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Scale up of *Cerrena unicolor* Laccase Production

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The effect of different carbon source in culture medium of wood–degrading basidiomycete *Cerrena unicolor* C–139 were investigated. The maximal growth and laccase synthesis in shaken flask was observed in mineral salts broth containing maltose as the carbon source and asparagine as the nitrogen source (C/N= 17.45). The maximal laccase activities in this condition (in the end of fungus exponential growth phase) was 4–times higher (28000 nkat/l) according to control conditions with glucose. The comparison of different inoculum age preparations for laboratory scale fermentor seeding display that the best results (154 078 nkat/l) were observed when the mycelium comes from the early exponential growth phase.

In the scale–up process in *SIP* type 40l fermentor controlled automatically by the cascade of agitation (200–500 rpm) and gas flow of air (2–3 SLMP) giving the dissolved oxygen concentration in the range of 35–100% of initial amount, (enriched from 2 to 6 day of cultivation by the sequential addition of 10 μ M cupric ions) the highest laccase activity in the medium with glucose (150,000 nkat/l) or maltose (145,000 nkat/l) at 8–th and 10–th day of cultivation were observed. The after culture fluids from these time were next concentrated and partially purified giving the crude laccase preparations Lac–G and Lac–M (from media with glucose or maltose respectively). The obtained preparations compared by ion–exchange HPLC, electrophoresis and cyclic voltammetry gave no significant differences in their properties.

Key words: *Cerrena unicolor*, laccase, wood degrading, fermentor

INTRODUCTION

The classical (blue ones) laccases (benzenediol: oxygen oxidoreductases; EC 1.10.3.2) are multicopper enzymes containing four atoms distributed in three different copper binding sites depending on their spectroscopic characteristics (Solomon *et al.*, 1996; Pereira *et al.*, 2005). All these copper ions are apparently involved in oxygen reduction to water and simultaneously perform a one–electron oxidation of many aromatic substrates (polyphenols, methoxy substituted monophenols and aromatic amines in the catalytic mechanism (Rogalski and Leonowicz, 2005). In the presence of low molecular mediators with higher potential than laccase, this enzyme is capable to oxidize also non–phenolic compounds (Bourbonais *et al.*, 1997; Banci *et al.*, 1999).

Laccases are widespread in plants, fungi, insects and bacteria; however, only the ligninolytic organisms like white–rot fungi are the best known laccases producers and the major source of these enzymes (Claus, 2004). The enzyme possess great biotechnological potential including polymer synthesis (Huttermann *et al.*, 2001), ethanol production (Larsson *et al.*, 2001), bioremediation (Riva, 2006), food industry (Minussi *et al.*, 2002)

forest product industry (Widsten and Kandelbauer, 2008), cosmetics and nanobiotechnology (Couto and Herrera, 2006). The fact that laccase has a broad specificity for the substrates makes it attractive potential candidate as a component of biosensors (Jarosz–Wilkolazka *et al.*, 2005; Odaci *et al.*, 2006) for the determination of total phenols (Quan *et al.*, 2006) and biofuel cell cathode (Klis *et al.*, 2007; Nazaruk *et al.*, 2010; Bilewicz *et al.*, 2011).

Recently, *Cerrena unicolor*, was determined as a new fungal source of extracellular laccase, excreting the enzyme under non–induced conditions with a rate similar to the best laccase producers. Several attempts to increase its production were undertaken, including optimization of the mediums composition and the physical parameters of the culture (Leonowicz *et al.*, 1997; Janusz *et al.*, 2007; Rogalski and Janusz, 2010). Reducing the costs of laccase production by optimising the fermentation process is the basic research for the industrial applications (Fenice *et al.*, 2003).

In the present study, the scale–up process of laccase production by the white–rot fungus *Cerrena unicolor* was investigated.

MATERIALS AND METHODS

Organism and culture conditions

Cerrena unicolor C–139 was obtained from the culture collection of the Regensburg University and deposited in the fungal collection at the Department of Biochemistry (Maria Curie–Skłodowska University, Poland) under the strain number 139. Stock cultures of

