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Solid-State Fermentation of Lignocellulosic Plant Residues from *Brassica napus* by *Pleurotus ostreatus*

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Abstract

Solid-state fermentation (SSF) of inedible parts of rapeseed was carried out using a white-rot fungus, *Pleurotus ostreatus*, to degrade lignocellulosic material for mycelial–single cell protein (SCP) production. This SSF system has the potential to be adapted to a controlled ecological life support system in space travel owing to the lack of storage space. The system for converting lignocellulosic material to SCP by *P. ostreatus* is simple; it can be carried out in a compact reactor. The fungal vegetative growth was better with a particle size of plant material ranging from 0.42 to 10 mm, whereas lignin degradation of the lignocellulose was the highest with particle sizes ranging from 0.42 to 0.84 mm. The addition of veratryl alcohol (3,4-dimethoxybenzyl alcohol), hydrogen peroxide, and glycerol promotes lignocellulose degradation by *P. ostreatus*. The enhancement of bioconversion was also observed when a gas-flow bioreactor was used to supply oxygen and to maintain the constant moisture of the reactor. With this reactor, approx 85% of the material was converted to fungal and other types of biomass after 60 d of incubation.

Index Entries: Lignocellulose; controlled ecological life support system; biodegradation; *Pleurotus ostreatus*; solid-state fermentation.

Introduction

Extended space travel may involve production of food from hydroponically grown plants. The inedible portions of the plants (stems, leaves, roots, and pods) are a disposal problem, since humans lack the hydrolytic enzymes to use any of the three major components of lignocellulosic plant

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fiber: cellulose, hemicellulose, and lignin. An efficient method for conversion of lignocellulosic materials to sugars or other usable forms, in an environmentally safe and cost-effective way, is necessary not only in a controlled ecological life support system (CELSS), but also for terrestrial applications *(1)*. The research conducted in our laboratory for NASA-sponsored research on CELSS has reemphasized a desire to meet this goal using *Brassica napus* (rapeseed), one of the candidate species in a CELSS as an oil crop *(2,3)*.

The physical arrangement of the three fiber components limits the access of the commercially available cellulases to the cellulose. Lignin surrounds the cellulose and inhibits the action of the cellulases and hemicellulases *(4)*, since cellulase systems do not exhibit ligninolytic activity. The crystalline form of cellulose, because of its tight and dense structure, also limits the access of cellulases and poses another physical impediment to cellulase enzyme. Therefore, an effective cellulose pretreatment should be able to enhance access of the enzyme systems to the substrate by eliminating the protective effects of lignin and crystallinity of the cellulose.

Lignin is degraded by a narrower array of microbes than any other biopolymer *(5)*. White-rot fungi (*Basidiomycetes*) are capable of degrading lignin *(6–13)*. *Pleurotus ostreatus* is able to degrade lignin as well as hemicellulose and cellulose, so it will increase the efficiency of recycling of the lignocellulosics, and less storage space will be required for inedible plant residue. Another advantage of using *P. ostreatus* is that it can produce fruiting bodies, i.e., oyster mushrooms. These flavorful mushrooms could be used to improve an otherwise bland diet on a planetary outpost *(1)*. The capability of lignin degradation gives these fungi competitive advantages by providing access to cellulose and hemicellulose *(14)*. Other possible advantages to this type of process include a low water requirement since growth would occur under solid-state fermentation (SSF) conditions, mild temperature or pH, and no need for agitation, although aeration may be necessary. For a CELSS, biological conversion of the lignin is an attractive alternative to wet oxidation, which combusts the material to CO_2 and water.

Phanerochaete chrysosporium is one of the best-characterized white-rot fungi with well-defined growth conditions *(4,15)*. These provide a reference base for the growth of *P. ostreatus*. We followed basic growth conditions for *P. ostreatus*, developed by Kaneshiro *(16)* and Lindenfelser et al. *(17)*, to pretreat lignocellulosic agricultural wastes in the present study.

Veratryl alcohol and hydrogen peroxide appear to have multiple roles in lignin degradation by *P. chrysosporium*. Veratryl alcohol apparently induces expression of lignin peroxidase (LiP) and protects LiP from inactivation by hydrogen peroxide. Also, veratryl alcohol has been shown to potentiate LiP-catalyzed oxidation of nonlignin peroxidase substrates, possibly as an enzyme-associated mediator or an electron-transfer agent *(18)*. Molecular oxygen increases the production of hydrogen peroxide and veratryl alcohol and results in increased LiP activity. The cation radicals

generated by LiP react with water and oxygen, resulting in enzymatic combustion. The main role of hydrogen peroxide is to oxidize and activate the resting enzyme, LiP. Glycerol is believed to inhibit primary growth, but it triggers secondary growth of the microorganism *(1)*.

