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The Conversion of Straw into Feed by Basidiomycetes*

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Summary. 1. The solid-state fermentation of straw by *Pleurotus cornucopiae, Pleurotus sp. Florida, Agrocybe aegerita,* and *Stropharia rugosoannulata* at 22°, 25°, and 30°C during 0–120 days was examined.

2. During the first stage of fungal growth (saprophytic colonization), the quantity of water soluble substances, reducing sugars, and in vitro digestibility of the straw-fungal mycelium mixture decreased.

3. After 20 days, the amount of water soluble substances and reducing sugars increased. Temperature strongly influenced the rate of substrate decomposition, particularly with cultures of *Pleurotus cornucopiae* and *Stropharia rugosoannulata.*

4. Of the fungal cultures examined, *Pleurotus cornucopiae* and *Stropharia rugosoannulata* showed the highest rate of straw decomposition and released the greatest amount of metabolic energy from the straw.

5. The heat of combustion of decomposed substrate decreased due to increasing ash content and varying degree of metabolism of cellulose and lignin.

6. The in vitro digestibility of wheat straw was strongly influenced by incubation temperature and increased during fermentation by *Pleurotus species* and *Stropharia rugosoannulata.*

7. *Agrocybe aegerita* exhibited good properties for production of fruiting bodies, but this species was not satisfactory for the conversion of plant residues to feed. *Stropharia rugosoannulata*, however, increased the digestibility of straw by 31.6%.

8. Some technical possibilities for using fungi for upgrading waste straw to animal feed are discussed.

Introduction

The digestibility of plant material by ruminants depends on the amount and quality of natural polymers (lignin and cellulose) and the amount of readily soluble substances

*To Prof. Dr. R. von Sengbusch on his 80th birthday

such as sugars and amino acids. Various chemical and biochemical methods for increasing the digestibility of plant waste have previously been examined. Treatment of straw by sodium or calcium hydroxide was proposed by Beckmann (1919), Magnus (1919), Engels (1948), and Donefer et al. (1969).

Various biological methods for upgrading plant residues to animal feed have been reported. Han Young (1974) and Han Young and Anderson (1975) used cultures of *Candida utilis, Aureobasidium pullulans,* and *Trichoderma viride* to ferment ryegrass which had been pretreated with 0.5 N H_2SO_4 and neutralized with 5 N NH_4OH. During this process, the digestibility of the ryegrass increased approximately 40% while the microbial mass increased 20 to 200-fold. Cultures of white-rot fungi are able to break down the cellulose-lignin complex (Falck, 1926, 1930; Findlay, 1940) and liberate digestible compounds (Schánĕl et al., 1966; Herzig et al., 1968; Zadražil, 1975, 1976).

In this investigation, a solid-state fermentation was conducted using wheat straw without chemical pretreatment or nitrogen amendments as a substrate for four physiologically different fungi. The influence of temperature and time of incubation on the quality of partially decomposed substrate were investigated.

Material and Methods

Microorganisms. Isolates of *Pleurotus cornucopiae* Paul ex Fr., *Pleurotus sp. Florida* (Eger, 1965), *Agrocybe aegerita* (Brig.) Sing., and *Stropharia rugosoannulata* Farlow ex Murr., were used as model organisms. The cultures were isolated from cultivated fruiting bodies during the last two years and were maintained on malt agar at 25°C.

Substrate. Winter wheat straw (25 g, mill size ca. 1 mm) was placed in 500 ml Erlenmeyer flasks with 75 ml distilled water and sterilized at 121°C for 30 min. Triplicates were inoculated with two agar plugs (7 mm) per flask. The cultures were incubated in the dark at 22°, 25°, and 30°C for 8, 16, 30, 44, 58, 79, 100, 120 days, then harvested, dried at 105°C, homogenized, and aliquots taken for analysis.

Analytical Methods. The loss of dry weight during the incubation was determined by weighing the dry samples at the beginning and end of the experiments. The pH of the straw-mycelium mixture was measured with a glass electrode 18 h after water had been added to the mixture creating a slury. Reducing sugars were determined by the anthron method (Mokras, 1954) and water soluble substances by extracting 1 g of substrate in 100 ml H_2O for 3 h at 80°C (Zadražil, 1976). The heat of combustion was determined using a IKA calorimeter. The loss of metabolic energy (entropy) was calculated by combusting samples before and after fermentation.

