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Methane production from wheat straw with anaerobic sludge by heme supplementation

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HIGHLIGHTS

- A cost-effective method of anaerobic digestion to produce methane was created.
- Heme was added in the anaerobic digestion process can improve methane production.
- Heme can improve reduce power of the whole fermentation system.
- The mechanism of heme as electron carriers was discussed.

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ABSTRACT

Wheat straw particles were directly used as substrate for batch anaerobic digestion with anaerobic sludge under 35 °C to evaluate the effects of adding heme on methane production. When 1 mg/l heme was added to the fermentation process with no agitated speed, a maximum cumulative methane production of 12227.8 ml was obtained with cumulative methane yield of wheat straw was 257.4 ml/g-TS (total solid), which was increased by 20.6% compared with 213.5 ml/g-TS of no heme was added in the reactor. Meanwhile, oxido-reduction potential (ORP) level was decreased, the activity of coenzyme F_{420} was significantly improved and $NADH/NAD^+$ ratio were the highest than other experimental groups. These results suggest that heme-supplemented anaerobic sludge with no agitated speed may be providing a more reductive environment, which is a cost-effective method of anaerobic digestion from biomass waste to produce methane with less energy consuming.

1. Introduction

Sustainable biogas production plays a decisive role in the ongoing fight against global warming and climate change, as it replaces fossil fuels, reduces the energy demand of waste treatment plants and can yield valuable organic fertilizers (Pohl et al., 2012; Weiland, 2010). As the largest agricultural country, China has abundant biomass resources. Crops straw is the residues after agricultural crop harvest, which is the main byproduct of agriculture production. Agricultural residues are considered as the most abundant renewable resource. It is necessary and important for the viable and sustainable biofuels production in the long run (Baker and Keisler, 2011).

Wheat straw is one of the main abundant crop residues in China. It is the third most abundant crop residue after rice straw and corn stover. The total annual output of wheat straw in China reaches 1.09×10^8 t, which is approximately 22% of the total crops straw resources in 2007, and the available resources amount 0.61×10^8 t (Yang et al., 2010). Biogasification shows great promise as a key technology for sustainable utilization of wheat straw as a renewable energy source. Wheat straw contains of 30–40% cellulose, 20–30% hemicelluloses and 10–20% ligin. After anaerobic digestion, the relative content of ligin increased significantly, and cellulose and hemi-cellulose contents decreased slightly. However, the sum of cellulose and hemi-cellulose content was still close to 50% and may be reused for biogas production (Chen et al., 2014). Many attempts have been tried to enhance the performance of anaerobic digestion of wheat straw to biogas production in anaerobic digestion, including physical, chemical (i.e. alkaline, acidic, oxidative) or biological pretreatment methods. Also, key parameter of process performances by process parameters adjustment such as total solids content (Motte et al., 2013; Li et al., 2011), reactor configuration (Kaparaju et al., 2009), structural and chemical analysis of process residue (Hansen et al., 2013), carbon/nitrogen (C/N)

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ratio (Liu et al., 2008), pH (Chen et al., 2007), loading rate (Mähnerta and Linkea, 2009) and so on. Their studies have focused on the pretreatment of wheat straw and some simple fermentation process control, which are expensive to implement on a large scale. The cost of the pretreatment is a critical factor for the development of biogas industry, especially in rural areas of developing countries. In fact, it is critical and urgent for the industry to improve the ability of anaerobic sludge to digest wheat straw to produce methane.

Biogas production may be artificially enhanced controlling the occurring biochemical reactions in order to maximize the methane production and consequently the energy recovery from the digested biomass. Anaerobic digestion as a typical anaerobic fermentation process, the lack of reducing power is existed in metabolic process. Under anaerobic growth, and in the absence of an alternate oxidizing agent, the regeneration of NAD^+ is achieved through fermentation by using NADH to reduce metabolic intermediates (Clark, 1989; De Graef et al., 1999). Therefore, in fermentation, alterations in the availability of NADH should have a profound effect in the whole metabolic network. To regulate the level of reducing power in the fermentation process, previous studies have focused on the following methods. Feeding different types of carbon sources with different oxidation state to regulate the level of reducing power (Du et al., 2006). The addition of an electronic mediator, such as methyl violet, to improve the level of reducing power (Liu et al., 2014). And finally, the use of molecular biology techniques to overexpress of certain genes to improve the reducing power (Sánchez et al., 2005).

In this study, the availability of direct anaerobic digestion of wheat straw with anaerobic sludge was investigated during 45-d anaerobic digestion, and the methane production was enhanced by heme utilized to supplement the anaerobic sludge.

