*Proc. 4th European Congress on Biotechnology 1987, Volume 2.*
edited by O.M. Neijssel, R.R. van der Meer and K.Ch.A.M. Luyben
Elsevier Science Publishers B.V., Amsterdam, 1987 — Printed in The Netherlands



# BIOCONVERSIONS IN AQUEOUS TWO-PHASE SYSTEMS

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## Introduction

Extractive bioconversion using cells or enzymes in aqueous two-phase system has attained increasing biochemical and technological interest in the last few years (1). The integration of production and separation of the product have been shown confer several advantages (2). However when using aqueous two-phase systems in large scale applications, it turns out that there is a need for less expensive phase forming polymers. We have studied the feasibility to use a two-phase system composed of polyethylene glycol (PEG 20M) and partially hydrolyzed starch.

## Materials and Methods

PEG 20 M (Union Carbide, USA) was purified prior to use (3). Waxy maize starch (Stadex, Sweden),150 g, was suspended in 350 ml water. Calcium acetate, 280 mg, and 150 mg alpha-amylase (Termamyl 60L, Novo,Denmark) was added to it . The suspension was kept at 90°C for 2 hours, after which the pH was adjusted to 3.5 to denature the enzymes. The solution was filtered (glass filter #3) and the pH was adjusted to 5.0.

The fermentation medium contained 60 g glucose, 2.5 g yeast extract, 0.25 g $NH_4H_2PO_4$, 0.025 g $MgSO_4 \cdot 7H_2O$ and 50 g PEG 20M in one liter volume. The PEG 20M was omitted in the control medium. *Saccharomyces cerevisiae* (Swedish Yeast Company) was used. The temperature in the fermenter was 35°C and the pH was maintained at 4.5 by titrating with 4N NaOH. Stirring speed was 400 rpm. The cell growth was monitored by measuring the optical density at 600 nm. The glucose consumed and alcohol formed at different time intervals were measured by HPLC and GLC, respectively. 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 ADH activity was determined as described earlier (4).

The hydrolysis in phase systems was performed at 37°C in 10 ml graduated centrifuge test-tubes. Different amounts of glucoamylase (SAN 150L, Novo, Denmark) were added to the test tubes in duplicate. Samples were withdrawn at certain time intervals from one of the tubes for the determination of glucose, and the amount of bottom phase was measured in the other tube.

The binodial of the two-phase system was determined by carrying out continuous spectrophotometric measurement of the turbidity at 600 nm. A phase system (5 ml) of known composition was stirred in a 30 ml beaker, pumped through the flow cuvette in the spectrophotometer and back to the beaker. The phase system was continuously diluted at constant flow rate either with water or with a polymer solution of known composition. The measured transmittance was recorded. After a certain dilution of the phase system the transmittance was increasing drastically until homogeneous mixture was obtained. The points on the binodial could then be calculated using the flow rate and the break though points .

## Results and Discussion

The phase diagram of starch-PEG 20M obtained according to the method described previously is shown in fig. 1. The shaded area indicates the transition from a two-phase state to a homogeneous solution.When varying amounts of glucoamylase were added to a starch-PEG (19% w/w starch and 10% w/w PEG 20M) two-phase system it was convenient to follow the enzymatic hydrolysis both as the amount of glucose formed and as a decrease in the relative phase volume of the starch rich bottom phase (Fig. 2).

These studies show that a cheap two-phase system can be obtained by using PEG and partially hydrolyzed starch even when the starch preparation is polydisperse.
To test the feasibility of performing fermentation of the glucose released in the starch-PEG two-

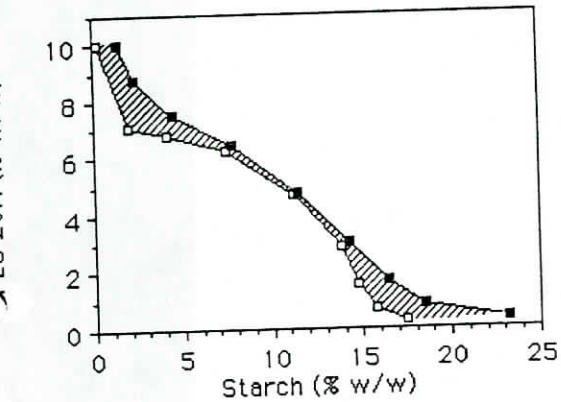


**FIGURE 1.** Binodial of the PEG 20 M-starch two-phase system. Shaded area is indicating the change from two-phase region to homogenuous solution.