Substrate modification and a better-controlled SSF system have been designed using known characteristics and mechanisms of the lignin-degrading system of white-rot fungi. In this article, we present the effects of *P. ostreatus* growth on lignocellulose degradation of inedible rapeseed plant components, and the recycling of inedible plant materials in the context of a CELSS.

Materials and Methods

Substrate

Inedible parts of hydroponically grown rapeseed plants were used as substrate for *P. ostreatus* growth. Stems, leaves, and pods of the harvested mature plants were separated, dried, and ground into desired particle size *(19)*. In these experiments, roots of the *Brassica napus* were not included in the lignocellulosic mixture, because we wanted to have a consistent and homogeneous substrate and there were not enough rapeseed roots to use throughout this research. Substrates used in the experiments were particles that passed through 40-mesh sieve ($d_p < 0.42$ mm), particles that passed through 20-mesh sieve but could not pass the openings on 40-mesh sieve ($0.42 < d_p < 0.84$ mm), and air-dried and hand-ground substrate ($d_p \sim 10$ mm). After the substrate was sieved, the same particle sizes of stems, pods, and leaves were combined in equal weights and mixed. Then, these three different sizes of substrates were stored in sealed containers at 4°C until use.

Preparation of Culture

P. ostreatus (NRRL 2366) was kindly provided by the Northern Regional Research Laboratory (NRRL) in Peoria, IL. The procedures of Kaneshiro *(16)* and Lindenfelser et al. *(17)* were followed to prepare nutrient solutions. Initial transfers and fungal pellet preparations were made as described by Sarikaya and Ladisch *(19)*.

SSF: Jar and Gas-Flow Bioreactors

The jar bioreactor is a 9-cm-diameter glass jar with a screw-on lid. Plant material (7.5 g) of each of the three different particle sizes was put into the jars. Water was added to each jar at a water:solids weight ratio of 5:1, which gave the optimal mycelial growth, using a hand atomizer. Sterilization of the water-substrate mixture was necessary before inoculation. Then the jars were autoclaved at 121°C for 20 min and allowed to cool to room temperature and inoculated with *P. ostreatus* pellets, which were growing in the nutrient solution. A single pellet was used to inoculate aseptically each jar at the center of the substrate, and 0.5 mL of water was

distributed on top of the plant material every day in order to keep the material sufficiently moist to maintain the fungal growth. Fermentations (mycelial growth) were carried out at 28°C for 32–50 d in the jars.

The fermentations were started at the same time by inoculating each jar with a single *P. ostreatus* pellet, except for the control, to which inoculum was not added. These were placed into an incubator at 28°C. After the fermentations were stopped, the contents of the bioreactors were combined with 100 mL of distilled water and occasionally mixed for 3 h, followed by filtering to separate mycelia from the remaining substrate. After washing in this manner, the substrate was dried overnight at 80°C. Component analysis was done on the dry material.

Specially designed gas-flow bioreactors were also used to control more closely the environmental conditions of humidity, temperature, and aeration of the fermentation in order to promote extensive fungal growth *(19)*. Fermentations (mycelial growth) were continued for 50 d, and the compositional analyses were carried out before and after the fermentations.

Effects of Nutritional Parameters and Oxygen on Lignin Degradation

Fermentations were carried out in jar and gas-flow bioreactors with the addition of 5 mL of 0.25 m*M* veratryl alcohol, 5 mL of 0.06 m*M* hydrogen peroxide, and 5 mL of 1% (v/v) glycerol *(18)* per gram of inedible plant material (6% moist) at the beginning of the fermentation to examine the effects of these components on lignin degradation. These concentrations corresponded to 0.02% (w/w) veratryl alcohol, 0.001% hydrogen peroxide, and 5.0% glycerol in the fermentation media. This concentration of the hydrogen peroxide should be high enough to activate peroxidase enzymes, but low enough not to deactivate these enzymes *(1)*. Required amounts of these substances were added to the water, which was five times the weight of substrate, before mixing it with the solid substrate.

Effects of pure oxygen or air were also determined, with the fermentations conducted in the gas-flow bioreactors. A gas flow of 0.2–0.4 mL/min through the substrate was used for both cases during the entire mycelial fermentation. Compositional analyses were performed after the fermentation was terminated.

Measurement of CO_2

The gas leaving each gas-flow bioreactor was bubbled through 50 mL of 0.1 *M* NaOH solution in order to trap CO_2; the CO_2 traps were replaced at intervals of 3 to 4 d. CO_2 trapped in NaOH solution was determined by carbonate precipitation with 0.1 *M* barium chloride followed by titration of the residual NaOH with 1 *M* HCl, using thymol blue as an indicator *(13)*.

