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Development of a stirred tank reactor system for the production of lignin peroxidases (ligninases) by *Phanerochaete chrysosporium* BKM-F-1767

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SUMMARY

Lignin peroxidases produced by *Phanerochaete chrysosporium* have several important potential industrial applications based on their ability to degrade lignin and lignin-like compounds. A stirred tank reactor system for the production of lignin peroxidases is described here. Included in this study is an examination of the mechanics of pellet biocatalyst formation and the optimization of an acetate buffered medium. Higher levels of lignin peroxidase were obtained with acetate buffer compared to the other buffer systems tested. Concentrations of 0.05% (w/v) Tween 80 and 0.4 mM veratryl alcohol gave optimal lignin peroxidase activity in acetate buffered medium. In shake flask cultures, mycelial fragments in the inoculum aggregated into pellets during the first eight hours of incubation and thereafter increased in size through the eighth day. The agitation rate in shake flask cultures affected pellet size, the number of pellets formed, and lignin peroxidase activity. Transfer of fungal pellets from shake flask culture to a continuously oxygenated baffled stirred tank reactor (STR) resulted in production of high lignin peroxidase titres comparable to those of shake flask cultures when the agitation rate, oxygen dispersion and foaming were closely controlled.

INTRODUCTION

Lignin peroxidases [19] are extracellular, hydrogen peroxide-dependent heme proteins which catalyze the oxidation of the C_α-C_β linkages in lignin, a major constituent of all vascular plants and the second most abundant renewable organic polymer in the biosphere. These enzymes are produced during idiophase in nitrogen or carbon-limited cultures of certain white rot fungi, as exemplified by the basidiomycete *Phanerochaete chrysosporium* [11]. Lig-

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nin peroxidases have several potential industrial applications in biopulping, detoxification of recalictrant organopollutants such as DDT and dioxins [3,4,7], degradation of pulp and paper mill effluents [10], and pretreatment of lignocellulosic biomass for efficient bioconversion to feeds, fuels and chemicals [6,18]. However, an important limitation affecting the development of applications is that lignin peroxidase is produced at low levels by the currently available strains of *P. chrysosporium* and culture conditions. Scaled up production of lignin peroxidases is a prerequisite for testing them in various industrial applications.

The first production of lignin peroxidases in the laboratory was in shallow ten ml stationary cultures of *P. chrysosporium* [19]. Initial attempts to produce lignin peroxidase in submerged cultures were unsuccesful due to the organism's sensitivity to agitation. Recently it has been shown that the addition of surfactants, particularly Tween 80, enables *P. chrysosporium* to produce lignin peroxidase under mildly agitated conditions [9]. This suggested that lignin peroxidases might be produced in stirred tank reactor systems. A rotating biological contactor has recently been used to produce lignin peroxidase from adsorbed fungal films [12]. However, fungal films are somewhat difficult to engineer and control beyond the laboratory scale. Production of lignin peroxidase also has been reported in a column reactor using mycelial pellets [17], in an air-lift reactor [16], and on polyurethane foam [14]. The development of a stirred tank reactor system for lignin peroxidase production has not been described to date. For many organisms, this type of reactor can be scaled-up readily beyond the laboratory size and culture parameters such as pH, dissolved oxygen and shear can be measured and controlled.

The objective of this study was to develop a stirred tank reactor as a first step in the large scale production of lignin peroxidases. This paper describes improvements to the culture media, the mechanism of pellet formation, and the effects of agitation on pellet formation and lignin peroxidase production.

MATERIALS AND METHODS

Organism and media

Phanerochaete chrysosporium strain BKM-F-1767 (ATCC #24724) was used because of its reported consistency in producing the lignin peroxidase enzyme system in high yield [9]. Cultures were grown in a nitrogen limited medium as described by Jager et al. [9] with ammonium tartrate as the nitrogen source (2.2 mM) and, where indicated, sodium acetate buffer (20 mM), tartrate (10 mM), oxalate (10 mM), succinate (10 mM), or citrate (10 mM) buffers. All the buffers were at pH 4.5. Where indicated, veratryl alcohol (# D13,300-0, Aldrich Chemical Co., 940 West St. Paul Ave., Milwaukee, WI, 53233), an inducer of lignin peroxidase [5], and Tween 80 (polyoxyethylene sorbitan monooleate, #P1754, Sigma Chemical Co., P.O. Box 14508, St. Louis, MO, 63178) were added. Sterile medium components were stored as individual solutions and were combined asceptically immediately prior to use.

