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Shifting product formation from xylitol to ethanol in pentose fermentations using *Candida tropicalis* by adding polyethylene glycol (PEG)

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Summary. When *Candida tropicalis* fermented xylose under oxygen limited conditions in the presence of increasing concentrations of polyethylene glycol (PEG), the ethanol production increased by a factor of two and the xylitol production was repressed by about 25%. Xylose assimilation and cell growth were not affected by the presence of PEG. The fermentation of glucose was not as strongly influenced by the presence of PEG as were xylose fermentations. The results are discussed in relation to the physico-chemical properties of a medium containing increasing concentrations of PEG. It is suggested that the presence of PEG might result in a fine-tuning of the aeration in the medium, necessary for ethanol production from xylose with *Candida tropicalis*.

Indroduction

When pentoses are fermented to ethanol with yeast the intermediate xylitol is formed as a major byproduct (Gong et al. 1981). The metabolic regulation behind this heterofermentative product formation is not yet fully understood. The present knowledge on pentose fermentation in yeast concerns mainly xylose metabolism. Experimental evidence supports the proposal that xylose is first reduced to xylitol by a NADPH-dependent reductase and that xylitol is then oxidized by a NAD-dependent dehydrogenase to xylulose, which is further metabolized through the pentose phosphate shunt (Chakravorty et al. 1962).

Candida sp. metabolize pentoses only under aerobic conditions, which has been suggested to be due to the fact that NADPH, necessary for xylose to xylitol conversion, is mainly regenerated via isocitrate dehydrogenase in the TCA cycle (Horecker 1962). Ethanol production is then formed under oxygen-limited conditions (Baillargeon et al. 1983).

In a previous study it was found the presence of a specific respiratory inhibitor, azide, enhanced ethanol formation two-fold and repressed xylitol formation by more than 90% under oxygen limited conditions (Lohmeier-Vogel and Hahn-Hägerdal 1985, this volume). In another study it was found that a decreased water activity in the medium (achieved by additions of dextran and PEG) increases the initial ethanol production rate in glucose fermentations with *S. cerevisiae* (Hahn-Hägerdal et al. 1982), which was understood as an increased maintenance metabolism at the expense of cell growth (Esener et al. 1981).

The present study was undertaken to investigate whether or not increasing concentrations of PEG can switch the metabolism of *Candida tropicalis* during xylose fermentation from xylitol production to ethanol production.

Materials and methods

Organism. Candida tropicalis, ATCC 32113, was maintained at 4° C on slants of agar (15 g/l) containing 5 g/l yeast extract, 10 g/l peptone, and 50 g/l xylose.

Fermentation media. For liquid cultivation, cells were grown on 20 g/l peptone, 10 g/l yeast extract, 10 mM $NaPO_4$, pH 5.5, containing 50 g/l carbon source. The carbon source was either xylose, glucose or glucose and xylose in a 7:5 ratio. Polyethylene glycol 1540, for GLC, was purchased from BDH Chemicals Ltd., Poole, England, and was added on a w/w basis.

Experimental conditions. Innocula consisted of log phase cells (10 g/l dry weight) grown in the desired carbon source. The cells were centrifuged in order to remove remaining medium and washed before being resuspended to the desired concentration in fresh medium.

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The cells were incubated under oxygen limited conditions: 50 ml medium in a 250-ml Erlenmeyer flask; 30° C and 160 RPM, shaking frequency, corresponding to an oxygen transfer rate of 10 mmol/l × h as estimated by the sulfite method (Cooper et al. 1944).

Analytical methods. At various time intervals, samples were withdrawn from the cultures. Cell growth was first estimated from the optical density at 620 nm, then the samples were centrifuged and the supernatants filtered through a 0.2 -μm filter (Sartorius, GmbH, Göttingen, FRG). Ethanol concentrations in the filtrates were determined enzymatically using alcohol dehydrogenase (Sigma; Kaplan and Ciotti 1957) and glucose, xylose, xylitol, and ethanol were assayed by HPLC on an Aminex HPX-87H Organic Acid analysis column (Biorad, München, FRG) using 0.01 N H_2SO_4 as eluent. Dilutions were made when needed. The two ethanol determination methods gave the same results.

Dry weight measurements were made by filtering 2 ml culture medium through preweighed 0.2-μm filters (Sartorius) in triplicate. A 15-ml distilled water wash followed. After drying at 110° C overnight, the cooled filters were weighed again and the difference used to calculate the dry weight in g/l of the cell mass.

Results and discussion

Xylose was first fermented with *C. tropicalis* under oxygen limited conditions in the presence of increasing amounts of polyethylene glycol (PEG) in the medium (Fig. 1).

