J Wood Sci (2013) 59:88–93
DOI 10.1007/s10086-012-1293-3

NOTE

Effect of increased harvests on saccharification ratio of waste mushroom medium from the cultivation of shiitake mushroom (*Lentinula edodes*)

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Received: 17 May 2012 / Accepted: 29 August 2012 / Published online: 16 September 2012

Abstract Waste mushroom medium (WM) was saccharized with cellulase to obtain glucose after shiitake fruiting bodies were harvested 3 and 5 times (WM-3 and WM-5, respectively). Glucose can be used as a feedstock for the production of bioethanol or other bioproducts. WM-3 and WM-5 were analyzed for the amounts of shiitake fruiting bodies harvested and chemical components. The fresh weight ratio of shiitake fruiting bodies from the 4th and 5th harvests relative to the total fresh weight of shiitake fruiting bodies until the 5th harvest was 4.1 %. The additional 2 harvests decreased WM dry weight to 78.0 % and slightly decreased acid-insoluble lignin and xylan contents from 11.9 and 12.0 % to 10.0 and 9.6 %, respectively. The additional 2 harvests did not decrease glucan content. WM-5 included 31.6 % of glucan relative to dry weight, and 54.5 % of the glucan was saccharized to glucose with Meicelase (5 FPU/g substrate) at 40 °C for 48 h without pretreatment. The saccharification ratio of WM-3 was 45.0 % under the same saccharification condition. The amounts of saccharized glucose in WM-3 and WM-5 were 155.1 ± 9.8 mg/g substrate and 191.3 ± 9.2 mg/g substrate, respectively.

Keywords Waste mushroom medium · *Lentinula edodes* · Number of harvest times · Bioethanol · Enzymatic saccharification

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Introduction

Renewable biomass resources have become an important alternative to fossil resources, and various methods for the conversion of lignocellulosic biomasses into bioethanol or other bioproducts have been suggested [1–3]. Shiitake mushroom (*Lentinula edodes*) is one of the most commonly produced edible mushrooms in the world [4]. Waste mushroom medium (WM) from the cultivation of shiitake mushrooms is more suitable as a feedstock for enzymatic saccharification than other lignocellulosic biomasses because resource collection costs are low, resource supplies are seasonally stable, and enzymatic saccharification is easy [5]. Most WM is believed to be composted or discarded [6, 7]. When WM is composted or discarded, most of the cellulose within it degrades to CO_2. Obtaining glucose, a chemical precursor of bioethanol or other bioproducts, from WM can lead not only to the effective use of the resource but also to improved profits for mushroom farmers. Shiitake fruiting bodies usually are harvested from a mushroom medium multiple times [8, 9], and many cases of 3 and 5 harvests have been reported [8–11]. When WM is stored, the shiitake mycelia degrade acid-insoluble lignin and xylan in the lignocellulose of the medium and increase the cellulose saccharification ratio [5]. An increase in the saccharification ratio may be possible by adding harvests and extending the use of a mushroom medium. However, reports of the relationship between harvests and saccharification have been limited. The objective of this study was to survey whether saccharification ratios increased with increasing harvests for the advanced use of WM. WM from 3 and 5 harvests of shiitake fruiting bodies (WM-3 and WM-5, respectively) was saccharized with cellulase, and lignin and sugar contents and amounts of fruiting bodies harvested were investigated.

