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Chromate-reducing activity of *Hansenula polymorpha* recombinant cells over-producing flavocytochrome b_2

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ABSTRACT

In spite of the great interest to studies of the biological roles of chromium, as well as the toxic influence of Cr(VI)-species on living organisms, the molecular mechanisms of chromate bioremediation remain vague. A reductive pathway resulting in formation of less toxic Cr(III)-species is suggested to be the most important among possible mechanisms for chromate biodetoxification. The yeast L-lactate:cytochrome *c*-oxidoreductase (flavocytochrome b_2, FC b_2) has absolute specificity for L-lactate, yet is non-selective with respect to its electron acceptor. These properties allow us to consider the enzyme as a potential candidate for chromate reduction by living cells in the presence of L-lactate.

A recombinant strain of thermotolerant, methylotrophic yeast *Hansenula polymorpha* with sixfold increased FC b_2 enzyme activity (up to 3 μmol min^{-1} mg^{-1} protein in cell-free extract) compared to the parental strain was used for approval our suggestion. The recombinant cells, stored in dried state, as well as living yeast cells were tested for chromate-reducing activity *in vitro* in the presence of L-lactate (as an electron donor for chromate reduction) and different low molecular weight, redox-active mediators facilitating electron transfer from the reduced form of the enzyme to chromate (as a final electron acceptor): dichlorophenolindophenol (DCPIP), Methylene blue, Meldola blue, and Nile blue. It was shown that the highest chromate-reducing activity of the cells was achieved in the presence of DCPIP.

The ability of chromate to catch electrons from the reduced flavocytochrome b_2 was confirmed using purified enzyme immobilized on the surface of a platinum electrode. The increasing concentration of Cr(VI) resulted in a decrease of enzyme-mediated current generated on the electrode during L-lactate oxidation. The shift and drop in amplitude of the peak in the cyclic voltammogram are indicative of Cr(VI)-dependent competition between reaction of chromate with reduced FC b_2 and direct electron transfer from the enzyme to the electrode surface.

The application of the chromate-reducing ability of FC b_2-over-producing recombinant cells of *H. polymorpha* toward chromate bioremediation and the construction of cells-based biosensor for chromate monitoring in the environment are discussed.

1. Introduction

Hexavalent chromium Cr(VI) is widely used in different branches of industry. Cr(VI)-containing products include chromate pigments in dyes, inks, and plastics (Pellerin and Booker, 2000). Chromates are used in the production of stainless steel, textile dyes, wood preservation, leather tanning, and as anti-corrosion agents (OSHA 3320-10, 2006). Thus, soluble Cr(VI)-pollutants are distributed in waste streams and poisonous fumes of different industrial factories (Blowes, 2002).

Cr(VI) is a strong oxidizing species with very toxic and carcinogenic influences on all living organisms, including human. Contact with Cr(VI)-compounds over a prolonged period of time is a risk factor for developing lung cancer (Salnikow and Zhitkovich, 2008), and inhalation of Cr(VI) can cause severe damage and irritation to the nose, throat, and lungs. Trivalent chromium Cr(III) is less harmful and a more common representative of chromium compounds as compared to Cr(VI) (IARC Monographs, 1999). Moreover, taking advantage of the structural and electrical similarity between chromate and sulfate ions, Cr(VI) penetrates into the cells via the sulfate transporters, while intracellular penetration of Cr(III) is rather impossible (Pereira et al., 2008).

Chromates are xenobiotics and normally have not occurred in nature, but could actively interact with living organisms being

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structural analogues of sulfate and possessing high oxidizing activity *in vitro* and *in vivo*. Living cells are capable of reductive detoxification of very toxic Cr(VI) to 100-fold less toxic Cr(III) via non-enzymatic and enzymatic pathways. Ascorbic acid, glutathione, and cysteine are efficient reducers of Cr(VI) to Cr(III) at physiological conditions. The mechanisms of enzymatic reduction of chromates are well studied in bacteria and may function under both aerobic and anaerobic conditions. For instance, Cr(VI)-resistant strains of *Enterobacter cloacae* reduce chromates under anaerobic conditions, using Cr(VI) as electron acceptors. Aerobic chromate reduction in *Pseudomonas* and *Aeromonas* is supported by NADH-and NADP(H)-dependent reductases (Park et al., 2000). A metal-reducing bacterium *Shewanella oneidensis* possesses both up-regulated and down-regulated proteins involved in chromate-induced stress response (Brown et al., 2006), including 13–16-fold up-regulation of haem and flavin-containing sulfite reductase. Additionally, the up-regulation of cytochrome genes was demonstrated via gene expression profiling of *Saccharomyces cerevisiae* after Cr(VI) treatment (Eide et al., 2005).

