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Amperometric detection of mono- and diphenols at *Cerrena unicolor* laccase-modified graphite electrode: correlation between sensitivity and substrate structure

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Abstract

Graphite electrode modified with laccase from *Cerrena unicolor* served as a biosensor for detection of 30 phenolic compounds with different structures. Some correlations of the sensor response to the structures of substrates are discussed. This biosensor responded to: (i) nanomolar concentrations of some of the selected phenolic compounds, e.g., 2,6-dimethoxyphenol, coniferyl alcohol, caffeic acid, DOPAC and hydroquinone, (ii) micromolar concentrations, e.g., ferulic acid, syringic acid, dopamine, 3,4-dihydroxybenzoic acid and DL-noradrenaline, and (iii) millimolar concentrations in the case of phenol and 4-hydroxybenzaldehyde. Among the *ortho*- or *para*-substituted phenols, the sensitivity of the *C. unicolor* laccase-modified electrode increased in the following order −H, $-CH_3$, −OH, $-OCH_3$ and $-NH_3^+$ but in the case of *para*-substituted phenols, the K_m^{app} values were lower. The sensitivity of the laccase electrode increased with an additional −OH group in *para*-substituted phenols. In the case of the selected compounds, kinetic data from electrochemical flow injection system were compared with those obtained from experiments in solution.

Keywords: Laccase; Electrodes; Amperometric detection; Phenolic compounds

1. Introduction

Laccase (EC1.10.3.2) is a copper-containing oxidase, which is widely distributed in fungi [1], higher plants [2], and in some bacteria [3]. It is able to oxidise many different substrates with the concomitant reduction of dioxygen to water and its specific affinity for oxygen as electron acceptor is very high [4]. The specificity of laccase towards its reducing substrates is rather low, and therefore, it has the ability to oxidise many phenolic and non-phenolic compounds [5]. Laccase catalyzes the removal of a hydrogen atom from the hydroxyl group of *ortho*- and *para*-substituted mono- and polyphenolic substrates [6]. Therefore, laccase has been applied to many industrial processes including decolourization of dyes [7], pulp delignification [8], oxidation of organic pollutants [9], microbial transformation of natural products [10] and the development of biosensors [11,12] or biofuel cells [13]. Transformation processes catalyzed by laccase can be accompanied by the appearance of electrochemically active products, which enable the use of standard electrochemical techniques for their determination.

Recently, a comprehensive study on the immobilization of fungal laccases on graphite electrodes and its use in a flow-injection system for amperometric detection of a large number of phenolic compounds both simple and complex, were performed [14–16]. We pointed out that laccase-modified electrodes might be used in detection even of highly polymerised phenolic compounds and the sensitivity of this analytical system depended on substrates' structures. For example, the position of only one OH group in the case of catechin and epicatechin determined completely different values of

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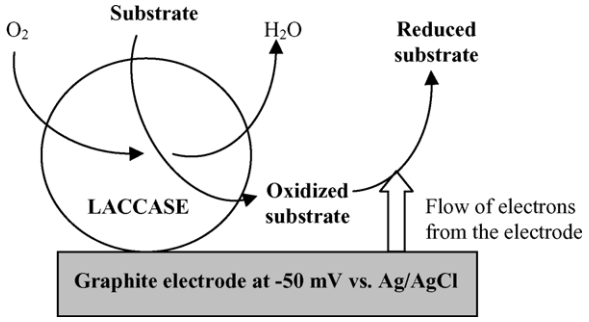

Fig. 1. Electron transfer mechanism of the reactions on the laccase biosensor.

Michaelis–Menten constant during oxidation of these compounds by laccase [17].

The laccase molecules at the surface of the electrode are oxidized by oxygen and then re-reduced by phenolic compounds, acting as electron donors for the oxidized form of the enzyme (Fig. 1). In this reaction, the phenolic compounds are converted into quinone and/or phenoxy radicals. These products can be reduced at the surface of the electrode at potentials below 0 V versus SCE [14], giving a reduction current which is proportional to the phenol concentration. Laccase-based sensors for phenolic compounds have the advantage that the applied potential is within the optimum potential range, where contributions to the response from compounds usually interfering in enzyme-based biosensors are small and the background current takes its smallest value. Another advantage with laccase-based sensors is that its re-oxidising agent, molecular oxygen, is already present in the carrier solution and needs not to be added. The other commonly used amperometric biosensors for the determination of phenolic compounds are based on tyrosinase [18], peroxidase [19], pyrroloquinoline quinone dependent glucose dehydrogenase (GDH) [20] or cellobiose dehydrogenase (CDH) [21].

