Maximizing the value of Korshinsk peashrub branches by the integration of *Pleurotus tuoliensis* cultivation and anaerobic digestion of spent mushroom substrate

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Abstract

Background: Korshinsk peashrub branches are agroforestry byproducts that accumulate in millions of tons per year and need to be rationally and efficiently utilized. To maximize the utilization value of Korshinsk peashrub branches, an integrated process was proposed and conducted by using Korshinsk peashrub branches as the main substrate ingredient for *Pleurotus tuoliensis* cultivation and performing anaerobic digestion of spent mushroom substrate for methane production in this study.

Results: It was found that the substrate including 45% Korshinsk peashrub branches obtained higher mushroom yields than that using a commercial substrate, and anaerobic digestion of spent mushroom substrate from *P. tuoliensis* cultivation achieved a high cumulative methane yield. Preliminary calculation suggested that, for every kg of Korshinsk peashrub branches consumption, 1.12 kg mushroom product and 8578 L methane were produced, obtaining actual economic value of RMB 27.5 Yuan. In addition, this integrated strategy has positive ecological and social benefits.

Conclusion: The value of Korshinsk peashrub branches can be maximized by *P. tuoliensis* cultivation and anaerobic digestion of spent mushroom substrate, which not only promotes the biological conversion of Korshinsk peashrub branches to a circular economy, but also is beneficial for the sustainable development of environment, energy conservation and edible fungi industry.

Background

Korshinsk peashrub (*Caragana korshinskii* Kom.), a drought-tolerant perennial mesquite shrub, is widely distributed in northeastern, northwestern, and northern parts of China [1-3]. The adaptability and survival rate of Korshinsk Peashrub are quite high and its strong resistance to coldness, drought, and barren lands have led to the widespread planting of this shrub in the loess plateau and desert region, to control soil erosion and restore vegetation [4, 5]. The ability of Korshinsk peashrub to branch and regenerate is extremely strong, and its branches must be chopped every four to five years. The production of Korshinsk peashrub branches (Korpb) are estimated to be approximately 550,000 tons every year, however, most of the branches are discarded or burned, not only leading to serious pollution, but also causing a great waste of resources. It becomes an important mission for government and technologists to find out a way to make effective use of Korpb to avoid a biomass resource waste, but also help realizing sustainable development of an economic society.

Edible fungi have the ability to degrade and utilize waste components such as cellulose, hemicellulose and lignin as a nutrient source [6, 7]. Therefore, edible fungi cultivation is an efficient biological method to convert agroforestry byproducts into mushroom products, providing a direct link between waste and human food [8, 9]. Korpb that contains a high amount of lignocellulose, including 16.63% cellulose, 26.43% hemicellulose and 11.20% lignin [10, 11], could be used to produce high value-added edible mushroom products. Few studies have examined the use of Korpb for mushroom cultivation, only limited to *Pleurotus* spp [11, 12]. Yang et al. (2011) reported that the addition of Korpb to the substrate for the production of *P. tuoliensis*, a commercially valuable edible fungus with extremely high nutritional and medicinal value [13, 14], did not influence the quality and flavor of fruiting bodies compared to the conventional cotton seed hull formula, but the yield and biological efficiency was low, probably due to an unsuitable substrate formula. *Pleurotus* spp are the second most widely cultivated genus worldwide, easily cultivated and cost-effective [15]. The production of *Pleurotus* spp using Korpb as the substrate ingredient could consume a large amount of Korpb, not only achieving efficient waste utilization, but also solving the shortage problem of conventional cultivation substrate such as cotton seed hull.

The edible fungi cultivation results in considerable quantities of spent mushroom substrate (SMS) (5 kg of spent mushroom substrate are generated from 1 kg of fresh mushrooms produced), which is long considered as a waste product [16, 17]. Therefore, SMS from *Pleurotus* spp cultivation based on the substrate with Korpb should also be reasonably developed and utilized. Many researches have demonstrated that *Pleurotus* spp SMS has good gas-productivity performance. Anaerobic digestion (AD) of *P. ostreatus* SMS could produce 263 L/kg volatile solids (VS) of methane and with the combination of SMS and milling, a higher methane production could be obtained in only 30 days of AD (165% higher than the control) [18, 19]. SMS from *P. tuoliensis* cultivation could produce 286 mL/g methane and 717.38 mL/g biogas at 1% total solids (TS) content via AD, higher than the gas yield from SMS of *P. eryngii* and *F. velutipes* [20], demonstrating that *P. tuoliensis* SMS has good gas productivity. However, the gas potential of SMS containing Korpb has not been accessed.

