agronomy

MDPI

Article

Evaluation of Using Spent Mushroom Sawdust Wastes for Cultivation of *Auricularia polytricha*

Chiu-Yeh Wu [1], Chih-Hung Liang [2] and Zeng-Chin Liang [3,*]

[1] Department of Culinary Arts, Chung Chou University of Science and Technology, Changhua 510, Taiwan; cywu4212@gmail.com
[2] Department of Food Science, Tunghai University, Taichung 407, Taiwan; liarng200@thu.edu.tw
[3] Department of Medicinal Botanicals and Health Applications, Da-Yeh University, Changhua 515, Taiwan
* Correspondence: zcliang@mail.dyu.edu.tw

Received: 29 October 2020; Accepted: 26 November 2020; Published: 29 November 2020

Abstract: The purpose of this study was to investigate the suitability of different spent mushroom sawdust wastes (SMSWs) and different proportions of SMSWs as potential substrates for the cultivation of *Auricularia polytricha* by evaluating yield and biological efficiency of the fruiting body. Nine SMSWs were respectively utilized as the main ingredient in the cultivation of *A. polytricha*. Then, spent *Pleurotus eryngii*, *Pleurotus cystidiosus*, and *Pleurotus ostreatus* sawdust wastes were screened among these nine SMSWs to be utilized as substrate and to determine the suitable proportion of SMSW in the cultivation of *A. polytricha* based on their yields and biological efficiencies. The highest yield and biological efficiency (total of two flushes) of *A. polytricha* cultivation on a single SMSW substrate was obtained with spent *P. eryngii* sawdust waste, followed by spent *P. cystidiosus* and *P. ostreatus* sawdust wastes. These three SMSWs were then applied in nine combination substrates, which were screened based on yield and biological efficiency for cultivation of *A. polytricha*. The combination substrate with the highest yield and biological efficiency of *A. polytricha* cultivation was *P. eryngii* (PES) + *P. cystidiosus* spent sawdust (PCYS) (235.4 g/bag yield and 58.85% biological efficiency); its yield was 1.62 folds higher than that of the control. From the results, we found that it was feasible to use spent sawdust wastes of *P. eryngii* and *P. cystidiosus* to replace sawdust for cultivation of *A. polytricha*.

Keywords: mushroom cultivation; spent sawdust waste; *Auricularia polytricha*; biological efficiency

1. Introduction

The rapid worldwide growth in mushroom production has resulted in about 13.6 million metric tons/y of spent sawdust wastes [1]. Similarly, over 140,000 metric tons of fruiting bodies are produced every year, which bring over 300,000 metric tons of spent sawdust wastes in Taiwan [2]. The large amount of waste is an environmental problem, and development of technologies for its treatment or reuse is underway. Although most of these spent sawdust wastes are used as organic fertilizer, some of sawdust wastes are left in the mushroom cultivation areas to be disposed of through decay.

In recent years, some spent sawdust wastes have been utilized as substrate for cultivation of mushrooms, such as mushroom sawdust wastes for cultivation of *Pleurotus citrinopileatus* [3]; *P. ostreatus* cultivated on *Hypsizigus marmoreus* spent mushroom substrate [4]; spent *Pleurotus* compost for production of *Agrocybe cylindracea* [5]; *Pleurotus*, *Ganoderma*, and *Lentinula* cultivated on spent oyster mushroom substrate [6]; the use of *Pleurotus ostreatus* and *Pleurotus sajor-caju* for the culture of *Agaricus blazei* [7]; spent *Flammulina velutipes* substrate as the main compost constituent for the cultivation of *Agaricus bisporus* [8]; and spent shiitake substrate for production of *Pleurotus sajor-caju* [9]. Spent mushroom sawdust wastes (SMSWs) could be a potential alternative substrate to cultivate other

mushrooms. However, it is unknown whether all spent sawdust wastes of different mushrooms are appropriate cultivation substrates for mushrooms.

Auricularia polytricha, also known as wood ear fungus, is a saprophytic fungus that is usually found on dead wood or on dead parts of living trees. It possesses many physiological effects, such as antioxidant activity [10], immunomodulatory activity [11], antitumor activity [12,13], antidementia properties [14], attenuation of inflammatory response, oxidative stress and lipid deposition [15], and antihypercholesterolemic effects [16]. Recently, strong consumer demand has stimulated increased production of *A. polytricha.* It is one of the five most cultivated edible mushrooms in Taiwan, and over 13,000 tons of fruiting bodies are produced each year [2]. Cultivation of this mushroom includes two important steps: (1) spawn preparation and (2) fruiting body production. Mycelia obtained from fruiting body by tissue culture were used to develop grain or sawdust spawns. The most commercial fruiting body production was acted by polypropylene bag method [17].

In order to decrease the environmental impacts resulting from mushroom production, effectively treat spent sawdust wastes, and determine suitable spent sawdust substrate for the cultivation of mushrooms, this study aimed to investigate the suitability of different SMSWs and different proportions of SMSWs as potential substrates for the cultivation of *A. polytricha* by evaluating yield and biological efficiency of the fruiting body.

2. Materials and Methods

2.1. Microorganism and Spawn Preparation

A strain of *A. polytricha* (obtained from Da-Nan Mushroom Farm, Puli, Nantou, Taiwan) was grown on potato dextrose agar (PDA; 200 g/L of diced potatoes, 20 g/L of glucose, and 15 g/L of agar) medium at 25 °C for regular subculture, and was maintained on PDA slants at 4 °C for a maximum of 3 months. Sawdust spawn was prepared according to our previous study [18]; 850 mL polypropylene plastic bottles were filled with 250 g of dry sawdust and supplemented with 10% rice bran and 1% calcium carbonate (*w*/*w*, in terms of dry weight). The water content of the mixture was adjusted to approximately 60% and then sterilized at 121 °C for 60 min. After cooling to 25 °C, the sterilized sawdust mixture in each bottle was inoculated with 9 cm^2 mycelial agar discs. The spawn was incubated at 25 °C until the substrate fully was colonized.