Inoculum preparation

Cultures were stored on malt extract slants [13]. Conida from 4-day-old malt extract plates in sterile deionized water were aseptically filtered through a cheese cloth, and resuspended in water to give approximately 4.0×10^7 conidia per ml. The spore concentration was measured microscopically using a haemocytometer as well as by measuring the optical density (OD) of the conidial suspension at 650 nm ($1.0\ cm^{-1}$ - 5×10^6 conidia per ml.) Unless otherwise indicated, approximately 2×10^7 conidia were used to inoculate 75 ml of low nitrogen medium (described above) without Tween 80 in a 3-l Fernbach flask. These cultures were incubated with no agitation at 39°C for 48 h and blended aseptically for 5 min at maximum setting in a Sorval blender (Sorval Omni-Mixer, model #17150; Ivan Sorval Inc., Newtown, Conn., 06470). The blended mycelial suspension was used as an inoculum for agitated cultures.

Culture conditions for optimization and pellet formation

Erlenmeyer flasks containing low nitrogen medium (45 ml/125 ml flask) were inoculated with 10% (v/v) blended mycelial suspension and were used for various optimization experiments. The 2-l Erenmeyer flasks containing 750 ml of inoculated medium were used to prepare mycelial pellets for inoculating the STR. These cultures were agitated in a rotary shaker bath (Gyrotary water bath, model G76, New Brunswick Scientific Co., Edison, NJ). Except where mentioned otherwise, shaker bath conditions were 200 rev min^{-1}, diameter of rotation of 1.25 cm, and a temperature of 39°C. The 2-l flask cultures were agitated at 125 rev min^{-1}. The agitation rate was measured using a phototachometer. Since oxygen has been shown to enhance lignin peroxidase activity [11], cultures were flushed daily with ten volumes of sterile 99.5% oxygen (Linde Air Products Co.) and closed with sterile rubber stoppers. Triplicate or quadruplicate cultures were used.

Lignin peroxidase production in a stirred tank reactor (STR)

After 48 h growth, the 2-l Erlenmeyer flask cultures were used to inoculate a stirred tank reactor (Bioflo Model C30, New Brunswick Sci. Co. model C30, New Brunswick, NJ operating volume 500 ml) operated at 39°C with an agitation rate of 100 rev min^{-1}. Unless otherwise mentioned, culture conditions of Jager et al. [9] were employed (0.05 wt % Tween 80, 0.4 mM veratryl alcohol and 2.5×10^5 spores ml^{-1}). Agitation was provided by a stainless steel agitator with four impeller blades mounted on a thick disk at the bottom of the reactor vessel. The agitator was powered by a magnetic stirrer located beneath the reactor vessel. The fermenter was continuously sparged with humidified, sterile oxygen (0.5 liter min^{-1}) via a gas dispersion tube with a fine glass frit located directly above the agitator. Antifoam (Thomas Antifoam spray, # 1130-D15 [silicone emulsion; Dow Corning FG-10; dilute], Thomas Scientific, 99 High Hill Rd at I-295, Swedesboro, NJ 08085) was used to reduce culture foaming.

Lignin peroxidase assay

Lignin peroxidase activity was assayed at room temperature by measuring the increase in absorbance at 310 nm due to the formation of veratraldehyde from veratryl alcohol [19]. The reaction mixture contained 500 μl culture filtrate, 400 μl tartaric acid (125 mM, pH 2.5), 50 μl veratryl alcohol (40 mM) and 50 μl H_2O_2 (8 mM). A unit of lignin peroxidase activity was defined as one μmole of veratryl alcohol oxidized to veratraldehyde per min ($\varepsilon = 9300\ M^{-1}\ cm^{-1}$).

Pellet size measurement

Size distributions of fungal pellets were determined using U.S.A. standard screen sieves with 2.0, 1.4, 1.18, 1.0, 0.85 and 0.212 mm opening sizes. Samples were added directly to stacked sieve trays and rinsed with deionized water to move the pellets through the openings. The number of pellets in each tray from a sample of known volume was counted. During the first 24 h, the pellets were too small to be counted using sieve trays so a microscope and haemocytometer were used to count pellets.

Protein estimation

The protein concentration of the culture filtrate was measured using the Bradford method [2] employing bovine serum albumin (BSA # B2518, Sigma Chemical Co., P.O. Box 14508, St. Louis, Mo. 63178) as a standard.

