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Cultivation of *Pleurotus florida* mushroom on rice straw and biogas production from the spent straw

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Rice straw, used as a substrate for three successive crops of the fruiting bodies of *Pleurotus florida* having 22% protein, had less cellulose but more nitrogen and ash than the original straw. *In vitro* digestibility using bacterial cellulase released 4.3-fold more reducing sugars per g cellulose from spent straw than from plain straw. There was 8-fold increase in biogas production from the spent straw compared with the original when used either in 3:1 (w/w) or 1:1 (w/w) combination with cattle dung.

La paille de riz, utilisée comme substrat pour trois récoltes successives de corps fruités de *Pleurotus florida*, à 22% de protéines, contenait moins de cellulose mais plus d'azote et de cendres que la paille originelle. La digestion *in vitro* par une cellulase bactérienne, relarguait 4.3 fois plus de sucres réducteurs par g de cellulose à partir de la paille résiduaire qu'à partir de la paille originelle. On observe un accroissement de 8 fois dans la production de biogas à partir de la paille résidualle par rapport à la paille originelle lorsque celles-ci sont utilisées en combinaison avec la bouse de vache dans les proportions soit de 3:1 soit de 1:1 (p/p).

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Rice is a major cereal crop in India. Increased rice production, because of improved varieties of rice and more land under irrigation, has led to a much larger quantity of rice straw becoming available (Dhillon *et al.* 1980). At present much of it is burnt rather than being collected (Han 1978). Rice straw can be subjected to methanogenesis when in combination with cow manure, but fungal pre-treatment of straw can make it a still better substrate for methanogenesis. Use of some fungi that produce edible fruiting bodies, i.e. mushrooms, can be important when considering an economical process for disposing of rice straw (Muller & Trosch 1986).

With these observations in mind, this study reports results on the growth of *Pleurotus florida* (an edible mushroom) on rice straw and the subsequent methanogenesis of the spent straw.

Materials and Methods

Organism

Pleurotus florida was obtained from the National Mushroom Research and Development Centre, Solan (India) and maintained on malt extract agar slants.

Substrate

Rice straw was sun-dried and ground to a particle size of 1 cm^2 or less.

Cellulase

Cellulase was obtained by growing *Bacillus* sp. PDV, a therophilic soil isolate, in minimal M9 medium (Maniatis *et al.* 1982) containing 0.5% (w/v) glucose for 24 h at 37°C (200 rev/min) (Sharma *et al.* 1987). The culture was then centrifuged and the supernatant used as the enzyme source. The enzyme activity was measured by estimating reducing sugars formed by the action of enzyme on carboxymethylcellulose (Reese & Mandels 1963): one unit of enzyme activity is defined as the amount of enzyme liberating one μmol reducing sugars/min.

Mushroom Cultivation

Pennisetum typhoides (millet) grains were boiled in an equal volume of water until they were soft and excess water was then removed. The grains were mixed with 2% (w/w) $CaSO_4$ and 3% (w/w) $CaCO_3$, based on boiled grain weight, and flat bottles containing 200 g of the mixture were sterilized for 1 h at 121°C and, when cool, inoculated with *Pleurotus florida*. Extensive growth of *P. florida* which occurred after 20 days' incubation served as spawn. Rice straw was soaked in tap water, containing 75 ppm formaldehyde, for 48 h and was thoroughly mixed with 10% spawn on a wet-weight basis. The substrate/spawn mixture (500 g) was packed into polythene bags (17.5 cm × 22.5 cm), which were held at room temperature (30°C). The compact mass was regularly sprinkled with water to keep it moist after the 18th day. Three crops of mushrooms were harvested.

Chemical Analysis of Spent Straw

After the final harvest of mushrooms, a portion of spent straw was dried at 105°C for 24 h and analysed for cellulose (Uppdegraff 1969), lignin (K. E. Eriksson, personal communication), nitrogen (Kjeldahl method) and ash content.

Cellulase Digestibility of Straw

Plain and spent straw (equivalent to 25 mg cellulose) were mixed with 4 ml phosphate buffer (0.08 M, pH 6.5) containing 0.01% sodium azide and cellulase (0.86 U/ml). The incubation was carried out in 100 ml conical flasks shaking at 100 strokes/min at 55°C. Reducing sugars released in the supernatant were estimated by the dinitrosalicylic acid method (Miller 1959).