It is still unknown whether enzymatic/non-enzymatic or intra-/extracellular reduction systems plays a decisive role in chromate-detoxification processes in eukaryotic microorganisms. Our previous work demonstrated that the extracellular pathway of chromate reduction plays a dominating role in detoxification of Cr(VI) in several yeast species (Ksheminska et al., 2006, 2008). Cr(III)-complexes were isolated as the final products of extracellular reduction of Cr(VI)-species. Moreover, the secretion of some metabolites possessing both Cr(VI)-reducing and Cr(III)-chelating ability by the cells was proved (Ksheminska et al., 2006). However, the possibility of some enzymatic activity contributing to reductive detoxification of chromate could not be excluded. Such an enzyme would have to meet several requirements: (1) high redox potential; (2) nonspecificity in electron acceptors (keeping in mind the xenobiotic nature of chromate, the evolution of an adapted, specific chromate reductive system in nature is implausible). According to these requirements, flavocytochromes are possible candidates for chromate-reducing catalysis. Ferrireductases from the flavocytochromes family are involved in reduction of different Fe(III)-complexes in baker's yeast (Yun et al., 2001; Philpott and Protchenko, 2008). However, such enzymes are poorly characterized. Yeast mitochondrial L-lactate:cytochrome *c* oxidoreductace (flavocytochrome b_2, FC b_2) was successfully isolated and characterized. FC b_2 is a homotetramer. FMN- and haem-containing domains are constituents of each monomer (Labeyrie et al., 1978; Silvestrini et al., 1993). L-lactate is a specific electron donor for the FMN-domain. On the contrary, the haem-domain is non-selective for electron acceptors. There are numerous inorganic and organic substances that can replace a natural electron acceptor (cytochrome *c*) in L-lactate oxidation (Capeillere-Blandin, 1995; Silvestrini et al., 1995). We suggest that chromate is a possible electron acceptor in FC b_2-catalyzed reduction of Cr(VI).

The aim of this study was to evaluate chromate-reducing activity of the constructed FC b_2 over-producing recombinant yeast *Hansenula polymorpha* (Dmitruk et al., 2008; Smutok et al., 2007). We describe the investigation of chromate reduction by FC b_2 using bioelectrochemical and photometric approaches. Purified recombinant FC b_2, as well as dried and living FC b_2 over-producing yeast cells were used in the study.

2. Materials and methods

2.1. Chemicals

L(+)-lactic acid was obtained from Acros Organics (Geel, Belgium); 2,6-dichlorophenolindophenol (DCPIP), Methylene blue, Nile blue, Meldola blue, phenazine methosulfate (PMS), SDS, Chromazurol S, diphenylcarbazide (DPC), 3-(N-morpholino)propanesulfonic acid (MOPS), cetylmethylammonium bromide (CTAB), acetone, H_2SO_4, NaOH, K_2CrO_4 were from Sigma (Deisenhofen, Germany); $(NH_4)_2SO_4$, Na_2HPO_4, KH_2PO_4, $MgSO_4$, $CaCl_2$ were obtained from Merck (Darmstadt, Germany).

The cathodic electrodeposition polymer (*CP8*) was synthesized in laboratory of *Elektroanalytik & Sensorik, Ruhr-Universität* (Bochum, Germany) following previously published procedures (Ngounou et al., 2004; Smutok et al., 2006).

All chemicals were of analytical grade, and all solutions were prepared using water purified using a Milli-Q system (Millipore). Solution of L-lactic acid was prepared in 50 mM phosphate buffer, pH 7.8 followed by neutralization using concentrated NaOH.

