Full utilization of nutrients in rice straw by integrating mushroom cultivation, biogas production, and fertilizer use

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Abstract: This study proposed an integrated process of *Pleurotus ostreatus* cultivation, anaerobic digestion of spent mushroom substrate (SMS), and fertilizer value evaluation of digestate for achieving multiple products and full utilization of nutrients in rice straw. The results showed that the cultivated mushroom yield from rice straw was 401 g/kg TS. Biomethane yield obtained from anaerobic digestion of SMS was 133.0 mL/g VS. The fertilizer value of the SMS based digestate reached the national standard of fertilizers from organic sources in China. Substance conversion of rice straw during the integrated process revealed that the process could achieve the full utilization of nutrients in the substrate. Energy assessment indicated that the process had the viability to be applied. Therefore, this study provided a feasible strategy to set up a complete recycling agricultural ecosystem.

Keywords: rice straw, *Pleurotus ostreatus*, anaerobic digestion, fertilizer value, full utilization
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1 Introduction

China is one of the biggest crop-producing countries, which contributes 28.7% of the total global rice output. In 2015 statistics showed that the total rice straw yield was over 230 million tons, which made China as one of the most leading countries in terms of rice straw resource in the world[1]. However, until today the traditional rice straw handling practices in China are open field burning and being throwing aside have been often carried out after harvest season. Such practices led to pose environmental and health threats, in addition to the large sum of energy and nutrient losses.

The common ways of rice straw utilization in China include papermaking, compost, and production of livestock forage[2,3]. However, the product value and energy recovery from rice straw through those methods were relatively low. On the other hand, the rice straw has been used in recent years for biological processes like mushroom cultivation and anaerobic digestion (AD) to generate value-added products such as mushroom and biomethane, respectively.

Cultivation of mushroom could be considered as an economically feasible way for converting the rice straw into protein-rich food[4]. Additionally, the spent mushroom substrate (SMS) provided an alternative substrate for microorganisms in the anaerobic environment, because mushroom cultivation improved the degradation of the crystallinity and hydrolysis of polymers in lignocellulosic compounds and the content of nitrogen in rice straw[5,6]. However, the loss in organic matter (OM) and other nutrients during mushroom cultivation resulted that SMS had lower nutritional worth for anaerobic digestion as compared to raw rice straw[7].

Anaerobic digestion (AD) is a process in which organic matter breaks down under natural action of anaerobic microorganisms to produce biomethane while stabilizing strong organic wastes[8]. Recent literature showed that the biomethane yield obtained using rice straw as AD substrate ranged from 135 L/kg VS to 180 L/kg VS, suggesting that rice straw has great potential to produce biogas[9-12]. At the end of 2010, 273 biogas plants including 47 medium- and large-scale plants using crop straws as the raw material had been built in China[13]. However, AD also produces a large amount of final biologically stable and partially hygienic organic byproduct called digestate, which might lead to negative environmental consequences if it is not disposed of well.

Previous studies showed that anaerobic digestate was considered a suitable organic fertilizers for soil conditioning and plant nourishment[14]. Evaluation of the fertilizer properties of anaerobic digestate revealed that the properties of anaerobic digestate primarily depends on the nature of the feedstock[15]. SMS contains abundant nutrients including organics, nitrogen (N) and phosphorous (P) which could be slowly released and consumed as their respective soluble forms[16]. Hence, application of digestate from anaerobic reactors using SMS as raw material could yield similar or higher crop products compared to digestate of raw straw[17].

Although rice straw has great potential to be used as a biomass source to produce different kinds of value-added products. So far, no single method that can achieve the full utilization of rice straw. Nowadays, green agriculture has already become a priority theme for sustainable development in China[18]. Previous researches focused on the fungal pretreatment reported that the degradation of

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lignin and biomethane production potential of the biomass averagely increased by 50%[19,20]. However, the fungi itself consume some amount of accessible carbon from raw material during simultaneous degradation of holocellulose, even though the species were highly selective to delignification, the loss of carbon from other components of lignocellulose was inevitable[21]. The carbon removed during this process was converted to either mycelium or carbon dioxide. Moreover, long pretreatment time is another common limitation of applying fungal pretreatment, because fungal degradation often occurs slowly until the optimum amount of lignin removed from the raw material. To cope with those limitations, choosing edible mushroom species could be a better option to substitute the function fungi because it could achieve a similar effect on delignification in the same cultivation time while producing high-value mushroom fruit. A few studies combined solid-state anaerobic digestion (SS-AD) and Shiitake cultivation for utilization of wood biomass and approached to a positive effect because fungal pretreatment is particularly efficient on the substrate with high lignin content[22,23]. However, as far as our knowledge concerned there is no report on the viability of combining edible mushroom cultivation, biomethane production, and fertilizer use for full utilization of nutrients in rice straw, and give a specific analysis of the conversion of mass, elements, and energy during the whole process. Hence, methods on full utilization and complete cycling of rice straw, which formed a biogas project linked eco agricultural engineering model, should become the focal point for researchers in the future. Consequently, this study proposed a feasible way to combine three independent processes (mushroom cultivation, biogas production, and fertilizer use) to generate multiple products, and introduce material and energy cycling from rice straw. Based on a perspective of full utilization of rice straw, this study aimed not only to provide energy but also to circulate biomass in an integrated rice-mushroom-biogas-fertilizer-rice process.

In this study, an integrated process was set up to present a small circulation system and achieve the full utilization of rice straw. Initially, the edible mushroom, *Pleurotus ostreatus* (*P. ostreatus*) was cultivated on rice straw as the first kind of value-added product. Then, SMS after mushroom cultivation was used as the substrate for anaerobic digestion to generate biogas as the second kind of product. Finally, after AD, the digestate was prepared for organic fertilizer. The production and properties of the *P. ostreatus*, biogas, and digestate generated from each process were analyzed and evaluated based on their specific parameters. Furthermore, the mass balance by the integrated rice-mushroom-biogas-fertilizer-rice process was investigated to account for the introduction of a complete cycle of the agricultural ecosystem.

