ELSEVIER

Bioresource Technology 76 (2001) 229–233

BIORESOURCE TECHNOLOGY

Influence of substrate wood-chip particle size on shiitake (*Lentinula edodes*) yield

Daniel J. Royse [a,*], Jose E. Sanchez-Vazquez [b]

[a] *Mushroom Research Center, Department of Plant Pathology, 316 Buckhout Laboratory, Pennsylvania State University, University Park, PA 16802, USA*

[b] *El Colegio de la Frontera Sur. Apdo. Postal 36, Tapachula, Chiapas 30700, Mexico*

Received 1 May 2000; received in revised form 28 July 2000; accepted 31 July 2000

Abstract

Wood chips from four commercial hardwood sawmills were screened with 10 US standard sieves (4–0.21 mm) to assess particle size distributions. 96–98% of wood chips were <4 mm while 95–99% of particles were >0.21 mm. The majority (mean = 64.5%) of wood chips passed through US standard sieve size 14 (<1.4 mm). Shiitake (*Lentinula edodes*) was grown in three crops to determine the effect of four particle size classes (1 = 2.8–4 mm; 2 = 1.7–2.8 mm; 3 = 0.85–1.7 mm; 4 = < 0.85 mm) on mushroom yield. Yields from substrates prepared with wood chips from class 4 (<0.85 mm) were lower by 27.7%, 12.4% and 2% (mean = 14.9%) for Crops I, II, and III, respectively, when compared to controls. Profiling of wood chips may help growers optimize their production media and reduce production costs.

Keywords: *Lentinula edodes*; Shiitake; Wood chips; Mushrooms

1. Introduction

Production of shiitake [*Lentinula edodes* (Berk.) Pegler] worldwide increased more than 7-fold in the 14-year period from 1983 (207 000 t) to 1997 (1 573 000 t; Chang, 1999). Most of this increase occurred in China where more than 10 million part- and full-time farmers cultivate shiitake (Luo, 1998; Wang, 1998). Shiitake is widely consumed in China, yet one-third of production is exported (Zhang and Lai, 1993; Chiu et al., 1999). In 1997, China produced approximately 88% of the total world output (Chang, 1999).

In the US, production of shiitake is a relatively new enterprise, having begun only in the late 1970s. In 1990, the US produced 1123 t of shiitake (USDA, 1992) and by 1999, production reached 3941 t (a 3.5-fold increase; USDA, 1999). This increase in production is due, in part, to increased production efficiency and to increasing consumer demand. Farmers have learned to provide the specialized management this crop requires, thereby reducing production costs (Royse et al., 1990). The amount of controlled-environment production surface devoted to growing shiitake on synthetic logs has increased 2.9-fold from 1990 to 1999 (74 200 to 212 400 m^2, respectively).

Sawdust is the most popular basal ingredient used in synthetic formulations of substrate for producing shiitake in the US (Miller and Jong, 1987; Royse, 1997a), but other basal ingredients may include straw, corn cobs, or both. Starch-based supplements (20–60% dry weight) such as wheat bran, rice bran, millet, rye, and maize may be added to the mix. These supplements serve as nutrients to provide a more optimum growth medium (Royse, 1996, 1997b).

Most sawdust used for shiitake production originates from sawmills, where wood is cut by band saws to make lumber. In the last few years, band saw blades have generally decreased in thickness due to availability of harder and stronger steel used for blade manufacture. Thus, wood chips generated by band saws have become finer as blade thickness has decreased. Furthermore, the number of teeth per mm of band length may also affect wood-chip particle size (Montgomery, personal communication).

Shiitake growers generally believe that finer sawdust is more desirable for use in synthetic logs because finer chips may allow for the faster breakdown of

* Corresponding author. Tel.: +1-814-865-7322; fax: +1-814-863-7217.

E-mail addresses: djr4@psu.edu (D.J. Royse), esanchez@tap-ecosur.edu.mx (J.E. Sanchez-Vazquez).

