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Biotransformation of aromatic aldehydes by *Saccharomyces cerevisiae*: investigation of reaction rates

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SUMMARY

The rate of production of L-phenylacetyl carbinol by *Saccharomyces cerevisiae* in reaction mixtures containing benzaldehyde with sucrose or pyruvate as cosubstrate was investigated in short 1 h incubations. The effect of yeast dose rate, sucrose and benzaldehyde concentration and pH on the rate of reaction was determined. Maximum biotransformation rates were obtained with concentrations of benzaldehyde, sucrose and yeast of 6 g, 40 g and 60 g/l, respectively. Negligible biotransformation rates were observed at a concentration of 8 g/l benzaldehyde. The reaction had a pH optimum of 4.0–4.5. Rates of bioconversion of benzaldehyde and selected substituted aromatic aldehydes using both sucrose and sodium pyruvate as cosubstrate were compared. The rate of aromatic alcohol production was much higher when sucrose was used rather than pyruvate. *o*-Tolualdehyde and 1-chlorobenzaldehyde were poor substrates for aromatic carbinol formation although the latter produced significant aromatic alcohol in sucrose-containing media. Yields of 2.74 and 3.80 g/l phenylacetyl carbinol were produced from sucrose and pyruvate, respectively, in a 1 h reaction period.

INTRODUCTION

L-Phenylacetyl carbinol, a precursor used in the manufacture of L-ephedrine, is produced by biotransformation of benzaldehyde using *Saccharomyces cerevisiae* [6]. The reaction, which is mediated by the pyruvate decarboxylase complex, involves decarboxylation of pyruvate, generated from hexose, to active acetaldehyde which then forms phenylacetyl carbinol in the presence of benzaldehyde as cosubstrate [4,10,13]. During this bioconversion, some of the benzaldehyde is usually reduced to benzyl alcohol and a low level of benzoic acid may also be produced [1,2,9,11,12].

S. cerevisiae also manifests the capacity to catalyse the conversion of a range of substituted benzaldehydes to corresponding L-acetyl aromatic carbi-

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nols and, under these conditions, substituted aromatic alcohols are also formed [7]. The role of purified yeast alcohol dehydrogenase in converting benzaldehyde and substituted benzaldehydes to the corresponding alcohols has been conclusively established [7].

In the biotransformation studies referred to above, prolonged incubations, usually of 6–18 h duration, were carried out in order to establish the product yields obtainable. Over these incubation periods, the rate of product formation decreases and eventually stops. The decline in reaction rate is not simply due to establishment of reaction equilibria, but also to inactivation of key enzymes [13].

We have attempted to investigate reaction rates ('initial rates') for these biotransformations by shortening the reaction time to 1 h. Shorter reaction periods are impractical because of possible delays in diffusion of substrate and product into and from the cell, respectively. The rationale for this study is to define optimal conditions, using early reaction rates, in order to later attempt to maintain reaction conditions close to optimum for prolonged periods in developing a process. This study is part of a project aimed at optimising product formation rates and yields for the production of L-phenylacetyl carbinol and ring-substituted L-acetyl aromatic carbinols.

MATERIALS AND METHODS

Yeast biotransformations

Unless otherwise indicated, fermentation media contained bacteriological peptone (Oxoid), 6 g/l, KH_2PO_4, 13.6 g/l or sodium citrate, 10.5 g/l as buffering agent and sucrose or sodium pyruvate as carbon source. Fermentations were carried out in 250 ml Erlenmeyer flasks, containing 150 ml of medium, inoculated with fresh pressed bakers yeast (dry weight 30%, w/w). Flasks were incubated at 30°C on an orbital shaker, set at 150 rpm. Following an equilibration period of 1 h, the fermentation was initiated by addition of aromatic aldehyde. The shake flask reaction was carried out for 1 h at 30°C. After this fermentation, yeast cells were removed by centrifugation at 7500 × *g* and the clarified broth was recovered for analysis.

L-Acetyl aromatic carbinol analysis

L-Acetyl aromatic carbinols in fermentation broths were determined using the colorimetric method of Groger and Erge [3]. The suitability of this method of determination was verified using the following extraction and analytical procedures. L-phenylacetyl carbinol was extracted from fermentation broth using a modification of the procedure reported by Smith and Hendlin [11]. Broth was extracted, in 50 ml aliquots, with ether (100 ml). The combined ether extracts were evaporated to approximately 100 ml and extracted with aqueous sodium carbonate (100 ml). The ether layer was removed and extracted with 10% sodium metabisulphite (3 × 100 ml). The combined aqueous extracts were washed with ether (2 × 100 ml) and treated with solid sodium bicarbonate until evolution of CO_2 ceased. The solution was extracted with ether (2 × 100 ml) and the combined ether extracts were dried using sodium sulphate. Removal of the ether gave a yellow oil. The product obtained gave an identical NMR spectrum to a chemically synthesised sample of (±)-phenylacetyl carbinol. A range of other L-acetyl aromatic carbinols were similarly extracted and characterised.