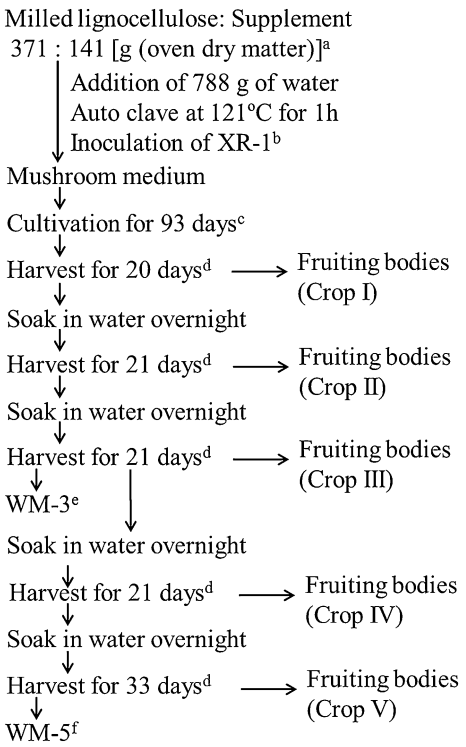

Fig. 1 Flow diagram for *Lentinula edodes* cultivation and production of fruiting bodies. [a]According to Hiyama et al. [5]. [b]Breed variety of shiitake mushroom; Mori, Gunma, Japan. [c]22 ± 1 °C, ≈70 % relative humidity (RH). [d]16 ± 1 °C, >85 % RH. [e]Waste mushroom medium after 3 harvests. [f]Waste mushroom medium after 5 harvests

Materials and methods

Materials

The design for the production of shiitake fruiting bodies, WM-3, and WM-5 is shown in Fig. 1. We produced 27 WM, 6 of which were randomly selected for WM-3 and 6 of which were randomly selected for WM-5. Fresh weights and numbers of fruiting bodies were measured in Crop I–V. After Crops III and V were harvested, WM-3 and WM-5 were roughly broken (<4 × 4 × 4 cm). The broken WM-3 and WM-5 were dried immediately at 50–60 °C for 1–3 days until moisture content was less than 10 %. The dried WM-3 and WM-5 were ground in a Wiley mill with 2-mm mesh for chemical component analysis and enzymatic saccharification. After milling, 3 samples were randomly selected from the 6 WM-3 substrates and 3 samples were randomly selected from the 6 WM-5 substrates for chemical component analysis and enzymatic saccharification.

Chemical component analysis

The chemical components of the substrates were analyzed according to our previous method [5], except for α-glucan (glucan that can be saccharized by α-amylase and glucoamylase). α-Glucan content was not measured because our previous study [5] indicates that the amounts were negligible.

Enzymatic saccharification

The enzymatic saccharification ratios of glucan, xylan, galactan, arabinan, and mannan (S_{glc}, S_{xyl}, S_{gal}, S_{ara}, and S_{man}, respectively) were examined with a commercial cellulase preparation (Meicelase; Meiji Seika, Tokyo, Japan) according to our previous method [5]. Meicelase (1.2 FPU) and sodium acetate buffer (12 ml, 0.1 M; pH 4.8) were added to a substrate (240 mg dry matter) in a tube at 5 FPU/g substrate. The tubes were subsequently incubated at 40 °C for 48 h on a shaker at 80 rpm. The amounts of saccharized glucose, xylose, galactose, arabinose, and mannose were analyzed using high-performance liquid chromatography, as described previously [5].

S_{glc}, S_{xyl}, S_{gal}, S_{ara}, and S_{man} were calculated using Eq. 1:

$$S(\%) = A/B \times 100 \quad (1)$$

where S is S_{glc}, S_{xyl}, S_{gal}, S_{ara}, or S_{man}; A is the amount of polysaccharide (glucan, xylan, galactan, arabinan, or mannan) hydrolyzed to monosaccharide (glucose, xylose, galactose, arabinose, or mannose) by Meicelase (mg); and B is the polysaccharide (glucan, xylan, galactan, arabinan, or mannan) content in a substrate (mg).

Statistical analysis

The homoscedasticity of total yield and the number of fruiting bodies in Crops I, II, and III of WM-3 and WM-5 were assessed using the F test. The difference between substrates with homoscedasticity that was assumable was examined using Student's t test. The differences in total yield and the number of fruiting bodies between Crops I–III and Crops I–V harvested from WM-5 were assessed using paired t test.

The homoscedasticity of chemical components and saccharification ratio of each polysaccharide between WM-3 and WM-5 was assessed using the F test. The difference between substrates with homoscedasticity that was assumable was examined using Student's t test. The difference between substrates with homoscedasticity that was not assumable was examined using Welch's t test.

