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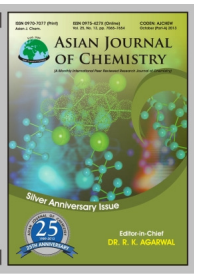

Chemical Composition of Spent *Pleurotus eryngii* Mushroom Substrate and Its Reuse for *Volvariella volvacea* Production

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The nutrient compositions, physical components and metal contents of spent *Pleurotus eryngii* mushroom substrate (SMS) and traditional wheat straw substrate (WS) were analyzed. The result show that SMS can be used for the cultivation of *V. volvacea*. The effects of compost of SMS, additive of WS, substrate moisture content and initials pH on yield, bioconversion efficiency, spawn growth and nutrition of fruit body were investigated. The cultivation result show that composted SMS was successfully used as basic substrate for *V. volvacea* production with adjusted the initial pH of 8 and 70 % of moisture content. In laboratory and large scale cultivation experiments, the average biological efficiency of the cultivated on SMS were 26.12 and 27.42 %, respectively, considerably higher than the corresponding values on traditional WS controls of 17.92 and 20.17 %. The proximate composition, amino acids, trace elements of the fruit body cultivation between SMS and WS media were analyzed. The result indicated that *V. volvacea* cultivate on SMS had a higher nutritional value than those grown on WS.

Key Words: Spent mushroom substrate, Chemical composition, Mushroom cultivation, Nutrition analysis.

INTRODUCTION

Mushroom cultivation is currently the biggest solid-state-fermentation industry in the world and a good bioconversion process for the utilization of a wide range of residues from the agro-industrial sector[1]. One of the major problems in the mushroom producing countries remains the treatment and disposal of the residues. After several mushroom cultivation cycles the productivity decreases and the residual by-product, known as spent mushroom substrate (SMS) has to be properly disposed of to avoid environmental problems, such as water and soil contamination, which result from the high level of organic and salt contents of the spent mushroom substrates.

It is generally acknowledged that SMS is a valuable material for improving soil structure in tilled soils owing to its highly organic nature[2] and increasing dry matter production on grassland soils[3]. As a lignocellulosic material, SMS could potentially be a source of reducing sugars for production of biofuels and other biomaterials[4-6]. Furthermore, the use of SMSs on biodegradation of pollutants has also been well documented[7-9]. However, there are nearly few studies on the uses of fresh SMS from one mushroom production for cultivation of another mushroom.

Volvariella volvacea Singer, the Chinese straw mushroom, is a commercially important edible mushroom cultivated in tropical and subtropical regions and ranks fifth among cultivated mushrooms in terms of annual world-wide production[10]. Unlike other cultivated edible mushrooms such as *Agaricus bisporus, Lentinula edodes* and *Pleurotus sajor-caju*, *V. volvacea* grows poorly on substrates with a high lignin content[11] and its biological efficiency is very low[12], because of its cellulolytic characteristics. Although some improvements have been achieved by replacing the traditional rice straw substrate with cotton waste, fruiting body yield is still lower than that of other popular cultivated mushrooms. One of the methods enhancing the productivity is to find a low-cost and high efficiency feedstock for mushroom cultivation.

The main objective of this work is to develop an economical bioprocess to produce *V. volvacea*. We also determined the effect of *V. volvacea* grown in different media of spent mushroom substrate in various ratios with straw, initial pH; moisture content and superphosphate concentrate on cultivation parameters in pilot scale experiment. Moreover, we analyzed composition of *V. volvacea* production from spent mushroom substrate and traditional straw substrate.