2.2. Spent Mushroom Sawdust Wastes Collection

Nine SMSWs were collected. *Agrocybe aegerita*, *Hypsizygus marmoreus*, *P. citrinopileatus*, and *P. ostreatus* spent sawdust wastes were obtained from Dong-Chin Farm (Yuchih, Nantou, Taiwan). *A. polytricha*, *Hericium erinaceus*, and *P. sajor-caju* spent sawdust wastes were obtained from Hsing-Tai Biotechnology Co., Ltd. (Yuanlin, Changhua, Taiwan). *Pleurotus eryngii* spent sawdust waste and *Pleurotus cystidiosus* spent sawdust waste were obtained from Q-Yo Biotechnology Farm (Puxin, Changhua, Taiwan) and a local private mushroom farm (Dacun, Changhua, Taiwan), respectively. All SMSWs were kept at 25 °C and then dried in the sun before substrate preparation.

2.3. Substrate Preparation and Analysis of the C/N Ratio

The first experiment screened for suitable spent sawdust wastes for cultivation of *A. polytricha* according to the yield and biological efficiency on the different substrates based on a single SMSW. The control substrate formulation (all ingredients based on dry substrate weight, *w*/*w*) consisted of 90% sawdust, 9.5% rice bran, and 0.5% calcium carbonate. Sawdust was replaced in the nine tested substrates with the same proportion of different SMSWs. The symbols and C/N ratios of substrate formulas are shown in Table 1.

Table 1. Single spent mushroom sawdust used as substrate and its mixture ratio and analysis.

Symbol of Single Spent Mushroom Sawdust Substrate [1]	Carbon (%)	Nitrogen (%)	C/N Ratio
Control [2]	47.78 ± 0.31	0.99 ± 0.03	48.26 ± 0.25
AAS	45.31 ± 1.25	0.92 ± 0.25	49.25 ± 1.00
APS	44.35 ± 2.27	1.35 ± 0.15	32.85 ± 1.66
HES	43.80 ± 2.21	1.10 ± 0.22	39.82 ± 2.05
LSS	42.03 ± 1.77	1.48 ± 0.17	28.40 ± 1.14
PCIS	45.60 ± 2.35	1.07 ± 0.15	42.62 ± 1.84
PCYS	46.22 ± 1.24	1.09 ± 0.16	42.40 ± 1.06
PES	45.83 ± 2.75	1.57 ± 0.14	29.19 ± 2.13
POS	47.77 ± 1.38	1.38 ± 0.12	34.62 ± 1.02
PSCS	41.38 ± 2.55	1.46 ± 0.19	28.34 ± 1.72

[1] All substrates also contained 9.5% rice bran and 0.5% calcium carbonate. [2] Control: sawdust; AAS: *Agrocybe aegerita* spent sawdust; APS: *Auricularia polytricha* spent sawdust; HES: *Hericium erinaceus* spent sawdust; LSS: *Lyophyllum shimeji* spent sawdust; PCIS: *Pleurotus citrinopileatus* spent sawdust; PCYS: *Pleurotus cystidiosus* spent sawdust; PES: *Pleurotus eryngii* spent sawdust; POS: *Pleurotus ostreatus* spent sawdust; PSCS: *Pleurotus sajor-caju* spent sawdust.

Table 2 is the second experiment tested for suitable ratios of SMSWs for cultivation of *A. polytricha*; various ratios (1:1, 1:2, and 2:1) of pairs of SMSWs were tested as substrates according to the three spent sawdust waste substrates with higher biological efficiencies (*P. eryngii*, *P. ostreatus*, and *P. cystidiosus*) in the first experiment.

Table 2. Mycelial growth of *Auricularia polytricha* on single spent mushroom sawdust substrates.

Substrate [1]	Mycelial Growth Rate (mm/d)	Total Colonization Time (d)	Time to First Primordia (d)
Control	3.72 ± 0.12c [2]	32.3 ± 2.4bc	36.2 ± 2.6bc
AAS	2.47 ± 0.08f	48.5 ± 3.5a	- [3]
APS	3.61 ± 0.14d	33.2 ± 2.9b	-
HES	3.43 ± 0.21e	35.0 ± 1.8b	40.1 ± 2.5a
LSS	3.62 ± 0.32d	33.1 ± 2.3b	37.2 ± 1.6b
PCIS	3.53 ± 0.27d	34.0 ± 2.8b	38.6 ± 2.8b
PCYS	3.98 ± 0.36b	30.2 ± 1.9c	34.3 ± 2.0c
PES	3.59 ± 0.24d	33.4 ± 1.4b	37.5 ± 2.7b
POS	4.41 ± 0.35ab	27.2 ± 1.6d	31.2 ± 2.7d
PSCS	4.79 ± 0.25a	25.1 ± 1.2e	30.5 ± 1.7d

[1] All substrates also contained 9.5% rice bran and 0.5% calcium carbonate. [2] Data were analyzed using Duncan's multiple range test. Each value is expressed as the mean ± SD ($n = 6$). Means within a column followed by the same letter are not statistically significantly different ($p < 0.05$). [3] No primordia.

The water content of the substrate was adjusted to about 65% (*w*/*w*). Then, each polyethylene bag was filled with 1 kg of substrate and sterilized at 121 °C for 120 min. After the substrates were cooled to 25 °C, they were inoculated with 5 g of sawdust spawn per bag.

For substrate analysis, samples were dried at 60 °C to a constant weight, and then ground into a coarse powder (8 openings per centimeter) using a mill. Carbon content was determined according to the report of Nelson and Sommers [19], and nitrogen content was determined using the Kjeldahl method [20]. Then, the C/N ratio of each substrate was calculated.

