

Assessing biodeterioration in wood using ATP photometry. Part III. Estimation of the fungal biomass of *Phanerochete chrysosporium* in decayed wood using ATP and energy charge measurements

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Abstract

Measurements of ATP and Energy Charge (EC) were used to estimate the changes in biomass of *P. chrysosporium* (white rot), grown in wood blocks. The wood blocks were inoculated with *P. chrysosporium* and incubated for 3 months at 25°C and 95% RH. The blocks were sampled every month for concentrations of nucleotides (ATP, ADP, and AMP), EC, and weight loss. The possible interference of wood chemicals with the nucleotide assay was studied. EC levels for the three fungi remained below 0.52. The ADP and AMP concentrations were at least 10-fold larger than the ATP. The ATP data were converted into biomass using a conversion factor (ATP/Biomass = 0.99 nM/mg of dry weight for EC values below 0.6). *P. chrysosporium* produced a larger biomass when grown on loblolly (167 µg/g of wood) than on birch (40 µg/g of wood) but the weight loss of the wood was larger in the birch blocks (16% vs 14.73%). © 1998 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Indirect chemical methods to estimate microbial biomass are based upon the measurement of a structural or functional chemical component, present in microbial cells. Fungal biomass can be estimated by using measurements of ergosterol (Newell et al., 1987) or chitin (Ride and Drysdale, 1972) as well as with methods used for bacteria (e.g., protein and total N). ATP measurements, together with the Energy Charge (EC) values of the mycelia, have been found to be a better estimate of fungal biomass than protein and chitin (Nieto et al., 1997b).

There are three different types of wood decay caused by fungi. White rot is caused by the degradation of lignin by Basidiomycete fungi. Some species of white rot decay both lignin and cellulose (Blanchette et al., 1990). Brown rot occurs when the cellulose and the hemicellulose of the cell wall is removed by other Basidiomycete fungi (Blanchette et al., 1990). Finally, soft rot is the result

of the degradation of cellulose from the wood, by Ascomycete or Fungi Imperfecti (Blanchette et al., 1990).

In this study ATP concentrations and EC were used to estimate the fungal biomass in wood decayed *in vitro*. Extracts from plant matter are rich in phenolic compounds which combine with proteins reversibly by hydrogen bonding and irreversibly by oxidation followed by covalent condensations (Loomis, 1974). This may result in inactivation of assay enzymes (e.g., luciferase). The interference of chemical compounds found in wood using the nucleotide assay was studied.

2. Materials and methods

2.1. Wood block test

Two types of wood (birch and loblolly) were used. The wood blocks were 2 cm × 2 cm × 1 cm. The wood blocks were dried in the oven at 60°C until the dry weight was constant. The dry weights were recorded, and the blocks were then rehydrated in distilled water overnight. Then they were dry-blotted with a paper towel. The blocks

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were placed in screw-capped jars containing 15 cm³ of vermiculite and 10 ml of distilled water. The jars were then autoclaved at 120°C for 45 min, and placed in a humidified chamber at 98% RH until inoculated. The water content of the wood was 50%.

Phanerochaete chrysosporium (Forest Products Laboratory BKMF 1767) (white rot), was grown for 4 days in Malt Extract Broth (MEB) at 25°C on a shaker. The liquid cultures were inoculated using a 1 cm plug from malt extract agar (MEA) plates. The 4-day-old mycelium was used to inoculate the wood blocks. Four blocks (three replicates and one control, uninoculated) were sampled after 1, 2, and 3 months, for nucleotide concentrations and for substrate degradation (loss of weight).

