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Spent bleaching clay (SBC) from oil refining as a substrate for the spawn production of shiitake mushroom (*Lentinula edodes*)

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While no effective (both in a technical and economic sense) methods exist so far to handle large quantities of untreated spent bleaching clay (SBC) in China, there are indeed great demands for low-cost alternatives for mushroom substrate. Hence, it is in the interest of both edible-oil-refining industries and mushroom producers to explore how partially substituting traditional shiitake substrate by SBC would influence the mycelial growth in shiitake spawn production. This paper conducted a comparative shiitake spawn production experiment with a conventional shiitake substrate formula comprising of 78% sawdust, 20% wheat bran, 1% magnesium sulfate, and 1% gypsum (percentage by weight, the same thereafter) as the control and substrate formulae with the substitution of SBC for part of sawdust or wheat bran as treatments to observe the mycelial growth. The results indicated that formulae with SBC had lower incidence of microbial contamination and higher mycelial growth speed, and the optimal formula was 15% SBC, 78% coarse sawdust, 5% wheat bran, 1% magnesium sulfate and 1% gypsum. Therefore, the utilization of SBC in shiitake spawn production not only considerably reduces the costs of shiitake production but also bring about substantial environmental benefits.

Key words: Spent bleaching clay, shiitake mushroom, *Lentinula edodes*, vegetable oils, spawn production, mycelial growth, components.

INTRODUCTION

Spent bleaching clay (SBC), is the by-product of edible oil refining industry, which mainly comprised of clay, neutral oil, phospholipids, proteins, saccharides and their breakdown products, mucous substances, and trace elements. Approximately, 3 million tons of SBC were generated annually only in China (Wang et al, 2006). Currently, SBC is mainly used to produce phosphatide (Wu et al., 2005; Kong et al., 2008; Wang, 2007), fatty acids and glycerol (Gu et al., 2001; Yue et al., 2006), biodiesel (Al-Widyan et al., 2002; Li et al., 2003; Keskin et al., 2008), chemical products such as soaps, water-proof bitumen and parting agents (Xu, 1997; Sun, 2001), fodder supple-

mentary (Zhao and Cao, 1999) or feed (Al-Zubaidy, 1992), and organic fertilizer. The former three usages need advanced technologies and have high requirements on production conditions, however, only result in low recovery rate and low quality products, the latter several usages can also only have low recovery rate and limited economic benefits. Thus, a huge amount of SBC is improperly disposed, which not only is a waste of resources but also imposes severe impacts on the environment. SBC starts composting and causing odor soon after being piled up for a few days in a warm environment, causing serious environmental pollution to the surrounding and underground water. Generally, it becomes invalid for reuse after being piled up for 3 to 7 days in summer (Li and zhang, 1996). Hence, it is compelling to explore new channels or develop new technologies to reusing this waste.

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Table 1. Ingredients of substrate in each treatment for shiitake spawn production (% dry weight).

Treatment	Sawdust	Wheat bran	SBC	Magnesium sulfate	Gypsum
Formula 1(CK)	78	20	0	1	1
Formula 2	78	15	5	1	1
Formula 3	78	10	10	1	1
Formula 4	78	5	15	1	1
Formula 5	78	0	20	1	1
Formula 6	58	0	40	1	1

With the increasing development of mushroom industry in China, it needs a great quantity of raw materials. Raw materials with lower cost will make the mushroom production more competitive in the world market (Royse and Sanchez, 2007; Ozcelik and Peksen, 2007; Fang and Sun, 2008; Liu et al., 2009). Amongst various mushrooms, shiitake is on the best sale list of mushrooms; in fact, it is entitled as 'the king of mushrooms' owing to its remarkable nutritional and medicinal value (Ruan et al., 2005; Chihara et al., 1969). According to Wang (2001), China has become the major producer of shiitakes, which implies the practical significance of locating feasible and cost-effective materials for shiitake cultivation. There have been no reports on the utilization of SBC in mushroom production yet. In order to ensure the safety application of SBC in mushroom production and, to further determine appropriate proportion of SBC in the substrate formulae for shiitake spawn production, the principal nutritional components and trace elements were analyzed before the experiment.

In this paper, we conducted a comparative shiitake spawn production experiment by using substrate formulae containing SBC (treatments) and a conventional substrate formula without SBC (control) to observe the effect of SBC on the shiitake mycelial growth and select an optimal proportion of SBC in the substrate formula of shiitake spawn production.