To better understand the interaction of laccase with phenolic compounds, a comparative kinetic study with fungal laccase from *Cerrena unicolor* adsorbed on graphite electrodes was carried out in a flow injection system for a series of phenols and other aryl analogues trying to correlate the sensor response to the structures of the substrates. In the case of the selected compounds kinetic data from electrochemical flow injection system were compared with those obtained from experiments in solution.

2. Experimental

2.1. Enzyme and chemicals

Fungal laccase (EC 1.10.3.2) from the white rot fungus *C. unicolor* (from the Fungal Collection (FCL) at the Department of Biochemistry, UMCS, Lublin, Poland) was purified using a previously described procedure [1]. Until use laccase was stored frozen at a temperature below −18 °C and after thawing and diluting with citrate buffer solution, it was used to prepare working laccase solution with a precise activity in the presence of syringaldazine as the substrate [22].

Phenol, 4-methoxyphenol, 4-chlorophenol, hydroquinone, vanillin, guaiacol, *p*-cresol and *o*-cresol were obtained from Merck (Darmstadt, Germany). DL-Noradrenaline and 2-aminophenol were obtained from Fluka (Buchs, Switzerland). Syringic acid, coniferyl alcohol, ferulic acid, adrenaline, 3,4-dihydroxyphenylacetic acid (DOPAC), L-3,4-dihydroxyphenylalanine (L-DOPA), dopamine, 2,6-dimethoxyphenol, acetovanillone, acetosyringone, caffeic acid, ABTS and syringaldazine were obtained from Sigma (St. Louis, MO, USA). Coniferylaldehyde, 4-aminophenol, 3,4-dihydroxybenzoic acid and 3,4-dihydroxybenzaldehyde were obtained from Aldrich (Steinheim, Germany). Catechol and vanillic acid were obtained from ICN Biomedical Inc. (Aurora, OH, USA) and 4-hydroxybenzaldehyde was obtained from Acros (Geel, Belgium). All chemicals were of analytical grade and used without further purification. All substrate solutions were prepared daily from 100 or 10 mM stock solution in a methanol/water (1:4) mixture. All aqueous solutions were prepared using water purified with a Milli-Q system (Millipore, Milford, CT, USA).

2.2. Electrode preparation and electrochemical analysis

The laccase-modified electrodes served as working electrodes, which were prepared from spectrographic graphite (Ringsdorff Werke GmbH, Bonn, Germany, type RW001, 3.05 diameter). The electrodes were adsorptively modified with the enzyme by placing 10 μl aliquot of the laccase solution (30.5 ± 2.52 mU/10 μl) according to Haghighi et al. [14]. The laccase-modified electrodes were stored at 4 °C in 0.1 M citrate buffer, pH 5.0 in a glass beaker covered with sealing film before using them for the electrochemical measurements. The enzyme electrode was fitted into a Teflon holder and inserted into a wall-jet amperometric flow-through cell. The Ag|AgCl (0.1 M KCl) electrode was used as the reference electrode and a platinum wire served as auxiliary electrode. Electrochemical measurements were carried out at −50 mV as earlier defined optimal potential [15]. The mechanism of the laccase-modified electrodes for detection of phenolic compounds is presented in Fig. 1.

3. Results and discussion

To better understand the interaction of laccase with phenolic compounds, we carried out a comparative kinetic study with *C. unicolor* laccase (CuL) modified electrode and a series of mono- and di- and methoxyphenols. After applying the potential to the CuL-modified electrode, a background current is observed due to the catalytic reduction of dissolved molecular oxygen in the carrier solution of flow injection

system. The adsorptively immobilized CuL catalyzes oxygen reduction to water through a direct electron transfer mechanism between the enzyme and graphite [18]. However, in a previous publication, an immobilization protocol was worked out minimizing the effect of electrocatalytic oxygen reduction at the laccase modified electrode through allowing the enzyme to adsorb for a prolonged time (15 h) [23]. During the injection of phenolic compounds into the flow, a transient current on the top of the background is observed due to the oxidation of phenolic compounds to their oxidized or radical forms by immobilized CuL. In these reactions, the electron transfer between the electrode and the enzyme occurs in a mediated electron transfer reaction sequence, in which the phenolic compound acts as an electron donor to the oxidized form of CuL (Fig. 1). The generation of cathodic current during the biosensor action is associated with electrochemical reduction of oxidized products that are produced during laccase-catalyzed substrate oxidation [16]. The behaviour of the laccase-modified electrodes in flow injection system was investigated in respect to different phenolic and non-phenolic compounds.