2.4. Inoculation, Incubation, and Harvest

The procedures of incubation and harvest for *A. polytricha* were the same as those in our previous study [18]. The inoculated substrates were kept in a spawn running room at 25 °C and 70% relative humidity under dark conditions. When the substrate was about to fully colonize by the mycelia, the height between the top substrate and bottom substrate (i.e., the height of mycelia) was calculated. The mycelial growth rate was determined as the height of mycelia in the colonized culture bag divided by the incubation time (d). After the surfaces of the substrates were entirely covered with mycelia, the bags were cut at about 5 cm of line to allow the development of fruiting bodies under a relative

humidity of 90% or greater. The fruiting bodies in each polyethylene bag were harvested when their margin showed a wave-like pattern. The harvested fruiting bodies were then counted and weighed. For all substrates, two flushes of fruiting bodies were harvested from each of the bags. For each flush, the harvested fruiting bodies were weighed. At the end of the harvest period, the accumulated data were used to calculate biological efficiency. Biological efficiency was the ratio of the weight of the fresh fruiting body (g) to the dry weight of substrate (g), which was expressed as a percentage. Thirty replicate polyethylene bags were used for each medium formula, and six bags were randomly sampled during sampling.

2.5. Statistical Analysis

Each data value was presented as the mean ± standard deviation. Differences between the means of individual groups were assessed using a one-way ANOVA with Duncan's multiple range test at a 95% confidence level.

3. Results

3.1. Substrate Analysis in the Screening for Suitable Spent Mushroom Sawdust Waste

The substrates in the first experiment to screen for suitable spent sawdust waste used single SMSW as the main ingredient. Carbon and nitrogen contents and the C/N ratio varied considerably among substrates (Table 1). The carbon content of single spent mushroom sawdust substrate ranged from 41.38 to 47.77, which was generally lower than that of the control, whereas the nitrogen content of the same substrate ranged from 0.92 to 1.57, which was generally higher than that of the control, except for sawdust (AAS). Therefore, the C/N ratios ranged from 28.34 to 49.25 and were lower than that of the control, except for AAS.

3.2. Mycelial Growth in the Screening for Suitable Spent Mushroom Sawdust Mastes

The mycelial growth rate, total colonization time, and time to first primordia of *A. polytricha* cultivated on different single spent mushroom sawdust substrates are shown in Table 2. Substrates *Pleurotus sajor-caju* spent sawdust (PSCS), *Pleurotus ostreatus* spent sawdust (POS), and *Pleurotus cystidiosus* spent sawdust (PCYS) showed a faster ($p < 0.05$) mycelial growth rate compared to that of the control and other substrates. The mycelia of *A. polytricha* completely colonized the substrates and were consistent with the mycelial growth rate, which corresponded with total colonization time and was within a period of 48.5 days of spawn run. The shortest period of mushroom colonization of the PSCS substrate was 25.1 days. The time to first primordia formation for the substrates was clearly different. The primordia started appearing for all the substrates after the spawn fully colonized the substrate. The shortest periods of primordia formation for PSCS and POS were 30.5 and 31.2 days, respectively, which were not significantly different ($p < 0.05$), whereas the longest period for *Auricularia polytricha* spent sawdust (HES) was 40.1 days. Meanwhile, the AAS and *Agrocybe aegerita* spent sawdust (APS) substrates showed no primordia formation.

3.3. Yield and Biological Efficiency in the Screening for Suitable Spent Mushroom Sawdust Wastes

The yields and biological efficiencies of mushrooms in different flushes and total biological efficiencies for spent mushroom sawdust substrates are shown in Figure 1. There were two flushes in the cultivation period. This indicated that the yield and biological efficiency of the second flush was clearly higher than that of the first flush for each substrate. In the first flush, the highest yield was obtained with *Pleurotus eryngii* spent sawdust (PES), which had 26.40% biological efficiency, followed by PCYS (22.63%) and POS (18.80%). In the second flush, the highest yield was also obtained with PES, with 28.58% biological efficiency, followed by PCYS (26.98%) and POS (21.38%). The total biological efficiencies of PES (54.98%), PCYS (49.61%), and POS (40.18%) were higher than that of the control

(34.51%) ($p < 0.05$). These spent mushroom sawdust substrates were the three most suitable substrates, and were selected to test for suitable ratios of pairs of SMSWs for *A. polytricha* cultivation.