2 Materials and methods

2.1 Spawn, feedstock, and inoculum

The spawn of edible mushroom (*P. ostreatus*) cultivated on sawdust was purchased from Institute of Microbiology, Hei Longjiang Academy of Sciences, Hei Longjiang, China. The purchased spawn was immediately inoculated on the rice straw within 24 h at room temperature.

Rice straw used in this study was collected from Wuqing District, Tianjin, China. The straw was air-dried until the moisture content was less than 10% and chopped into 30-40 mm in length, and then ground into the size of 20-mesh by a hammer mill (YSW-180, Yanshan Zhengde Co, Beijing, China). Then the prepared straw was sub packaged in ziplock plastic bags at room temperature until used for the next test.

The inoculum was collected from continuously operated stable biogas plant for swine manure treatment in Shunyi District, Beijing, China. The main characteristics of rice straw and inoculum were listed in Table 1.

Table 1 Characteristics of rice straw and inoculum*

Items	Rice straw	Inoculum
TS^a/%	94.8±0.3	13.3±0.0
VS^a/%	85.9±0.1	7.3±0.1
$MLSS^a$/$g{\cdot}L^{-1}$	--	141.3±0.0
TC^b/%	40.4±0.1	29.2±0.7
TN^b/%	0.7±0.0	2.8±0.0
TP^b/$mg{\cdot}(kg\ TS)^{-1}$	8740.5±180.8	34 370.8±703.6
TK^b/$mg{\cdot}(kg\ TS)^{-1}$	1076.8±70.5	14 228.6±369.6
C/N^b	56.2	10.5
$Cellulose^b$/%	34.4±0.2	--
$Hemicellulose^b$/%	22.5±0.8	--
$Lignin^b$/%	5.4±0.3	--

Note: * Values are means±SD (n=3); ^{a} Content of fresh matter; ^{b} Content of dry matter.

2.2 Experimental set-up

2.2.1 *P. ostreatus* cultivation

Rice straw was used to cultivate *P. ostreatus* as a mono-substrate. Deionized water was added until the moisture content of the rice straw reach 65%, which was believed to be the optimum moisture content for the growth of *P.ostreatus*. The initial pH of moisturized rice straw (6.1) was adjusted to 7.5-8.0 using lime. Then the substrate was mixed as evenly as possible and soaked overnight following a recipe for *P.ostreatus* production[24]. Mixed substrate (1 kg) was loaded into each polypropylene bag (17 cm×33 cm×5 cm) with an additional plug and plastic ring; then compacted manually and autoclaved at 121°C for 2 h. The prepared substrates were cooled to room temperature before inoculation.

60 g of the spawn (3%-8% of the weight of the substrate) was added to each bag which contains the thoroughly mixed rice straw substrate. These ready bags were incubated at 25°C-28°C and 40%-50% humidity. After 30 days of spawn run time, the mushroom primordia formed and initiated reproductive growth of the species. When the fruiting bodies were formed, the bags were opened and transferred to a shelf in a sterilized room at 30°C and over 90% humidity. The mushrooms were only harvested for one flush after 10 d.

2.2.2 Anaerobic digestion

After harvesting the mushroom, the SMS was mixed evenly prior to sampling for characteristic analysis and AD. Anaerobic batch tests were carried out on blue cap bottles with total volume and working volume of 1 L and 0.8 L, respectively. The organic loading of the substrate and inoculum were set at 50 g TS/L and 15 g MLSS/L, respectively. The initial pH of each bottle was 7.5±0.3. Afterward, the bottles were incubated in a water bath at 35°C±2°C with manual shaking for twice a day in a uniform frequency for 45 d of AD period. The daily biogas production and methane content were monitored every day to evaluate the biomethane production performances.

2.2.3 Fertilizer value evaluation

The fertilizer properties of digestate generated after AD were analyzed according to the People’s Republic of China agricultural industry standards (NY 525-2012)[25].

2.3 Analysis methods

Total solid (TS), volatile solid (VS) and mixed liquid suspended solids (MLSS) were measured according to the APHA standards[26]. The pH was measured with a pH meter (3-Star, Thermo Orion, America). Ammonia nitrogen (NH_4^+-N) concentration was measured by Kjeldahl analyzer (KT-260, Foss, Danmark). Total carbon (TC), total hydrogen (TH), and total nitrogen (TN) were determined by the elemental analyzer (Vario EL/micro cube elemental analyzer, Germany). Total oxygen (TO) was calculated by the elementary formula ($C_nH_aO_bN_eS_f$) of the organic matter. Total potassium (TK), total phosphorus (TP), and heavy metals (Cr, As, Cd, Hg and Pb) were determined by the elemental analyzer (IRIS INTREPID Ⅱ, Thermo Elemental, USA, ICPS-7500, Shimadzu, Japan). Organic matter, available N, P, and K were measured using the methods described by Ma et al.[27]

The characteristics and nutritional value of mushrooms were measured according to the methods adopted from Colavolpe and Albertó[28]. Biological efficiency (BE) was used to evaluate the yields, %, and was calculated as follows:

$$BE=FW/DW\times 100 \quad (1)$$

where, FW is fresh weight, g, of the mushroom yield and DW is the dry weight of the substrate, g[29]. The content of cellulose, hemicellulose, and lignin were measured using fiber analyzer (A2000I, ANKOM, USA).

