Cultivation of *Pleurotus ostreatus*, a Potential Candidate for Biogas Residues Degradation

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Biogas projects are rapidly expanding in China, but there is insufficient cropland to degrade these biogas residues. Mushroom cultivation has been used to degrade various agricultural wastes. In this study, to screen the feasibility of utilizing biogas residues as potential substrates for the cultivation of *Pleurotus ostreatus*, seven different concentrations (0 to 60%) of biogas residue mixed with cottonseed hull, wheat bran, and lime were used to cultivate *P. ostreatus*. The mycelial growth rate, mycelial colonization time, yield, biological efficiency, chemical compositions, and content of four heavy metals, Cd (cadmium), Pb (lead), Hg (mercury), and As (arsenic), were analyzed. The results showed that 10 to 30% of biogas residue mixed in the substrates induced the growth of *P. ostreatus* mycelia faster than the others. A lower percentage (10 to 20%) of biogas residue added to the substrates is beneficial to the production and nutrient components of *P. ostreatus*, and the fruiting bodies produced on biogas residue-containing substrates conform to the safety standards for edible mushrooms. Although the total harvest is not significantly increased when biogas residue is added, the utilization of cheap biogas residues can conceivably reduce the practical cost and benefit the environment.

Keywords: Biogas residue; Oyster mushroom; Heavy metals; Chemical content; Substrate amendment

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INTRODUCTION

The global energy demand is rapidly growing, and approximately 88% of the current demand is fulfilled by fossil fuels (Weiland 2010). The non-regenerative energy sources, such as oil, coal, natural gas, and other fossil fuels, are increasingly exhausted (Mao *et al.* 2013). Moreover, the consumption of fossil fuels causes serious pollution, leading to climate change (Jiang *et al.* 2011). Therefore, it is necessary to develop clean and renewable energy sources to replace fossil fuels (Mao *et al.* 2013). Among these renewable energy sources, biogas has gained increasing attention since the beginning of the 21st century (Zirkler *et al.* 2014). Based on the progress of China's 2016 to 2020 National Rural Biogas Construction Plan, 1.11 million new biogas pools and 28,650 new medium and large-scale biogas plants will be built by 2020. Therefore, millions of tons of biogas residues have to be dealt with in the next few years. Currently, most biogas residues are mainly used as fertilizers in many developed countries in order to return nutrients back to the soil ecosystem (Svensson *et al.* 2004; Odlare *et al.* 2011; Quakernack *et al.* 2012). However, in China, there are insufficient croplands or ponds

around the biogas projects that deplete these residues. Thus, secondary pollution by biogas plants is rampant due to the wasting of the residues (Song *et al.* 2014).

In recent years, the bioconversion of agricultural and industrial wastes into edible products has attracted attention worldwide (Ingale and Ramteke 2010). Mushroom cultivation provides an effective and ecofriendly method to convert biogas and other agricultural wastes into proteinaceous food (Ananbeh and Al-Momany 2008; Amuneke *et al.* 2011; Alananbeh *et al.* 2014; Marlina *et al.* 2015; Kamthan and Tiwari 2017). Many types of mushrooms have been recently used to decrease the cellulose and lignin contents in the undigested residues and produce nutritious food (Banik and Nandi 2004; Chen *et al.* 2010; Al-Momany and Ananbeh 2011; Ashwath *et al.* 2016; Gupta *et al.* 2016; Malayil *et al.* 2016; Atila *et al.* 2017). Because the lignocellulosic substrates are partially degraded during anaerobic fermentation, biogas residues are expected to serve as a good starter and stimulant for improved mushroom cultivation in terms of nutrient content as well as productivity (Banik and Nandi 2004).

Pleurotus (Fr.) P. Kumm. (also called oyster mushrooms), the second most grown edible mushroom worldwide after *Agaricus bisporus* (J.E. Lange) Imbach (Sánchez 2010), is one of the easiest, fastest, and cheapest to cultivate (Mandeel *et al.* 2005). Naturally, *Pleurotus* spp. mostly grow on dead parts of plants, which are generally poor in nutrients and vitamins (Naraian *et al.* 2009). However, a wide range of carbon compounds can be utilized to cultivate mushrooms, particularly the decomposition products of cellulose and hemicellulose (Chang and Hayes 1978). *Pleurotus* can efficiently degrade lignin selectively from nonwoody lignocellulosic materials (Martínez *et al.* 1994; Ananbeh and Almomany 2005). Various agricultural wastes, such as oak sawdust (Estrada and Royse 2007), water hyacinth grape (Chen *et al.* 2010), marc plus cotton gin trash (Koutrotsios *et al.* 2014), date-palm leaves (Alananbeh *et al.* 2014), *Ficus* leaves (Alemu and Fisseha 2015), tea waste (Yang *et al.* 2016), cottonseed shells, corn cob particles (Lin *et al.* 2017), and olive mill waste (Avni *et al.* 2017), are recently being used as substrates to cultivate different kinds of edible mushrooms.