EXPERIMENTAL

The *P. eryngii* SMS used in the present study were obtained from a factory farming named Rong-zhen mushrooms Co., LTD. located in the city of Chengdu (Sichuan province), which is the largest industrialized mushroom cultivation company in southeast China. This SMS was composted and formulated with 50 % sawdust, 25 % cotton seed hulls and 25 % wheat bran, supplemented with gypsum ($CaSO_4 \cdot 5H_2O$) and $CaCO_3$ and supported 3-4 fruiting cycles. After drying SMS was ground to a particle size of 800 μm and then stored in air-tight container at 4 °C for further use. *V. volvacea* strain V27, original strain, is preserved in Rong-zhen mushrooms Co., LTD. and was acquired for CGMCCC (China General Microbiological Culture Collection Center). The culture media used for routine culture and storage purposes were potato dextrose agar (PDA) and complete yeast medium (CYM). Grain spawn of species was prepared in 500 mL Erlenmeyer flasks filled with 180 g of boiled wheat (Triticum durum) grains supplemented with 3 % calcium carbonate and 1.5 % gypsum (w/w, in terms of dry weight). The contents of flasks were thoroughly mixed and autoclaved at 121 °C for 0.5 h. Each flask was inoculated with mycelium scraped off the surface of a growing colony in a Petri dish (about 9 cm diam). Flasks were incubated at 26 °C for 3 weeks, in the dark, with periodical shaking.

The lignin content of the raw spent mushroom substrate and acid hydrolysis residues were determined[13-15] according to National Renewable Energy Laboratory Procedures LAP 001, 003 and 004. The carbohydrate composition of SMS was determined by High Performance Liquid Chromatography (Waters 2795, Waters corp., USA) with Evaporative Light-scattering Detector (All-Tech ELSD 2000, All-tech., corp., CA) using an Aminex HPX-87-Pb column (Bio-Rad, Hercules, USA) at 80 °C and flow rate of 0.6 mL/min with distilled water as eluent. Nitrogen gas was used as carrier at the pressure of 3.2 Bar and draft temperature was 110 °C for the ELSD detector. Protein concentration was determined by the Kjeldahl method. Lipids concentration was determined by gravimetric analysis after solvent extraction with hexane (Soxhlet method). Ashes were quantified by gravimetric analysis after burning samples at 550 °C for 5 h. Fibers concentration was calculated by difference. Moisture content was determined by gravimetric analysis after drying at 105 °C to constant weight. The C, H, N was analyzed by CHN Analyzer with automatic sampler (Carlo-Erba elemental analyzer, Model 1106, Italy). The sample was digested in 5:1 nitric: perchloric acids and analyzed for As, Cr, Cd, Hg, Pb, Na, K, Ca, Mg, P, Cu, Mn, Zn and Fe using inductively coupled plasma atomic emission spectrometer (ICP-AES, Model IRIS 1000, Thermo Fisher, USA).

Substrate preparation: Non-composted substrate of SMS was prepared as follows: The *P. eryngii* SMS with particles size of 3 cm were soaked in water for 24 h. After the surplus water had been drained off, substrates were mixed with 5 % of limestone (dw) to obtain a pH value of 7 and were supplemented with wheat straw according to experimental design. The moisture content of the sterilized substrates was 60-75 % on the basis of requirement. Composted substrate of SMS was prepare as follows: About 300 kg of SMS from *P. eryngii* cultivation were filled in the drum, watered thoroughly, allowed to drain to obtain 65 % moisture and subjected to a 15 day composting process. Mixing and aeration was achieved by periodic drum revolutions. Along with composting, temperature and moisture level were monitored daily just before turning and water was added as necessary to maintain 65-70 % moisture content. Temperature raised at 65-70 °C in first 4 days, kept in the range of 50-65 °C for the following 6 days and gradually dropped to 40 °C by the completion of the process.

Culture of *V. volvacea* by the *P. eryngii* SMS: Polypropylene-autoclavable bags were filled with 2.5 kg of substrate and were sterilized twice for 1 h at 121 °C. Inoculation was carried out along the central vertical axis of bag, at a rate of 3 % (w/w). Colonization of the substrates took place in growth chambers, in the darkness, at 37 °C. After complete colonization of the substrate, polypropylene bags were removed and environmental conditions (temperature, relative humidity, aeration and light intensity) were adjusted for basidiomata induction and maintained at the appropriate levels during the entire length of production cycles. During fructification, the light intensity was set at 700 lux (12h/day, fluorescent lamps), air exchange rates were controlled to maintain low CO_2 level (< 1200 ppm), relative air humidity was adjusted at 90 % and temperature was set at 30 °C. Three flushes of *V. volvacea* mushrooms at the egg or elongation stage were harvested during the cropping period that lasted from 30-50 days, depending on the experiment. For fructification and productivity evaluation, three replicates per substrate were used. For all replicates, the time needed for complete substrate colonization and appearance of primordial was also recorded.