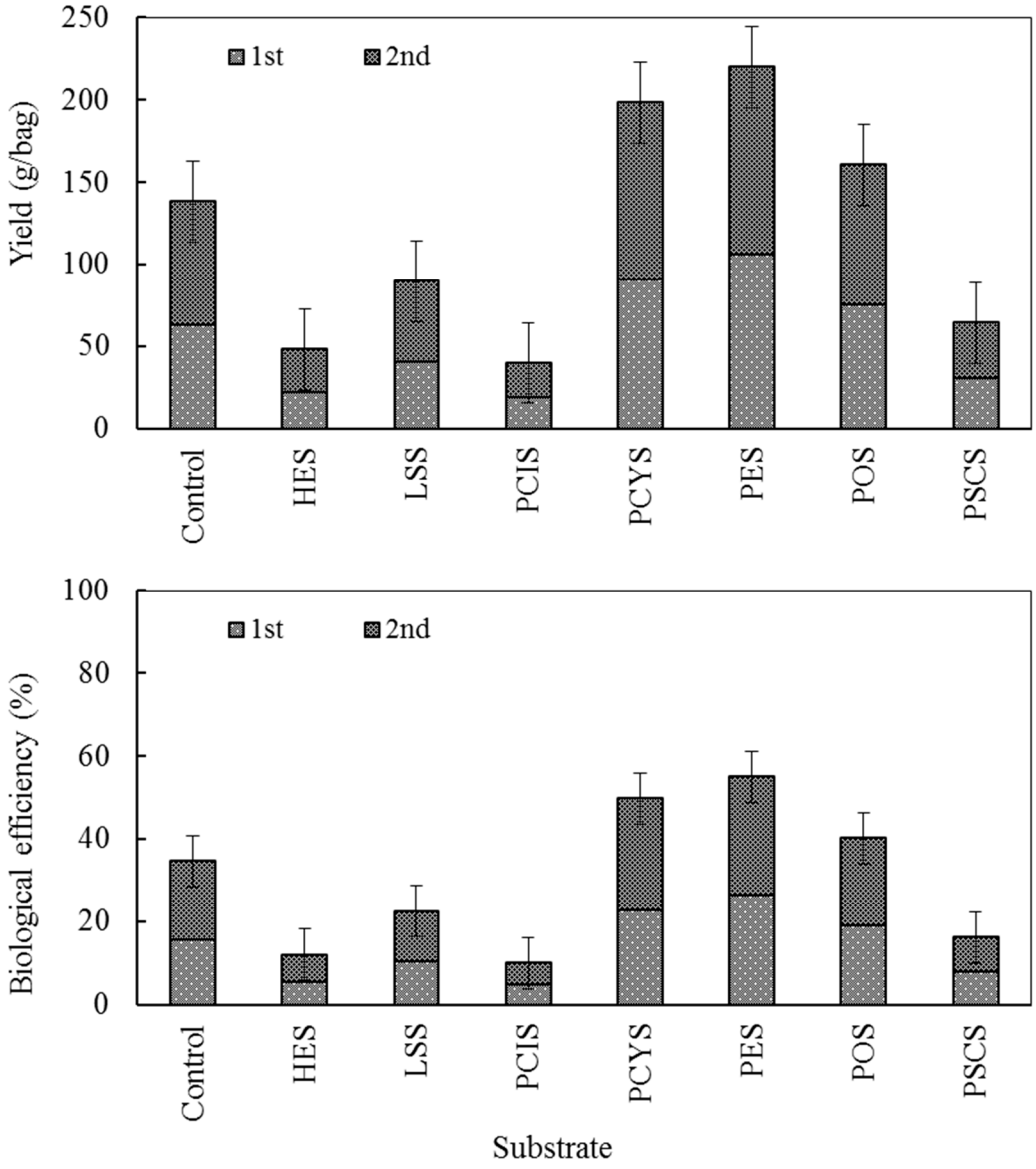

Figure 1. Yields and biological efficiencies of the first and second flush of *Auricularia polytricha* grown on single spent mushroom sawdust substrates.

3.4. Substrate Analysis in the Test for Suitable Ratios of Pairs of Spent Mushroom Sawdust Wastes

Carbon, nitrogen, and the C/N ratio of the substrates with different proportions of SMSWs are shown in Table 3. The carbon content of the substrates with different proportions of SMSWs ranged from 46.08 to 47.86, which was generally lower than that of the control, whereas the nitrogen content of the same substrate ranged from 1.25 to 1.46, which was generally higher than that of the control. Therefore, the C/N ratios were lower than that of the control. The C/N ratios ranged from 32.24 to 37.42 and were lower than that of the control.

Table 3. Carbon, nitrogen, and C/N ratio of *Auricularia polytricha* cultivated on substrates with different proportions of spent mushroom sawdust.

Substrate and Mixture Ratio by Weight [1]	Symbol	Carbon (%)	Nitrogen (%)	C/N Ratio
Control [2]	Control	48.80 ± 1.25	1.01 ± 0.05	48.32 ± 0.98
PES:POS = 1:1	PES + POS	46.75 ± 0.95	1.45 ± 0.13	32.24 ± 0.52
PES:POS = 1:2	PES + 2POS	47.86 ± 1.24	1.46 ± 0.24	32.78 ± 0.64
PES:POS = 2:1	2PES + POS	47.02 ± 1.36	1.45 ± 0.31	32.43 ± 1.05
PES:PCYS = 1:1	PES + PCYS	46.08 ± 1.54	1.35 ± 0.25	34.13 ± 1.09
PES:PCYS = 1:2	PES + 2PCYS	46.40 ± 1.34	1.32 ± 0.34	35.15 ± 0.94
PES:PCYS = 2:1	2PES + PCYS	46.60 ± 2.05	1.31 ± 0.15	35.57 ± 1.26
PCYS:POS = 1:1	PCYS + POS	47.52 ± 1.63	1.27 ± 0.07	37.42 ± 1.15
PCYS:POS = 1:2	PCYS + 2POS	46.78 ± 1.34	1.25 ± 0.06	37.42 ± 0.95
PCYS:POS = 2:1	2PCYS + POS	47.22 ± 1.87	1.28 ± 0.09	36.89 ± 1.35

[1] All substrates also contained 9.5% rice bran and 0.5% calcium carbonate. [2] Contained 90% sawdust.

3.5. Mycelial Growth in the Test for Suitable Ratios of Pairs of Spent Mushroom Sawdust Wastes

The mycelial growth rate, total colonization time, and time to first primordia of *A. polytricha cultivated on* the substrates with different proportions of SMSWs are shown in Table 4. The most suitable substrate for mycelial growth was 2PES + POS, followed by 2PES + PCYS and PES + PCYS; their mycelial growth rates were higher than that of the control ($p < 0.05$). The mycelia of *A. polytricha* completely colonized the substrates and were consistent with the mycelial growth rate and time to first primordia. In this experiment, the mycelial growth rate of PCYS + 2POS was slower than that of the control; however, they were not significantly different ($p < 0.05$). The shortest period of primordia formation was obtained with 2PES + POS, followed by 2PES + PCYS and PES + PCYS. The times to first primordia of the latter two substrates were not significantly different ($p < 0.05$).

Table 4. Mycelial growth of *Auricularia polytricha* on substrates with different proportions of spent mushroom sawdust.