With the increasing prices of the cottonseed hulls, corncob, wheat bran, sawdust, and other materials, it is necessary to identify some economical candidates to cultivate mushrooms. The biogas residual slurry manures are rich in mineral nutrients and are very effective for increasing the yield of oyster mushrooms (Banik and Nandi 2004). In this investigation, biogas residues were used as substitutes for cottonseed hulls to cultivate *P. ostreatus* (Jacq.) P. Kumm. To screen the feasibility of utilizing biogas residues as potential substrates for the cultivation of oyster mushrooms, mycelial growth rate, fruiting body production, chemical components, and heavy metal (lead, mercury, cadmium, and arsenic) contents were investigated in this study.

EXPERIMENTAL

Materials

Fungus and raw materials

Pleurotus ostreatus (trade name Huimei No. 2) used in this study was preserved in the Beijing Engineering Research Center for Edible Mushroom, Beijing Academy of Agriculture and Forestry Sciences (Beijing, China).

The biogas residues, which mainly consisted of chicken excrements and wheat straws, were provided by Daxing Biogas Station and generally handled through a short

fermentation process to degrade the ammonia. During fermentation, the moisture in biogas residues was maintained at 70% at the beginning, and the pile was turned at every two-day interval over a 10-day period. Other raw materials, such as the cottonseed hulls and wheat bran, were obtained from Beijing Lvyuanyongle Agricultural Development Co., Ltd. (Beijing, China). Carbon (C) and nitrogen (N) contents of biogas residues, cottonseed hulls, and wheat bran were analyzed following the method described by Dundar *et al.* (2009), as shown in Table 1. The content of heavy metals (lead, mercury, cadmium, and arsenic) in biogas residues, cottonseed hulls, wheat bran, lime, and water used in this experiment were analyzed by Pony Testing International Group (Beijing, China), and this analysis is shown in Table 2.

Table 1. Carbon (C) and Nitrogen (N) Analysis of Cottonseed Hull, Wheat Bran, and Biogas Residue Used for *Pleurotus ostreatus* Cultivation

Material	C (%)	N (%)	C/N
Cottonseed hull	38.06	0.57	66.77
Wheat bran	36.92	1.98	18.65
Biogas residue (before fermenting)	25.81	2.03	12.71
Biogas residue (after fermenting)	18.57	1.65	11.25

Table 2. Heavy Metal Contents of Raw Materials Used in this Study

Material	Heavy Metal Contents (mg/kg or mg/L) *			
	Cadmium	Lead	Mercury	Arsenic
Cottonseed hull	0.0815 ± 0.0005 b	0.5930 ± 0.0250 c	0.0019 ± 0.0001 c	0.0420 ± 0.0010 c
Wheat bran	0.0975 ± 0.0015 a	0.0865 ± 0.0350 d	0.0018 ± 0.0001 c	0.1350 ± 0.0050 b
Biogas residue (before fermenting)	0.0665 ± 0.0005 c	2.6800 ± 0.0500 b	0.0225 ± 0.0005 a	0.6800 ± 0.1000 a
Biogas residue (after fermenting)	0.0635 ± 0.0015 c	2.7600 ± 0.0300 b	0.0210 ± 0.0000 b	0.6550 ± 0.0050 a
Lime	0.0435 ± 0.0025 d	3.0950 ± 0.0450 a	0.0001 ± 0.0000 d	0.6600 ± 0.0200 a
Water	0.0003 ± 0.0000 e	0.0015 ± 0.0001 e	0.0001 ± 0.0000 d	0.0007 ± 0.0000 d

* Values are the mean of three replicates. Means in the column followed by the same superscripts are not statistically different at $P < 0.05$ according to Duncan's multiple range test.

Methods

Substrate preparation, and mushroom cultivation and harvesting

The strain used in this study was incubated on potato dextrose agar (PDA, 200 g/L of diced potatoes; 20 g/L of glucose; 15 g/L of agar) medium at 25 °C for the regular subculture. The spawn preparation was conducted as described by Xu *et al.* (2016).

All materials used in this study were insolated to ensure no mold contamination (Xu *et al.* 2016). The control (CK) and six treatments (T1 to T6) with different combinations of substrates were used (Table 3). The water content of the final mixed substrates was adjusted to 65% (w/w).

The prepared substrates weighing 1.2 kg were placed in the polypropylene bags (17 cm × 33 cm × 0.04 cm) and sterilized at 121 °C for 2 h. All the sterile substrates were inoculated with 2% (w/w) spawn on the surface of substrate under aseptic conditions on September 8, 2015. Sixty bags were used and equally divided into three replicates for each treatment.

