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Cyclodextrin-aided microbial transformation of aromatic aldehydes by *Saccharomyces cerevisiae*

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Summary. Biotransformation of benzaldehyde and vanillin by growing cells of *Saccharomyces cerevisiae* was performed in an aqueous medium containing either α- or β-cyclodextrin at the same molar concentration as the substrate. The yeast fermentative activity, as reflected by CO_2 evolution, and bioconversion to the corresponding alcohols were both faster and greater in the presence of the cyclic dextrins. Clearly, cyclodextrins were shown to significantly alleviate the inhibitory effects of the aromatic aldehydes.

Introduction

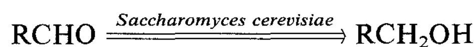
Microbial transformation of sparingly soluble organic compounds often meets with serious, sometimes unsurmountable, difficulties (Bar 1988). These are (a) a limited substrate accessibility to the biocatalyst and (b) inhibition or toxicity of both substrate and product exerted upon the microbe. Attempts to overcome these shortcomings include the use of surfactants and water-miscible or immiscible solvents. Inhibitory and toxic effects introduced by these surfactants and organic solvents may again partly or fully inactivate the biocatalyst.

A different type of bioconversion medium is an essentially aqueous medium into which an inherently non-toxic ingredient is added to solubilize the lipophilic substrate. Along these lines. Bar (1988) recently suggested a liposomal medium for bioconversion of sterols and steroids. Cyclodextrins are also biocompatible and capable of solubilizing lipophilic organics in an aqueous medium.

Cyclodextrins (Bender 1986) are cyclic oligomers of 1.4-α-D-linked glucose units. The main cyclodextrins consist of 6.7 or 8 glucose molecules forming α-, β- and γ-cyclodextrins, respectively. Their molecular structures are doughnut-shaped with a hydrophilic shell and a somewhat hydrophobic cavity. Consequently, the cyclic dextrins are water-soluble and capable of forming water-soluble inclusion compounds via complexing of lipophilic chemicals in their cavities. Furthermore, cyclodextrins are known to be harmless to enzymes and microbes (Szejtli and Pagington 1986).

Reports on microbial transformations in a cyclodextrin medium appear to be very scarce. One recent study (Hesselink et al. 1986) concluded that initial rates of microbial transformation of cholesterol increased in the presence of β-cyclodextrin.

In this article, an investigation of a yeast-mediated reduction of aromatic aldehydes in an aqueous broth of cyclodextrins is reported:



$$RCHO \xrightarrow{\textit{Saccharomyces cerevisiae}} RCH_2OH$$

where RCHO is vanillin or benzaldehyde. With respect to this specific microbial transformation, *S. cerevisiae* is particularly advantageous in that it does not excrete amylases, so that enzymatic degradation of the cyclodextrins is not possible.

Materials and methods

Materials. Both α- and β-cyclodextrins were purchased from Senn Chemicals (Dielsdorf, Switzerland). Vanillin was recrystallized from water and dried in vacuo. Benzaldehyde was purified by distillation under an argon atmosphere and subsequently stored under argon in the dark at 0° C. All aromatic aldehydes and alcohols were purchased from Sigma, St. Louis, USA.

Microorganisms. Baker's yeast was used in the form of active dry granules. Its real biomass content was experimentally de-

termined by drying (60° C, 24 h) a prewashed and preweighed amount of the crude yeast and was found to be 66%. A defined strain of *S. cerevisiae* FU 63 was obtained from the local collection of microorganisms of the Department of Applied Microbiology, The Hebrew University, Israel. It was maintained on agar slants which consisted of 20 g glucose, 3 g Bactopeptone (Difco, Detroit, USA), 3 g yeast extract (Difco), 20 g Bactoagar (Difco) and 1000 ml deionized water.