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the fungus were stored at 4°C on malt agar slants. For inoculations, pieces of mycelium overgrowing agar were grown using Lindeberg & Holm (1952) medium (pH 5.5) in stationary conical flasks for 7 days at 28°C. The mycelial mats were subsequently collected, broken in a Waring blender (three times for 15 s at 10,000 rpm), and homogenates were used as inocula in aerated bioreactor cultures. For the development of a pilot scale inoculum the bioreactor-scale cultivations were performed at 28°C in a 3.5 l glass fermenter (BioFlo III, New Brunswick Scientific, Edison, NY, USA) containing 2.0 l of the optimized Lindeberg & Holm medium (Janusz *et al.*, 2007). The fermenter equipped with pH-, temperature and pO_2 sensors was sterilized (121°C, 40 min) and seeded with mycelial suspension (10% of total volume). The fungal culture was run for 14 days at the aeration rate of 1 l air min^{-1} with air and stirrer speed of 150 or 300 rpm. Antifoam B emulsion (Sigma-Aldrich Fine Chemicals, St. Louis, MO, USA) was used periodically to break the foam and the pH was not regulated. The pilot scale fermentation was done in the SIP class 40l fermenter type 510 (New Brunswick Scientific, Edison, NY, USA) connected to the steam generating unit WA-42 (ALUX, Bielsko-Biała, Poland). The fermenter vessel contained 25L of the optimized Lindeberg & Holm medium and was automatically sterilized at 121°C by 45 min. For the inoculation the mycelial suspension from BioFlo III fermenter (2.5L) was added into the vessel via sterilized by 30 min port. The fungal culture was run for 14 days at the aeration rate of 1–3 l air min^{-1} , 0.01 MPa overpressure, 28°C and stirrer speed of 200–500 rpm. Antifoam B emulsion, used to break the foam, as well the samples of the medium were taken *via* sterilized ports. The fermenter was equipped with pH-, temperature and pO_2 sensors. In the case of pilot scale fermentation the pre-boiled earlier tap water was used.

The effect of different carbon sources on laccase synthesis

To study the different carbon sources on laccase production, *Cerrena unicolor* was grown in 50 ml wide-mouth Erlenmeyer flasks with 15 ml optimized earlier Lindenbergl & Holm medium (Janusz *et al.*, 2007) containing 10 g/l galactose, fructose, lactose, sucrose, maltose or cellobiose (all from Merck, Darmstadt, Germany), instead glucose. The flasks were incubated on orbital rotary shaker Multitron (Inforce HT, Bottmingen/Basel, Switzerland) at 28°C and 160 rev/min for a period of 14 days.

The biomass determination

Microbial dry cell weights (DCW) were determined by filtering 30 ml samples through predried and weighted quantitative filter papers – 41 ashless grade (Whatman, Meidstone, Kent, UK). The filter was then rinsed with distilled water (3×10 ml) prior to drying in an oven at 70°C for 24 h. The results presented are the mean of DCW obtained in two separate cultivations

Enzyme purification

The after culture liquid was centrifuged at 10,000×g on CEPA LE Benchtop centrifuge (New Brunswick Scientific, Edison, NY, USA) with the flow rate 15 L/h. The supernatant was next concentrated about 25 times on the ultrafiltration system Prep/Scale TFF-6 (0.54 m²) cartridges (Millipore, Bedford, USA) with PT polyether-sulfone membrane (10 kDa cut off), desalted on Sephadex G-25 column, distributed into lyophilisation vials and lyophilised in Labconco FreeZone 12 (Labconco, Kansas, MO, USA).

Laccase activity and protein measurement

Laccase activity in culture supernatant was measured spectrophotometrically at 525 nm in Shimadzu UV-Vis 160A spectrophotometer (Tokyo, Japan) or at BioTek ELx800 Absorbance Microplate Reader (Winooski, USA) controlled by KC-Junior (v. 1.41.8) software using syringaldazine as a substrate (Leonowicz and Grzywnowicz 1981). One nano katal (nkat) of laccase activity was defined as the amount of enzyme catalyzing the production of one nano mol of coloured product (quinone, $\epsilon^M=65,000 M^{-1} cm^{-1}$) per second at 25°C and pH 5.5, and expressed as nano katals per litre of culture (nkat/l). The protein concentration was determined using the Bio-Rad Protein Assay Reagent using bovine serum albumin (BSA) as standard (Bradford, 1974) or fluorometrically on Qubit 2 with Qubit Protein Assay Kit (Ahnert *et al.*, 2007).

