



Co-digestion of Hybrid *Pennisetum* and Peanut Shell After Adding TiO₂ Nanoparticles: Focusing on the Synergistic Effects on Methane Production

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Received: July 20, 2021

Published: August 13, 2021

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Abstract

Anaerobic digestion is a widely accepted method to treat wastes such as peanut shell. The energy and nutrients are simultaneously recovered by this method. The objective of this study was to elucidate the effect of TiO₂ nanoparticles in co-digestion of hybrid *Pennisetum* and peanut shell under mesophilic conditions. The results demonstrated the methane (CH₄) production was improved by adding the TiO₂ nanoparticles. The cumulative gas production is best (up to 11,133.3 mL) by adding 0.15% nano-TiO₂ particles. The microbial community analysis showed that *Methanobacterium* and *Methanosarcina* were enriched in the presence of TiO₂ nanoparticles indicating that TiO₂ can improve CH₄ production by stimulating the growth of methanogens.

Keywords: Anaerobic Fermentation; Methane Production; TiO₂ Nanoparticles; Hybrid *Pennisetum*; Peanut Shell

Introduction

The total annual output of peanuts in China is 5 million tons. Peanut shells (PS) are food waste, and food waste constitutes 30–50% of municipal solid waste [1,2]. Approximately 60 million tons of food waste was produced last year in China alone. Food waste production is expected to keep increasing while maintaining social/economic growth as well as population growth.

Hybrid *Pennisetum* (HP) is herbaceous plant with great energetic potential. It has a high biological yield, strong regeneration ability, and multiple rounds of harvest [3,4]. Many researchers have focused on methane production from anaerobic fermentation of hybrid *Pennisetum*. However, *Pennisetum* is not fully utilized when used as a carbon source. Therefore, significant effort is

needed to handle ever-increasing peanut shell waste and use it to produce biogas via *Pennisetum*.

Aerobic digestion has been developed and is well recognized as a promising tool for waste stabilization and energy recovery in the form of biogas. It has two processes: wet anaerobic digestion and dry anaerobic digestion. The total solid of wet anaerobic digestion system is less than 15%, and the total solid of dry anaerobic digestion system is higher than 15% [5,6]. Dry anaerobic digestion (DAD) is increasingly popular. This requires less water, and it does not cause more pollution than a wet digestion system [7-9]. In addition, co-digestion can greatly improve specific methane yields and methane production rates versus mono-digestion because of the superior nutrient availability and synergistic microbiomes.

Nanoparticles offer unique physiochemical properties and widespread applications [10]. Their effects on the environment have been investigated, but most studies focused on soil and wastewater toxicity [11,12]. The effect of nanomaterials on wastewater treatment has been reported [13-15]. The adsorption of activated sludge was reported to be the main mechanism of nanoparticles. Nevertheless, the effects of metal oxide nanoparticles (such as TiO₂ nanoparticles) on anaerobic digestion for HP and PS are rarely investigated. TiO₂ nanoparticles have no significant toxicity on the viability of bacterial cells and show no inhibitory effects on waste-activated sludge digestion.

In this study, dry anaerobic experiments were conducted under mesophilic conditions with different mixing ratios of HP and PS.

Purpose of the Study

The purpose was to investigate methane production with different ratios of HP and PS, investigate the effects of five dosages of the TiO₂ nanoparticles on methane yield, and assess the influences of TiO₂ nanoparticles on flora.

Materials and Methods

Feedstock and inoculum

The surface part of hybrid *Pennisetum* used here was from the experimental farm of Yunnan Agricultural University, Kunming, Yunnan Province. The samples were cut into 2 - 3 cm. The dehydrated sludge was taken from the fifth water purification plant in Kunming, Yunnan Province, and appeared brown. The total solids (TS) content was 15.46%, and the volatile solid (VS) content was 41.54%.

Nanoparticles synthesis

Nano-TiO₂ powder was synthesized according to a reported route [16]. Here, 0.1600g (0.84 mmol) of dopamine (3,4-dihydroxy-β-phenylethylamine) and 30 mL of benzyl alcohol were added to an Erlenmeyer flask and stirred vigorously for 20 minutes. We then added 1.5 mL of TiCl₄ dropwise. This was stirred vigorously at room temperature for 2h. The temperature was then increased to 80°C, and the mixture was transferred to a polytetrafluoroethylene reactor at 80°C for 3 days. After cooling, the resulting red-brown mixture was centrifuged, the supernatant was decanted, and the precipitate was washed three times with chloroform and dried at 60°C to obtain a large amount of dark red nano-TiO₂ powder.

DNA extraction and PCR amplification

DNA extraction of different digestion period samples was detected by a MIO-BIO Power Soil DNA Isolation Kit. The remaining steps for DNA extraction were performed via the DNA isolation kit protocol. Subsequently, the V4-V5 variable region of the bacterial 16S rRNA gene was amplified using primers 515F and 926R through polymerase chain reactions (PCRs). PCR was performed in a 10 μL volume containing 1× PCR buffer, 1 μL dNTPs, 1 μL primer, 1 unit taq DNA polymerase, and 5 - 50 ng template DNA under the two cycling conditions: Bacteria had pre-denaturation at 94°C for 2 min, 22 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s or 50°C for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 5 min. Archaea had pre-denaturation at 94°C for 2 min, 30 cycles of denaturation at 94°C for 30s, annealing at 50°C for 30s, extension at 72°C for 30s, and a final extension at 72°C for 5 min. Finally, the PCR products were tested through high-throughput sequencing using the Illumina Miseq 2x300bp platform.