Mushroom Production

To promote the formation of mushrooms, the incubation temperature was lowered to 20–22°C, and lids of the bioreactors were opened after 50 d

of incubation. At this point, the mycelial growth was visible throughout the substrate as a white mat with the lignocellulosic material having been bleached from brown to a light tan. Moisture contents of the fermentation medium were kept higher during this part of the fermentation; two milliliters of water/day was sprayed on top of the mycelial mat to promote the formation of fruiting bodies. Within 7–10 d, pin formation was observed. Within 2 to 3 additional days, fully grown mushrooms could be harvested. Once the fermentations were completed, the contents of the bioreactor was washed and the remaining substrate was dried before compositional analyses were conducted.

Compositional Analysis

Microanalysis of Carbon, Hydrogen, Nitrogen, and Sulfur

Carbon, hydrogen, and nitrogen were analyzed by standardized methods *(20)* with a Perkin-Elmer 240°C Elemental Analyzer in the Chemistry Department, Purdue University, by Hsiu-Pu D. Lee. Sulfur was determined as follows. Samples were digested in an oxygen flask to convert sulfur in samples into sulfate. Alkaline ions, which would interfere with the titration of sulfate, were removed with a cation-exchange resin (AG50W-X12, hydrogen form; Bio-Rad Laboratories, Berkeley, CA). Sulfates free from alkaline ions were then titrated with a standardized barium solution, and sulfur contents were calculated accordingly *(20)*.

Fiber Analysis

Neutral detergent fiber (NDF), which is based on extraction of the feed with a hot neutral solution of sodium lauryl sulfate, and acid detergent fiber (ADF), which is obtained by heat treatment of the sample with 0.5 *M* sulfuric acid containing cetyltrimethyl-ammonium bromide, assays were conducted according to Goering and Van Soest *(21)* and Van Soest and Wine *(22)*, to determine the carbohydrate composition of the plant material before and after the growth of *P. ostreatus* on the lignocellulosic material. The amount of lignin was determined by using the permanganate lignin assay of Van Soest and Wine *(23)*, on the material remaining after ADF determination, as shown in Fig. 1 *(24)*. ADF comprises lignin, cellulose, and insoluble minerals, whereas the NDF also includes hemicellulose. Therefore, subtracting the ADF value from the NDF value gives the weight of the hemicellulose in the sample. The standard procedure for lignin analysis requires about 1 g of dry sample for ADF analysis. Prepared ADF is treated with permanganate solution to oxidize lignin. Weight loss, measured after drying the permanganate-treated sample at 100°C overnight, corresponds to the lignin content of the fiber. Depending on the amount of substrate remaining after the fermentation (especially for gas-flow bioreactors), if there was not enough material left for both microanalysis and fiber analysis, carbon, hydrogen, and nitrogen analyses were performed instead of fiber analysis. This result alone showed that lignin seal was removed effectively and therefore hemicellulose and cellulose components were utilized