2.2. Enzyme

L-lactate:cytochrome *c*-oxidoreductase (flavocytochrome b_2) (EC 1.1.2.3) was isolated and purified from the constructed recombinant strain "tr1" of the methylotrophic yeast *H. polymorpha* (Dmitruk et al., 2008). The enzyme was purified to a specific activity of 9 U mg^{-1} and stored as a suspension in 30%-saturated ammonium sulfate, pH 7.2 according to previous protocols (Smutok et al., 2005). One unit of the enzyme activity was defined as that amount of the enzyme which oxidizes 1 μmol of L-lactate in 1 min under standard conditions of the assay (20 °C; 30 mM phosphate buffer, pH 7.5; 0.33 M L-lactate, 0.83 mM $K_3[Fe(CN)_6]$, 1 mM EDTA) (Appleby and Morton, 1959).

2.3. Yeast strains

Mutant strain of thermotolerant methylotrophic yeast *H. polymorpha* C-105 (*gcr1 catX*) defective in glucose catabolite repression and depleted of catalase activity (Gonchar et al., 1998, 2002) and its derivative FC b_2-over-producing recombinant strain "tr1" were constructed earlier (Dmitruk et al., 2008) and used in this study.

FC b_2-activity of the cells of both recombinant and parental strains was estimated by the method described before (Appleby and Morton, 1959; Gaida et al., 2003). The FC b_2-activity in cell-free extracts of the recombinant strain "tr1" was approximately 3.2 U mg^{-1} protein and was found to be 6-fold higher than that of the initial strain (activity 0.5 U mg^{-1}).

2.4. Cultivation of the yeast cells

Cultivation of the C-105 mutant and its derivative recombinant was performed in flasks on a shaker (200 rpm) at 37 °C until the end of the exponential growth phase (~32 h) in a medium containing (g L^{-1}): $(NH_4)_2SO_4$ – 3.5; KH_2PO_4 – 1.0; $MgSO_4 \times 7H_2O$ – 0.5; $CaCl_2$ – 0.1; yeast extract – 2. A mixture of glucose (10 g L^{-1}) and L-lactate (2 g L^{-1}) was used as a carbon and energy source. Yeast cells were washed twice with 50 mM phosphate buffer, pH 7.8 and harvested by centrifugation. Concentration of cells suspension was determined spectrophotometrically at 540 nm using a pre-established gravimetrical calibration and was expressed in mg of dried cells per mL of a culture medium (mg mL^{-1}). After washing, the cells were suspended in 50 mM phosphate buffer (pH 7.8) containing 1 mM PMSF and 1 mM EDTA with subsequent drying at room temperature.

2.5. Permeabilization of the yeast cells

To assay FC b_2-activity of the yeast cells, freshly-prepared or dried cells were re-suspended to 30 mg mL^{-1} in 50 mM phosphate buffer, pH 7.8 supplemented with 1 mM EDTA. The same volume of

permeabilizing reagent CTAB (2 mg mL^{-1}) was added to the cell suspension. The resulting solution was mixed at 30 °C in a water bath for 15 min. The permeabilized cells were washed by centrifugation (3200 g, 5 min) in 50 mM phosphate buffer, pH 7.8. The cells were re-suspended to 50 mg mL^{-1} in the same buffer solution and used no later than the day of preparation. For storage, the permeabilized cells were lyophilized.

2.6. Estimation of Michaelis–Menten constant (K_M) of the yeast cells toward L-lactate

The K_M of L-lactate was estimated at ambient temperature. DCPIP was used as electron donor (66 μM) and PMS (200 μM) as mediator in the presence 0.1 M phosphate buffer pH 7.0, and L-lactate ranging in concentrations from 0.01 to 20 mM (Pohanka and Zboril, 2008). K_M was calculated from the initial reaction rates, depending on substrate concentration, via the equation:

$$V = \frac{V_{max}[S]}{K_M + [S]}, \quad (1)$$

where V – initial rate of the reaction, S – substrate concentration, V_{max} and K_M – kinetic constants. Constants were calculated by a double-reciprocal method of Lineweaver and Burk (Lineweaver and Burk, 1934) using program Microcal Origin 6.0.