The L-acetyl aromatic carbinols extracted from the broth were assayed, over a range of concentrations, colorimetrically using acetylbenzoyl (1-phenyl-1,2-propanedione) as a standard and a correction factor of 1.3 [3] and also by gas chromatography (see below). The two sets of results were consistent.

Gas chromatography

Gas chromatography was used to determine aromatic alcohol levels in fermentation broths and to confirm the validity of the Groger and Erge [3] colorimetric method for L-acetyl aromatic carbinol determination. Analysis was carried out using a Hewlett Packard GC model 5830A, using a glass 6 ft long × 0.25 in o.d. column packed with 30% silicone elastomer E301 on Chromosorb WHP 60-80. Peaks were separated using a temperature programme from 155 to 195°C changing at a rate of

3°C/min. Aqueous aromatic alcohol standards (Aldrich, Milwaukee), aqueous purified L-acetyl aromatic carbinols and fermentation broth (10 ml) were extracted with ether (2 × 10 ml) and the ether extract was made up to 20 ml. 1 μl volumes were injected.

RESULTS

The effect of benzaldehyde concentration on the rate of L-phenylacetyl carbinol production was determined using standard fermentation conditions. Yeast dose rate was 30 g/l pressed yeast. The medium contained sucrose, 50 g/l and KH_2PO_4, 13.6 g/l. Other components were as in Materials and Methods. The reaction mixture had a pH of 4.5. The results are presented in Fig. 1. The rate of L-phenylacetyl carbinol production increases with increasing benzaldehyde concentration up to 6 g/l. Using this level of benzaldehyde, the effect of yeast dose rate on L-phenylacetyl carbinol conversion was investigated. All other conditions were as described above. The results are presented in Fig. 2. The rate of L-phenylacetyl carbinol production increased with increasing yeast dose rate up to 60 g/l. Using a benzaldehyde concentration of 6 g/l and yeast dose rate of 60 g/l, the effect of sucrose concentration on L-phenylacetyl carbinol was determined. As is indicated in Fig. 3, the rate of product formation in-

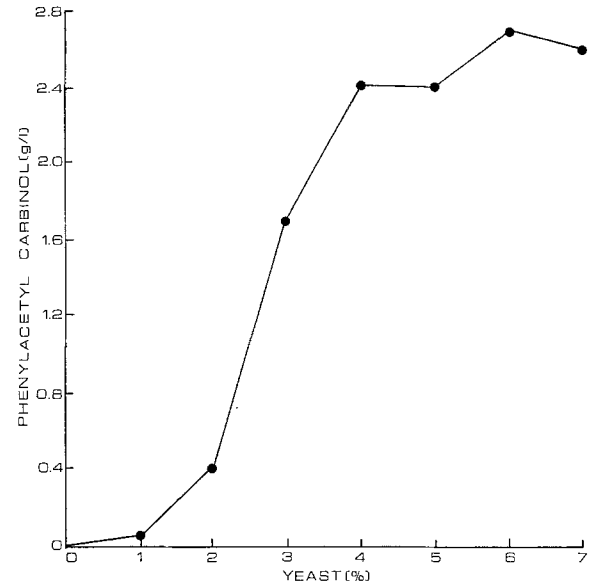

Fig. 2. Effect of yeast dose rate on production of phenylacetyl carbinol.

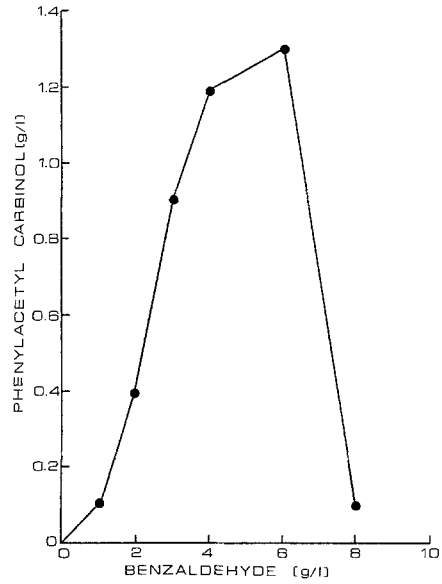

Fig. 1. Effect of benzaldehyde concentration on the rate of phenylacetyl carbinol production.

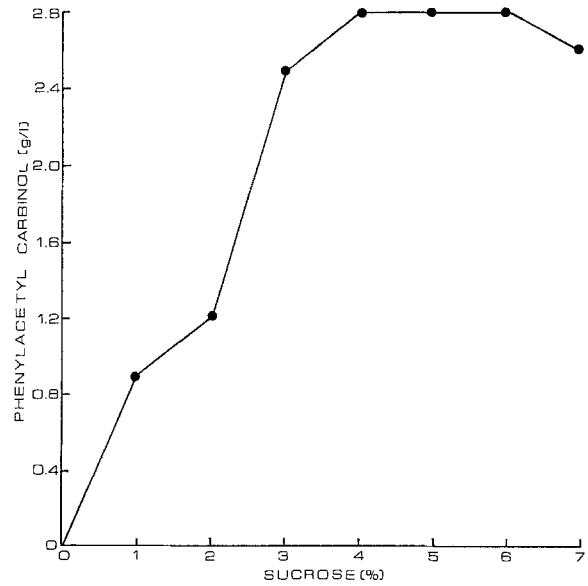

Fig. 3. Effect of sucrose concentration on rate of phenylacetyl carbinol production.

