Establishment of the white rot fungus *Phanerochaete chrysosporium* on unsterile straw in solid substrate fermentation systems intended for degradation of pesticides

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**Summary**

The effects of different inoculum-loading rates and pre-treatment of wheat straw with formic acid and hot water (50 °C) on the establishment of *Phanerochaete chrysosporium* on unsterile straw were studied in laboratory scale and in a 1.5-m³ bioreactor. The establishment of *P. chrysosporium* on unsterile straw was satisfactory. *Phanerochaete chrysosporium* and other fungi, which developed simultaneously, were able to produce the activity necessary to degrade two herbicides, bentazon and MCPA (4-chloro-2-methylphenoxacyetic acid) in 20 days (65 and 75%, respectively). The decrease of both herbicides coincided with the presence of the activity of the lignin-degrading enzymes lignin peroxidase and manganese peroxidase/laccase. Extensive growth of *P. chrysosporium* or other lignin-degrading fungi on unsterile straw would be excellent for inexpensive solid substrate systems intended for degradation of pesticides.

**Abbreviations:** DMAB – 3-(dimethylamino)benzoic acid; LiP – lignin peroxidase; MBTH – 3-methyl-2-benzothiazolinone hydrazone; MnP – manganese peroxidase; MEA – malt extract agar; SSF – solid substrate fermentation.

**Introduction**

The extensive use of improperly managed pesticides and other chemicals leaves residues in surface and ground water as well as in sewage (Kreuger 1996, 1997). The white rot fungus *Phanerochaete chrysosporium* is well known for its ability to degrade lignin, through the action of lignin peroxidase (LiP), manganese peroxidase (MnP) and a peroxide-producing system, which attacks lignin unspecifically (Kirk & Farrell 1987). Lignin biodegradation is part of a secondary metabolism, which is triggered when carbon, nitrogen or sulphur becomes limiting. Lignin is unable to serve as the sole carbon and energy source, so, in order for lignin degradation to proceed an additional, more readily available, source of carbon is required by the fungus.

It has been demonstrated that the same system also, at least in part, degrades diverse organic compounds. Highly persistent compounds such as benzo(a)pyrene, lindane, DDT, dioxins and PCBs are among the compounds degraded by the fungus (Bumpus et al. 1985).

In an earlier paper (Castillo et al. 2000) we reported on the degradation of herbicides by *P. chrysosporium* supported by straw in solid substrate fermentation. The system was based on the growth of the fungus on autoclaved straw on a laboratory scale. Possible biotechnological application of this system may require the use of unsterile straw, because the preparation of large amounts of sterile substrate is energy-consuming and expensive.

The aim of the present work was to study the establishment of the fungus *P. chrysosporium* on unsterile straw. The effect of inoculum-loading rate and pre-treatment of the straw with hot water or formic acid was studied. In addition, the usefulness of the technique was tested by following: (a) the growth of *P. chrysosporium* on unsterile straw; (b) the degradation of the herbicides bentazon and MCPA; and (c) the activity of ligninolytic enzymes, in a 1.5-m³ bioreactor.

**Materials and methods**

**Fungi and inocula**

*Phanerochaete chrysosporium* BKM-F-1767 was kindly obtained from T.K. Kirk, Forest Products Laboratory,
Madison, Wisconsin and maintained at room temperature on 2% malt agar slants. Subcultures were routinely made every 30–60 days. The fungus was grown on Petri dishes with 3% malt extract agar (MEA) at 37 °C for 1 week. The inoculum was prepared by placing wheat grains in water and boiling for 15 min. The grain (200 g) was then weighed into a 500 ml Erlenmeyer flask, CaCO₃ (2 g) was added and the flask was autoclaved for 30 min at 121 °C. After cooling, the grain was inoculated with agar plugs (1.3 cm diameter) cut from the malt agar plates. The flasks were kept at 37 °C for 10 days and flushed for 30 min daily with humidified air. The inoculum for the 1.5-m³ bioreactor was prepared in the same way, but 5-L Erlenmeyer flasks with 1 kg wet wheat grains and 10 g of CaCO₃ were used.

**Chemicals**

MBTH (3-methyl-2-benzothiazolinone), DMAB (3-(dimethylamino) benzoic acid) and veratryl alcohol (3,4-dimethoxybenzyl alcohol) were supplied by Aldrich Chemical Co., Germany. Bentazon (3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one2,2-dioxide) and MCPA (4-chloro-2-methylphenoxyacetic acid) (Figure 1) were supplied by Dr. Ehrenstorfer GmbH, Germany. Basagran-MCPA was supplied by BASF, Germany. Cellulose, in form of ground newspaper, was used as a more readily utilizable carbon source. All other chemicals were supplied by KEBO AB, Sweden.

**Cultivation of P. chrysosporium on unsterile straw in laboratory scale**

For testing the effect of the inoculum-loading rate, polyethylene bags were filled with wheat straw (50 g dry matter, 3–5 cm long), 200 ml tap water (4 ml/g straw), 0.46 g of diaminotetramine tartrate, 0.50 g CaCO₃ and 2.5 g cellulose. The bags were plugged with a cotton stopper and left overnight. Different amounts of inoculated wheat grains were added: 0, 5, 10, 30 and 40% (w/w of dry weight). All bags were incubated at 20 °C and flushed for 30 min daily with humidified air. Sampling was made at days 0, 5, 10, 20, 30, 40 and 60.

To the study of the effect of formic acid, wheat straw was soaked overnight with 4 ml H₂O + 9 µl formic acid per gram dry weight. Soaked straw (60 g dry matter), 2.5 g cellulose, 0.22 g diaminotetramine tartrate and 40% inoculum (w/w dry matter) were mixed in polyethylene bags, incubated at 20 °C and flushed for 30 min daily with humidified air. Sampling was made at days 0, 7, 14, 20 and 30.

To the study of the effect of hot water, the wheat straw was rinsed with hot tap water (50 °C). An additional 1 ml H₂O per gram of straw was added and left over night. Soaked straw (60 g dry matter) was packed in plastic bags with 2.5 g cellulose, 0.22 g diaminotetramine tartrate and 40% inoculum (w/w dry matter). The bags were incubated at 20 °C and flushed for 30 min daily with humidified air. Samples were taken at days 0, 7, 14, 20 and 30.

The bioreactor used in this experiment was used previously for composting of liquid manure (Thyselius 1986). Figure 2 shows a diagram of the plant used. The reactor had a diameter of 0.8 m, a height of 3.0 m and a total volume of 1.5 m³. An auxiliary tank for storage of the recirculation liquid was used. A total of 31.5 kg (0.734 m³) wheat straw (89.4% dry matter, 5–10 cm long) was fed to the bioreactor (day 0). Water (170 l) was added and recirculated 1 day before addition of 21 cow manure (4 g nitrogen per liter). The reactor was flushed with air for 2 h every day. The hydraulic detention time was 4 h. After 18 days the reactor was inoculated with 40% (w/w dry matter) of wheat grains with *P. chrysosporium*. At day 72, after the fungus was established on the straw, the herbicide Basagran-MCPA was added to the recirculation liquid at an initial concentration of 220 mg/l for bentazon and 110 mg/l for MCPA. Liquid samples were taken within a period of 20 days and tested for degradation of bentazon and

![Figure 1. Chemical structure of bentazon and MCPA.](image1)

![Figure 2. A diagram of the 1.5-m³ bioreactor.](image2)