MATERIALS AND METHODS

Experimental materials

The conventional substrate, comprising of sawdust, wheat bran, magnesium sulfate, and calcium sulfate, was used as the control, and the experimental substrate contained sawdust, wheat bran, and SBC. In the experimental substrate, SBC substituted part of the sawdust but other ingredients remained in the same proportion.

SBC raw material was bought from Sichuan Gaoda Oil Refinery Factory, Chengdu, China. Samples of SBC were analyzed by the Analysis and Testing Center of Sichuan Academy of Agricultural Sciences, China. The analysis was conducted according to correspondent standards and literature: protein by Kjeldahl method (Ministry of Health of China, 2010), organic matter, phosphorus, potassium and heavy metals according to GB18877-2002 (General Administration of Quality Supervision, Inspection and Quarantine of China, 2002), humic acid according to NY 1106-2006 (Ministry of Agriculture of China, 2006), and amino acid according to GB/T 5009.124-2003 (Ministry of Health of China, 2003).

Shiitake mushroom strain

Shiitake strain L931, a popular variety in shiitake production in China, was provided by the Soil and Fertilizer Research Institute, Sichuan Academy of Agricultural Sciences, China. Its characteristics are as follows: The pileus was big, round and nice-looking; the texture was plump; the color was tan to dark brown; the stipe was of medium size and grew right in the middle of the pileus; the strain was of great adaptability and stability; and the fruiting temperature was 15 to 30 °C.

Substrate formulae for shiitake spawn production

With the aim of exploring the feasibility of reusing SBC for the spawn production of shiitake mushroom, as well as identifying the optimal substrate formula containing SBC, we designed a comparative experiment with a conventional shiitake substrate formula comprising of 78% sawdust, 20% wheat bran, 1% magnesium sulfate, and 1% gypsum as the control and substrate formulae with the substitution of SBC for 5, 10, 15, 20 and 40% of sawdust or wheat bran as treatments (Table 1). Each treatment has 10 samples (bottles). Before the experiment, we pretreated SBC with special complex microorganisms and nitrogen additives.

Experimental methods and data analysis

The spawn production experiment was conducted in December of 2009. Firstly, the materials used for shiitake substrate were thoroughly mixed separately for different treatments, and then moistened till the water content was at 65 to 70 °C; secondly, the prepared substrates were packed into 750 ml glass bottles, all bottles contain substrate with the same weight and same compactness; thirdly, the bottles were sterilized under the temperature of 121 °C for 3 h, and then transferred to the inoculation room, and inoculated on December 18th, 2009 with the same amount of stock culture of shiitake mushroom strain L931 when the temperature dropped to around 25 °C; finally all the inoculated spawn bottles were incubated in a clean culture room with normal room temperature and relative humidity of 60 to 70 °C. Six treatments were set with 10 replicates (10 bottles) for each treatment. So the experiment totally has 60 bottles of shiitake spawn. Mycelial growth was carefully observed and the spawn germination, colonization, and run time, as well as the color of the mycelia were recorded.

The length of spawn run was measured during 25 to 30 days after the incubation, in every 3 days till the mycelia covered the whole bottle. The length of spawn run was converted to the growth rate of mycelia. The experiment ended on January 28th, 2009 when the substrate in all bottles was covered by mycelia. Statistical significance was evaluated using the Statistical Package for the Social Sciences (SPSS) 11.5 software, more specifically, the One-way Analysis of Variance (One-way ANOVA) and the Least Significant Difference (LSD) test.

Table 2. Main nutrients and heavy metal concentrations in SBC.

Parameter	Content						
	Nutrients	Protein (%) 1.32	OM (%) 31.25	HM(%) 19.48	Total AC (%) 0.40	Avail. P (mg/kg) 59	Avail. K (mg/kg) 601
Heavy metals	Cd (mg/kg) 0.26	Pb (mg/kg) 12.9	Hg (mg/kg) 0.82	As (mg/kg) 1.65	Cr (mg/kg) 8.03		

OM, Organic matter; HM, humic matter; AC, amino acid, P, phosphorus, K, potassium.

Table 3. Growth details of Shiitake Mushroom L931 on six different formulae.

Formulae	Average growth rate (cm/d)	Days for spawn run	Features of mycelia	Contamination rate (%)
1 (CK)	0.38	40	Grayish white, sturdy and thick	10
2	0.40*	36	Grayish white, sturdy and thick	0
3	0.41*	35	Grayish white, sturdy and thick	0
4	0.42*	33	Grayish white, sturdy and thick	0
5	0.39	36	Grayish white, sturdy and thick	0
6	0.38	38	Grayish white, sturdy and thick	0

The significance level was at $P = 0.05$.