Cultivation of cells. Saccharomyces cerevisiae FU 63 was cultivated in a medium which consisted of (per litre) 20 g glucose, 3.0 g yeast extract (Difco), 3.0 g Bactopeptone (Difco), 4.0 g $(NH_4)_2SO_4$, 1.0 g KH_2PO_4, 1.0 g $K_2HPO_4 \cdot 2H_2O$, 0.8 g $MgSO_4 \cdot 7H_2O$ and 0.06 g $CaCl_2 \cdot 2H_2O$. The initial pH was 6.6. Cell cultures, inoculated from a slant culture, were incubated in shake flasks at 30° C, harvested in mid-exponential phase and washed once with sterile deionized water. The wet biomass was kept frozen at −12° C. The dry weight of this frozen biomass was experimentally determined to be 20% of the wet weight. This frozen biomass of the defined yeast strain and the commercial dry baker's yeast served as inocula for biotransformation cultures.

Microbial transformation. A sterile 250-ml round-bottomed flask, equipped with a magnetic bar, was loaded with 50 ml sterile medium, identical with the cultivation medium, and was placed in a thermostatted water bath at 30° C. The whole flask system was gassed with argon for 10 min under magnetic stirring, and then an accurately measured amount of the aldehyde in 0.5 ml ethanol was added dropwise to the stirred medium. Subsequently, a weighed inoculum of either baker's yeast or frozen biomass of the defined strain was placed into the medium and the flask was connected to a gas burette which measured the volume of gas evolved by displacing acidified (5 ml conc H_2SO_4/l) water.

For the purpose of microbial conversion in the presence of cyclodextrin, this was first dissolved in the medium and the alcoholic aldehyde solution was added dropwise under an argon blanket. Following magnetic stirring for 20 min the flask was inoculated, its headspace flushed again with argon and finally conected to the gas burette.

The gas evolution due to anaerobic yeast fermentation was monitored with time, and intermittently an aliquot of 0.2 ml broth was withdrawn for analysis.

Assays. The broth samples were immediately centrifuged in an Eppendorf centrifuge (Model 5415, Hamburg) at 14000 RPM for 5 min at room temperature (in order to avoid precipitation of crystals). The supernatant of the vanillin broth was assayed at room temperature at 254 nm using a Merck RP-8 HPLC column (Merck, Darmstadt, FRG) with an eluant consisting of 700, 300 and 2.5 parts of water, methanol and conc H_2SO_4 respectively. The retention times of vanillyl alcohol, vanillic acid and vanillin, at an eluant flow rate of 0.7 ml/min were 6.65, 12.5 and 14.1 min respectively. The quantitative determination of the three components was made from their respective peak areas using the predetermined molar relative response factors of 1.00:2.26:6.78 for the alcohol:acid:aldehyde.

The benzaldehyde supernatant was assayed by HPLC under the same conditions but with methanol replaced by acetonitrile. The retention times for benzyl alcohol, benzoic acid and benzaldehyde were 7.1, 9.4 and 13.2 min respectively. Owing to the presence of an unidentified product (phenyl acetyl carbinol?) with a retention time of 7.6 min (its relative surface area did not exceed 5%) and of a small amount of benzoic acid formed (not more than 1%), only the main product, benzyl alcohol, was determined by the method of external standard. For this purpose, vanillyl alcohol, which had a retention time of 3.8 min, was chosen as an external standard.

It is important to note that the presence of cyclodextrins did not alter either the UV absorbances or the retention times of the aldehydes and their products injected into the liquid chromatograph.

Results and discussion

The kinetics of gas evolution in the presence and absence of 1.0 g/l vanillin in a broth with 7.45 g/l β-cyclodextrin are illustrated in Fig. 1. This figure clearly shows the inhibitory effect of vanillin on fermentation metabolism as reflected by gas evolution. It is precisely this inhibition which has limited other investigators such as de Wulf et al. (1986) to a lower substrate concentration (0.5 g/l) despite the much higher aqueous solubility of 13.2 g/l at 30° C (Cohen and Lach 1963). Indeed, the fermentation was complete after 25.5 h in the presence of the aromatic aldehyde but after only 11.9 h in its absence. In addition, fermentation with vanillin present was faster in the presence of the cyclodextrin than in its absence. Furthermore, the latter fermentation experienced a longer lag time of 10 h. One may then conclude that the equimolar amount of β-cyclodextrin significantly alleviated the inhibitory effect of vanillin. This could be rationalized by a reduction of the effective free concentration of vanillin and its products as a result of them complexing with the cyclodextrins. This interaction between vanillin and β-cyclodextrin has previously been investigated by Cohen and Lach (1963).

