

## Short Communication

### An Inexpensive Pretreatment of Cellulosic Materials for Growing Edible Oyster Mushrooms

#### Abstract

Artificial cultivation of oyster mushrooms on ligno-cellulosic materials involves the pretreatment of the substrate with hot water to ward off the competing molds during the spawn run. An inexpensive anaerobic pretreatment of the cellulosic materials has been suggested as an alternative to the conventional hot-water treatment. Copyright © 1996 Elsevier Science Ltd.

#### INTRODUCTION

Cultivation of oyster mushrooms on various crop residues is well known and it has been shown that *Pleurotus* spp. can be successfully grown on rice straw, wheat straw, cotton stalks and other cellulosic materials (Bano & Srivastava, 1962; Zadrazil, 1978; Balasubramanya, 1981; Chang & Quimio, 1982; Chang & Miles, 1989; Balasubramanya & Khandeparkar, 1989). It has also been suggested that *Pleurotus* is best suited for commercial cultivation in tropical and sub-tropical countries. The most important step for the successful cultivation of this mushroom is the preparation of the cellulosic material and its pasteurisation process. The cut materials are subjected to soaking in cold water followed by hot-water treatment (80°C) for 2–4 h to ward off the saprophytic fungi which normally compete with the mushroom fungus during the spawn run. In addition to this, it has also been suggested that bavistin and formaldehyde can be used for successful cultivation (Vijay, 1990). We report, in this communication, an inexpensive anaerobic treatment at room temperature to reduce/kill the competing molds during the spawn run, which could replace the conventional hot-water treatment.

#### METHODS

##### Substrates

Rice straw (*Oryza sativa* Linn.), wheat straw (*Triticum* sp.) and cotton stalks (*Gossypium hirsutum* Linn.) were obtained from the experimental farm from Akola, Maharashtra, India.

##### Culture

*Pleurotus sajor-caju* was obtained from the National Centre for Mushroom Research and Training (NCMRT), Solan, Himachal Pradesh, India, and maintained on potato dextrose agar (PDA) slants by monthly transfers.

##### Anaerobic treatment

Polythene tubes of 30 cm width were used for subjecting the materials to anaerobic treatment. Cellulosic materials cut to 3–5 cm size (1 kg in each case) were transferred in the tubes and both the ends were tied with polythene tapes and the bags were punctured with a needle at random in order to facilitate the entry of liquid in the digester. Such bags were placed in the anaerobic digester with a mixed microbial consortium maintained in cow-dung slurry (Balasubramanya *et al.*, 1994). The bags were removed after 7 days and the materials were placed in a bucket and washed with fresh tap water by shaking followed by decantation, and then seeded with grain spawn (Garcha, 1979) in the conventional bag technique. After the spawn run, the mycelial entangled bags were hung for the production of mushroom sporophores (Balasubramanya, 1981).

##### Hot-water treatment

Cellulosic materials were soaked in tap water overnight and the excess water was removed by decantation. Hot water (at 80°C) was added and allowed to cool for 2 h and thereafter the excess water was removed by decantation again. The pasteurised materials were seeded with grain spawn and mushroom sporophores were harvested as per the method detailed under anaerobic treatment.

##### Microbial enumeration

Bacteria, fungi and actinomycetes were enumerated from the samples, before and after hot-water treatment, on nutrient agar, rose Bengal streptomycin sulphate agar and Kuster's agar, respectively, using the standard dilution plate technique. Anaerobically treated materials before and after water wash were also included for microbial counts. Cellulosic materials without any treatment were directly taken in sterile water for microbial counts and served as controls.

## RESULTS AND DISCUSSION

The results on the microbial numbers after different treatments of the cellulosic materials are given in Table 1. It is clear that the microbial numbers during anaerobic treatment of cotton stalks increased, indicating the multiplication of the native flora on the cotton stalks. This could be due to the higher nitrogen content of cotton stalks (0.8%) than of rice (0.4%) or wheat straws (0.6%) (Balasubramanya & Bhatawdekar, 1988). The interesting feature of this inexpensive anaerobic treatment is that the resident microflora which normally cannot be easily dislodged from the surface of the cellulosic materials by soaking overnight in water are removed with the digester fluid. The organisms, if any, adhering loosely on the surface at the end of the treatment are removed by a simple water wash. Thus, the numbers of organisms are reduced to a significant extent (Table 1).

