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EFFECT OF POLY (ETHYLENE GLYCOL) ON *SACCHAROMYCES CEREVISIAE* WITH RESPECT TO GROWTH AND ETHANOL PRODUCTION

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Alterations of the physico-chemical environment of microorganisms cause changes in their metabolism. This report deals with the effect of poly (ethylene glycol) (PEG) of varying concentration and molecular weight on production of ethanol in *Saccharomyces cerevisiae*. It was found that the PEG with the highest molecular weight (MW 17,500) affected the greatest increase in ethanol production at the expense of cell mass production. Similar effects were observed for higher concentration of low molecular weight PEG. Cell grown on PEG-rich media produced ethanol more efficiently and showed an increased level of alcohol dehydrogenase.

INTRODUCTION

Immobilized cells may exhibit a different metabolic behavior as compared to free living cells. It has been hypothesized that reduced water activity and a changed oxygen supply are the main reasons for the changes observed [1]. However, the hypothesis only dealt with the effects on the cellular level and discussed changes in metabolic fluxes. It was based on over-all observations of metabolic fluxes and did not deal with changes on the molecular level, e. g. concentrations of enzymes. On

immobilization, cells are exposed to a polymer rich microenvironment. It has been proven efficient to expose them to media containing soluble polymers to simulate the immobilized situation [2,3]. The present investigation deals with cultivation of *Saccharomyces cerevisiae* in media containing poly (ethylene glycol) of varying concentrations and molecular weights.

MATERIALS AND METHODS

Purification of poly (ethylene glycol) : Two different poly (ethylene glycols), purchased from Union Carbide, U. S. A, having molecular weights of 17,500 (PEG 20 M) and 8000 (PEG 8000) were purified prior to use [4].

PEG 20 M or PEG 8000 (100g) was dissolved in 2 litres of warm acetone. The mixture was cooled to room temperature and 1 litre of diethyl ether was added. It was then left over night in a fume hood at room temperature. The crystals formed were filtered off using a sintered funnel and washed with 1 liter of acetone-ether (2 : 1) mixture, and the residue was air dried.

PEG 1540 (lot. no. 04076, Polysciences Inc., USA) of pharmaceutical grade was used without purification.

ANALYTICAL METHODS

Determination of glucose by HPLC : Glucose was determined by HPLC (Shimadzu LC 6A) equipped with a refractive index detector (Shimadzu RID 2A). An ion exchange column (300 * 6.5 mm, Sugar Pak^R; Waters, Millipore, U. S. A.) packed with microparticulated silica (cation exchanger) gel in calcium form was used. The column was maintained at 90°C. The mobile phase was deionized water (stored at 90°C to prevent air dissolving in the water) pumped at a rate of 0.5 ml/min.

The samples (10µl) were injected using an auto injector (Shimadzu SIL 6A). A stock glucose solution (glucose monohydrate 66 mg/ml) was used as a standard.

Determination of ethanol by GLC : The gas chromatograph (Shimadzu GC9 AM) was equipped with a flame ionization detector. The column (2 6 mm i.d and 2.1 m long) was packed with chromosorb 20M (stationary phase; Cat No. 1-1994; Lot No. T 26852, 10% carbowax^R 20M on 80/100 chromosorb^R; Supelco, Inc.). The carrier gas was N_2 at a flow rate of 35 ml/min.

A stock ethanol solution (15 g/l) was used as standard. Samples (10µl) were injected by an auto injector (Shimadzu SCL 6A). The column temperature was maintained at 160°C.

FERMENTATIONS IN SHAKE FLASKS

Preparation of the inoculum : *Saccharomyces cerevisiae* was obtained from

Jastbolaget, Sweden. The medium was composed of glucose monohydrate (66.05 g/l), yeast extract (2.5 g/l), $NH_4H_2PO_4$ (0.25 g/l), and $MgSO_4 . 7H_2O$ (0.025 g/l). The pH was adjusted to 4.5. The solution was autoclaved for 30 min at 120°C.

The autoclaved medium was cooled and inoculated with the yeast, and was incubated in a thermostatted water bath equipped with shaking facilities at a speed of 400 r. p. m. at 35°C. The cells were cultivated for 18 hours to reach their lag phase. and cell growth was monitored by measuring tha optical density at 600 nm. The harvested cells intended to be used as inoculum for the further studies were divided and frozen in portions of 2ml and 15ml.