Anaerobic digestion tests

Experimental set-up

The digestion device consists of a 1 L reaction bottle, gas bottle, water bottle, and constant- temperature water bath. According to the experimental design, different ratios of substrates and TiO₂ nanoparticles were added to the reactors. All reactors were incubated at 37(± 1°C).

Feedstock

The carbon to nitrogen ratio (C/N) plays an important role in dry AD. If the C/N is higher, then the gas yield may be lower. The methanogenic bacteria will be toxic if the C/N is lower. According to Weiland and Richa, a C/N ratio in the range of 20% - 30% is the best. The C/N ratio of hybrid *Pennisetum* is 31.15 (C 47.35% and N 1.52%). The C/N of peanut shell was also measured (Table 1). The C/N ratio is 25. We choose the hybrid *Pennisetum* and peanut shell as feedstocks.

| Sample | The content of elements | | | |
|--------|-------------------------|-------|-------|-------|
| | C | H | N | 元素C/N |
| PS | 60.60% | 5.90% | 2.40% | 25.25 |
| HP | 47.35% | 5.89% | 1.52% | 31.15 |

Table 1: Analysis of the content of HP and PS elements.

Experimental design

The digestion process contains two steps. First, to determine the best ratio, the HP:PS mixing ratios were selected as 0:4, 1:3, 2:2, 3:1, and 4:0. Each sample group was set up in triplicates. Second, different quality scores of TiO₂ nanoparticles were added to the reactors under the best ratio of HP:PS.

Analytical methods and characterization

The volatile solids (VS) and the total solids (TS) were measured by standard methods [17,18]. Total carbon (TC) and total nitrogen (TN) were measured with an elemental analysis instrument (VARI-OEL III, Germany). The 16s rRNA gene amplification and sequencing used NGS Illumina MiSeq 2 x 300 bp.

Results and Discussion

Cumulative Methane production and daily methane yield

Figure 1 shows 40-day methane yields for different ratios of mixed feedstock and cumulative methane production. The pH values in reactors ranged from 6.5 to 7.5 because they were suitable for methanogenesis. The changing trend of daily methane yield and cumulative methane production are similar. However, the peak values of daily methane yield were different. The sample at 3:1 shows the highest daily methane yield on the 17th day and the highest value at 323 mL. The highest cumulative production of the sample of 3:1 is 10,405 mL. The results suggested that the best ratio of HP and PS is 3:1.

Impact of nano-TiO₂ on methane production

The effects of TiO₂ nanoparticles on anaerobic digestion are shown in figure 2a. The pH values in the reactors also ranged from 6.5 to 7.5. Five sets of experiments with different amounts of TiO₂ starting from 0 to 2% were performed. The results showed that the addition of TiO₂ had an encouraging impact on methane production. Under TiO₂ nanoparticles addition, the cumulative methane production increased significantly. When 0.15% TiO₂ was added, the cumulative methane production increased by 23.7%. However, the methane production decreased when the concentration of TiO₂ was 0.20%. This indicated that the high concentration of TiO₂ NPs inhibits anaerobic digestion. The COD curves of different samples on anaerobic digestion are shown in figure 2b. The trend of COD decreased after 10 days. The nitrogen and carbon content is exhausted along with the aging of the microbial cells. Moreover, the

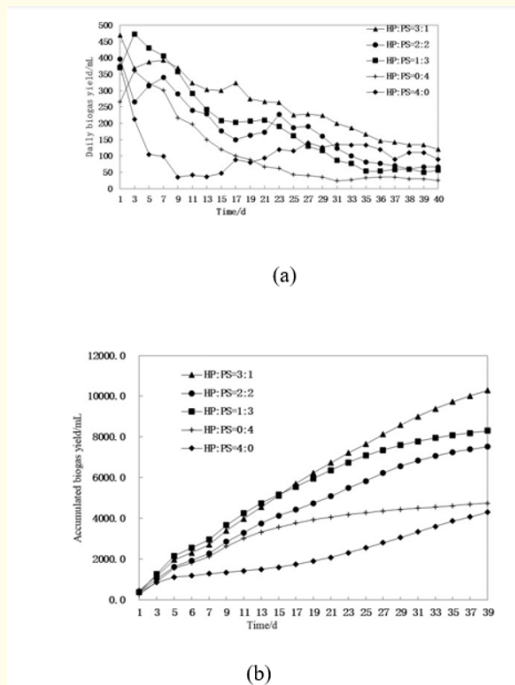


Figure 1: (a) Daily methane yield and (b) cumulative methane production.

COD removal efficiency was gradually increased, and the order of COD removal rates was blank (57%) < 0.05% TiO₂ (60%) < 0.1% TiO₂ (62%) < 0.15% TiO₂ (65%) < 0.2% TiO₂ (67%).