Calculations: Closed (lamellae not exposed) mushrooms were harvested, counted and weighed daily. At the end of each break, yield and bioconversion efficiency were determined as the ratio of fresh mushrooms (g) harvested per dry substrate weight (g) and expressed as a percentage. Yield was expressed in kg/100 kg substrates. Average mushroom productivity rate (kg/100 kg/day) was calculated as the total yield of fresh mushrooms (kg/100 kg) over the total harvest period (day).

RESULTS AND DISCUSSION

Chemical composition of spent *Pleurotus eryngii* mushroom substrate: As shown in Table-1, initial pH, average moisture content, C/N rate, protein concentration, ashes, fibers and lipids of SMS dry matter (and rice straw substrate) were approximately 6.8 (6.7), 21.5 % (18.1 %), 41.74 (36.84), 3.61 % (1.42 %), 6.02 % (8.86 %), 62 % (37.45 %) and 0.54 % (0.12 %) (w/w) respectively. The C, H and N of SMS dry matter (and rice straw substrate) were approximately 59.59 % (48.12 %), 7.93 % (5.24 %) and 1.64 % (1.02 %), respectively. The nutrient composition and physical component description between the SMS and traditional rice straw substrate for *V. volvacea* production were varied, where it is evident that SMS has a considerable supply of plant nutrients.

As shown in Table-2, As, Cr, Cd, Hg, Pb, Na, K, Ca, Mg, P, Cu, Mn, Zn and Fe of SMS dry matter were approximately 0.16, 0.12, 0.83, 0.00006, 2.11, 244, 3927, 4671,1391, 1262, 2.9, 13.1, 5.6 and 19.5 (μg/g), respectively. Though no legal heavy metal limits or standards have been published for SMS or indeed for any general compost produced in China, yet there

TABLE-1
NUTRIENT COMPOSITIONS AND PHYSICAL COMPONENTS OF SMS AND RICE STRAW SUBSTRATE

Substrate	pH	Moisture (%)	C/N	Protein (%)	Ashes (%)	Fibers (%)	Lipids (%)
SMS	6.8±0.2	21.5±0.6	41.74±2.1	3.61±0.11	6.02±0.22	62±3.04	0.54±0.05
Rice straw	6.7±0.2	18.1±0.5	36.84±1.55	1.442±0.05	8.86±0.13	37.45±2.25	0.12±0.02

TABLE-2
METAL CONTENTS OF SPENT *Pleurotus eryngii* MUSHROOM SUBSTRATE

Metal element	Concentration (μg/g)	Metal element	Concentration (μg/g)
As	0.16±0.001	Ca	4671±68.09
Cr	0.12±0.001	Mg	1391±20.87
Cd	0.83±0.025	P	1262±45.62
Hg	0.00006	Cu	2.9±0.05
Pb	2.11±0.03	Mn	13.1±0.25
Na	244±10.25	Zn	5.6±0.1
K	3927±109.22	Fe	19.5±0.35

are standards implemented in many other European countries for general compost. These limits for lead range from 120 to 1200 mg/kg, from 1 to 105 μg/g for cadmium, 70 to 750 μg/g for chromium, nickel from 20 to 400 μg/g, copper from 90 to 1750 μg/g and zinc from 280 to 4000 μg/g[16]. According to our study, most of the heavy metals are passable and the values obtained are well within the recommended range in EU countries and should not be a cause for concern when applied to land, as elevated heavy metal concentrations negatively affect plant growth.