0960-8524/01/$ - see front matter
PII: S0960-8524(00)00110-3

lignocellulosic particles by mycelium. This concept has not been tested, however, in controlled experiments using particle size ranges commonly found in present-day sawdust. The purpose of this work, therefore, was to characterize (US standard sieves) commercial sawdust piles for particle size ranges and to examine the effect of various particle sizes on mushroom yield.

2. Methods

2.1. Particle size distribution of commercial sawdust

Samples (400 g) were collected from four commercial sources of sawdust. Four random samples from each pile were collected at a site approximately 25 cm under the surface layer of each pile. Samples were pooled for each pile and then dried in an oven at 80°C for 48 h. Samples then were screened with 10 US standard sieves and the percentages of wood chips remaining between screens were determined.

2.2. Substrates and preparation

Six substrates (two controls and four sawdust particle size classes) were prepared from mixed hardwood sawdust collected from a commercial sawmill in Centre County, PA. The predominant species found in the sawdust was the Northern red oak (*Quercus rubra* L.). The sawdust was collected in the fall of 1998 and was stored in an enclosed building until it was used. The moisture content of the fresh sawdust was approximately 37% by weight. Four sawdust particle size ranges (2.8–4.0; 1.7–2.8; 0.85–1.7, and <0.85 mm) were obtained by sieving sawdust through the appropriate US standard sieves. The general substrate formulation (all ingredients based on oven dry substrate weight) consisted of 45% sawdust, 30% millet, 15% wheat bran and 10% rye.

Mixed substrate ingredients were pasteurized, cooled, inoculated and bagged with a 0.283 m^3 paddle mixer. Injecting live steam into the mixer and allowing the substrate to heat to 111°C pasteurized the substrate. This temperature was maintained for 20 min with continuous agitation to insure uniform substrate heating. After pasteurization, the substrate was rapidly cooled by passing cold tap water through a jacket fitted to the mixer. Sterility of the mixture was maintained by injecting filtered air into the mixer during cool-down to create a positive airflow. When the substrate had cooled to below 27°C, it was spawned with 210 g rye grain spawn contained in a 500 ml Erlenmeyer flask. When the spawn was thoroughly mixed with the substrate, the resulting mixture was bagged in unused polyethylene bags (20.3 cm $\times$ 12.7 cm $\times$ 50.8 cm) and closed with a twist-tie. The amount of substrate was weighed at time of filling with a digital scale placed under the bagging port of the mixer. Each bag contained 2.5 kg spawned substrate at 59% moisture (1.03 kg oven dry weight). Dry substrate weight was determined by drying 100 g of the processed substrates in an oven for 48 h at 80°C. Dry weight subsequently was used to determine the % biological efficiency (BE – ratio of fresh mushrooms harvested per dry substrate weight and expressed as a percentage).

2.3. Experimental design and statistical treatment

The experiments were a completely randomized design with 12–14 replicates per treatment. The general linear models procedure was used to perform an analysis of variance (SAS Institute, 1998). The Waller–Duncan k-ratio t-test was used to separate treatment means (Steel et al., 1997). The experiments were repeated three times and designated Crops I, II, and III.

2.4. Isolate and spawn

Isolate R26 was selected because it is a commercially used cultivar. The isolate was maintained on potato-dextrose yeast-extract agar as outlined previously by Jodon and Royse (1979). Spawn of R26 was prepared as outlined previously by Royse and Bahler (1986).

2.5. Spawn run, log browning and soaking

After a spawn run of 7 days, 20 slits (5 mm each) were made on the top of each bag with a sharp scalpel to provide gas exchange. At the end of 22 days incubation at 22 ± 1°C, the plastic bags were removed and the synthetic logs moved to a "browning room". In the browning room (93–98% relative humidity, 18 ± 1°C air temperature), the synthetic logs were hand-watered lightly with a 600-hole roseface nozzle each day. At the end of 14 days in the browning room, all logs were rotated 180° to provide a more uniform browning of the logs' surface. 3 h of light were provided daily by cool-white fluorescent bulbs. Sufficient air changes were maintained to hold CO_2 levels below 1900 ppm (1200 μl/l). At the end of 28 days in the browning room, the logs were soaked in cool water (13 ± 2°C) until each weighed approximately 2.3 kg. After each flush of mushrooms was harvested, logs were re-soaked to increase log weight to 2.3 kg.