Biogas production was measured by the water displacement method. Biogas composition (H_2, N_2, CH_4, and CO_2) was detected by gas chromatography (SP2100, BeiFenRuiLi, Beijing, China) with a TDX-01 column (2 m×3 mm stainless-steel column) and a thermal conductivity detector (TCD). The operational temperature of injection, column, and detector was 150°C, 140°C, and 150°C, respectively. Argon was used as the carrier gas at a flow rate of 30 mL/min. Daily methane production was calculated after transforming to standard conditions (STP).

The content of total VFAs in digestate was determined using the gas chromatograph (GC2014, Shimadzu, Japan) equipped with a flame ionization detector (FID) and a DB-WAX123-7032 capillary column. Nitrogen was used as the carrier gas. The operational temperatures of injector, detector and column were kept at 250°C, and increased from 100°C to 180°C at a rate of 5°C/min, respectively.

Each analytical result was reported as the mean value of three replicate sample measurements. The standard deviations and statistical differences were analyzed by Microsoft Excel 2016. All figures in this paper were drawn using OriginPro 9.0 software.

2.4 Energy assessment

An energy assessment of the integrated process was set up to evaluate its scalability. To make the assessment, the scale of the process had to be increased. The parameters for the process were estimated from experimental data, using 1 t TS rice straw. The parameters for the mushroom cultivation, AD and fertilizer use were adapted from previous studies[30-34]. More specifically, the energy assessment included input, output and energy balance assessment.

The input energy of mushroom cultivation include heat preservation, electricity and water were calculated based on the energy coefficient from the previous study[30].

The energy inputs for anaerobic digestion were the energy for heating and electricity for mixing. Where its calculations were done according to Equations (2) and (3) in a modified manner as described in the literature[31].

$$E_{i,h}=86.4\rho Q\gamma(T_d-T_a)+kA(T_d-T_a) \quad (2)$$

where, $E_{i,h}$ is input heat, kJ; ρ is density, kg/m^3; Q is biomethane production, m^3; γ is specific heat, kJ/(kg^{-1}·°C); T_d is anaerobic digestion temperature, 35°C; T_a is ambient temperature, 25°C; k is heat transfer coefficient, W/(m^2·°C); A is surface area of the reactor wall, m^2.

$$E_{i,e}=V\omega \quad (3)$$

where, $E_{i,e}$ is input electricity, MJ; V is useful volume, m^3; ω is electricity consumption for mixing, MJ/m^3.

Normally, the digestate had to be dewatered to make the content of TS up to 30% before its fertilizer use[35]. Its energy input (Equation (4)) was calculated using the modified equation[32].

$$E_{i,d}=3600Q_dk_d \quad (4)$$

where, $E_{i,d}$ is the energy input of digestate dewatering, MJ; Q_d is the quantity of total dry solid for digestate, kg; k_d is the specific energy consumption, 35×10^{-3} kWh/kg.

The energy output from the mushroom cultivation, anaerobic digestion, and fertilizer use were calculated from the mushroom yield, methane yield, and the estimated crops yield after the land application of the dewatered digestate.

Finally, energy assessment was expressed as net energy output (ΔE), energy ratio (R), and energy conversion efficiency of the degraded substrate (η) was calculated based on Equations (5)-(7)[33]:

$$\Delta E = E_o - E_i \quad (5)$$

$$R = E_o/E_i \quad (6)$$

$$\eta=\Delta E/OLRVR_s \quad (7)$$

where, ΔE is the net energy output, MJ; E_o is the energy output, MJ; E_i is the energy input, MJ; R is the energy ratio; η is the energy conversion efficiency of degraded organic matter, MJ/kg Degraded VS; OLR is organic loading rate, kg VS/m^3; V is useful volume, m^3; R_s is the degradation rate of organic matter, %.

3 Results and discussion

3.1 *P. ostreatus* cultivation

The morphological structures of fruiting bodies on rice straw at different growth stages were shown in Figure 1. It took 30 d to grow mycelium on the rice straw. The matured fruiting bodies were formed 10 d later after the formation of the primordium. The pileus of the fruiting bodies harvested from all bags were white in color. The average pileus diameter, stipes length, and stipe diameter of fruiting bodies on rice straw was 6.3 cm, 3.9 cm, and 0.6 cm, respectively (Table 2). The morphological structures of fruiting bodies harvested from different rice straw bags were as almost similar to that of oyster mushroom cultivar on rice and wheat straw[36].

In this study, the fruiting bodies were harvested just once so that the majority of the remaining organic matter (SMS) reserved for the AD process. The average weight of the fruiting body yield and BE of one flush was 133.1 g and 40.1%, respectively (Table 2), which was in line with the results reported by Vieira[37].

Nutritional value of the dried fruiting bodies cultivated on rice straw was tested. Based on the results, the moisture and ash contents were 88.8% and 4.2%, respectively (Table 2), indicating that the fruiting bodies harvested from rice straw were fresh and had high moisture content[38]. Crude polysaccharides and crude protein were the main compositions of mushroom, the cultivated *P. ostreatus* were proved to have a relatively high content of those among other mushroom species[39]. The results showed that the crude polysaccharides, protein, fiber and fat content of fruiting bodies were 34.9%,18.7%, 8.1%, and 5.4%, respectively (Table 2), which were comparable to previous reports[38].

a. *P. ostreatus* cultivation on rice straw

b. Mature mushroom fruiting body

Figure 1 Mature mushroom fruiting body of *P. ostreatus* on rice straw

Table 2 Morphological structure and nutritional values of *P. ostreatus* mushrooms*

