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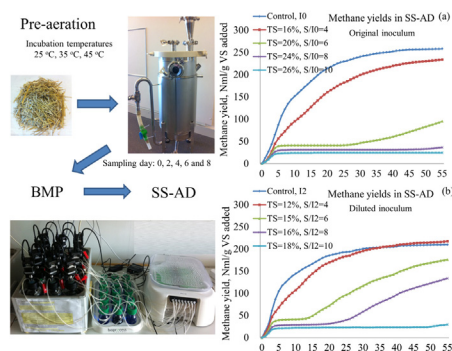
## The effects of pre-aeration and inoculation on solid-state anaerobic digestion of rice straw

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### HIGHLIGHTS

- Impact of pre-aeration on the degradation of rice straw in SS-AD was investigated.
- Pre-aerated straw for 2 d/35 °C showed the highest hydrolytic efficiency and BMP.
- VFAs accumulation at high S/I ratio and TS content led to a reduced methane yield.
- Lower inoculum concentration was priority choice for rapid initiation in SS-AD.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Pre-aeration was investigated for enhancing biodegradation of recalcitrant lignocellulosic structure of rice straw under various low temperatures regimes (25, 35 and 45 °C) and aeration durations (0, 2, 4, 6 and 8 days). It was demonstrated aerated rice straw for 2 days at 35 °C resulted in highest hydrolytic efficiency and biochemical methane potential (BMP) ( $355.3 \pm 18.7$  ml CH<sub>4</sub>/gVS). Furthermore, both methane yields and initiation speeds of the solid-state anaerobic digestion (SS-AD) were inversely proportional to substrate-to-inoculum ratios due to the accumulation of volatile fatty acids (VFAs) and poor mass transfer. The highest methane yield achieved under SS-AD was 234 ml CH<sub>4</sub>/gVS at TS of 16% which 72% of the BMP. Inoculum dilution with recycled water improved buffering capacity and mitigated accumulation of VFAs, resulting in an improved SS-AD performance. The combined pre-aeration and SS-AD was therefore established as a viable option to accelerate methane production for lignocellulosic biomass.

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## 1. Introduction

Nowadays, increasing attention has been devoted on various strategies for the bioconversion of biomass into methane-rich biogas due to increased global warming, the need for sustainable waste management and high energy costs (Li et al., 2016b). The

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anaerobic digestion (AD) or biomethanation is an attractive approach to biodegradable waste treatment which has a dual-advantage of volumetric reduction of organic wastes in the oxygen-free condition and renewable energy generation such as biogas, containing 60–70% of methane (Yan et al., 2015). AD of bio-waste and sludge is a well-developed technique in European countries. In Germany, which is the leading country in this field, >50% of the biogas potential results from energy crops treated in over 7000 biogas plants (Li et al., 2011). Furthermore, it was reported by Swedish Energy Agency that there are over 260 biogas plants in

Sweden, which facilitates the utilization of sewage sludge, manure, agricultural crops and food waste (IGR, 2015). Compared to liquid-state anaerobic digestion (L-AD), solid-state anaerobic digestion (SS-AD) has several predominances including smaller digester volume, higher solid loading capacity with total solids (TS) content of 15%–35%, free of floating and stratification of fibrous materials, reduced energy requirements for agitation, minimal material wear by fewer detachable machine parts to handle. SS-AD may also be less susceptibility to detrimental substances and over acidification especially when the new substrate is mixed with the digestate from the bottom of the reactor (Weiland, 2010). SS-AD has been used to manage municipal solid waste since the 1990s and preferred over conventional L-AD (Karthikeyan and Visvanathan, 2013). Moreover, various types of organic wastes with lignocellulosic structure of high solid contents are also treated by SS-AD to produce biogas, avoiding the subsequent slurry treatment (Li et al., 2011). It has also been reported that there is no significant difference in methane yields in both SS-AD and L-AD when treating lignocellulosic biomasses such as wheat straw, switch grass and corn stover (Brown et al., 2012).

Rice straw is one of the major agricultural wastes and the dry content of global rice straw reached approximately 741 million tons in 2014 (FAO, 2016). The waste management of rice straw via incineration or landfilling can raise serious environmental problems such as greenhouse gas emission. Several studies have reported the co-digestion of rice straw with animal manure or waste water to produce biogas (Mussoline et al., 2012; Ye et al., 2013). However, one of the drawbacks for rice straw as co-substrate is that pre-processing may be required, including material particle size reduction and pre-mixing prior to digestion. Also, it is complicated to adjust substrates ratio in co-digestion and the outcome can either be synergetic, leading higher methane yields, or antagonistic, leading to even lower yields (Li et al., 2017). It is noteworthy that L-AD is not preferred to anaerobically digest rice straw since rice straw cannot be pumped or homogenized with conventional digester without grinding due to its high TS contents. AD of rice straw in L-AD is often plagued with clogging of tubing, stratification and scum formation, and floatation of biomass (Li et al., 2011). On the other hand, the recalcitrant lignocellulosic structure and nutritive deficiency are the major problem of digestion using rice straw as the mono-substrate in SS-AD. The reason is that hydrolysis becomes the limiting step of biogas production due to the highly crystalline and compact structure (Li et al., 2016b).