The in vitro digestibility was determined using the method of Tilley and Terry (1963) as modified by Rohr (1976). Substrate (0.5 g) was suspended in 40 ml of a phosphate-carbonate buffer containing 5 ml of a 0.3% urea solution. Rumen fluid (10 ml) was added to the sample in 100 ml centrifuge tubes, gassed with CO_2, and fermented at 38°C for 48 h. After 48 h, the flasks were centrifuged at 4000 g for 25 min and pellet mixed with 50 ml of a 0.1 N HCl-pepsin solution for 24 h at 38°C, centrifuged again, and the supernate filtered through a glass filter. The residue was dried at 105°C and

weighed. The ash content was determined after combustion at 550°C. The portion of organic matter lost during digestion is expressed as the in vitro digestibility.

Results

Temperature Effect on Straw Decomposition. The differences in the ability of the tested fungi to degrade straw during 120 days of fermentation at three different temperatures are shown in Figure 1. Both *Pleurotus cornucopiae* and *P. sp. Florida* had short lag periods in relationship to *Agrocybe aegerita* and *Stropharia rugosoannulata.* After 120 days at 30°C, *S. rugosoannulata* and *P. cornucopiae* decomposed approximately 60% of the organic matter, whereas the amount decomposed by *P. sp. Florida* and *A. aegerita* was only 40% and 20%, respectively. Large differences in the rate of straw decomposition due to temperature were observed for *P. cornucopiae* and *S. rugosoannulata.* However, for the other two fungi, temperature did not strongly effect the rate of straw decomposition (Fig.1). Fruiting bodies appeared in cultures of *P. cornucopiae* and *A. aegerita* after 25 days at 22° and 25°C,but no fructification occurred at 30°C. *P. sp. Florida* and *S. rugosoannulata* did not form fruiting bodies under the given experimental conditions.

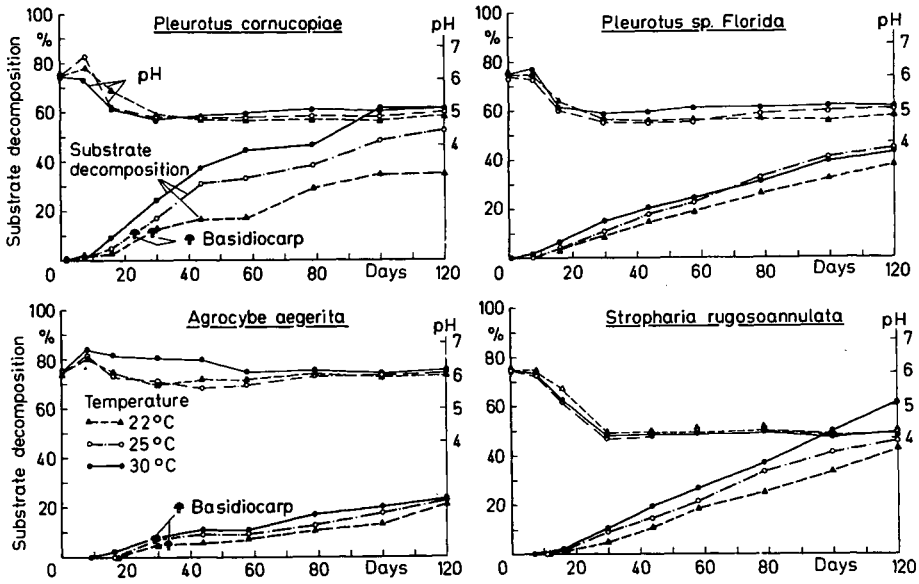

Fig. 1. Wheat straw decomposition (in % dry weight) and pH changes during solid-state fermentation at 22° (▲), 25° (○), and 30°C (●) over 0–120 days

pH of the Straw Substrate. Fungal growth caused changes in the pH (Fig.1) of the straw-mycelium mixture. *A. aegerita* initially caused a slight increase in pH which later stabilized at pH 6. With the two *Pleurotus* species, the pH initially increased but

then decreased to pH 5.1 over the course of fermentation. With *S. rugosoannulata*, the pH initially dropped to 3.9–4.0, which is optimal for this culture. This pH decrease in the substrate correlated directly with the decomposition activity of *S. rugosoannulata*.

Soluble Substances. During the initial incubation period, the amount of soluble substances and reducing sugars in the straw either decreased or remained constant (Fig.2 and 3). After 20 days, however, high amounts of easily soluble substances were produced by cultures of *P. cornucopiae* and *S. rugosoannulata*. For these two species, temperature had an important influence on substrate solubilization, whereas for the other two cultures the differences were less significant.