2.7. Electrochemical instrumentation and measurement

Platinum working electrodes (2 mm diameter) were obtained from CH Instruments Inc. (Austin, USA), and pre-treated by polishing on aluminium paste, followed by thorough washing with buffer. Further, the biosensor was constructed by immobilization of 2 μL suspension of FC b_2 with enzyme activity 9 U mg^{-1} and protein concentration 5 mg mL^{-1} onto the electrode surface and following electroinduced deposition by means of cathodic paint *CP8* (ratio 1/2; 20 cycles of deposition (Smutok et al., 2007).

The properties of resulting biofunctionalised electrodes were tested by constant-potential amperometry in a three-electrode configuration electrochemical cell, using a Ag/AgCl 3 M KCl reference electrode and Pt-wire as a counter electrode. Amperometric measurements were carried out using a potentiostat CHI 1200A (IJ Cambria Scientific Ltd., Burry Port, UK) and performed in batch mode under continuous stirring using a standard 40 mL cell at room temperature. After the background current stabilized, the experiments commenced by addition of L-lactate and K_2CrO_4 aliquots using stock solutions prepared in 50 mM phosphate buffer, pH 7.8.

Electrochemical characterization of low molecular redox-active mediators was accomplished using potentiostat AUTOLAB 302N FRA ECD (Metrohm Autolab B.V., Utrecht, The Netherlands).

2.8. Photometric monitoring of chromate reduction

2.8.1. Testing mediator activity of the dyes

The mediator activity of the dyes was tested in FC b_2-catalyzed L-lactate oxidation by spectrophotometric monitoring the rate of their reduction. For this aim, the set of mixtures was prepared: 25 mM acetate buffer, pH 6.3, 10 mM L-lactate, suspension of previously dried cells of *H. polymorpha* "tr1" 0.5 mg mL^{-1} and 0.2 mM dye (DCPIP, Meldola's blue, Methylene blue or Nile blue). To avoid the spontaneous oxidation of the reduced dyes by oxygen, the mixtures were incubated in closed vials (pseudoanaerobic conditions). After incubation at 37 °C during fixed time (10 min), cells were separated via centrifugation. The extinction coefficients of resulting supernatants were measured at proper wavelength (λ) (DCPIP λ = 600 nm, Meldola blue λ = 540 nm, Methylene blue λ = 668 nm and Nile blue λ = 628 nm). The degree of discoloration of the dyes was used as an index of their mediator activity.

2.8.2. Monitoring of chromate-reducing activity of the dyes reduced in FC b_2-catalyzed reaction

After removing the cells, the supernatants of the aforementioned mixtures were incubated in the presence of 0.5–1 mM K_2CrO_4. The reduction of chromate was monitored by the measurement of the Cr(VI)-residual content (Marchart, 1964).

2.8.3. Testing of chromate bioremediation activity of the yeast cells

The ability for bioremediation of chromium (VI) was tested for previously dried, as well as for freshly-prepared living *H. polymorpha* cells at the following conditions: 25 mM acetate buffer, pH 6.3; cells – 0.5–3.0 mg mL^{-1}; 3 mM L-lactate; 0.2 or 2 mM DCPIP; room temperature (24 °C); different concentrations of chromate (0.01–0.5 mM).

Photometric measurements were carried out on spectrophotometer *SHIMADZU UV-1650 PC* using standard spectroscopic software *UVProbe 2.20.* Experimental data were processed with Microcal Origin 6.0.

3. Results and discussion

3.1. Biosensor analysis

The high redox potential of chromate (E^0 = + 1.33 V) suggests it has the ability to oxidize the reduced form of flavocytochrome b_2 produced in the reaction of L-lactate oxidation. To support this hypothesis, electrochemical experiments were carried out using immobilized homogeneous FC b_2 isolated from the recombinant *H. polymorpha* srtain "tr1" (Dmitruk et al., 2008). As reported in our previous work, reduced FC b_2 is able to direct electron transfer from L-lactate to the surface of a carbon electrode, although some synthetic mediators essentially increase the efficacy of this process (Smutok et al., 2005). In this study, the immobilization of FC b_2 was made using cathodic polymer precipitated on the surface of working platinum electrode according to (Smutok et al., 2007). Electrochemical cells contained 50 mM phosphate buffer, pH 7.8, 2 mM L-lactate and chromate in concentration in the range 0–70 μM, without addition of any electron transfer mediators.