Growth of *Saccharomyces cerevisiae* in media containing poly (ethylene glycol) : Six solutions of PEG 1540, 8000 and 20M (10 g and 20 g) were prepared in deionized water to a final weight of 100g. To each of the six flasks was added 100 g of the inoculum medium (double concentrated), so that each flask contained 5 or 10% (w/w) of the PEG respectively. A control with no polymer was also prepared.

The pH in all these media was adjusted to 4.5 and they were autoclaved for 30 min. at 120°C.

The flasks were cooled and inoculated with 2 ml of the inoculum, and inoculated media were incubated in a water bath (400 r. p. m.) at 35°C.

Aliquots (5 ml) were withdrawn and the optical density at 600 nm was measured to monitor the growth of the yeast.

The samples were also centrifuged at 6000 r. p. m. in a bench centrifuge and the supernatants were diluted twice with deionized water containing 0.04% NaN_3. These were then analysed for glucose and ethanol content.

FERMENTER EXPERIMENTS

Growth of *Saccharomyces cerevisae* using inoculum without PEG : The inoculum was prepared as in the shaker flask experiments. The medium (5 l, same composition as above) was transferred to a fermenter having a working volume of 10 litre. The fermenter was autoclaved for 3 hours at 120°C. The medium was cooled and inoculated with 50ml of the inoculum (1%). The temperature in the fermenter was maintained at 35°C while mixing at a speed of 400 r. p. m. and pH was maintained at 4.5 by titrating with 4N NaOH.

Samples were withdrawn and the cell growth was monitored. After centrifugation (6000 r. p. m.) in a bench centrifuge the supernatants were analysed for contents of glucose and ethanol.

The final cell mass was measured in

terms of dry weight and viable cell count. The cells were disintegrated in a bead mill (Dyno-mill, WAB, Switzerland) and the level of alcohol dehydrogenase (ADH) activity was determined [4].

The experiment was repeated with cells growing in a medium containing PEG 20M (50 g/l).

Growth of *Saccharomyces cerevisiae* in medium containing PEG 20M using an inoculum grown in PEG containing medium : The composition of the medium for the inoculum was same as described above with the addition of 50 g/l PEG 20M. The medium was autoclaved at 120°C for 30 min., cooled and inoculated with 1 ml (1%) of the inoculum used above. The medium was incubated at 35°C in a water bath at a speed of 400 r. p. m. for 18 hours. The fermentation using this inoculum was performed as described above.

RESULTS AND DISCUSSION

Small scale batch experiments (200 ml) were performed to evaluate the effect of the presence of different poly (ethylene glycols) in the cultivation medium on growth and metabolism of *Saccharomyces cerevisiae*.

The optical density in the control medium was in all cases higher than in those media containing PEG (Fig. 1-3). Furthermore, the effects were more pronounced at higher polymers concentrations in the media. The effect of the different PEG-preparations varied. PEG 8000 had less overall influence than PEG 20M and PEG 1540. However, media containing PEG 20M gave the lowest cell densities. It has been reported in the literature [6] that PEG

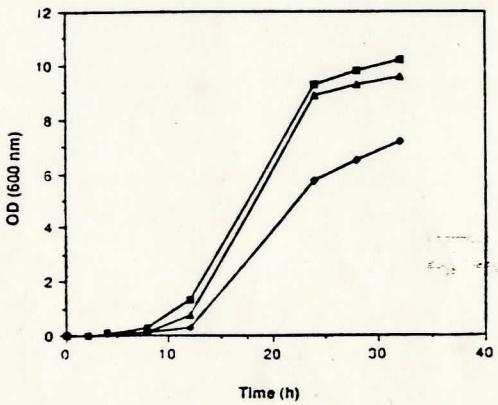


Fig 1. Optical density (OD) as a function of time in control medium (squars), in 5% PEG 1540 (triangles) and in 10% PEG 1540 (stars).

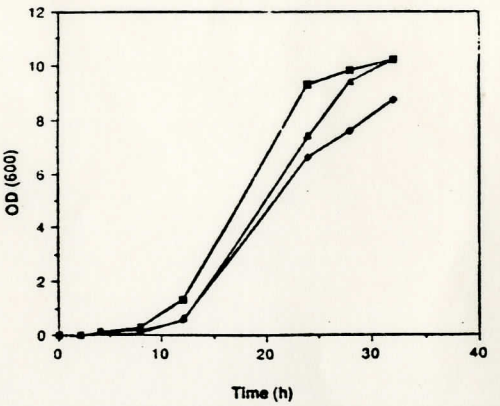


Fig. 2. Optical density (OD) as a function of time in control medium (squares), in 5% PEG 8000 (triangles) and in 10% PEG 8000 (stars).

is inhibitory to the growth of *Saccharomyces cerevisiae*. The effect was interpreted as a result of a decrease in water activity.