Combining *P. tuoliensis* cultivation and anaerobic digestion of SMS would be a plausible strategy for utilizing Korpb and solving the problem of SMS accumulation where they represent a pollution risk, which would promote the transition of Korpb to a circular economy and bring considerable economic benefit.

Therefore, the purpose of this study was as following:

1. Evaluate the feasibility of Korpb for *P. tuoliensis* cultivation.
2. Determine the gas potential of SMS containing Korpb via anaerobic digestion.
3. Assess the economic, ecological, and social benefits of this integration strategy.

Results And Discussion

Suitability of Korshinsk peashrub branches as substrate ingredient for P. tuoliensis production

Korpb are shrub wastes that accumulate continuously throughout the year and contain high amounts of cellulose, hemicellulose, and lignin, as well as crude protein and crude fat [21]. As shown in Table 1S, Korpb had a total carbon content of 32.2% and a total nitrogen content of 2.24%. The C/N ratio (14.391:1) was comparable to the C/N ratio in wheat bran (11.956:1), but significantly lower than the C/N ratio in corn cob (52.821:1), cotton seed hull (28.088:1), and maize powder (31.725:1), which are all conventional substrate materials for edible fungus production [22, 23]. The above result showed that Korpb are a substrate material with high nitrogen content and can be an ideal raw material for edible fungus production.

To determine if the lignocellulose byproduct, Korpb, are suitable as a substrate ingredient for *P. tuoliensis* cultivation, eight substrate formulas containing various proportions of Korpb were designed.

Effect of Korshinsk peashrub branches on the growth of P. tuoliensis mycelium

The mycelial growth rate in different substrate formulas varied significantly (Fig. 1). The mycelial growth rate in T7 was the highest, followed by that in T3 and T4, all of which were significantly higher than that in CK. While the mycelial growth rate in T2, T5, and T6 was comparable to that in CK. However, the mycelial growth rate in T1, which contained 65% Korpb, was the lowest.

Previous studies have shown that the mycelial growth rate depends mainly on the C/N ratio in the cultivation substrate [24]. The optimum C/N ratio of *P. tuoliensis* mycelium was in the range of 20:1 - 30:1. Too much nitrogen will inhibit mycelial growth and delay the formation of fruiting bodies [25]. Formula T1 had a C/N ratio of 19:1 and a very high nitrogen content, which can well explain why the growth rate of mycelium in T1 was the lowest.

Effects of Korshinsk peashrub branches on the yield and biological conversion rate of P. tuoliensis

As shown in Table 1, the yield and biological efficiency (BE) of formula T2, which contained 45% Korpb, 20% cottonseed hull, and 15% wheat bran, were the highest, at 380 ± 65 g per bag and 52.6%, respectively, significantly higher than those of CK by 5.26% and 9.02%, respectively ($p < 0.05$). The BE and yield of T3 and T6 were similar to those of CK, while those of T7 without cotton seed hull were lowest. The yield and BE of T2 were the highest, demonstrating that the formula composition of T2 can provide more nutrients for mushroom growth and well promote the bioconversion of the substrate to mushroom products.

Table 1 Agriculture traits of *P. tuoliensis* cultivated on substrates with various proportions of Korpb

Formulas	Average yield (g/bag)	Biological efficiency (%)	Pileus length (mm)	Pileus width (mm)
CK	361±66bc	48.0±3.56bc	161±36a	119±27a
T1	344±69bcd	46.3±2.54bc	153±25a	122±22a
T2	380±65a	52.6±4.28a	157±24a	119±25a
T3	359±55bc	50.0±3.79bc	160±31a	120±23a
T4	357±49bc	48.0±4.21bc	159±28a	118±15a
T5	330±60cd	46.0±3.12cd	157±19a	123±22a
T6	363±69bc	50.1±5.42bc	160±24a	120±20a
T7	311±47d	43.5±5.11d	153±22a	116±23a

Values followed by the same letter are not significantly different (ANOVA; Tukey's test, $p < 0.05$).