2.6. Harvesting and determination of BE

Mushrooms were harvested from the substrates at the same time each day, when the veil had broken and the gills were fully exposed. The mushrooms were then counted and weighed. At the end of the harvest period (63 days), the accumulated data were used to calculate the BE. The substrate dry weights were used to calculate the percentage of BE ([weight of fresh mushrooms harvested/substrate dry matter content] × 100).

3. Results

Particle size distributions of four commercial sawdust sources are shown in Table 1. Considerable variation in wood chip size was found among the commercial sources. Approximately 96–98% of all sawdust particles were smaller than 4 mm, while 95–99% of particles were larger than 0.21 mm. The majority (mean = 64.5%) of the wood chips passed through a US standard sieve size 14 (<1.4 mm). This value ranged, however, from 50.4% for commercial source B to 79% for commercial source C.

Percentage distribution of four particle size classes for the four commercial sources is shown in Table 1. Three out of four sources contained the highest percentage distribution for sawdust class 3 (0.85–1.7 mm). However, these values ranged from 39% for source D to 51.4% for source A (mean = 44.4%). The next highest percentage distribution (mean = 34.5%) was for class 4 (<0.85 mm). Greater variation was observed for this class, where distributions ranged from 22.5% for source B to 50.5% for source C.

Yield for Crop I was significantly lower for a wood-chip particle size of <0.85 mm (Table 2). While mushroom yield was highest for the substrate containing a particle size distribution of 0.85–1.7 mm (1133 g/log), there was no significant difference in any of the other treatments and the control treatments (Table 2). BEs ranged from 79.6% for the <0.85 mm particle size to 110.5% for the 0.85–1.7 mm particle size.

Results for Crop II were similar to Crop I, where mushroom yields for the substrate containing particle sizes of <0.85 mm gave the lowest yield (Table 2). Yields for the control treatments and the treatment containing particles of 0.85–1.7 mm were highest but not significantly different from each other. Crop II had the highest overall yields of the three crops. BEs ranged from 107.2% for the <0.85 mm particle size to 121.8% for the 0.85–1.7 mm particle size.

Overall yields and BE for Crop III were the lowest of the three crops (Table 2). However, results were similar to Crops I and II where a substrate containing wood chips of <0.85 mm gave the lowest yields. A substrate containing wood chips of 2.8–4 mm gave the highest mushroom yield but yield was not significantly different than one of the control treatments (control <4.0 mm). BEs ranged from 74.6% for the <0.85 mm particle size to 93.7% for the 2.8–4.0 mm particle size.

Mean yields and BEs for all three crops are presented in Table 2. Mean BEs ranged from 87.1% for the treatment containing particles <0.85 mm to 107.4% for the treatment containing particle sizes of 0.85–1.7 mm. Means for the two control treatment BEs were 102% and 103.8%.

4. Discussion

The trend toward greater use of synthetic media compared to natural logs (USDA, 1999) to produce shiitake in the US has been attributed to greater yield potential and reduced time required to produce a crop on synthetic media (Royse and Bahler, 1986; Royse et al., 1990; Royse, 1996). Most synthetic media used commercially are composed of approximately 50% each of wood chips and nutrient supplements such as millet, wheat bran and rye. In the present study, we characterized particle size distribution of commercial sources

Table 1
Particle size distribution (class no., sieve no. and sieve size) for wood chips (sawdust) collected from four commercial sources used for production of shiitake

Class no.	Sieve no.	Sieve size (mm)	Wood chips remaining between screens (%)			
			Commercial source			
			A	B[a]	C	D
1	5	>4.00	1.2	1.2	1.1	3.7
	7	2.80	1.5	4.1	1.4	6.2
2	8	2.36	3.3	10.3	1.8	7.5
	10	2.00	2.4	5.2	1.7	4.5
	12	1.70	8.0	10.4	2.8	6.4
3	14	1.40	13.7	18.4	12.2	13.1
	16	1.18	14.8	10.5	10.1	10.3
	20	0.85	22.9	17.4	18.4	15.6
4	35	0.50	22.5	15.7	28.5	17.3
	70	0.21	8.4	5.8	17.6	11.2
	Pan	<0.21	1.3	1.0	4.4	4.2

[a] Wood chips from source B were used for all treatments as listed in Table 2.