Community diversity of archaea

Archaeal community diversity of three samples with different fermentation periods is shown in table 2. ACE and Chao1 indexes were used to compare the species richness, and the Shannon index was used to compare community diversity in different samples. The un-fermented (NF) samples had highest species richness with ACE and Chao 1 values of 57 and 58 followed by pre- fermented (FQ) samples (ACE of 57 and Chao1 of 55) and middle-fermented (FM) samples with the lowest ACE (51) and Chao1 (50). The Shannon index also decreased in fermentation progresses.

Archaeal flora distribution of different samples

As many as 14 archaeal flora were identified in the three different fermentation periods (Figure 3). In the un-fermented period,

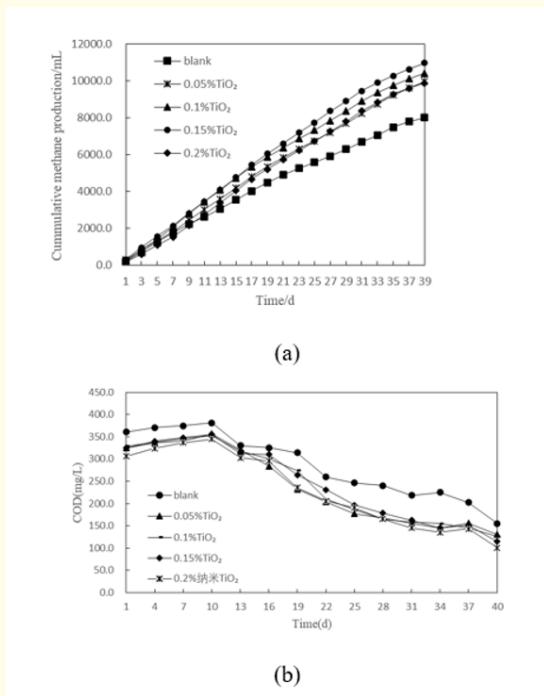


Figure 2: The curves of cumulative methane production (a) and COD (b).

| Sample | Number of sequences | OTUs | ACE | Chao 1 | Shannon | Coverage |
|--------|---------------------|------|-----|--------|---------|----------|
| NF | 10302 | 56 | 57 | 58 | 2.69 | 99.98% |
| FQ | 8284 | 52 | 57 | 55 | 2.52 | 99.93% |
| FM | 18361 | 50 | 51 | 50 | 2.32 | 99.99% |

Table 2: Comparison of species richness and community diversity estimators of the Archaeal communities in different samples.

the dominant archaea are *Methanobacterium* (35.20%), *Methanosaeta* (19.37%), *Methanosporillum* (18.00%), and *Methanobrevibacter* (7.61%). In pre-fermented period, the dominant archaea are *Methanobacterium* (52.90%), *Methanosaeta* (14.18%), *Methanosporillum* (7.69%), *Methanobrevibacter* (9.11%), and *Metgabisarcina* (7.40%). In the middle-fermented period, the dominant archaea are *Methanobacterium* (40.40%), *Methanosaeta* (20.47%), *Methanosporillum* (23.06%), and *Metgabisarcina* (5.23%).

Therefore, the categories of major archaea genera were similar, but their relative abundances were different. The archaea of *Methanobacterium* and *Metgabisarcina* were enriched with the addition of TiO₂ nanoparticles. These results indicate that TiO₂ played an important and positive role in CH₄ production.

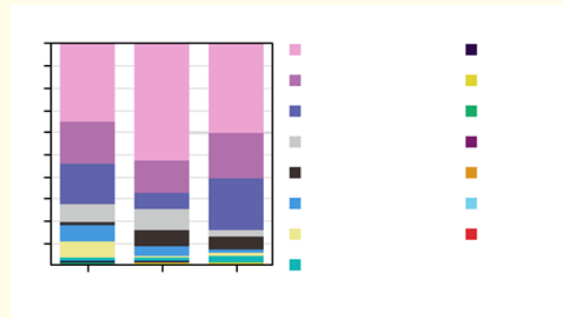


Figure 3: Distribution of various stains at the genus level during anaerobic digestion.

Conclusion

This study indicated that the addition of TiO₂ nanoparticles promoted methane production in the system of anaerobic co-digestion of hybrid *Pennisetum* and peanut shell. The archaea of *Methanobacterium* and *Metgabisarcina* can be enriched upon addition of TiO₂ nanoparticles. This study expands our knowledge of the role of TiO₂ nanoparticles in mixed dry anaerobic fermentation. In our future work, the effect of nanoparticles on fermentation will be further investigated, and biological flora will be studied in the fermentation process.

Acknowledgments

This work was supported by Engineering and Research Center for Industrial Biogas Technology of Yunnan Province University. The archaeal diversity analysis was performed using the Omicshare tools-a free online platform for data analysis (<http://www.omicshare.com/tools>.) We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript.

Funding Information

The work received financial support from Agricultural Union Youth Program of Yunnan Provincial Science and Technology Department (2018FG001-102).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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Volume 5 Issue 9 September 2021

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