The biological conversion of substrate to mushroom product can also be reflected on the utilization rate of lignocellulose biomass in the substrate. The growth and development of edible fungus rely on the degradation of lignocellulose by lignocellulosic degrading enzymes secreted from mycelia [40]. In our study, due to the addition of various proportions of Korpb, the different substrate formulas exhibited varied cellulose (15-28%), hemicellulose (7-14%), and lignin (13-21%) contents. After 110 days of *P. tuoliensis* cultivation, the degradation degree of cellulose and hemicellulose in the cultivation substrates were in the range of 13.52-56% and 8.5-55%, respectively, while lignin contents were only slightly changed (Table 2S), which corresponds to the results of [26], who demonstrated that *Pleurotus* spp. were the best laccase producers, but the weakest lignin degraders. These results also demonstrated that the lignocellulose biomass in cultivation substrates has been successfully converted into *P. tuoliensis* food.

Effects of Korshinsk peashrub branches on agriculture traits and nutritional composition of P. tuoliensis

The ideal commodity traits of *P. tuoliensis* include a big fan pileus and a short stipe [27]. As shown in Table 1 and Fig. 2, the pileus length and width of T1 - T7 were not significantly different from the regular formula (CK). This data demonstrates that the addition of Korpb in the cultivation substrate did not affect the agricultural traits of *P. tuoliensis* fruiting bodies.

The analysis index, such as crude protein, fat, ash, sugar, and fiber, are conventional indicator to evaluate the nutritional quality of edible fungi [27]. As shown in Table 2, no significant differences in crude protein content were detected in fruiting bodies grown in formulas CK, T1, T2, T3, T4, and T6, whereas crude protein content in T5 and T7 was significantly lower compared to CK. Similarly, the crude fiber content of fruiting bodies in CK, T1, T2, T3,

and T6 were high, whereas the crude fiber contents in other formulas were slightly lower, with that for T7 being the lowest. The highest fat content was detected in T2 fruiting bodies, which was twice that of CK. In the CK formula, which did not include Korb, the ash content was higher than that in other formulas. Crude sugars, including reducing and non-reducing sugars, were higher in T1, T5, and T7 than in CK; whereas that of other formulas were slightly lower. Nutritional index analysis showed that the addition of Korb to substrate did not influence the nutritional value of *P. tuoliensis* fruiting body.

Table 2 Nutritional composition of *P. tuoliensis* cultivated on substrates with various proportions of Korb

Formula	Crude protein g/100g	Crude fiber g/100g	Fat g/100g	Ash g/100g	Crude sugar g/100g
CK	22.25±0.25a	6.28±0.02d	2.91±0.01c	6.35±0.01a	8.38±0.06e
T1	22.05±0.25a	6.67±0.05b	2.92±0.01c	6.00±0.01d	9.98±0.03c
T2	22.50±0.20a	6.14±0.07e	5.21±0.01a	5.94±0.01e	7.24±0.00g
T3	21.80±0.10b	6.48±0.01c	2.76±0.01d	6.02±0.01c	7.44±0.09g
T4	21.50±0.80c	5.29±0.03g	2.71±0.01f	5.78±0.01f	7.85±0.02f
T5	19.85±0.25d	5.70±0.04f	2.68±0.01g	5.70±0.01h	11.71±0.03a
T6	22.75±0.15a	6.90±0.10a	3.62±0.01b	6.33±0.02b	8.76±0.07d
T7	18.95±0.15e	4.48±0.01h	2.51±0.01h	4.92±0.01i	10.95±0.04b

Values followed by the same letter are not significantly different (ANOVA; Tukey's test, $p < 0.05$).

Based on the comparison of growth and development indices of *P. tuoliensis*, such as mycelium growth rate, yield, biological convention rate, agriculture traits, and nutritional analysis, the formula T2 including 45% Korb, 20% cotton seed hull, 15% corncobs, 10% wheat bran, 5% maize powder, 4% lime and 1% gypsum was considered as an optimized formula for the cultivation of *P. tuoliensis*. The main component of this formula is Korb waste, therefore, the promotion and using of Korb for *P. tuoliensis* production could consume a large number of Korb waste to solve the problem which is availability low of Korb. In addition, this formula can replace 40% cotton seed hull in a conventional substrate, vastly alleviating the shortage of traditional raw material and saving raw material costs. Using Korb as the main substrate ingredient for edible fungi cultivation would have excellent economic and ecological value.

