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Biotransformation of alcohols to aldehydes by immobilized cells of *Saccharomyces cerevisiae* PTCC5080

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Abstract

Saccharomyces cerevisiae was immobilized in polyacrylamide gel beads. The effect of each gel constituents on whole cell alcohol dehydrogenase activity of yeast were studied. Prior to entrapment the cells were permeabilized by cetyltrimethylammonium bromide (*CTAB*) where 27% increase in activity was obtained. The permeabilized entrapped cells were treated with different detergents. CTAB was found to increase the gel permeability. The optimum concentration of nicotinamide adenine dinucleotide (NAD) was determined to be 30 μM. The optima concentration of ethanol for permeabilized cells, permeabilized and entrapped yeast cells were 3 and 2 M, respectively. Entrapped yeast cells converted almost 32.4 and 45% ethanol and 2-butanol to their respective aldehydes.

Keywords: Alcohols; Aldehydes; *Saccharomyces cerevisiae*

1. Introduction

Biotransformations are reactions of organic compounds performed by either isolated enzymes or whole cell biocatalysts. Biocatalysts are applied in industry for pharmaceutical, agrochemical, chemical, fragrance and flavour, nutritional and bioremediation process. Henceforth, proteases, lipases, estereases and yeast cells like *Saccharomyces cerevisiae* can be used in non-conventional media in order to solubilize substrate and/or products. They can also be employed for limiting water molecular activity to obtain kinds of biotransformation, which are either difficult or quite impossible to perform in water [1,2]. During biotransformation many parameters affect the stability and activity of biocatalysts [3–5]. To render such circumstances employment and immobilization of whole cell biocatalysts can be beneficial and immobilization of such biocatalyst conventionally performs biotransformation [6]. Entrapment of whole cell containing enzyme intracellularly (the enzyme does not require large molecular weight substrate) in either synthetic or naturally occurring polymeric matrices, can become a method of choice [7]. In this paper attempts are made to entrap permeabilized cells of *S. cerevisiae* PTCC5080 in the lattice of polyacrylamide gel beads in order to study biotransformation of alcohols to aldehydes.

2. Materials and methods

2.1. Microorganism

S. cerevisiae PTCC5080 was obtained from the Persian Type Culture Collection, Tehran, Iran. It was maintained on Saboraud's dextrose agar (Difco). It was sub cultured every 4–6 weeks.

2.2. Chemicals

Acrylamide, bis-acrylamide, *N*,*N*,*N*′,*N*′-tetramethylene diamine (TEMED), and tris were of Sigma. Oxidized nicotinamide adenine dinucleotide (NAD) and its phosphate salt, cetyltrimethylammonium bromide (CTAB) were obtained from Merck. Other reagents used were of analytical grade.

2.3. Permeabilization of S. cerevisiae

S. cerevisiae PTCC5080 was cultivated in Rough bottles containing Saboraud's dextrose agar. They were incubated at 25 °C for 24 h. After incubation time, sterile phosphate

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buffer pH 7 was added to the bottles containing yeast cells. The bottles were then slowly swirled. The cell suspensions were collected in sterile centrifuge tubes, and centrifuged at 4000 rpm, 4 °C for 15 min. The obtained cake was re-suspended in sterile buffer containing 0.2% (w/v) CTAB. This was then kept on a rotary shaker at 150 rpm, 25 °C for 30 min. The cells were recovered by centrifugation and were washed several times with sterile buffer. The cake was stored at 4 °C.

2.4. Immobilization of S. cerevisiae PTCC5080

Permeabilized yeast cells (0.2 g wet weight) were added to 6.0 ml of Tris–HCl buffer, 0.2 M, pH 7.8, containing 105 mg acryl amide, 3 mg bis-acrylamide. These were dropped into 100 ml of oil containing 84 mg ammonium persulfate and 84 μl TEMED, at a flow rate of 0.1 ml/min. The polymerization was allowed to take place at room temperature for 24 h. The formed beads were washed several times with the same buffer. The uniformly obtained flat shaped polyacrylamide gel beads were stored at 4 °C in the above buffer.