Carbohydrate determination

The carbohydrate concentration was analyzed by the HPLC method on a VP chromatographic system (Shimadzu, Tokyo, Japan) composed of a LC-10 AD pump, a RID-10A refractive index detector, a SCL-10A controller, a CTO 10-AS oven (all of which were controlled by Class VP 5.03 Workstation Software; Shimadzu, 1999) and sampling valve Model 7725 (Rheodyne, Berkeley, USA) with a 20 μ L loop. The mobile phase (Milli Q water) was run at a flow rate of 0.6 mL/min through a Rezex RPM-monosaccharide column (7.8×300 mm; 1 μ m; Phenomenex) at 75°C. The calibration of the column was carried out using the sets of sugar and sugar alcohol standard for chromatography A and B (Merck, Darmstadt, Germany).

HPLC ion exchange chromatography

The protein profiles in after culture liquids were determined in the HPLC gradient chromatograph (Shimadzu, Tokyo, Japan) composed of a Photodiode Array UV-VIS detector (SPD M10A), LC-9A pumps (all controlled by Class-M10A; v.1.64 software) and sampling valve Model 7125 (Rheodyne, Berkeley, USA) with 100 μ L loop. The Protein-Pak DEAE 5PW column (7.5×75 mm; 10 μ m; Waters-Millipore, Milford Massachusetts, USA) was stabilised by elution of buffer B (0.1 M TRIS-HCl buffer, pH 6.5). All analyses were run with gradient elution by using Buffer B and buffer A (0.1 M TRIS-HCl, pH 6.5 containing 0.5 M NaCl). The gradient was partially linear, buffer A concentration increase as follows: 0%

(0 min), 0% (10 min), 100% (20 min) and 100% (25 min). The analysis time was 30 min, the flow rate was 1 ml/min at the temperature 20°C.

Electrophoresis and gel staining

The SDS-PAGE was run on a Mini-Protean Tetra (Bio Rad, Berkeley, CA, USA) camera in Mini-Protean TGX (4–15%) acrylamide gels (1×83×73 mm) using molecular weight markers Precision Plus Standard (Bio Rad, Berkeley, CA, USA), according to (Laemmli, 1970). The proteins bands in the gels were visualized by staining method (Wong *et al.*, 2000). The gels staining for laccase activities, were done as in (Kirk *et al.*, 1968) using 100 ml 0.1 M Mc Ilvaine buffer pH 5.5 with 1% guaiacol in 96% ethanol.

Electrochemical measurements

Electrochemical experiments were done in three electrode arrangement with Ag/AgCl (KCl sat.) reference electrode, platinum foil as the counter electrode and glassy carbon electrode (GCE, BAS) as the working electrode with surface area of 0.071 cm². Cyclic voltammetry experiments were carried out using ECO Chemie Autolab potentiostat. All electrochemical measurements were done at 22±2°C. All current densities were calculated using geometrical area of the electrode. The working electrodes were modified by depositing 72 micrograms of multi walled pristine carbon nanotubes (MWCNTs) (90 microliters of a suspension prepared from 12 ml ethanol and 8 mg nanotubes) and 48 micrograms of single walled carbon nanotubes with covalently attached naphthalene (suspension prepared in an analogous manner). Electrodes were placed in a solution of laccase, which was prepared from 20 mg of protein in 500 microliters of Mc Ilvaine buffer solution, pH=5.3 and incubated for 8 hours at 4°C.

Statistics

Each experiment was run at least twice; the standard deviation of analysis was less than 10% of the mean. Other methodological details are given in tables and figures.

RESULTS AND DISCUSSION

White-rot basidiomycete *Cerrena unicolor* C-139 represents a new fungal source of extracellular laccase (Rogalski *et al.*, 1999). In the previous study, we showed that the production of laccase in shaking condition was considerably enhanced by the addition of micromolar concentrations of Cu²⁺ into carbon and nitrogen-sufficient medium (C/N=16.69). The fermentor laboratory scale cultivation of *C. unicolor* resulted in higher production of crude laccase than observed in submerged cultures (Janusz *et al.*, 2007).

The white-rot fungi display a wide diversity in their response to carbon source and their concentration in nutrient media (Galhaup *et al.*, 2002; Elisashvili *et al.*, 2006; Wang *et al.*, 2008).