At optimal conditions for electrochemical registration of catechol found earlier [15], the amperometric response of the laccase-modified electrode for a number of selected phenolic compounds was recorded in the flow injection mode. The limit of detection (LOD, calculated as three times the noise to signal ratio), linear dynamic range and sensitivity obtained for different mono- and diphenolic compounds are presented in Table 1. The *C. unicolor* laccase-based graphite electrodes responded to (i) nanomolar concentrations of some of the selected phenolic compounds, e.g., 2,6-dimethoxyphenol, coniferyl alcohol, caffeic acid, DOPAC and hydroquinone, (ii) micromolar concentrations, e.g., ferulic acid, syringic acid, dopamine, 3,4-dihydroxyenzoic acid and DL-noradrenaline or even (iii) millimolar concentrations as in the case of phenol and 4-hydroxybenzaldehyde (Table 1).

The current–concentration dependencies of the analyzed compounds were fitted to the electrochemical Michaelis–Menten equation and Michaelis–Menten constants K_m^{app} have been calculated. The calculated values of K_m^{app}, according to the different substituents in analyzed compounds, are presented in Tables 2 and 3. Among the *ortho*- or *para*-substituted phenols, the sensitivity of the *C. unicolor* laccase-modified electrode increased in the following order $-H$, $-CH_3$, $-OH$, $-OCH_3$ and $-NH_3^+$ but in the case of *para*-substituted phenols, the K_m^{app} values were lower (Table 2). Increase in sensitivity indicates that the enzymatic oxidation products (i.e., phenoxy radicals) of the *ortho*-substituted phenols are more rapidly produced and rereduced at the elec-

Table 1
Calibration data for different compounds using *Cerrena unicolor* laccase-modified electrode in FI amperometric measurements ($n = 5$)

Substrate	Detection limit (μM)	Linear dynamic range (μM)	Sensitivity (nA μM^{-1})	r
2,6-Dimethoxyphenol	0.091 ± 0.002	0.1–2	202.09	0.999
Coniferyl alcohol	0.35 ± 0.05	0.2–6	98.7	0.999
Ferulic acid	1.56 ± 0.09	1–40	69.63	0.999
Caffeic acid	0.56 ± 0.03	1–10	57.92	0.999
2-Aminophenol	0.95 ± 0.02	1–8	53.96	0.995
DOPAC	0.23 ± 0.01	1–10	52.11	0.999
Hydroquinone	0.58 ± 0.02	1–10	50.99	0.999
Syringic acid	1.27 ± 0.02	1–40	44.65	1
ABTS	0.58 ± 0.03	1–10	38.63	0.999
4-Aminophenol	0.61 ± 0.05	1–10	32.4	0.999
Acetosyringone	0.40 ± 0.02	1–10	29.0	0.999
Catechol	0.89 ± 0.04	1–20	24.4	0.999
Guaiacol	0.90 ± 0.03	1–20	22.38	0.999
3,4-Dihydroxybenzoic acid	3.8 ± 0.1	1–40	14.7	0.998
Coniferyl aldehyde	0.68 ± 0.05	1–20	14.03	0.999
Dopamine	1.51 ± 0.02	1–60	11.08	0.999
4-Methoxyphenol	7.9 ± 0.2	1–100	9.09	0.998
DL-Noradrenaline	1.9 ± 0.1	1–80	6.26	1
3,4-Dihydroxybenzaldehyde	5.2 ± 0.3	10–150	5.76	0.999
L-DOPA	0.49 ± 0.02	1–40	5.13	0.999
Vanillic acid	9.48 ± 0.35	10–100	3.45	0.997
Adrenaline	10.0 ± 0.5	1–150	3.16	0.998
Syringaldazine	3.1 ± 0.2	20–100	2.5	1
p-Cresol	39 ± 1	10–1000	0.44	0.999
Acetovanillone	6.1 ± 0.4	10–100	0.42	0.999
o-Cresol	54 ± 1	10–1000	0.289	0.999
Vanillin	31 ± 1	10–400	0.251	0.998
Phenol	296 ± 10	1000–10000	0.011	1
4-Chlorophenol	346 ± 10	1000–10000	0.02	1
4-Hydroxybenzaldehyde	783 ± 17	1000–10000	0.018	0.998

Table 2
Effect of substituents in *ortho*- or *para*-substituted phenols and *para*-substituted 2-OH phenols on the apparent Michaelis–Menten constant