3. Results and discussion

Biotransformation of ethanol to acetaldehyde in the presence of oxidized NAD was studied by employing *S. cerevisiae* PTCC5080 in three different forms namely unpermeabilized cells, permeabilized cells and permeabilized cells entrapped in polyacrylamide gel. The formed beads were in flat shape. It was found that alcohol dehydrogenase activity of permeabilized cells of *S. cerevisiae* was higher than those of unpermeabilized and immobilized (Table 1) [8]. The low activity of immobilized cells might be due to inhibitory effect of TEMED on whole cell alcohol dehydrogenase activity (Table 2) and diffusional restriction imposed by immobilization. These were further improved by including 0.1% (w/v) bovine serum albumin in the reaction mixture leading to the formation of polyacrylamide beads. The entrapped cells were treated separately with Tween-80, Triton X-100 and CTAB. It was observed that CTAB improved alcohol dehydrogenase activity of immobilized yeast cells (Table 3). The effect of different concentration of co-factor on the initial rate of biotransformation of ethanol to acetaldehyde was studied. It was found that NAD at 30 μM concentration was optimum. Different concentrations of ethanol were used to find out the optima concentration of substrates for both forms of yeast cells. It was observed that permeabilized free cells catalyzed conversion of alcohol up to 8 M but showing highest activity at 3 M ethanol concentration while the immobilized yeast cells were active upto 4 M ethanol concentration. The optimum ethanol concentration for catalytic activity of immobilized cells was found to be 2 M (Fig. 1). The low activity of immobilized cells were at lower ethanol concentration could be related to diffusional limitation caused by recondensation of water to the immobilized cells [9]. Banza et al. [10], Lamare and Legoy [11], and Young and Russel [12] reported the same effect while studying oxidation of ethanol to acetaldehyde by alcohol dehydrogenase, lipase of *Candida cylindracea*, catalyzing esterification reaction and yeast alcohol dehydrogenase to catalyze conversion of acetone to isopropanol. The effect of immobilized yeast cell mass on initial rate of biotransformation was studied. The initial reaction rate was linear with the immobilized cell mass. This linearity indicates that neither deactivation of immobilized cells nor production of acetaldehyde attributed to the rate of biotransformation (Fig. 2). The relative rates of biotransformation using different alcohols like methanol, 1-propanol, 2-butanol and 1-pentanol were studied. It was observed that the relative

Table 1
Relative activity of three different forms of whole cell alcohol dehydrogenase activity

Types of cell	Relative activity (%)
Permeabilized cell	100
Unpermeabilized cell	73
Permeabilized and immobilized	75

Table 2
Effect of different gel constituents on whole cell alcohol dehydrogenase activity

Reagents	Relative activity (%)
Acrylamide	100
Bis-acrylamide	100
Ammonium persulfate	100
TEMED	78.2
Control	100

The control did not contain any reagents and its activity is taken as 100%.

Table 3
Activation of permeabilized immobilized whole cell alcohol dehydrogenase activity at various time

Detergents	Time of treatment (min)	Activity (u/g immobilized cell)
Tween-80	25, 35, 45, 55, 65	0, 0.2, 0.2, 0.2, 0.216
Triton X-100	25, 35, 45, 55, 65	0, 0.167, 0.187, 0.187, 0.187
CTAB	25, 35, 45, 55, 65	0, 0.166, 0.187, 0.218, 0.228

Table 4
Effect of permeabilized, immobilized and activated whole cell alcohol dehydrogenase activity on different alcohols

Alcohols	Relative activity (%)
Methanol	41
Ethanol	100
1-Propanol	67
2-Butanol	174

