



Formulation of a liquid medium with wheat bran for the production of laccase by *Trametes versicolor* in an air-lift bioreactor

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Abstract

The aim of this work was to formulate a liquid medium with wheat bran for the production of *Trametes versicolor* laccase in an air-lift bioreactor. Univariate experiments were carried out in order to optimize the contents of CuSO₄ (0-2 mM), glucose (0-50 g/L), peptone (0-25 g/L) and fermentation parameters as pH (3-7), inoculum (40-360 mycelium plugs/L) and aeration (0-1 v/v/m). After the optimization an optimized experiment was accomplished (pH 3, inoculum of 40 mycelium plugs/L, CuSO₄ concentration of 1.2 mM, 10 g/L of peptone and the aeration of 0.5 v/v/m) in an air-lift reactor. The highest activity (32,000 UI/L) was reached in the 10th experimental day.

Key words: Laccase, enzyme production, air-lift bioreactor.

Introduction

Laccases (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) are the most extensively studied group of enzymes among oxidases. They belong to the family of blue multicopper oxidases, which catalyze the one-electron oxidation of four reducing-substrate molecules concomitant with the four-electron reduction of molecular oxygen to water ¹.

These enzymes were known to catalyze the oxidation of a wide range of phenolic compounds and aromatic amines. In the last years, due to their potential application in biotechnological processes, laccase utilization was investigated for toxic-pollutant², industrial-dye degradations ³ and wood-pulp delignification ⁴.

White rot fungi are the best-known laccase producers, and there are also certain bacteria and actinomycetes that are known to produce laccases. Owing to vivid biotechnological applications, studies on laccase producing organisms have been intensified in the recent years and the optimization of laccase production from different microorganisms is being carried out by several groups. Among basidiomycetes, *Trametes versicolor* was reported to produce laccase as the predominant lignolytic enzyme. This white-rot fungus has been used in investigations of dyes decolorization and in soil bioremediation ^{5,6}.

Experiments for reducing the costs of laccase production are essential for the increase of its industrial application. Another approach is the overproduction of laccase in a suitable host. A good strategy to increase the productivity of the laccase fermentation process is the optimization of the fermentation medium and then enhancing laccase activity by using inducers. In particular, the selection of an appropriate carbon source is crucial in the development of an efficient and economic process; 2,5-xylidine, veratryl alcohol and ethanol are inducers that resulted in

a 20-fold increase of laccase production in a *Trametes versicolor* submerged bioprocess ⁷.

Wheat bran is an abundant agricultural residue that has been used in bioprocess experiments. This agricultural residue is rich in carbohydrates, proteins and polyphenols and can be used for the development of filamentous fungi without any nutritional supplementation ⁷.

The air-lift bioreactor has a good oxygen transference and agitation. These conditions are important for laccase production. Ryan *et al.* ⁸ developed an air-lift reactor, which allowed the production of high levels of laccase from the white-rot fungus *Trametes pubescens*.

The aim of this work was to formulate a liquid medium from an aqueous extraction of wheat bran for production of laccase by *Trametes versicolor* in an air-lift bioreactor.

Materials and Methods

Microorganism: *Trametes versicolor* (CCT 4521) was obtained from the fungi collection from University of Campinas, São Paulo, Brazil. This strain was maintained on MEG agar slants (5 g/L malt extract, 10 g/L glucose, 20 g/L agar) at 4°C.

Submerged fermentation (SmF) in air-lift bioreactor: The culture medium was prepared using the water soluble components (80°C) of wheat bran (30 g), pH 5, inoculum 150 mycelium plugs (5 mm diameter mycelium discs obtained from MEG agar slants previously inoculated with *T. versicolor*, 7 days) and aeration of 0.5 v/v/m (air volume per reactor volume per minute) of filtrated air (4 µm). The air-lift bioreactor used in this study employs an internal tube (riser) and an annular space. The working volume of this bioreactor was

350 mL and the temperature (25°C) was controlled by water circulation through the annular jacket surrounding the reaction zone. The aeration rate was measured by a flow meter.