Table 1

Effect of pH on production of phenylacetyl carbinol

Initial pH	Final pH	Phenylacetyl carbinol (g/l)
2.0	2.3	0.1
3.0	3.0	2.0
4.0	4.1	2.5
4.5	4.4	2.5
5.0	4.9	2.1
5.5	5.4	2.3
6.0	5.7	2.0

creased with increasing sucrose concentration and was maximal at 40 g/l.

The effect of pH on reaction rate was determined by varying the initial pH in the range 2–6. The fermentation contained peptone, 6 g/l; sodium citrate, 10.5 g/l; sucrose, 50 g/l; yeast, 60 g/l; and benzaldehyde, 6 ml/l. The results are presented in Table 1. Substantial conversion rates were observed in a broad pH range, 3–6, with a maximum bioconversion rate of 2.5 g/l at pH 4.0–4.5.

Comparative reaction rates for benzaldehyde and selected substituted aromatic aldehydes using both sucrose and sodium pyruvate as cosubstrate were investigated. General reaction conditions are the same as those described in Table 1. Initial aromatic aldehyde concentrations were 6 ml/l. The results are presented in Table 2. The rate of aromatic alcohol production was much higher when sucrose was used rather than pyruvate. *o*-Tolualdehyde and 1-chlorobenzaldehyde were poor substrates for aromatic carbinol formation although the latter substrate produced significant aromatic alcohol in sucrose-containing media.

DISCUSSION

The observation that phenylacetyl carbinol production is negligible at benzaldehyde concentrations of >6 g/l is most probably related to the protein-denaturing capacity of the aldehyde. Vojtisek and Netrval [13] concluded that a glycolytic enzyme in the metabolic sequence between hexose and pyruvate is denatured during phenylacetyl carbinol production from benzaldehyde. In separate studies, we have observed that increasing the benzaldehyde concentration reduces yeast cell viability. In prolonged 24 h reactions, the highest optimal benzaldehyde concentration for phenylacetyl carbinol production was reported to be 1.71 g/l, with complete inhibition of the process at 2.14 g/l benzaldehyde [1]. When reaction times are prolonged, benzaldehyde is usually added incrementally in order to achieve the final maximum yield [2,8].

We observed a culture pH optimum of 4.0–4.5 for production of phenylacetyl carbinol by whole yeast from sucrose and benzaldehyde. Smith and

Table 2

Rate of formation of aromatic carbinol and aromatic alcohol from aromatic aldehyde and sucrose or pyruvate

	Sucrose		Pyruvate	
	aromatic carbinol	aromatic alcohol	aromatic carbinol	aromatic alcohol
Benzaldehyde	2.74	2.72	3.80	0.39
o-Tolualdehyde	0.00	0.32	0.10	0.06
m-Tolualdehyde	0.45	0.87	1.35	0.20
p-Tolualdehyde	1.71	1.78	1.86	0.08
2-Chlorobenzaldehyde	<0.05	1.00	<0.10	0.18
3-Chlorobenzaldehyde	0.43	0.95	0.10	0.20
4-Chlorobenzaldehyde	1.46	1.90	0.64	0.17

Hendlin [10] investigated the nature of the enzyme reaction for phenylacetyl carbinol production in acetone powders of yeast supplemented with co-factors and observed a pH optimum between 4.5 and 5.5.

Ose and Hironaka [9] observed that acetone powders of bakers yeast converted benzaldehyde to benzyl alcohol only when NADH was present. The higher levels of aromatic alcohols which we obtained in sucrose/aromatic aldehyde reactions are likely to be due to the presence of NADH produced by glyceraldehyde-3-phosphate dehydrogenase and the necessary reoxidation of the NADH to maintain glycolysis. NADH is not generated in the pyruvate/benzaldehyde biotransformation reaction and the low level of benzyl alcohol observed probably reflects the endogenous NADH level. Bisulphite, nicotinic acid analogues and nicotinamide may be used to suppress reduction of benzaldehyde to benzyl alcohol [5].

As was observed with tolualdehyde and chlorobenzaldehyde in the 1 h enzymatic reactions described above, we also noted that a variety of aromatic aldehydes having substitutents in the 2- or *ortho* position were poor substrates for L-acetyl aromatic carbinol production in 6 h incubations [7]. Typical reported yields of phenylacetyl carbinol produced during prolonged fermentations (10–18 h) were 4.5–10.2 g/l [2,5,8]. Our yields of 2.74 and 3.80 g/l phenylacetyl carbinol, produced from sucrose and pyruvate respectively in a 1 h reaction period, illustrate the potential which exists for improving the efficiency of this biotransformation.

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