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Immobilized formaldehyde-metabolizing enzymes from *Hansenula polymorpha* for removal and control of airborne formaldehyde

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ABSTRACT

Formaldehyde (FA)-containing indoor air has a negative effect on human health and should be removed by intensive ventilation or by catalytic conversion to non-toxic products. FA can be oxidized by alcohol oxidase (AOX) taking part in methanol metabolism of methylotrophic yeasts. In the present work, AOX isolated from a *Hansenula polymorpha* C-105 mutant (*gcr1 catX*) overproducing this enzyme in glucose medium, was tested for its ability to oxidize airborne FA. A continuous fluidized bed bioreactor (FBBR) was designed to enable an effective bioconversion of airborne FA by AOX or by permeabilized mutant *H. polymorpha* C-105 cells immobilized in calcium alginate beads. The immobilized AOX having a specific activity of 6–8 U mg^{-1} protein was shown to preserve 85–90% of the initial activity. The catalytic parameters of the immobilized enzyme were practically the same as for the free enzyme (k_{cat}/K_m was 2.35×10^3 M^{-1} s^{-1} *vs* 2.89×10^3 M^{-1} s^{-1}, respectively). The results showed that upon bubbling of air containing from 0.3 up to 18.5 ppm FA through immobilized AOX in the range of 1.3–26.6 U g^{-1} of the gel resulted in essential decrease of FA concentration in the outlet gas phase (less than 0.02–0.03 ppm, i.e. 10-fold less than the threshold limit value). It was also demonstrated that a FBBR with immobilized permeabilized C-105 cells provided more than 90% elimination of airborne FA. The process was monitored by a specially constructed enzymatic amperometric biosensor based on FA oxidation by NAD^+ and glutathione-dependent formaldehyde dehydrogenase from the recombinant *H. polymorpha* Tf 11-6 strain.

1. Introduction

Formaldehyde is a very important commercial chemical, and is among the most toxic pollutants used in many industries. It is used as an adhesive material in pressed wood products, as a preservative in paints and coatings, in the production of fertilizer, paper, plywood and urea–formaldehyde resins and in numerous other applications (Yocom and McCarthy, 1991; Khoder et al., 2000; Otson and Fellin, 1992). FA was introduced into medical practice as a disinfectant and tissue hardener and is used extensively for preserving tissue specimens in hospitals and laboratories (Walker, 1964; Cox, 1994). It is also used as a sterilizer and a preservative in vaccine production (Geier and Geier, 2004; Offit and Jew, 2007).

In vivo FA is detoxified principally via the action of FdDH (EC 1.2.1.1), a specific enzyme that catalyzes the conversion of FA into S-formylglutathione (finally, into formic acid) and NADH in the presence of GSH and NAD^+ (Pourmotabbed and Creighton, 1986; Uotila and Mannervik, 1979). FdDH is widely used for bioanalytical purposes (Ali et al., 2006; Gonchar et al., 2002; Hämmerle et al., 2010; Herschkovitz et al., 2000; Kawamura et al., 2005; Korpan et al., 1993, 2000; Vastarella and Nicastri, 2005; Winter and Cammann, 1989).

Hydrated form of FA can be easily oxidized into formic acid without any exogenous cofactor by AOX (EC 1.1.3.13), an enzyme which is responsible *in vivo* for the first reaction of methanol metabolism in methylotrophic yeast (Kato et al., 1976; Klei van der et al., 1990).

It is regarded that exposure to 20 ppm FA in air is dangerous to life and health. Several methods have been proposed for the

Abbreviations: FA, formaldehyde; AOX, alcohol oxidase; FdDH, formaldehyde dehydrogenase; GSH, reduced glutathione; MB, meldola blue; MBTH, 3-methyl-2-benzothiazolinone hydrazone hydrochloride; TLV, threshold limit value; MSDS, material safety data sheets; PMSF, phenylmethylsulfonyl fluoride; CTMAB, cetyltrimethylammonium bromide; FBBR, fluidized bed bioreactor.

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removal of FA from indoor air. Physical adsorption of FA with activated carbon (Boonamnuayvitaya et al., 2005; Tseng et al., 2005), various fractions of karamatsu bark (Takano et al., 2008) and zeolites (Cazorla and Grutzeck, 2006) were demonstrated to yield good to high results. However, simple adsorption cannot provide a radical solution to the problem, since FA is not decomposed, but is only transferred from one phase (air) to another (solid). Attempts at physical FA decomposition with the help of photocatalytic, negative ion and ozone air cleaners resulted in only up to 50% elimination of FA and failed to supply an FA level that meets WHO guidelines (0.08 ppm) (Tseng et al., 2005). Chemical decomposition of FA by composite silica particles functionalized with amine groups and platinum nanoparticles demonstrated a very high capacity for removing FA (Lee et al., 2008). However, this method is expensive. Another approach to chemical elimination of airborne FA was developed by Sekine, where manganese dioxide was shown to be effective for FA oxidation (Sekine, 2002; Tian and He, 2009). Combustion of a formaldehyde–methanol mixture in an airstream on Mn/Al_2O_3 and $Pd–Mn/Al_2O_3$ catalysts was shown to afford total conversion of organic compounds (Álvarez-Galván et al., 2004). The chemical approach to FA decomposition exhibits high efficiency, but the solid wastes that remain after these processes usually contain harmful toxic components which cause subsequent utilization problems.