The ethanol production is enhanced by a factor of two from 2.7 g/l to 5.5 g/l at the highest concentration of PEG. In contrast to what was found for glucose fermentation with *S. cerevisiae* (Hahn-Hägerdal et al. 1982), the initial ethanol production rate appears to be the same at the four different PEG concentrations investigated. Maximum ethanol concentration is reached after 48–72 h after which the ethanol is reassimilated due to the presence of oxygen in the medium.

In Fig. 1 only one curve respectively is shown for xylose consumption and cell growth. This is due to the fact that it was not possible to discriminate differences in the growth curves for the various concentrations of PEG.

In the next experiment xylose, glucose/xylose (7:5) and glucose fermentations with *Candida tropicalis* were compared at increasing concentrations of PEG, up to 21% (Table 1). The ethanol yield, the ethanol output per gram of cell dry weight and the xylitol yield were recorded. Again the increased concentrations of PEG increased the ethanol yield by a factor of almost two in xylose fermentations. This was not true for glucose fermentations, where at most an increase by a factor of 1.3 was found. The glucose/xylose fermentations showed an intermediate increase by a factor of 1.5.

The ethanol output per gram of cell dry weight follows closely the ethanol yield for the three

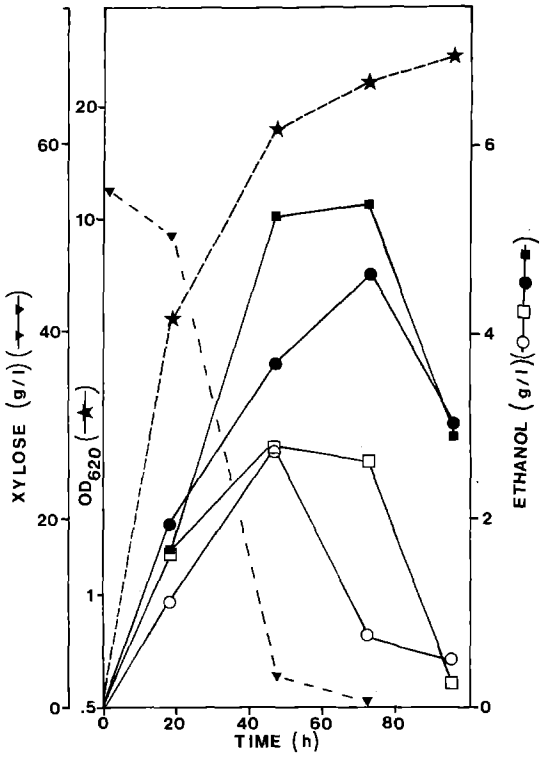

Fig. 1. Ethanol production under oxygen limited conditions during xylose fermentation (55 g carbon source/l) with *Candida tropicalis* having various concentrations of PEG in the medium. (○) control, no PEG; (□) 6% w/w PEG; (●) 12% w/w PEG; (■) 18% w/w PEG; (▼) xylose; (★) OD_{620}

Table 1. Influence of PEG on ethanol and xylitol production in glucose, glucose/xylose and xylose fermentations with *Candida tropicalis* under oxygen limited conditions[a]

Sugar	PEG conc. (w/w)	g ethanol / g sugar	g ethanol / g dry wt cells	g xylitol / g xylose
Glucose	Control	0.25	0.88	NA[b]
	6%	0.29	1.17	NA
	12%	0.32	1.15	NA
	18%	0.26	1.13	NA
	21%	0.21	0.90	NA
Glu/xyl	Control	0.32	0.48	ND[c]
	6%	0.35	0.58	ND
	12%	0.44	0.61	ND
	18%	0.49	0.65	ND
	21%	0.35	0.58	ND
Xylose	Control	0.083	0.223	0.447
	6%	0.109	0.312	0.424
	12%	0.126	0.335	0.425
	18%	0.131	0.353	0.378
	21%	0.139	0.407	0.337

[a] Culture conditions were the following: 50 g of medium (± PEG) containing approximately 10 g/l dry weight cells (initial) in a 250-ml Erlenmeyer flask incubated at 30° C with 160 RPM shaking speed

[b] NA = Not applicable

[c] ND = Not determined

different fermentations, which is in agreement with the first experiment (Fig. 1) where it was found that cell growth was not influenced by the presence of PEG under the experimental conditions chosen.

The xylitol yield was only measured for the xylose fermentations and at most a 20% decrease in xylitol output was found.