FPLC protein profile

The extra-cellular fluid from various cultures was collected on the day of maximum activity (usually the 6th day of incubation), concentrated, dialyzed, and applied to a Mono Q column (Pharmacia, Uppsala, Sweden) at a flow rate of 1 ml/min using a gradient of acetate buffer (pH 6.0) from 10 mM to 1.0 M [12]. Heme proteins were detected by continuous monitoring at 409 nm.

RESULTS AND DISCUSSION

Effect of buffer

The buffer, 2,2-dimethyl succinate (DMS), has been used previously by several investigators. How-

ever, this buffer is expensive and alternative buffer systems were evaluated in this study. An effective and less expensive buffer would reduce media costs for ligninase production. A pH of 4.5 has been shown to be optimal for the expression of ligninolytic activity for *P. chrysosporium* in DMS buffer [13]. Less costly buffers with a p*K*a between 4.2 and 4.8 are citrate, oxalate, acetate, tartrate and succinate. Acetate buffer has been reported to be toxic to *P. chrysosporium* [13], however, acetate toxicity was not observed in this study.

The comparison between these buffers was made with shake flask cultures using the oxidation of veratryl alcohol as the test for lignin peroxidase activity. Generally, two to three-fold greater activity was observed in a medium buffered with acetate as compared to that buffered with DMS (Fig. 1). Tartrate and oxalate buffered cultures produced no detectable lignin peroxidase activity whereas citrate and succinate buffered cultures gave slightly lower activities than the DMS buffered cultures. In all subsequent optimization experiments, acetate buffer was used in place of DMS buffer.

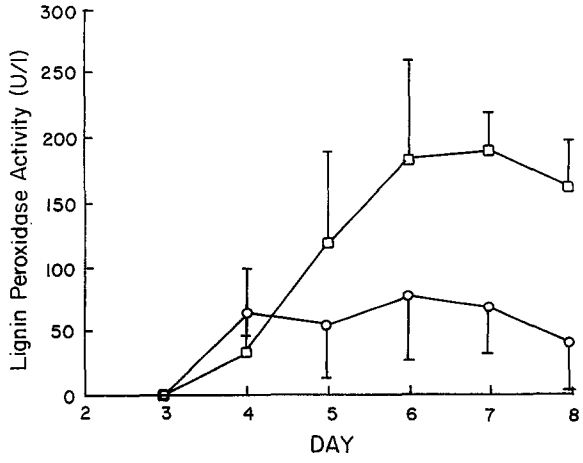

Fig. 1. Comparison of lignin peroxidase activity in low nitrogen medium (see Methods) buffered with acetate (20 mM, pH 4.5) verses 2,2-dimethyl succinate (10 mM, pH 4.5). The curves are drawn through average values and the limit bars represent + one standard deviation for acetate and − one standard deviation for DMS. Symbols: □: Acetate buffer; ○: 2,2-Dimethyl succinate buffer. Experiments were conducted in 125-ml Erlenmeyer flasks containing 45 ml of nitrogen-limited medium at 39°C and an agitation rate of 200 rev min^{-1}. Triplicate cultures on two separate occasions were used.

Optimization of acetate buffered media

The concentrations of conidia, Tween 80 and veratryl alcohol which support optimal lignin perox-