	Items	Values
Morphological structure	Fruit bodies yield/g	133.1±4.6
	Biological efficiency/%	40.1±1.4
	Pileus diameter/cm	6.3±0.8
	Stipe length/cm	3.9±0.6
	Stipe diameter/cm	0.6±0.0
Nutritional value	Moisture[a]/%	88.8±0.0
	Ash[a]/%	4.2±0.0
	Crude polysaccharides[b]/%	34.9±0.6
	Crude protein[b]/%	18.7±0.3
	Crude fiber[b]/%	8.1±0.5
	Crude fat[b]/%	5.4±0.2

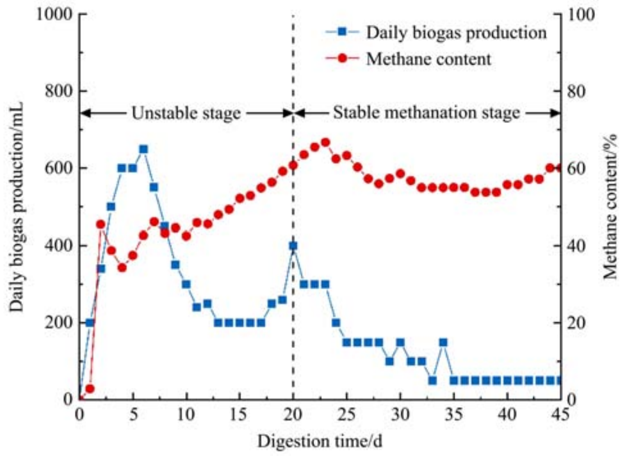
Note: *Values are means±SD (n=3); [a] Content of fresh matter; [b] Content of dry matter.

3.2 Anaerobic digestion

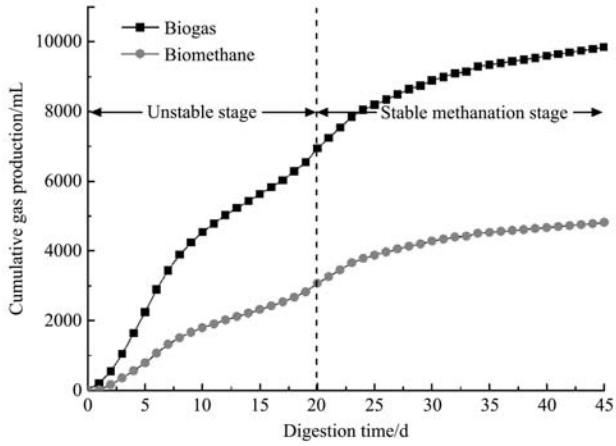
Daily biogas production (DBP) of SMS is shown in Figure 2a. The experiment showed two peaks of DBP which were gradually approached to zero during the last days of digestion time. More specifically, for SMS, the first peak appeared on day 6 with the DBP of 400 mL/d whereas the second DBP peaks reached 650 mL/d on day 20. The process performed successfully without any inhibition from VFAs during the digestion time. Reports showed that biogas production in solid-state anaerobic digestion using SMS as raw material was relatively low at the initial phase, which was due to the effect of acidification[40,41]. However, in this study, the moisture content of the AD system was 90%, which made SMS more feasible to use as a sole feedstock because the VFAs could be diluted in a liquid system. Compared to solid-state anaerobic digestion, using SMS as feedstock in liquid-state anaerobic digestion shortened the digestion time and increased the biogas production due to avoiding of acidification, which could be worth enough to consider the technological process for the industrial level.

The methane content (MC) of SMS is shown in Figure 2a. During AD of SMS, the MC increased steadily, achieved its 50% of production at day 14, and then remained at the level that average methane contents ranged between 50% and 60% throughout the digestion time. Reports in the literature showed that methane content in the biogas from rice straw slowly increased and fluctuated at the beginning of digestion time due to acidification of the reactors[42,43]. This shows that using SMS as AD feedstock can make methanogenesis predominant at the initial stage of AD, which could be due to the decomposition of the lignin-carbohydrate complex during mushroom cultivation which made the SMS substrate more readily degradable than rice straw. So that, SMS was more utilized by anaerobic microorganism during AD than rice straw.

Cumulative biogas production (CBP) and cumulative biomethane production (CMP) showed in Figure 2b was 9840 mL and 4812 mL for SMS, respectively. CBP and CMP of SMS showed a similar trend, which was gradually increased in the first 20 days and approached a constant value during the last days of digestion time. Additionally, this result was consistent with the trend of DBP and MC of the anaerobic digestion process in this study. The VS biomethane yield was 133.0 mL/g VS. Feng et al.[44] reported that the methane production potential of *P. eryngii* spent compost material was 194 L/kg VS; *P. ostreatus* pretreated rice straw was 167 L/kg VS[19]. This indicates that SMS still has great potential for methane conversion, and is a considerable substrate for biogas production.



a. Daily biogas production and methane content



b. Cumulative biogas and biomethane production

Figure 2 Daily biogas production, methane content, and cumulative biogas and biomethane production during AD of SMS

The time required to produce 80% of the maximal biomethane production (T_{80}) is used to evaluate the digestion performance of AD processes[45]. The T_{80} for SMS was 25±1 days and the shorter digestion time (10.7% shorter than that of the control test in previous studies)[46] could be associated to delignification of the substrate by mushroom cultivation.

3.3 Fertilizer value evaluation

Anaerobic digestate contains a high proportion of nutritional components such as nitrogen (N), phosphorus (P), and potassium (K), which were necessary nutrients source for crops. Some parameters of the digestate after anaerobic digestion of SMS to evaluate the fertilizer properties are shown in Table 3. Data showed in this section was the contents based on dry matter. N is an important nutrient and is the most common plant growth necessary factor of crops. Previous test showed that NH_4^+ in digestate would convert to NO_3^- and used by crops. Moreover, the net organic N and P in digestate also be partly used for crops growth[47]. In the integrated process, mushroom cultivation on rice straw has an advantage in decreasing the C/N ratio to the optimum level for AD of SMS, meanwhile, increasing the TN content of digestate. Transformation of TN from SMS to digestate was 99.75%, suggesting most of the N was preserved. During AD, complex organic N compounds were mineralized and formed as available N, such as NH_4^+-N (417.7 mg/L) and allowed its immediate utilization by crops. The presence of NH_4^+ could also lead to the formation of $(NH_4)_2CO_3$ and increase the pH of digestate. It is a useful property to solve the problem of soil acidification.