Name	Substituents[a]	K_m (mM)	Error (mM)
ortho-substituted phenols			
2-Aminophenol	2-NH_3^+	0.00759	0.00082
Guaiacol	2-OCH_3	0.051	0.0022
Catechol	2-OH	0.246	0.023
o-Cresol	2-CH_3	3.69	0.15
Phenol	2-H	43.4	2.03
para-substituted phenols			
4-Aminophenol	4-NH_3^+	0.0135	0.0016
Hydroquinone	4-OH	0.019	0.0012
4-Methoxyphenol	4-OCH_3	0.0883	0.0061
p-Cresol	4-CH_3	2.16	0.066
4-Hydroxybenzaldehyde	4-CHO	10.8	1.1
4-Chlorophenol	4-Cl	22.4	1.1
4-Hydroxybenzoic acid	4-CO_2^-	16.78	2.2
Phenol	4-H	43.4	2.0
para-substituted 2-OH phenols			
DOPAC	4-$CH_2CO_2^-$	0.0127	0.00058
Caffeic acid	4-$CHCHCO_2^-$	0.0274	0.0027
3,4-Dihydroxybezoic acid	4-CO_2^-	0.0553	0.0042
Dopamine	4-$CH_2CH_2NH_3^+$	0.0611	0.0043
DL-Noradrenaline	4-$CHOHCH_2$ NH_3^+	0.125	0.015
L-DOPA	4-$CH_2CHCO_2^-NH_3^+$	0.146	0.005
3,4-Dihydroxybenzaldehyde	4-CHO	0.167	0.071
Catechol	4-H	0.264	0.023
Adrenaline	4-$CHOHCH_2NHCH_3$	0.302	0.030

[a] The charge states are referred to that at pH 5.

trode, viz., an increase in the amplification reaction cycle. Laccases are classified as *p*-diphenol oxidases, but are generally considered to be remarkably non-specific as to their substrates, being able to oxidize in vitro a range of aromatic substances such as polyphenols, substituted phenols, synthetic amines, thiols, dyes, etc. [4,5,24]. Since the catalyzed reaction consists in withdrawing one electron from the substrate, it is conceivable that the electron density at the level of the oxidizing group plays an important role in determining the rates of oxidation of the substrates. Garzillo et al. [24] indicate that the polar effect of non-bulky substituent groups on the aromatic ring is the most relevant factor for determining the rates of substrate oxidation by laccase form *Trametes trogii*. In a comparison of a series of *ortho*- and *para*-substituted phenols presented by Xu [5], it was indicated that the presence of electron-withdrawing substituents decreased the activity of phenol towards recombinant laccase from *Polyporus pinsitus*. These substituents reduce the electron density at the phenoxy group, thus making it more difficult to be oxidized, less reactive in surrendering an electron to the T1 copper of laccase and less basic.

Based on the results obtained in this work it is shown that the sensitivity of the laccase electrode increases with an additional –OH group in *para*-substituted phenols. In the

Table 3
Effect of different substituents in *para*-substituted 2-methoxy and 2,6-dimethoxyphenols on the apparent Michaelis–Menten constant

Name	Substituents[a]	K_m (mM)	Error (mM)
para-substituted 2-OCH_3 phenols			
Coniferyl alcohol	4-$CHCHCH_2OH$	0.00783	0.00045
Coniferyl aldehyde	4-CHCHCHO	0.0467	0.006
Guaiacol	4-H	0.0511	0.0022
Ferulic acid	4-$CHCHCO_2^-$	0.0948	0.014
Vanillic acid	4-CO_2^-	0.203	0.013
Acetovanillone	4-$COCH_3$	0.714	0.032
Vanillin	4-CHO	3.57	0.813
para-substituted 2,6-$(OCH_3)_2$ phenols			
2,6-Dimethoxyphenol	4-H	0.00654	0.00071
Acetosyringone	4-$COCH_3$	0.0224	0.0016
Syringic acid	4-CO_2^-	0.0969	0.011
Syringaldazine	4-$CHNNCHC_6H_2(OCH_3)_2OH$	0.280	0.055

[a] The charge states are referred to that at pH 5.