Table 3. CK and Six Treatments Formula Used for *Pleurotus ostreatus* Cultivation (% by Dry Weight)

Material	CK	Treatment Group					
		T1	T2	T3	T4	T5	T6
Cottonseed hulls	80	70	60	50	40	30	20
Biogas residue (after fermenting)	0	10	20	30	40	50	60
Wheat bran	18	18	18	18	18	18	18
Lime	2	2	2	2	2	2	2
C/N	45.66	38.45	32.65	27.89	23.91	20.53	17.62

The inoculated substrates were incubated in the spawn running room at an ambient temperature of 25 °C and 65 to 70% relative humidity. The mycelial growth rate was determined following the method of Gregori *et al.* (2008), and the results are shown (Table 4).

After a complete spawn run, the bags were moved to the greenhouse and maintained at 22 to 25 °C and 85 to 90% relative humidity. When the first primordia of *P. ostreatus* appeared, plugs were ripped out from the bags to make fruiting bodies grow. Mushrooms were harvested when the cap surfaces were flat to slightly up-rolled at the margins.

Three flushes were obtained for all treatments, and mushroom harvesting was finished on the 90th day after inoculation. The fruiting bodies of *P. ostreatus* were manually harvested and measured daily. At the end of the yielding period, the accumulated weights were used to calculate the biological efficiency (BE, Table 5), as follows,

$$\text{BE (\%)} = (\text{Weight of fresh mushroom fruiting bodies/weight of dry substrates}) \times 100 \quad (1)$$

Chemical and statistical analyses

The fruiting bodies of *P. ostreatus* harvested at the first flush were dried in the oven at 60 °C to obtain a constant weight and then kept at 4 °C (Xu *et al.* 2016). Compositions (Table 6) of moisture, dietary fiber, ash, protein, fat, carbohydrate, amino acids, and the four heavy metals in the biomass were analyzed by PONY Testing International Group (Beijing, China).

Data obtained from two consecutive harvests and chemical biomass composition analyses were subjected to a one-way analysis of variance. Differences among the means of six treatments were assessed using Duncan's multiple range tests at the 95% confidence level. All statistical analyses were performed using IBM SPSS Statistics V22.0 (IBM, Chicago, IL, USA).

RESULTS AND DISCUSSION

Results

Carbon and nitrogen content of different substrates

As shown in Table 1, the biogas residue contained less carbon and more nitrogen than cottonseed hull and wheat bran. After fermentation with aerobic microorganisms, the percentage of carbon and nitrogen and the C/N ratio were significantly decreased. Although the concentration of the four heavy metals slightly decreased during the fermentation process, there was no significant difference between biogas residue before and after fermenting. The cadmium concentration in wheat bran was significantly higher than that in other residues, while the lead content in lime was much higher than that in other materials. The biogas residue contained much more mercury and arsenic than cottonseed hull and wheat bran. Among all the seven mixed substrates, CK was found to have the highest C/N ratio (45.66) while T6 had the lowest (17.62) (Table 3).

Mycelial Growth and Colonization on Different Treatment Substrates

The mycelial growth rate, mycelial colonization, and primordial initiation time of *P. ostreatus* cultivated on substrates exposed to different treatments are shown in Table 4. The mycelial colonization time on all the substrates ranged from 21 to 25 days, and the primordial initiation time of the seven treatments ranged from 32 to 34 days. Compared to the other treatments, T2 resulted in significantly faster mycelial growth. T1 and T3 also resulted in a markedly faster mycelial growth than the CK. However, when the substrates had a > 30% biogas residue concentration (T4 to T6), the mycelial growth on treated substrates was slower than that on CK substrates.

Table 4. Comparison of Mycelial Growth Rate, Mycelial Colonization Time, and Primordium Initiation Time of *P. ostreatus* Cultivated on Different Treatment Groups

Treatment Group	Growth Rate (mm/d) mean ± SD *	Mycelial Colonization Time (days)	Primordial Initiation Time (days)
T2	5.69 ± 0.14 a	21 to 22 a	32 to 33 a
T1	5.35 ± 0.25 b	21 to 23 ab	32 to 33 a
T3	5.30 ± 0.11 b	21 to 23 ab	32 to 33 a
CK	5.07 ± 0.26 c	22 to 24 b	33 to 34 bc
T4	4.54 ± 0.18 d	22 to 24 b	33 ab
T6	4.42 ± 0.24 d	22 to 24 b	34 to 35 c
T5	4.11 ± 0.14 e	23 to 25 c	34 c

* Values are the mean of three replicates. Means in column followed by the same superscripts are not statistically different at P < 0.05 according to Duncan's multiple range test.