Biogas production by anaerobic digestion of spent mushroom substrates containing various proportions of Korshinsk peashrub branches

To further promote the conversion of Korb to circular economy and solve the SMS accumulation, the gas potential of SMSs containing Korb via AD were evaluated. The parameters of eight kinds of SMS and inoculum for AD are shown in Table 3. After drying at 105 °C for 4 h, the TS contents of SMS ranged from 56.13 - 64.53%. The VS contents of these feedstocks were in the range of 27.51 - 33.88%. Their total carbon and total nitrogen contents ranged from 29.10 - 36.28%, and 1.57-1.88%, respectively, which resulted in C/N ratios of 15.88-22.75, indicating rich nitrogen content in SMS. In addition, cellulose, hemicellulose, and lignin contents varied among these SMSs, which may cause significant differences in their degradability.

Table 3 Characterization of SMS and inoculum

Material	CK	T1	T2	T3	T4	T5	T6	T7	Inoculum
Total solids (%)	63.63	57.37	56.13	59.03	59.23	63.3	63.1	64.53	5.46
Volatile solids (%)	27.91	31.17	33.33	31.58	33.88	28.72	29.68	27.51	3.1
Total carbon (%)	35.52±0.04	29.80±0.17	30.21±0.58	32.22±0.23	36.28±0.24	33.29±0.25	29.34±0.77	29.10±0.19	35.87±0.10
Total nitrogen (%)	1.57±0.13	1.88±0.02	1.88±0.01	1.71±0.07	1.7±0.03	1.83±0.02	1.81±0.05	1.62±0.02	2.92±0.02
C/N ratio	22.75±0.82	15.88±0.17	16.08±0.27	18.82±0.58	21.34±0.48	18.16±0.25	16.19±0.05	18.00±0.20	10.77±0.05
Cellulose (%)	18.40±0.17	12.25±0.23	10.90±0.07	14.45±0.15	19.57±0.23	13.87±0.53	9.59±0.19	9.25±0.34	
Hemicellulose(%)	12.26±0.05	5.70±0.07	6.13±0.27	6.88±0.16	9.79±0.09	7.38±0.66	5.56±0.19	6.23±0.02	
Lignin (%)	22.27±0.13	15.26±0.16	14.73±0.08	18.95±0.04	21.28±0.14	17.21±0.06	13.82±0.24	14.80±0.47	

Mono-digestion of SMS with 1% and 3% TS content

Eight kinds of SMS were used as a mono-substrate for AD in batch tests with 1% and 3% TS to evaluate the performance of SMS mono-digestion. The pH of all of the reactions was in the range of 6.5–8.0, which is within the suitable range (6.5–8.2) for digestion (Fig. 3a and 3b).

Usually, an increase in the volume of biogas corresponds to an increase in the volume of methane [20]. The highest cumulative methane yield from eight kinds of SMS were all obtained with 3% TS. The methane yield of CK SMS was the highest (687.82 mL/g TS), but not significantly higher than that of T4 SMS (628.65 mL/g TS). The methane yields of T5, T6, and T7 SMSs significantly decreased by 1.5- to 2-fold compared to that of CK and T4 SMSs. These SMSs exhibited the same methane-producing performance at 1% TS (Table 4).

Table 4 Biogas yield and methane yield for the digestate obtained after mono-digestion

SMS	TS (%)	Biogas yield (mL/g TS)	Methane yield (mL/g TS)
CK	1	847.88	366.93
	3	1511.71	687.82
T1	1	666.96	212.75
	3	825.84	381.32
T2	1	696.85	230.37
	3	1017.92	416.01
T3	1	690.40	207.81
	3	1215.92	510.36
T4	1	774.77	297.84
	3	1304.38	628.65
T5	1	672.42	215.65
	3	834.73	353.90
T6	1	585.65	187.51
	3	668.70	291.95
T7	1	624.43	186.77
	3	758.99	371.88

The methane yield of CK and T4 SMSs were the highest, likely due to appropriate C/N ratio of SMSs, 22.75 ± 0.82 and 21.34 ± 0.48 , respectively. The C/N ratio of feedstock for biogas production should be in the range of 20 to 30 [28], which is not only beneficial for long-lasting and stable production of biogas, but also for the regulation of acidity and alkalinity of reactants, which is conducive to biogas residue.