Table 2
Yield (g/log), percentage BE and means for three crops of *L. edodes* grown on various wood chip sizes

Treat no.	Wood-chip particle size range (mm)	Crop I		Crop II		Crop III		Means (I, II, III)	
		BE	Yield	BE	Yield	BE	Yield	BE	Yield
1	2.8–4.0	103.2	1057 a[A]	115.2	1181 ab	93.7	1018 a	104.0	1085
2	1.7–2.8	109.4	1121 a	114.2	1171 ab	90.0	978 ab	104.5	1090
3	0.85–1.7	110.5	1133 a	121.8	1248 a	89.9	977 ab	107.4	1119
4	<0.85	79.6	816 b	107.2	1097 b	74.6	811 c	87.1	908
5	Control[B]	108.2	1109 a	121.3	1243 a	76.6	833 bc	102.0	1062
6	Control <4.0	107.3	1100 a	119.6	1226 a	84.4	917 abc	103.8	1081

[A] Means followed by the same letter in the same column are not significantly different according to the Waller–Duncan *k*-ratio *t*-test.
[B] Wood chips used directly from pile (no sieving).

of sawdust and examined the effect of particle size classes on mushroom yield.

Particle size distribution for commercial sawdust varied by as much as 300% within a sieve size. For example, US standard sieve size 70 retained 17.6% of particles from source C while only 5.8% was retained for source B. Thus, substantial variation is present in various commercial sources of sawdust. Considering the potential impact of fine particles in substrates, growers may want to consider profiling their sawdust at the source prior to purchase. Such profiling could help reduce production dips when sub-optimal sawdust is used for production.

Commercial mushroom growers generally believe that smaller particle size is beneficial to yield because of greater physical and enzymatic accessibility of the fungus. Nisikado et al. (1941) found that vegetative mycelial growth of shiitake was greater on large (2–3 mm)- and medium (1.5–2 mm)-sized particles than on small (<1.5 mm) particles. However, Nisikado et al. (1941) examined only vegetative growth, not mushroom yield. The rate of vegetative growth is not always a good predictor of mycelial biomass development. For example, Ohga (1990) demonstrated that the vegetative growth rate of shiitake mycelium was different at the surface compared to the interior of substrate prepared with various wood-chip particle sizes. As particle size decreased, the radial mycelial extension rate increased while mycelial biomass decreased. Ohga (1990) suggested that oxygen (O_2) depletion was the cause of reduced mycelial biomass development in substrates containing smaller particle size. However, none of the above-mentioned studies examined the effect of particle size on mushroom yield. Donoghue and Dennison (1995, 1996) demonstrated that O_2 and CO_2 levels in the airspace above the incubating substrate were correlated with subsequent mushroom yields. Thus, gas-exchange restriction in substrates prepared with smaller particle sizes (<0.85 mm) may be responsible for sub-optimum yields observed in this study. Small air spaces present in small particle size media may slow gas exchange from deep within the interior of the synthetic log to the surface.

This study demonstrated that substrates containing only wood chips of <0.85 mm are not optimum for production of shiitake. Further study is needed to determine the effect of blending known mixtures of various particle sizes on mushroom yield and BE. As shiitake production becomes more competitive and profit margins are squeezed, growers willing to optimize their production media may have an advantage in the marketplace. Ultimately, consumers will benefit from increased mushroom yields by having shiitake available at a lower price.

Acknowledgements

We wish to thank Vija Wilkinson for spawn making and Tom Rhodes, Doug Keith and Henry Shawley of the Mushroom Research Center for technical assistance.

References

Chang, S.T., 1999. Production of cultivated edible mushrooms in China with emphasis on *Lentinula edodes*. ISMS Newsletter 78 (4), 3–6.