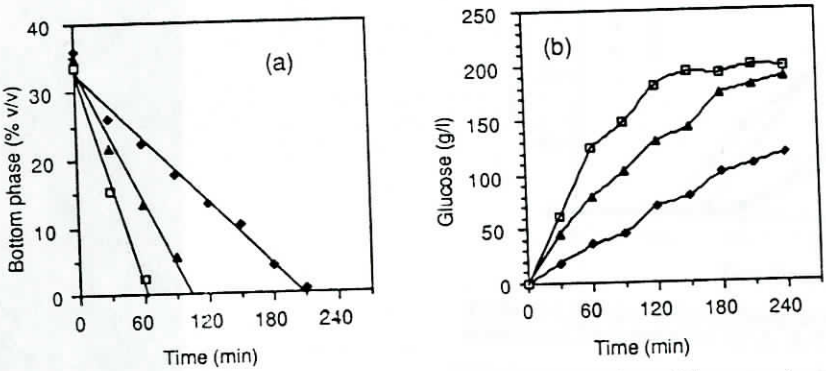


**FIGURE 2.** Decrease in bottom-phase volume (Fig. a). Glucose formed from starch versus time (Fig. b). Different amount of glucoamylase was used, 12 µl (stars), 24 µl (triangles) and 48 µl (squares).

phase system , four different systems were studied :

A) Control without PEG, normal inoculum.
B) Medium containing 5% PEG 20M, normal inoculum.
C) As B, inoculum grown in 5% PEG 20M.
D) As B, under aerobic conditions (0.05 VVM).

Figure 3 shows the growth expressed as optical density (O.D). The end values of dry weight, OD and viable cell count 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 containing PEG (C). The cell growth was faster when the medium was aerated (D).

As can be seen in Table 1 the dry weight and viable cell count are lower in the media containing PEG, but glucose is completely consumed in all the 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 observed (Fig. 4). These observations indicate an altered metabolism.

The activity of alcohol dehydrogenase (Table 2) in the cells increased in PEG media by a factor of 2. However, after aeration the protein content and alcohol dehydrogenase activity were approximately 10 times higher than in the anaerobic situation. Further studies on changes in the enzyme pattern will reveal if there are any specific points where the effect is more pronounced.

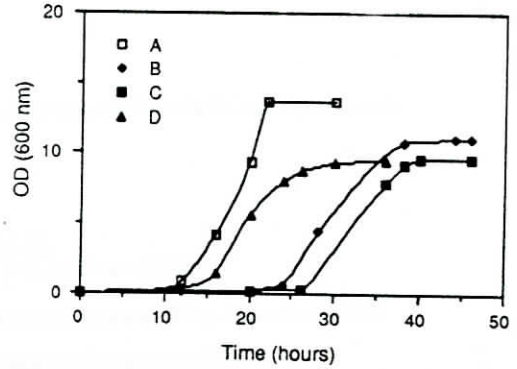


**FIGURE 3.** Growth of *Saccharomyces cereviciae* under standard conditions (A) and in PEG containing media (B, C and D). Conditions are given in the text.

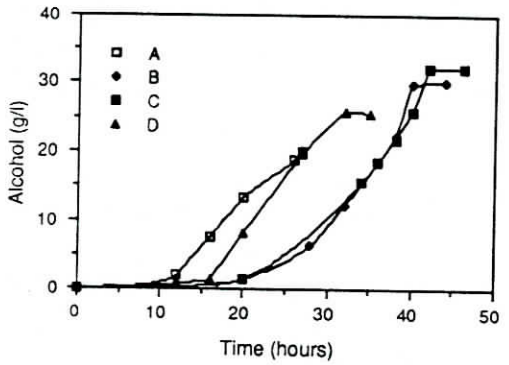


**FIGURE 4.** Patterns of alcohol formation under standard conditions (A) and in PEG containing media (B, C and D). Conditions are given in the text.

**TABLE 1.** The dry weight, optical density and viable cell count were measured at the end of ea experiment. The conditions for A, B, C and D are the same as in the text.

| | Dry weight (g/l) | Optical density (600 nm) | Viable cell count ($X 10^7$) |
|---|---|---|---|
| A | 6.4 | 13.7 | 3.1 |
| B | 5.0 | 11.6 | 2.1 |
| C | 3.5 | 9.6 | 2.0 |
| D | 3.4 | 9.5 | 2.1 |

**TABLE 2.** The protein content and the alcohol dehydrogenase activity at the end of cultivations measured after 6 min of homogenization in the bead mill. A, B, C and D as in Table 1.

| | Protein (mg/ml) | ADH activity (U/mg protein) |
|---|---|---|
| A | 2.9 | 0.021 |
| B | 2.5 | 0.050 |
| C | 0.9 | 0.077 |
| D | 9.1 | 0.254 |

Acknowledgements

This project was supported by the National Swedish Board for Technical Development and The Swedish Institute.

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