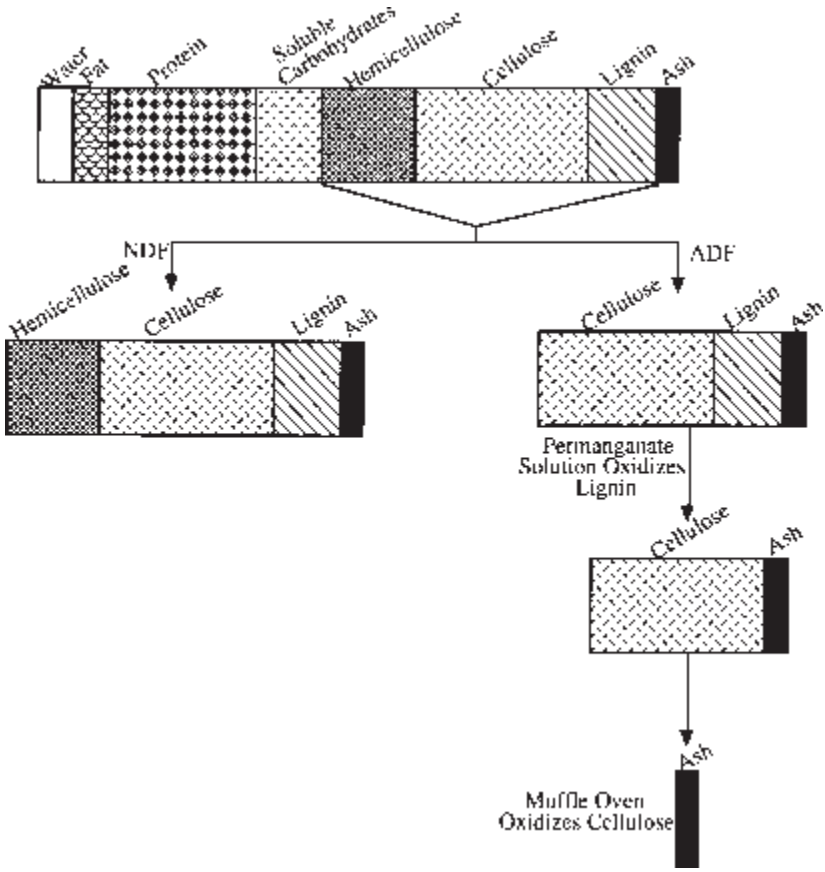

Fig. 1. Composition of inedible plant material. (Reproduced with permission from ref. *24*.)

to a great extent during fermentation in a flow bioreactor. Carbon and nitrogen composition of the remaining material was required for the material balances.

Results and Discussion

The general purpose of lignocellulose pretreatments is to remove the protective effect of lignin and moderate the recalcitrant nature of the crystalline cellulose. In this context, selective lignin degradation could be beneficial *(3)*. If the purpose is to recycle the inedible plant material as a whole and to obtain edible mushrooms at the end of the fermentation, then the degradation of all three components of lignocellulose would be necessary. In the context of a CELSS, the complete recycling of inedible rapeseed material was studied. Inedible plant material, obtained from hydroponically grown *B. napus*, was composed of 10% hemicellulose, 23% cellulose, and 15% lignin. The remaining portion (52% of the dry substrate) consisted of protein, fat, and ash. Since the plants were grown hydroponically, ash

Table 1
Effect of *P. ostreatus* Treatment on Lignocellulose Composition of Rapeseed Inedibles for Fermentations Performed in Jar Bioreactors

Sample number	Sample[b]	Decrease in weight[a] Total weight (%)	Hemicellulose (%)	Cellulose (%)	Lignin (%)
1	Sterilized control (32 d); d_p: 0.42–0.84 mm	32.3	25.0	0.0	0.0
2	*P. ostreatus* (32 d); hand-ground, d_p: 1 cm	39.0	41.0	35.9	0.0
3	*P. ostreatus* (32 d); d_p: <0.42 mm	37.6	25.7	31.6	15.0
4	*P. ostreatus* (32 d); d_p: 0.42–0.84 mm	40.7	25.0	37.0	36.0
5	*P. ostreatus* (50 d); d_p: 0.42–0.84 mm	51.6	82.6	78.1	84.1

[a]Dry wt basis, measured after washing with water. Values are based on the average of three runs with a standard deviation (SD) range of 1.1–3.0%.
[b]d_p = particle diameter of the substrate.

content was high, about 22% (dry wt basis). Parameters such as particle size of the substrate; addition of veratryl alcohol, hydrogen peroxide, and glycerol; and controlled moisture and oxygen supply throughout the fermentation process were studied. The efficiency of lignocellulose degradation and material balances were determined under these different conditions.

Particle Size Effects in Jar Bioreactors

It has been observed previously that mycelial growth was better for the substrates with particle sizes from 0.42 mm to 1.0 cm than with particle sizes <0.42 mm *(19)*.

Table 1 shows the fiber analysis of the rapeseed samples after *P. ostreatus* growth was performed in glass jars. The control in Table 1 was treated in the same way as the fermented substrates, except the control was not inoculated with the microorganism. All runs in the jars were carried out in triplicate, and an average value was reported. Whereas the control sample did not show any loss in lignin or cellulose content, a 25% decrease in hemicellulose was noted. This finding indicated that a fraction of the hemicellulose was water soluble at room temperature. This is probably owing to partial autohydrolysis of the hemicellulose, which is known to occur in biomass materials at 121°C (i.e., sterilization) *(25)* with solubilized hemicellulose components being removed during the wash step. There was about a 32% decrease in the total dry wt of the substrate for the control experiment. The removed portion included part of the hemicellulose and some other soluble components that were solubilized during the sterilization and washing steps.

Results obtained after 32 d of fermentation with samples 2–4 in Table 1 indicated that an additional disappearance of hemicellulose occurred only for 1-cm particles. The cellulose degradation was similar for the three different particle sizes. The results show that *P. ostreatus* growth is associated with a proportional decrease in the cellulose and lignin components for the intermediate-sized particles (sample 4). Lignin degradation is absent for the largest particles (sample 2) and attains an intermediate range for the smallest particles (sample 3). Lignin degradation was the highest, at 36% after 32 d of fermentation in the glass jar bioreactors for the substrates with particle sizes between 0.42 and 0.84 mm (sample 4). Therefore, substrates with particle sizes between 0.42 and 0.84 mm were used for the fermentations. After 50 d of mycelial growth, the disappearance of the cellulose, hemicellulose, and lignin components exceeded 80%, whereas the total weight loss was 51.6%.

