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Fungal pretreatment of rice straw with *Pleurotus ostreatus* and *Trichoderma reesei* to enhance methane production under solid-state anaerobic digestion

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HIGHLIGHTS

- Solid state anaerobic digestion of fungal treated rice straw for biogas production.
- Fungal pretreatment caused 33% of lignin loss led to methane yield increased by 120%.
- Moisture content and incubation time significantly affected the lignin degradation.
- Methane yield increased linearly with selectivity value in fungal pretreatment.

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ABSTRACT

Rice straw was subjected to fungal pretreatment using *Pleurotus ostreatus* and *Trichoderma reesei* to improve its biodegradability and methane production via solid-state anaerobic digestion (SS-AD). Effects of moisture content (65%, 75% and 85%), and incubation time (10, 20 and 30 d) on lignin, cellulose, and hemicellulose degradation during fungal pretreatment and methane yield during anaerobic digestion were assessed via comparison to untreated rice straw. Pretreatment with *P. ostreatus* was most effective at 75% moisture content and 20 d incubation resulting in 33.4% lignin removal and a lignin/cellulose removal ratio (selectivity) of 4.2. In comparison *Trichoderma reesei* was most effective at 75% moisture content and 20 d incubation resulting in 23.6% lignin removal and a lignin/cellulose removal ratio (selectivity) of 2.88. The corresponding methane yields were 263 and 214 L/kg volatile solids (VS), which were 120% and 78.3% higher than for the untreated rice straw, respectively.

1. Introduction

In 2014, the world energy consumption was 12928.4 Mtoe (541.3 EJ), of which oil, natural gas coal, nuclear energy, hydroelectricity and other renewable energy consumption were 32.6%, 23.7%, 30.0%, 4.4%, 6.8% and 2.4%, respectively [1]. Fossil fuel consumption accounted for 86.3% of the world energy consumption, whereas renewable energy consumption accounted for 9.2%. At present world energy consumption is growing by 2% per year. Consumption of fossil fuels is recognized as the main cause of global warming and climate change with associated adverse environmental effects as a result. The strong dependency on fossil fuels further carries the risk of significant changes in energy costs due to variations in fossil fuel prices, which in turn may result in economic and political challenges [2]. Therefore, alternative energy sources with low greenhouse gas emissions and more stable prices should be developed, to meet the growing energy demand, reduce greenhouse gas emissions and secure more stable energy prices [3].

Lignocellulosic biomass, is one of the world's largest sources of biomass-based renewable energy with an annual production of 200 billion tons, but is at present underutilized [4]. Lignocellulosic biomass is known to be one of the best sources for inexpensive production of carbohydrates, and has been used to produce biofuels such as biogas and bioethanol using anaerobic treatment [4,5]. Rice straw is the most abundant lignocellulosic biomass in China with an annual production ranging between 180 and 270 million tons [6]. Rice straw contains 32–47% cellulose, 19–27% hemicellulose and 5–24% lignin [7]. Cellulose (the main component of rice straw) can be hydrolyzed into glucose which may be readily con-

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verted into biogas or bioethanol. The presence of lignin and hemicellulose reduces the rate of hydrolysis especially under anaerobic conditions, resulting in lower rice straw conversion efficiency [8].

To reduce the difficulty of lignocellulosic biomass decomposition, different methods of pretreatment such as physical (particle size reduction), thermal (application of high temperature and pressure), chemical (application of strong acids or bases) and biological (application of microorganisms to decompose lignin and lignocellulose) have been widely studied and applied in recent years [9–12]. These methods can alter the chemical composition and physical structure of lignocellulosic materials, breaking the linkage between polysaccharides and lignin and consequently making cellulose and hemicelluloses more accessible to hydrolytic enzymes [13]. Biological pretreatment is more environmentally safe compared to other methods as it consumes less energy and chemicals. It is further carried out under moderate environmental conditions (temperature, pressure and pH), reducing production of potentially inhibiting compounds which could affect anaerobic digestion negatively [10].

Two groups of fungi; ascomycetes (represented by *Trichoderma reesei*) and basidiomycetes, white rot fungi (represented by *Pleurotus ostreatus*), were studied because of their ability to degrade the polymers of lignocellulose material. Some ascomycetes are known to degrade cellulose and hemicellulose, but have little ability to degrade lignin while white rot fungi can degrade cellulose, hemicellulose and lignin at equal rates [14,15]. Biological pretreatment using fungi is one of the most effective and extensively applied methods for lignocellulosic biomass conversion [16]. Taniguchi et al. [17] pretreated rice straw with four different strains of white-rot fungi (*Trametes versicolor*, *Phanerochaete chrysosporium*, *P. ostreatus*, and *Ceriporiopsis subvermispora*). *P. ostreatus* was found the most successful fungus as it was able to selectively decompose the lignin fraction but not the holocellulose component. To achieve good fungal pretreatment (selective lignin removal) efficiency, knowledge about the factors affecting fungal growth and metabolism (moisture content, feedstock particle size, oxygen concentration, and incubation time) is critical, [16].

