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Anaerobic Biotransformation of Guaiacol to Catechol by Growing and Non-growing Harvested Cells of *Acetobacterium woodii* DSM 1030

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Abstract: *Acetobacterium woodii*, an anaerobic acetogenic bacterium was used in this study to investigate the biotransformation of guaiacol in batch culture via O-demethylation reaction. The culture was grown anaerobically using 250 mL conical flask containing guaiacol in the presence or the absence of fructose. Results showed that limiting fructose concentration for the highest cell growth was 2.0 g L^{-1}. It is also found that 94% of guaiacol was transformed to catechol (0.56 g L^{-1}) when 2.0 g L^{-1} of fructose and 0.6 g L^{-1} of guaiacol were used as carbon source for growth. In cultures containing 2 g L^{-1} fructose (with addition of 0.6 g L^{-1} guaiacol after 20 h incubation), the catechol production was 0.5 g L^{-1} (82% transformation), while in cultures containing only guaiacol, the catechol production was 0.4 g L^{-1} (62% transformation). In the experiment using harvested cells, the highest catechol production was 0.47 g L^{-1} (79% transformation). The highest transformation was obtained using non-growing harvested cells from cultures containing 2 g L^{-1} fructose with 0.6 g L^{-1} guaiacol addition suggesting that this is the best combination of carbon source for high production of catechol. It is also suggested that high cell concentration in culture containing limited concentration of fructose will resulted in high concentration of catechol believed to be due of stimulation of the enzyme for O-demethylation reaction.

Key words: *Acetobacterium woodii*, guaiacol, catechol, O-demethylation, anaerobic

INTRODUCTION

Biotransformation is a chemical reaction mediated by living cells or enzymes which known as biocatalyst. This technology has been used widely in industry for the synthesis of fine and commodity chemicals, pharmaceutical and agrochemical intermediates, as well as drug substances[1-3]. The commercial benefits of using biocatalyst in chemical synthesis are, fewer side reactions, easier products separations and less pollution, all of which translate to lower cost[4].

Therefore, the present study was conducted to determine the suitability of *A. woodii*, an anaerobic acetogenic bacterium as biocatalyst for synthesis of chemical with commercial value. Bache and Ffennig[5] reported that *A. woodii* was able to grow with methoxy aromatic compound as substrate anaerobically. The methyl substituent was cleaved and metabolized further to generate energy in the form of ATP through acetyl Co-A pathway[6]. Interestingly, the demethylated product was not metabolized further and accumulated naturally[7]. Whole cell biocatalyst was used to catalyze the O-demethylation reaction of guaiacol to catechol, as the enzyme involved was an intracellular enzyme[8].

The aim of this study is to enhance catechol production by time determination of guaiacol addition into the medium and finding the limiting fructose concentration for maximum cell growth.

MATERIALS AND METHODS

Microorganism and culture condition: *Acetobacterium woodii* DSM 1030 was obtained from DSM, Germany. The bacterium was grown and maintained anaerobically in liquid medium using fructose (2 g L^{-1}) as the organic carbon source[9]. In biotransformation experiment, cultures were grown either with guaiacol (0.6 g L^{-1}) in the presence or absence of fructose or grown with fructose followed by addition of guaiacol (0.6 g L^{-1}) after 20 h incubation. All media preparation and inoculation were executed in an anaerobic chamber (Labconco, USA). This chamber can achieved up to 99% anaerobic condition. Resazurin was used as an indicator of the anaerobic condition of the chamber.

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Samples were taken at 6 h time intervals and cell growth were determined by measuring the optical density of the culture at 660 nm using a spectrophotometer (UV-1201V Shimadzu).

Determination of fructose limiting concentration: *A. woodii* was grown in Balch medium containing different concentrations of fructose (0.5, 1.0, 2.0 and 3.0 g L^{-1}). The cultures were incubated anaerobically at 30°C[10] and samples were taken at 6 h time intervals for cell growth determination. When the growth reached stationary phase, fresh fructose was added to each cultures. The cultures were then incubated at the same condition for another 18 h.

Preparation of non-growing harvested cells: *A. woodii* was grown in Balch medium containing 1 and 2 g L^{-1} fructose with 0.6 g L^{-1} guaiacol or the addition was made after 20 h incubation. After 48 h cells were harvested by centrifugation at 13,000 rpm for 5 min. The pellet was washed using 20 mM phosphate buffer at pH 7 and concentrated 10 times with the same buffer. Guaiacol (0.6 g L^{-1}) was added to the concentrated cells for biotransformation experiment. Samples were taken after 15 h incubation for product (catechol) analysis.