Chiu, S.W., Wang, Z.M., Chiu, W.T., Lin, F.C., Moore, D., 1999. An integrated study of individualism in *Lentinula edodes* in nature and its implication for cultivation study. Mycol. Res. 103, 651–660.

Donoghue, J.D., Denison, W.C., 1995. Shiitake cultivation: gas phase during incubation influences productivity. Mycologia 87, 239–244.

Donoghue, J.D., Denison, W.C., 1996. Commercial production of shiitake (*Lentinula edodes*) using whole-log chip of *Quercus*, *Lithocarpus*, and *Acer*. In: Royse, D.J. (Ed.), Proceedings of the Second International Conference on Mushroom Biology and Mushroom Products, University Park, PA, 9–12 June, pp. 265–275.

Jodon, M.H., Royse, D.J., 1979. Care and handling of cultures of the cultivated mushroom. P. Agric. Exp. St. Bull. 258, 4.

Luo, X.C., 1998. Mushroom genetic resource, evaluation and utilization in China. In: Lu, M., Gao, K., Si, H.-F., Chen, M.-J. (Eds.), Proceedings of the 98 Nanjing International Symposium on Science and Cultivation of Mushrooms. JSTC-ISMS, Nanjing, China, pp. 142–152.

Miller, M.W., Jong, S.C., 1987. Commercial cultivation of shiitake in sawdust filled plastic bags. In: Wuest, P.J., Royse, D.J., Beelman, R.B. (Eds.), Developments in Crop Science, vol. 10, Cultivating Edible Fungi. Elsevier, Amsterdam, pp. 421–426.

Nisikado, Y., Kimura, K., Miyawaki, Y., 1941. Studies on the effect of kinds of tree in culture medium upon the growth of *Cortinellus berkeleyanus* I. The mycelial growth in pure culture on the sawdust medium prepared of various kinds of tree. Ber. Ohara Inst. Landwirtsch. Forsch. 9, 39–60.

Ohga, S., 1990. Growth rate of mycelium of shiitake, *Lentinus edodes*, in relation to water potential of medium. J. Fac. Agric, Kyushu Univ. 34, 413–420.

Royse, D.J., Bahler, B.D., 1986. Effects of genotype, spawn run time, and substrate formulation on biological efficiency of shiitake. Appl. Environ. Microbiol. 52, 1425–1427.

Royse, D.J., Bahler, B.D., Bahler, C.C., 1990. Enhanced yield of shiitake by saccharide amendment of the synthetic substrate. Appl. Environ. Microbiol. 56, 479–482.

Royse, D.J., 1996. Yield stimulation of shiitake by millet supplementation of wood chip substrate. In: Royse, D.J., (Ed.), Proceedings of the Second International Conference on Mushroom Biology and Mushroom Products, University Park, PA, 9–12 June, pp. 277–283.

Royse, D.J., 1997a. Cultivation of Shiitake on Synthetic and Natural Logs. College of Agricultural Sciences, Cooperative Extension, Pennsylvania State University, University Park, PA, USA, pp. 12.

Royse, D.J., 1997b. Specialty mushrooms and their cultivation. Hortic. Rev. 19, 59–97.

SAS Institute. 1998. SAS user's guide: statistics. SAS Institute Statistical Analysis System, Cary, NC.

Steel, R.G.D., Torrie, J.H., Dickey, D.A., 1997. Principles and Procedures of Statistics – a Biometrical Approach. McGraw-Hill, NY.

United States Department of Agriculture (USDA), 1992. Mushrooms. National Agricultural Statistics Service, Agricultural Statistics Board, Washington, DC.

United States Department of Agriculture (USDA), 1999. Mushrooms. National Agricultural Statistics Service, Agricultural Statistics Board, Washington, DC.

Wang, N.L., 1998. Present condition and prospects of edible mushroom industry in China. Edible Fungi of China 17 (5), 3 (in Chinese).

Zhang, S., Lai, M., 1993. The Culture and Cultivation History of the Chinese Shiitake. Shanghai Science and Technology Press, Shanghai (in Chinese).