Substrate [1]	Mycelial Growth Rate (mm/d)	Total Colonization Time (d)	Time to First Primordia (d)
Control [2]	3.26 ± 0.51d [3]	36.8 ± 4.7a	37.6 ± 2.5a
PES + POS	4.04 ± 0.92cd	29.7 ± 4.7b	32.5 ± 1.5b
PES + 2POS	4.24 ± 1.10c	28.3 ± 5.3b	30.4 ± 1.6b
2PES + POS	6.13 ± 0.45a	19.6 ± 1.4d	22.3 ± 1.1d
PES + PCYS	5.09 ± 0.73bc	23.6 ± 2.3c	25.6 ± 1.4c
PES + 2PCYS	4.35 ± 0.96c	27.6 ± 3.1b	29.5 ± 2.1bc
2PES + PCYS	5.31 ± 0.48b	22.6 ± 2.4c	25.1 ± 1.4c
PCYS + POS	3.61 ± 0.22d	33.2 ± 2.5ab	36.0 ± 2.4ab
PCYS + 2POS	3.21 ± 0.52d	37.4 ± 3.6a	38.1 ± 2.6a
2PCYS + POS	3.44 ± 1.15d	34.9 ± 3.8ab	37.2 ± 2.8a

[1] All substrates also contained 9.5% rice bran and 0.5% calcium carbonate. [2] Contained 90% sawdust. [3] Data were analyzed using Duncan's multiple range test. Each value is expressed as the mean ± SD ($n = 6$). Means within a column followed by the same letter are not statistically significantly different ($p < 0.05$).

3.6. Yield and Biological Efficiency in the Test for Suitable Ratios of Pairs of Spent Mushroom Sawdust Wastes

The yields and biological efficiencies of mushrooms in the two flushes for the substrates with different proportions of SMSWs are shown in Figure 2. This indicated that the yield and biological efficiency of the second flush was higher than that of the first flush for every substrate. In the first flush, the highest yield was obtained with PES + PCYS with 27.55% biological efficiency, followed by PES + 2PCYS (26.38%) and 2PES + PCYS (26.33%); however, they were not significantly different ($p < 0.05$). In the second flush, the highest yield was also obtained with PES + PCYS with 31.30% biological efficiency, followed by PES + 2PCYS (29.68%) and 2PES + PCYS (28.25%); however, they were also not significantly different ($p < 0.05$). The most suitable substrate for high biological efficiency was PES

+ PCYS (58.85%), followed by PES + 2PCYS (56.05%) and 2PES + PCYS (54.58%), which had higher biological efficiencies than the control (36.40%).

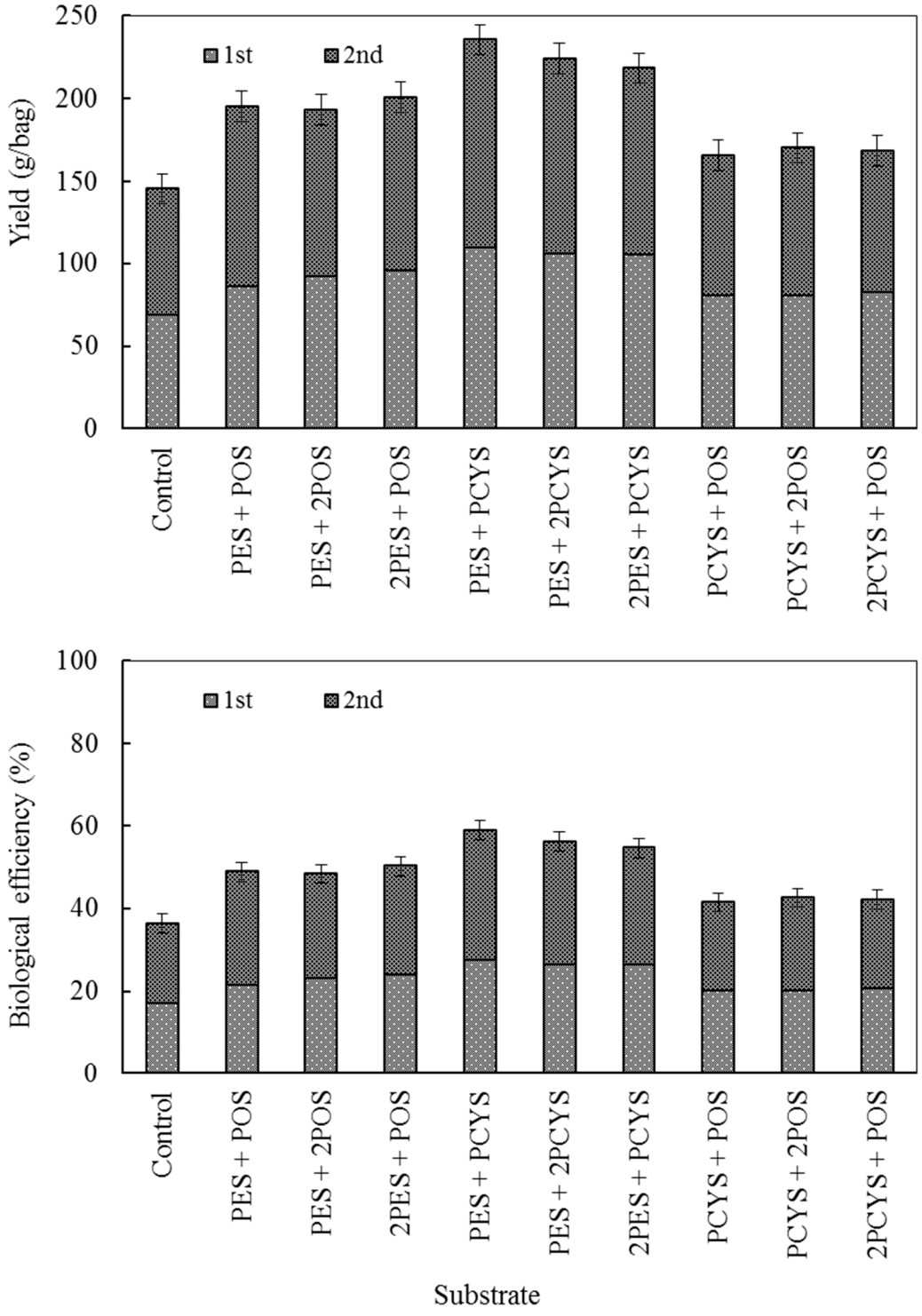

Figure 2. Yields and biological efficiencies of the first and second flush of *Auricularia polytricha* cultivated on substrates with different proportions of spent mushroom sawdust.