Table 3 Fertilizer properties of digestate*

Items	Content	Organic fertilizer (NY 525-2012)[c]
Organic matter[a]/%	59.5±1.3	≥45.0
Total N[a] g/kg TS	16.4±2.1	-
P_2O_5[a] g/kg TS	24.0±1.1	-
K_2O[a] g/kg TS	15.0±1.5	-
Nutrients (Total N+P_2O_5+K_2O)/%	5.5	≥5.0
Moisture[b]/%	95.7±2.1	≤30.0
pH[b]	7.2±0.0	5.5-8.5

Note: * Values are means±SD (n=3); [a] Content of dry matter; [b] Content of fresh matter; [c] People's Republic of China agricultural industry standards.

Phosphorous was another important plant nutrient in digestate. The amount of P in the form of P_2O_5 in digestate was 10.5 g/kg TS. A study showed that fungal could transform P from the organic state into a soluble form[29], indicating that P in SMS will be easily used by microorganism during AD. Anaerobic microorganisms also enhanced hydrolyzable of P containing substrates during degradation processes of AD and transferred it into a liquid fraction, which was consistent with the result of the available P content in this study.

The content of K (K_2O) in digestate was 12.5 g/kg TS. The high content of K in digestate has a positive aspect because it could be used as a nutritional supplement to improve the property of the digestate as fertilizer. The amount of K remained after AD of the SMS was 92.6%, indicating that almost all the K in the SMS remained in digestate. This result assures that the digestate of SMS could highly increase the content K in the soil when used as organic fertilizer.

Available N includes ammonium nitrogen, nitrate nitrogen, amino nitrogen, amide nitrogen and N from some simple peptide and protein compounds, which are soluble and easy to be absorbed by the plant[48]. Available P and K are the P and K components that can be absorbed by plants, including all water-soluble P, K partially adsorbed phosphorus and organic P. In this study, available N, P, and K in liquid fractions of digestate were also analyzed to evaluate the fertilizer property of digestate obtained from integrated processes (Table 4). The results showed that the available nutrients content of the digestate is 3.3% of its total nutrients content. Moreover, the ratio of available P/ available K was approximately 1:3, which was good for the growth of grains[49]. Based on this result, the integrated process could be a practical method to improve the nutrient's availability during AD and promote the quick-release of those elements as nutrients supplement for soil.

In the organic fertilizer used in China, the total contents of N, P_2O_5, and K_2O compounds should be greater than or equal to 5% based on dry weight[25]. Hence, digestate of SMS was acceptable and achieved the complete circulation of nutrients via the rice-mushroom-biogas-fertilizer-rice process.

In the AD process, heavy metals might arise from input feedstock and remain in the digestate[50]. The content of five kinds of heavy metals in digestate of SMS was also analyzed in this study (Table 4). Cr was found as the most prevalent heavy metal in the digestate, followed by Pb and As, however, there were no traces of Cd or Hg detected. National regulations in China prohibit the use of digestate if the concentration of one or more heavy metal exceeds a certain limit. As People's Republic of China agricultural industry standards, the maximum permissible limit has been set for Cr, As, Cd, Hg, and Pb, which are 150, 15, 3, 2, and 50 mg/kg TS, respectively[25]. It was observed that the content of heavy metals in SMS based digestate in this study had no harmful health implications of their applications.

Table 4 Available N, P, and K in liquid fractions and heavy metals content of digestate*

Items	Content
Available N[a] /mg·(kg TS)$^{-1}$	0.4±0.0
Available P[a] /mg·(kg TS)$^{-1}$	0.2±0.0
Available K[a] /g·(kg TS)$^{-1}$	0.7±0.0
Cr[a] /mg·(kg TS)$^{-1}$	11.2±0.2
As[a] /mg·(kg TS)$^{-1}$	4.4±0.1
Cd[a] /mg·(kg TS)$^{-1}$	ND[b]
Hg[a] /mg·(kg TS)$^{-1}$	ND[b]
Pb[a] /mg·(kg TS)$^{-1}$	6.2±0.1

Note: * Values are means±SD (n=3); [a] Content of fresh matter; [b] ND represents non-detected.

3.4 Mass balance

Conversion of compositions in rice straw was used to assess the AD performance and calculate the utilization rate of rice straw after three processes.

3.4.1 Conversion of TS and VS

TS and VS conversion of rice straw in the integrated processes are given in Figure 3a. The calculation was based on that the initial TS value of rice straw was 1000 g. The conversion of TS of rice straw to mushroom, biogas, and digestate was 4.5%, 41.8%, and 44.9%, respectively. On the hand, the degradation of TS and VS of rice straw after cultivation of mushroom was 13.30% and 16.43%, respectively. This was due to the decomposition of rice straw components by *P. ostreatus* as nutrients for the growth of mycelium to develop fruiting bodies. Moreover, the combination of mushroom cultivation and AD increased the removal of the total TS and VS reduction to 55.1% and 60.8%, respectively (Figure 3a).

More specifically, the total TS and VS reduction during the integrated process was increased by 35.6% and 47.0% compared to only AD, This TS and VS reduction rate was 1.3% and 24.0%, respectively higher than the findings from Dai et al.[51] which used AD of 6% NaOH pretreated rice straw. In another sense, the integrated process improved the total solid reduction of the process and converted to mushroom and biogas.