FA removal from air using biological decomposition is not well developed. Several biofilters and biotrickling filters containing natural microorganisms were tested for the treatment of a mixture of formaldehyde and methanol (Prado et al., 2004, 2006). A maximum FA elimination capacity of 180 g m^{-3} h^{-1} was reached.

To the best of our knowledge, enzyme-based bioreactors have not been tested as a potential tool for FA bioremediation of indoor air. The goal of the present work was to study a possibility to use the enzyme AOX, as well as mutant yeast *H. polymorpha* cells overproducing this enzyme, to design continuous bioreactor for catalytic oxidation of airborne FA. For quantitative monitoring of this process, FA-selective amperometric biosensor based on recombinant NAD^+- and GSH-dependent FdDH was to be constructed.

2. Materials and methods

2.1. Chemicals

DEAE-Toyopearl 650M was purchased from Toyo Soda (Tokyo, Japan); para-formaldehyde, Triton X-100, PMSF, chromotropic acid, MBTH, MB, and sodium alginate were obtained from Sigma–Aldrich (USA); CTMAB and GSH were purchased from Fluka (Buchs, Switzerland). NAD^+ and NADH were obtained from Gerbu Biotechnik (Gailberg, Germany), 1% Nafion solution in ethanol (Aldrich, Germany) and the cathodic electrodeposition paint "GY 83-0270 0005" was purchased from BASF Farben und Lacke (Munster, Germany).

All chemicals were of analytical reagent grade and all solutions were prepared using HPLC-grade water. FA solution (1 M) was prepared by hydrolysis of the corresponding amount of paraformaldehyde (Sigma–Aldrich, USA) in water (300 mg; 10 ml water) by heating the suspension in a sealed ampoule at 105 °C for 6 h.

2.2. Mutant and recombinant yeast strains

The permeabilized cells of two *H. polymorpha* mutants were used as a bioactive material for construction of cell bioreactors: (1) CSA-33 (*gcr1*) ("constitutive synthesis of alcohol oxidase") with impaired glucose catabolite repression of AOX synthesis (Sibirny et al., 1993) and (2) C-105 (*gcr1 catX*) with additional mutation inactivating catalase (Gonchar et al., 2001, 2002). The latter strain was also used as a source for AOX isolation.

The recombinant *H. polymorpha* Tf 11-6 strain constructed by us previously was used for isolation of thermostable NAD^+- and glutathione-dependent FdDH (Demkiv et al., 2005).

2.3. Assay of enzyme activities

AOX activity was measured as described previously (Shleev et al., 2006).

FdDH activity was determined by the rate of NADH formation monitored spectrophotometrically at 340 nm (Schutte et al., 1976) under the following conditions: 25 °C, 1 mM FA, 1 mM NAD^+, and 2 mM GSH in PB (50 mM phosphate buffer, pH 8.0). Instant activity of FdDH (A) was calculated as the difference between A^{+FA} (in the presence of FA) and A^{-FA} (without addition of FA).

One unit (1 U) of activity for both enzymes was defined as the amount of enzyme which forms 1 μmol of the product per 1 min under standard conditions of the assay.

Protein concentration was estimated by the Lowry method.

2.4. Isolation and purification of the enzymes

2.4.1. AOX from mutant overproducing H. polymorpha yeast cells

AOX was isolated from cell-free extracts of the yeast *H. polymorpha* C-105 (*gcr1 catX*) strain cultivated in glucose medium (Gonchar et al., 2001; Shleev et al., 2006). Purification of the enzyme was carried out using a two-step precipitation with ammonium sulfate (at 40 and 60% saturation) in the presence of 1 mM EDTA and 0.4 mM PMSF to inhibit proteases. At 40% saturation, the protein precipitate was discarded, and the AOX precipitate obtained at 60% saturation was collected by centrifugation. The final activity of the enzyme preparations used for the bioreactor was 6–8 U mg^{-1} protein.

2.4.2. Recombinant FdDH

For enzyme isolation, cells of the recombinant *H. polymorpha* Tf 11-6 strain were cultivated in 1% methanol medium in the presence of 5 mM FA (Demkiv et al., 2007).