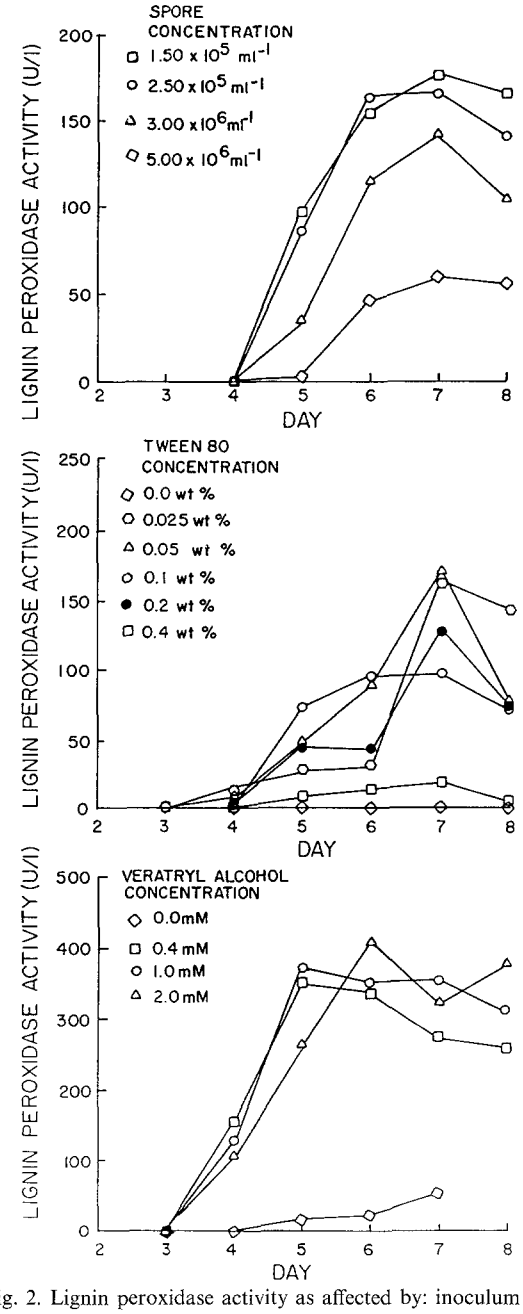

Fig. 2. Lignin peroxidase activity as affected by: inoculum size (A), Tween 80 concentration (B), and veratryl alcohol concentration (C). The points presented are averages for triplicate or quadruplicate cultures. Experiments were conducted in 125-ml Erlenmeyer flasks containing 45 ml of nitrogen-limited media buffered with acetate. Flasks were agitated at a rate of 200 rev min^{-1}.

idase activity in acetate buffered agitated flask cultures of *P. chrysosporium* were determined (Fig. 2). The amount of conidial inoculum added to Fernbach flasks appeared to affect ligninase activity (Fig. 2a). Maximal peroxidase activity was obtained when a conidial inoculum of 1.5 to 2.5 $\times$ 10^5 spores ml^{-1} was used. Perhaps the more concentrated spore inoculum leds to more rapid depletion of nutrients and affected the age of the starter cultures at the time of blending.

Tween 80 has been shown to enhance lignin peroxidase production in agitated cultures and was shown to give maximal lignin peroxidase activity in DMS buffered cultures at a concentration of 0.05 wt % [1,9]. The optimal Tween 80 concentration for lignin peroxidase production was 0.05 wt % in acetate buffered cultures also; no activity was seen in the absence of Tween 80. The differences in lignin peroxidase activity at 0.2 wt % and 0.025 wt % concentrations of Tween 80 were not significant (Fig. 2b). For these experiments, one standard deviation generally equalled 20% of the plotted average value.

Veratryl alcohol is thought to be an inducer of lignin peroxidase activity [5]. In DMS buffered cultures, a concentration of 0.4 mM has been reported to be saturating for lignin peroxidase production [5]. In Fig. 2c, the effect of increasing veratryl alcohol concentration is presented. High levels of lignin peroxidase activity were observed at veratryl alcohol concentrations of 0.4 mM; there were no appreciable differences in lignin peroxidase activities at veratryl alcohol concentrations of 1 mM or 2 mM. Only traces of lignin peroxidase activity were observed in the absence of veratryl alcohol in the medium. Concentrations of 0.05 wt% Tween 80, 0.4 mM veratryl alcohol and 5.0 $\times$ 10^5 spores ml^{-1} were used in all subsequent experiments.

Pellet formation and growth

Lignin peroxidase production in agitated cultures is associated with the formation of mycelial pellets. The formation and growth of mycelial pellets of *P. chrysosporium* has not been previously studied. Since lignin peroxidase expression by pellets occurs only under certain conditions, a better understanding of the mechanism of pellet formation and growth by *P. chrysosporium* may be useful for the development of large scale lignin peroxidase production systems. The mechanism of pellet formation was studied by observing the concentration of mycelial fragments in the inoculated Erlenmeyer flasks as a function of time. After inoculation, hyphal fragments in the agitated cultures aggregated to form pellets. This is shown by a semi-log plot of the number a hyphal fragments vs. time for cultures in both 125-ml and 2-l Erlenmeyer flasks (Fig. 3). The hyphal fragments, which numbered approximately 1 $\times$ 10^7 per liter initially, aggregated to 2.0 – 4.0 $\times$ 10^4 pellets per liter in less than 8 h. Each pellet on the average was an aggregate of 2 $\times$ 10^2 to 6 $\times$ 10^3 hyphal fragments. Once formed, the pellets remained intact throughout the fermentation and did not break apart even at relatively high agitation rates. A schematic representation of the observed mechanism of pellet formation is presented in Fig. 4.