3.4.2 Conversion of organic matter

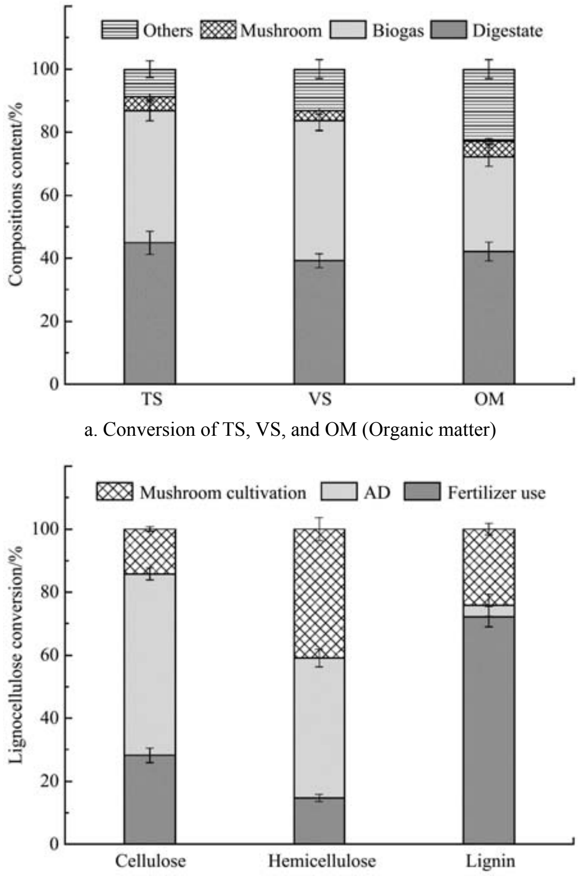
Conversion of organic matter (OM) in rice straw (major components are carbohydrates, lignocellulose N-containing compounds, fat, resin, wax, and tannin)[52,53] during three experimental processes is given in Figure 3a. In the first stage of mushroom cultivation, 4.9% and 72.2% of the organic matter from rice straw converted to mushroom and SMS, respectively. The loss of the organic matter was mainly due to the metabolism during mushroom growth. The digestate was characterized by relatively low dry matter content and 42.2% (deducted inoculum) of the organic matter was remained because of the methane conversion (30.0%) during the AD stage. Organic matter in digestate contains 81.0 mg/L of total volatile fatty acid which could be decomposed within a few days and could increase the soil organic matter content. Hence, it was suitable for using the digestate as fertilizer and for soil amendment.

3.4.3 Conversion of lignocellulose

The degradation of cellulose, hemicellulose, and lignin of *P. ostreatus* cultivation in the first step of mushroom cultivation were 14.2%, 40.9%, and 24.1%, respectively (Figure 3b). The degradation rate of hemicellulose and lignin was significantly higher than that of cellulose ($p<0.05$). *P. ostreatus* was proved to have high relative selectivity on lignin degradation of softwood substrates due to the presence of enzymes like laccase, Mn-peroxidase, and Lignin peroxidase[54, 55]. The results of this study showed that edible mushroom (*P.ostreatus*) also had significant selectivity on lignin degradation, which made the SMS highly digestible compared to the rice straw. The degradation of hemicellulose was highest among the three compositions, showing that during the cultivation period hemicellulose was the main carbon source for mycelium growth and metabolism. The loss of cellulose in this test (14.2%) was relatively lower than that in the report (32.0%) by Taniguchi et al.[56]

The presence of high cellulose and hemicellulose content in rice straw made the_raw material rich in main carbon sources for anaerobic microbes during the production of methane in the AD process[57]. Lignin is poorly degradable, and holocellulose (cellulose and hemicellulose) is hardly accessible by anaerobic microbes owing to interactions and linkages with lignin networks[58]. During AD of SMS, the maximum degradation of cellulose and hemicellulose were 67.1% and 75.2%, respectively. These results showed that cellulose and hemicellulose in SMS were easily biodegradable, which had a great advantage for methane production from SMS. Although lignin is recalcitrant to AD, however, in this study certain amount of lignin degraded during the AD process. The conversion of lignin from SMS was 4.9%, which could be due to the mushroom cultivation destroyed the structure and the extended digestion time enhanced the breaking of the lignin network[59].

The total conversion rate of cellulose, hemicellulose, and lignin by the combined process of mushroom cultivation on rice straw and followed by AD of SMS was 71.8%,85.3%, and 27.8%, respectively. The cellulose/lignin ratio of digestate has been suggested to be an indicator of the degree of humification of the organic matter. The value of the cellulose/lignin (0.5) represent to the threshold that distinguishes between the fresh and mature substrate, and when the value is higher than 0.5 represent the substrate is fresh[60]. In this study the ratio was 2.3, indicating the digestate was the fresh substrate. It represented that the digestate could be used as organic fertilizer after compost, and the integrated process could improve the utilization rate of lignocellulose in rice straw.



a. Conversion of TS, VS, and OM (Organic matter)

b. Conversion of lignocellulose

Figure 3 Conversion of TS, VS, and organic matter (OM), and lignocellulose during the integrated processes

3.4.4 Transformation of carbon, hydrogen, and oxygen

Transformation of carbon, hydrogen, and oxygen from rice straw to multiple products during the integrated process was the key parameter to evaluate the efficiency of mass utilization and bioenergy conversion (Figure 4). The amount of carbon, hydrogen, and oxygen in biogas were calculated by assuming that those elements containing compounds in biogas were carbon dioxide and methane gas. In this study, 8.1%, 8.1%, 5.7%; and 39.0%, 44.9%, 34.0% of the carbon, hydrogen, and oxygen were used to form mushroom and biogas, which brought economic efficiency and energy valorization by elements utilization form update. Moreover, 52.9%, 47.0%, and 60.3% of carbon, hydrogen, and oxygen were remained in digestate, respectively. As carbon, hydrogen, and oxygen were the main constituent elements of organic matter, this result was consistent with the trend of organic matter conversion.

