



# Integration of Shiitake cultivation and solid-state anaerobic digestion for utilization of woody biomass



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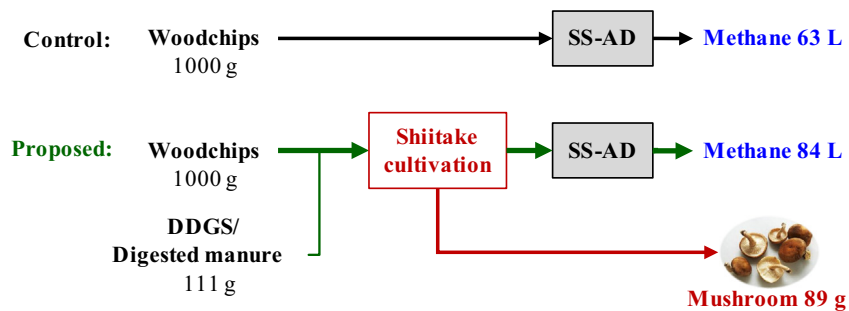
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## HIGHLIGHTS

- Woodchips were pretreated by Shiitake cultivation for enhanced biogas production.
- Shiitake cultivation was used as pretreatment for anaerobic digestion of woodchips.
- Mushroom yields comparable to those using a commercial substrate were obtained.
- Enzymatic digestibility of mushroom substrates were increased by at least 1.5 times.
- Both methane production and solid waste reduction were significantly improved.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Pretreatment technologies that can not only reduce the recalcitrance of woody biomass but also achieve a high benefit-cost ratio are desirable for bioenergy production from woody biomass. In this study, an integrated process was proposed and conducted by pretreating woodchips via Shiitake cultivation for improved methane yield during solid-state anaerobic digestion (SS-AD), and simultaneously producing mushrooms as a high-value co-product. Shiitake cultivation using woodchips as the main substrate ingredient obtained mushroom yields comparable to those using a commercial substrate. Enzymatic digestibility and cumulative methane yields ( $133\text{--}160\text{ L kg}^{-1}\text{ VS}$  during 62 days of SS-AD) of pretreated substrates (spent mushroom substrate) were at least 1.5 times as high as those of untreated woodchips. Compared to a sole SS-AD process, the integrated Shiitake cultivation/SS-AD process increased methane production and solid waste reduction per kilogram of woodchips by about 1.5 and 8 times, respectively.

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## 1. Introduction

Woody biomass has been recognized as an important source for energy production. In 2010, about 65% of the world's renewable energy was supplied from woody biomass (Lauri et al., 2014).

The potential energy from available woody biomass has been estimated to be about  $1\text{--}4 \times 10^{20}\text{ J}$  per year in 2050, which could meet 10–40% of the global primary energy consumption (Lauri et al., 2014).

Solid-state anaerobic digestion (SS-AD) is a technology that is capable of converting woody biomass into bioenergy in the form of biogas (De, 2000; Schäfer et al., 2006). Biogas is a renewable energy source that can be used for cooking through direct combus-

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**Table 1**  
Composition of substrate ingredients and inoculum (grain spawn).

Parameters	Woodchips	DDGS	Digested dairy manure	Grain spawn
Total solids (%) <sup>a</sup>	89.2 ± 0.2	88.4 ± 0.3	15.3 ± 0.1	52.8 ± 0.9
Volatile solids (%) <sup>b</sup>	99.2 ± 0.3	92.8 ± 0.7	79.9 ± 0.1	97.9 ± 1.8
Total carbon (%) <sup>b</sup>	47.5 ± 0.1	52.3 ± 1.9	44.4 ± 0.6	43.0 ± 0.3
Total nitrogen (%) <sup>b</sup>	0.34 ± 0.04	5.14 ± 0.01	5.94 ± 0.13	1.71 ± 0.08
C/N ratio	140 ± 16	10.2 ± 0.3	7.5 ± 0.2	25.3 ± 1.1
Extractives (%) <sup>b</sup>	7.1 ± 0.0	44.1 ± 0.1	15.1 ± 0.0	16.1 ± 1.8
Protein (%) <sup>b</sup>	1.7 ± 0.0	31.9 ± 0.4	17.7 ± 0.9	10.1 ± 0.7
Cellulose (%) <sup>b</sup>	33.0 ± 0.4	10.8 ± 1.7	14.0 ± 0.2	57.5 ± 0.0
Hemicellulose (%) <sup>b</sup>	13.9 ± 0.1	12.9 ± 2.1	10.9 ± 0.2	6.7 ± 0.2
Lignin (%) <sup>b</sup>	25.2 ± 0.3	3.6 ± 0.2	27.0 ± 0.3 <sup>c</sup>	4.4 ± 0.9
pH	4.87	4.50	8.38	–

<sup>a</sup> Based on wet weight.