Productivity evaluation of P. ostreatus

Table 5 shows the biological efficiencies (BEs) and total yields of the first two flushes of *P. ostreatus* cultivated on different treatment substrates. The maximum average wet weight (6.22 ± 0.27 kg) and BE (64.76 ± 2.77%) of fruiting bodies was obtained in T2. However, these values were not significantly different from those for T1 and CK. When the percentage of biogas residue mixed in the substrates was higher than 30%, the total yield significantly decreased. T6 resulted in the lowest total weight (3.14 ±

0.11 kg) and BE (32.70 ± 1.13%); these values were nearly half of those for CK, T1, and T2.

The three flushes (the third flush was observed 14 days after the second flush was harvested) for all the treatments are shown in Fig. 1. In the first flush, fruiting bodies from all treatments had excellent quality traits with good mushroom shapes, and no malformed mushrooms were observed. In the second flush, treatments CK, T1, and T2 resulted in good yield and fruiting body quality traits for *P. ostreatus*, and only a few malformed mushrooms were harvested. T3 also resulted in well-shaped mushrooms, but the harvest was significantly decreased. When substrate had a biogas residue concentration higher than 30%, the cultivated mushrooms were significantly malformed. Most of the fruiting bodies in treatments T5 and T6 had grayish-white pileal surfaces and longer stipes. In the third flush, the mushrooms grew slowly in all seven treatment groups and most of the fruiting bodies were malformed; therefore, the total weights and biological efficiencies were not analyzed at all.

Table 5. Comparison of Yield, Biological Efficiency, and Water Content of *P. ostreatus* on Different Treatment Groups in 20 Bags (mean ± SD) [a]

Treatment Group	Fresh Weight of Mushrooms by Flushes (kg)			BE (%)
	First	Second	Total	
CK	3.29 ± 0.18 ab	2.83 ± 0.27 a	6.12 ± 0.25 a	63.78 ± 2.63 a
T1	3.27 ± 0.18 ab	2.89 ± 0.2 a	6.16 ± 0.32 a	64.13 ± 3.31 a
T2	3.65 ± 0.28 a	2.57 ± 0.23 a	6.22 ± 0.27 a	64.76 ± 2.77 a
T3	3.15 ± 0.13 bc	2.17 ± 0.17 b	5.31 ± 0.15 b	55.32 ± 1.59 b
T4	2.8 ± 0.22 c	1.82 ± 0.16 b	4.62 ± 0.37 c	48.14 ± 3.89 c
T5	2.66 ± 0.19 c	1.43 ± 0.13 d	4.09 ± 0.32 c	42.66 ± 3.28 c
T6	2.32 ± 0.09 d	0.82 ± 0.11 e	3.14 ± 0.11 d	32.70 ± 1.13 d

[a] Values are the mean of three replicates. Means in column followed by the same superscripts are not statistically different at $P < 0.05$ according to Duncan's multiple range test.

Chemical biomass compositions of P. ostreatus

To determine the chemical compositions of *P. ostreatus* cultivated on the seven different substrate combinations, this study analyzed the concentration of 18 amino acids, total proteins, carbohydrates, four heavy metals, and several other chemicals in fruiting bodies from the first flush using 100 g dry matter (Table 6).

Generally, an increase in the biogas residue concentration in the substrate resulted in an increase in the amino acid content of the *Pleurotus* fruiting bodies. The highest total amino acid content was detected in T6 (18.91 ± 0.05 g/100 g), while the lowest was in CK (14.10 ± 0.10 g/100 g). Arginine and lysine contents were both significantly lower in mushrooms grown on CK than in those grown on biogas residue-containing substrates. Interestingly, there was no significant difference between CK and other treatments in the content of cysteine among all the 18 amino acids. Similar to the total amino acid content, an increase in the substrate biogas residue content also led to an increase in the protein content of the mushrooms. The moisture content of *P. ostreatus* initially decreased and then increased as the biogas residue content of the substrates increased, and the highest and lowest moisture contents were found in T6 and T2, respectively.

In contrast to the moisture content results, the ash content in *P. ostreatus* fruiting bodies initially increased and then decreased when the substrate biogas residue content increased, and the highest and lowest contents were found in T1 and T5, respectively. However, there was no specific relevance of the total fat content to the substrate biogas residue concentration. The fat content in CK was much lower than that in T1, but significantly higher than those in the other five treatments. Dietary fiber content was much higher in mushrooms from T4 than in those from other treatments, while the lowest content was found in mushrooms from T5. *Pleurotus ostreatus* fruiting bodies from T5 produced the most carbohydrates (47.30 ± 0.25 g), followed by those from treatments T2, T3, and T1. However, for CK, the mushroom carbohydrate content was higher than those in treatments T4 and T6.