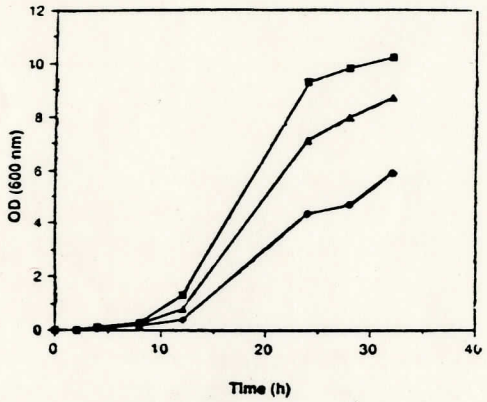


Fig. 3. Optical density (OD) as a function of time in control medium (squares), in 5% PEG 20 M (triangles) and in 10% PEG 20 M (stars).

Glucose consumption was faster in the cases where PEG was used in the medium than in the control medium (Fig. 4-6). The

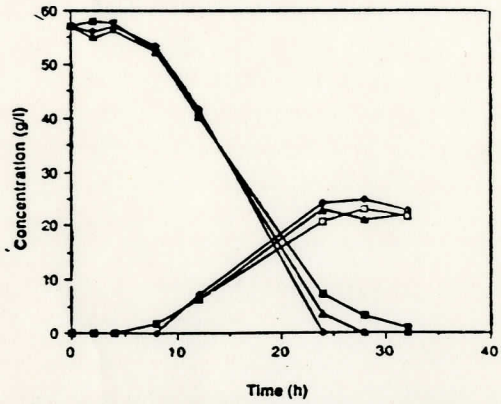


Fig. 4. Glucose consumption (filled symbols) and ethanol production (open symbols) in control medium (squares), 5% PEG 1540 (triangles) and 10% PEG 1540 (stars).

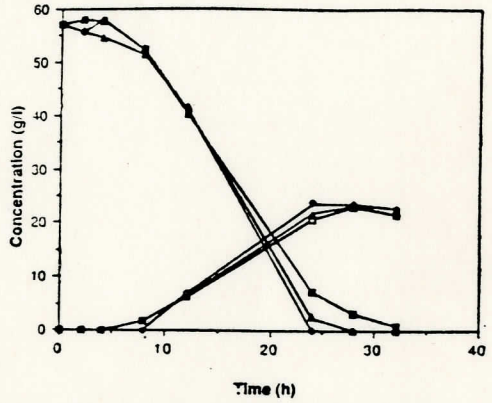
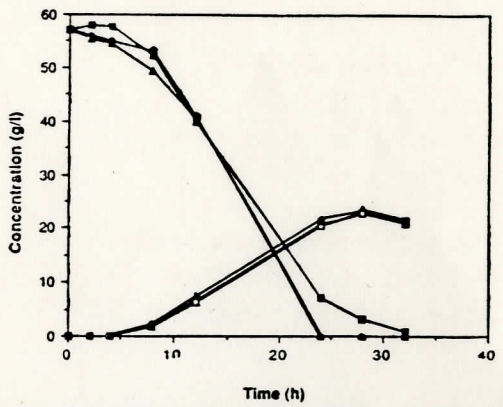


Fig. 5. Glucose consumption (filled symbols) and ethanol production (open symbols) in control medium (squares) 5% PEG (8000) triangles and 10% PEG 8000 (stars).

glucose was consumed within 24-28 hours in the PEG-containing media and in 32 hours hours in the control. Ethanol was produced more efficiently in the PEG-containing media than in control media.



Fi6. 6. Glucose consumption (filled symbols) and ethanol ptoduction (open symbols) in control medium (squares) 5% PEG 20 M (triangles and 10% PEG 20 M (stars).

Similar observations have been reported earlier [1]. It is interesting to note that despite the fact that cell growth is hampered in the PEG-rich medium, ethanol production is as efficient or better than in the control medium.

In all cases where the fermentations took place in shaking flasks the conditions were not strictly anaerobic. The conditions were similar but the glucose consumption was faster and the alcohol production was more efficient when PEG was used. The effect was concentration dependent for the two smaller polymers, whereas no such clear observations were obtained for PEG 20M.