Heat of Combustion. The loss of substrate energy during fungal fermentation is shown in Figure 4. The heat of combustion of the partially degraded substrate was determined at different times during the fermentation process. This parameter decreased only slightly during the fermentation, due perhaps to differences in the degradation rates of cellulose and lignin. Lignin has a higher heat of combustion (ca. 5200 cal/g) than does cellulose (ca. 4030 cal/g). The loss of metabolic energy caused by fungal metabolism correlates with the decomposition of the straw.

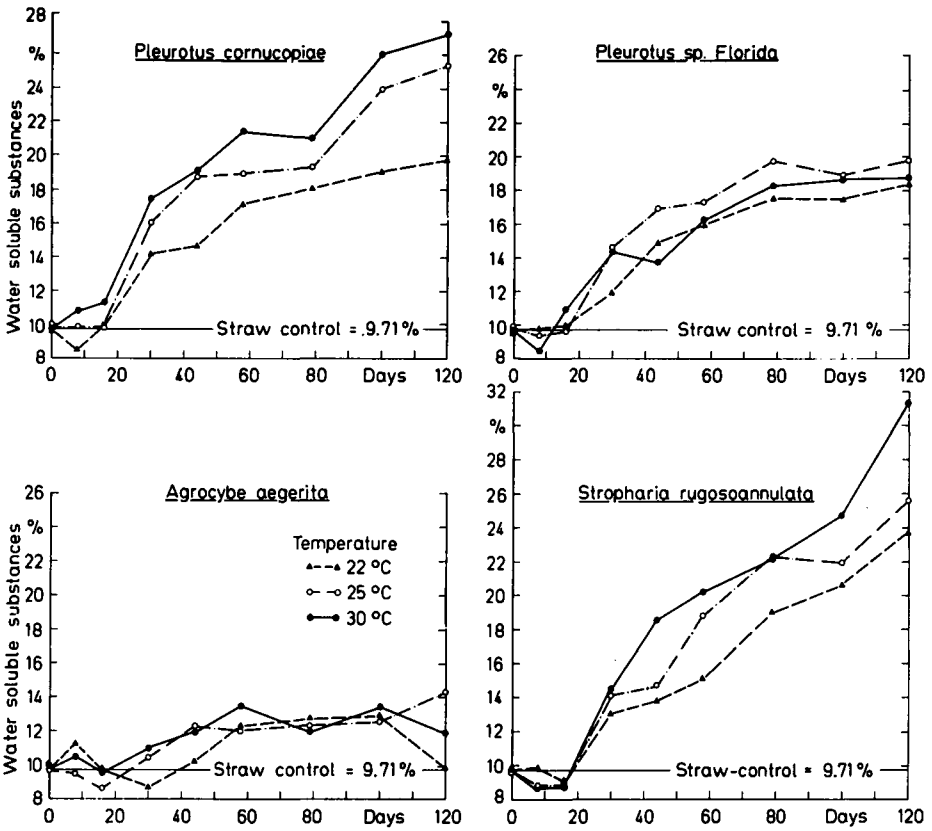

Fig. 2. Liberation of water soluble substances (in % dry weight) from wheat straw during fungal fermentaion at various temperatures