2.2. Interference by wood constituents

Two different types of wood, birch (hardwood), and loblolly pine (softwood) were used. The wood was ground in a Wiley mill to pass through a size 60 sieve (2 mm). *P. chrysosporium* was cultured in 20 ml of malt extract broth (MEB) in 25 cm² tissue flasks for 6 days at 25°C. The flasks were inoculated using plugs of stock cultures (1 cm in diameter) maintained in malt extract agar (MEA). Five hundred milligrams of ground wood were added to the cultures (approximately a 10:1 ratio of wood to mycelium). The nucleotides were then extracted overnight with 40 ml of 5% TCA. The control cultures also were extracted using 40 ml of 5% TCA, but wood was not added. The nucleotide concentrations were measured as described in Nieto et al. (1997b). Uninoculated blanks and controls without wood were also extracted. Three more replicates were used to measure the dry weight of the cultures.

2.3. Wood decay

The wood decay tests were disassembled. The blocks were dried in a convection oven at 60°C to a constant dry weight. The weight loss was calculated by subtracting the dry weights before and after incubation.

2.4. Nucleotide measurement

Each block was cleaned of superficial fungal growth using a heat-sterilized razor blade and then ground in a Wiley Mill to pass through a size 60 sieve (2 mm). The nucleotides were extracted from the ground wood using 20 ml of 5% TCA; extraction was overnight at 0°C (Nieto et al., 1997a). A 1 ml aliquot was removed and stored until assayed. The assay procedure for the measurement of the ATP, ADP, and AMP concentrations has been already described in Nieto et al. (1997b).

3. Results

3.1. Synergism by wood constituents

There was more interference in birch samples than those with loblolly pine (Figs 1 and 2). The differences between the control and experimentals were tested using a one-way ANOVA. No significant differences between the controls and the experimentals were found for either of the nucleotides and wood types ($\alpha=0.05$).

3.2. Wood block tests

The total ATP of the mycelium of *P. chrysosporium* increased with time which was correlated with increases in biomass of the mycelium (Figs 3 and 4). The largest ATP concentrations were found in *P. chrysosporium* grown in loblolly pine (ATP ranged from 1.53×10^{-12} to 3.22×10^{-10} mol/g of wood). The concentrations of ADP and AMP were at least 10-fold greater than the ATP. The EC values found were less than or equal to 0.52, which indicates low activity of the mycelium (Fig. 5).

The ATP concentrations were converted into biomass using a conversion factor for this particular range of EC (ATP/dry weight = 0.99 nM/mg of dry weight) (Nieto et al., 1997b). The biomass produced by *P. chrysosporium* was larger when grown in loblolly than in birch (325.25 and 87.58 μg of dw/g of wood, respectively; Fig. 6).

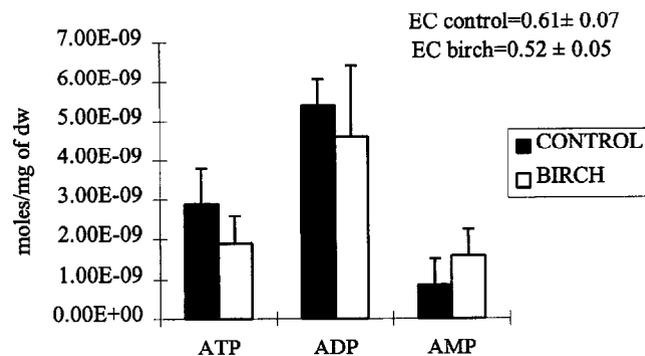


Fig. 1. Wood interference with the nucleotide assay (birch).

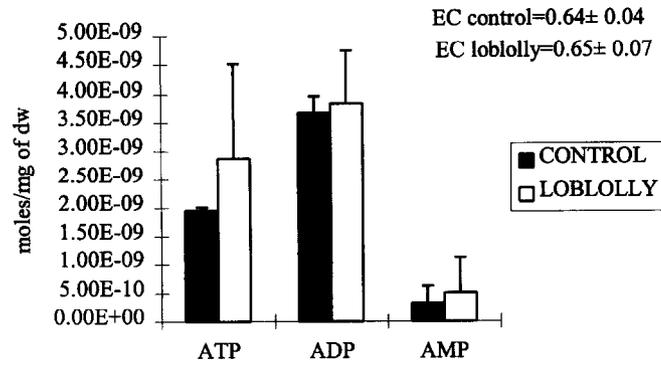


Fig. 2. Wood interference with the nucleotide assay (loblolly).