Optimization of submerged fermentation (SmF): Uni-variates studies were carried out in order to optimize the content of CuSO₄ (0-2 mM), glucose (0-50 g/L) and peptone (0-25 g/L) and also pH (3-7), inoculum (40-360 mycelium plugs /L) and aeration (0-1 v/v/m). The optimization experiments lasted 72 hours and the optimized experiment lasted 19 days.

Enzyme assay: Laccase activity was determined by measuring the oxidation of 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) at 420 nm ($\epsilon = 3.6 \times 10^4 \text{ cm}^{-1} \text{ M}^{-1}$). The reaction mixture contained 0.4 ml of 1 mM ABTS and 1.2 mL of 0.1 M glycine-HCl buffer pH 3.5 and 0.8 ml aliquots of appropriately diluted culture fluid. One laccase activity unit was defined as the amount of enzyme that oxidized 1 mmol ABTS per min. The activities were expressed in U/mL. All the values are the means of duplicate experiments⁹.

Results

In the optimization experiment (Fig. 1), pH of 3.0 and 4.0 were the most appropriated for laccase production, however, pH 7.0 presented a negative influence on biomass. Inoculum of 40, 120 or 200 plugs/L presented the similar enzyme levels, however, 360 plugs/L decreased the enzyme levels. Glucose inhibited the laccase production and, as expected, caused biomass augmentation. The best content for laccase production was 10 g/L, although it did

not have influence on biomass production. Cu²⁺ of 1.25 mM was the best concentration for the enzyme production, but concentrations higher than 0.4 mM reduced the biomass production. Aeration presented an important condition for biomass production, levels of 0.5 and 1 v/v/m was the best condition for enzyme and biomass production.

After these optimization, an experiment was accomplished in an air-lift bioreactor using pH 3, inoculum of 40 "mycelium plugs"/L, CuSO₄ concentration of 1.2 mM, 10 g/L of peptone and the agitation of 0.5 v/v/m. The bioprocess in the air-lift reactor produced maximal activities of about 32,000 U/L, in the 10th experimental day (Fig. 2).

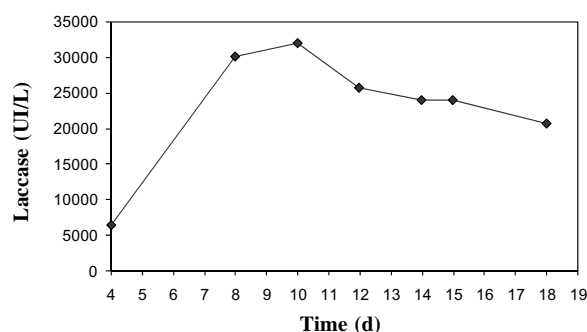


Figure 2. Optimized experiment for laccase production.