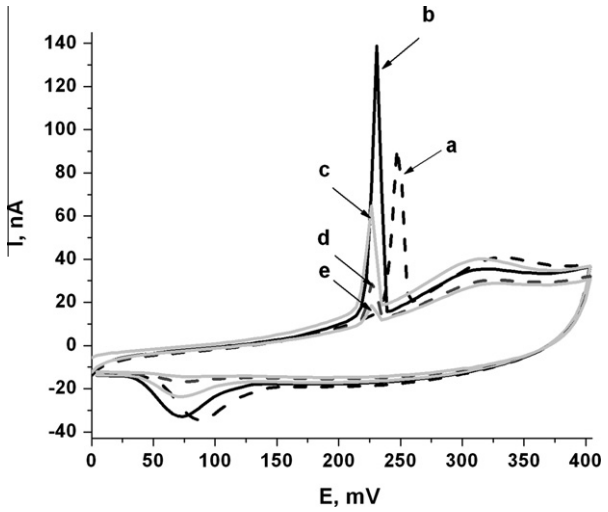

Fig. 1. Cyclic voltammograms of direct electron transfer of FC b_2-modified electrode (a) in presence of 2 mM L-lactate (b) and different chromate concentrations: (c) 9.6 μM; (d) 28 μM; (e) 47.2 μM. The working electrode: 2 mm diameter platinum disk; scan rate 10 mV s^{-1} vs. Ag/AgCl.

Increasing concentrations of Cr(VI) resulted in the decrease of enzyme-mediated current generated on the electrode during L-lactate oxidation. The shift and drop in amplitude of the current peak in the cyclic voltammogram indicated Cr(VI)-dependent competition between reaction of chromate with reduced FC b_2 and direct electron transfer from the enzyme to the electrode surface (Fig. 1). However, if Cr(VI) was removed away, the normal peak of L-lactate oxidation on the cyclic voltammagram have been restored. The hypothetical scheme of electron competition between electrode and Cr(VI) is represented in Fig. 2A.

The observed competition is tightly dependent on Cr(VI) concentration (Fig. 2B). Chromate (10 μM) causes approximately a 50% decrease in the current produced. The obtained data revealed rather effective electrochemical communication of chromate with reduced form of FC b_2. The observed phenomenon could be used as a platform for construction of biosensors for quantitative determination of chromate pollutants in the environment, as well as for bioremediation of chromate-containing wastes using FC b_2 overproducing recombinant yeast cells.

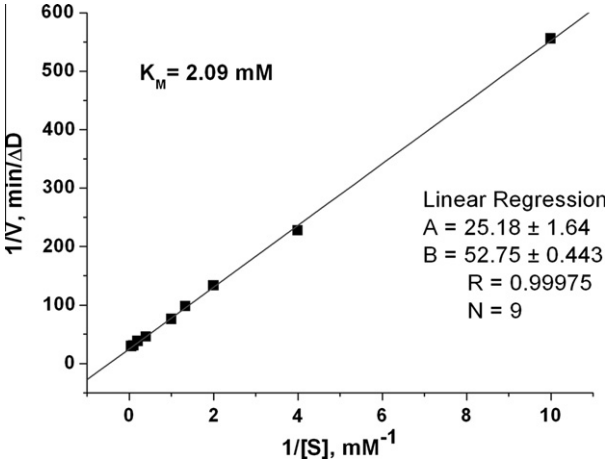

Fig. 3. Estimation of Michaelis–Menten constant (K_M) toward L-lactate for the recombinant cells of *H. polymorpha* "tr1" in the presence of DCPIP.