Table 4
Oxidation rates of aromatic compounds by laccases from different sources (K_m (mM))

Substrate	*Cerrena unicolor* (a)	*Cerrena unicolor* [29]	*Cerrena maxima* [25,27]	*Coriolus hirsutus* [26,27]	*Coriolus zonatus* [27]	*Phlebia radiata* [28]
2,6-Dimethoxyphenol	0.00654	0.0078	ND	0.053	ND	ND
ABTS	0.0166	ND	ND	0.0567	ND	ND
Hydroquinone	0.0190	ND	0.095	ND	0.086	ND
Acetosyringone	0.0224	ND	ND	0.0605	ND	ND
Caffeic acid	0.0274	0.0364	ND	ND	ND	0.34
Guaiacol	0.051	0.116	0.255	0.011/0.065	0.091	1.76
Ferulic acid	0.0948	0.0887	0.034	0.017/0.031	0.025	ND
Syringic acid	0.0969	0.08	ND	ND	ND	0.21
Vanillic acid	0.203	0.172	ND	ND	0.165	0.68
Catechol	0.246	ND	0.122	0.0399	0.197	ND
Syringaldazine	0.280	ND	ND	0.1427	ND	ND

(a) This work, (ND) not determined.

case of –COOH (–RCOOH), –CHO, –H, $-CH_3$ ($-RCH_3$) an increased sensitivity is observed for the group of diphenols when compared with their respective monophenols. Only in the case of $-NH_3^+$ or $-RNH_3^+$, the enzyme preferentially oxidizes the *para*-substituted monophenol (4-aminophenol) than *para*-substituted diphenols (dopamine, DL-noradrenaline, L-DOPA) but this difference was not very significant. An additional –OH group with at least one single pair of electrons on the atom adjacent to the aromatic ring allows the –OH group to be oxidized efficiently by the enzyme. In the case of *para*-substituted diphenols, the enzyme oxidizes more preferentially substances with –COOH and –RCOOH group (DOPAC, caffeic acid, 3,4-dihydroxybenzoic acid) than compounds with an $-RNH_3^+$ group in *para*-position (dopamine, DL-noradrenaline, L-DOPA).

Within the groups of *para*-substituted 2-methoxyphenols (guaiacol) and 2,6-dimethoxyphenols, it was observed that the presence of an additional $-OCH_3$ group allows laccase to oxidize more effectively dimethoxyphenols (Table 3). Comparing the K_m^{app} values indicates that 2,6-dimethoxyphenol is the preferred phenolic substrate among those observed. This result is not surprising, if it is considered that lignin comprises more than 90% 4-hydroxy-3-methoxycinnamyl units. Further substituting *ortho*-diphenols in the *para*-position with $-CH_2-$ or $-CH_2CH_2-$ groups (DOPAC, dopamine and L-DOPA) or *ortho*-methoxyphenols in the *para*-position with a –CH=CH– group (coniferyl alcohol, ferulic acid and coniferylaldehyde) causes the sensitivities of the biosensor modified with laccase from *C. unicolor* to increase for those phenolic compounds (Tables 2 and 3).

It should be noted here that the current obtained during laccase-biosensor action depends not only on the rate of substrate oxidation by enzyme but also on the thermodynamic and kinetic of electrochemical re-reduction of substrate oxidized form at the electrode surface. The increase of response of laccase biosensor to some compounds may be result of cyclic conversion of substrates at the modified electrode surface. It was shown that cyclic conversion of substrates increases the sensitivity of biosensors [16].

Table 4 presents a comparison of Michaelis–Menten constants for some selected aromatic compounds by native laccases from different sources in solution with data obtained in this work using laccase from *C. unicolor* adsorbed on graphite electrode. Our data using immobilized laccase are comparable to Michaelis–Menten constants obtained with laccases from other white rot fungi [25–28].

In conclusion, it can be stated that laccase is an enzyme, which is able to communicate with the electrode through direct electron transfer. Phenolic compounds can mediate this transfer of electrons between electrode and enzyme. The results, we have obtained on *C. unicolor* laccase adsorbed on graphite electrode confirm that this multicopper oxidase has a wide range of substrate specifity in vivo. Hydroxylation and methoxylation of substrates may represent a strategy to use laccase-modified sensors in the determination of various aromatic compounds. The similarity of Michaelis–Menten constants obtained for different phenolic compounds with laccases in homogeneous solutions and *C. unicolor* laccase immobilised on the electrode surface points to the fact that the enzyme retains its native structure, i.e., it is not considerably denatured due to physical adsorption at graphite surface.