With respect to the heavy metal content, the highest lead content (1.1550 ± 0.0550 mg/kg) was detected in T1, followed by CK and T2; however, these differences were not significant. The other four treatments resulted in lower lead contents and displayed no significant difference from each other. T3 showed the highest cadmium content (0.1400 ± 0.0100 mg/kg), while the lowest content was observed in T6 (0.0550 ± 0.0000 mg/kg). The concentration of the other two metals analyzed, mercury and arsenic, increased with an increase in the substrate biogas residue content; the highest and the lowest contents were both found in T6 (0.0074 ± 0.0000 mg/kg for Hg and 0.1650 ± 0.0050 mg/kg for As) and CK (0.0030 ± 0.0002 mg/kg for Hg and 0.0530 ± 0.0020 mg/kg for As), respectively.

Discussion

Biogas production is a fast-growing market in many parts of the world (Weiland 2010), and fully developed biogas technologies, integrated utilization patterns, and management abilities have been formed and enhanced in China (Chen *et al.* 2012). Biogas residues, which exist in the insoluble or organic and inorganic solid forms that are difficult to decompose, cannot be depleted because of insufficient croplands or ponds around most biogas projects (Song *et al.* 2014). Moreover, with the popularity of biogas projects, there is a need for tests gauging environmental risks to determine the environmental friendliness of the heavy metals found in fermentation residues (Feng *et al.* 2011). The present study aimed to use biogas residue to substitute the cottonseed hull substrate in *P. ostreatus* cultivation.

Chicken manure is considered to contain 21.4 to 58.6% total organic carbon and 2.1 to 6.57% total nitrogen, with a C/N ratio between 6.57 and 13.4 (Gao *et al.* 2010; Shen *et al.* 2011; Wang *et al.* 2012, 2014; Yan *et al.* 2018). The manure has a high ash content of 56.8% and is rich in many mineral nutrients, such as N (Nitrogen), P (Phosphorous), K (Potassium), Na (Sodium), Ca (Calcium), Cu (Copper), Mg (Magnesium), Mn (Manganese), Ni (Nickel), and Zn (Zinc) (Banik and Nandi 2004; Insam *et al.* 2015). Chicken manure biogas residue contains the highest content of Hg and As of all the materials used in this study. The Pb content of biogas residue is less than that of lime, but much more than that of cottonseed hull and wheat bran. Before mixing the biogas residue with other materials, the residue was allowed to ferment for ten days to decompose the ammonia within. After the fermentation period, the carbon and nitrogen contents and C/N ratio were significantly decreased; this result is in agreement with those of Huang *et al.* (2016) and Ma *et al.* (2017).

Fig. 1. The three flushes of fruiting bodies of *Pleurotus ostreatus* for all the treatments. –1, –2, and –3 indicate the first, second, and third flush, respectively.

Table 6. Comparison of Chemical Compositions from 100 g Dry Matter of *P. ostreatus* on Different Treatment Groups * (mean ± SD, n = 2)