The purification procedure included preparation of a cell-free extract and a two-step column chromatography on anion-exchange sorbent DEAE-Toyopearl 650M (Demkiv et al., 2007). The final specific activity of the enzyme used for the biosensor construction was 18 U mg^{-1} protein.

2.5. AOX-based bioreactor

2.5.1. Immobilization of AOX in calcium alginate gel

Aliquots of 1 ml AOX solutions in 0.05 M PBS, pH 7.5 (buffer A) with activity of 2, 10, 20 or 40 U ml^{-1} were mixed with 2 ml of 3% (w/v) sodium alginate. The mixtures were dropped into a 2.5 mM $CaCl_2$ aqueous solution using a syringe with a 21G needle under stirring at room temperature and kept for 45 min for bead formation. The obtained gel beads were washed twice with 20 ml of buffer A.

2.5.2. Immobilization of the yeast cells in calcium alginate gel

Suspensions of 30 mg permeabilized mutant *H. polymorpha* C-105 cells (specific AOX activity 0.2 U mg^{-1}) and CSA-33 (activity 0.08 U mg^{-1}) were diluted in 1 ml of buffer A, mixed with 2 ml of 3% (w/v) alginate and treated as described above for immobilization of AOX.

Permeabilization of the yeast cells was carried out by their treatment with 0.1% CTMAB at 30 °C for 30 min. The permeabilizing agent was removed by centrifugation and washing the cells with

10 mM phosphate buffer, pH 7.0. Before use, the permeabilized cells were lyophilized or dried at room temperature.

2.5.3. Determination of enzymatic parameters of free and immobilized AOX

Michaelis–Menten constants, K_m, and catalytic constants, k_{cat}, of free and alginate immobilized AOX were determined using the routine Lineweaver–Burk method.

2.5.4. FA oxidation and assay of FA in gaseous and liquid phases by continuous FBBR

The gel beads with immobilized AOX (1.3–26.6 U g^{-1}) or cells (20 mg g^{-1}) were applied onto a 1 cm × 30 cm column (1.5 g of beads in 20 ml of buffer A) connected to an air source with a known FA concentration. The 0.3–18.5 ppm FA concentrations in air were obtained by bubbling airflow of 7–132 ml min^{-1} using a multi-channel Ecoline peristaltic pump (Ismatec, Switzerland) through a 2.7–100 mM aqueous FA solution at 25 °C. The scaled-up FBBR was designed on the basis of 10 cm × 25 cm column, containing 37.5 g of the AOX containing alginate gel beads (6.6 U g^{-1}) in 750 ml of buffer A connected to an air source with a 18.5 ppm FA bubbled with airflow of 152 ml min^{-1}. The FA concentration in the gaseous phase at the inlet to the column was calculated based on Henry's law (Seyfioglu and Odabasi, 2007) and tested with a Formaldehyde Gas Detector (Model FP-40 Riken Keiki, Japan). The FA concentration in the gaseous phase at the outlet of the column was tested with the same detector. The FA concentration in the liquid phase in the FBBR was assayed by a standard photometric method using a reaction with 1% chromotropic acid (Sawicki et al., 1961), as well as by the amperometric FdDH-based biosensor described in this paper. The control column contained beads without AOX or cells.

2.6. FdDH-based amperometric biosensor

2.6.1. Construction of FdDH-modified bioelectrodes

Platinized screen-printed gold electrodes (type C220AT, 4.0 mm diameter) from DropSens (Oviedo, Spain) were used as working electrodes. Platinization of the gold electrodes was carried out in a 6 mg ml^{-1} solution of hexachloroplatinum (IV)-acid hexahydrate in water by cyclic voltammetry (−0.6 to 0.4 V *vs* Ag/AgCl/KCl at a scan rate of 50 mV s^{-1}, 3–4 cycles). After platinization, the electrodes were rinsed with 20 mM phosphate buffer, pH 8.2.

Electrodeposited MB was used as a mediator for providing the optimal electron transfer from reduced FdDH through coenzyme to working electrode. Immobilization of MB on the surface of platinized electrodes was performed by means of cyclic voltammetry (−0.4 to 0.4 V *vs* Ag/AgCl/KCl at a scan rate of 50 mV s^{-1}, 4–5 potential cycles). After electrodeposition of MB, the MB-modified electrodes were rinsed with 20 mM phosphate buffer, pH 8.2.