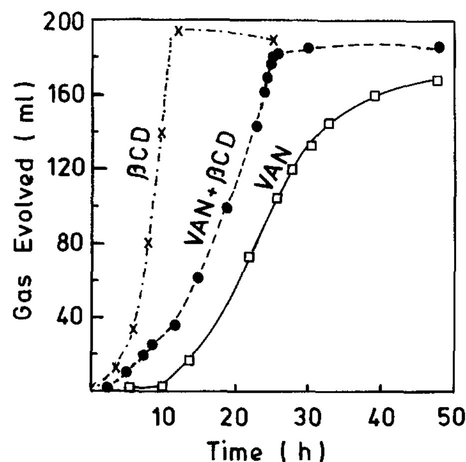


Fig. 1. Effect of vanillin (VAN, 1 g/l) and/or equimolar amount of β-cyclodextrin (βCD) on CO_2 evolution from an anaerobic fermentation of baker's yeast (inoculum of 0.66 g dry wt/l)

Table 1. Product distribution in mole % from vanillin (1 g/l) bioconverted by baker's yeast (inoculum of 0.66 g dry wt/l) in a closed anaerobic system at 30° C in the absence (control) and presence of an equimolar amount of β-cyclodextrin (βCD)

Substrate and products	Time					
	24 h		48 h		72 h	
	Control	βCD	Control	βCD	Control	βCD
Vanillyl alcohol	59.9	54.1	82.4	86.0	83.0	83.1
Vanillic acid	0.0	3.2	11.3	13.0	15.0	16.9
Vanillin	40.1	42.7	6.4	1.0	2.0	0

The extent of bioconversion of vanillin at 24, 48 and 72 h in the presence and absence of β-cyclodextrin is shown in Table 1. Interestingly, the amount of vanillic acid formed was not negligible despite the anaerobic conditions; de Wulf et al. (1986), who performed vanillin reduction by *S. cerevisiae* under anaerobic conditions also reported the presence of the acid. Table 1 reveals that β-cyclodextrin did not introduce a substantial change in either vanillin conversion or product distribution. This was not however the case for benzaldehyde bioconverison.

Figure 2a shows that benzaldehyde at the maximal aqueous solubility of 3 g/l inhibited fermentation from the start and finally brought it to a halt after 30 h. Addition of an equimolar quantity of α-cyclodextrin (27.5 g/l) enabled gas evolution to be complete in 26 h. Again, omission of benzaldehyde from the cyclodextrin-containing broth yielded faster rates of gas evolution, thus demonstrating the inhibitory effects of benzaldehyde on the yeast cells. Benzaldehyde was also previously shown (Bowen et al. 1986) to completely inhibit enzymic activity of a purified yeast alcohol dehydrogenase even at the much lower aldehyde concentration of 0.21 g/l. The complexing of both benzaldehyde and benzyl alcohol, which is well established (Szejtli 1982), apparently alleviates considerably the inhibitory toxic effects of both the substrate and product.

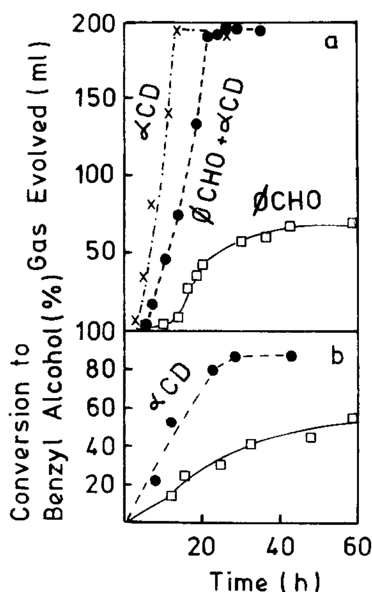


Fig. 2. Effect of benzaldehyde (φCHO, 3 g/l) and/or an equimolar amount of α-cyclodextrin (αCD) on **(a)** CO_2 evolution from an anaerobic fermentation of *Saccharomyces cerevisiae* (inoculum, 1 g/l) and **(b)** benzaldehyde bioconverison to benzyl alcohol (mole %)