Parameter	Treatments						
	CK	T1	T2	T3	T4	T5	T6
Asparagine (g)	1.73 ± 0.01 d	1.80 ± 0.03 cd	1.82 ± 0.01 cd	1.86 ± 0.07 c	2.06 ± 0.03 b	2.13 ± 0.02 b	2.33 ± 0.00 a
Threonine * (g)	0.74 ± 0.00 e	0.79 ± 0.02 de	0.80 ± 0.01 de	0.79 ± 0.03 cd	0.86 ± 0.01 c	0.92 ± 0.01 b	1.00 ± 0.00 a
Serine (g)	0.71 ± 0.01d	0.76 ± 0.03 cd	0.80 ± 0.01 bc	0.77 ± 0.01 c	0.83 ± 0.02 b	0.90 ± 0.01 a	0.95 ± 0.00 a
Glutamate (g)	2.37 ± 0.02 d	2.60 ± 0.06 c	2.38 ± 0.01 d	2.52 ± 0.07 c	2.76 ± 0.03 b	3.01 ± 0.03 a	3.08 ± 0.03 a
Proline (g)	0.66 ± 0.03 cd	0.68 ± 0.04 cd	0.68 ± 0.01 cd	0.63 ± 0.01 d	0.72 ± 0.01 bc	0.78 ± 0.02 ab	0.82 ± 0.02 a
Glycine (g)	0.73 ± 0.00 d	0.78± 0.01 c	0.78 ± 0.01 c	0.76 ± 0.03 cd	0.84 ± 0.01 c	0.89 ± 0.01 b	0.95 ± 0.01 a
Alanine (g)	0.87 ± 0.00 f	0.93 ± 0.02 de	0.97 ± 0.00 d	0.92 ± 0.02 ef	1.03 ± 0.01 c	1.12 ± 0.02 b	1.23 ± 0.02 a
Cysteine (g)	0.20 ± 0.01 a	0.18 ± 0.01 a	0.18 ± 0.01 a	0.17 ± 0.01 a	0.17 ± 0.02 a	0.17 ± 0.01 a	0.20 ± 0.03 a
Valine * (g)	0.76 ± 0.01 d	0.79 ± 0.02 d	0.79 ± 0.00 d	0.79 ± 0.03 d	0.87 ± 0.02 c	0.93 ± 0.02 b	1.00 ± 0.01 a
Methionine * (g)	0.24 ± 0.01 bc	0.24 ± 0.01 bc	0.21 ± 0.01 c	0.25 ± 0.02 b	0.26 ± 0.01 ab	0.29 ± 0.00 a	0.29 ± 0.01 a
Isoleucine * (g)	0.54 ± 0.01 c	0.58 ± 0.02 c	0.57 ± 0.01 c	0.56 ± 0.02 c	0.64 ± 0.01 b	0.67 ± 0.02 b	0.74 ± 0.01 a
Leucine (g)	0.96 ± 0.03 d	1.05 ± 0.03 c	1.02 ± 0.00 cd	1.01 ± 0.03 cd	1.13 ± 0.01 b	1.16 ± 0.03 b	1.29 ± 0.03 a
Tyrosine * (g)	0.33 ± 0.03 c	0.42 ± 0.03 ab	0.37 ± 0.01 bc	0.36 ± 0.02 bc	0.39 ± 0.00 bc	0.40 ± 0.00 ab	0.47 ± 0.03 a
Phenylalanine * (g)	0.81 ± 0.01 d	0.86 ± 0.02 cd	0.87 ± 0.03 bc	0.81 ± 0.05 d	0.87 ± 0.01 bc	0.91 ± 0.01 b	1.00 ± 0.01 a
Lysine * (g)	0.97 ± 0.01 e	1.04 ± 0.01 d	1.05 ± 0.00 d	1.03 ± 0.03 d	1.14 ± 0.01 c	1.2 ± 0.01 b	1.30 ± 0.02 a
Histidine * (g)	0.36 ± 0.01 d	0.39 ± 0.01 c	0.38 ± 0.01 cd	0.38 ± 0.02 cd	0.42 ± 0.00 b	0.44 ± 0.00 b	0.48 ± 0.00 a
Tryptophan (g)	0.33 ± 0.01 c	0.34 ± 0.01 c	0.34 ± 0.00 c	0.34 ± 0.01 c	0.43 ± 0.01 a	0.39 ± 0.01 b	0.44 ± 0.01 a
Arginine (g)	0.82 ± 0.02 e	0.91 ± 0.01 d	0.88 ± 0.01 d	0.90 ± 0.03 d	0.99 ± 0.01 c	1.13 ± 0.01 b	1.31 ± 0.02 a
Total amino acids (g)	14.10 ± 0.10 e	15.11 ± 0.07 d	14.87 ± 0.09 d	14.81 ± 0.01 d	16.38 ± 0.06 c	17.42 ± 0.04 b	18.91 ± 0.05 a
Moisture (g)	10.16 ± 0.08 c	9.86 ± 0.02 d	9.58 ± 0.02 e	11.34 ± 0.09 b	11.44 ± 0.11 b	11.75 ± 0.09 a	11.87 ± 0.12 a
Ash (g)	5.91 ± 0.03 b	6.25 ± 0.06 a	5.99 ± 0.04 b	5.99 ± 0.02 b	5.90 ± 0.04 b	5.56 ± 0.06 c	5.99 ± 0.05 b
Fat (g)	1.88 ± 0.00 b	2.05 ± 0.03 a	1.69 ± 0.01 cd	1.73 ± 0.02 c	1.35 ± 0.03 e	1.63 ± 0.02 d	1.63 ± 0.02 d
Protein (g)	24.80 ± 0.43 d	25.76 ± 0.50 d	25.94 ± 0.27 d	28.01 ± 0.20 c	29.76 ± 0.46 b	29.45 ± 0.72 bc	32.86 ± 0.40 a