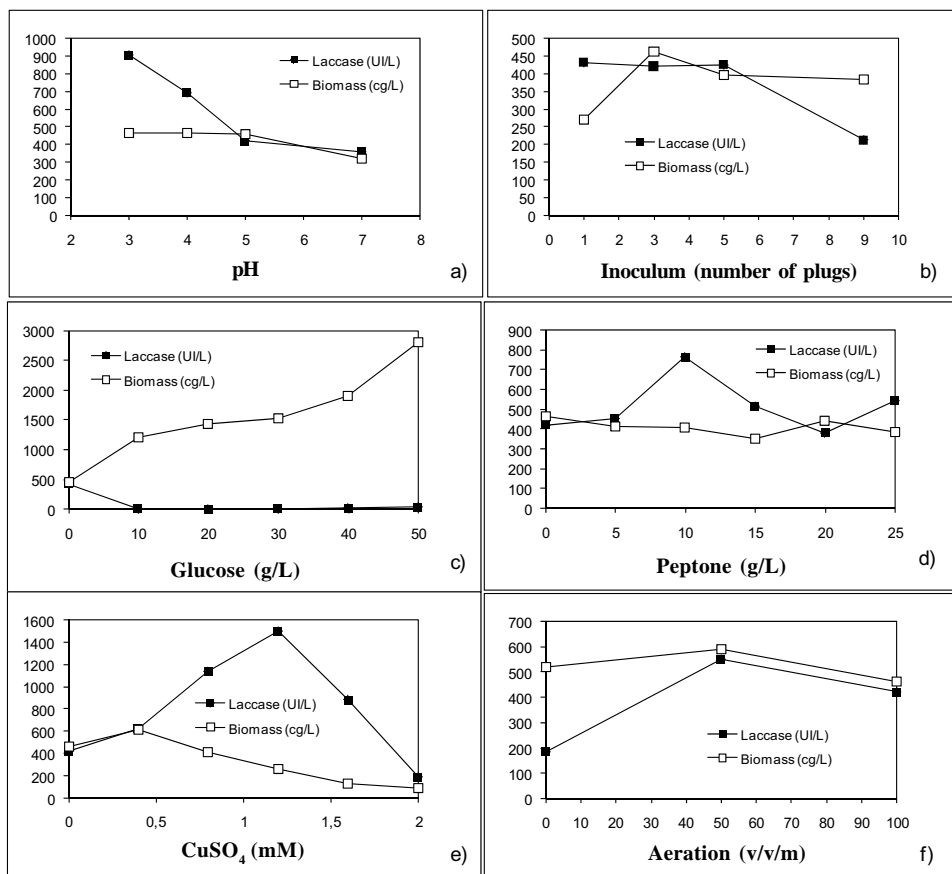


Figure 1. Results from the optimization experiments for laccase production.

Discussion

The information established in the literature shows that mild agitation, carbon source control, phenolic inducers and the addition of copper to the culture medium favor the laccase production at reactor scale^{5,6}.

In this investigation, carbohydrates and phenolic compounds from wheat bran were the main carbon source and laccase inducers, respectively. This study pointed out that the nature of lignocellulosic material and the method of fungi cultivation were factors determining the expression of laccase.

The starch, cellulose and other carbohydrates, presented in the wheat bran, are the same carbon sources that white rot fungi find during the wood degradation. However, during the wood degradation it is necessary to remove/transform lignin for the progress of the fungi in new substrates. The oxidative enzymes, including laccase, have been described as responsible for this action. The excellent results obtained with wheat bran for laccase production can be explained by its composition, wheat bran supports enough starch that is necessary for the fungal development and allows the contact between cellulose/aromatic compounds with the white rot fungi during the stationary phase inducing the laccase production.

Laccase has copper in its composition and the copper addition to the culture medium enhanced laccase production at reactor scale. Among the studied factors, CuSO₄ content showed the strongest positive influence. The glucose inhibited the laccase production and it can be explained because this sugar makes longer the exponential growth phase of the fungi delaying the laccase production that occurs in the stationary phase.

The typical reported strains produce laccase activity in the range of 4,000-100,000 U/L¹⁰⁻¹⁴. Cordi *et al.*¹⁶ using this same strain and a synthetic medium containing 2,5-xylidine and copper sulfate achieved a maximum laccase activity of 40,774 U/L on the 12th day. In our study, using an inexpensive natural medium and the air-lift bioreactor, laccase activity of 32,000 U/L was reached.

On the whole, it can be asserted that the combination of environmental conditions (suitable inducers, feeding medium composition, aeration, agitation system, etc.), culture technique and bioreactor design should be taken into account to produce high amounts of laccase¹⁶⁻¹⁹. In these experiments, the utilization of wheat bran as substrate and the utilization of an air-lift bioreactor was adequate to get high levels of laccase.

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