Rapid Paper

Production of Acrolein,
Acetaldehyde and Propionaldehyde
by Cells of a Methanol Yeast,
Candida boidinii S2

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Heat-treated cells of a methanol yeast, showing high alcohol oxidase activity, were used for the production of aldehydes, *i.e.*, acrolein, acetaldehyde and propionaldehyde, from the corresponding alcohols, allyl alcohol, ethanol and 1-propanol, respectively. As high a level as 7.5% acetaldehyde was obtained, in the reaction mixture, from 8.0% ethanol as a substrate. The acrolein concentration reached 3% after 2-hr reaction, from 5% allyl alcohol as a substrate. The difference in molar yields of these aldehydes is discussed on the basis of inactivation of alcohol oxidase by reaction products.

The use of microbial cells for the production of fundamental chemicals would be attractive because 1) the narrow specificity of the enzymatic reaction could reduce the amount of a by-product, and 2) the milder reaction conditions than those of usual chemical processes might reduce energy costs.¹⁾ In preceding studies,^{2~7)} we constructed a reaction system for formaldehyde production from methanol using cells of a methanol yeast, *Candida boidinii* S2. In chemostat-grown cells under appropriate culture conditions, alcohol oxidase comprised nearly 50% of the total soluble proteins.⁴⁾

Acrolein is now mainly industrially produced through the direct oxidation of propylene. However, the recovery of the product is low and the reaction product contains large amounts of acetaldehyde and acetone as by-products. Acetaldehyde is used for the synthesis of a number of chemicals and is produced through ethylene oxidation. Kierstan

proposed that alcohol oxidase could be used for the oxidation of ethanol to acetaldehyde, as a recovery system for ethanol in fermentation medium.⁸⁾ However, the yield of acetaldehyde has not been reported.

In this study, allyl alcohol, ethanol or 1-propanol was oxidized for the production of acrolein, acetaldehyde or propionaldehyde, respectively, with heat-treated cells of a methanol yeast showing high alcohol oxidase activity.

MATERIALS AND METHODS

Chemicals. Acrolein was purchased from Tokyo Chemical Industry Co., Ltd. The concentration of acrolein in the sample was calculated to be 98.9%, which was determined by gas-liquid chromatographic analysis.

Strain and cultivation. A methanol yeast, *C. boidinii* S2 strain AOU-1.²⁾ was used in this study. Cells were grown in a methanol-limited chemostat culture at the dilution rate of 0.075 hr⁻¹ as described previously.⁴⁾

Reaction for aldehyde production. Chemostat-grown cells were harvested and then heat-treated as described previously.⁴⁾ The reaction mixture contained 90 mg (as dry cell weight) of heat-treated cells, 1 mmol of potassium phosphate buffer, pH 6.5, for acrolein production, and pH 7.5 for acetaldehyde and propionaldehyde production, respectively, and alcohol as a substrate in a final volume of 3 ml. The reaction mixture was put in a 30-ml Erlenmeyer flask, which was equipped with a rubber balloon to supply pure oxygen, and then shaken reciprocally at 170 rpm. The reaction was terminated by removing the cells by centrifugation at 4°C. The resultant supernatant was subjected to gas-liquid chromatographic analysis.

Cation M2-treated cells were prepared as described previously.⁷⁾

Gas-liquid chromatographic analysis. The reaction mixture was applied to a Shimadzu gas-liquid chromatography GC7-A, equipped with a flame ionization detector. A glass column, FAL-M, 2 m, was used for assaying allyl alcohol, acrolein, 1-propanol and propionaldehyde. For the allyl alcohol and acrolein assays, the carrier gas was He 38 ml/min, and the column and injection temperatures were 100°C and 130°C, respectively. For the 1-propanol and propionaldehyde assays, the carrier gas was He 20 ml/min, and the column and injection temperatures were 102°C and 130°C, respectively. To determine the amounts of ethanol and acetaldehyde, a column of Porapak Q, 2 m, was used; the carrier gas was N₂ 50 ml/min, and the column and injection temperatures

were 160°C and 190°C, respectively. Integration and calibration of peak areas were carried out with a Shimadzu Chromatopack C-R1B.

The substrate and product concentrations were expressed as % (w/v).

RESULTS AND DISCUSSION

Heat- or Cation M2-treated cells and intact cells were tested as to acrolein production at various temperatures. As shown in Fig. 1, heat-treated cells showed slightly higher acrolein productivity than Cation M2-treated or intact cells. At 4°C, heat-treated cells accumulated 3.5-fold more acrolein than those at 28°C, the optimum temperature for alcohol oxidase.³⁾ The consumption of allyl alcohol paralleled the acrolein production at 4°C and 28°C. After similar experiments, heat-treated cells were selected also for acetaldehyde and propionaldehyde production. The optimum temperature for aldehyde production was 4°C in all cases tested,³⁾ though the highest formaldehyde productivity was obtained with Cation M2-treated cells.³⁾

The effect of the initial pH of the reaction mixture on aldehyde production was investigated using 0.33 M potassium phosphate buffer. As shown in Fig. 2, both the production of acrolein and the consumption of allyl alcohol were maximum at pH 6.5. On the other hand, a rather alkaline pH 7.5~8.0, was suitable for acetaldehyde or propionaldehyde production.