Dietary fiber (g)	16.44 ± 0.31 b	14.4 ± 0.68 c	12.88 ± 0.08 d	11.44 ± 0.16 e	18.94 ± 0.19 a	10.49 ± 0.27 f	11.56 ± 0.00 e
Carbohydrate (g)	42.80 ± 0.00 e	43.60 ± 0.10 d	46.00 ± 0.20 b	44.60 ± 0.00 c	40.95 ± 0.00 g	47.30 ± 0.25 a	41.85 ± 0.15 f
Lead (mg/kg)	1.1250 ± 0.0350 a	1.1550 ± 0.0550 a	1.0350 ± 0.0450 a	0.8700 ± 0.0100 b	0.7850 ± 0.0350 b	0.8400 ± 0.0200 b	0.8300 ± 0.0300 b
Cadmium (mg/kg)	0.1200 ± 0.0000 b	0.1060 ± 0.0030 b	0.1150 ± 0.0050 b	0.1400 ± 0.0100 a	0.0875 ± 0.0065 c	0.0860 ± 0.0040 c	0.0550 ± 0.0000 d
Mercury (mg/kg)	0.0030 ± 0.0002 e	0.0050 ± 0.0000 d	0.0050 ± 0.0000 d	0.0051 ± 0.0004 d	0.0058 ± 0.0001 c	0.0066 ± 0.0001 b	0.0074 ± 0.0000 a
Arsenic (mg/kg)	0.0530 ± 0.0020 e	0.0710 ± 0.0000 d	0.0960 ± 0.0020 c	0.07450 ± 0.0025 d	0.0960 ± 0.0020 c	0.1260 ± 0.0025 b	0.1650 ± 0.0050 a
* Values are the mean of three replicates. Means in column followed by the same superscripts are not statistically different at $P < 0.05$ according to Duncan's multiple range test.							

In *Pleurotus* cultivation, supplementation of biogas residue at a lower ratio is effective for the growth rate and mushroom productivity. Analogous results have also been reported by Banik and Nandi (2004), Gou *et al.* (2007), Yu *et al.* (2012, 2014), and Chen *et al.* (2010). When the percentage of biogas residue in the substrate reached 20%, *P. ostreatus* showed the highest mycelial growth rate and the highest fresh weight of mushrooms at the first flush. Moreover, treatments T1 and T3 also resulted in higher mycelial growth rates than that of CK; this could be attributed to inorganic nutrients, such as Mg^{2+} or PO_4^{3-}, other trace elements, and the vitamin, effective for mycelial growth and initiation of fruiting body formation (Chang and Miles 2004). However, when the biogas residue content in the substrate exceeded 30%, the mycelial growth rate of *P. ostreatus* distinctly decreased; this may be partially attributable to the higher doses of nitrogen-rich supplements that caused an increase in temperature (thermogenesis) sufficient to kill the mycelia (Lelley and JanBen 1993) or to the residual ammonia gas, which could have inhibited mycelial growth. An appropriate amount of biogas residue in the substrate can promote efficient growth of mycelia through the breakdown of macromolecular organic matter (such as starch, fat, and protein) into small molecular substances (such as volatile fatty acids and amino acids) through decomposition reactions during fermentation (Wang *et al.* 2016).

However, the growth rate in the present study does not correspond with the total yields and BEs, and this result is consistent with the viewpoint that mycelial growth and the yield of mushrooms have different requirements (Oei 1991). Tokimoto and Kawai (1975) concluded that fruit body growth depends on the carbon source in the substrate; *i.e.* the more concentrated the sugar, the better the fruit body produced. In this study, at the first flush, T2 yielded about 3.65 kg of fresh mushrooms for every 20 bags, whereas CK and T1 produced about 3.3 kg of fresh mushrooms; these harvest values are much higher than those obtained by other treatments. At the second flush, CK and treatments T1 and T2 also produced the highest number of fruiting bodies among all treatments. However, when the percentage of biogas residue in the substrate was higher than 20%, fewer mushrooms were harvested. Generally, when the content of the biogas residue in the substrate exceeds 20%, as more of the residue is added to the substrate, fewer fruiting bodies are harvested during cultivation. Fruiting bodies harvested from treatments T5 and T6 had whitish pilei and long stipes and lacked commodity attributes. At the third flush, most fruiting bodies were malformed, and their numbers increase with increasing biogas residue supplementation. This result corroborates the view that high concentrations of nitrogen are inhibitory in mushroom cultivation (Chang and Hayes 1978).

Sturion and Oetterer (1995) previously reported that the fungal nutritional value can be greatly affected by the cultivation substrate. The yield, protein, and mineral contents of *P. sajor-caju* were significantly improved in the Indian subcontinent or in other regions with similar climatic conditions when mushrooms were cultivated on substrates mixed with biogas residue (Banik and Nandi 2004). Similarly, the contents of proteins and almost all the amino acids in *P. ostreatus* fruiting bodies growing on substrates without biogas residue were significantly lower than those growing on substrates with biogas residue, possibly due to the abundant nitrogen and amino acids included in the biogas residue. This result is in agreement with the analyses of *P. geesteranus* cultivated on substrates mixed with biogas fluid-soaked water hyacinth reported by Chen *et al.* (2010). The ash content of oyster fruiting bodies found to be unaffected between CK and other treatments, but the content is lesser than that of oyster