Analytical method: Catechol produced was determined by using 4-aminoantipyrine reagents as described by La Rue[11] while fructose concentration was measured by a method described by Mirza *et al.*[12].

RESULT AND DISCUSSION

Determination of limiting fructose concentration: The limiting concentration of fructose for maximum growth of cell was determine due to the fact that excess concentration of fructose will inhibit cell growth. Four different concentrations (0.5, 1.0, 2.0 and 3.0 g L^{-1}) were used to investigate this matter. The results showed that cells growth was increased after stationary phase in cultures containing 0.5, 1.0 and 2.0 g L^{-1} fructose when fresh fructose was added (Fig. 1) but no growth increment was observed in cultures with 3.0 g L^{-1} fructose. Results suggested that concentration at 2 g L^{-1} or lower were indeed limited for cell growth and 3.0 g L^{-1} of fructose seems to be excessive. Excessive fructose in the medium will inhibit growth and cells lost its ability to survive due to accumulation of acetate[13].

Among the limiting concentrations of fructose used, 2.0 g L^{-1} fructose produced the highest cells concentration (0.3 g L^{-1}), which was 17 and 7% higher than those with 0.5 and 1.0 g L^{-1} fructose, respectively.

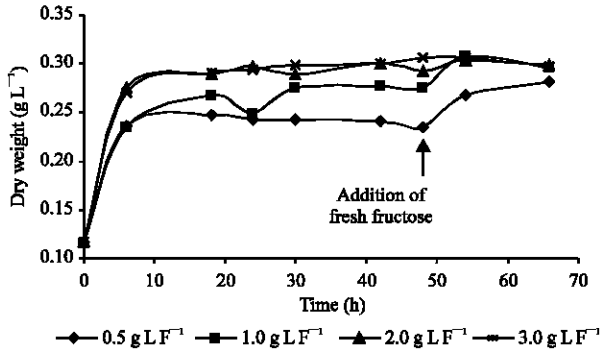

Fig. 1: Cells dry weight in g L^{-1} produced with 4 different concentrations of fructose. Fresh fructose was added into the culture after 48 h

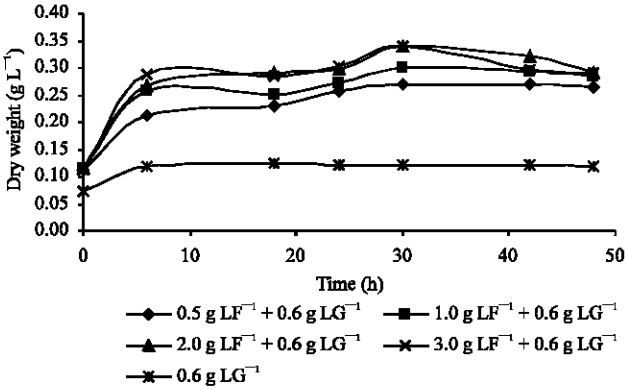

Fig. 2: Concentration of cell produced in cultures containing guaiacol in the presence or absence of fructose

Catechol production in cultures grown with guaiacol alone: The concentration of cells produced in cultures containing guaiacol alone was low (Fig. 2). The maximum concentration of cells was 0.12 g L^{-1}. It was also found that only 62% guaiacol was transformed to catechol (0.4 g L^{-1} catechol produced) in cultures with guaiacol alone (Fig. 3). These results suggested that guaiacol alone is not suitable for production of high concentration of cells or catechol.

Catechol production in cultures grown with fructose and guaiacol: Cells production in cultures containing guaiacol and fructose was high. The highest concentration of cell produced was 0.34 g L^{-1} in cultures containing 3 g L^{-1} fructose with 0.6 g L^{-1} guaiacol. This concentration was only 0.2% higher than the cell concentration produced in cultures containing 2 g L^{-1} fructose with 0.6 g L^{-1} guaiacol and was 12 and 21% higher than cell production in cultures containing 1.0 and 0.5 g L^{-1} fructose with 0.6 g L^{-1} guaiacol, respectively (Fig. 2).