Pre-treatment is a critical step for improving the biodegradation of recalcitrant structure of lignocellulosic feedstocks. Numerous types of pretreatments ranging from physical (eg. size reduction), biological (eg. enzyme) to chemical (eg. alkaline) have been reported in literatures with the aim of improving the biogas or methane yield (Dehghani et al., 2015; Li et al., 2016a; Mussoline et al., 2012). Among biological methods, pre-aeration can be considered as a simple and easy operated pretreatment. Pre-aeration was introduced in a number of lab-scale studies to improve the start-up condition and initial performance in SS-AD. Yan et al. (2015) used composting pretreatment to facilitate the bioconversion efficiency. Nguyen's research showed a positive effect in methane production through micro-aeration (2007). The reason for this might be derived from better hydrolysis/acidification during the start-up of AD process and the provision of substrate for methanogens. Additionally, short time aeration is also used as the pre-step in the industrial scale in biogas plants which have numerous dry fermentation techniques and brands such as BEKON and GICON by using lignocellulosic biomass. A major concern with pre-aeration though is the finding a balance as to avoid the toxic effect of oxygen on the slow growing, fastidious methanogens. It is thus worthy to study the effect of different pre-aeration regimes on hydrolysis and subsequent methane generation.

Compared to L-AD, SS-AD usually has inadequate mass transfer and tends to be more difficult to start up and control, thus inoculation is a principal factor for this process (Le Hyaric et al., 2012). A primary parameter that drives SS-AD is substrate to inoculum (S/I) ratio. S/I ratio has been reported to have significant impact on the methane yield (Di Maria et al., 2012; Motte et al., 2013a; Xu et al., 2016). These studies showed that increasing the quantity or concentration of inoculum could strengthen the active microorganisms for a quick start-up, shortened digestion time and improved efficiency of the SS-AD. Comparison of inoculum from solid anaerobic digesters and dewatered effluent from liquid anaerobic digesters with different S/I ratios was studied by using yard trimmings with high TS content (Xu et al., 2016). This study concluded that dewatered effluent as inoculum reduced the start-up time due to higher concentration of methanogens which reduced the risk of volatile fatty acids (VFAs) accumulation in the initial stage of SS-AD, but prolonged lag phase and even inhibition was observed with high S/I ratio. Inoculum with proper concentration provides sufficient beneficial microorganisms and prevents process inhibition from VFAs accumulation (Schievano et al., 2010). It should be observed, though, that these studies were conducted in batch reactor by mixing the substrate and effluents from liquid or SS-AD process, without comparing the impact from inoculum concentration. The inoculum concentration has also been considered as a critical factor for performing effective SS-AD. At low inoculum concentration, diluted microbial community exhibits lower metabolic activity and weakens methane production. On the other hand, lower inoculum concentration ameliorates mass transfer of solutes in the solid matrix (Bollon et al., 2013).

From the above, it is clear that the effects of pre-aeration and the role of inoculum in SS-AD require further investigation. For example, Yan et al. (2015) used composting pretreatment as a prelude to SS-AD of rice straw. However, the impact of composting on the biodegradability of the rice straw mixture was not investigated. In this study, low temperature pre-aeration pretreatment regimes (temperatures of 25, 35, 45 °C and duration of 0, 2, 4, 6, 8 days) were evaluated to improve the biodegradability of rice straw. To facilitate a process study of SS-AD, the effects of inoculum concentration via dilution using recycled water and S/I ratio were evaluated. The study was performed via a three phase configuration: (1) pre-aeration to improve hydrolysis of rice straw (2) biochemical methane potential assay to evaluate the effects of the pre-treatment on methane production and (3) SS-AD to test the feasibility of digestion of rice straw under the solid-state mode.

## 2. Material and methods

### 2.1. Substrates and inoculum

The rice straw was collected from Caisang Lake Village (29°53'60"N 112°75'32"E) in Yueyang, China where it is prevalently cultivated with high productivity. It was packed outdoor and air-dried for 2 months after harvest, then transported to Lund University, Sweden at the end of October 2015 and stored at 4 °C prior to use. The TS of the rice straw was 93.0% and a volatile solid (VS) was 80.2%. The rice straw was ground with a grinder (Grindomix 200, Retch USA) to pass through a 2-cm for homogeneity. The aerobic sludge was collected from the secondary sedimentation tank at a wastewater treatment plant (WWTP, Källby, Lund, Sweden). The aerobic sludge was statically placed for 24 h and the supernatant was collected to mix with rice straw in order to adjust moisture content and enrich microbial diversity for aeration treatment.

The anaerobic sludge (inoculum) was collected from the anaerobic digester at the same WWTP. The inoculum was pre-incubated

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