Production of Yeast Invertase from Sauerkraut Waste

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Sauerkraut waste was found to be a favorable medium for the production of invertase (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26) by Candida utilis.

Sauerkraut waste presents a serious treatment problem because of its extremely high biochemical oxygen demand (BOD), low pH, and high NaCl content (4). Hang et al. (3) found sauerkraut waste to be a more favorable medium for cultivating yeasts than malt extract broth and as good or better than peptone-dextrose broth. Of the species tested, Candida utilis grew most rapidly and gave the highest cell yield. Dworschack and Wickerham (2) demonstrated that C. utilis produced exceptionally large amounts of extracellular and total invertase (β-D-fructofuranoside fructohydrolase, EC. 3.2.1.26) in a medium containing 3% sucrose, 0.5% peptone, and 0.3% yeast extract. The objective of this work was to study the capability of C. utilis to produce invertase in sauerkraut waste.

Sauerkraut waste was obtained from a nearby factory and contained the following, expressed as milligrams per liter: BOD, 12,400; total acid as lactic, 7,400; Kjeldahl nitrogen, 620; total phosphorus, 81; and NaCl, 18,600. Experiments were carried out in 500-ml Erlenmeyer flasks containing 100 ml of autoclaved sauerkraut waste incubated at 25 C on a rotary shaker at a speed of 200 rpm. All flasks were inoculated with a 22-h-old yeast culture at a 1% level (vol/vol). The methods used to determine the 5-day BOD, total acid as lactic, Kjeldahl nitrogen, total phosphorus, and NaCl were described previously (4). Dry cell weight was determined by filtering, washing with distilled water, and drying at 105 C overnight. Reducing sugar was measured by the method of Clark (1). Total invertase was determined directly on the whole culture. The supernatant fluid, after centrifugation at 2,000 rpm for 10 min, was used for extracellular invertase assay. One unit of invertase is defined as the quantity of enzyme which catalyzes the formation of 1 μmol of reducing sugar per 5 min at pH 4.5 and 25 C. All samples were prepared in duplicate and the reported data are average values.

Invertase production approximately paralleled the amount of yeast growth (Fig. 1). The most rapid production of invertase occurred between 12 and 24 h during the exponential growth phase. The ratio of total invertase to extracellular invertase was approximately 5 to 1.

Dworschack and Wickerham (2) used peptone-sucrose-yeast extract broth to produce yeast invertase. However, we found in this work that the 48-h-old washed cells of C. utilis grown in sauerkraut waste produced 672,000 U of invertase per g of dried yeast, whereas those grown in peptone-sucrose-yeast extract broth

Fig. 1. Production of yeast invertase in sauerkraut waste.

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produced only 307,500 U of invertase per g of dried yeast. *C. utilis* thus produced twice as much invertase in sauerkraut waste as in peptone-sucrose-yeast extract broth. This could be attributed to the presence of some stimulating substances in the waste water. It is also possible that the lactic acid that is present is a more favorable carbon source for invertase production than is sucrose. Dworschack and Wickerham (2) showed that *C. utilis* produced high yields of invertase whether the carbon source was sucrose, glucose, maltose, or xylose, and still higher yields with lactic acid, glycerol, and ethyl alcohol.

The yeast completely neutralized the waste acid, and reduced the BOD, Kjeldahl nitrogen, and total phosphorus to 1,400, 168, and 8 mg/liter, respectively. Our data thus indicate that *C. utilis* could be used to reduce the strength of sauerkraut waste with the concomitant production of yeast invertase.

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