Gas-Flow Bioreactors with Air or Oxygen, and Controlled Moisture

A fermentation system in which a moisturized gas stream would flow through the substrate was designed in order to study the effects of microbial growth at saturated humidity *(19)*. Two bioreactors were used. Pure oxygen was passed through the substrate in one bioreactor; air was used in the other. Slow flow rates, 0.2–0.4 mL/min, were used in both cases. Both air and oxygen were maintained at saturated humidity by bubbling the gas through water.

The controlled, saturated atmosphere increased the primary growth rate of our microorganism. At the end of 50 d of fermentation, the percentage decrease in dry wt of the inedible plant material was 67.4% when air was passed through the substrate and 72.4% for oxygen, compared to 51.6% for the jar bioreactor. Primary mycelial growth covered the surface of the substrate in 5 d in both cases, compared to 8 d when the jar bioreactor was used.

Hence, 35% less time was needed for the gas-flow bioreactor, although the incremental improvement was obtained when oxygen was substituted for air (Table 2).

Effects of Veratryl Alcohol, Hydrogen Peroxide, and Glycerol on Biodegradation

Lignin degradation by white-rot fungi is observed during the secondary growth phase of the microorganism when readily available nutrient sources are depleted. Veratryl alcohol, a secondary metabolite of basidiomycetes, is a substrate for the oxidized form of the enzyme LiP. Veratryl alcohol radicals obtained from this reaction react with lignin, initiating the bond cleavage. Hydrogen peroxide is required for ligninase activity to oxidize LiP. Oxygen is believed to play a role in producing hydrogen peroxide and producing active oxygen species, which are capable of promoting extensive autooxidation of lignin through binding to veratryl alcohol cation radicals. Glycerol is also believed to improve lignin degradation by

Table 2
Effect of Moisturized Gas Streams on Degradation
of Different Components of Substrate in a Flow Bioreactor

Sample[a]	Decrease in weight[b] Weight (%)	C (%)	H (%)	N (%)
P. ostreatus (50 d); d_p: 0.42–0.84 mm	51.6	47.0	49.0	79.0
P. ostreatus (50 d); d_p: 0.42–0.84 mm; purge gas = air at saturated humidity, 28°C	67.4	72.0	71.0	76.0
P. ostreatus (50 d); d_p: 0.42–0.84 mm; purge gas = O_2 at saturated humidity, 28°C	72.4	77.5	78.0	82.0

[a]d_p = particle diameter of the substrate.
[b]Dry wt basis, measured after washing with water.

Table 3
Effect of Oxidation Potentiators
on Degradation of Inedible Plant Material in a Jar Bioreactor[a]

Oxidation potentiator(s)	Decrease in weight[b] Weight (%)	C (%)	H (%)	N (%)
No potentiator added	35.0	35.0	39.0	62.0
Veratryl alcohol	40.0	41.0	30.0	65.0
Veratryl alcohol + hydrogen peroxide	45.0	47.5	33.0	68.0
Glycerol	43.0	43.0	49.0	69.0

[a]Fermentation = 32 d; d_p = 0.42–0.84 mm.
[b]Dry wt basis.

triggering the secondary, lignin-degrading phase of the white-rot fungi *(1)*. Therefore, the effects of veratryl alcohol, hydrogen peroxide, and glycerol on lignocellulose degradation were studied in combination with moisture control.

Jar Bioreactors

The percentage decrease in dry wt of the substrate (45% after 32 d of fermentation) was higher when hydrogen peroxide and veratryl alcohol were combined together than when either was used alone. A similar effect (45% decrease) was observed when glycerol was used. The percentage decrease in substrate weight was about 35% in the absence of veratryl alcohol, hydrogen peroxide, or glycerol (Table 3).