The homoscedasticity of the amounts of monosaccharide obtained from WM-3, WM-5, and WM-5 calculated on the basis of the dry weight of WM-3 was assessed using Bartlett's test. The difference in the amount of monosaccharide with homoscedasticity that was assumable was examined using Dunnett's test, and the amount of monosaccharide obtained from WM-3 was assumed as a control.

Table 1 Fresh weight (g) and number of shiitake fruiting bodies harvested from WM-3 and WM-5

							Total	
	Substrate[a]	Crop I	Crop II	Crop III	Crop IV	Crop V	Crop I–III	Crop I–V
Yield of fruiting bodies (g/medium)[b]	WM-3	347.2 ± 11.6	111.3 ± 20.1	46.7 ± 45.5	–	–	505.2 ± 39.0	–
	WM-5	365.5 ± 11.3	80.8 ± 51.8	48.2 ± 53.7	0.0 ± 0.0	21.0 ± 32.8	494.4 ± 39.8	515.4 ± 49.0
Number of fruiting bodies	WM-3	30.0 ± 6.1	3.8 ± 2.6	1.2 ± 1.2	–	–	35.0 ± 8.3	–
	WM-5	37.7 ± 5.3	1.8 ± 1.3	1.2 ± 1.3	0.0 ± 0.0	0.5 ± 0.8	40.7 ± 5.0	41.2 ± 4.8

Values represent means of 6 repetitions ± SD

[a] WM-3 and WM-5, waste mushroom medium from 3 and 5 harvests, respectively

[b] Fresh weight of shiitake fruiting bodies per 1,300 g fresh weight of mushroom medium

The homoscedasticity of obtained glucose amount based on glucan except for α-glucan in mushroom medium between WM-3 and WM-5 was assessed using the F test. The difference between substrates with homoscedasticity that was assumable was examined using Student's t test.

$P < 0.05$ was considered significant.

Results and discussion

Production of fruiting bodies on sawdust-based medium

The yields and numbers of fruiting bodies harvested from WM-3 and WM-5 mushroom medium are shown in Table 1. The total yields of fruiting bodies from each 512 g (dry matter) of WM-3 and WM-5 mushroom medium were 505.2 and 515.4 g, respectively. No significant difference in total yields and total numbers of fruiting bodies in Crops I, II, and III were detected ($P < 0.05$, Student's t test) between WM-3 and WM-5. Therefore, an accurate random selection of WM-3 and WM-5 from the substrates was assumed. The total fresh weight and number of fruiting bodies in Crops IV and V of WM-5 were only 21.0 ± 32.8 and 0.5 ± 0.8 (mean ± SD), respectively. The ratio of the total fresh weight of fruiting bodies in Crops IV and V of WM-5 relative to that in Crops I–V of WM-5 was only 4.1 %. No significant difference was detected in the total yield and the total number of fruiting bodies between Crop I–III and Crop I–V harvested from WM-5 (paired t test). Little amounts of fruiting bodies were harvested in Crops IV and V under this cultivation condition. XR-1, the breed variety of the shiitake mushroom used in this study, is known to adapt to short-term cultivation, and shiitake fruiting bodies of XR-1 are usually harvested 3 times [8]. The low production of fruiting bodies in Crops IV and V likely are an attribute of XR-1.

Chemical component analysis

Moisture contents of WM-3 and WM-5 were 69.8 ± 3.5 and 58.9 ± 3.1 (mean ± SD, $n = 6$), respectively.

Lignin and sugar contents in each substrate and changes in dry weight are shown in Table 2. Acid-insoluble lignin and xylan contents were lower in WM-5 than in WM-3. No significant difference in glucan contents was detected between WM-3 and WM-5. These results indicate that shiitake mushrooms preferentially degrade acid-insoluble lignin and xylan over glucan. No significant difference in acid-soluble lignin, galactan, arabinan, and mannan contents was detected between WM-3 and WM-5. The addition of 2 harvests decreased dry weight to 78.0 % in WM-3.