Adding PEG to a fermentation medium might have a number of different effects on the metabolism of microbial cells. In a previous study where the influence of increasing concentrations of polymers on ethanol production in *S. cerevisiae* was studied (Hähn-Hägerdal et al. 1982), it was suggested that the increased output of ethanol was primarily a function of a decreased water activity in the medium. This suggestion was based on findings for *Klebsiella pneumoniae,* which at low water activity switched to maintenance metabolism at the expense of cell growth (Esener et al. 1981). However, for *Candida tropicalis* fermenting xylose at water activities (calculated according to Hahn-Hägerdal et al. 1982) ranging from 0.995 with no PEG added to 0.989 with 18% (w/w) PEG, no differences in growth rate and cell mass production were observed (Fig. 1). Thus, the decreased water activity can not be the only answer to the increase in ethanol production.

Polyethylene glycol has a chemical structure quite similar to Tween 80, a non-ionic detergent frequently used in fermentations to improve the release of extracellular enzymes to the culture filtrate (Tangnu et al. 1981). Both compounds are surface active in that they lower the surface tension of the medium to some extent depending on concentration. The possibility might therefore exist that PEG acts as an extractant for ethanol.

Data which point in that direction have recently been reported (Mattiasson et al. 1984). The influence of PEG and sodium chloride were compared with respect to their ability to cause the formation of extracellular glycerol with the halotolerant alga, *Dunaliella parva.* When compared at the same water activity, PEG addition resulted in four times more glycerol being produced than with sodium chloride.

Finally, PEG, being a polymer causes an increase of the viscosity of the medium which will in turn lower the oxygen transfer rate (OTR). On the other hand, because it is somewhat surface active, PEG will also improve the OTR. Since it is not possible to measure the oxygen transfer rate with the sulfite method in the presence of PEG we made an attempt to verify whether or not PEG can fine-tune aeration by using a pH electrode in order to measure the redox potential. The experiments were performed at pH 5.5 in the medium in the presence of xylose and varying concentrations of PEG, but in the absence of cells. It was in fact found that the redox potential decreased linearily from 59 to 50 mV with increasing PEG concentrations.

In the preceeding study on the influence of various metabolic inhibitors on ethanol production from xylose with *C. tropicalis* it was found that only azide, a specific inhibitor of cytochrome aa_3 would increase ethanol production and repress xylitol formation (Lohmeier-Vogel and Hahn-Hägerdal 1984). The results were interpreted in terms and of a "fine tuning" of respiratory activity necessary for ethanol production from xylose with this organism.

The present investigation would thus imply that the presence of PEG could achieve a fine tuning of aeration, giving a similar result. However, since PEG is not as specific as azide, the xylitol levels in this study have only been lowered by 25%, at most, compared to the 90% reduction in the presence of azide.

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References

Baillargeon MW, Jansen NB, Gong C-S, Tsao GT (1983) Effect of oxygen uptake on ethanol production by a xylose-fermenting yeast mutant, Candida sp *XF 217.* Biotechnol Lett 5: 339-344

Chakravorty M, Veiga LA, Bacila M, Horecker BL (1962) Pentose metabolism in *Candida.* II. The diphosphopyridine nucleotide specific polyol dehydrogenase of *Candida utilis.* J Biol Chem 237: 1014–1020

Cooper CM, Fernstrom GA, Miller SA (1944) Performance of agitated gas-liquid contactors. Ind Eng Chem 36: 504–509

Esener AA, Bol G, Kossen NWF, Roels JA (1981) Effect of water activity on microbial growth. In: Moo-Young M, Robinson CW, Vezina C (eds) Adv Biotechnol I, Pergamon Press, Toronto, p 339

Gong C-S, Chen LF, Tsao GT (1981) Quantitative production of xylitol from D-xylose by a high-xylitol producting yeast mutant of *Candida tropicalis* HPX2. Biotechnol Lett 3: 130–135

Hahn-Hägerdal B, Larsson M, Mattiasson B (1982) Shift in metabolism towards ethanol production in *Saccharomyces cerevisiae* using alterations of the physical-chemical enviroment. Biotechnol Bioeng Symp No 12: 199–202

Horecker BL (1962) Pentose metabolism in bacteria. John Wiley and Sons, New York

Kaplan NO, Ciotti MM (1957) Enzymatic determination of ethanol. In: Colowich SP, Kaplan No (eds) Methods in enzymology III. Academic Press, New York, pp 253

Lohmeier-Vogel E, Hahn-Hägerdal B (1985) The utilization of metabolic inhibitors for shifting product formation from xylitol the ethanol in pentose fermentations using *Candida tropicalis.* Appl Microbiol Biotechnol 21: 167–172

Mattiasson B, Larsson M, Hahn-Hägerdal B (1984) Metabolic behaviour of immobilized cells – Effects of some microenviromental factors (submitted for publication)

Tangnu SK, Blanch HW, Wilke CR (1981) Enhanced production of cellulase, hemicellulase and beta-glucosidase by *Trichoderma reesei* (Rut C-30). Biotechn Bioeng, vol XXIII, pp 1837–1849

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