Bioconversion of airborne formaldehyde by immobilized formaldehyde dehydrogenase from the recombinant methylotrophic yeast

_Hansenula polymorpha_

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Abstract—Formaldehyde (FA) is a very toxic pollutant. FA in indoor air has a negative effect on human health and should be removed by ventilation or by conversion to non-toxic products. The formaldehyde dehydrogenase (FdDH), a NAD- and glutathione-dependent enzyme from recombinant methylotrophic yeast _Hansenula polymorpha_, was tested for its ability to oxidize airborne FA. A continuous fluidized bed bioreactor was designed to enable a bioconversion of airborne FA by FdDH or by recombinant yeast cells immobilized in calcium alginate beads. The results demonstrated that such the bioreactor with immobilized FdDH provides 97-100% elimination of airborne FA and the bioreactor with immobilized cells eliminates 94-100% of FA.

Keywords - formaldehyde; bioconversion; _Hansenula polymorpha_; recombinant formaldehyde dehydrogenase; enzyme immobilization; fluidized bed bioreactor.

I. INTRODUCTION

Formaldehyde (FA) has a negative effect on human health, especially on the central nervous, circulatory and immune systems. Anatomists, technicians, medical or veterinary students and embalmers are among the people who are at great risk for FA toxicity. FA can also be found in food and in some skin treatment products. FA is found in the smog in the lower atmosphere, and therefore in the air people breathe every day. The exhaust of cars that do not have catalytic converters or that use oxygenated gasoline also contains FA [1]. In domestic settings, FA is emitted by cigarettes and other tobacco products, gas cookers, and open fireplaces. It is found in many products used every day around the house, such as antiseptics, cosmetics, dish-washing liquids, fabric softeners, shoe-care agents, carpet cleaners, glues, lacquers, paper, plastics, and some types of wood products [2]. When inhaled, FA primarily affects the respiratory system, and the severity and extent of the physiological response depends on its concentration in the air.

FA is classified as a mutagen and a possible human carcinogen and is considered one of the chemical mediators of apoptosis [3]. FA induces mutations and DNA damage in bacteria. DNA-protein cross-links, DNA single-strand breaks, chromosomal aberrations, sister chromatid exchanges and gene mutations are induced in human and rodent cells. Although it has not been definitively established, FA is assumed to be a potential cause for carcinoma in the nasal cavity of humans, whereas such carcinogenic effects have already been proven in rats.

FA is concomitantly a naturally occurring metabolite which is produced in very small amounts in our bodies as part of our normal everyday serine, glycine, methionine and choline metabolism as well as by the demethylation of _N_, _S_- and _O_-methyl compounds [4]. It is estimated that the endogenous FA concentration in blood is close to 0.1 mM. FA is detoxified principally via the action of glutathione (GSH)-dependent formaldehyde dehydrogenase (FdDH, EC 1.2.1.1), a specific enzyme that catalyzes the conversion of FA into S-formylglutathione and NADH in the presence of GSH and NAD⁺ [5]. S-formylglutathione (GSH=O) is finally hydrolyzed into free formic acid:

a) \( \text{CH}_3\text{O} + \text{GSH} \leftrightarrow \text{GS} - \text{CH}_2\text{OH} \);
b) \( \text{GS} - \text{CH}_2\text{OH} + \text{NAD}^+ \leftrightarrow \text{GSH} = \text{O} + \text{NADH(H}^+) \);
c) \( \text{GSH} = \text{O} + \text{H}_2\text{O} \rightarrow \text{GSH} + \text{HCOOH} \)

Since FdDH is a GSH-dependent enzyme, the pool of GSH available for FA binding is important in the regulation of FdDH activity. FA can then be metabolized into formate and can enter the one-carbon pool for incorporation into cell constituents [6]. FdDH is a key enzyme of FA metabolism and is widely used for bioanalytical purposes [7, 8].

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Several methods have been proposed for the removal of FA from indoor air. Physical adsorption of FA with activated carbon [9] and zeolites [10] demonstrated 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 photo-catalytic, 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) [9]. Chemical decomposition of FA by composite silica particles functionalized with amine groups and platinum nanoparticles had a very high capacity for removing FA [11]. 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 [12, 13]. Combustion of a formaldehyde-methanol mixture in an airstream on Mn/Al2O3 and Pd-Mn/Al2O3 catalysts afford total conversion of organic compounds [14]. 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 hardly described. Several biofilters and biotrickling filters containing natural microorganisms were tested for the treatment of a mixture of formaldehyde and methanol [15]. A maximum FA elimination capacity of 180 g m⁻³ h⁻¹ was reached.