Enzymatic saccharification

The amounts of monosaccharide obtained from WM-3 and WM-5 are shown in Table 3. The amounts of glucose, mannose, and total monosaccharides obtained from WM-5 were significantly more than those from WM-3. No significant difference was detected in the amounts of xylose, galactose, and arabinose between WM-3 and WM-5. The amounts of galactose, arabinose, and mannose were low because the contents of them in WM-3 and WM-5 were comparatively low. To compare the influence of dry weight loss of a substrate by the addition of 2 harvests with increase of the amount of monosaccharides, we calculated the amount of monosaccharide in WM-5 per the dry weight of WM-3. The amounts of xylose and arabinose obtained from calculated WM-5 were lower than those of WM-3. No significant difference in glucose, galactose, mannose, and total monosaccharides between WM-3 and calculated WM-5 was detected. The addition of 2 harvests increased the amount of glucose based on dry weight of each WM after final harvest. However, when the amount of glucose was calculated based on dry weight of WM after Crop III, the yield of glucose was not increased. The amounts of xylose and arabinose obtained from calculated WM-5 were lower than those of WM-3.

Before shiitake was inoculated, a mushroom medium whose dry weight was 512 g contained 145.7 g of glucan except for α-glucan [5]. Shiitake cultivation during 3 and 5

Table 2 Lignin and sugar contents in the substrates and changes in dry weight

Substrate[a]	Components[%(w/w)][b]								Changes in dry weight [%(w/w WM-3)]
	Acid-insoluble lignin	Acid-soluble lignin	Glucan	Xylan	Galactan	Arabinan	Mannan	Other components[c]	
WM-3									
m	11.9	6.1	31.0	12.0	1.6	2.4	3.3	31.8	100.0
sd	0.4	0.2	0.6	0.5	0.4	0.5	0.6	2.8	6.5
	*	ns	ns	**	ns	ns	ns		
WM-5									
m	10.0	6.0	31.6	9.6	1.2	2.1	3.5	35.9	78.0
sd	1.0	0.1	1.1	0.4	0.1	0.0	0.3	1.2	1.2

Differences in acid-insoluble lignin, acid-soluble lignin, glucan, xylan, galactan, and mannan contents between WM-3 and WM-5 were statistically analyzed using Student's *t* test

Difference in arabinan content between WM-3 and WM-5 was statistically analyzed using Welch's test

m mean value of 3 repetitions, *sd* standard deviation, *ns* no significant difference

Asterisks show significant difference (** $P < 0.01$, * $P < 0.05$)

[a] See Table 1

[b] Percentage of lignin and sugar weights based on dry weight of each substrate

[c] Dry weight minus the sum of acid-insoluble lignin, acid-soluble lignin, glucan, xylan, galactan, arabinan, and mannan

Table 3 Amounts of monosaccharide obtained from WM-3 and WM-5

Substrate[a]	Monosaccharide (mg/g substrate)					
	Glucose	Xylose	Galactose	Arabinose	Mannose	Total
WM-3	155.1 ± 9.8	27.5 ± 1.2	4.5 ± 0.6	2.9 ± 0.2	2.3 ± 0.5	192.2 ± 9.6
WM-5	191.3 ± 9.2**	28.1 ± 0.9ns	4.7 ± 0.4ns	3.0 ± 0.1ns	3.5 ± 0.4*	230.6 ± 9.5**
WM-5 calculated[b]	149.3 ± 9.4ns	22.0 ± 0.9**	3.7 ± 0.4ns	2.4 ± 0.1*	2.7 ± 0.3ns	180.0 ± 10.1ns

Values represent means of 3 repetitions ± SD

ns no significant difference

Asterisks show significant difference to Dunnett's test; amount of monosaccharide obtained from WM-3 was assumed as a control (** $P < 0.01$, * $P < 0.05$)

[a] See Table 1

[b] Amounts of monosaccharide obtained from each constituent polysaccharide in WM-5 calculated of the basis of the mean of the dry weight of WM-3

times harvest consumed 53.1 and 63.8 % of glucan except for α-glucan in mushroom medium, respectively. After cultivation and enzymatic saccharification, 234.9 ± 26.0 and 219.3 ± 13.7 (mean ± SD) mg glucose/g glucan except for α-glucan in mushroom medium before cultivation were obtained from WM-3 and WM-5, respectively. No significant difference in obtained glucose amount based on glucan except for α-glucan in mushroom medium between WM-3 and WM-5 was detected (Student's *t* test).