The amount of carbon in three phases of experimental lines was calculated from biogas, liquid fraction of digestate (LFD), and solid fraction of digestate (SFD) (carbon from inoculum was deducted). The distribution of carbon in gas, liquid, and solids of the AD process was not shown in this paper. The transformation of carbon into gas depends on the amount of methane and carbon dioxide produced during the conversion process which was an important parameter to evaluate the AD performance[61]. The results revealed that the total amount of carbon transformed into gas was 37.0%. The amount of carbon remained in solid was 44.68%, indicating that the SFD obtained from all processes could supplement carbon for the amendment of low carbon agricultural soils. Totally, the remaining 18.4% of carbon was transferred to LFD; this could be due to the mushroom cultivation which improved the conversion of organic matter in SMS from insoluble organics to soluble substrates like VFAs, and low carbon molecules during AD process[62].

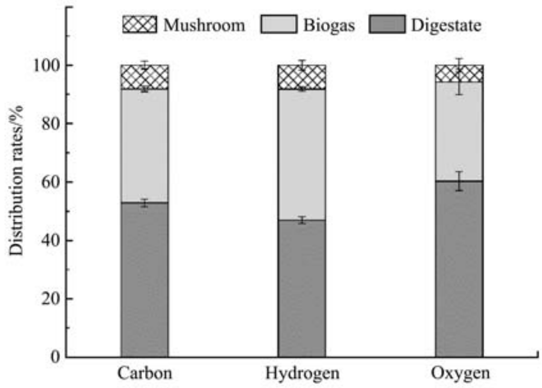


Figure 4 Distribution rates of carbon, hydrogen, and oxygen in mushroom, biogas, and digestate

3.5 Energy assessment

In this study, the energy assessment of the integrated process of mushroom cultivation, anaerobic digestion, and fertilizer use were carried out to evaluate its viability. To make the assessment, two traditional approaches and the integrated process such as: process 1 (mushroom cultivation then SMS composting), process 2 (AD then the use of digestate as fertilizer) and process 3 (mushroom cultivation combines AD and fertilizer use of digestate), respectively (Figure 5) applied and the results are summarized in Table 5.

Usually, mushroom cultivation combines with the composting process for the subsequent treatment of the SMS. The input energy of mushroom cultivation and SMS composting was 16772 MJ and 315 MJ, respectively. The output energy of process 1 was from the calories of edible mushroom and crops. Since mushroom and crops were both low-calorie foods, as a result, the net energy output (NEO) of process 1 was negative. Additionally, in the scale-up process, a large amount of diesel fuel was often consumed for the heating and humidity purpose by substituting electricity, which was the main reason for the negative net energy. Literature result also made the same conclusion that in mushroom production, which stated that the change in energy was negative[30].

Anaerobic digestion is a renewable energy generation process and often followed with the digestate treatment system for organic fertilizer production. As the results summarized in Table 5, the input energy of AD and fertilizer use was 17 980 MJ and 93 MJ, respectively. The high energy input for AD are attributed to the energy applied for reactor heating and biomass mixing. On the other hand, dewatering is also the main energy consuming process for digestate treatment to increase its solids content. Total energy input of process 2 is higher than that of process 1. However, the total energy generation of process 2 (22 800 MJ) was much higher than that of process 1 due to the high energy value of biomethane. NEO of process 2 was 4727 MJ, and the positive value indicates net energy gain in this stage.

In this study, process 3 which is designed as an integrated process was set up to combine processes 1 and 2. Results showed that the NEO of this process was 1150 MJ, indicating that the energy generation from the AD process can meet the energy consumption of the integrated process, especially the energy deficit during the mushroom cultivation. The energy balance analysis (Table 5) showed the ΔE, R, and η of process 3 are higher than those of process 1 while those of process 2 is highest. The results suggest the integrated process can recover energy from the rice straw after mushroom and biogas production, and land application of organic fertilizer. Although when compared to process 2 the net energy production and energy conversion efficiency of process 3 decreased, the high economic value of mushroom could be an offset.