Immobilization of FdDH together with its cofactor (NAD^+) was performed using a commercial cathodic paint (GY 83-0270 0005; pH 5.5) in order to form the biorecognition layer. 2 μl of FdDH suspension (30 U ml^{-1}; pH 8.2) and 2 μl of 100 mM NAD^+ were dropped on the surface of the MB-modified platinized electrodes, followed by addition of 8 μl of a 10-fold dilution of the cathodic paint solution. After drying, a well-adhering polymer film was formed. For co-entrapment of the second cofactor, glutathione, and covering of the sensing layer by a Nafion membrane, 4 μl of a 50 mM GSH solution neutralized to pH 8.0 were dropped on the top of the FdDH and NAD^+-modified electrodes. After drying (2–4 min), 5 μl of a 1% Nafion solution (the commercial ethanol solution was diluted with water) were placed on the sensor surface. The Nafion membrane was allowed to dry for 20–25 min at +4 °C. Before use, the constructed bioelectrodes were rinsed with 20 mM phosphate buffer, pH 8.2.

2.6.2. Amperometric measurements

The properties of the FdDH-based amperometric biosensor were evaluated using constant-potential amperometry in a three-electrode configuration with an Ag/AgCl/KCl (3 M) reference electrode and a Pt-wire 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 20 ml cell at room temperature. After the background current stabilized, the experiments were commenced by addition of formaldehyde aliquots using stock solutions prepared in 20 mM phosphate buffer, pH 8.2 (for calibration) and aliquots of the outlet fluid from the bioreactors.

The modified electrodes were stored between experiments in 20 mM phosphate buffer, pH 8.2, at 4 °C.

2.7. Photometric FA measurements

Chemical assays of FA were performed by two methods, using either chromotropic acid (Polska Norma, 1988) or MBTH (Sawicki et al., 1961). The previously developed Formatest kit was used for enzymatic assays of FA (Demkiv et al., 2007).

3. Results and discussion

We tested the ability of AOX isolated from mutant *H. polymorpha* C-105 cells overproducing this enzyme to oxidize FA that was immobilized in calcium alginate beads, based on its relatively high stability and ease of preparation. The isolated AOX preparation had a specific activity in the range of 6–8 U mg^{-1} protein and was shown to preserve 85–90% of the initial activity after incorporation into the calcium alginate gel. This activity was proven to remain unchanged for up to twelve months upon storage of the immobilized enzyme at 4 °C, which is essential for potential practical applications. The immobilized enzyme can be stored for a year before the use. The permeabilized mutant *H. polymorpha* CSA-33 and C-105 cells were also used as a bioactive material in bioreactors.

The enzymatic parameters K_m and k_{cat} of free and calcium alginate-immobilized AOX were determined from the Lineweaver–Burk plot (Table 1). As can be seen, the immobilization had almost no effect on the AOX affinity for FA or its kinetic ability.

The FBBR was constructed based on a glass column filled with gel beads suspended in buffer A with immobilized AOX or cells. A column filled with gel alone was used as control. FA-containing air was bubbled through the columns from the bottom to the top (Fig. 1).

FA in air was generated by bubbling fresh air through an aqueous solution with various concentrations of FA. The FA concentration in the gaseous phase was calculated by the Henry law: $P_A = X_A H_A(T)$, where P_A is the partial pressure of FA in the gaseous phase, X_A is the mole fraction of FA in water and $H_A(T)$ is a temperature-dependent Henry constant calculated using the formula: $\ln H_A$ (L atm mol^{-1}) = −(1641.3/T) − 3.089 (Seyfioglu and Odabasi, 2007). Thus, gaseous phases with concentrations of 0.3–18.5 ppm FA were built. In the cases of low inlet FA concentrations (under 0.6 ppm) they were also confirmed by measurement with the Formaldehyde Gas Detector. This range of concentrations was chosen because it reflects the level of FA in the air in industrial areas under working regimes and because these concentrations are dangerous to human

Table 1
Enzymatic characteristics of the free and immobilized AOX.

Enzyme	K_m (mM)	k_{cat} (s^{-1})
Free AOX	10.7 ± 0.8	30.9 ± 0.9
Calcium alginate-immobilized AOX	12.4 ± 1.5	29.2 ± 1.5

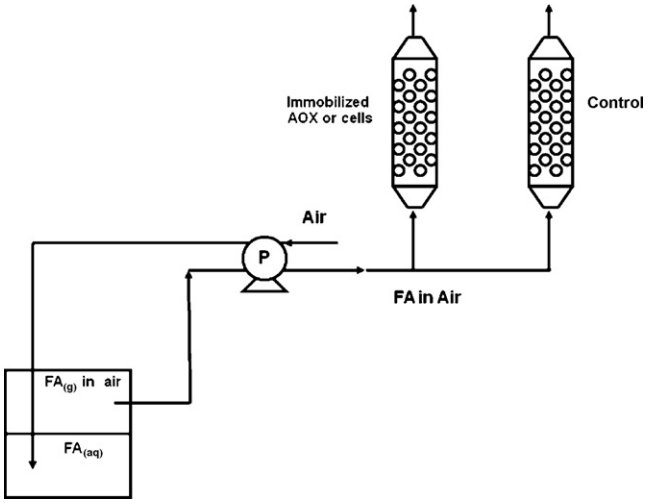

Fig. 1. Scheme for oxidation of airborne FA by AOX immobilized in calcium alginate or cells in a continuous FBBR. The control column contained gel beads without immobilized material.

health (Philp, 2001; Wartew, 1983). According to WHO standards, the threshold limit value (TLV) of FA in air is 0.3 ppm. However, it should be noted that most people feel consequences of exposure even at a FA level of 0.25 ppm (Holness and Nethercott, 1989). According to the MSDS, exposure to 20 ppm FA in air is regarded as posing immediate danger to life and health.

