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Combinations of fungal and milling pretreatments for enhancing rice straw biogas production during solid-state anaerobic digestion

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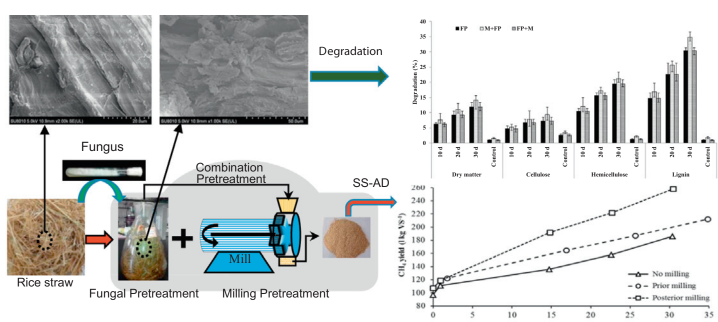
HIGHLIGHTS

- Solid state anaerobic digestion of treated rice straw by combination pretreatments.
- Methane yield increased linearly with lignin degradation in fungal pretreatment.
- Incubation time during fungal pretreatment strongly affected total methane yield.
- Combinations of fungal and milling pretreatment improve the methane yield by 165%.
- Milling after fungal treatment increases methane yield compared to prior milling.

GRAPHICAL ABSTRACT



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ABSTRACT

Rice straw was pretreated by different combinations of physical (milling) and biological (incubation with *Pleurotus ostreatus* fungus) treatment to improve its biodegradability and biogas production during solid-state anaerobic digestion (SS-AD). Effects of milling ($\leqslant$2 mm) and incubation time (10, 20 and 30 d), on lignin, cellulose, and hemicellulose degradation during fungal pretreatment and methane yield during digestion were assessed by comparison with untreated rice straw. Both incubation time and milling had significant impacts on both lignin removal during fungal pre-treatment and methane yield during digestion. A combination of fungal pretreatment at 30 days followed by milling prior to anaerobic digestion resulted in 30.4% lignin removal, the highest selectivity value (the ratio between relative lignin removal and relative cellulose removal) of 4.22, and the highest methane yield of 258 L/kg VS. This was equivalent to a 165% increase in methane yield from SS-AD compared to untreated rice straw.

1. Introduction

Concerns over greenhouse gas emissions caused by fossil fuel combustion and the associated impacts on global climate have, during recent decades, resulted in an increased interest in alternative and more sustainable sources of energy, such as biomass (Vasco-Correa and Li, 2015). Rice straw is one of the world's most readily available sources of renewable energy, and one of the most abundant lignocellulosic agricultural crop wastes in China, with an annual production ranging between 180 and 270 million tons (Zhao et al., 2010). In China, rice straw is used or disposed of in a number of ways, such as animal feeding, fuel for house heating or cooking, and as a fiber for paper production. Unfortunately,

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the largest part of the produced rice straw is disposed of by open burning in the field or by incorporation into the soil (Gadde et al., 2009; Glissmann and Conrad, 2000). Open combustion of one ton of rice straw releases 3 kg particulate matter, 60 kg CO, 1460 kg CO_2, and 2 kg SO_2 to the atmosphere (Sahni and Phutela, 2013). These substances cause lung and respiratory diseases and therefore, have a strongly negative effect on public health (Wang and Christopher, 2003). Thus it is necessary to find more sustainable and environmentally friendly alternatives for the management of rice straw. Production of renewable energy and biochemical products from agricultural waste biomass is believed to be one of the key paths toward a more sustainable development. Biogas is one of the most important sources of renewable energy that can be produced from organic waste such as lignocellulosic materials (Wang et al., 2015). Depending on the total solids content of the substrate, anaerobic digestion (AD) can be classified as liquid AD (L-AD) which operates at total solids (TS) contents of 15% or less and solid-state AD (SS-AD), which is generally operated at TS contents of 15% or more (Brown et al., 2012). In case of low solid state wastes L-AD is preferred as it produces more methane per unit mass of organic material fed to the digester, and it is easier to operate and maintain (Güelfo et al., 2010). However, for wastes with higher solids contents such as municipal solid wastes and crop residues, L-AD is not the best choice as it requires addition of large amounts of liquids (such as water or liquid manure) and digesters with large volumes. Recently therefore, the use of SS-AD for treatment of these high solid content wastes have become more widespread (Brown and Li, 2013). Because SS-AD requires smaller reactor volumes it produces more methane per volume of reactor consumes less energy for reactor heating per mass of waste treated, and needs less transportation of waste material mass to and from the plant. On the other hand SS-AD requires higher quantities of inoculum to ensure fast start-up and efficient biogas production (Li et al., 2011).