***V. volvacea* grown on composted and non-composted SMS:** Many species of *Pleurotus* including *P. eryngii* for commercial are cultivated using a substrate consisting of various mixtures of wheat straw, hay, corncobs, seed hulls and meal, horse manure, chicken manure, inorganic fertilizer, calcium sulfate, peat moss and ground limestone[17-21]. After mushroom harvest, the residual substrate is pasteurized and either discarded or sold as spent mushroom compost. In order to assess the potential of SMS as a feedstock for *V. volvacea* production, the mycelium growth, total mushroom yield and bioconversion efficiency were determined on composted and non-composted SMS. As shown in Table-3, the composted substrates supported better substrate colonization for *V. volvacea* than non-composted substrate, *e.g*. on composted SMS the mycelium growth, total yield weight and bioconversion efficiency were reduced by 63.12, 41.65 and 37.08 % respectively. In previous reports, there were contradiction results about the growth of *V. volvacea* on composted and non-composted SMS, expecially for different strain of *V. volvacea*[22,23]. In this study, it is concluded that composted SMS from *P. eryngii* cultivation achieved better growth of *V. volvacea* than non-composted one.

TABLE-3
EFFECT OF *V. volvacea* GROWN ON COMPOSTED AND NON-COMPOSTED

Substrate	Mycelium growth rates (mm/d)	Bioconversion efficiency (%)	Total yield weight (Kg)
Composted SMS	12.7±0.25	17.17±0.68	18.48±0.73
Non-composted	4.6±0.14	10.16±0.45	11.64±0.51

***V. volvacea* grown on different media of composted SMS in various ratios with wheat straw:** In order to compare composted SMS and traditional wheat straw as substrate for *V. volvacea* production, the fruit body yield, bioconversion efficiency, harvest cycle and spawn grown have been analyzed. As shown in Table-4, the SMS was blended with wheat straw (WS) at 0, 5, 10, 15, 25 and 95 % on a wet basis and including 5 % limestone. Six treatments included SMS 95 %, SMS 90 % + WS 5 %, SMS 85 % + WS 10 %, SMS 80 % + WS 15 %, SMS 70 % + WS 25 % and WS 95 %. According to our study, bioconversion efficiency of *V. volvacea* from adding some SMS was higher than those under traditional wheat straw substrate condition. Perhaps because SMS have amount of fermentable nutrient which comes from the hydrolase activity of *P. eryngii* that could been easily utilized. Philippoussis *et al.*[23] reported that *V. volvacea* grow better on cotton waste than WS and peanut shells. In addition positive correlation was also detected between cellulose content and mushroom yield for *V. volvacea* strain. In the case of our research, high content of fiber was detected in SMS (62 % of fiber in total dry matter) rather than WS (37.45 % of fiber in total dry matter), which may the reason for better bioconversion efficiency on SMS.

Furthermore, the mushrooms produced from 1st flush commonly have best appearance and qualities. Adding amount of SMS could remarkable increased the yield of 1st flush, the mushroom yield with SMS was about 50 % higher than with wheat straw. In all substrate with adding SMS, an average mushroom productivity rate yield was 16.44 g/d, considerably higher than the corresponding values for WS controls of 12.8 g/d. Fasola *et al.*[24] evaluated the mycelial growth of *Volvariella speciosa* (Fr. Ex. Fr.) Singer on synthetic and semi-synthetic

TABLE-4
SUBSTRATE CONDITION AND RESULT OF *V. volvacea* CULTIVATION ON WHEAT STRAW AND SMS

Substrate (w/w)	Spawn grown	Mushroom yield distribution (%)			Total yield (g)	Total harvest period (day)	Bioconversion efficiency (%)	Productivity (g/d)
		1st Flush	2nd Flush	3rd Flush				
SMS 95 %	+	56.10±2.56	30.2±1.98	13.66±1.86	653.0±18.73	36	26.12±0.75	18.14±0.52
SMS 90 % + WS 5 %	++	53.80±0.91	30.3±0.96	15.8±2.15	610.0±23.45	35	24.40±0.94	17.40±0.67
SMS 85 % + WS 10 %	+++	52.20±2.39	30.4±0.72	17.4±1.77	537.5±11.69	33	21.50±0.46	16.30±0.35
SMS 80 % + WS 15 %	+++	50.30±3.31	29.8±0.92	19.9±0.50	490.0±19.33	31	19.60±0.77	15.80±0.62
SMS 70 % + WS 25 %	++++	52.00±2.43	30.9±3.38	17.1±1.22	465.8±20.57	32	18.63±0.82	14.60±0.64
WS 95 %	++++	49.07±4.97	33.3±2.33	17.6±0.54	448.0±15.52	35	17.92±0.62	12.80±0.44