The FA concentrations were tested for about two weeks. The results showed that the outlet gas phase contained less than 0.02–0.03 ppm FA when using immobilized AOX in the range of 1.3–26.6 U g^{-1} of the gel upon bubbling of air containing 0.3 ppm FA (Fig. 2a). The FA concentration in the gas phase at the outlet from the control column without the enzyme was essentially higher (0.09 ppm), but also relatively low compared to the input level (Fig. 2a), evidently due to FA dissolution in the liquid phase of the column (Fig. 2b) and possibly also due to adsorption by the gel. Parallel testing of the FA concentration in the aqueous phases of the columns by chemical methods (MBTH and chromotropic acid), as well as by FdDH-based methods ("Formatest" and FdDH-based biosensor), confirmed the difference between the AOX-containing gels and blank gels – FA concentration in the reactor aqueous phase was 0.9–3.5 mM (1 mM of FA in aqueous solution equals 30 ppm), whereas in the control column – up to 10.5 mM (Fig. 2b and Table 2).

Other reference methods were also used in order to provide an accurate FA analysis in the corresponding samples, in addition to the biosensor: standard photometric methods (Sawicki et al., 1961; Polska Norma, 1988) and the FdDH-based enzymatic method using the "Formatest" kit (Demkiv et al., 2007) (Table 2).

When the FA concentration in air was increased to 18.5 ppm, the system still proved its ability to decompose the FA. As can be seen from Fig. 3a, the outlet FA concentration was less than 0.03 ppm,

Table 2
FA content in analyzed samples as determined by different methods.

Sample/method	FA concentration, mM			
	Chemical methods		FdDH-based methods	
	MBTH	Chromotropic acid	"Formatest"	Biosensor
Alginate gel column without AOX	10.1 ± 0.6	9.58 ± 1.4	10.8 ± 0.50	10.2 ± 0.23
AOX-bioreactor	3.3 ± 0.13	3.86 ± 0.21	3.42 ± 0.16	3.2 ± 0.13

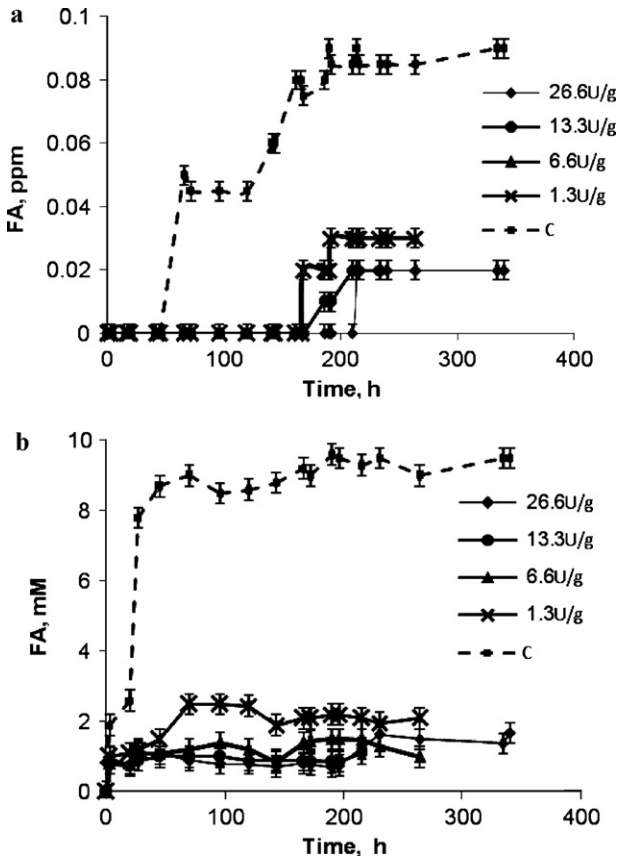

Fig. 2. FA concentration in the gaseous phase at the outlet (a) and in the aqueous phase (b) of the continuous FBBR upon oxidation of FA in the 7 ml min^{-1} air flow with inlet FA concentration of 0.3 ppm by immobilized AOX (1.3–26.6 U g^{-1} of the gel). C (control): calcium alginate gel alone.