The methane yields produced by SMSs from *P. tuoliensis* cultivation in our study were 10-80% higher than that reported by [20], who showed that the methane production of *P. tuoliensis* SMS via AD was only 159.25 ml/g TS at 3% TS. The difference in the methanogenic performance of *P. tuoliensis* SMS in different studies is mainly due to differences in substrate ingredients and their proportions. At present, thirteen species of edible fungi SMSs have been used as materials for the generation of biogas and methane, and SMS from *Pleurotus* spp. have been demonstrated to be excellent lignocellulose biomass for biogas production [19, 29, 30].

Co-digestion of SMS and dairy manure at 3% TS

Co-digestion can increase biogas production, balance the whole system, and diluted inhibitors [31, 32]. Dairy manure (DM) is an excellent co-digestion material, due to its superior buffering capacity and rich microbial and nutrient content [33, 34]. To compare the mono-digestion and co-digestion performance of SMS from *P. tuoliensis*, further batch tests were performed using co-substrates (SMS and DM with the ratio of 1:1) at 3% TS.

Similar to mono-digestion of SMS, the pH of all co-digestion reactions were in the range of 6.5-8.0, demonstrating the suitable systemic buffering capacity of these reactions (Fig. 3c). As shown in Fig. 4, the cumulative methane production of mono-digestion using DM only was 70.72 mL/g TS, which was the lowest among these tests. The high nitrogen content of DM likely caused ammonia poisoning during the anaerobic digestion [35]. The methane yields for co-digestions using T1, T5, T6, or T7 SMS and DM were 457.18 mL/g TS, 424.47 mL/g TS, 364.30 mL/g TS, and 379.75 mL/g TS, respectively. These methane yields were 19.89%, 19.94%, 24.78%, and 2.11% higher than digestion using SMS alone. However, the co-digestion methane yields for CK, T2, T3, or T4 SMSs and DM were not higher than the mono-digestion with these SMSs.

There are many confounding factors influencing the co-digestion effects, including the C/N ratio, dilution of toxic compounds, buffering capacity of the anaerobic system, and changes in the structure of the microbial community [36]. The positive synergistic effects of SMS and DM were probably due to the substrates providing adequate nutrition for the microorganism present in each component[37]. The addition of DM probably improved the buffering capacity of the reaction and reduced the influence of VFA accumulation[38]. While the appearance of negative synergistic effects was likely due to an improper C/N ratio in the reaction system after the addition of DM, which is an important parameter determining the synergistic effect in AD [39].

Based on the gas-productivity results of mono- and co-digestion of SMS, we found that the methane yield of SMS was gradually decreased with the increase of Korp content in substrate, showing that the presence of Korp in SMS is not conducive to the production of biogas. Although the methane yield of SMS containing Korp were lower than the control SMS with 65% cotton seed hull in our study, but higher than that in other reported research, in which only 286 mg/mL methane produced from *P. tuoliensis* SMS [20].

Benefit analysis

Our study demonstrated that Korp can be efficiently converted to high-valued edible products. Meanwhile, anaerobic digestion experiments indicate that SMS, containing Korp, can produce higher yield of methane than that reported in the previous study. Therefore, the utilization of Korp for mushroom and biogas production via an integrated *P. tuoliensis* cultivation and AD process would have remarkable economic, ecological, and social benefits.

Economic benefit analysis

The cultivation formula with the top yield (T2) demonstrates that the addition of 1 kg Korp in the substrate can produce 1.12 kg *P. tuoliensis*, which brings a direct economic benefit of RMB 33.6 Yuan and generates 5.6 kg SMS. If these SMS is anaerobic digested, 8578 L methane will be generated for a direct economic benefit of RMB 22.25 Yuan. In general, removing the raw material and labor cost, each kg of Korp consumed can generate economic benefit of RMB 27.5 Yuan.