The aggregation process ceased eight hours after inoculation, that is, the number of pellets was constant. However, the pellet size increased due to cellular growth (Fig. 5). The Sauter mean diameter [8] of the pellets was calculated according to equation 1.

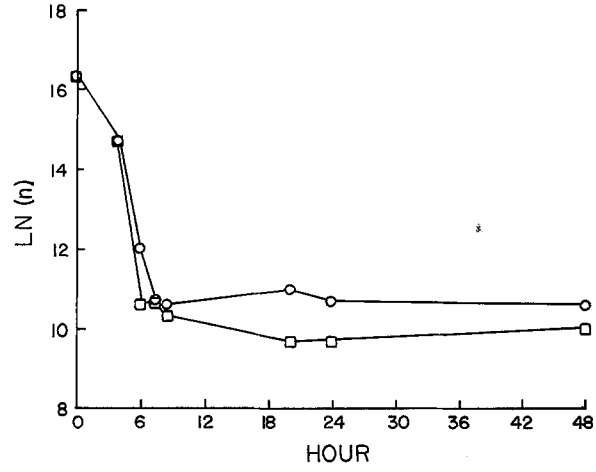

Fig. 3. Semi-log plot of number of hyphal agglomerations (n) vs. time. □: 45-ml shake flask culture (200 rev min^{-1}), ○: 750-ml shake flask culture (125 ml rev min^{-1}). Experiments were conducted in nitrogen-limited medium buffered with acetate.

$$D_{32} = \text{Sauter Mean Diameter} = \frac{\sum_{1}^{k}(d_k^3 n_k)}{\sum_{1}^{k}(d_k^2 n_k)} \quad (1)$$

where D_{32} is the mean Sauter pellet diameter, d is the pellet diameter measured using screen sieves, n is the number of pellets and k is the index of screen sieve sizes (Fig. 5). The Sauter mean diameter averages particle size based on the ratio of volume to surface area, which is appropriate for catalyst applications. The pellet size distribution indicated that a fairly narrow range of pellets was formed. The average pellet size was 1.29 ± .21 mm after eight days of growth, at 200 rev min^{-1}.

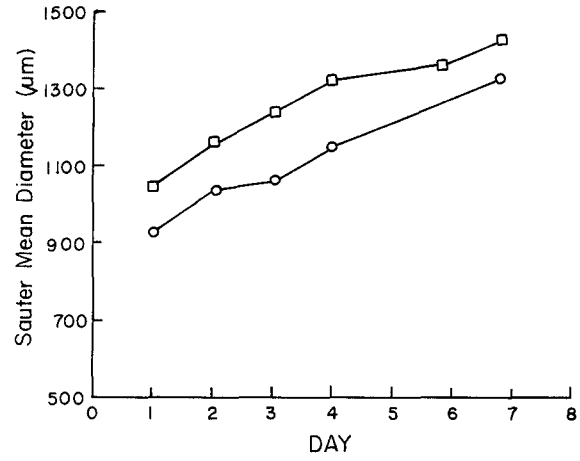

Fig. 5. *P. chrysosporium* pellet size vs. incubation time (days). Pellet size is expressed as the Sauter mean diameter in micrometers. □: 45-ml shake flask culture; ○ : 500-ml STR. Experiments were conducted in nitrogen-limited medium buffered with acetate.

Effect of agitation

It has been reported that agitation and pellet size can affect lignin peroxidase enzyme production in agitated cultures of *P. chrysosporium* [13]. In shallow (10 ml/150 ml flask volume) cultures of *P. chrysosporium*, Asther et al. [1] found that agitation rates greater than 50 rev min^{-1} reduced lignin peroxidase activity. Leisola et al. [15] used different flask and inoculum sizes to produce pellets of various sizes and concentrations. Pellets which were between 1 and 2 mm in diameter produced the highest lignin peroxidase activity. In cultures with slightly larger or smaller pellets, lignin peroxidase activity was reduced by 50%. Wase et al. [20] have reported that in disc turbine agitated cultures of cellulase producing mutants of *Aspergillus niger*, increasing the agitation rate from 100 to 300 rev min^{-1} caused a decrease in enzyme activity and an increase in extra-cellular protein concentration. They suggested that mycelium was being disrupted by shear forces generated by the impeller, liberating protein into the medium. They also detected protease activity, albeit at low levels, in the extracellular fluid.