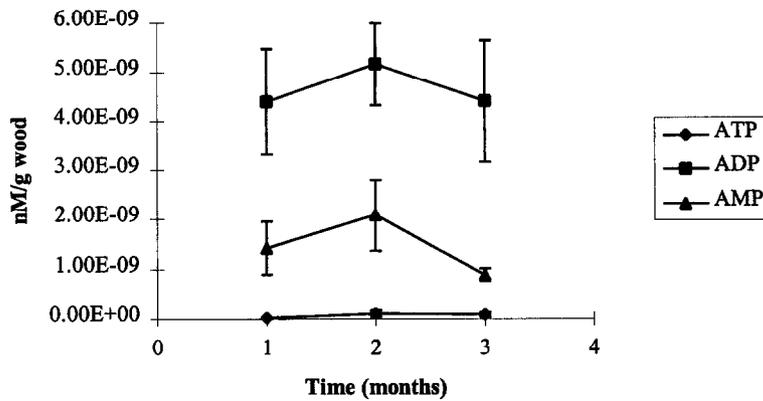


Fig. 3. Changes in nucleotide concentrations in *P. chrysosporium* grown on birch.

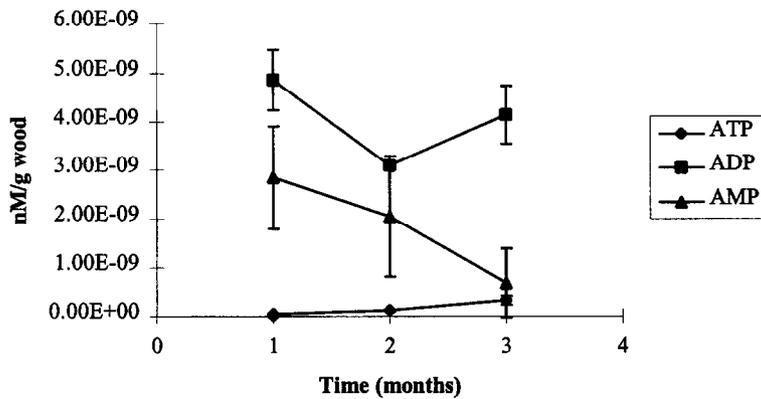


Fig. 4. Changes in nucleotide concentrations in *P. chrysosporium* grown on loblolly pine.

3.3. Weight loss

The wood colonized by *P. chrysosporium* lost 5% of its weight per month. *P. chrysosporium* degraded more birch than it did loblolly pine (16% vs 14.73%; Table 1 even though its biomass was larger in loblolly (325.25 μg of dw/g wood; Fig. 6) than it was on birch blocks (87.58 μg of dw/g wood; Fig. 6).

4. Discussion

Fungal biomass estimates calculated using ATP values were in consonance with the data on weight loss. Larger weight losses were associated with larger biomass estimates for a given wood type. The amount of wood decayed by *P. chrysosporium* was significant (5% loss per month). Although there was greater biomass of *P.*