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References

[1] J. Luterek, L. Gianfreda, M. Wojtaś-Wasilewska, J. Rogalski, M. Jaszek, E. Malarczyk, A. Dawidowicz, G. Ginalska, A. Leonowicz, Acta Biochim. Pol. Acta 46 (1997) 297.
[2] G. Gramss, K.-D. Voigt, B. Firsche, Chemosphere 38 (1999) 1481.
[3] G. Alexandre, I.B. Zhulin, Trends Biotechnol. 18 (2000) 41.
[4] C.F. Thurston, Microbiology 140 (1994) 19.
[5] F. Xu, Biochemistry 35 (1996) 7608.

[6] A.I. Yaropolov, O.V. Skorobogatko, S.S. Vartanov, S.D. Varfolomeyev, Appl. Biochem. Biotechnol. 49 (1994) 257.
[7] E. Abadulla, T. Tzanov, S. Costa, K.-H. Robra, A. Cavaco-Paulo, G.M. Gubitz, Appl. Environ. Microbiol. 66 (2000) 3357.
[8] C. Crestini, D.S. Argyropoulos, Bioorg. Med. Chem. 6 (1998) 2161.
[9] A. Lante, A. Crapisi, A. Krastanov, P. Spettoli, Process Biochem. 36 (2000) 51.
[10] M. Hosny, J.P.N. Rosazza, J. Agric. Food Chem. 50 (2002) 5539.
[11] R.S. Freire, N. Duran, L.T. Kubota, Anal. Chim. Acta 463 (2002) 229.
[12] R.S. Freire, N. Duran, L.T. Kubota, Talanta 54 (2001) 681.
[13] G. Tayhas, R. Palmore, H.-H. Kim, J. Electronanal. Chem. 565 (1999) 110.
[14] B. Haghighi, L. Gorton, T. Ruzgas, L. Jönsson, Anal. Chim. Acta 487 (2003) 3.
[15] A. Jarosz-Wilkolazka, G. Janusz, T. Ruzgas, L. Gorton, E. Malarczyk, A. Leonowicz, Anal. Lett. 37 (2004) 1497.
[16] J. Kulys, R. Vidziunaite, Biosens. Bioelectron. 18 (2003) 319.
[17] A. Jarosz-Wilkolazka, T. Ruzgas, L. Gorton, Enzyme Microb. Technol. 35 (2004) 238.
[18] J. Wang, F. Lu, S.A. Kane, Y.K. Choi, M.R. Smyth, K. Rogers, Electroanalysis 9 (1997) 1102.
[19] F.D. Munteanu, A. Lindgren, J. Emneus, L. Gorton, T. Ruzgas, E. Csoregi, A. Ciucu, R.B. van Huystee, I.G. Gazaryan, L.M. Lagrimini, Anal. Chem. 70 (1998) 2596.
[20] J. Szeponik, B. Moller, D. Pfeiffer, F. Lisdat, U. Wollenberger, A. Makower, F.W. Scheller, Biosens. Bioelectron. 12 (1997) 947.
[21] A. Lindgren, T. Ruzgas, L. Stoica, F.D. Munteanu, L. Gorton, Cellobiose dehydrogenase and peroxidase biosensors for determination of phenolic compounds, in: A. Mulchandani, O.A. Sadki (Eds.), Chemical and Biological Sensors for Environmental Monitoring, ACS Symposium Series, 762, 2000, p. 113.
[22] A. Leonowicz, K. Grzywnowicz, Enzyme Microb. Technol. 3 (1981) 55.
[23] M.R. Tarasevich, A.I. Yaropolov, V.A. Bogdanovskaya, S.D. Varfolomeev, Bioelectrochem. Bioenerg. 6 (1979) 393.
[24] A.M.V. Garzillo, M.C. Colao, C. Caruso, C. Caporale, D. Celletti, V. Buonocore, V. Appl. Microbiol. Biotechnol. 49 (1998) 545.
[25] O.V. Koroleva, I.S. Yavmetdinov, S.V. Shleev, E.V. Stepanova, V.P. Gavrilova, Biochemistry (Moscow) 66 (2001) 618.
[26] K.-S. Shin, Y.-J. Lee, Arch. Biochem. Biophys. 384 (2000) 109.
[27] S.A. Smirnov, O.V. Koroleva, V.P. Gavrilova, A.B. Belova, Biochemistry (Moscow) 66 (2001) 774.
[28] J. Rogalski, A. Leonowicz, A. Hatakka, E. Jozwik, J. Mol. Catal. A-Chem. 95 (1995) 99.
[29] J. Rogalski, A. Dawidowicz, E. Jozwik, A. Leonowicz, J. Mol. Catal. B-Enzyme 6 (1999) 29.