*All the substrate were added 5% (w/w) limestone

media, as well as different agro-industrial wastes and found among the different agricultural substrates used, growth was poor on fresh cow dung and poultry droppings and enhanced mostly on maize and andropogon gianus straw but greatest mycelial density was obtained with cassava peels. Likewise, the growth of mycelial was poor with the substrate adding SMS. This low growth may be due to high containing of ammonia, comes from residual of *P. eryngii* cultivation which may inability of *V. volvacea* to secrete hydrolyzing enzyme, which would convert the substrate to utilizable amino acids and carbon compounds necessary for growth, are toxic to mycelial growth of fungi[25].

Effect of pH and moisture content on *V. volvacea* cultivation by spent mushroom substrate: The mushroom yield, including 1st flush, 2nd flush and 3rd flush for different test conditions are given in Figs. 1 and 2. The test runs 1-5 and 6-10 were carried out as three separate cultivation runs to determine the effect of pH and moisture content on the mushroom growth on SMS 95 % and limestone 5 %. According to the results presented in Figs. 1 and 2, the difference of pH and moisture content caused a difference in total bioconversion efficiency.

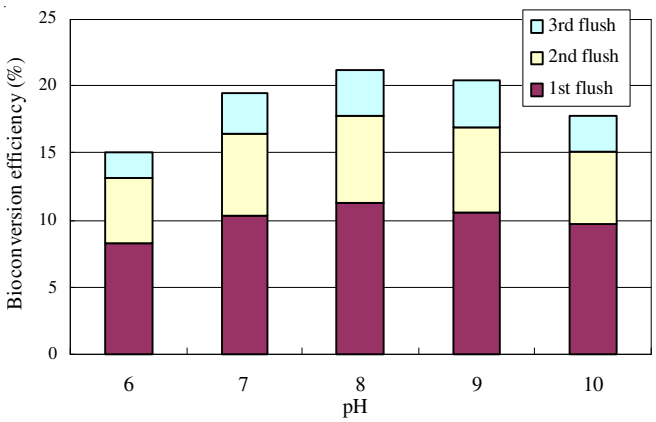

Fig. 1. Effect of pH on *V. volvacea* cultivation by SMS

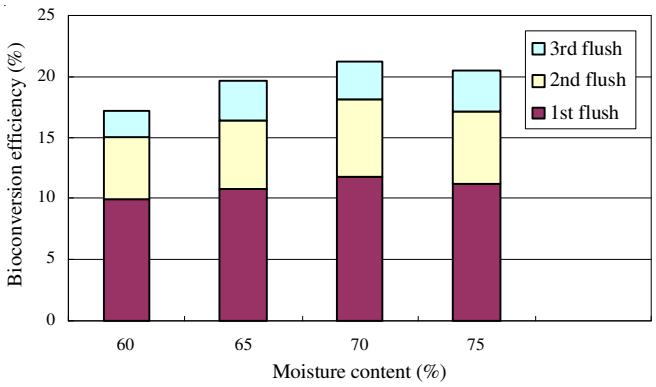

Fig. 2. Effect of moisture content on *V. volvacea* cultivation by SMS

As shown in Fig. 1, the mycelial growth of *V. volvacea* was obtained in the pH range 6 to 10. It was observed that the best bioconversion efficiency (22.6 %) was observed in the slightly alkaline pH of 8 while good growths (18.9 % and 20.1 %) were also obtained at pH 7 and 9. This result implies that *V. volvacea* prefers alkaline pH values, tending toward neutrality. This result is similar to those obtained by Fasidi[26] (for *V. esculenta*) and Jonathan[27] (for *L. procera*). Generally, vegetative growth of this mushroom was reduced at high acidic and alkaline pH. Hopkins[28] reported that, at high acidic or alkaline pH, cell wall and tonoplast of plants may corrode and selective permeability function of the cell membrane may be impaired. This may be the reason why there were no good growths at pH 6 and 10.