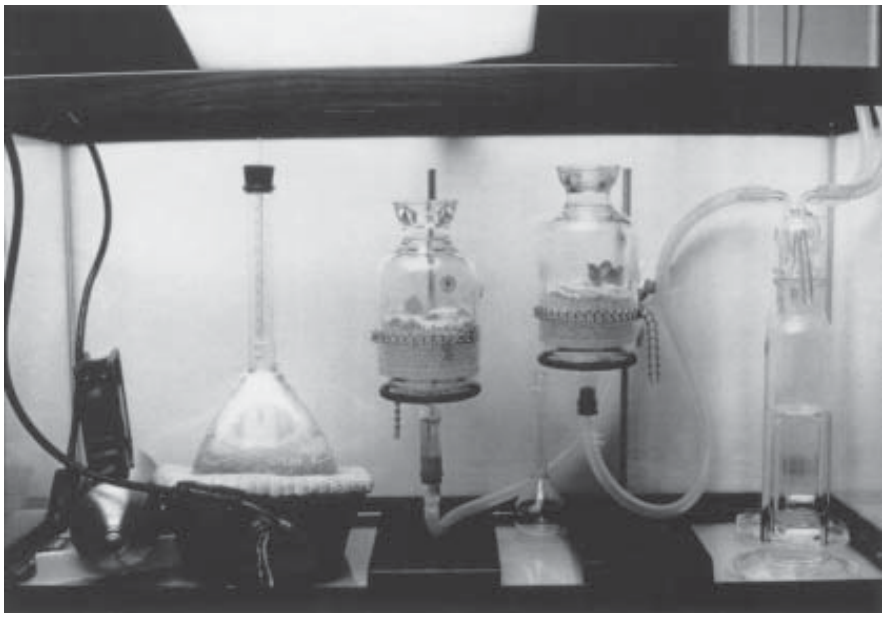

Fig. 2. Gas-flow bioreactor system.

About 8 d were required to cover the surface of the substrate with mycelia when a jar was used as the bioreactor to degrade the plant material with a particle size of 0.42–0.84 mm and a water:substrate ratio of 5:1. Similarly, primary growth of the microorganism required 8 d when hydrogen peroxide and veratryl alcohol at concentrations of 0.05 and 0.21 m*M*, respectively, were added. But when glycerol was used at a concentration of 0.1 *M*, it took 12 d instead of 8 d for the same surface to be covered with mycelial growth, showing that glycerol inhibits primary growth of the microorganism on the lignocellulosic material.

Gas-Flow Bioreactors

Based on a 32-d run in the jar-bioreactor experiments, we combined all three components—veratryl alcohol, hydrogen peroxide, and glycerol—since all had a positive effect on lignin degradation, for the gas-flow bioreactor experiments. The experiments were conducted by combining 7.5 g of lignocellulosic plant material, at 6% moisture, with 37.5 mL of water together with veratryl alcohol, hydrogen peroxide, and glycerol at the same concentrations used in the jars, and passing oxygen through the substrate at a flow rate of 0.2–0.4 mL/min (Fig. 2). Gas-flow bioreactor experiments were conducted in duplicate, and average values are presented herein. After 60 d of fermentation (vegetative + fruiting-body formation), the thickness of the substrate decreased from 1.5 to 0.4 cm, and it had a white, rubbery structure (behaving like a bendable mat). Table 4 gives the elemental composition of the remaining material.

Table 4
Elemental Analysis of Rapeseed Inedibles (d_p = 0.42–0.84 mm) After 60 d of Fermentation (vegetative + fruit-body formation) in Gas-Flow Bioreactor

	Weight (%)[a]		
Sample	C	H	N
No treatment (original)	35.49	5.33	3.44
0.21 m*M* veratryl alcohol, 0.05 m*M* hydrogen peroxide, 0.1 *M* glycerol, 0.2–0.4 mL/min O_2	27.73	4.19	1.06

[a]Dry wt basis. Values are based on the average of two runs with an SD range of 0.11– 1.20%.

Material Balance

Equal weights of 0.42–0.84 mm (particle size) stems, pods, and leaves of rapeseed material were mixed. Microanalyses were applied to determine the carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) composition of the mixture, which was 36.8% C, 5.3% H, 3.4% N, and 1.6% S with a C/N mole ratio of 12.6. After 50 d of mycelial growth in a sealed-jar bioreactor, 48.4% of the dry substrate was left with a composition of 40.2% C, 5.6% H, 1.5% N, and 0.3% S. The C/N mole ratio for the untreated rapeseed mixture was 31.3 after 50 d of vegetative growth in the sealed-jar bioreactors.

The decrease in the relative amounts of nitrogen indicated that a disproportionate consumption of protein occurred compared to carbon and hydrogen, whose relative compositions did not change. At the end of the fermentation, when the substrate was analyzed without removing the mycelia, the amount of nitrogen was approximately the same as the untreated substrate, showing that nitrogen was incorporated into the mycelial mass. Decreases in weights of the rapeseed material (dry wt basis) were 47% for C, 50% for H, and 79% for N after 50 d of mycelial growth. Further utilization of carbon (about 80%) was observed when fermentation was continued an additional 10 d to obtain the fruiting bodies of *P. ostreatus*. The percentage decrease in dry wt of the substrate was 85%. After the fermentation was over, it was not possible to completely wash out the mycelia from the remaining substrate. Degradation of the cellulosic biomass was >85%, because the mycelia were included in the remaining dried materials.

