Synthesis of Xylitol by Reduction of Xylulose with the Combination of Hydrogenase and Xylulose Reductase

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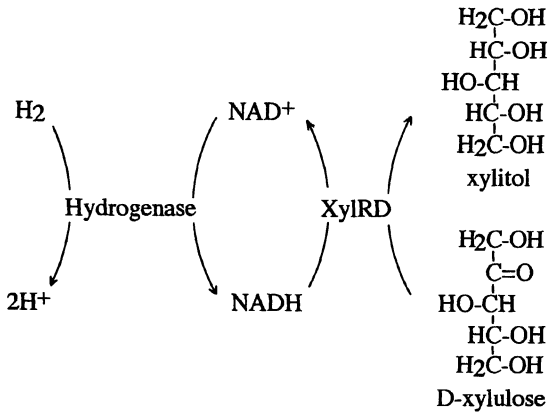
Xylitol synthesis by reduction of xylulose was performed by the combination of NADH regeneration system and xylulose reductase. The conversion of xylulose to xylitol was 98% after 34 h and the turnover number of NAD was 1017.

An enzymatic NADH regeneration system has been established using hydrogenase from *Alcaligenes eutrophus* with hydrogen gas as a reducing agent.[1] Enzymatic systems are great advantage in producing compounds with high selectivity. Xylitol is an useful sugar for the activation of sugar metabolism for diabetic patients. Xylitol has been produced by the reduction of xylulose using nickel or sodium amalgam as a catalyst under high pressure of hydrogen gas. If the NADH regeneration system with hydrogenase is combined with xylulose reductase (XylRD), xylitol will be produced from xylulose under mild reaction conditions. In this study an attempt to synthesize xylitol was tried by a combination of above regeneration system and XylRD, as depicted in the following scheme.



The hydrogenase from *A. eutrophus* was partly purified according to the literature.[2] The activity (1 unit) of hydrogenase was to reduce 1 umol of NAD for 1 min. XylRD from *Candida parapsilosis* IFO 0708 was partly purified according to the following method. *C. parapsilosis* was aerobically cultured at 30 °C. The medium (100 ml) contains glucose (1.0 g), polypeptone (0.5 g), yeast extract (0.3 g) and malt extract (0.3 g). A part of cytoplasm was collected from sonicated bacterial solution by the centrifugation (28000 x g), and then it was purified partly using ammonium sulfate (20% saturation). The solution was deionized by the ultrafiltration and it was used as the xylulose reductase. The activity (1 unit) of XylRD was to reduce 1 μmol of xylulose for 1 min.

The xylitol formation was carried out as follows. The sample solution containing hydrogenase, NAD, XylRD, D-xylulose in phosphate buffer (pH 8.0), was deaerated by repeated freeze-pump-thaw cycles. The reaction was carried out at 30 °C by the introduction of hydrogen gas into the above system. Xylitol was analyzed by HPLC (LC-6A, Shimadzu Co.) with Shimpack CLC-NH2(M) column using acetonitrile - water (3:1 vol%) eluent at 25 °C. The sample solution was deprotenized with acetic acid-heat method in advance.

When hydrogen gas was introduced into the system containing hydrogenase, NAD, xylulose, and XylRD, xylulose was reduced

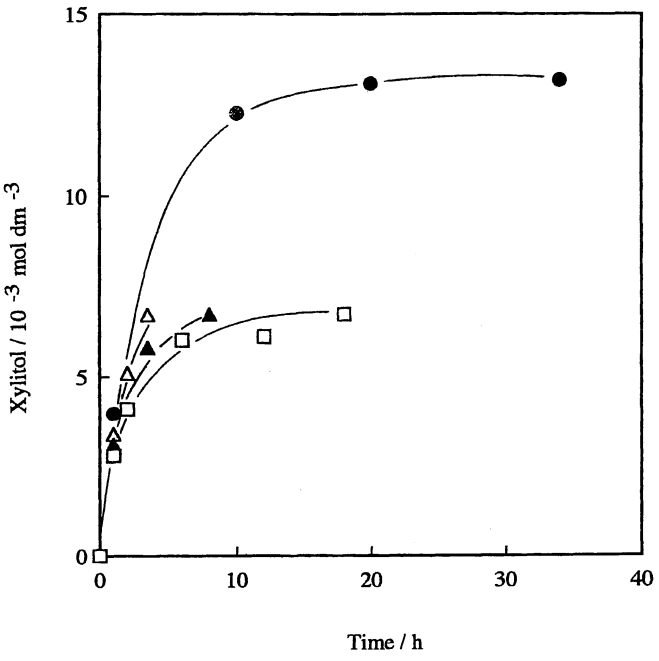


Figure 1. Time dependence of xylitol formation. The sample solution contains hydrogenase, xylu-lose reductase, xylulose in 5.0 x 10^{-3} mol dm^{-3} phosphate buffer (pH 8.0). The reaction was carried out under 300 torr of hydrogen pressure at 30 °C.
Reaction conditions.
△: hydrogenase, 16 U; XylRD, 1.3 U; xylulose 6.7 x 10^{-3} mol dm^{-3}; NAD, 4.0 x 10^{-3} mol dm^{-3}
▲: hydrogenase, 16 U; XylRD, 1.3 U; xylulose 6.7 x 10^{-3} mol dm^{-3}; NAD, 6.7 x 10^{-4} mol dm^{-3}
□: hydrogenase, 16 U; XylRD, 1.3 U; xylulose 6.7 x 10^{-3} mol dm^{-3}; NAD, 1.6 x 10^{-4} mol dm^{-3}
●: hydrogenase, 90 U; XylRD, 2.0 U; xylulose 1.3 x 10^{-2} mol dm^{-3}; NAD, 1.3 x 10^{-5} mol dm^{-3}

and xylitol was formed. No by-product was observed. Time dependence of xylitol formation at various reaction conditions are shown in Figure 1. In every time course, xylitol formed almost linearly with time at the beginning and then the formation rate gradually decreased with time. Under these reaction conditions, the initial xylitol formation rate did not depend on NAD concentration. At the optimum condition as shown by closed circles in Figure 1, the conversion of xylulose to xylitol was 98% after 34 h and the turnover number of NAD was 1017.

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References and Notes

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