The yearly quantity of Korp in China is estimated to be around 550,000 t, and the utilization rate is just 10%-20%. If 400 large-scale mushroom farms were to adopt the optimal formula for the production of *P. tuoliensis*, 5% of annual Korp will be utilized, 48125 t edible mushroom products will be produced, resulting in an enormous economic benefit of RMB 1.44×10⁹ Yuan. Meanwhile, 240625 t SMS will be generated that can be converted into 3.4 × 10⁸ L methane with an economic benefit of RMB 8.8×10⁵ Yuan. The total economic value after removing production costs is estimated as 9.5×10⁸ (Table 5).

Table 5 The economic value of Korshinsk peashrub branches for *P. tuoliensis* cultivation and anaerobic digestion of SMS

<i>P. tuoliensis</i> cultivation and anaerobic digestion		
Small-scale cultivation	Korp (kg)	1.0
	Fruiting body (kg)	1.12
	Direct economic value (yuan)	33.6
	Pt SMS (kg)	5.6
	Methane (L)	8578
	Direct economic value (yuan)	22.25
	Actual economic value (yuan)	27.5
Large-scale cultivation	Korshinsk peashrub branches (t)	27500
	Fruiting body (t)	48125
	Direct economic value (yuan)	1.44×10 ⁹
	Pt SMS (t)	240625
	Methane (L)	3.4×10 ⁸
	Direct economic value (yuan)	8.8×10 ⁵
	Actual economic value (yuan)	9.5×10 ⁸

Korp: Korshinsk peashrub branches

Ecological benefit analysis

At present, the utilization rate of chopped Korp is extremely low. A great number of Korp are burned or incorporating into the soil and sand. The conversion of Korp into high quality edible health food can not only avoid the waste of Korshinsk peashrub branche resources, but can also solve the environmental pollution problem. In addition, spent mushroom substrate, the remaining lignocellulose biomass, can be used as a biological energy source for biogas production, to meet the energy needs of people in their daily lives. Therefore, the utilization of Korp for mushroom and biogas production, via an integrated *P. tuoliensis* cultivation and AD process, results in minimum energy waste and substance discharge. This integrated process meets the objectives of agriculture in a sustainable economic society by saving energy and reducing emissions.

Social benefit analysis

Korpb are cheap and easy to obtain, and the high efficient conversion capability of Korpb into the *P. tuoliensis* product can greatly arouse the enthusiasm of the edible fungi-producing enterprises and peasants. Meanwhile, the resulting edible fungi residue can be used as a biological energy source for biogas production, which can solve the environmental pollution problem caused by discarding the SMS. An integrated *P. tuoliensis* cultivation and biogas production process, with the utilization of Korpb, can form a firm circular chain by promoting mutual development. This new avenue can efficiently solve employment problems for surplus rural labors and significantly increase peasants' incomes to build a new socialist countryside and realize agricultural modernization.

Conclusion

P. tuoliensis cultivation using Korpb as the main substrate ingredient can achieve higher mushroom yields to that obtained using conventional substrates. Anaerobic digestion was employed to add value to SMS, and a higher cumulative methane yield was obtained. In terms of economic benefit, for every kg of Korpb consumption, 1.12 kg mushroom product and 8578 L of methane were produced, which bring a actual economic value of RMB 27.5 Yuan. The integrated *P. tuoliensis* cultivation and AD processes are very effective way to maximate the utilization rate of Korpb and beneficial for the sustainable development of the environment, energy conservation, and edible fungus industry.

Methods

Strain

P. tuoliensis strain (ACCC 50869) was provided by the Agricultural Culture Collection of China. For all experiments, mycelia were grown on potato-dextrose agar (PDA) medium at 25 °C in the dark for 10 d before use.

The Korpb were obtained from Yuanfeng Grass Industry Co., Ltd., Yanchi County, Ningxia Hui Autonomous Region, China. These branches were chopped into small pieces (1–2 cm) with pruning shears and ground to pass through a 2 cm inner screen.