RESULTS

Components of SBC

The determined results (see Table 2) showed that the SBC contains rich nutrients which can provide carbon and nitrogen nutrients for the mycelial growth of shiitake mushroom. Its high organic matter and humic matter content can stimulate mycelial growth. In addition, it contains rich available phosphorus and potassium necessary for mycelial growth. Furthermore, its heavy metal concentration is lower than the limitation standards of mushroom cultivation using raw materials.

Mycelial growth of different treatments

All samples started to germinate, colonize and grew 2 days after being inoculated. As can be seen in Table 3, all mycelia grown on substrates of different formulae showed the same characteristics of being grayish white, sturdy and thick. Their spawn run time is 33 to 40 days. The mycelial growth rate appeared to be different among formulae. According to the statistical analysis, there were significant differences in mycelial growth rate between Formula 1 (CK) and Formula 2, 3 and 4, but no significant differences between Formula 2, 3 and 4, nor between Formula 5 and 6. More precisely, the mycelial growth rate of Formula 1, 2, 3, 4, 5 and 6 were 0.38, 0.40, 0.41, 0.42, 0.39 and 0.38 cm/d, respectively. Besides, microbial contamination was spotted in the control group, the

incidence of which was up to 10%.

DISCUSSION

Safety of reusing SBC in shiitake mushroom production

The determined concentrations of heavy metals such as mercury, lead, cadmium, arsenic, chromium in SBC are much lower than the stipulated heavy metal limitation concentrations in China, EU and several other countries (see Table 4). Therefore, it is safe to conclude that the application of SBC in mushroom production will not cause any heavy metal contamination.

Effect of SBC as a substrate to substitute part of sawdust-wheat bran in traditional substrate formula on the mycelial growth

The experiment indicated that under the same incubation conditions, mycelia grew well on all sets of formulas, but grew faster in formulae with 5 to 20% of SBC than the other two, namely CK and Formula 6. Moreover, the mycelial growth rate saw an upward trend along with the increase of the proportion of SBC, which reached the peak of 0.42 cm/d in Formula 4 with 15% of SBC. Following that, the mycelial growth rate started to drop, down to 0.38 cm/d, the same growth rate of CK when the substitution rate reached 40% (in Formula 6). As a result,

Table 4. Required limitation concentrations of heavy metals (HM, mg/kg) for rubbish and sewage sludge in several countries or regions (Ministry of Environmental Protection of China, 1988, Matthews, 1996).

HM	China	EU	France	Italy	Holland	Belgium	Switzerland
Cd	3	20 to 40	2	20	1.25	1	5
Pb	100	750 to 1200	100	750	100	50	500
Hg	5	16 to 25	1	10	0.75	1	5
As	30	NA	NA	NA	15	NA	NA
Cr	300	1000 to 1500	150	NA	75	100	500

NA, Not applicable.

15% SBC in the substrate is the optimal substitution rate if productivity is the major concern. But in terms of waste reuse, more SBC consumed would deliver better environmental benefits. So substitution rates of 20 to 40% are as well worth to consider, depending on the amount of waste.

An ideal alternative to substitute traditional substrate for shiitake mushroom

The nutritional component analysis and the comparative spawn production experiment demonstrated that SBC contains abundant nutrients such as protein, organic substance, amino acid, phosphorus, potassium, and carbon and nitrogen source, which can be an essential supplement to traditional substrate in shiitake production. In addition, the pH value of SBC was 3.52, right dropping into the range of pH value (3 to 5) that is required by mycelia of shiitake mushrooms.

Substantial economic and environmental benefits of reusing SBC in growing shiitake mushroom

The present market price for sawdust and wheat bran is 800 and 1800 RMB/ton in China, whereas the cost of SBC, including pre-treatment and transport cost, is merely no more than 200 RMB/ton, implying considerable price discrepancy on the market. In addition, the comparative experiment indicated that treatments added with SBC experienced lower incidence of microbial contamination and needed less spawn incubation days, which suggests reduced uncertainty and less loss caused by spawn contamination. Besides, the above economic benefits, the technology we invented in this paper is also greatly beneficial to the environment. As illustrated earlier, large quantities of SBC are currently discharged without proper treatment in China, thus, reusing SBC in mushroom spawn production provides not only a way to "get rid of" them, but also a way to actually make benefits out of them.

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