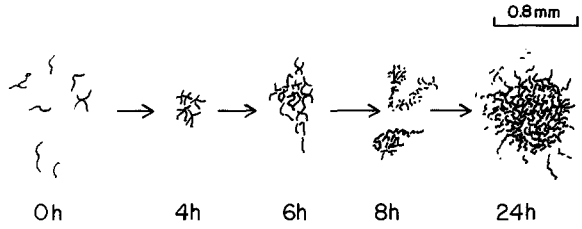

Fig. 4. Schematic illustration of pellet formation by aggregation of hyphal fragments of *P. chrysosporium* as observed microscopically (schematics are not to scale).

In shake flask cultures of *P. chrysosporium*, we have found that the size and number of pellets, and the amount of lignin peroxidase produced is affected by the rate of agitation (Table 1). At an agitation rate of 100 rev min^{-1}, pellets formed were fewer in number but much larger than pellets formed at higher agitation rates. These pellets produced no detectable lignin peroxidase activity and little extracellular protein. Upon cut section, the pellets were found to be hollow spheres with a mycelial shell approximately 1 mm thick. The low number of pellets at 100 rev min^{-1} were smaller in size, much higher in number and produced substantial levels of lignin peroxidase activity as compared to the pellets formed at 150 rev min^{-1}. Also, the pellets formed at rates higher than 150 rev min^{-1} were solid spheres. In cultures agitated at 200 rev min^{-1}, lignin peroxidase production was optimal (Fig. 6). Pellet size was smaller and pellet number was larger than in

Table 1

Culture parameters for 8 days old cultures of *P. chrysosporium* at four agitation rates

RPM	Lignin peroxidase activity (U/l)	Extra-cellular protein (μg/ml)	Mean pellet diameter (mm)	Total number of pellets per l
100	0 ± 0	2.8 ± 0.3	6.63 ± 1.06	360 ± 90
150	341 ± 73	9.2 ± 1.0	1.83 ± 0.32	10000 ± 7000
200	376 ± 33	10.9 ± 1.4	1.29 ± 0.21	32600 ± 3700
260	62 ± 100	11.9 ± 2.8	1.27 ± 0.23	36500 ± 8500

cultures agitated at 100 or 150 rev min^{-1} and comparable to cultures agitated at 260 rev min^{-1}. The lignin peroxidase activity was greatly reduced in cultures agitated at 260 rev min^{-1}.

The above results indicate that higher agitation rates, in general, result in smaller diameter pellets and greater number of pellets per liter. This is probably a result of increasingly greater shear forces at higher agitation rates affecting the aggregation of hyphae into pellets. Extra-cellular protein increases at higher agitation rates suggest that some cell disruption might be occurring at these rates. Lignin peroxidase activity presumably decreases at high agitation rates due to shear effects. Thus, the production of lignin peroxidase by *P. chrysosporium* appears to be sensitive to the rate of agitation.

Shear rate is often correlated with the size of pellets formed. A characteristic shear rate for the liquid in a rotating flask is given by rotation rate (N) times the diameter of the flask at the liquid fill level. Fig. 7 shows the natural log of the pellet Sauter mean diameter (D_{32} in μm) vs. rotation rate for cultures in 125-ml Erlenmeyer flasks. A simple empirical model for the relationship between pellet sizes and rotation rates is:

$$D_{32} = 2.1 \times 10^4 N^{-.52} \quad (2)$$

The correlation coefficient for eq. 2 is 0.70.

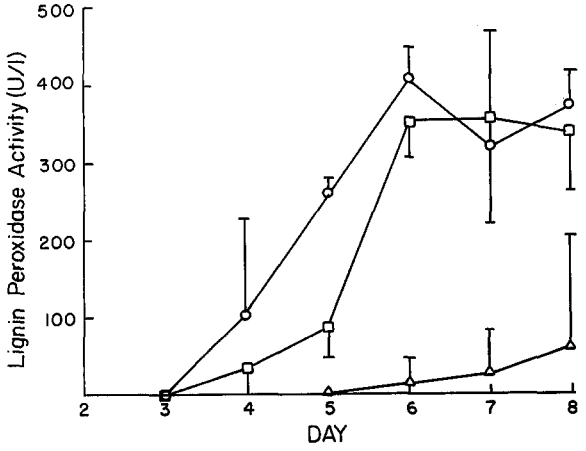

Fig. 6. Effect of agitation rate on lignin peroxidase activity. Experiments were conducted in 125-ml Erlenmeyer flasks containing 45 ml of nitrogen-limited medium. The curves are drawn through the average values and the limit bars represent + or − one standard deviation. Triplicate cultures were used. □: 150 rev min^{-1}; ○: 200 rev min^{-1}; △: 260 rev $minmin^{-1}$.