2. Methods

2.1. Chemicals and materials

All chemicals were of reagent grade and were purchased from either Sinochem (Shanghai, P.R. China) or Fluka Chemical (Buchs, Switzerland). CO_2, H_2 and N_2 were obtained from Nanjing Special Gases Factory (Nanjing, P.R. China).

2.2. Feedstock and inoculum

Wheat straw was freshly collected from a farm yard in Luhe District, Nanjing, Jiangsu Province, China at the end of May, 2013, and cut into approximately particles of 2–3 mm by using a grinder (Hummer 900). After being air-dried, the straw particles were stored at 4 ± 0.5 °C until use. The wheat straw particles contained 39.2% cellulose, 28.3% hemicelluloses, 13.2% lignin, and 5.6% ash. Specifically, Table 1 shows the chemical parameters of wheat straw and anaerobic sludge.

The inoculum of anaerobically digested sewage sludge was taken from a wastewater treatment plant in Yangzi Petrochemical Co., Ltd, Nanjing, China. The glucose was fed in the sludge with 1.5 g/l per day at 35 ± 1 °C for 1 month, and then the feeding of glucose was stopped. When no biogas production observed for 1 week, the seed culture was thoroughly mixed and filtered through a screen with a pore size of 833 μm (20 meshes). This was carried out to ensure the removal of easily degradable organic matter still present in the inoculum and to remove the dissolved methane.

2.3. Batch assay methane fermentation setup

All the experiments were conducted in sequencing batch model with total volume of 1000 ml and the active volume of the reactor was 800 ml. The reactors were fed with wheat straw with anaerobic sludge mainly containing total solids contents of 6%. The experiments were carried on mesophilic temperature of 35 ± 1 °C. After the feedstock was added into the reactors, sealed immediately with butyl rubber stoppers, the batch assay methane fermentation reactors were carefully checked for any leakage and flushed with pure nitrogen (99.9%) for 3 min in order to ensure anaerobic condition. An outlet in the stopper was used for collecting biogas in gas tight glass jars. Each digester of static culture was manually mixed once a day to avoid stratification. The initial carbon-to-nitrogen ratio (C/N) of 30:1 was maintained by the addition of carbamide in the each reactor. Heme was dissolved in 1 M KOH or NaOH solution and the initial concentration was 1 mg/l in the reactor by adding an appropriate volume.

Batch experiments were conducted in triplicate to determine the biogas production rates of wheat straw for 45 days. During anaerobic digestion (AD), the biogas samples were collected daily while the liquid samples were measured at 3-day intervals from the control digester for process stability investigation.

2.4. Analytical methods

2.4.1. Characteristics of gas

The daily biogas production was directly obtained from the volume of displaced saturated $NaHCO_3$ solution in the graduated cylinder after the mixture being stirred manually. Methane concentration in the biogas was analyzed using a gas chromatograph (GC 9890A, Renhua, China) equipped with a TCD, a TDC-01column (Φ4 mm × 1 m, Shimadzu, Japan) and hydrogen as the carrier gas. The injector, oven and detector temperatures were 100, 150 and 120 °C, respectively. Flow rate of carrier gas was 50 ml/min, and the injection volume of sample was 0.5 ml.

2.4.2. Chemical composition analyses

The parameters of total solid (TS) and volatile solid (VS) were performed in accordance with standard methods of APHA (American Public Health Association, 1998). Total carbon (TC) and total nitrogen (TN) were analyzed by the CHN (carbon, hydrogen, nitrogen) analyzer vario EL (Perkin Elmer, USA made). The protein content was calculated with a conversion factor of 6.25. Carbohydrate was calculated as the fraction of VS remaining after the subtraction of protein and lipids (Li et al., 2009). The pH value was directly measured from liquid samples with a digital pH meter (FE20K, Mettler-Toledo, Switzerland). Lipids were determined by a Soxhlet system at 65 °C with more than 60 circulations using methylene dichloride as an extractive reagent (Miao et al., 2013).

Table 1
The characteristics of wheat straw and anaerobic sludge used in the reactors.

Substrate	Total solids (TS/%)	Volatile solids (VS/%)	Total carbon (mg/g-TS)	Total nitrogen (mg/g-TS)	Carbohydrate (/TS)	Protein (/TS)	Lipids (/TS)
Wheat straw	90.01 ± 2.03[a]	89.26 ± 0.63	479.83 ± 0.02	5.34 ± 0.18	54.62 ± 0.37	3.41 ± 0.32	6.23 ± 0.21
Anaerobic sludge	4.36 ± 0.06	67.42 ± 1.07	491.60 ± 0.01	13.41 ± 0.09	NA[b]	NA	NA

[a] Each value is an average of three parallel replicates and is represented as mean ± standard deviation.
[b] No analysis.