PRODUCTION OF FRUCTOSE DIPHOSPHATE BY BIOCONVERSION OF
MOLASSES WITH Saccharomyces cerevisiae CELLS.

C. Compagno, A. Tura, B. M. Ranzi and E. Martegani*
Dipartimento di Fisiologia e Biochimica Generali, Universita' di Milano, via Celoria 26, 20133 Milan,
Italy.

SUMMARY
Sugar beet molasses was used as carbon source for Saccharomyces cerevisiae growth and as
substrate for bioconversion to fructose diphosphate. The highest level of fructose diphosphate (26.6
g/L) was reached after 10 h incubation of permeabilized cells under appropriate molasses and
phosphate to cell ratio and represented a 64% yield of bioconversion.

INTRODUCTION
One of the methods commonly used for manufacturing fructose 1,6-diphosphate (FDP), an
important glycolytic intermediate whose salts find clinical applications (Markov et al. 1981), is based
on the enzymatic phosphorylation of glucose with inorganic phosphate using permeabilized
brewer's yeast cells (Bisso and Melelli 1986; Melelli et al. 1989). Previously we reported that a
substantial improvement in the yield of the glucose-FDP bioconversion can be achieved using
fed-batch grown Saccharomyces cerevisiae S288C cells (Compagno et al. 1992). Molasses is one
of the cheapest sources of carbohydrates due to its relative abundance and renewable nature. The
major carbohydrate in molasses is sucrose, which is metabolized by yeast after the
invertase-catalyzed hydrolysis into the glucose and fructose. The yeast Saccharomyces cerevisiae,
mainly used in industrial processes, possess at least six unlinked genes all encoding invertase
(SUC1, SUC2, SUC3, SUC4, SUC5 and SUC7) which confer the ability to ferment sucrose (Carlson,
1987). In this report we show that an efficient bioconversion and cheaper production of FDP from
molasses can be obtained using Saccharomyces cerevisiae S288C cells grown in molasses. In
order to obtain both higher yields and rates of bioconversion, appropriate molasses and phosphate
to cell ratio have been optimized.

MATERIALS AND METHODS
Strain and growth condition. The haploid strain S288C (MAT, gal2, SUC2, MAL) of S. cerevisiae was
used. Yeast cells were grown in batch at constant temperature (30 C) in rich media containing 1%
yeast extract, 2% peptone and 2% sucrose or 1% yeast extract, 2% peptone and sugar beet
molasses at a final concentration of 2% sugar. The molasses, given by Dr. V. Cavazzoni (University of
Milan) was prepared by dilution of a concentrated solution (total sugar 50g/100g) with distilled water.
Yeast cells were grown at a final concentration about 2x10^8 cells/ml, centrifuged and resuspended in
the bioconversion medium.
Bioconversion cycles. Yeast cells were resuspended in 40 ml medium to give different cell
concentrations (175 g or 375 g wet weight/L). Sucrose medium solution contained 5% sucrose and
7.5% NaH2PO4; molasses media contained 5% total sugar (sucrose + glucose + fructose) and
7.5% NaH₂PO₄. Cells were permeabilized by addition of 10% toluene and vigorous shaking of the suspension. The cultures were then stirred in a water bath at 30 C. 

**Analytical methods.** Supernatant samples of the suspension taken at different times were deproteinized by adding 1 ml of 1M perchloric acid to 1 ml sample and allowing to stand for 15 min at 0 C. After neutralization with 0.5 ml of 2.5M KHCO₃ for 15 min on ice, the samples were centrifuged and the supernatants used for metabolites determination. FDP and ethanol were determined according to Bergmeyer (1984). The sucrose, glucose and fructose assays were performed by an enzymatic kit method (Boehringer Mannheim, FRG). The FDP yield was calculated as moles of FDP formed from moles of disaccharide consumed during the bioconversion.

**RESULTS AND DISCUSSION**

Since sucrose is the major carbohydrate in molasses we started our experiments using purified sucrose as the bioconversion substrate. The *Saccharomyces cerevisiae* S288C cells were also grown in batch on sucrose medium. Previously we observed that the best bioconversion was achieved by an appropriate glucose and phosphate to cell ratio (Compagno et al., 1992). Thus to optimize the conditions for sucrose-FDP bioconversion we performed experiments at different concentrations of sucrose, phosphate and cells permeabilized with 10% toluene. The best results were achieved by using a cell concentration of 375 g/L, 5% sucrose and 7.5% phosphate. In these conditions (Fig.1), sucrose was completely hydrolyzed in 3 h, glucose and fructose were metabolized simultaneously but at different rates. The maximum FDP production was 24 g/L at 13 h from the beginning of bioconversion, which represents a 58% yield, (the process yield is one mole of FDP and two moles of ethanol produced per mole of sucrose consumed).

![Fig.1. Time-course of sucrose-FDP bioconversion.](image-url)