Moisture content is critical in nutrient transfer during fungal pretreatment, thus sufficient moisture is required for healthy fungus growth and ligninolytic activity [18,19]. Optimum moisture content for fungal growth varies with fungus strain and type of biomass [20]. However, previous studies suggests that *P. ostreatus* degrades lignin well for moisture contents ranging from 60% to 85% by weight [21]. Also incubation time is important and optimal incubation time varies with biomass composition and fungus strain. Requirements for long incubation time, due to low lignin removal rates, is one of the main obstacles for the application of fungal pretreatment on a large scale [16,22]. Therefore, optimization of moisture content and incubation time in combination during fungal pretreatment is necessary to maximize lignin removal efficiency.

There is, however, a lack of research and knowledge on fungal pre-treatment of rice straw and the authors are not aware of any studies that have investigated the interactive effects of moisture content and incubation time and their combined influence on lignin removal efficiency during fungal pretreatment of rice straw and its effect on subsequent methane production. The objectives of this study were therefore to: (1) investigate the effect of fungus type, moisture content and incubation time on degradation of dry matter, cellulose, hemicellulose, and lignin during fungal pretreatment of rice straw; (2) evaluate the effect of fungal pretreatment on the biogas and methane yield of rice straw; (3) evaluate the relationship between methane yield and lignin degradation and lignin selectivity value (lignin degradation/cellulose degradation).

2. Materials and methods

2.1. Feedstock and inoculum preparation and characterization

Rice straw was obtained from a farm in Haiyan, Zhejiang Province, China. It was initially air dried for one week to a moisture content of less than 10%, subsequently cut into pieces 2–3 cm in length and stored at room temperature until further use.

Anaerobic sludge obtained from the effluent of a mesophilic biogas plant (with cow manure as feedstock) at Lin'an Zhengxing Farm, Hangzhou, Zhejiang Province, China was used as inoculum. Prior to sampling, the digester stirring was stopped for 1 day to increase the dry matter content in the inoculum. After sampling, the sludge was stored in an airtight container at room temperature (about 25 °C) until use. The concentrations of total solids (TS), volatile solids (VS) and ash content of untreated rice straw and inoculum were measured according to the Standard Methods [23]. Estimation of cellulose, hemicellulose and lignin contents were conducted by the detergent method [24]. Total carbon and nitrogen contents were evaluated by an elemental analyzer (EA 1112, CarloErba, Italy). All chemical analysis were conducted in triplicate. Characteristics of raw rice straw and inoculum are shown in Table 1. The surface structure of untreated rice straw was further examined employing field launch scanning electron microscope (SEM) using an SU8010 microscope (Hitachi, Japan). Before examination, sample particles were coated with gold film. The launching voltage of the electron microscope was 10.0 kV. Specific surface area of raw and pretreated rice straw was measured based on nitrogen adsorption using a static nitrogen absorption instrument (JW-BK, Beijing). Measurements were conducted in a liquid nitrogen environment at −196 °C for ten different nitrogen adsorption pressure intervals using about 0.8 g dried sample. The Brunauer-Emmett Teller (BET) adsorption isotherm was used to approximate the data and calculate specific surface area.

2.2. Fungus preparation

Two types of fungi were used in this study, the white-rot fungus *P. ostreatus* (DSM 11191) and *Trichoderma reesei* (QM9414). The fungi were obtained from the Department of Food Science and Nutrition and the Department of Plant Protection, Zhejiang University, Hangzhou, China, respectively. It was cultured on potato dextrose agar (PDA) plates in an incubator at 28 °C for 8 days. Four pieces of agar medium (about 5 mm in diameter) with fungus mycelium were placed in one 125 mL Erlenmeyer flask containing 25 mL of potato dextrose liquid medium. The flask was subsequently closed with a cotton stopper and incubated for 8 days at 28 °C with agitation. The fungus was separated from the liquid medium by centrifuging at 3000 rpm for 5 min using a Heal Force,

Table 1
Characteristics of rice straw and inoculum.

Parameters	Rice straw	Inoculum
TS (%, w.b.)	89.9 ± 0.2	12.1 ± 0.1
VS (%, w.b.)	80.6 ± 0.2	6.4 ± 0.1
VS/TS (%)	89.7 ± 0.1	52.9 ± 0.1
Total carbon (%, d.b.)	41.4 ± 0.3	30.1 ± 0.3
Total nitrogen (%, d.b.)	1.3 ± 0.4	2.2 ± 0.5
C/N	31.8 ± 0.4	13.7 ± 0.6
pH	ND	7.85 ± 0.1
Cellulose (%, d.b.)	37.8 ± 0.2	ND
Hemicellulose (%, d.b.)	29.6 ± 0.7	ND
Lignin (%, d.b.)	14.8 ± 0.4	ND
Ash content (%, d.b.)	10.3 ± 0.5	47.1 ± 0.2

Note: w.b., wet base; d.b., dry base; ND, not determined.
Data are mean values ± standard error of three replicates.