4. Discussion

In this study, among the nine single spent mushroom sawdust substrates for cultivation of *A. polytricha*, PSCS provided the fastest mycelial growth, shortest total colonization time, and shortest

time to first primordia, whereas AAS provided the slowest mycelial growth and longest total colonization time. Mycelia of *A. polytricha* grew on AAS and APS, but these substrates showed no primordia formation or fruiting body yield. This indicated that most of the spent mushroom sawdust of *Pleurotus* mushrooms supported the mycelial growth of other mushrooms. This result was similar to results of previous studies on the suitability of spent *P. eryngii* sawdust as a substrate for mycelial growth of *Agrocybe chaxingu* [21] and spent *P. ostreatus* sawdust as a substrate for mycelial growth of *Ganoderma resinaceum* [6].

In the use of single spent mushroom sawdust substrate for cultivation of *A. polytricha*, PSCS, POS, and PCYS showed faster mycelial growth rates compared to that of the control, which corresponded with the C/N ratio between 28.34 and 42.40. The C/N ratios of APS, HES, LSS, and PES were also in this range; however, their mycelial growth rates were slower than that of the control. This did not correspond with the viewpoint that higher C/N ratios favor mycelial growth for mushrooms [22,23]. The higher yields and biological efficiencies of single spent mushroom sawdust substrates were in the order of PES, PCYS, and POS, which corresponded with the C/N ratios between 29.19 and 42.40. This also did not correspond with the viewpoint that lower C/N ratios favor the growth of fruiting bodies [23].

In the test for suitable ratios of pairs of SMSWs, all the C/N ratios of experimental substrates were between 32.24 and 37.42, which were clearly lower than that of the control (48.32). The faster mycelial growth rate, total colonization time, and time to first primordia of substrates were in the order of 2PES + POS, 2PES + PCYS, and PES + PCYS; they were also clearly faster than those of single spent mushroom sawdust substrates, such as PES, PCYS, and POS. The C/N ratios of the former three substrates were concentrated between 32 and 38; however, those of the latter three substrates were distributed over 28–43. The mycelial growth of *A. polytricha* cultivated on spent mushroom sawdust substrate might not need a specific C/N ratio. In previous studies, we demonstrated that the hot water extract from spent *P. citrinopileatus* sawdust could enhance the mycelial growth of some mushrooms, such as *Antrodia camphorata*, *H. erinaceus*, *Lepista sordida*, *Phellinus linteus*, *P. citrinopileatus*, *P. eryngii*, and *P. ferulae* [24], and that the hot water extract from spent *P. eryngii* sawdust could enhance the mycelial growth of *Ganoderma lucidum*, *Hypsizigus marmoreus*, *Lentinula edodes*, *P. linteus*, and *P. sajor-caju* [25]. Mycelium growth and fruiting body production of *Pleurotus* spp. are affected by cellulose/lignin and carbon/nitrogen ratios [26]. SMSW does not produce good yield when it is used again in mushroom production as a substrate, due to subsequent utilization of nutrients by mycelium resulted in depletion of nutrients [27]. Compared with uncultivated sawdust, most SMSWs (Table 1) with reduced carbon content but increased nitrogen content can still grow other mushrooms and increase the yield. In this study, the mycelial growth and yield of fungus did not show a high correlation with a specific carbon/nitrogen ratio. It is likely that spent sawdust of some mushrooms have promotive substances for mycelial growth of other mushrooms. In addition, the characteristics of hot water extracts from spent mushroom sawdust should be further studied.

In the tests using single spent mushroom sawdust substrates and in the tests to determine the suitable ratios of pairs of SMSWs for cultivation of *A. polytricha*, the fastest mycelial growth rate did not correspond with the highest yield and biological efficiency. These results were similar to those of our previous studies on the cultivation of *P. citrinopileatus* [28], *P. pulmonarius* [29], and *A. polytricha* using grass plants to partially replace sawdust [18], and on the cultivation of *A. polytricha* using agrowastes to partially replace sawdust [30]. These results were also consistent with those of other reports, such as for *P. ostreatus* cultivated on different lignocellulosic by-products [31], *P. ostreatus* cultivated on different proportions of agrowastes [23], and *Agrocybe cylindracea* cultivated on spent *Pleurotus* compost [5]. Additionally, the yields and biological efficiencies of the second flush were almost higher than those of the first flush in the same two tests. These results were also similar to those in our previous report on the use of grass plants for cultivation of *P. citrinopileatus* [28]. However, some reports were not consistent with these results, such as the results of *A. cylindracea* and *P. ostreatus* cultivated on nine

agro-industrial and forestry by-products [32], *P. ostreatus* cultivated on spent *H. marmoreus* substrate [4], and *A. polytricha* cultivated on different proportions of agrowastes [30].

In the test to determine the suitable ratios of pairs of SMSWs for cultivation of *A. polytricha*, mycelial growth rates on the substrates that contained spent *P. eryngii* sawdust waste were faster than those of the control and of the substrates containing spent *P. cystidiosus* and *P. ostreatus* sawdust wastes. The same results were also shown for the mushroom yield and biological efficiency; the substrates that contained spent *P. eryngii* sawdust waste generally had higher yields and biological efficiencies than other substrates without spent *P. eryngii* sawdust waste. These results were in good agreement with those of a previous study, in which the hot water extract of spent *P. eryngii* sawdust waste containing promotive substances enhanced mycelial growth [25].