In our previous work a bioreactor based on immobilized alcohol oxidase from mutant *Hanselula polymorpha* yeast was shown to be a powerful tool for FA bioremediation of indoor air [16, 17]. The aim of the present work was to design a continuous bioreactor based on an immobilized FA-oxidizing NAD⁺- and GSH-dependent FdDH and on recombinant *Hanselula polymorpha* yeast.

II. MATERIALS AND METHODS

*Mutant and recombinant yeast strains*

The recombinant *H. polymorpha* Tf 11-6 strain constructed by us previously was used for isolation of thermostable NAD⁺- and glutathione-dependent FdDH [18].

*Assay of enzyme activity*

FdDH activity was determined by the rate of NADH formation monitored spectrophotometrically at 340 nm [19] at 25°C in 50 mM phosphate buffer, pH 7.5, containing 1 mM NAD⁺ and 2 mM GSH (buffer I) in the presence of 1 mM FA. Instant activity of FdDH (A) was calculated as the difference between $A_{\text{OA}}$ (in the presence of FA) and $A_{\text{FA}}$ (without addition of FA). One unit (1 U) of activity was defined as the amount of the enzyme which forms 1 μmole of the product per 1 min under standard conditions of the assay. Protein concentration was estimated by the Lowry method.

*Isolation and purification of 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 [20]. The purification procedure included preparation of a cell-free extract and a two-step column chromatography on anion-exchange sorbent DEAE-Toyopearl 650 M [20]. The final specific activity of the enzyme was 18 U mg⁻¹ protein.

Immobilization of FdDH in calcium alginate gel

Aliquots of 2 ml FdDH in buffer I with activity of 13.3 U ml⁻¹ were mixed with 2 ml of 3% (w/v) sodium alginate. The mixtures were dropped into a 2.5 mM CaCl₂ 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 50 mM phosphate buffer, pH 7.5 (buffer II).

Immobilization of the yeast cells in calcium alginate gel

30 mg of recombinant *H. polymorpha* Tf 11-6 cells (specific FdDH activity 0.6 U mg⁻¹) were suspended in 2 ml of buffer II, mixed with 2 ml of 3% (w/v) alginate and treated as described above for immobilization of FdDH, except for the final washing, which was fulfilled by the buffer II.

*FA oxidation by continuous fluidized-bed bioreactor*

The fluidized-bed bioreactor (FBBR) was designed and operated as follows: the gel beads with immobilized FdDH (6.6 U g⁻¹ of the gel) or cells (20 mg g⁻¹ of 12 U mg⁻¹ of the gel) were applied onto a 1x30 cm column (1.5 g of beads in 20 ml of buffer I in the case of FdDH and of buffer II in the case of the cells), the bottom of which was 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 7-130 ml min⁻¹ using a multichannel Ecoline peristaltic pump (Ismatec, Switzerland) through a 2.7-100 mM aqueous FA solution at 25°C. The FA concentration in the gaseous phase at the inlet to the column was calculated based on Henry’s law [16, 21] and also 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 FBBR was assayed by a standard photometric method using a reaction with 1% chromototropic acid [22].

III. RESULTS AND DISCUSSIONS

We examined the ability of FdDH isolated from recombinant *H. polymorpha* Tf 11-6 cells overproducing this enzyme and immobilized in calcium alginate beads to oxidize FA, in order to develop an efficient and specific tool for biocconversion of FA. FdDH was shown to preserve more than 85% of the initial activity after incorporation into the calcium alginate gel. The recombinant *H. polymorpha* Tf 11-6 cells were also immobilized and used in a bioreactor.

The FBBR was constructed using a glass column filled with gel beads suspended in buffer I, which contained the cofactors GSH and NAD⁺, and immobilized FdDH. In the case of immobilized yeast cells, the gel beads were suspended in buffer II without cofactors. A column filled with calcium alginate gel alone was used as control. The two columns (with and without immobilized biomaterial) were always tested in parallel. Air containing FA was bubbled through the columns from the bottom to the top as described previously [16]. The FA
0.3-18.5 ppm of FA in air were generated by bubbling fresh air through an aqueous solution with various concentrations of FA, as described previously by us [16]. The chosen FA concentration range was between the threshold limit value (TLV) of FA in air and a concentration that poses an immediate threat to health and life [16].