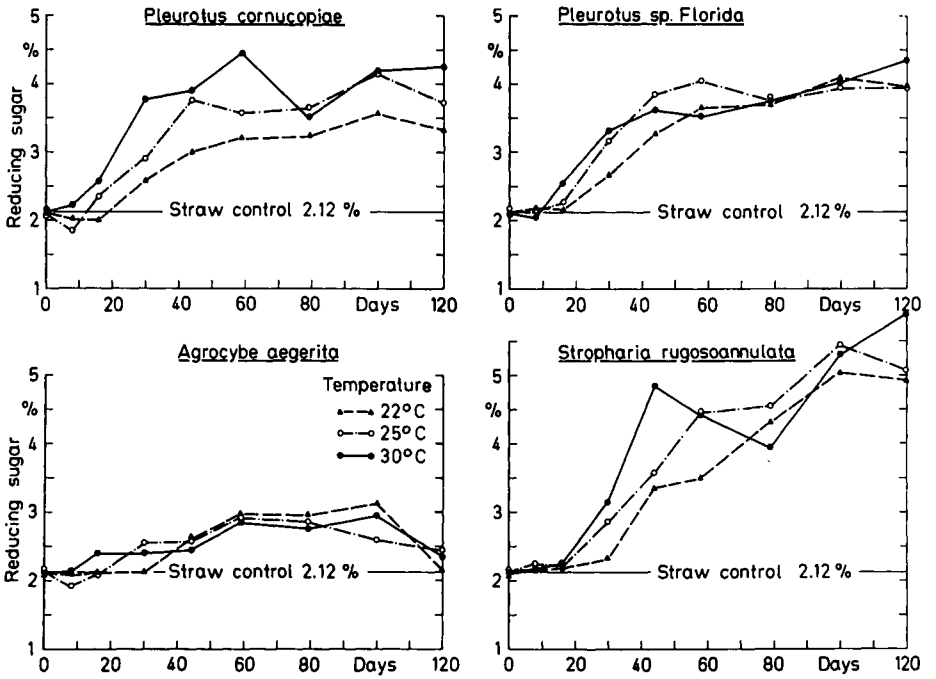

Fig. 3. Liberation of reducing sugars (in % dry weight) from wheat straw during fungal fermentation at various temperatures

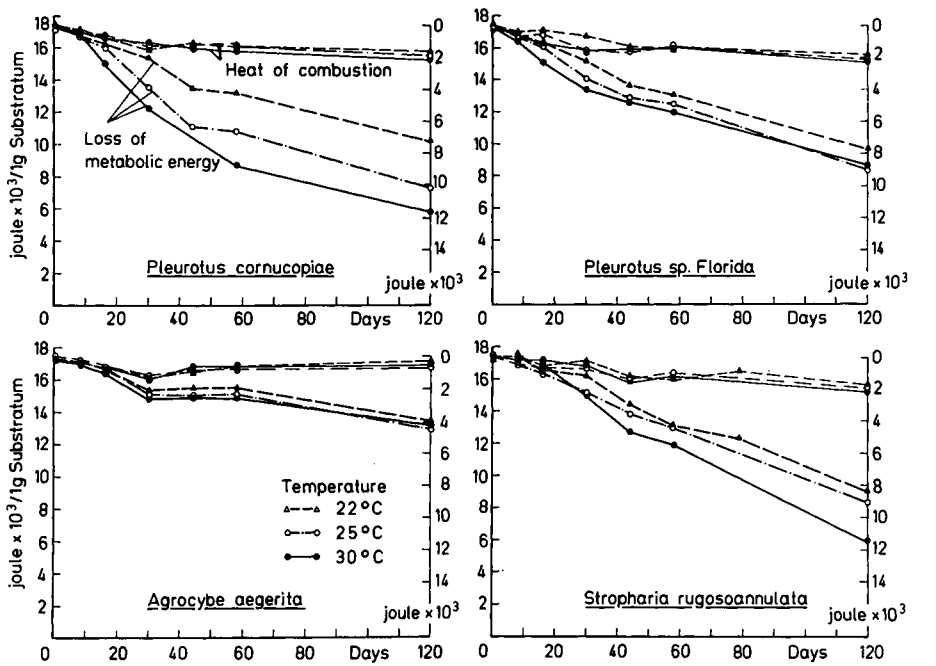

Fig. 4. Changes in the heat of combustion (joule/g substrate) and the amount of substrate energy (joule/g original substrate) of wheat straw fermented at various temperatures

Table 1. Nitrogen concentration in the straw substrate during different stages of fungal decomposition at various temperatures (reported in % of dry matter)

Fungus	Temperature	Days of fermentation		
		44 days	79 days	120 days
Stropharia rugosoannulata	22°	0.55	0.58	0.75
	25°	0.56	0.60	0.81
	30°	0.56	0.63	1.13
Pleurotus sp. Florida	22°	0.55	0.59	0.73
	25°	0.56	0.62	0.79
	30°	0.57	0.60	0.78
Pleurotus cornucopiae	22°	0.56	0.60	0.71
	25°	0.60	0.64	0.94
	30°	0.62	0.65	1.13
Agrocybe aegerita	22°	0.52	0.56	0.57
	25°	0.54	0.55	0.59
	30°	0.53	0.56	0.63

Nitrogen Accumulation During the fermentation, the amount of nitrogen in the substrate relatively increased due to carbon dioxide loss. The greatest differences in the relative nitrogen accumulation were detected after 120 days with *S. rugosoannulata* and *P. cornucopiae* (Table 1). The increase in nitrogen, however, did not correlate with the in vitro digestibility.