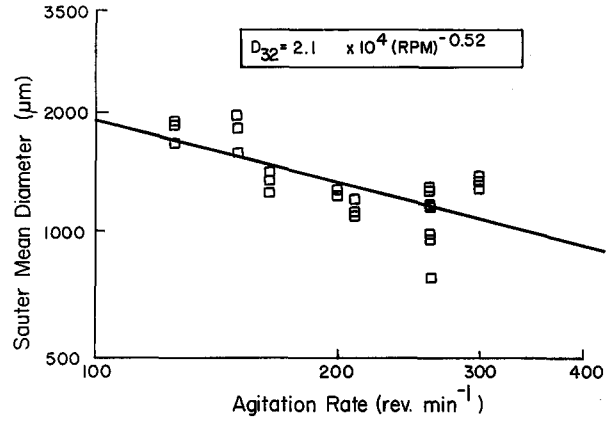

Fig. 7. Logarithm of the Sauter mean pellet diameter (eq. 1) versus logarithm of the shaker flask agitation rate. The experiments were conducted using 45 ml of nitrogen limited medium in 125-ml Erlenmeyer flasks agitated in a shaker bath with a diameter of rotation of 1.25 cm.

Operation of the stirred tank reactor

Production of lignin peroxidase in a stirred tank reactor system has not previously been described. Using the optimized culture media, and a knowledge of pellet formation, a bench scale stirred tank reactor system was developed for the production of lignin peroxidase. Several factors were critical to obtaining lignin peroxidase activity in the stirred tank reactor (STR): reactor mixing, oxygen dispersion, mycelial adsorption to reactor surfaces and foaming. Improvements in operating techniques for each area led to lignin peroxidase activities in the STR comparable to those obtained in shake flasks.

Two methods for making pellet biocatalysts were used: inoculation of blended mycelia directly into the STR and transfer of pellets to the STR from 2-l shake flask cultures. Inoculation of blended mycelia in to the STR did not give detectable lignin peroxidase activity. Some of the mycelia formed pellets and some adhered to reactor surfaces, forming large, irregular clumps. The pellets formed under these conditions failed to produce detectable amounts of lignin peroxidase. Removing the reactor baffles reduced the absorption of mycelia and the amount of irregular agglomerates, but gave large pellets which did not produce lignin peroxidase in detectable amounts. Pellets transfered to the STR after pellet formation in a 2-l shake flask culture for 48 h produced lignin peroxidase at times and levels similar to shake flask cultures. The agitation rate was adjusted to provide adequate suspension of the pellet biocatalysts (100 rev min^{-1}). High agitation rates in the STR (200 rev min^{-1}) resulted in no measurable lignin peroxidase production.

The oxygen sparging method affected the lignin peroxidase activity in the STR. In the bench scale STR, oxygen was introduced above the agitator using a fine glass frit. When a glass tube was used as a sparger, no lignin peroxidase activity was detected. The small bubbles produced by the frit tube created foam which did not disperse easily. At an oxygen flowrate of 0.2 liter min^{-1}, foam overflowed the reactor. At 0.05 liter min^{-1}, foaming was roughly static, and below 0.05 liter min^{-1} little foam was created. Silicone antifoam (Dow Corning FG-10 10% emulsion) was added in order to obtain an adequate oxygen supply rate in the STR (1.0 v/v/min). The antifoam enabled STR operation without foaming at an oxygen flowrate of 0.5 liter min^{-1} (1.0 v/v/min) and did not inhibit culture growth or enzyme activity.

A typical plot of the time course of lignin peroxidase activity and extracellular protein for a STR agitated at 100 rev min^{-1}, oxygenated at 0.5 liter min^{-1} and with 5 ml of silicone antifoam emulsion added is presented in Fig. 8. One day after pellet biocatalyst transfer, the extracellular fluid became yellowish in color. On the third day the pellets turned a dark brown color (which is generally associated with lignin peroxidase production) and lignin peroxidase activity was detected. Lignin peroxidase activity peaked on the fourth day. Six days after transfer, the pellets began to collect around the reactor components and coagulate in the top third of the operating volume. This coagulation of pellets corresponded with the appearance of long filaments growing from the pellets. Variations in the lignin peroxidase activity for cultures on different occasions are similar to the variation seen in shake flask cultures.