mushrooms grown on substrates mixed with straw and biomanure (Banik and Nandi 2004), grape marc plus cotton gin trash, olive mill by-products, pine needles (Koutrotsios *et al.* 2014), and paper (Fernandes *et al.* 2015). Mushrooms have a low total fat content but a high proportion of polyunsaturated fatty acids (72 to 85%), mainly due to the presence of linoleic acid (Dundar *et al.* 2008). In the present study, the fat content in the fruiting bodies of CK is much higher than that produced in fruiting bodies from other treatments except T1, but these results are much lower than those obtained from oyster mushroom cultivated on banana straw and rice straw (Bonatti *et al.* 2004), straw and biomanure (Banik and Nandi 2004), and wheat stalk (Dundar *et al.* 2008). Our analysis showed that the *Pleurotus* fruiting bodies are rich in carbohydrates, and the substrate mixed with the biogas residue can increase the content of carbohydrates. Oyster mushrooms cultivated on a substrate mixed with 10 to 30% biogas residue have significantly increased carbohydrate content; these data are lower than those obtained for oyster mushrooms cultivated on substrates mixed with paper (Fernandes *et al.* 2015), but higher than the results obtained for mushrooms grown on straw and poultry litter biomanure, straw and cowdung biomanure, and straw and jute caddis biomanure (Banik and Nandi 2004).

Dietary fiber has multiple proven benefits, such as reduction in the risk for cardiovascular disease (Papathanasopoulos and Camilleri 2010) and diabetes (Weickert and Pfeiffer 2018) and an increase in gut bacterial diversity (Tap *et al.* 2015). The data showed that there was a generally decreasing trend of dietary fiber content as more biogas residue was added in the substrate; this decrease results in the loss of a few beneficial functions in the oyster mushroom. However, the total dietary fiber content reported in this study is significantly higher than those of *Agaricus bisporus* (8.0 to 10.4%), *Boletus edulis* (8.0%), *Lentinula citrinus* (7.3 to 8.0%) (Crisan and Sands 1978), *Hericium erinaceus* (7.8%), and *Tricholoma giganteum* (4.5%) (Mau *et al.* 2001).

Interestingly, the Pb content in oyster mushrooms decreases with the increase in the proportion of biogas residue in the substrate. A similar conclusion was also found by Yu *et al.* (2017) for *Coprinus comatus* cultivation on a substrate mixed with biogas residue. This may be because the Pb in the biogas residue, compared to that in cottonseed hull, is much more difficult to assimilate by *P. ostreatus*. As more biogas residue was mixed with the substrate, the proportion of Cd in the cultivation substrate was reduced, and a decreasing trend of this heavy metal in dried fruiting bodies was obtained; this result conforms well to that of Yu *et al.* (2017). In contrast, the Hg and As contents of *P. ostreatus* were significantly increased with the increasing proportion of biogas residue in the cultivation substrate.

According to the Green Food—Edible Mushroom (NY/T 749—2012; 2012), Pb, Cd, Hg, and As contents should not exceed 2.0, 1.0, 0.2, and 1.0 mg/kg, respectively, in dried mushrooms. The contents of these metals in all the *P. ostreatus* fruiting bodies produced from cultivation on substrates mixed with biogas residue were in accordance with the standard NY/T 749—2012. Moreover, in the China National Standard for Food Safety—Maximum Levels for Certain Contaminants in Foodstuffs (GB 2762—2017; 2017), the maximum limits for Pb, Cd, Hg, and As in fresh mushroom fruiting bodies are 1.0, 0.2, 0.1, 0.5 mg/kg, respectively. Although the Pb contents in the fruiting bodies obtained from CK, T1, and T2 were higher than 1.0 mg/kg, it should be noted that these data were measured from dried oyster mushrooms in this study, and if these data were calculated for fresh mushrooms, the Pb contents would be within these limits. Therefore,

mushrooms cultivated on the substrate mixed with biogas residue in this study should be generally acceptable for consumption.

CONCLUSIONS

1. *Pleurotus ostreatus* can be cultivated on substrates mixed with chicken manure biogas residue, indicating that biogas residue can be used as a candidate and partly replace cottonseed hulls in *P. ostreatus* cultivation.
2. Supplementation of a lower percentage of biogas residues is effective in improving the nutritional quality of oyster mushroom in terms of protein, amino acid, ash, and carbohydrate contents.
3. A biogas residue proportion of 10 to 20% in substrates can promote the mycelial growth rate and formation of fruiting bodies that conform to the safety standards of edible mushrooms.
4. Although there was no significant increase in the harvest when a small quantity of biogas residue was added, such a utilization of biogas waste could conceivably reduce the practical cost of mushroom cultivation and biogas residue disposal and benefit the environment.

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