Substrate preparation, inoculation, and culture

The cultivation substrate was primarily composed of Korpb, cottonseed hull, and ground corncobs, supplemented with commercially available auxiliary materials, including wheat bran, maize powder, lime, and gypsum. The carbon (C) and nitrogen (N) content of each raw material were analyzed using the method described by [40].

Eight formulas were designed using Korpb, cottonseed hull, and ground corncobs (Table 6). The control formula (CK) was 65% cottonseed hull, 15% ground corncobs, 10% wheat bran, 5% maize powder, 2.5% lime, and 2.5% gypsum, with 65% water content, which is a regular formula commonly used for *P. tuoliensis* cultivation in China. In T1 - T7, the amounts of Korpb, cottonseed hull, and ground corncobs varied in proportion, while the amounts of auxiliary materials remained unchanged. The C/N ratio of T1 - T7 are in the range of 19:1 to 28:1.

Table 6 Eight substrate formulas containing various proportions of Korshinsk peashrub branches for *P. tuoliensis* cultivation (wt%)

Formula	Korpb	Cotton seed hull	Corncobs	Wheat bran	Maize powder	Lime	Gypsum	C/N
CK	-	65	15	10	5	4	1	28:1
T1	65	-	15	10	5	4	1	19:1
T2	45	20	15	10	5	4	1	22:1
T3	25	40	15	10	5	4	1	24:1
T4	5	60	15	10	5	4	1	27:1
T5	55	-	25	10	5	4	1	21:1
T6	45	-	35	10	5	4	1	24:1
T7	35	-	45	10	5	4	1	27:1

Korpb: Korshinsk peashrub branches

The well-mixed substrates were placed in poly ethylene bags (1400 ± 50 g per bag), sterilized at 110 °C for 4 h, and then inoculated under aseptic conditions. The inoculated bags were incubated at 25 °C in the dark until the cultivation substrate was fully covered by *P. tuoliensis* mycelia. The mycelia were cultured under scattered light for an additional 35 days to induce physiological maturation of mycelia and then the primordia stage was initiated. To stimulate the formation of primordia, the bags were subjected to a temperature of 15/4 °C (day/night) for 10 days. Then, these bags were maintained at a temperature ranging from 12-14 °C and a relative humidity of 85–90%. When the pilei of the fruiting bodies had not been completely unfolded, the fruiting bodies were harvested and weighed using an electronic balance with an accuracy of 0.01 g. Only a single flush of fruiting body yield was recorded. The BE was calculated based on the following formula: BE (%) = weight of fresh mushrooms harvested per bag/dry weight per bag × 100. Pileus thickness and diameter of the fresh fruiting bodies were measured with a slide caliper.

Chemical analysis

Fruiting bodies of *P. tuoliensis* from eight different formulas were dried in an oven at 60 °C to a constant weight. The dried fruiting bodies were then ground through a 200-mesh sieve and sampled for nutrient analysis. The total sugar content was determined using the sulfuric acid-anthrone colorimetric method. The protein content ($N \times 4.38$) was estimated using the macro Kjeldahl method (Kjeltec™ 8000, Foss, Hilleroed, Denmark). The crude fiber content was measured using the traditional Van Soest method [41]. The fat content was determined using a Soxhlet extractor system (Automatic Fats Analyzer, model 2050, Foss, Hilleroed, Denmark). The ash content was determined by incineration at 600 ± 15 °C. These analyses were performed by the PONY Testing International Group (Beijing, China).

SMS from eight different formulas were naturally air-dried and then weighed for lignocellulose content analysis. Cellulose, hemicellulose, and lignin contents in SMS were determined using a fiber analyzer (Model ANKOM220, USA). Non-inoculated cultivation substrate was used as a control.

Feedstock and inoculum

After harvesting the fruiting bodies, SMS from these eight formulas were weighed for solid-state anaerobic digestion. Dairy manure was collected from a dairy farm in Doudian, Beijing. The inoculum was obtained from a mesophilic liquid anaerobic digester fed by manure and maize. The inoculum was kept at 4 °C for one night and then domesticated at 37 ± 2 °C for 7 days before the beginning of anaerobic digestion experiment.

