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Co-digestion of tobacco waste with different agricultural biomass feedstocks and the inhibition of tobacco viruses by anaerobic digestion

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HIGHLIGHTS

- The methane yield of tobacco stalk was 0.163 m^3 CH_4·kg VS^{-1}.
- Cucumber Mosaic Virus can be inactivated by mesophilic anaerobic digestion.
- Tobacco Mosaic Virus can be inactivated by thermophilic anaerobic digestion.

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ABSTRACT

Tobacco is widely planted across the world especially in China, which means that a large amount of tobacco waste needs to be treated. This study investigated the biogas fermentation of tobacco stalks co-digested with different biomass feedstocks and the inactivation of Tobacco mosaic virus (TMV), Cucumber mosaic virus (CMV) by anaerobic digestion. Results showed that the maximum methane yield of tobacco stalks at 35 °C was 0.163 m^3 CH_4·kg VS^{-1}, which was from the co-digestion of tobacco stalks, wheat stalks and pig manure. The largest VS removal rate of tobacco stalks was 59.10%. Proven by indicator paper stripe, half-leaf lesion and RT-PCR, CMV could be inactivated by mesophilic and thermophilic anaerobic digestion, whereas TMV could be only inactivated by thermophilic anaerobic digestion over 20 days. These results suggested that using tobacco stalks as feedstock for anaerobic digestion and applying the digested residue and slurry to Solanaceae crop land are feasible.

1. Introduction

China is the world's largest tobacco producer and consumer, and contributes about 40% of the world's total tobacco production (Eriksen et al., 2015). This considerable production of tobacco leads to large quantities of tobacco waste (tobacco stem and discarded tobacco leaf), which is harmful to the environment and is generated during the cultivation and manufacturing processes (Meng et al., 2014; Zhong et al., 2010; Zi et al., 2013). The conventional methods for dealing with tobacco waste are direct burning or reusing it as organic fertilizer after composting (Kopčić et al., 2014; Yang et al., 2012b). Moreover, some chemical compounds, such as nicotine and solanesol, which are used as pesticides, can also be extracted from tobacco waste (Piotrowska-Cyplik et al., 2009). Over the last few years, more attention has been paid to the thermo-chemical conversion of tobacco waste for energy production using processes such as pyrolysis or co-combustion with other fuels (Li et al., 2011; Yang et al., 2012b; Zhang et al., 2013).

In contrast to the treatment methods above, anaerobic digestion is a more renewable and sustainable solution. Most biomass types can be used as feedstock in an anaerobic digester, including animal manure, crop residues, municipal waste, aquatic biomass etc. (Wellinger et al., 2013). The cellulose contents in tobacco waste can vary between 43.4% and 68.4% (Ye et al., 2013b), which is good for anaerobic digestion. Meher et al. (1995) obtained a biogas yield of 0.169–0.282 m^3 kg TS^{-1} of tobacco waste using a semi-continuous experiment at temperatures between 23.5 °C and 36.0 °C. González-González et al. (2014, 2013) obtained a methane yield of 53.84 ± 14.48 Nm^3 CH_4·t^{-1} from fresh tobacco under mesophilic conditions using a 16 day degradation period. Nonetheless, using tobacco waste, especially tobacco leaves, as anaerobic digestion feedstock is controversial due to its high nicotine content. A previous research report indicated that tobacco leaves can inhibit the metabolism of anaerobic microbes and cannot be used as a

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mono-feedstock to start up the anaerobic digestion process (Yang et al., 2012a). Nevertheless, the nicotine content in tobacco stalks is much lower than in leaves, so it may be feasible to use tobacco stalks as feedstock for anaerobic digestion. Moreover, some drawbacks to the mono-digestion of crop stalks can be solved by co-digestion with biomass that contains high levels of nitrogen (Mata-Alvarez et al., 2014). Therefore, the anaerobic digestion efficiency of tobacco stalks co-digested with other biomass types is a significant research topic.

However, the existence of tobacco viruses in feedstock plants may lead to virus infected digested slurry, which would prevent the reuse of the digested slurry as organic fertilizer on Solanaceae crop land. Tobacco plants can be attacked by a wide range of diseases, and a great number of viruses can infect them and other Solanaceae crops either naturally or experimentally (Shew and Lucas, 1991). The most harmful tobacco viruses are Tobacco mosaic virus (TMV), Cucumber mosaic virus (CMV), Potato virus Y (PVY) and Tobacco etch virus (TEV) (Chatzivassiliou et al., 2004). In particular, TMV is known to be hard to inactivate because it has a very stable structure, even when using ultraviolet disinfection or aerobic composting (Alonso et al., 2013). Anaerobic digestion is an effective pathogen removal method even under mesophilic conditions (Horan et al., 2004; Ward et al., 2008). However, there have been no reports as to whether or not anaerobic digestion could inactivate tobacco viruses.