In vitro Digestibility. Changes in the in vitro digestibility of the straw after fungal fermentation at various temperatures are shown in Table 2. During the first growth period (20 days), the easily soluble substances of the straw were used for fungal biomass, producing a substrate more difficult to digest than the initial straw. Subsequently, fungal enzymes were released and easily soluble substances accumulated.

Table 2. Changes in the amount (g) of digestible substance in 100 g wheat straw substrate after fermentation with different fungi. Digestibility of untreated straw, which was 40%., was set equal to zero for calculation

Fungus	Temperature	Days of fermentation			
		30 days	44 days	79 days	120 days
Stropharia rugosoannulata	22°	− 5.1	3.2	15.1	20.4
	25°	− 0.7	3.3	21.1	30.5
	30°	− 0.5	14.6	28.6	31.6
Pleurotus sp. Florida	22°	− 3.9	3.3	8.3	10.7
	25°	5.4	5.9	12.0	13.1
	30°	− 2.1	− 1.6	1.0	5.0
Pleurotus cornucopiae	22°	− 7.0	− 1.7	12.7	19.8
	25°	− 4.0	− 7.4	10.6	17.0
	30°	0.2	− 2.2	− 1.6	−10.6
Agrocybe aegerita	22°	−18.9	−21.6	−17.0	−25.8
	25°	−18.1	−18.8	−19.1	−12.9
	30°	−21.9	−23.6	−24.6	−23.5

S. rugosoannulata caused the largest increase in the digestibility of straw. After 120 days of fermentation at 30°C, the digestibility of straw increased by 31.6% in comparison to the control. With *P. cornucopiae*, the largest increase in digestibility occurred at 22°C (19.8%), and with *P. sp. Florida* at 25°C (13.1%). With *A. aegerita*, the in vitro digestibility decreased at all examined temperatures. This fungus commonly has a lower rate of metabolism and yield of fruiting bodies than the other fungal species examined.

Discussion

Because of the high incrustation of cellulose with lignin, the digestibility of various types of straw and other plant materials by ruminants is very low (Millett et al., 1970; Faist et al., 1970; Baker, 1973; Han Young and Callihan, 1974). The aim of this investigation was to improve the digestibility and the feed value of plant residues by increasing the availability of carbohydrates, rather than by production of fungal protein. In this and in previous research (Zadražil, 1975, 1976), white-rot fungi, *Pleurotus cornucopiae, P. sp. Florida,* and *Stropharia rugosoannulata*, exhibited promising properties for the decomposition of lignin-cellulose containing materials and for increasing the feed value of these feeds. Lindeberg (1949) and Rypáček (1966), examined a large number of wood and soil inhabiting Basidomycetes and found that many of them were able to preferentially degrade lignin, but Lindeberg reported that lignin decomposers grew slower than cellulose-lignin decomposers.

From a technical point of view, high growth rates and high competitive saprophytic ability are desirable properties for fungi that are to be used for upgrading plant wastes to animal feed. It is apparent that fungal species, temperature, and duration of the fermentaion are important in determining the quality and in vitro digestibility of fermented wheat straw. The enhanced digestibility results from an increase in the concentration of easily soluble substances, as well as physical and other chemical changes in the straw.

The increase in the digestibility of straw during fermentation with *S. rugosoannulata, P. cornucopiae,* and *P. sp. Florida* occurred simultaneously with the liberation of water soluble substances. *Agrocybe aegerita*, however, can probably only use the readily accessible ingredients of the straw and does not sufficiently attack the cellulose-lignin structure. Straw fermented with *Stropharia rugosoannulata* (44 days at 30°C, or 79 and 120 days at all three temperatures) as well as with *Pleurotus cornucopiae* (120 days at 22 and 25°C) attained the digestibility of an average quality hay (60–70% digestibility). The most promising results were obtained with *S. rugosoannulata*, a culture that can be grown under very simple conditions (Püschel, 1971; Zadražil und Schliemann, 1975). Therefore, the technological characteristics of this fungus should be studied in regard to large scale applications since, with the large amount of sugars and other soluble substances released, the straw substrate is susceptible to colonization by other competitive microorganisms.

The results presented here do not represent the optimal conditions for using fungi to convert straw to feed, but show that if might be possible to use fungal cultures for improving the feed value of plant wastes. The use of fermented wood called 'Huempe' or 'Palo podrido', by natives in Chile for food (Phillippi, 1893) as well as for feed

(Knoche et al., 1929; Kühlwein, 1963; Grinbergs, 1975), indicates the feasibility of the suggested technique.

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