Solid-state anaerobic digestion

This research mainly involved in (1) mono-digestion of eight kinds of SPt with two TS contents (1% and 3%) and (2) co-digestion of dairy manure with eight kinds of SPt at 1:1 ratios with 3% TS. The inoculum was added at 5% TS. The batch experiment was performed using 500 mL conical flasks with 300 g of mixed feedstock, inoculum, and tap water. Inoculum only with water was used as a control. All reactors were purged with N₂ for 3-5 min to remove O₂ and then tightly closed with rubber stoppers. Each reactor experiment was carried out in duplicate at mesophilic temperature (35 °C). The experiment was terminated when there was no obvious gas production.

The basic properties of the material for solid-state anaerobic digestion

The samples were dried in a crucible at 105 °C for 6 h to determine their dry matter content. The TS, VS, total nitrogen and total carbon in these samples were measured in triplicate according to standard methods [42]. Samples for pH measurements were prepared by suspending 10 g of sample in 100 ml of deionized water. The supernatant was measured using a compact pH meter (Model B-212, Horiba, Japan).

Biogas volume was monitored daily at a fixed time using a BMP-Test System pressure gauge (WAL Messund Regelsysteme GmbH, Oldenburg, Germany). The composition of biogas (Carbon dioxide, Methane and Nitrogen) were analyzed by gas chromatography (GC-2014, Shimadzu, Kyoto, Japan) equipped with a stainless steel column (2 m × 0.004 m) and a Thermal Conductivity Detector (TCD). Hydrogen was used as a carrier gas at a flow rate of 25 mL/min. Temperatures of the column and detector were set at 40 and 100 °C, respectively. To prevent the reactors from exploding, the biogas generated in the reactors was immediately released through water once the measurements were over. The volume of biogas was calculated using the following ideal gas law [43].

$$V_{\text{biogas}} = \Delta P \times V_{\text{head}} \times C / (R \times T)$$

where V_{biogas} represents the daily biogas volume (mL), ΔP is the absolute pressure difference (kPa), V_{head} refers to the volume of the headspace (mL), C is the molar volume (22.41 L/mol at 273.15 K, 101.325 kPa), R means the universal gas constant (8.314 J/K/mol), and T stands for the absolute temperature (K).

Benefit analysis of Korshinsk peashrub branches for P. tuoliensis cultivation and biogas production

To evaluate the feasibility of using Korpb in edible fungi cultivation and biogas production, the sustainable development of the process was analyzed in terms of the economic, ecological, and social benefits.

Statistical analysis

Data processing, graph construction and statistical analyses were performed using Microsoft Excel 2010 (Microsoft Co., Redmond, WA, USA) and Statistical Package for Social Sciences (SPSS) version 19.0. All reported values were the average measurements from three experimental repetitions, except for the value of methane yield, which was the average of duplicate results.

Declarations

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Author's contribution

FD performed the cultivation of *P. tuoliensis* and AD of SMS, and was major contributor in the writing of the manuscript. QQ performed AD of SMS and the statistical analysis of all data; QH provided guidance and checking in design of experiments and was contributor in the writing of the manuscript; YZ provided guidance and checking in design of experiments and was contributor in the writing of the manuscript; GH performed the cultivation of *P. tuoliensis*; DD performed the AD of SMS; XY provided guidance and checking in the design of experiments. All authors read and approved the final manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Abbreviations

Korpb: Korshinsk peashrub branches; AD: anaerobic digestion; SMS: spent mushroom substrate; VS: volatile solids; TS: total solids; PDA: potato-dextrose agar; C: carbon; N: nitrogen; CK: control formula; BE: biological efficiency; TCD: Thermal Conductivity Detector; SPSS: Statistical Package for Social Sciences; DM: Dairy manure;

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Figures

Figure 1

The growth rate of mycelia in substrate formulas including various proportion of Korshinsk peashrub branches. Values followed by the same letter in the bars with similar pattern are not significantly different (ANOVA; Tukey's test, $p < 0.05$).

Figure 2

The morphological characteristics of fruiting bodies in each formula.

Figure 3

pH from anaerobic digestion of spent mushroom substrate. A. pH from mono-digestion of SMS at 1% TS. B. pH from mono-digestion of SMS at 3% TS. C. pH from co-digestion of SMS and DM at 3% TS.

Figure 4

The comparison of mono-digestion and co-digestion of SMS at 3% TS for methane production.

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