These observations of decrease in cell number and increased productivity may be interpreted as a result of a decrease in water activity of the medium[5,6].

To test the feasibility of performing fermentations in PEG rich media with more strict control of the conditions regarding pH and oxygen level, three different systems were studied.

A) Control fermentation without PEG, normal inoculum.

B) Medium containing 5% PEG 20M, normal inoculum.

C) As B, inoculum grown in 5% PEG 20M.

The growth was measured as optical density (OD) and is plotted vs time in Figure 7.

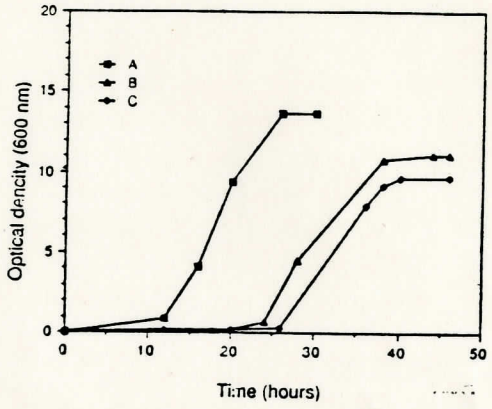


Fig. 7. Growth of *Saccharomyces cerevisiae* under standard conditions (A), in medium containing 5% PEG 20 M, normal inoculum (B) and in medium containing 5% PEG 20 M, inoculum grown in 5% PEG 20 M (C).

The end values of dry weight, OD, viable cell count, protein content and the level of alcohol dehydrogenase activity are shown in Table 1. The cell mass and the growth rate were higher in the control medium (A), whereas the cell growth was delayed in the PEG containing media. Almost no difference was observed between the growth in media with the normal inoculum (B) and inoculum grown on media containing PEG (C).

As can be seen in Table 1 the dry weights and viable cell counts are lower in the media containing PEG. However, glucose is completely consumed in all cases.

Since the growth is slow in PEG-containing media a larger proportion of the glucose is converted to ethanol and thus a higher ethanol yield is obtained (Fig. 8).

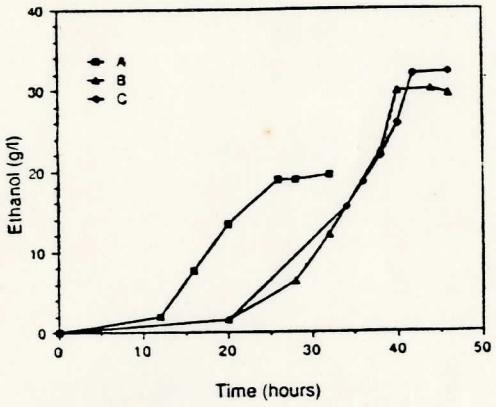


Fig. 8. Patterns of alcohol formation under standard conditions (A), in medium containing 5% PEG 20 M, normal inoculum (B) and in medium containing 5% PEG 20 M inoculum grown in 5% PEG 20 M (C).

These observations indicate an altered metabolism. To demonstrate such an effect we studied the level of alcohol dehydrogenase in the different preparations. The alcohol dehydrogenase activity of the cells grown in PEG media increased by a factor of 2, and in the experiment using an inoculum grown in PEG-rich media the activity level increased by a factor of 4 (Table 1).

Further studies on changes in the enzyme pattern will reveal if there are any specific points where the effect is more pronounced.

The results reported here fully confirm earlier observations that additions of polymers to a medium changes the metabolism of yeast cells. These results were earlier interpreted in terms of a compensation of the cells to changing conditions in the environment. It is apparent that the response is a function of changes in the protein synthesis pattern, favouring the key enzyme in the process studied.

TABLE 1. Dry weight, optical density and viable cell count were measured at the end of each experiment. The protein content and the alcohol dehydrogenase activity were measured after 8 min of homogenization in the bead mill, at the end of cultivations. A; Control fermentation without PEG, normal inoculum. B; Medium containing 5% PEG 20M, normal inoculum. C; As B, inoculum grown in 5% PEG 20M.

	Dry weight (g/l)	Optical density (600 nm)	Viable cell count (X 10^7)	Protein (mg/ml)	ADH activity (U/mg protein)
A	6.4	13.7	3 1	2.9	0.017
B	5.0	11.6	2.1	3.1	0.031
C	3.5	9.6	2.0	2.5	0.066

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