Under the condition of cultivation in this study, the increases in both the yield of shiitake fruiting bodies and sugar due to the addition of 2 harvests were not detected. Therefore, the addition of 2 harvests under this condition is economically inefficient because the addition of 2 harvests makes mushroom farmers need managerial resources; for example, the space of cultivation, manpower, and cost of air-condition.

Saccharification ratios of WM-3 and WM-5 are shown in Table 4. Around half of glucan in WM-3 and WM-5 were saccharized with comparatively [12–15] smaller Meicelase (5 FPU/g substrate) without pretreatment (only rough pulverization). The significant differences in S_{glc} and

Table 4 Saccharification ratios of WM-3 and WM-5

Substrates[a]	Saccharification ratio to each constituent polysaccharide (%)									
	Glucan		Xylan		Galactan		Arabinan		Mannan	
WM-3	45.0 ± 3.4	*	20.2 ± 0.6	*	26.0 ± 3.2	ns	10.7 ± 2.4	ns	6.6 ± 2.2	ns
WM-5	54.5 ± 2.9		25.8 ± 0.6		35.6 ± 5.8		12.5 ± 0.7		8.9 ± 1.0	

Values represent means of 3 repetitions ± SD

* Significant difference between WM-3 and WM-5 according to Student's t test ($P < 0.05$)

ns no significant difference

[a] See Table 1

S_{xyl} values between WM-3 and WM-5 were detected though the significant differences in S_{gal}, S_{ara}, and S_{man} values between WM-3 and WM-5 were not detected. However, S_{gal}, S_{ara}, and S_{man} values, especially S_{gal} values, of WM-5 appeared to be higher than those of WM-3. Because galactan, arabinan, and mannan contents were low (Table 2), the concentrations of galactose, arabinose, and mannose in saccharified solution were low. Thus, the experimental data varied widely. As a result, the no significant differences in S_{gal}, S_{ara}, and S_{man} values may be detected. S_{glc} and S_{xyl} values in WM-5 were about 1.2 and 1.3 times higher, respectively, than S_{glc} and S_{xyl} values in WM-3. The addition of 2 harvests increased S_{glc} and S_{xyl} values. Although S_{xyl} value in WM-5 was higher than that of WM-3, the amount of xylan obtained from WM-5 was at the same level as from WM-3 because of the lower content of xylan in WM-3.

It is known that removing xylan increase S_{glc} value [16, 17]. During the additional 2 harvests decreased the dry weight of each chemical component, the declining rates of acid-insoluble lignin and xylan were larger than that of glucan (Table 2). Shiitake mycelia disrupted the balance of cellulose–hemicellulose–lignin. It was indicated that the parts of rigid structure of cellulose–hemicellulose–lignin in lignocellulose of mushroom medium were damaged and parts of cellulose were exposed. The major reason of the improvement of S_{glc} value in this study was probably the increase of accessibility of cellulase to cellulose.

The improvement of S_{glc} value possibly increases glucose concentration in the enzymatic saccharification solution. In a sugar platform biorefinery, maintaining a high concentration of sugar in the solution is important. This is because, for example, during distillation of bioethanol, low sugar ant ethanol concentrations must be raised to desirable levels, which requires large quantities of energy [2, 18–20]. With regard to this point, WM-5 has more advantages than WM-3.

In this study, the yield of shiitake fruiting bodies in Crops IV and V was little under the cultivation condition; the breed variety, composition of mushroom medium, and cultivation method. However, under some cultivation conditions, the yields of shiitake fruiting bodies in Crops IV and V are not low [8, 11, 21]. Our next interest is to survey saccharification ratio of WM derived from the cultivation condition suitable for long-term cultivation.