The starting point of these studies was the selection

of different carbon sources as monosaccharides (galactose, fructose) and disaccharides (cellobiose, sucrose, lactose and maltose) instead glucose in Lindeberg & Holm medium for effective production of extracellular laccase by *C. unicolor*. Titres of laccase were measured for 14 days in shaken flask cultures. The results (Table 1) indicated that the enzyme activity reached its maximum (over 3400 nkat/l on day 12) in the medium containing maltose. But all tested disaccharides were consumed by *C. unicolor* as a carbon source that suggest the production by this fungus the enzymes degrading sucrose (β -fructofuranosidase), lactose (β -galactosidase), cellobiose (β -glucosidase) and maltose (α -glucosidase). The best carbon source (maltose) concentration in the medium was next optimized. The concentration of carbon (added as maltose) varied from 1 to 50 g/l (2.92–146 mM) while that of nitrogen (added as L-asparagine) was 1.5 g/l (11.34 mM) as in (Janusz *et al.*, 2007). The C/N molar ratio in the respective media ranged from 1.54 to 77.30 or from 3.54 to 79.41 when L-asparagine was taken into account as an additional carbon source. The results (Fig. 1) indicated that the enzyme activity reached its maximum (over 28,000 nkat/l on day 12) in cultures with a C/N ratio of 17.45 when calculated as carbon moles in maltose and L-asparagine to nitrogen moles in L-asparagine, i.e., containing 20 g/l maltose (58.40 mM) as a carbon source and 1.5 g/l L-asparagine (11.34 mM) as a nitrogen source.

Table 1. Laccase synthesis during *C. unicolor* growth on different carbon sources

Carbon source	*HLA [nkat/l]	Time of HLA [day]	Decrease according to glucose [fold]
Galactose	3000	8	1.83
Fructose	1860	8	2.95
Cellobiose	3340	12	1.65
Sucrose	2730	12	2.01
Lactose	2270	12	2.42
Maltose	3400	12	1.62

*HLA-highest laccase activity

The dynamics of culture parameters was presented in (Fig. 2). The maximal laccase activities was observed in the end of *C. unicolor* exponential growth phase as well as in the time where maltose concentration drop down to 10% of initial amount and the pH increase to the over 7.5 level. The obtaining laccase activity was 4-times higher as that obtained in the medium with glucose (Rogalski *et al.*, 1999).

In the submerged cultivation of *Cerrena unicolor* C-139 in the medium with glucose as a carbon source the highest extracellular laccase activities were observed in a carbon-sufficient and nitrogen-sufficient culture medium (Janusz *et al.*, 2007). The synthesis of

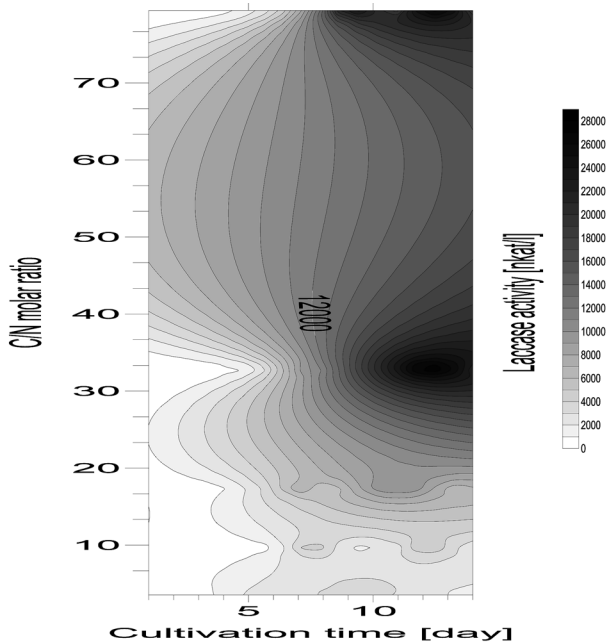


Fig. 1. The relationship between extracellular laccase synthesis and carbon/nitrogen ratio in the *C. unicolor* cultivation media.

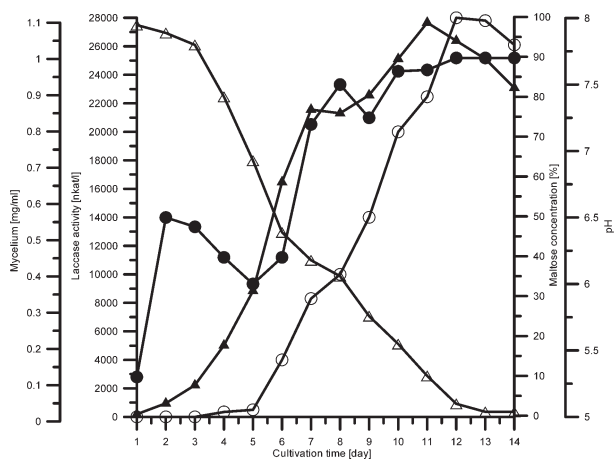


Fig. 2. Dynamics of culture parameters during *C. unicolor* grown in shaken flask cultures in the Lindeberg & Holm medium containing the optimised maltose concentration (2%). Mycelial growth—(▲); laccase activity—(○); pH—(●); maltose concentration—(Δ)