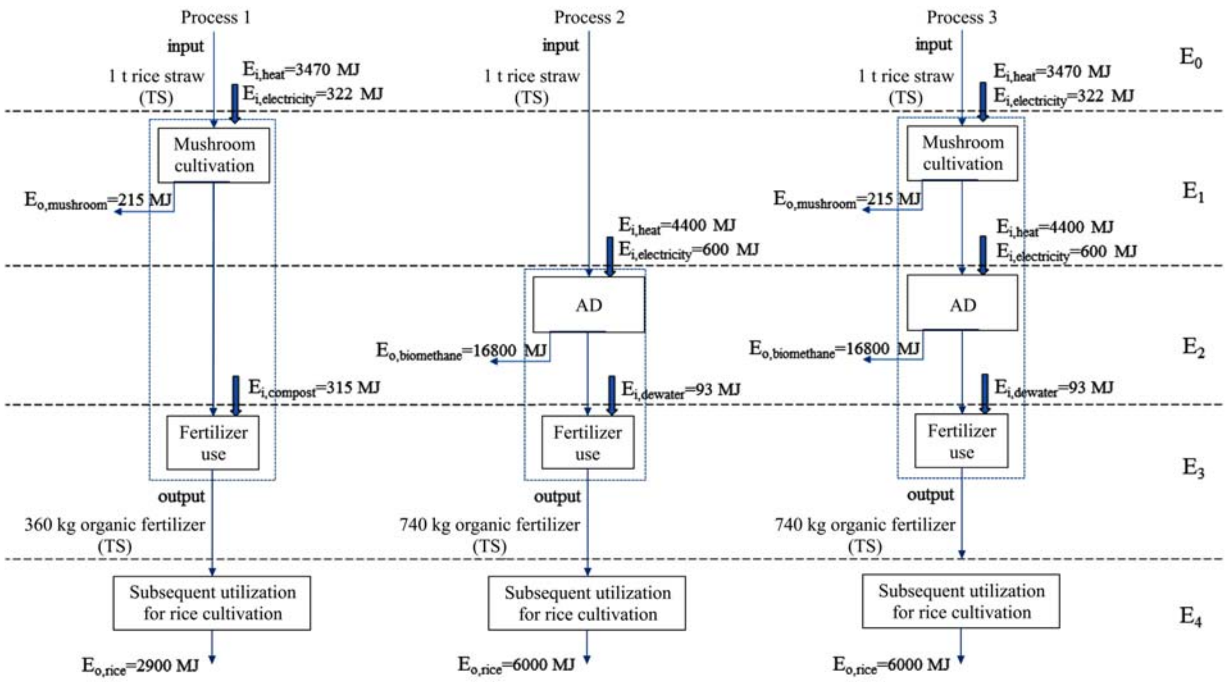


Figure 5 Energy assessment of three processes after scale-up

Table 5 Energy assessment of three processes after scale-up

	Item/MJ	Process 1	Process 2	Process 3
Input				
Feedstock	E_0			
Rice straw	$Q_{\text{rice straw}}^{a}$	12 980	12 980	12 980
Mushroom cultivation	E_1			
Heat preservation	$E_{i,\text{heat}}$	3470	-	3470
Electricity	$E_{i,\text{electricity}}$	320	-	320
Water	$E_{i,\text{electricity}}$	2	-	2
AD	E_2			
Heat preservation	$E_{i,\text{heat}}$	-	4400	4400
Electricity	$E_{i,\text{electricity}}$	-	600	600
Fertilizer use	E_3			
Dewatering	$E_{i,\text{dewater}}$	-	93	93
Composting	$E_{i,\text{compost}}$	315	-	-
Total energy consume	$E_{i,\text{total}}$	17 087	18 073	21 865
Output				
Mushroom	$Q_{o,\text{mushroom}}^{a}$	215	-	215
Biogas	$Q_{o,\text{biogas}}^{a}$	-	16 800	16 800
Crops	$Q_{o,\text{crops}}\ (E_4)^{a}$	2900	6000	6000
Total energy generation	$Q_{o,\text{total}}^{a}$	3115	22 800	23 015
Energy assessment				
NEO	ΔE^{b}	-13 972	4727	1150
Energy ratio	R^{b}	0.2	1.3	1.1
Energy conversion efficiency	η^{b}	-21.8	8.6	2.1

Note: [a] Quantity of heat; [b] No unit.

On the other hand, the distribution of total input energy in the form of renewable (biomass) and non-renewable energy (diesel fuel and electricity) groups in process 3 was 59.4% and 40.6%. Based on this study, even though the share of renewable energy was higher than that non-renewable energy in process 3, it still has the gap of the improvement. For example, the diesel fuel can be replaced with an alternative source of energy such as biomethane generated from the AD during the process. The water consumption for mushroom growth was a big concern cannot be ignored by the integrated process application. The moisture content of the culture substrate is an important factor to absorb and transport nutrients for the growth of mycelium and fruiting body, and also removing metabolic wastes during the cultivation of edible fungi. Therefore, the addition of sufficient water at all stages of growth and development of edible fungi was necessary. Managing water consumption of mushroom cultivation could be achieved by maintaining the suitable moisture content of culture substrate (60%-70% moisture) and surrounding (80%-90%) for the mycelium development and fruit body formation stage, respectively. However, some measures can be taken to reduce unnecessary wastage of water. For instance, using polypropylene bag to cultivate mushroom and adding water absorbing resin as water retaining agent could keep the moisture of the substrate and avoid frequent addition of water to the culture substrate. On the other hand, the water saving irrigation technique such as a combination of micro-sprinkler and centralized water injection could be used instead of manual spray to reduce water consumption and keeping the environment humid. Furthermore, when using SMS as the feedstock of AD it does not need adding much extra water to the reactor because SMS has already 60% moisture content. For these reasons, the integrated process applied in this study can be considered as a cleaner production process, which has the feasibility to be applied after integrating and optimizing.

Generally, the integrated process has economic benefits that are important indicators to evaluate the efficiency of the process of biomass utilization (mushroom cultivation, methane production, and organic fertilizer) apart from waste treatment during the process. Although the complex process could add the capital and operation costs in large-scale, which may further decrease the economic benefits of the process, it could be overcome by updating technology operations and maintenance to increase the production renewable energy during the process. Based on that, the integrated process had the potential to be an appropriate strategy to benefit the community by producing: healthy food (mushroom), clean energy, and organic fertilizer through the complete biomass circulation.

4 Conclusions

In this study, an integrated process as a small reusing system was set up and the full utilization of substances in rice straw was achieved. The biological efficiency of mushroom yield cultivated on rice straw was 40.1%. During the AD process, the biomethane yield from SMS was 133.0 L/kg VS. The fertilizer value of the digestate after AD was confirmed that in the range of the national standard of organic fertilizers in China. The integrated process also achieved a high utilization rate of nutritional compositions in rice straw. The potential of benefits generated during the process was appropriate for practical application. This study provides a significant strategy for full utilization of nutrients in rice straw.

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