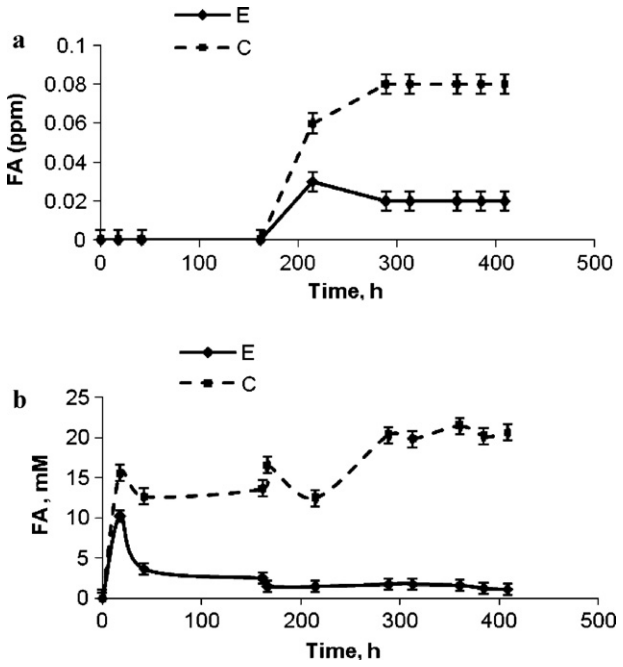

Fig. 3. FA concentration in the gaseous phase in the outlet (a) and in the aqueous phase (b) of the continuous FBBR upon oxidation of FA in the 7 ml min^{-1} air flow with inlet FA concentration of 18.5 ppm by immobilized AOX (13.3 U g^{-1} of the gel) (E). C (control): calcium alginate gel alone.

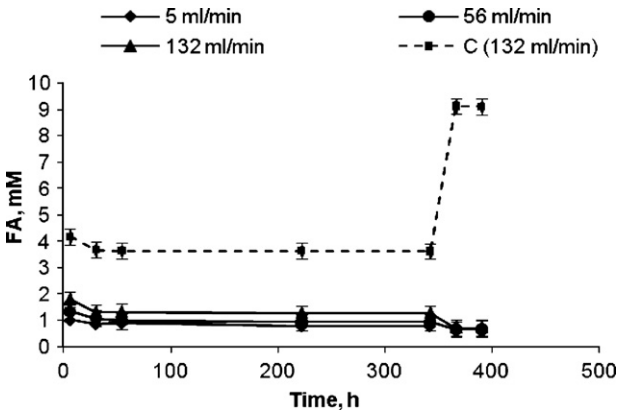

Fig. 4. FA concentration in the aqueous phase of the FBBR upon oxidation of FA in the 5–132 ml min^{-1} air flow with 0.3 ppm FA inlet concentration by immobilized AOX (13.3 U g^{-1} of the gel). C (control): calcium alginate gel alone which was tested at an air flow of 132 ml min^{-1}.

i.e. 10-fold less than the TVL. The FA concentration in the bioreactor liquid phase was ca. 1.5 mM (Fig. 3b). In the control experiment the outlet gas phase contained 0.08 ppm FA and the liquid phase almost 20 mM. It can be concluded that the proposed bioreactor is suitable for treating air containing various FA concentrations.

The bioreactor's efficiency was tested in a wide range of gas flows. The results of this experiment are presented in Fig. 4, which demonstrates the capability of immobilized AOX to decompose FA at air flows from 5 ml min^{-1} to 132 ml min^{-1} at an inlet FA concentration of 0.3 ppm. The FA concentration in the aqueous phase at all tested flows did not exceed 1.5 mM, whereas in the control system the FA accumulated and its concentration in the outflow increased with time. The outlet gas contained negligible concentrations of FA (less than 0.01 ppm) in all bioreactors, and in the control column the outlet FA concentration was 0.04 ppm. FA was almost totally removed in both cases, but only bioconversion led to safe FA levels in the gaseous and liquid phases. After the experiment ended, the FA concentration in the column's aqueous phase continued to be monitored, and after 16 days it decreased to 0.4 mM, i.e. it reached wastewater safety levels and was only ca. 13 times higher than the permitted concentration in drinking water (0.03 mM or 0.9 ppm). The FA concentration in the control column remained high.

The obtained results demonstrate that AOX successfully oxidizes FA in the aqueous phase. In the control system, a positive effect is observed only in the initial process stages due to simple FA extraction and absorption from the gaseous phase by liquid and solid phases, but at advanced stages the FA accumulation leads to FA release into the gas phase, i.e. the potential use of such a scheme is limited to several hours only. The results of FA bioconversion by the immobilized AOX are very promising. This system enables not only the absorption of the bubbled FA into the water phase, but also the decrease in the FA concentration in the water to low levels. Thus, such a system affords guaranteed safe FA levels even after prolonged use in both water and gas phases at the outlet. The efficiency of FA removal from the gaseous phase ranged between 93.0 and 99.9% in all described cases. In the scaled-up FBBR the obtained results were even better—the outlet air contained no FA, and in the aqueous phase the FA concentration did not exceed 0.9 mM after 3 weeks of observation.