manganese-dependent peroxidase also increase in medium where cofactor C/N was relatively low (65.3) (Rogalski *et al.*, 2006). In the case of *Pleurotus ostreatus*, *Lentinula edodes* and *Agaricus blazei* growing in solid state cultivation the highest laccase activities were observed in the media with soybean hulls and ammonium sulphate or urea giving the ratio of C/N on the level 5. Increase of carbon amount in the medium increase the grow of tested fungi but the laccase activities drop down in these cases (D'Agostini *et al.*, 2011). *Ganoderma* genus belonging to the wood rot fungi and extensively used in Asian traditional medicine can also synthesis laccase. Production of this enzyme was extensively checked by the selection of growing media

(Simakumar *et al.*, 2010; Ding *et al.*, 2012). In the case of *Ganoderma* sp. the activity of laccase increase about 2 and 10 times respectively in the case when mannitol, and starch at 2% concentration were used (Simakumar *et al.*, 2010) whereas for *Ganoderma lucidum* the results were opposite. In all tested carbon sources (in 2 and 8% concentrations) the laccase activities drop down according to the medium containing glucose (Ding *et al.*, 2012).

One of the factors limiting the efficient production systems of the enzymes at bioreactor scale are the different morphological growth of filamentous fungi that have a significant effect on the rheology of the fermentation broth and the performance of the bioreactor (Couto and Toca-Herrera, 2007). The effect of broth rheology on mass, momentum and heat transfer within a bioreactor have been well studied (Charles, 1985; Funahashi *et al.*, 1988). The most important stage in fermentor cultivation as well as in scale-up processes are inoculation. Among several fungal physiological properties, the age and size of the mycelial inoculum may play an important role in fungal pellets development (Glazebrook *et al.*, 1992; Petre *et al.*, 2005).

In the next stage of experiments the cultivation in laboratory scale fermentor for the optimization of *Cerrena unicolor* inoculum in 2-litre batches in a 3.5 l fermentor were made. Biosynthesis conditions were fixed, taking into account the previous results obtained in agitated flask cultures (Janusz *et al.*, 2007). During the first stage, we compared the effect of increasing the agitation rate of the stirrer speed from 150 to 300 rpm on biomass amount, dO_2 and laccase production by mycelia of *C. unicolor* in bioreactor cultures (Fig. 3). The laccase activity attained its maximum (about 8,000 nkat/l) after 10 days incubation at an agitation of 300 rpm (Fig. 3B). In the same time the glucose concentration drop down to the 5% and dissolved O_2 obtained level about 70% of initial amount. In the case of cultivation at 150 rpm stirrer speed, the laccase activities increase linearly from 9 to 14 days of cultivation to the level of 6,000 nkat/l (Fig. 3A). The consumption of the oxygen in this condition was much intensive, drooping down in the end of cultivation to the 20% of initial level. The mycelium growing curves observed in the cultivation at 150 rpm stirrer speed was more stretched in time according to the obtained at 300 rpm. During the cultivation at 300 rpm the mycelium in a large amount adsorbed on the glass vessel as well as on metallic elements of the fermentor. The production of erythromycin by *Saccharopolyspora erythracea* significantly effected by fungus clump morphology (Ghojavand *et al.*, 2011). The morphology of the mycelium depends of its growing phase as well as from the amount of oxygen availability in the medium (Park *et al.*, 2002; Petre *et al.*, 2005; Goma and Bialy, 2009).

For these cause in the next stage of optimization the variant of the inoculum production of *C. unicolor* growing in the medium with glucose as a carbon source in 3.5 l fermentor at 150 rpm was used. For the inoculation of a 3.5 l fermentor the obtained earlier mycelium