This study investigated the methane yield and VS removal increase when tobacco (flue-cured tobacco) stalks were co-digested with different biomass feedstocks that are easily found around tobacco growing areas, under mesophilic (35 °C) anaerobic digestion conditions. Subsequently, we chose the best co-digestion group as the feedstock for anaerobic digestion under mesophilic (35 °C) and thermophilic (55 °C) conditions for 20 days, and evaluated TMV and CMV inactivation by anaerobic digestion.

2. Methods

2.1. Feedstock and inoculum collection and preparation

Healthy tobacco stalks without any virus infections (HTS) and tobacco leaves with virus infections (TL) were obtained from a test field and a laboratory at the Tobacco Research Institute of Chinese Academy of Agricultural Sciences (CAAS). Wheat stalks (WS), rape stalks (RS), pig manure (PM) and cow manure (CM) were collected from a local farm in Jintang County, Chengdu, China. All the stalks were cut with a grinder and then sieved with a 40-mesh screen. Pig manure and cow manure were stirred separately by an agitator in order to homogenize the components. The above feedstocks, which were used in the biogas production test, were frozen at −18 °C until needed. Before use, tobacco leaves infected with TMV and CMV for the inhibition test were ground and diluted with distilled water at 20 times the mass of tobacco leaves. The inoculum was the anaerobic sludge collected from anaerobic digestion experiment carried out in a laboratory at the Biogas Institute of Ministry of Agriculture (BIOMA). Characteristics of the feedstock and inoculum before dilution are shown in Table 1.

2.2. Biogas fermentation of tobacco stalks

The digesters were made of plastic with a working weight of 800 g. The fermentation process was under mesophilic conditions (35 °C), which was maintained by a water bath. The mix ratios of the total solid content of feedstock and the inoculum for the different treatments are listed in Table 2. Distilled water was used to fill the digesters and was thoroughly mixed with fresh feedstock. Each treatment was repeated three times. The experiment was stopped 44 days after start up because no significant biogas production was detected for any treatment at that point. Biogas output was analyzed once a day, and total solids (TS) and volatile solids (VS) were tested at the end of the experiment. The methane volume fraction in the biogas was tested every day for the first 12 days. From day 13 to day 32, the methane volume fraction in the biogas was tested once per five days because the biogas output was too low to measure every day. The final test was carried out on the last day of the experiment.

2.3. Viruses inactivation by anaerobic digestion

The digesters were 35 mL working volume serum bottles sealed with rubber plugs. The feedstock was composed of wheat stalk (0.60 g), pig manure (2.15 g), a solution of tobacco leaves infected with TMV (5 mL), solution of tobacco leaves infected with CMV (5 mL) and inoculum (8.78 g) (the best co-digestion group identified by the tobacco stalk fermentation experiment). The feedstock was added to 35 mL distilled water. Control solutions contained the solution of tobacco leaves with TMV (5 mL) and the solution of tobacco leaves with CMV (5 mL).The experiment was operated under mesophilic (35 °C) and thermophilic (55 °C) conditions for 20 days. Each treatment was carried out in triplicate. Biogas output and virus activity were measured at the end of the experiment.

2.4. Analytical methods

TS, VS and TOC (total organic carbon) detection were carried out according to the procedures described in the Standard Methods (APHA, 2012). Cellulose, hemi-cellulose and lignin were detected by a cellulose test instrument (FIWE 3, VELP Scientifica, Innovative Analytical Instruments, Italy). Nicotine and TN (total nitrogen) were detected by an auto-analyzer (SEAL AutoAnalyzer 3, Seal Analytical, Germany). In the tobacco stalk fermentation experiment, biogas output was tested by the water volume replaced by the biogas generated in the digester. Biogas outputs by the substrates were the difference between the biogas output for each treatment and that of the control (44.67 mL in total). For the virus inhibition experiment, biogas output was tested with a milligascounter (MGC-1 V3.2 PMMA, Dr.-ing Ritter ApparatebauGmbh & Co. kg, Germany), and methane content was measured with a gas analyzer (Biogas 401, ADOS GmbH Instrumentation and Control, Germany). MS Excel 2007 and Origin 8.0 were used to analyze the data.

2.5. First-order rate equations

Methane production curves could be modeled by two first-order rate equations (Eqs. (1) and (2)). Eq. (2) gave a better fit result than Eq. (1) because of the heterogeneous feedstock and the presence of both rapidly and slowly degrading fractions (Wellinger et al., 2013).

$$Y = Y_{max}(1 - e^{-kt}) \tag{1}$$

$$Y = Y_{max}\left[1 - Pe^{-k_1^t} - (1 - P)e^{-k_2 t}\right] \tag{2}$$

where Y is the cumulative methane yield at a given time t ($m^3\ CH_4 \cdot kg\ VS^{-1}$), Y_{max} is the ultimate cumulative methane yield ($m^3\ CH_4 \cdot kg\ VS^{-1}$), k is the first-order rate constant, k_1 is the first-order rate constant for the readily degradable substrates, k_2 is the first-order rate constant for less readily degradable substrates, and P is the proportion of readily degradable substrates.