Biogas Production from Rice Straw

Both spent and plain rice straw were supplemented with cattle dung and then used for methane production. The fermentations were performed in 2 l aspirator bottles with a 1.5 l working volume. All solids were added in such an amount that the total dry matter in each bottle was 4% (w/v). Unsterilized tap water was added to make the volume 1.4 l, followed by 100 ml of slurry from an operating biogas digester. The temperature was maintained at 38°C. The biogas produced was collected in an inverted bottle. Gas analysis was done with 30% (w/v) KOH for CO_2 and pyrogallol/water (1:4, v/v) for O_2.

Results

Pleurotus florida was grown in polythene bags containing 500 g wet weight of rice straw. After 15 days, the straw became whitish and cottony, due to the profuse growth of the fungus. The bags were opened to the atmosphere after 18 days and the first batch of the mushrooms was harvested after 21 days. The second and third crop of mushrooms were harvested after 31 days and 43 days. The maximum amount of mushrooms produced was 171 g/bag and the average was 141 g. *Pleurotus florida* fruiting bodies had 22% protein on a dry weight basis (3.5% nitrogen content). After the final harvest of mushrooms, the cellulose content of the spent straw had fallen by 65% (w/w) with a concomitant increase in lignin, nitrogen and ash content (Table 1).

Table 1. Chemical composition of spent and plain rice straws.

Component	Plain straw (%)	Spent straw* (%)
Cellulose	32	11
Lignin	25	35
Nitrogen	0.4	1.5
Ash	15	30

* After supporting growth of mushrooms (3 crops) for 42 days.

Enzymatic degradation of cellulose in both spent and plain straw was examined. After 24 h incubation with a cellulase preparation (see Materials and Methods) at 50°C, spent straw, containing 25 mg cellulose, released 7.8 mg glucose compared with 1.8 mg glucose from plain straw containing the same amount of cellulose.

The daily gas production from a slurry containing plain or spent straw mixed with cattle dung (3:1, w/w) was studied (Figure 1A). Maximum gas production from spent straw took place between the 17th and the 28th day, with an average of 262 ml/day. The corresponding average gas volume from plain straw was only

