calculated from the highest cumulative methane yield (experimental methane yield, EMY) and TMY, as shown in Eq. (4):

$$BD = \frac{EMY}{TMY} \times 100\% \quad (4)$$

2.6. Synergistic effect index

The synergistic effect index (SEI) for the anaerobic co-digestion proposed in this study was calculated as shown in Eq. (5).

$$SEI = \frac{EMY_{co} - (X_1 \times EMY_1 + X_2 \times EMY_2)}{(X_1 \times EMY_1 + X_2 \times EMY_2)} \times 100\% \quad (5)$$

In Eq. (5), EMY_{co} is the EMY of a co-digestion. EMY₁ and EMY₂ are the EMYs of the mono-digestions of SD and waste paper, respectively. X₁ and X₂ are the VS fractions of SD and waste paper in the co-digestion, respectively.

2.7. Kinetic model

The modified Gompertz model (Eq. (6)), which has been widely applied in simulating and predicting anaerobic digestion performance [21,24], was used in this study.

$$B = B_0 \exp \left\{ -\exp \left[\frac{\mu_m e}{B_0} (\lambda - t) + 1 \right] \right\} \quad (6)$$

In Eq. (6), B represents the simulated cumulative methane yield (mL/g-VS); B₀ refers to the simulated maximum cumulative methane yield (mL/g-VS); μ_m means the maximum methane production rate (mL/g-VS/day); e is equal to 2.718; λ stands for the lag phase time (day); t is the digestion time (day).

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