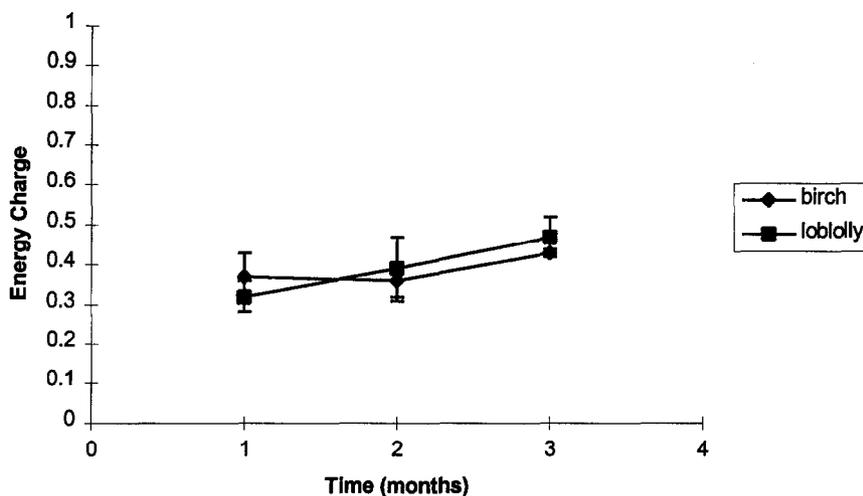
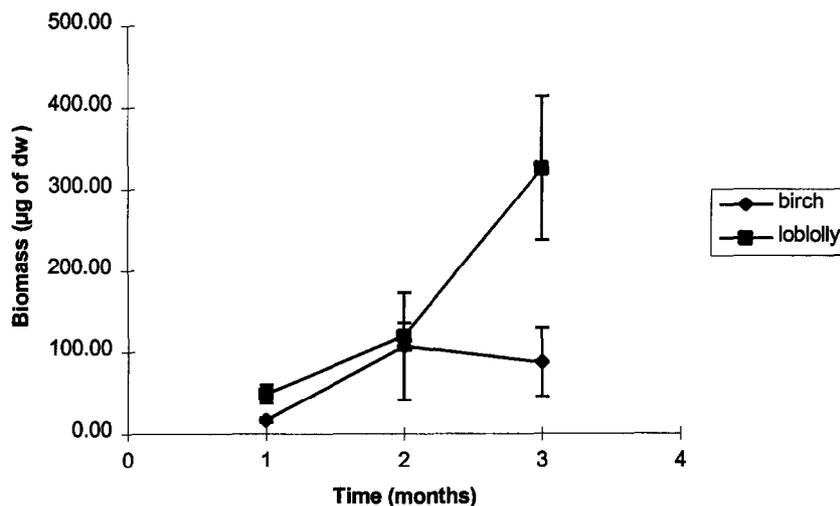
Fig. 5. Energy charge changes of *P. chrysosporium*.Fig. 6. Changes in biomass of *P. chrysosporium*.

Table 1
Percent weight loss of wood incubated with *P. chrysosporium*

	1 month	2 months	3 months
Birch	5.22 ± 0.15	11.33 ± 1.53	16.00 ± 1.73
Loblolly	3.94 ± 1.31	9.23 ± 1.33	14.73 ± 3.35

chrysosporium when it grew in loblolly pine than when it grew in birch, the weight loss was smaller.

Neither of the types of wood interfered with the nucleotide determinations. This is not always the case, and in a previous study it was found that the presence of wood interfered with a chromatographic assay of nucleotides

(Nieman et al., 1978). ATP assay and EC estimation together provide a rapid and accurate estimate of the biomass and its phase of growth. Fungi with EC values below 0.7 are still active. In spite of EC as low as 0.36 *P. chrysosporium* was capable of decaying the wood block at a rate of 5% per month. EC values are important in understanding the estimates of fungal biomass. The weight loss for loblolly pine wood blocks incubated with *P. chrysosporium* was smaller than that of the birch blocks. Softwoods have a higher lignin content, and it is thought to be a defense against decay.

There have been other attempts to estimate fungal biomass in natural samples. Jellison and Goodell (1989) used ELISA to detect and quantify fungal biomass in wood. Their procedure involves the preparation of mono-

clonal antibodies against every species of fungi involved. Although they were able to detect the fungus (*Poria placenta*) they could not get rid of cross-reactions with other species. More recently Johnston and Aust (1994) have used PCR and restriction analysis to detect *P. chrysosporium* in soil samples. They also experienced difficulties in differentiating between species within the genus *Phanerochaete*. These methods are time-consuming, expensive and require a well equipped laboratory. The method described in this paper is rapid, inexpensive and provides an accurate estimation of total biomass. Therefore, we conclude that nucleotide measurements are a rapid and accurate method to quantify fungal biomass in wood.

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