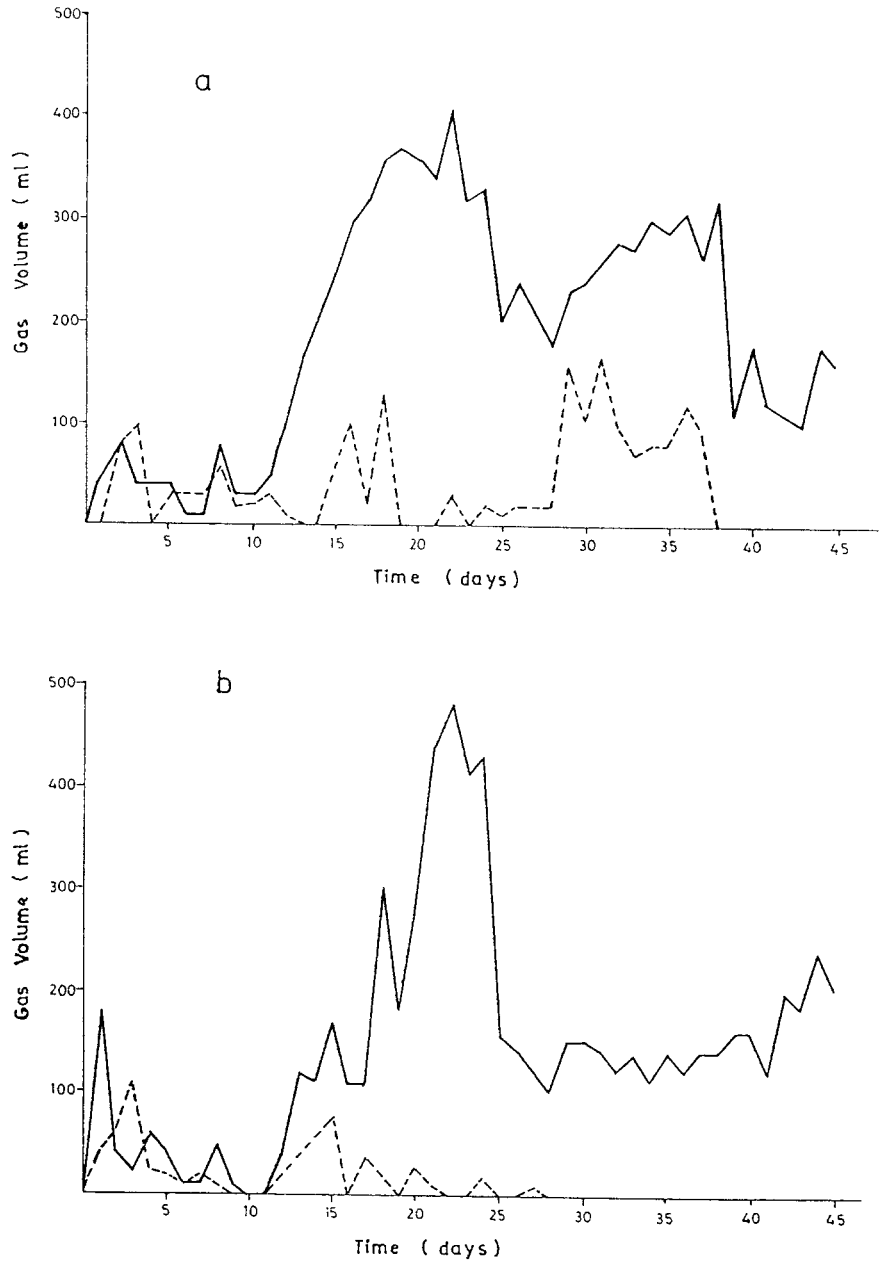

Figure 1. Daily gas production from a slurry containing spent straw and dung in the ratio of (a) 3:1 (w/w); (b) 1:1 (w/w). ——— spent straw; - - - - plain straw.

9.1 ml/day. Beyond 28 days there was no further gas production from plain straw, but with spent straw, the gas volume was 100 to 200 ml and lasted up to 45 days. Gas production from the slurry containing plain or spent straw and cattle dung (1:1, w/w) showed three peaks of activity with each type of straw (Figure 1B). Though the peaks almost coincided in the two types of straw, the total gas volumes were much lower with plain straw. The total volume of gas produced was more from the 1:1 straw/dung combination than from the 3:1 combination.

Spent straw produced 6690 ml of gas compared with 630 ml from plain straw from the slurry containing 3:1 straw/dung (Table 2). The corresponding yields from spent straw and plain straw were 8920 ml and 1820 ml, respectively, from the 1:1 straw/dung combination. The biogas produced consisted of 20% CO_2 and no discernable O_2.

Table 2. Comparison of gas yields from plain and spent straw mixed in two different concentrations with dung.

Combination	Ratio of components (w/w/dry wt basis)	Total gas yield (ml)	Gas yield/g of cellulose† (ml)	Gas yield/g of dry matter (ml)
Dung with spent straw*	3:1	6690	779	111
	1:1	8920	844	148
Dung with plain straw*	3:1	630	90	10
	1:1	1020	108	30

* Total dry matter = 60 g.

† Cellulose contents of spent straw, plain straw and dung were 11% (w/w), 32% (w/w) and 24% (w/w), respectively.

Discussion

Rice straw is a suitable substrate for growing *Pleurotus florida* mushroom with a good yield and a high protein content. This process could therefore be used in rural areas of India. Spent straw, after supporting fungal growth, had less cellulose than the original material; this has also been noticed by Bisaria *et al.* (1983) and Muller & Trosch (1986). Langer *et al.* (1980) obtained similar results when *Agaricus bisporus* was grown on wheat straw. The apparent increase in nitrogen and lignin content is due to overall loss of cellulose from the spent straw thus changing the relative proportion of the remaining constituents.

In the cellulase digestibility test, spent straw liberated 4.3 times more glucose upon treatment with cellulolytic enzymes than untreated straw, indicating it to be more hydrolysable and thus potentially more useful for subsequent microbial digestion than the initial rice straw. Indeed, spent straw proved eight times better for biogas production in an admixture with cow dung than unprocessed straw. Similarly, Bisaria *et al.* (1983) found that biological treatment of rice straw with *Pleurotus sajorcaju* enhanced the biogas yield by 54%, and a 30% increase in gas yield was obtained by using spent wheat straw after growth of *Pleurotus florida* (Muller & Trosch 1986).

The production of methane from lignocellulosic residues consists of three major phases (Bryant 1979): an initial lag phase (days 0 to 10 or 11), when polysaccharides are degraded into lower-molecular-weight substances, a major phase of gas production occurs (10 to 30 days approx.) when the smaller molecules are degraded into methane and H_2 and, finally, low gas yields beyond the peak period are due to the residual polysaccharides slowly breaking down. The peak of biogas production in this final phase was highest for the 1:1 dung/straw combination and is probably due to solids within the dung and not the rice straw, as this activity was seen with both treated and untreated straw.

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