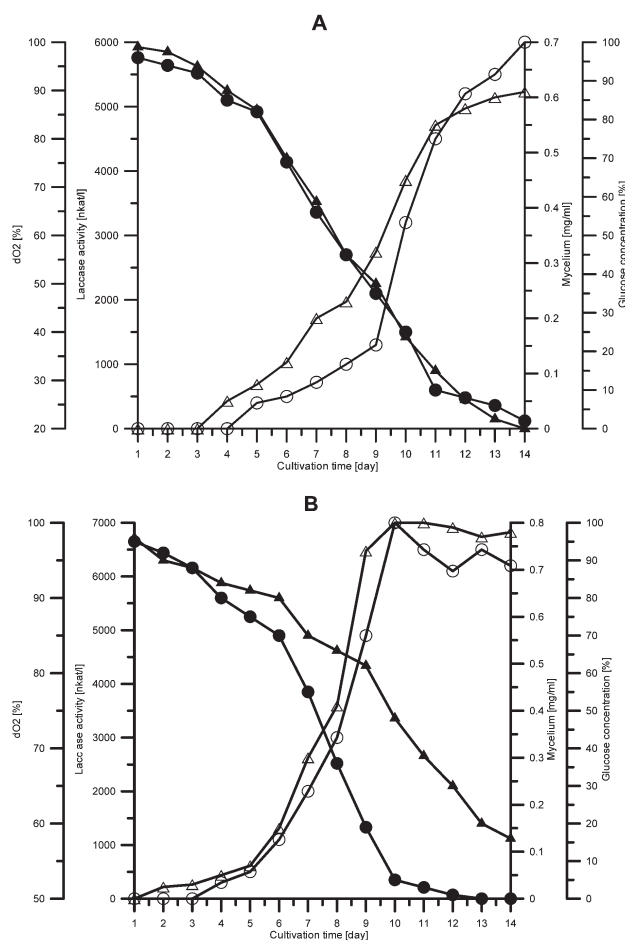


Fig. 3. Time course of laccase production, glucose consumption, dO_2 concentration and mycelium growth during aerated (11/min) fermenter cultivation at 150 rpm (A) and 300 rpm (B) of *C. unicolor* mycelium. Mycelial growth –(Δ); laccase activity –(\circ); dO_2 concentration –(\blacktriangle); glucose concentration –(\bullet)

Table 2. The effect of different inoculum age on *C. unicolor* laccase production in laboratory scale fermentor

Inoculum age [day]	*HLA [nkat/l]	Time of HLA [day]	**Increase of laccase activity [fold]
5	154,078	12	25.68
10	99,834	12	16.64
12	69,280	14	11.55

*HLA – highest laccase activity

** – increase of laccase activity according to maximal obtained during the inoculum preparation (6,000 nkat/l)

from early (5d) or late (10d) exponential growth phase, and stationary phase (12d) were used. During the cultivation the sequential addition of 10 μ M cupric ions were doses from 2 to 6 day of growth (Table 2). These activities were from 11 to 26 times higher than in the Cu^{2+} – free culture (production of inoculum). The highest titres of activity was observed on 12 day of cultivation when the inoculum come from early exponential phase

(154,078 nkat/l). Using as an inoculum mycelium from late exponential phase caused about twice drop down of activity (99,834 nkat/l–12 days) and 2.5–times when mycelium comes from stationary growing phase (69,280 nkat/l–14 days).

Cupric ions had been reported to be strong stimulants of laccase activity also by Giardina *et al.* (1999) and Galhaup *et al.* (2002), in whose experiments up to 50 times higher levels of the enzyme were obtained in induced, compared to non-induced, cultures. The sequential supplementation after 3, 6 and 9 days of incubation resulted in markedly increased laccase titres. The optimal copper dose for the enzyme production by *C. unicolor* C–139 in shaken flask cultures was found to be 10 μ M (Janusz *et al.*, 2007). The optimal Cu^{2+} dose was significantly lower than that (2.0 mM, added after 4 days of incubation) reported by Galhaup and Haltrich (2001) for submerged cultures of *T. pubescens*, but was still within the range of 2 to 600 μ M used in typical cultivation media for the production of laccase both in wild-type and recombinant strains of different basidiomycete fungi (Dittmer *et al.* 1997; Farnet *et al.* 1999; Palmieri *et al.* 2000; Chen *et al.* 2003). It had also been reported (Palmieri *et al.* 2000) that the induction of laccase in *P. ostreatus* occurred when the fungus was cultivated in a nutrient-rich medium supplemented with 150 μ M $CuSO_4$ at the time of inoculation.