<sup>b</sup> Based on dry weight.

<sup>c</sup> Contain other acid insoluble organic matter besides lignin.

**Table 2**  
Substrate ingredients for Shiitake cultivation.

Treatment No.	Woodchips (%)	DDGS (%)	Digested dairy manure (%)	TC (%)	TN (%)	C/N
1	86	14	0	48.2	1.0	47.7
2	87	7	6	47.7	1.0	47.2
3	90	10	0	48.0	0.8	58.6
4	90	7	3	47.8	0.8	56.7
5	83	17	0	48.3	1.2	41.9
6	83	12	5	48.0	1.2	40.1
7	78	22	0	48.6	1.4	34.8
8	80	8	12	47.6	1.4	34.1
Control	Sawdust (78%), wheat bran (19.7%), calcium sulfate (1%), sucrose (1%) and urea (0.3%)			48.8	1.0	48.7

tion, generating heat and electricity through a combined heat and power unit, or producing compressed natural gas or liquefied natural gas after upgrading. SS-AD of woody biomass has recently been studied by several researchers, but methane yields and total solids (TS) degradation were generally low (Brown and Li, 2013; Liew et al., 2012; Lin et al., 2014; Zhao et al., 2014a,b). Compared to non-woody biomass, woody biomass is recalcitrant and difficult to digest (Zhu and Pan, 2010). In order to reduce the recalcitrance of woody biomass, various pretreatment technologies, including physical, chemical, and biological methods, have been studied (Zheng et al., 2014). Currently existing pretreatment technologies require additional energy and/or chemicals, while no value-added products are produced during the pretreatment process. Therefore, a common limitation of these strategies is that the benefit-cost ratio of pretreatment is low.

*Lentinula edodes* (Shiitake) is an edible mushroom with high value, and it is usually cultivated on hardwood sawdust with grains as the nitrogen source (Pire et al., 2001; Rossi et al., 2003; Royse, 1997). Shiitake cultivation generates considerable amounts of spent mushroom substrate (SMS), a solid waste which must be treated properly. A substantial amount of the lignin in the substrate is decomposed and permeated by mycelia during Shiitake cultivation, making the SMS highly digestible compared to untreated mushroom substrate (Bisaria et al., 1983, 1990; Lin et al., 2014; Sharma et al., 1989).

Combining SS-AD and Shiitake cultivation technologies is a plausible strategy for utilizing woody biomass. This process would integrate (1) Shiitake cultivation on woodchips for mushroom production, and (2) SS-AD of the woodchips pretreated by Shiitake fungi for biogas production. The major advantage of this strategy is that the cost of pretreatment can be offset by producing a value-added co-product (mushrooms). SS-AD of sawdust-based SMS has recently been studied; however, its high digestibility made it unsuitable as a sole feedstock for SS-AD due to accumulation of volatile fatty acids (VFAs) which caused an inhibitory effect

on SS-AD (Lin et al., 2014). As a result, sawdust-based SMS had to be co-digested with low-digestible feedstocks, such as yard trimmings and wheat straw, in order to successfully produce biogas via SS-AD (Lin et al., 2014). Woodchips have been used to replace sawdust as the main substrate ingredient for Shiitake cultivation (Royse, 1996). Since woodchips have much larger particle sizes than sawdust, digestibility of woodchip-based SMS may not be as high as that of sawdust-based SMS (Zhang et al., 2012). Therefore, it is reasonable to envisage that woodchip-based SMS could possibly be used as the sole feedstock for SS-AD, although few studies reported SS-AD of woodchip-based SMS. To the best of the authors' knowledge, there have been no studies on using Shiitake cultivation as a pretreatment method for enhanced bioenergy production from woodchips.

In order to make the integrated process more cost-effective, alternative nitrogen sources are desirable to replace grains for Shiitake cultivation. Dried distillers grains with solubles (DDGS), a co-product from corn-ethanol production that uses a dry-mill process, has traditionally been used as a nitrogen source for the animal feed industry (Bonnardeaux, 2007). Digested dairy manure is the digestate from anaerobic digestion of dairy manure (Informa Economics, 2013). Both DDGS and digested dairy manure are rich in nutrients, although neither has been investigated as a nitrogen source for Shiitake cultivation.

The overall objective of this study was to evaluate the feasibility of utilization of woodchips for mushroom and biogas production via an integrated Shiitake cultivation and SS-AD process. Shiitake cultivation was conducted using woodchips as the main substrate ingredient, and DDGS and digested dairy manure as nitrogen sources. Effects of nitrogen loadings on mushroom production and degradation of cellulose, hemicellulose, and lignin in the substrates were examined. SS-AD of woodchips, raw substrates, and SMS were carried out, and biogas production and degradation of cellulose, hemicellulose, and volatile solids (VS) in these feedstocks during SS-AD were investigated. At last, nutrient requirements,

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