It has been known that the substrate moisture content is the important factor affecting mycelia growth and fruit body yield, in addition to chemical components of substrate[29,30]. Fig. 2 shows the variation of bioconversion efficiency in relation to moisture content by SMS. The bioconversion efficiency value increased with the moisture content and peaked at about 70 %. These results suggest the optimum moisture content of *V. volvacea* cultivation by SMS is around 70 % and this result is similar to the earlier observation of Alofe[31] who reported that *L. subnudus* and *P. tuber-regium* grew on agricultural wastes at different moisture contents.

Nutrition analysis of *V. volvacea* produced from SMS and traditional substrate: To evaluate the nutritional value of *V. volvacea*, the proximate composition, amino acids, trace elements of the fruit body cultivation between SMS and WS media were analyzed. As shown in Table-5, the cultivation on SMS had a greater potential to improve the accumulation of TKN, K, Ca, P and Na than did on WS substrate. This difference is in agreement with the analyses of *V. volvacea* cultivated on different substrate, composed of orange juice waste, bagasse, rice straw, horse manure, molasses and urea. In addition, the type of substrate also affected the amino acid profile, even though protein contents were similar in SMS and WS substrate. As shown in Table-6, the fruit bodies harvested from SMS contained all the essential amino acids, which comprised 39.8 % of total Kjeldahl protein. This result may attribute to not only the initial amount but also the nature of nitrogen source present in substrate influences the protein content of fruit bodies, which is in accordance with the literatures[32,33]. Furthermore, as regard shelf life, harvested sporophores grown on SMS showed a higher degree of color change from brown to black than those grown on traditional WS media (pictures not showed). This phenomenon was significantly reduced by packaging sporophores under a polyethylene film and need to be further research.

Conclusion

According to the results, it could be concluded that SMS from *P. eryngii* production is an attractive feedstock for the *V. volvacea* cultivation, especially in China which is the world's largest mushroom producer. Since composited SMS could provided the necessary nutrient element and the appropriate heavy metal concentrations for the fermentation of other mushroom such as *V. volvacea*, there was no need for supplementing these additionally. Moreover, the total mushroom yield, bioconversion efficiency, productivity, nutrition analysis and amino composition of fruit body cultivated on SMS were comparable to traditional wheat straw based substrate cultivation from both the economic standpoints and environmentally friendly.

TABLE-5
CHEMICAL COMPOSITION OF THE FRUIT BODY OF *V. volvacea* PRODUCED FROM SMS AND TRADITIONAL SUBSTRATE

Substrate	Nutrient composition (%)							
	TKG	Lipid	TRS	Ash	K	Ca	P	Na
SMS	30.23±1.08	1.13±0.05	0.49±0.02	9.01±0.18	6.083±0.15	0.360±0.002	1.185±0.02	0.169±0.003
WS	28.02±0.59	1.25±0.03	0.53±0.01	9.00±0.41	5.862±0.23	0.325±0.001	1.087±0.02	0.156±0.001

TABLE-6
AMINO ACID COMPOSITION OF THE FRUIT BODY OF *V. volvacea* PRODUCED FROM SMS AND TRADITIONAL SUBSTRATE

Substrate	Amino acid composition (%)								
	Leucine	Isoleucine	Lysine	Methionine	Phenylalanine	Threonine	Valine	Tryptophan	Total
Ideal protein	7	4	5.5	3.5	6	4	5	1	36
SMS	7.1±0.22	3.8±0.05	9.6±0.34	1.3±0.02	4.4±0.1	4.3±0.13	7±0.17	2.3±0.04	39.8±1.07
WS	6.9±0.08	4.3±0.13	9.8±0.26	1.2±0.04	2.6±0.12	3.4±0.12	6.5±0.09	1.2±0.05	35.9±0.89

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