All atoms of carbon, hydrogen, oxygen, and nitrogen are incorporated into new cells or excreted as products, mainly as CO_2 and water in our case, during the growth of *P. ostreatus* on lignocellulosic rapeseed material. The material balance helps to confirm the consistency of experimental data (Table 5). It also gives an idea about some parameters such as product yield, biomass yield, and respiratory quotient. It would help us to know the required carbon, sulfur, nitrogen, and oxygen for the process to be able to formulate the nutrient medium.

Table 5
Carbon and Nitrogen Balances for *P. ostreatus* Treatment (50 d of mycelial growth + 10 d of mushroom production) of Lignocellulosic Rapeseed Material in a Gas-Flow Bioreactor

	Carbon (g)	Nitrogen (g)
In		
Stream 1	2.5	0.243
Out		
Stream 2	0.2	0.043
Stream 3	0.9	0.196
Stream 4	1.2	—
Total	2.3	0.239

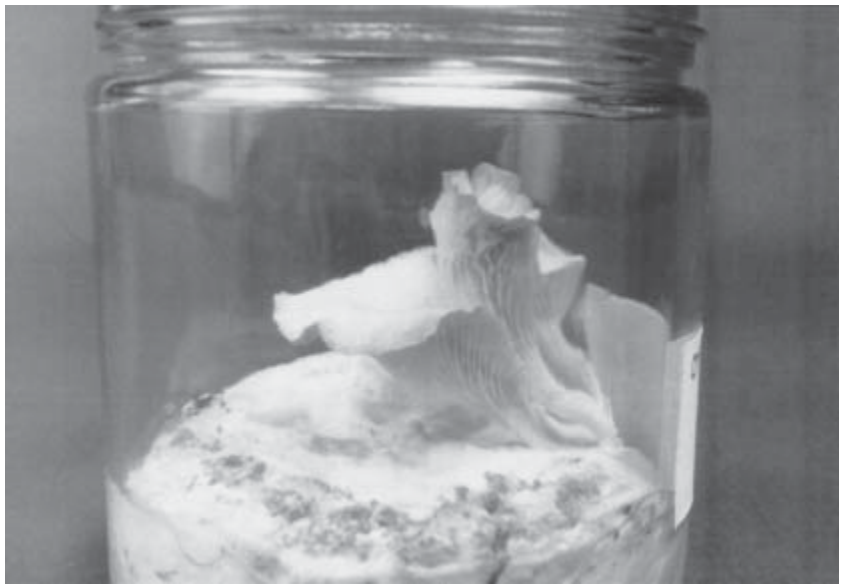

Fig. 3. Jar bioreactor system.

The gas-flow bioreactor design allowed us to measure the CO_2 evolving from the fermentor, which would give an idea about the amount of carbon usage for the microbial metabolism. Figure 3 shows the cumulative amount of CO_2 collected during the vegetative growth of microorganism in the bioreactor for about 50 d with added veratryl alcohol, hydrogen peroxide, and glycerol, and when oxygen was passed through the substrate at a flow rate of 0.2–0.4 mL/min throughout the fermentation. The total amount of CO_2 measured at the end of 50 d of fermentation was 4.367 g (from 7.05 g of dry substrate with a 2.5 g of carbon content).

Figure 4 shows a material balance diagram for the controlled fermentation process. Vegetative growth was for 50 d, and mushroom production

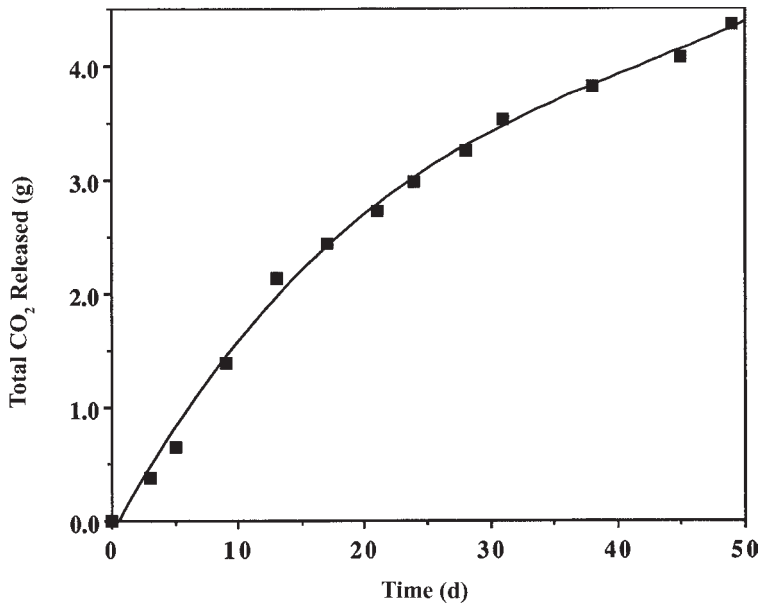

Fig. 4. Cumulative amount of CO_2 evolved during vegetative *P. ostreatus* growth on lignocellulosic rapeseed material.