Another observation was that the usual molds encountered as competitors during the spawn run on hot-water-processed materials, species of *Trichoderma*, *Penicillium*, etc., were not recovered on anaerobically processed materials during the spawn run. The bacterial types recovered were mostly facultative, Gram-negative forms. Fungal forms were mostly non-sporulating ones. *Streptomyces* sp. was the sole actinomycete recovered in the study.

It is well known that cellulosic materials are softened during anaerobic treatment. Harder materials like cotton stalks and straws of rice and wheat could be profitably used after anaerobic treatment for seeding oyster mushroom spawn without hot-water treatment. The 7 day anaerobic treatment is very close to (Table 1) the conventional hot-water treatment with regard to the microbial load and hence could be adopted.

The yield of fleshy mushroom fruiting bodies of *P. sajor-caju* on hot-water-pretreated cotton plant stalks is about 500 g/kg of the dry material (Balasubramanya, 1981). The yield on rice straw and wheat straw has also been reported to be about 500 g/kg of the substrate (Bano & Srivastava, 1962; Patil *et al.*, 1989). The same yield of 500 g of mushrooms was achieved in the present investigation on the substrates cotton stalks, rice straw and wheat straw after processing through 7 days of anaerobic digestion. However, the yield decreased to 300 g/kg of the material in the case of cotton stalks when the anaerobic treatment was extended to 14 days. Rice straw and wheat straw also exhibited poor growth of *P. sajor-caju* during the spawn run on 14 days anaerobically digested materials, the yield being drastically reduced to around 200 g/kg of the material. Anaerobic treatment of the cellulosic materials beyond 14 days significantly affected the spawn run on all the three substrates and hence the anaerobic treatment should be restricted to just 7 days.

The anaerobic treatment being ecofriendly, farmers could adopt this technology on the farm itself. Depending upon the raw material availability, the size of the anaerobic digesters can be decided. For example, if one wants to spawn 100 kg of cotton plant stalks every day, it is necessary to have seven digesters of 1000 l capacity so that every day 100 kg of the material can be charged in the digesters, which means one lot of digester material is available every day for seeding with grain spawn. The requirement of 1000 l of water at 80°C to pasteurise 100 kg of cotton stalks every day can be dispensed with. This suggests that the initial investment incurred on the construction of anaerobic digesters could be recovered in less than 1 year, since the energy to heat the water to 80°C is totally avoided. This could make the anaerobic pretreatment inexpensive. Since

Table 1. Microbial number from different cellulosic materials<sup>a</sup>

Materials		Bacteria <sup>b</sup>	Fungi <sup>b</sup>	Actinomycetes <sup>b</sup>
Cotton stalks	1	40 × 10 <sup>d</sup>	6 × 10 <sup>f</sup>	40 × 10 <sup>d</sup>
	2	123 × 10 <sup>e</sup>	69 × 10 <sup>f</sup>	135 × 10 <sup>f</sup>
	3	4 × 10 <sup>c</sup>	—	—
	4	12 × 10 <sup>c</sup>	12 × 10 <sup>c</sup>	6 × 10 <sup>c</sup>
Rice straw	1	36 × 10 <sup>e</sup>	6 × 10 <sup>f</sup>	14 × 10 <sup>f</sup>
	2	146 × 10 <sup>d</sup>	139 × 10 <sup>d</sup>	15 × 10 <sup>f</sup>
	3	22 × 10 <sup>c</sup>	—	—
	4	6 × 10 <sup>d</sup>	2 × 10 <sup>c</sup>	3 × 10 <sup>c</sup>
Wheat straw	1	60 × 10 <sup>f</sup>	64 × 10 <sup>f</sup>	60 × 10 <sup>e</sup>
	2	89 × 10 <sup>f</sup>	29 × 10 <sup>f</sup>	77 × 10 <sup>e</sup>
	3	39 × 10 <sup>c</sup>	—	—
	4	26 × 10 <sup>d</sup>	4 × 10 <sup>c</sup>	2 × 10 <sup>c</sup>

<sup>a</sup>Expressed as number per g of material on moisture-free basis.

<sup>b</sup>Average of three replications.

<sup>c</sup>Control, untreated materials.

<sup>d</sup>Anaerobic treatment (AT).

<sup>e</sup>Hot-water treatment.

<sup>f</sup>AT+water wash.

—No organisms.

the competing molds during the spawn run do not pose problems on anaerobically pretreated cellulosic materials, the pesticide requirement can be eliminated, thus preventing the problem of pesticide residues in the mushroom sporophores. The technology could, therefore, be taken up even by commercial mushroom growers, who could save energy spent on the pasteurisation process.

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