Under the optimal reaction conditions established, acrolein production was performed at various substrate, allyl alcohol, concentrations (Fig. 3). On the oxidation of 5% allyl alcohol, the concentration of acrolein in the reaction mixture reached 3% after 2 hr, and there after the reaction did not proceed any more. In the resultant reaction mixture, about 1.7% allyl alcohol remained, and no other volatile substrates could be detected on gas-liquid chromatography. This amount of acrolein corresponded to that in the case of crude acrolein solution obtained with the chemical process, which contained nearly 10% acetaldehyde (bp 21°C) and acetone (bp 56.5°C). When the great difference in boiling point

FIG. 1. Effect of the Reaction Temperature on Acrolein Production.

The initial acrolein concentration was 3%, and the reaction was performed at the indicated temperatures for 2 hr with: ○, intact cells; ●, heat-treated cells; △, Cation M2-treated cells. Other reaction conditions are given under MATERIALS AND METHODS.

FIG. 2. Effect of the Initial pH of the Reaction Mixture on Acrolein Production.

The initial acrolein concentration was 3%. The reaction was performed under the conditions given under MATERIALS AND METHODS using 0.33 M potassium phosphate buffer at the indicated pHs.

between acrolein (bp 53°C) and allyl alcohol (bp 96°C) is taken into account, our microbial process would exceed in purity of volatile products. Reactions for acetaldehyde and propionaldehyde production were also performed with various concentrations of ethanol and 1-propanol, respectively. As shown in Table I,

2% ethanol was completely oxidized to acetaldehyde after 1.5 hr. As high a level as 8% ethanol could be converted to 7.5% acetaldehyde, with a yield of nearly 1.0 (>0.98), on

18-hr reaction. On the other hand, 0.5% 1-propanol was completely converted to 0.48% propionaldehyde on 1.5 hr reaction.

FIG. 3. Effect of the Allyl Alcohol Concentration on Acrolein Productivity.

Acrolein production was performed for 2 hr, from the indicated concentrations of allyl alcohol. Other reaction conditions are given under MATERIALS AND METHODS.

TABLE I. EFFECT OF THE INITIAL ETHANOL CONCENTRATION IN THE REACTION MIXTURE ON ACETALDEHYDE PRODUCTIVITY

Acetaldehyde production was performed from various concentrations of ethanol. Other reaction conditions are given under MATERIALS AND METHODS.

Initial concn. of ethanol (%)	Acetaldehyde produced (Yield) (%)	
	Reaction time	
	1.5 hr	18 hr
0.5	0.48 (>0.99)	—
1.0	0.95 (>0.99)	—
2.0	1.92 (>0.99)	—
3.0	2.47 (0.86)	2.78 (0.97)
4.0	2.75 (0.72)	3.80 (>0.99)
5.0	2.89 (0.60)	4.78 (>0.99)
6.0	2.54 (0.44)	5.75 (>0.99)
7.0	2.50 (0.37)	6.65 (0.99)
8.0	2.53 (0.33)	7.48 (0.98)
9.0	2.65 (0.31)	7.88 (0.92)
10.0	2.84 (0.30)	7.87 (0.82)

TABLE II. PRODUCTION OF VARIOUS ALDEHYDES WITH CELLS OF A METHANOL YEAST

Substrate Product	Cells	Optimum		Concn. of		Yield	Reaction time	Complete oxidation
		Temp.	pH	Substrate	Product			
Methanol* Formaldehyde	Heat-treated	4°C	6.0	3.0 M (9.6%)	1.09 M (3.27%)	0.36	10 hr	No
	Cation M2-treated		7.0		1.16 M (3.48%)	0.39	7 hr	No
					1.38 M (4.14%)	0.46	20 hr	No
Ethanol Acetaldehyde	Heat-treated	4°C	7.5~8.5	0.43 M (2.0%)	0.43 M (1.89%)	>0.99	1.5 hr	Yes
				1.74 M (8.0%)	1.71 M (7.52%)	0.98	18 hr	Yes
<i>n</i> -Propanol Propionaldehyde	Heat-treated	4°C	7.5~8.5	83 mM (0.5%)	83 mM (0.48%)	1.00	1.5 hr	Yes
				333 mM (2.0%)	283 mM (1.64%)	0.85	19 hr	No
Allyl alcohol Acrolein	Heat-treated	4°C	6.5	862 mM (5.0%)	536 mM (3.0%)	0.62	2 hr	No

* See refs. 6 and 7.

These results are summarized in Table II for comparison of the characteristics of the production of each aldehyde. The purified alcohol oxidase from *C. boidinii* S2 AOU-1 showed the following relative activities toward the following alcohols: methanol, 100%; ethanol, 75%; 1-propanol, 30%; and allyl alcohol, 49%, as reported previously.²⁾ However, the molar yield of acetaldehyde was the highest and that of acrolein was the lowest among the aldehydes investigated. Also, the complete consumption of alcohol was observed on the oxidation of ethanol and 1-propanol, whereas some methanol or allyl alcohol remained, even when they were added at low concentrations, *i.e.*, less than 1.0%, as substrates.^{3,7)} Thus, a good substrate for enzyme activity is not always a good substrate for aldehyde production. The inactivation of purified alcohol oxidase by H_2O_2 were reported.^{9,10)} The differences in molar yields between aldehydes suggested that formaldehyde and acrolein were much more inhibitory than H_2O_2 in our reaction system containing an adequate amount of catalase.^{4,5)} Recently, Geissler *et al.* reported that the k_{cat}/k_{inact} ratio varied with different alcohols.¹¹⁾ It seems that ethanol/

acetaldehyde has the minimum and allyl alcohol/acrolein the maximum inhibitory effect on the reaction. The optimum temperature of 4°C for aldehyde production could prevent such inactivation of alcohol oxidase during the reaction.³⁾

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