Permeabilized mutant yeast cells overproducing AOX – *H. polymorpha* C-105 (*gcr1 catX*) and CSA-33 (*gcr1*) entrapped in Ca-alginate gel were also used for FA bioconversion in continuous gas flow. As shown by us previously (Gonchar et al., 2002), chemical modification of the cells resulted in increased permeability of the cellular barrier to low-molecular weight compounds. As in the case of the immobilized AOX at the outlet of the columns containing both

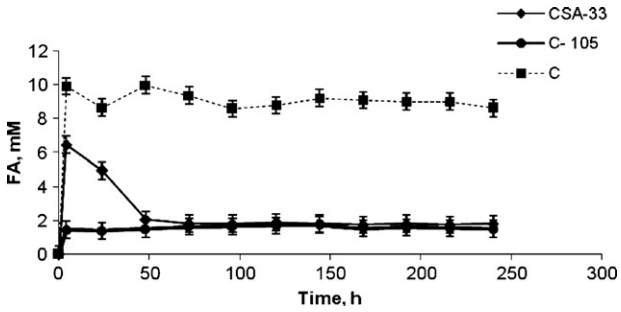

Fig. 5. Concentration of FA in the aqueous phase in FBBR with permeabilized C-105 cells (*gcr1 catX*) and CSA-33 (*gcr1*) (20 mg (DCW) g^{-1} of the gel) immobilized in calcium alginate gel or alginate gel alone (C) during bubbling of FA-containing air through the columns. Air flow was 7 ml min^{-1} and the inlet FA concentration was 0.3 ppm.

types of cells, the FA concentration in the gas phase was 0.02 ppm. Mutant C-105 cells showed better capability for decomposition of the FA in the column liquid phase during first two days than CSA-33 cells, but at advanced stages both cell types showed almost the same performance (Fig. 5). These data can be explained by various initial AOX activities inside the cells (2.5-fold difference—see Section 2.5.2). Upon continuous exposure of the CSA-33 cells to FA, there is probably a positive influence of catalase which degrades the H_2O_2 produced during FA oxidation (C-105 cells lack catalase).

As can be seen from Figs. 2–5, upon bubbling through the aqueous phase, 70–95% of the airborne FA is absorbed by dissolving in water and by gel absorption, which leads to a decrease of the air-

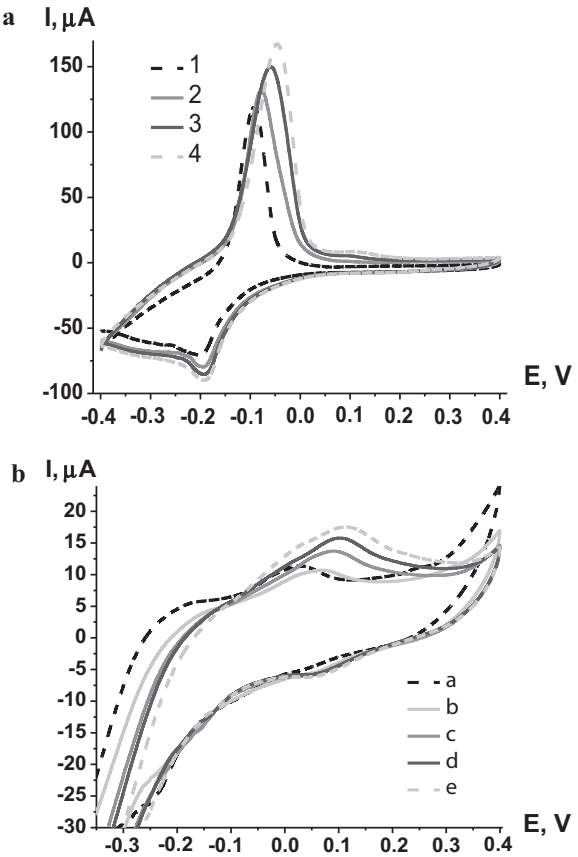

Fig. 6. Cyclic voltammograms of electroinduced deposition of MB on the top of a 4 mm platinized carbon electrode (1, 2, 3, 4—the cycles) (a) and electroinduced NADH oxidation on the top of a MB-modified electrode (a: MB-modified electrode; b: 0.5 mM NADH; c: 1 mM NADH; d: 1.5 mM NADH; e: 2 mM NADH) (b).