For pilot scale laccase production the SIP type 401 fermentor with 25 l batches containing 1% glucose or 2% maltose inoculated by the mycelium from early exponential growing phase were made (Fig. 4). The dissolved oxygen level (dO_2) was automatically controlled by the cascade of agitation (200–500 rpm) and gas flow of air (2–3 SLPM – standard liters per minute) giving the dissolved oxygen concentration in the range of 35–100% of initial amount. During the cultivation the sequential addition of 10 μ M cupric ions were doses from 2 to 6 day of growth via sterilized port. In the case of the using the glucose as the main carbon source, the maximal laccase was observed in 8 day of cultivation (150,000 nkat/l) and for maltose in 10 day (145,000 nkat/l). There were a progressive rise in pH through the fermentation, from about 7.0 to above 8. The maximal laccase activities are connected with reaching the stationary growing phase. In both cases the culture fluids showing the highest laccase activities were taken out from the fermentor and centrifuged for removing the mycelium. The supernatants containing laccase were next concentrated by ultrafiltration, purified by ion-exchange chromatography on DEAE–Sephacrose (fast flow) and lyophilized. The obtained preparations from media containing glucose (LAC–G) and maltose (LAC–M) were compared by ion-exchange HPLC on Protein–Pak DEAE 5PW column (Fig. 5) and electrophoresis (Fig. 6). The HPLC profiles show almost identical peaks corresponding to the flow out from the column at 0.2 M NaCl (laccase proteins). In the electrophoresis comparison the both laccase preparations (LAC–G and LAC–M) showed the same activity bands in gel (Fig. 6). In the last stage of this investigation both laccase samples were used in

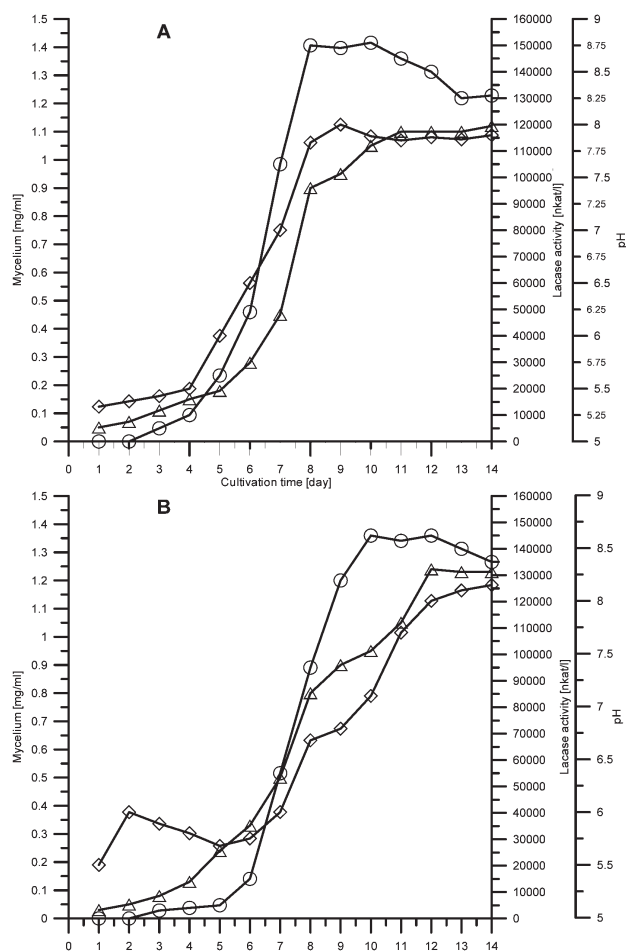


Fig. 4. The course of a typical *Cerrena unicolor* pilot-scale fermentation in the medium with glucose (A) and maltose (B) Mycelium growth (Δ); pH (\diamond); laccase activity (\circ)

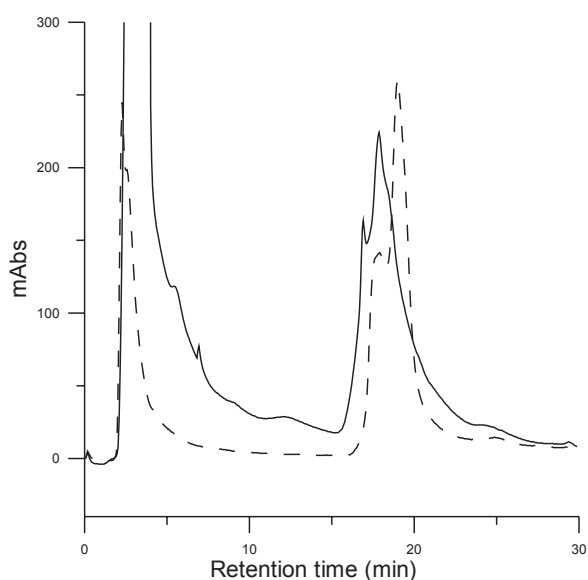


Fig. 5. The HPLC chromatogram of *C. unicolor* laccase preparations; LAC-G (solid line) and LAC-M (dashed line)