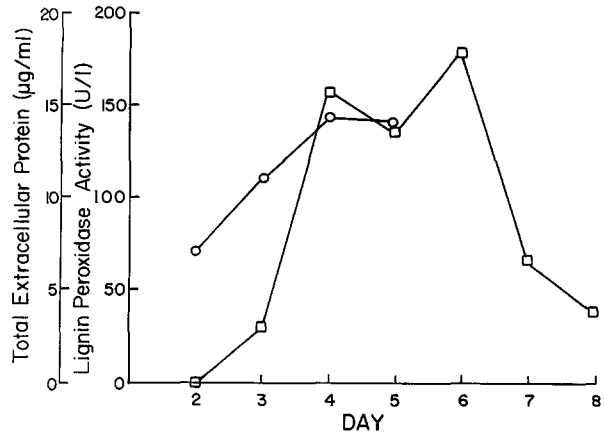

Fig. 8. Time course of lignin peroxidase activity (U/l; □) and total extracellular protein (μg/ml; ○) in a 500 ml volume stirred tank reactor (STR) agitated at 100 rev min^{-1} and oxygenated at 0.5 liter min^{-1}. Incubation time is given in days after transfer of the 48 h old fungal pellets to the reactor. The experiment was conducted using nitrogen-limited medium buffered with acetate. (5 ml of a 10% silicone antifoam was added to reduce reactor foaming).

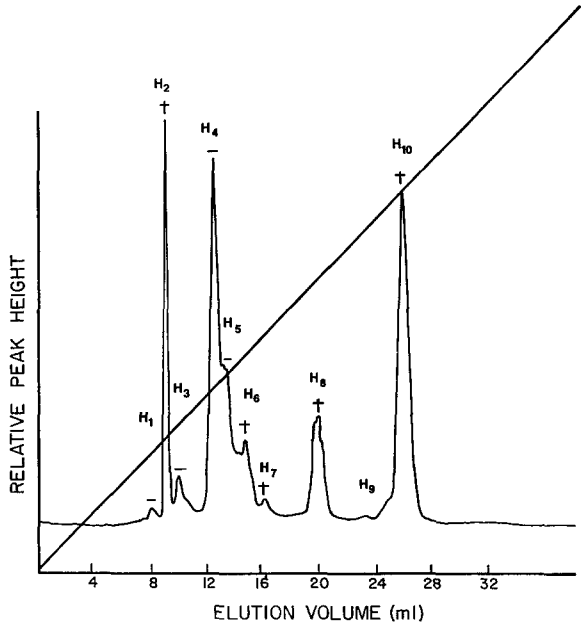

Fig. 9. FPLC profile of extracellular heme proteins in 38 ml of extra-cellular fluid from a 4 day old STR culture of *P. chrysosporium*. Experiments were conducted using nitrogen-limited medium buffered with acetate (see methods).

An FPLC protein profile of the heme proteins in extracellular fluid from an STR culture is presented in Fig. 9. Kirk et al. [12] have reported ten heme-protein peaks from six day old stationary cultures of BKM-F-1767 buffered with dimethyl succinate: six lignin peroxidase enzymes and four manganese peroxidases. These ten protein peaks were designated H_1 to H_{10} based on elution order. We have adopted this nomenclature system (Fig. 9). There are some similarities between the heme-protein profiles of these two studies. In both studies, no lignin peroxidase activity was detected in peaks H_3, H_4, and H_9. Lignin peroxidase activity was associated with the H_2, H_6, H_7, H_8, and H_{10} peaks. The H_2 peak had the highest lignin peroxidase specific activity. However, there were differences between the two profiles. In the STR profie, peaks H_2 and H_8 accounted for 50% of the total lignin peroxidase activity while peak H_{10}, the dominant peak, accounted for approximately 30% of the total activity. Kirk et al. [12] reported that peaks H_2 and H_8 accounted for over 80% of the lignin peroxidase activity. In the STR profile, peaks H_4 and H_5 were significantly larger than those observed by Kirk et al. [12]. Large differences in heme-protein profiles in lignin degrading cultures of *P. chrysosporium* have been reported to occur in response to variations in culture conditions such as age or agitation [9], the presence of veratryl alcohol [12] and strain differences [5]. The differences between our STR profile and that of Kirk et al. [12] may be due to different buffers, agitation rates and/or age.

The results of this study provide starting points for the scale-up of lignin peroxidase production with respect to agitation, adsorption of mycelia to reactor surfaces, oxygen dispersion and foaming. This study also demonstrates the feasibility of producing lignin peroxidases in a stirred tank reactor system.

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