According to the results, spent *P. eryngii* sawdust waste was most effective substrate among the nine SMSWs; its yield was 1.59 folds higher than that of the control for cultivation of *A. polytricha*. The PES + PCYS substrate was the most suitable substrate among different proportions of SMSWs; its yield was 1.62 and 1.07 folds higher than those of the control and spent *P. eryngii* sawdust substrate for cultivation of *A. polytricha*, respectively.

5. Conclusions

In this study, nine SMSWs were utilized as the main ingredient in the cultivation of *A. polytricha*, and spent *P. eryngii* sawdust substrate showed the highest yield and biological efficiency. In the next experiment, spent *P. eryngii*, *P. cystidiosus*, and *P. ostreatus* sawdust wastes were utilized to determine the suitable proportion of SMSWs for the cultivation of *A. polytricha*, and the PES + PCYS substrate showed the highest yield and biological efficiency. The results indicated that spent *P. eryngii* and *P. cystidiosus* (1:1) mushroom sawdust was a suitable alternative substrate to cultivate *A. polytricha*. From these results, we concluded that spent *P. eryngii* and *P. cystidiosus* sawdust wastes can be used as the materials for *A. polytricha* cultivation.

Author Contributions: C.-Y.W. and C.-H.L. carried out the analytical experiments and discussed the results. Z.-C.L. conducted experiments, performed data collection and wrote the original draft. All authors contributed to discussion and writing of the paper. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Agriculture and Food Agency, Council of Agriculture, Taiwan grant number 101AS-9.2.2-FD-Z2.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Lin, Y.; Ge, X.; Li, Y. Solid state anaerobic co-digestion of spent mushroom substrate with yard trimmings and wheat straw for biogas production. *Bioresour. Technol.* **2014**, *169*, 468–474. [CrossRef] [PubMed]
2. Chen, T.M.; Lue, Y.S.; Shih, H.D. The current situation of production of mushroom industry in Taiwan. In Proceedings of the Symposium on Biotechnology Industry of Mushroom, Taiwan Agricultural Research Institute, Council of Agriculture, Executive Yuan, Taichung City, Taiwan, 28 October 2016; pp. 13–28.
3. Liang, Z.C.; Wu, C.Y.; Wang, J.C. The evaluation of using mushroom sawdust wastes for cultivation of *Pleurotus citrinopileatus*. *Fung. Sci.* **2005**, *20*, 27–34.
4. Wang, S.; Xu, F.; Li, Z.; Zhao, S.; Song, S.; Rong, C.; Geng, X.; Liu, Y. The spent mushroom substrates of *Hypsizigus marmoreus* can be an effective component for growing the oyster mushroom *Pleurotus ostreatus*. *Sci. Hortic.* **2015**, *186*, 217–222. [CrossRef]
5. Noonsong, V.; Puttakun, N.; Tinsirisuk, M.; Seephueak, P. Recycling of spent *Pleurotus* compost for production of the *Agrocybe cylindracea*. *Mycosphere* **2016**, *7*, 36–43. [CrossRef]
6. Economou, C.N.; Diamantopoulou, P.A.; Philippoussis, A.N. Valorization of spent oyster mushroom substrate and laccase recovery through successive solid state cultivation of *Pleurotus*, *Ganoderma*, and *Lentinula*. *Appl. Microbiol. Biotechnol.* **2017**, *101*, 5213–5222. [CrossRef]
7. Gern, R.M.M.; Libardi, N.; Patricio, G.N.; Wisbeck, E.; Chaves, M.B.; Furlan, S.A. Cultivation of *Agaricus blazei* on *Pleurotus* spp. spent substrate. *Braz. Arch. Biol. Technol.* **2010**, *53*, 939–944. [CrossRef]