3.2. Photometric monitoring of chromate reduction

FC b_2 in living yeast cells is located in the intermembrane space of mitochondria (Daum et al., 1982). Therefore, FC b_2-mediated Cr(VI) reduction *in vivo* seems to require low molecular weight, free defusing electron transfer mediators enabling transfer of an electron from the enzyme, reduced by L-lactate, to Cr(VI). The recombinant protohaem IX-containing domain of cytochrome b_2 of *S. cerevisiae* has a redox potential of −31 mV, which is very close to the previously published value of −28 mV determined for the proteolytically released cytochrome b_2 core (Brunt et al., 1992). A similar low mid-point potential E_h = −19 mV was fixed for FC b_2-haem from *Hansenula anomala* (Capeillere-blandin et al., 1986). A low redox potential of FC b_2 is a favorable feature for chromate reduction which is characterized by particularly high redox potential (E^0 = +1.3 V).

FC b_2 catalysis is substantially enhanced by low molecular electron transfer mediators increasing electrochemical communication between reduced haem-binding domain of FC b_2 and the electrode surface (Smutok et al., 2005; Garjonyte et al., 2006; Smutok et al., 2007; Shkil et al. 2009). The mediator acceptability for four dyes (DCPIP, Meldola blue, Methylene blue, Nile blue) were tested using *H. polymorpha* cells of the recombinant strain "tr1" with 6-fold enhanced FC b_2-specific activity (up to 0.38 μmole min^{-1} mg^{-1} dried cells) (See Fig. 3).

The recombinant *H. polymorpha* yeast cells revealed K_M = 2.09 mM toward L-lactate in the presence of DCPIP. The same K_M value was determined by biosensor analysis for purified recombinant FC b_2 in the presence of PMS (Smutok et al., 2007). Therefore, recombinant cells could be considered as a proper model for study of the mediator activity of different dyes for FC b_2-catalyzed reaction.

The tested dyes differ significantly in the value of standard redox potential at pH 7.0 ($E^{0'}$) possessing similar absorbance peaks (Table 1). Theoretically, DCPIP with the highest positive potential is expected to be the best electron donor for reduced FC b_2. On the other hand, the reduced form of DCPIP can be readily oxidized by chromate (E^0 = + 1.3 V), maintaining the reduction of Cr(VI).

The reduction of chromate was initially monitored by measuring the absorbance increase related with oxidation of the reduced dyes by Cr(VI) after removing the cells. The initial incubation mixture included 25 mM acetate buffer, pH 6.3; 0.2 or 2 mM dye; 3 mM L-lactate, and, finally, *H. polymorpha* "tr1" cells in concentration 0.5 mg mL^{-1}. The addition of the cells stimulated discoloration of the dye within 2 min. After completed discoloration of the mixture, the yeast cells were removed by centrifugation and 0.5 mM K_2CrO_4 was added to the solution with further incubation for

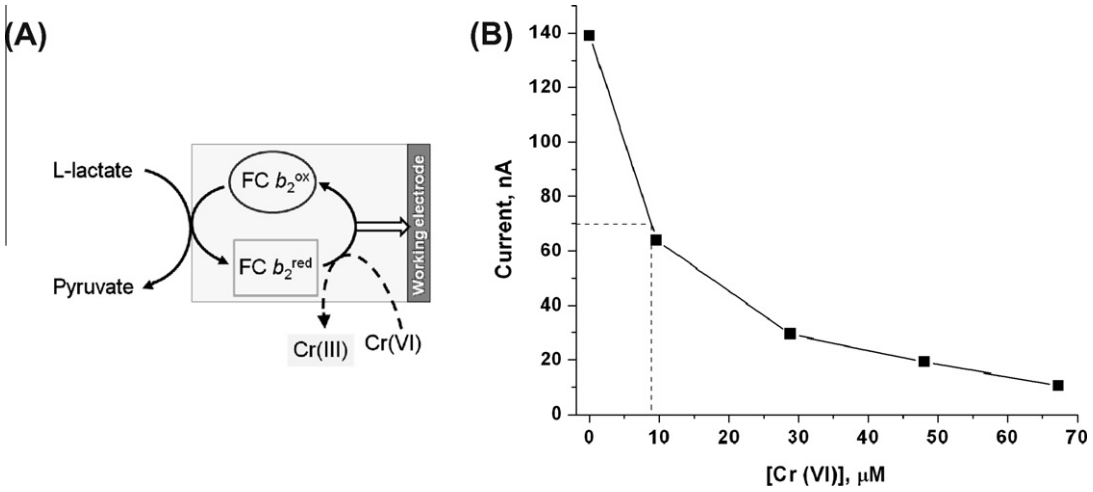

Fig. 2. (A) The scheme of competitive action of chromate on the direct electron transfer during FC b_2-catalyzed L-lactate oxidation. (B) Competition effect of chromate on FC b_2-mediated current generation during L-lactate oxidation.