The effect of α-cyclodextrin on the bioconversion of benzaldehyde by the defined *S. cerevisiae* strain is shown in Fig. 2b. In the absence of the dextrin, the substrate bioconversion to alcohol was clearly slower and stopped at a value of 55% after 60 h. On the other hand, in the presence of α-cyclodextrin, all the aldehyde reacted after 28 h whilst 87% of it was converted to benzyl alcohol. The effect of an equimolar amount of cyclodextrin was much more dramatic when 5 g/l benzaldehyde in an anaerobic broth inoculated with 4 g dry wt/l were fully converted in 22 h, 73% of the product being benzyl alcohol. In the control experiment with no dextrin, neither gas evolution nor bioconversion took place.

The mere presence of cyclodextrins may not however assure a substantial degree of bioconversion. The inoculum size was found to markedly determine the extent of bioconversion. Table 2 shows the cyclodextrin-aided conversion of 3 g/l benzaldehyde to benzyl alcohol for several inoculum sizes of *S. cerevisiae*. These results were obtained from experiments in air-open shake flasks with no initial blanket of inert gas, since the complexing of benzaldehyde by α-cyclodextrin is known to protect it from air oxidation (Szejtli 1982). However, comparison of Table 2 and Fig. 2b shows that the presence of air in the shake flasks apparently led to poorer bioconversion

Table 2. Bioconversion of benzaldehyde (3 g/l) to benzyl alcohol (in mole %) in air-open shake flasks by various inoculum sizes of *Saccharomyces cerevisiae* with and without an equimolar amount of α-cyclodextrin (αCD) at 30° C

Inoculum size (g dry wt/l)	αCD (g/l)	Time		
		12 h	24 h	48 h
1.0	27.5	14.0	16.1	21.2
2.0	27.5	22.6	37.4	33.6
3.0	27.5	32.6	49.9	43.3
4.0	27.5	44.2	67.4	62.9
4.0	0	14.2	15.4	17.8

yields both in the presence and absence of cyclodextrin. Table 2 reveals that the extent of bioconversion increased with increasing inoculum size but decreased significantly in the absence of α-cyclodextrin even with the largest inoculum used.

Finally, it is worth noting that no attempt was made in the present study to maximize the bioconversion per se by either enzyme induction, mutant selection or optimization of growth conditions (pH, temperature, etc.). The primary goal of the present study was to highlight the advantage of cyclodextrins in alleviating inhibitory and toxic effects on metabolic activity and bioconversion of substrates to corresponding alcohols.

References

Bar R (1988) Liposomal solution for microbial catalysis in organic solvents. Trends Biotechnol 6:55–56

Bender H (1986) Production, characterization and application of cyclodextrins. Adv Biotechnol Processes 6:31–71

Bowen WR, Pugh SYR, Schomburgk JD (1986) Inhibition of horse liver and yeast alcohol dehydrogenase by aromatic and aliphatic aldehydes. J Chem Technol Biotechnol 36:191–196

Cohen I, Lach IL (1963) Interaction of pharmaceuticals with schardinger dextrins II. J Pharm Sci 52:137–142

Hesselink PGM, de Vries H, Witholt B (1987) Enhancement of microbial steroid. Side chain cleavage by addition of cyclodextrins. In: Neijssel OM, van der Meer RR, Luyben KCAM (eds) Proceedings of 4th European Congress on Biotechnology 1987, vol 2. Elsevier Science, Amsterdam. pp 299–302

Szejtli I (1982) Cyclodextrins and their inclusion compounds. Akademiae Kiado, Budapest

Szejtli I, Pagington IS (eds., 1986) Cyclodextrin News, vol 1, p 3

Wulf O de, Thonart Ph, Gaignage PH, Marlier M, Paris A, Paquot M (1986) Bioconversion of vanillin to vanillyl alcohol by *Saccharomyces cerevisiae*. Biotechnol Bioeng Symp 17:605–616

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