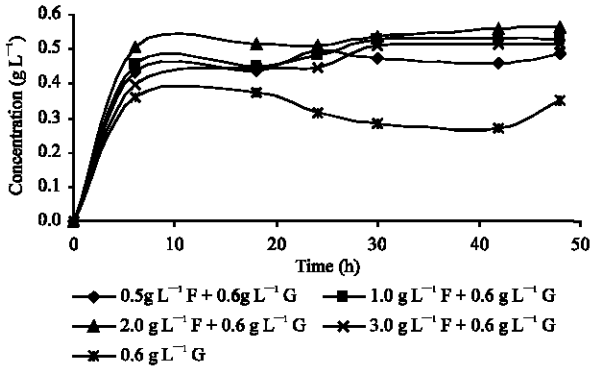

Fig. 3: Production of catechol in cultures containing guaiacol in the presence or absence of fructose

The highest concentration of catechol produced was 0.56 g L^{-1} (94% of guaiacol was transformed to catechol) in cultures containing 2 g L^{-1} of fructose with 0.6 g L^{-1} guaiacol, which was 6 and 13% higher than catechol production in cultures containing 1.0 and 0.5 g L^{-1} fructose with 0.6 g L^{-1} guaiacol, respectively. The lowest concentration of catechol produced was 0.52 g L^{-1} (86% transformation of guaiacol to catechol) in cultures containing 3 g L^{-1} fructose with 0.6 g L^{-1} guaiacol which is 8% lower than the highest concentration of catechol produced (Fig. 3)

Catechol produced in cultures grown with fructose and guaiacol (added after 20 h incubation): The maximum concentration of cell produced was 0.5 g L^{-1} in cultures containing 3 g L^{-1} fructose with 0.6 g L^{-1} guaiacol, which was 2, 5 and 19% higher than cell produced in cultures containing 2.0, 1.0 and 0.5 g L^{-1} fructose with 0.6 g L^{-1} guaiacol, respectively.

The highest transformation of guaiacol to catechol was 82% transformation (0.5 g L^{-1} catechol produced) in cultures containing 2 g L^{-1} fructose with 0.6 g L^{-1} guaiacol. The transformation was 4 and 3% higher than transformation of guaiacol in cultures containing 1.0 and 0.5 g L^{-1} fructose with 0.6 g L^{-1} guaiacol, respectively. The lowest transformation was 51% transformation (30% lower than the highest one) in cultures containing 3 g L^{-1} fructose and 0.6 g L^{-1} guaiacol.

Catechol production by harvested cells: Table 1 showed that harvested cells which grown with fructose with guaiacol from early stage of experiment catalyzed higher transformation of guaiacol after 20 h incubation to catechol then the harvested cells from cultures with guaiacol added after 20 h incubation. The highest transformation is 79% (0.47 g L^{-1} catechol produced) by harvested cells from cultures containing 2 g L^{-1} fructose with 0.6 g L^{-1} guaiacol. While harvested cells from cultures containing 2 g L^{-1} fructose with 0.6 g L^{-1} guaiacol (added after 20 h incubation) catalyzed 76% (0.46 g L^{-1}) transformation of guaiacol to catechol.

Table 1: Concentration of catechol produced (g L^{-1}) and percentage of transformation using harvested cells

	F + G		G added after 20 h incubation	
[F] g L^{-1}	1.0000	2.0000	1.0000	2.0000
Dry weight g L^{-1}	0.2789	0.2807	0.2889	0.3351
[K] g L^{-1}	0.4573	0.4740	0.4495	0.4569
%	76.2000	79.0000	74.9000	76.2000

[F] Concentration of fructose, [K] concentration of catechol

In cultures containing guaiacol alone as organic carbon source, the cell and catechol production were low. Therefore in the next experiment, fructose and guaiacol was used together in culture for high cell and catechol production. Cell production was higher in cultures containing fructose and guaiacol added after 20 h incubation then cell production in cultures containing fructose with guaiacol. But catechol production was lower in first culture than the after. The same result was obtained when the experiment was repeated using non-growing harvested cells. From this finding we suggested that although cell concentration was high but for higher production of catechol intracellular enzymes were needed to be stimulated to perform maximum activity.

From present finding, limited fructose for maximum growth of cells was found at 2.0 g L^{-1} of fructose. Resulted in maximum production of catechol, 2 g L^{-1} of fructose with 0.6 g L^{-1} of guaiacol was shown as the optimum concentration of carbon source to be used in production culture. It may be suggested that high cell concentration with stimulated intracellular enzymes will produced high concentration of catechol.

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