Table 1
Characteristics of the tested electron transfer mediators (Atta-ur-Rahman, 1998; Norris and Ribbons, 1970).

Dyes	$E^{0'}$, mV at pH = 7.0	λ_{max} (nm)
DCPIP	+217	600
Methylene blue	+71	650
Meldola blue	−8	570
Nile blue	−142	645

30 min at 24 °C in closed vials to avoid contact with ambient air. Oxidation of the reduced dyes by chromate was monitored photometrically at the absorbance maximum peak for each dye. The highest activity was reached in the presence of DCPIP (Fig. 4) while the Cr(VI) reduction with other reduced mediators was rather poor (data not shown).

Resulting oxidation of reduced DCPIP by Cr(VI) is expressed as a linear function of time and the rate of this process is 2.54 μM min^{-1} (Fig. 4). Only negligible oxidation of the reduced DCPIP was observed in the control experiment without chromate.

The reduction of chromate in the whole system containing the cells, L-lactate, DCPIP and chromate was shown also by direct photometric monitoring of residual Cr(VI) content in the incubation mixture (Fig. 5). Simultaneous measurement of Cr(III) content in the mixture was not possible because of very close spectra of two products in the tested system: oxidized DCPIP and the complex Cr(III)-chromazurol S (Honchar et al., 2008).

As shown in Fig. 5, fast and slow stages are observed in the kinetic curves for Cr(VI) reduction. Calculation of the rates for both stages at different concentration of DCPIP (inset table in Fig. 5) revealed a strong dependence of Cr(VI) reduction on mediator concentration at the first stage of the process. The mechanism of this phenomenon remains to be determined. Perhaps, at initial steps the rate is fast because the pre-formed $DCPIPH_2$ quickly reduces the Cr(VI) (fast rate). Once most of the $DCPIPH_2$ fraction becomes oxidized, then Cr(VI) reduction slows (slow rate, stage two).

The rate of Cr(VI) bioreduction was strongly dependent on the concentration of enzymatically-reduced DCPIP that in turn is tightly correlated with the FC b_2-activity of the yeast cells.

At the final stage of our experiments, the ability for bioremediation of Cr(VI) by intact living cells of FC b_2-over-producing recombinant strain of *H. polymorpha* "tr1" was studied (Fig. 6).

Increasing chromate concentration resulted in a decrease of Cr(VI) reduction apparently due to detrimental effect of the toxic compound to the yeast cells. The efficacy of bioremediation for recombinant strain "tr1" was significantly higher as compared to the parental strain. 42% of Cr(VI) is reduced by recombinant cells while parental strain reduced only 30% of the pollutant when initial concentration of chromate was 50 μM (Fig. 6). Derepression of the gene *CYB2* in the recombinant strain and the resulted increase in specific FC b_2-activity appeared to be crucial for efficient Cr(VI) bioremediation.

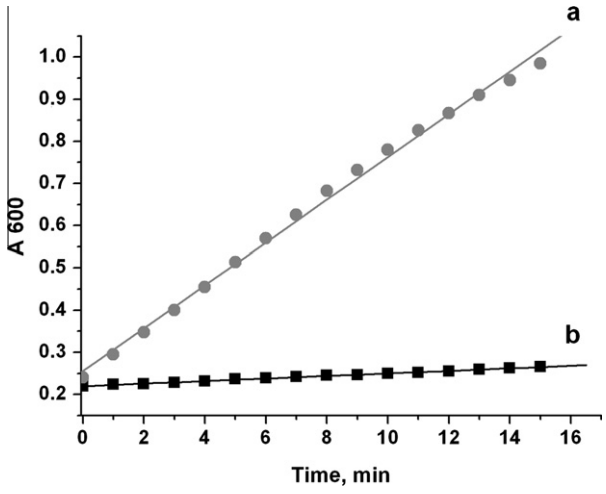

Fig. 4. The kinetics of the oxidation of DCPIP (0.2 mM) previously reduced by recombinant yeast cells (after drying) in the presence of L-lactate with addition (a) or absence (b) of 0.5 mM Cr(VI) in acetate buffer, pH 6.3.