The reactors were operated for about two weeks. The results showed that when the bubbled air contained 0.3 ppm FA at 7 ml min⁻¹ flow, the outlet gas phase contained no more than 0.01 ppm FA in the case of immobilized FdDH (97% elimination of FA). At the same time, the FA concentration at the outlet of the control column was 0.05-0.06 ppm (Fig. 1a). This value was also less than at the input, due to FA dissolution in the aqueous phase of the column and possibly also due to adsorption by the gel. Determination of the FA concentration in the aqueous phases of the columns confirmed the difference between the FdDH-containing gel and the blank gel. The FA concentration in the FBBR was 1.5-2 mM, whereas in the control column it reached 16 mM (Fig. 1b). Increasing the flow rate to 130 ml min⁻¹ had no negative effect on the bioreactor's efficiency. FA bioconversion remained at least 97% (data not shown).

Interestingly, increasing the inlet FA concentration to 18.5 ppm did not impair the performance of the FBBR (Fig. 2). The outlet FA concentration in the bioreactor still did not exceed 0.01 ppm (Fig. 2a), which corresponded to more than 99.9% FA bioconversion. The difference between the bioreactor and the control column can be clearly seen in Fig. 2b. The FA concentration in the aqueous phase of the blank gel reached 50-52 mM, whereas in the FBBR column it was less than 4 mM. These results indicate the ability of immobilized FdDH to continuously bioconvert FA. The bioreactor demonstrated very high efficiency in various regimes in a wide range of FA concentrations and air flow rates.

We have previously demonstrated similar performance of FBBR with immobilized alcohol oxidase (AOX) from mutant H. polymorpha cells [16, 17]. AOX has certain advantages over FdDH in continuous applications, since it does not require any cofactors. However, FdDH is known to have higher specificity for FA than AOX, so that use of the former is preferable in all the cases in which air pollution is caused by FA.

We also tested the ability of immobilized recombinant H. polymorpha cells containing AOX to convert airborne FA. The whole cells contain both needed for FdDH functioning cofactors, so there was no need to introduce them into the reactor buffer. The results are presented in Fig. 3. The FA concentration in the gas phase at the outlet of the immobilized cell-containing column in a continuous regime did not exceed 0.02 ppm. The immobilized cells thus demonstrated up to 94% FA bioconversion efficiency. We conclude that the immobilized cells were slightly less efficient than the immobilized FdDH, despite the higher FdDH activity used in the case of the cells. This fact can be easily explained by diffusion limitations. FA as well as the reaction products concentration was monitored in the gas phase at the inlet and outlets of the system, as well as in the aqueous phase of the reactors.

![Figure 1](image1.png)  
Figure 1. 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 air by FdDH immobilized in 1.5% calcium alginate gel. The air flow was 7 ml min⁻¹, initial FA concentration in air was 0.3 ppm. The air was bubbled through a 1x30 cm column with 1.5 g of gel beads, containing FdDH 6.6 U g⁻¹ of the gel in buffer (E), C (control) - alginate gel alone. FA analysis in the gaseous phase was carried out using a Formaldehyde Gas Detector. The FA concentration in the aqueous phase was monitored by a standard photometric method using a reaction with chromotropic acid.

![Figure 2](image2.png)  
Figure 2. 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 air by FdDH immobilized in 1.5% calcium alginate gel. The air flow was 7 ml min⁻¹, initial FA concentration in air was 18.5 ppm. The air was bubbled through a 1x30 cm column with 1.5 g of gel beads, containing FdDH 6.6 U g⁻¹ of the gel in buffer I (E), C (control) - alginate gel alone. FA analysis in the gaseous phase was carried out using a Formaldehyde Gas Detector. The FA concentration in the aqueous phase was monitored by a standard photometric method using a reaction with chromotropic acid.
probably do not pass easily through the yeast cell membrane. On the other hand, an application using immobilized cells instead of the enzyme could lower the cost of the FA bioconversion process considerably.

Based on the results of the present study, it can be concluded that bioconversion of airborne FA can be performed successfully by immobilized FdDH or by FdDH-containing cells. By using a continuous regime, these systems enabled the achievement of over 94% FA bioconversion efficiency.

REFERENCES