Rice straw is a lignocellulosic biomass that contains about 19–27% hemicellulose, 32–47% cellulose, and 5–24% lignin (Karimi et al., 2006). Most of the cellulose is associated with lignin in the form of lignocellulose and therefore, difficult for anaerobic microorganisms to digest due to its special resistant structure (Imai et al., 2004). The chemical composition, physical properties and chemical structure of rice straw can be changed using various pretreatment methods that increase the accessibility of holocelluloses to enzymes, which in turn will improve the anaerobic digestion of rice straw and enhance methane production (Zhang et al., 2015). Digestibility can be improved by pretreating the straw by physical (particle size reduction), chemical (high or low pH), thermal (high temperature) and/or biological (microbial degradation) methods prior to digestion (Bolado-Rodriguez et al., 2016; Chen et al., 2014; He et al., 2009; Motte et al., 2014; Zhong et al., 2011). Particle size reduction by milling is a commonly used physical method for improving digestibility by increasing the specific surface area of the feedstock. Biological pretreatment has been extensively studied because it is very inexpensive, less energy consuming, and safer compared with other pretreatment methods (Mutschlechner et al., 2015). This method uses microorganisms to selectively degrade lignin and make the biomass more accessible to subsequent digestion. Basidiomycetes such as the white rot fungi (*Pleurotus ostreatus*), have been widely studied because of its ability to degrade lignin (Arantes et al., 2012; Liers et al., 2011). Pre-treatment with *Pleurotus spp.* have been shown to increase biogas production from rice or wheat straw by up to 30% (Feng et al., 2013) and *P. ostreatus* has further been shown as one of the most effective fungi to degrade lignin compared to other fungi (Taniguchi et al., 2005). Mustafa et al. (2016) pretreated rice straw with two types of fungi (*Pleurotus ostreatus* and *Trichoderma reesei*). *P. ostreatus* was better than *T. reeseias* and was able to selectively decompose the lignin and hemicellulose. For *P. ostreatus* treatment lignin degradation was 33.4% with a selectivity value of 4.30 at 75% moisture content and 20 d incubation time, resulting in a 120% increase in methane yield compared to untreated rice straw.

For improved efficiency, biological pretreatment may be combined with thermal, chemical or physical treatment methods either before or after biological pretreatment (Narayanaswamy et al., 2013). Using steam explosion before biological pretreatment with *P. ostreatus* for rice straw reduced the necessary incubation time from 60 to 36 days with 33% net glucose yield (Taniguchi et al., 2005). Lignin loss for corn stalks increased from 75.67% to 80% by alkaline treatment after biological pretreatment with Irpex lacteus (Yu et al., 2010). A summary of methane and biogas production from rice straw by various pretreatment methods is given in Table 1. Most of the studies listed in Table 1 combined size reduction (as extrusion or milling) and hydrothermal pretreatment prior to chemical (as alkali or acidic) pretreatment (Chandra et al., 2012; Gu et al., 2015; Zhang et al., 2015). In general, pretreatment increases methane production (from 20% to 125%) from pretreated rice straw compared with untreated samples (Chandra et al., 2012; Chen et al., 2014; Dehghani et al., 2015; Gu et al., 2015; Kaur and Phutela, 2016; Mustafa et al., 2016; Zhang et al., 2015). Research on the effect of milling (size reduction) before or after fungal pretreatment on lignin removal and methane yield, however is very limited. The objective of this study was therefore to assess the effect of fungal pretreatment incubation time in combination with milling (before or after fungal pretreatment) on removal of dry matter, cellulose, hemicellulose, and lignin during pre-treatment and methane yield during subsequent anaerobic digestion of rice straw.

2. Materials and methods

2.1. Feedstock and inoculum

The rice straw used in this study was collected from a farm in Zhejiang Province, China. After being air dried for one week, it was chopped into small pieces 2–3 cm in length (labeled RS). Approximately one third of the chopped rice straw was milled to a maximum particle diameter of $\leqslant$2 mm (labeled RS+M). The two materials were then stored separately in plastic bags at room temperature until further use. Anaerobic sludge obtained from the effluent of a mesophilic biogas plant (with cow manure as feedstock) in Hangzhou, China was used as inoculum. One day before the sampling, the digester mixing was stopped to increase the dry matter content of the inoculum. After sampling, the sludge was stored at room temperature (about 25 °C) in an airtight container. The total solids (TS) and volatile solids (VS) of raw rice straw and anaerobic sludge were estimated according to the APHA standard methods (APHA, 2005). The cellulose, hemicellulose and lignin contents were measured by the method of detergent (Van Soest et al., 1991). The contents of total carbon and nitrogen were conducted using an elemental analyzer (EA 1112, CarloErba, Italy). All chemical analyses were carried out in triplicate. Properties of untreated rice straw and anaerobic sludge are shown in Table 2. Due to its low TS content, the inoculum was dewatered by evaporation using a drying oven set to the same temperature of fermentation (37 °C) for 16 h to achieve a TS content of approximately 20%. According to reports to get rapid start-up for SS-AD systems, up to 50% of inoculum in the biomass mix on the reactor is essential (Brown and Li, 2013; Li et al., 2011). The surface structure of raw rice straw was scanned by scanning electron microscopy (SEM) using an SU8010 microscope (Hitachi, Japan) with an accel-