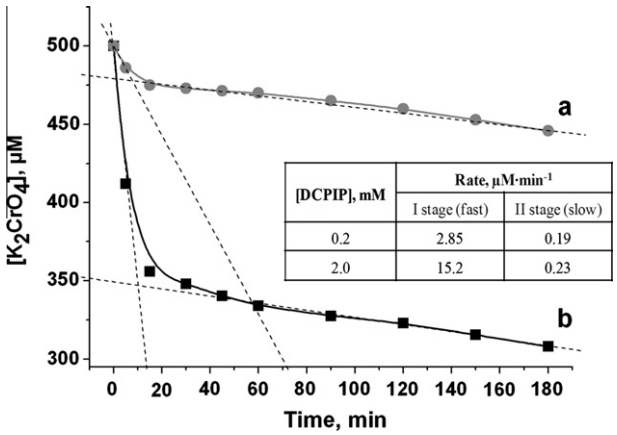

Fig. 5. The kinetics of Cr(VI) reduction in the system containing dried yeast cells of *H. polymorpha* "tr1" (0.5 mg mL^{-1}), 0.2 mM (a) or 2 mM (b) reduced DCPIP, 3 mM L-lactate, 0.5 mM chromate in 25 mM acetate buffer, pH 6.3. The rates for fast and slow stages of the process are shown in the integrated table.

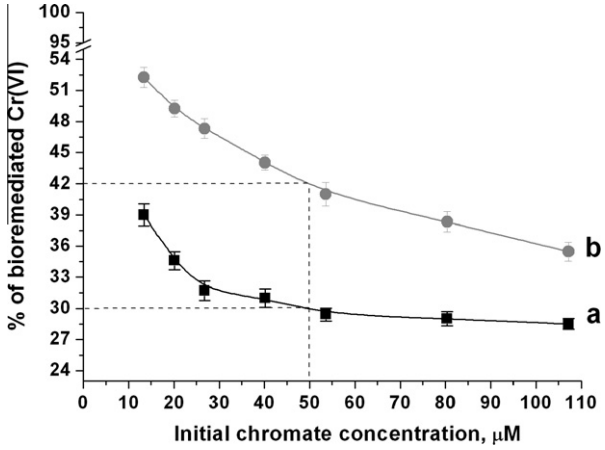

Fig. 6. The efficacy of Cr(VI) reduction by the living yeast cells at different initial concentrations of pollutant. (a) Parental strain C-105 (FC b_2-activity 1.53 U mL^{-1}); (b) recombinant "tr1" (enzyme activity 4 U mL^{-1}). Conditions: 25 mM acetate buffer, pH 6.3; cells – 3 mg mL^{-1}; 3 mM L-lactate; 2 mM DCPIP; 20 min at 24 °C.

4. Conclusions

In the present work, bioremediation of very toxic chromate Cr(VI) to less toxic Cr(III) by recombinant yeast *H. polymorpha* cells over-producing flavocytochrome b_2 was studied using bioelectrochemical approaches and photometric analysis. The phenomenon of the competitive effect of chromate on current generation during oxidation of L-lactate based on the model of bioelectrode prepared by immobilization of FC b_2 on a platinum surface was observed. This phenomenon can be used in the construction of chromate-selective biosensors. The ability of methylotrophic yeast cells to reduce chromate in the presence of L-lactate as an electron donor and synthetic dyes as electron transfer mediators was

demonstrated. Among tested mediators, the highest chromate-reducing activity of cells was achieved in the presence of DCPIP. The capacity of living cells for Cr(VI) bioremediation via the reductive pathway of recombinant yeast over-producing FC b_2 was established. The tested recombinant yeast cells could be readily used in chromate detoxification using dairy industry wastes as a cheap source of L-lactate.

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