References

1. Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M, Ladisch M (2005) Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour Technol 96: 673–686
2. Galbe M, Zachi G (2002) A review of the production of ethanol from softwood. Appl Microbiol Biotechnol 59:618–628
3. Amidon TE, Liu S (2009) Water-based woody biorefinery. Biotechnol Adv 27:542–550
4. Shang ST (1999) World production of cultivated edible and medicinal mushrooms in 1997 with emphasis on *Lentinula edodes* (Berk.) Sing. in China. Int J Med Mushroom 1:291–300
5. Hiyama R, Gisusi S, Harada A (2011) Evaluation of waste mushroom medium from cultivation of shiitake mushroom (*Lentinula edodes*) as feedstock of enzymic saccharification. J Wood Sci 57:429–435
6. Rinker DL (2005) Recycling spent shiitake substrate. http://www.alohamedicinals.com/book2/chapter-7-01.pdf. Accessed 9 March 2012
7. Inagaki H, Yamaguchi A (2009) Spent substrate of shiitake (*Lentinula edodes*) inhibits symptoms of anthracnose in cucumber. Mushroom Sci Biotechnol 17:113–115
8. Nozawa Y (2010) Plantsworld Co. Ltd (eds) 2010 nendoban Kinokonenkan (in Japanese). Plantsworld, Tokyo, pp 134–140
9. Royse DJ, Shen Q (2005) Shiitake bag cultivation http://www.alohamedicinals.com/book2/chapter-4-01-02.pdf. Accessed 12 March 2012
10. Royse DJ, Sanchez-Vazquez JE (2001) Influence of substrate wood-chip particle size on shiitake (*Lentinula edodes*) yield. Bioresour Technol 76:229–233
11. Uchiyama H, Kumada Y, Kumada A, Hasegawa T, Takehara T (2006) Shiitake kinshosaibai gijyutsu. Bull Fukushima Pref For Res Center 39:61–78 (in Japanese)
12. Asada C, Asakawa A, Sasaki C, Nakamura Y (2011) Characterization of the steam-exploded spent Shiitake mushroom medium and its efficient conversion to ethanol. Bioresour Technol 102: 10052–10056

13. Shimokawa T, Nakamura M, Nagasawa N, Tamada M, Ishihara M (2007) Effect of gamma-ray irradiation on enzymatic hydrolysis of spent corncob substrates from edible mushroom, enokitake (*Flammulina velutipes*) cultivation. Bull FFPRI 25(6):27–34
14. Shimoda T, Shirouchi T, Suzuki A, Morikawa Y, Nishibori K (2011) Storage of maitake mushroom (*Grifola frondosa*) culture medium after harvesting fruit bodies is an effective pretreatment for ethanol conversion. J Wood Sci 58:342–351
15. Irawati D, Yokota S, Niwa T, Takashima Y, Ueda C, Ishiguri F, Iizuka K, Yoshizawa N (2012) Enzymatic saccharification of spent wood-meal media made of 5 different tree species after cultivation of edible mushroom *Auricularia polytricha*. J Wood Sci 58:180–183
16. Öhgen K, Bura R, Saddler J, Zacchi G (2007) Effect of hemicellulose and lignin removal on enzymatic hydrolysis of steam pretreated corn stover. Bioresour Technol 98:2503–2510
17. Bura R, Chandra R, Saddler J (2009) Influence of xylan on the enzymatic hydrolysis of steam-pretreated corn stover and hybrid poplar. Biotechnol Prog 25:315–322
18. Balat M, Balat H, Öz C (2008) Progress in bioethanol processing. Prog Energy Combust 34:551–573
19. Wyman CE (2007) What is (and is not) vital to advancing cellulosic ethanol. Trends Biotechnol 24. http://www.cert.ucr.edu/research/ses/wymanpublications/What%20Is%20Vital%20to%20Advancing%20Cellulosic%20Ethanol.pdf. Accessed 4 April 2012
20. Komata K, Kikuchi S, Yoshii K (2012) Life cycle assessment of small-scale bioethanol production system using cultivated willows (in Japanese) https://www.jstage.jst.go.jp/article/ilcaj/2011/0/2011_0_28/_pdf. Accessed 31 July 2012
21. Ayuzawa S, Fujita H (2010) 2010 nendoban Kinokonenkan (in Japanese). Plantsworld, Tokyo, pp 141–147