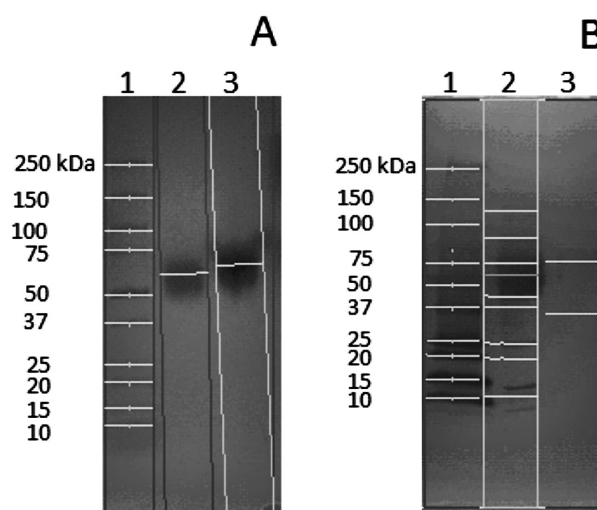


Fig. 6. The electrophoresis of purified *C. unicolor* laccases stained for activity (A) and SDS-PAGE stained for proteins (B) 1– molecular weight markers, 2 – LAC-G, 3 – LAC-M

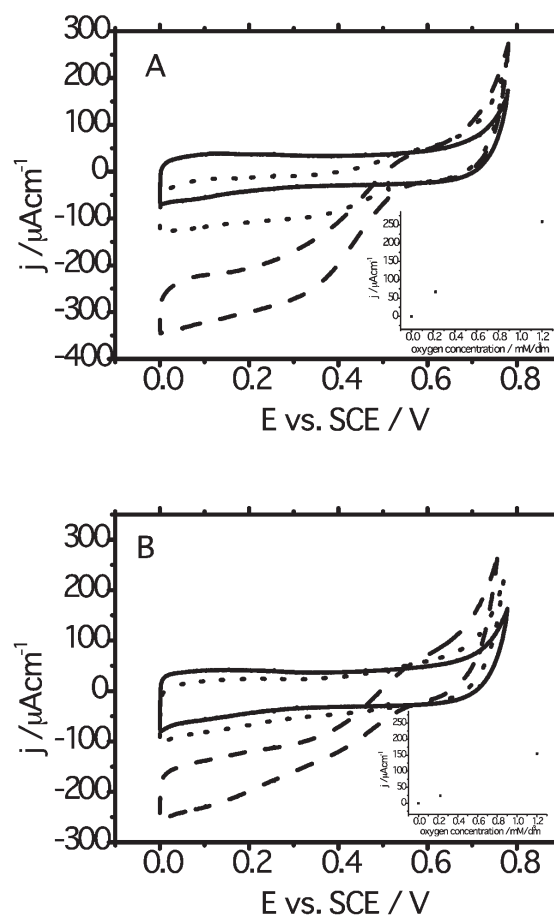


Fig. 7. Cyclic voltammograms recorded a scan rate of 1 mV/sec with *C. unicolor* laccase (Lac-G (A) and Lac-M (B) in 0.1M citrate-phosphate buffer, pH 5.3: 1–(solid line) – argon saturated buffer, 2–(pointed line) – air saturated buffer, 3–(dashed line) – oxygen saturated buffer. Insert: Dependence of catalytic current density on the oxygen concentration

electrochemical experiments (Fig. 7). The electrode covered with arylated carbon nanotubes and laccase was catalytically active in $4e^-$ reduction of oxygen. Catalytic wave increases with the increasing amount of catalyst on the electrode and with increasing concentration of oxygen in the solution.

Conclusions

1. The other carbohydrates as monosaccharides (galactose, fructose) and disaccharides (lactose, cellobiose, saccharose or maltose) can be used for cultivation of *C. unicolor* as the sources of carbon instead glucose.
2. The optimization of maltose concentration in the medium (2%) allow to reached the high level of laccase activity (28,000 nkat/l).
3. The process of inoculum preparation showed that the best variant was obtained when mycelium can be taken from early expotential *C. unicolor* growth phase at 150 rpm stirrer speed.
4. The described above cultivation of *C. unicolor* in pilot scale fermentor on glucose or maltose as an only carbon sources gave the high activity laccase preparations.
5. The comparison of both laccase preparations did not show any important differences in laccase isoforms, HPLC profiles and electrochemical properties.

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