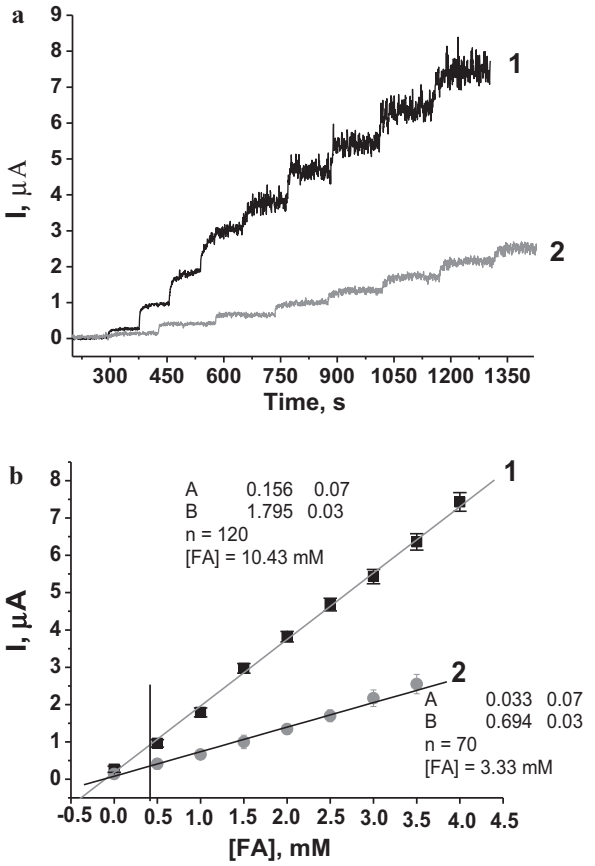

Fig. 7. Chronoamperograms (a) and calibration curves (b) for standard addition tests using a FdDH-based sensor for the FA assay in AOX-base bioreactor outflows. (1) Alginate gel alone and (2) AOX-containing alginate gel. The outflows were obtained by passing the aqueous phase of FA through a column with gel beads, containing 5 AOX U g^{-1} of the gel.

borne FA concentration in control columns from 0.3 to 18.5 ppm at the inlet to 0.08 to 0.09 ppm at the outlet. At the same time, 80–95% of the physically absorbed FA is decomposed by immobilized AOX or by AOX-containing cells, leading to 1–1.5 mM FA in the aqueous phase against 9–20 mM in control experiments. The overall effect of the physical absorption and of the bioconversion causes a 90–99% reduction in the airborne FA concentration.

It is very important to use a relevant analytical device in order to monitor the efficiency of FA removal by bioreactors. For this aim we constructed an amperometric enzyme-based biosensor. NAD^+- and glutathione-dependent FdDH isolated from the recombinant strain Tf 11-6 (Demkiv et al., 2007) was used as a selective biorecognition unit. Enzymatically generated NADH was re-oxidized at the electrode surface using electroimmobilized MB as a suitable redox mediator in order to optimize an electron-transfer pathway in the FdDH-based biosensor from an immobilized FdDH to the electrode surface. Immobilization of MB on the surface of platinized electrodes was performed by means of cyclic voltammetry (−0.4 to 0.4 V *vs* Ag/AgCl/KCl at a scan rate of 50 mV s^{-1}, 4–5 potential cycles) (Fig. 6a).

As can be seen in Fig. 6b, NADH oxidation peak in the presence of MB as a mediator accounted for +0.1 V (*vs* Ag/AgCl). However, oxidation of this co-enzyme is also observed at the zero potential. The improved selectivity of the sensor is feasible using low operating potentials. Therefore, we selected +0 V (*vs* Ag/AgCl) as a working potential for further research.

FdDH as a biorecognition element in the presented biosensor requires the simultaneous presence of two low-molecular cofactors: NAD^+ and glutathione. Because of this, a sophisticated sensor architecture is proposed consisting of using an additional negatively charged Nafion membrane, which prevents leakage of the cofactors due to charge interactions.

The FA content in the outflow liquid of the bioreactors was determined using a FdDH-modified amperometric bioelectrode in combination with the multiple standard addition method (Fig. 7).

The proposed method for FA removal from indoor air by the enzyme AOX and permeabilized cells entrapped in alginate gel provides not only an effective bioconversion of FA in the gas phase, but also a safe FA level in the liquid phase of the continuous FBBR. After termination of the process the content of the bioreactor can be used as organic fertilizer, since the gel beads together with the liquid phase are free of hazardous components. The entire process can therefore be considered as totally environmentally friendly.

4. Conclusion

The presented data confirm the possibility of exploiting the developed continuous bioreactors based on AOX or methylotrophic yeast cells overproducing this enzyme for effective (93.0–99.9%) oxidation of airborne FA. Immobilized in the calcium alginate gel AOX was shown to preserve 85–90% of the initial activity and to keep its catalytic parameters. It was demonstrated that an amperometric biosensor constructed on the base of recombinant FdDHase can be successfully used for a control of FA bioremediation in indoor air.

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