occurred in the subsequent 10 d. The mushroom, harvested once, gave a dry yield of 7.1 g of mushroom/100 g of the initial substrate (dry wt basis). The CO_2 evolved during mycelial growth was 4.4 g, which corresponds to 1.2 g of carbon (48.0% of the initial amount of carbon in the substrate). The respiratory quotient during mycelial growth was 0.11 (mol of CO_2 produced/mol of O_2 consumed). During the mushroom production stage, with the unsealed bioreactor, CO_2 evolved during the last 10 d of the fermentation was not measured.

A mycelial mat weight of 2.6 g remained after the mushrooms were picked. This mat consisted primarily of mycelia, and there was only a small amount of substrate left. Therefore, it was difficult to separate the mycelia from the remaining substrate by washing. After the mycelia was washed out, the remaining material (which included some mycelia) weighed 1 g, which corresponds to 15% of the starting substrate (Fig. 5).

The total nitrogen in the mushrooms obtained from bioreactor experiments was 8.66% (dry wt basis). From the material balance (Fig. 4), the amount of substrate disappearing in the form of CO_2, water, heat, and some other volatiles such as aldehydes and ketones should be about 3.95 g.

The amount of carbon introduced into our system through veratryl alcohol and glycerol was 0.161 g. Therefore, the total carbon component going into the bioreactor was 2.661 g. The sum of all the carbon components leaving the bioreactor was 2.3 g. The remaining 0.361 g of carbon is assumed to have been lost as CO_2 during mushroom production and as other volatile substances during the overall fermentation process.

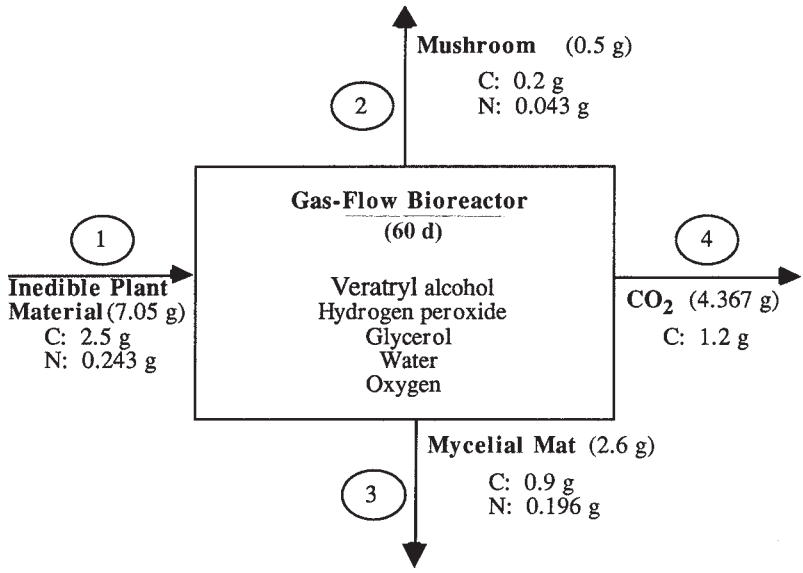

Fig. 5. C and N balances for controlled SSF process.

Conclusion

The white-rot fungi, *P. ostreatus* effectively degrades lignocellulosic plant material from hydroponically grown rapeseed inedibles (stems, pods, and leaves), into CO_2, water, and mycelial matter. The most extensive disappearance of mass occurred for plant material having a particle size range of 0.42–0.84 mm, with the gas-flow bioreactor giving more rapid degradation than a sealed jar bioreactor. Lignin degradation by white-rot fungi on the 0.42- to 0.84-mm particles in the gas-flow bioreactor was accelerated by the addition of veratryl alcohol, hydrogen peroxide, and glycerol. Substrate moisture was controlled by the bioreactor design, which provided a small but constant flow of saturated oxygen or air through the substrate material throughout the fermentation.

Material balances showed that bioconversion of lignocellulosic material by using *P. ostreatus* was efficient. The controlled fermentation process in the gas-flow bioreactor required 35% less time than a jar bioreactor to achieve the same extent of degradation. The results show that the use of *P. ostreatus* in a CELSS could be beneficial in biodegradation of inedible plant material.

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