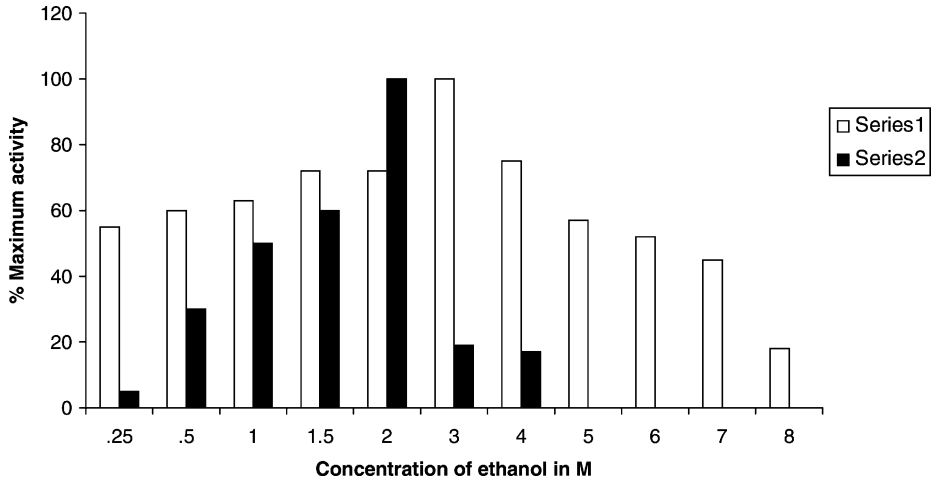

Fig. 1. Effect of permeabilized and permeabilized immobilized and activated whole cell alcohol dehydrogenase on different concentration of alcohols. Black bar: permeabilized cells. White bars: permeabilized, immobilized and activated cells.

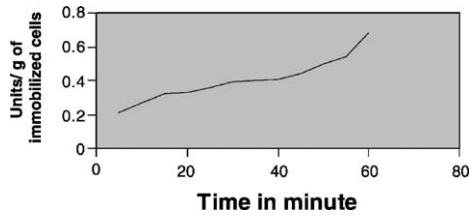

Fig. 2. Biotransformation of ethanol to acetaldehyde in relation to time, using immobilized *S. cerevisiae*.

activity of whole cell immobilized alcohol dehydrogenase was the highest when 2-butanol was employed as a substrate (Table 4). This indicates that higher relative activity towards 2-butanol is due to the presence of 2° alcohol dehydrogenase [13,14]. Finally, the immobilized cells were packed into two different double jacketed glass columns where the temperature of both the columns were maintained at $25 \pm 1\,^{\circ}C$ by circulating warm water through the jackets. The flow rate of the column was adjusted to 0.2 ml/min using a peristaltic pump. The rates of biotransformation of ethanol and 2-butanol by immobilized cells were calculated to be 32.4 and 45.4%, respectively. Finally, about 50% reduction in the rates of biotransformation of ethanol and 2-butanol was observed after 6 h of continuous operation. After each cycle, the cells were washed and treated with appropriate buffer and detergent in order to regenerate the columns. Fig. 3 shows the recycling of whole yeast cell containing alcohol dehydrogenase activity immobilized in polyacrylamide gel beads in packed columns with the ability of catalyzing conversion of ethanol and 2-butanol to their respective aldehydes. In conclusion, this simple system can be applied to obtain reduced NADH/NADPH or the reduction/oxidation of 4–6 (may be more) carbon containing alcohols/aldehydes.

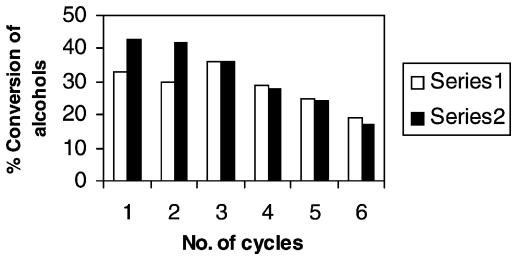

Fig. 3. Continuous biotransformation of ethanol and 2-butanol to their respective aldehydes. White bars: ethanol. Black bars: 2-butanol.

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