8. Zhang, W.; Niu, X.; Zhang, W.; Liu, Z.; Yuan, S. The cultivation of *Agaricus bisporus* on the spent substrate of *Flammulina velutipes*. *Afr. J. Agric. Res.* **2013**, *8*, 4860–4863.
9. Royse, D.J. Recycling of spent shiitake substrate for production of the oyster mushroom, *Pleurotus sajor-caju*. *Appl. Microbiol. Biotechnol.* **1992**, *38*, 179–182. [CrossRef]
10. Mau, J.L.; Chao, G.R.; Wu, K.T. Antioxidant properties of methanolic extracts from several ear mushrooms. *J. Agric. Food Chem.* **2001**, *49*, 5461–5467. [CrossRef]
11. Sheu, F.; Chien, P.J.; Chien, A.L.; Chen, Y.F.; Chin, K.L. Isolation and characterization of an immunomodulatory protein (APP) from the Jew's Ear mushroom *Auricularia polytricha*. *Food Chem.* **2004**, *87*, 593–600. [CrossRef]
12. Yu, M.; Xu, X.; Qing, Y.; Luo, X.; Yang, Z.; Zheng, L. Isolation of an anti-tumor polysaccharide from *Auricularia polytricha* (jew's ear) and its effects on macrophage activation. *Eur. Food Res. Technol.* **2009**, *228*, 477–485. [CrossRef]
13. Song, G.; Du, Q. Structure characterization and antitumor activity of an α β-glucan polysaccharide from *Auricularia polytricha*. *Food Res. Int.* **2012**, *45*, 381–387. [CrossRef]
14. Bennett, L.; Sheean, P.; Zabaras, D.; Head, R. Heat-stable components of wood ear mushroom, *Auricularia polytricha* (higher Basidiomycetes), inhibit in vitro activity of beta secretase (BACE1). *Int. J. Med. Mushrooms* **2013**, *15*, 233–249. [CrossRef] [PubMed]
15. Chiu, W.C.; Yang, H.H.; Chiang, S.C.; Chou, Y.X.; Yang, H.T. *Auricularia polytricha* aqueous extract supplementation decreases hepatic lipid accumulation and improves antioxidative status in animal model of nonalcoholic fatty liver. *BioMedicine* **2014**, *4*, 29–38. [CrossRef] [PubMed]
16. Zhao, S.; Rong, C.; Liu, Y.; Xu, F.; Wang, S.; Duan, C.; Chen, J.; Wu, X. Extraction of a soluble polysaccharide from *Auricularia polytricha* and evaluation of its anti-hypercholesterolemic effect in rats. *Carbohydr. Polym.* **2015**, *122*, 39–45. [CrossRef]
17. Priya, R.U.; Geetha, D.; Darshan, S. Biology and cultivation of black ear mushroom–*Auricularia* spp. *Adv. Life Sci.* **2016**, *5*, 10252–10254.
18. Liang, C.H.; Wu, C.Y.; Lu, P.L.; Kuo, Y.C.; Liang, Z.C. Biological efficiency and nutritional value of the culinary-medicinal mushroom *Auricularia polytricha* cultivated on a sawdust basal substrate supplement with different proportions of grass plants. *Saudi J. Biol. Sci.* **2019**, *26*, 263–269. [CrossRef]
19. Nelson, D.W.; Sommers, L.E. Total carbon, organic carbon, and organic matter. In *Methods of Soil Analysis Part 2, Chemical and Microbiological Properties*; Page, A.L., Miller, R.H., Keeney, D.R., Eds.; American Society of Agronomy; Soil Science Society of America: Madison, WI, USA, 1982; pp. 539–579.
20. Philippoussis, A.; Diamantopoulou, P.; Israilides, C. Productivity of agricultural residues used for the cultivation of the medicinal fungus *Lentinula edodes*. *Int. Biodeterior. Biodegrad.* **2007**, *59*, 216–219. [CrossRef]
21. Zeng, X.L.; Han, F.; Ye, J.L.; Zhong, Y.M. Recycling spent *Pleurotus eryngii* substrate supplemented with *Tenebrio molitor* feces for cultivation of *Agrocybe chaxingu*. *Int. J. Recyc. Org. Waste Agric.* **2017**, *6*, 275–280. [CrossRef]
22. Bellettini, M.B.; Fiorda, F.A.; Maieves, H.A.; Teixeira, G.L.; Ávila, S.; Hornung, P.S.; Júnior, A.M.; Ribani, R.H. Factors affecting mushroom *Pleurotus* spp. *Saudi J. Biol. Sci.* **2019**, *26*, 633–646. [CrossRef]
23. Yang, W.J.; Guo, F.L.; Wan, Z.J. Yield and size of oyster mushroom grown on rice/wheat straw basal substrate supplemented with cotton seed hull. *Saudi J. Biol. Sci.* **2013**, *20*, 333–338. [CrossRef] [PubMed]
24. Liang, Z.C.; Wu, C.Y. Stimulation of mycelial growth in several mushroom species by the hot water extract from *Pleurotus citrinopileatus* spent sawdust waste. In Proceedings of the 13th Conference on Biochemical Engineering, Yuan Ze University, Taoyuan, Taiwan, 27–28 June 2008; p. 133.
25. Liang, Z.C.; Lo, W.T.; Wu, C.Y. Growth promotion of mycelia of mushroom by the hot water extract from *Pleurotus eryngii* spent sawdust waste. In Proceedings of the 14th Conference on Biochemical Engineering, National Chung Hsing University, Taichung, Taiwan, 5–6 June 2009; p. 85.
26. Philippoussis, A.; Zervakis, G.; Diamantopoulou, P. Bioconversion of agricultural lignocellulosic waste through the cultivation of edible mushroom *Agrocybe agerita*, *Volvariella volvacea* and *Pleurotus* spp. *World J. Microbiol. Biotechnol.* **2001**, *17*, 191–200. [CrossRef]
27. Ashrafi, R.; Mian, M.H.; Rahman, M.M.; Jahiruddin, M. Recycling of spent mushroom substrate for the production of oyster mushroom. *Res. Biotechnol.* **2014**, *5*, 13–21.
28. Liang, Z.C.; Wu, C.Y.; Shieh, Z.L.; Cheng, S.L. Utilization of grass plants for cultivation of *Pleurotus citrinopileatus*. *Int. Biodeterior. Biodegrad.* **2009**, *63*, 509–514. [CrossRef]

29. Lian, Z.C.; Wu, K.J.; Wang, J.C.; Lin, C.H.; Wu, C.Y. Cultivation of the culinary-medicinal lung oyster mushroom, *Pleurotus pulmonarius* (Fr.) Quél. (Agaricomycetideae) on grass plants in Taiwan. *Int. J. Med. Mushrooms* **2011**, *13*, 193–199. [CrossRef]
30. Wu, C.Y.; Liang, C.H.; Wu, K.J.; Shih, H.D.; Liang, Z.C. Effect of different proportions of agrowastes on the culinary-medicinal mushroom *Auricularia polytricha* yield and nutritional composition. *Int. J. Med. Mushrooms* **2017**, *19*, 377–385. [CrossRef]
31. Obodai, M.; Cleland-Okine, J.; Vowotor, K.A. Comparative study on the growth and yield of *Pleurotus ostreatus* mushroom on different lignocellulosic by-products. *J. Ind. Microbiol. Biotechnol.* **2003**, *30*, 146–149. [CrossRef]
32. Koutrotsios, G.; Mountzouris, K.C.; Chatzipavlidis, I.; Zervakis, G.I. Bioconversion of lignocellulosic residues by *Agrocybe cylindracea* and *Pleurotus ostreatus* mushroom fungi—Assessment of their effect on the final product and spent substrate properties. *Food Chem.* **2014**, *161*, 127–135. [CrossRef]