The use of fed batch cultivation for achieving high cell densities in the production of a recombinant protein in *Escherichia coli*

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**Abstract:** The production of the fusion protein staphylococcal protein A/*E. coli* β-galactosidase in *Escherichia coli* was studied in batch and fed batch cultivations. Batch cultivation of a recombinant *E. coli* strain yielded a final cell dry weight of 16.4 g l⁻¹ with a final intracellular product concentration of recombinant protein corresponding to approximately 38% of the cell dry weight. Fed batch cultivation made it possible to increase the final cell dry weight to 77.0 g l⁻¹. The intracellular product concentration (25%) was lower as compared to batch cultivation resulting in a total concentration of recombinant protein of 19.2 g l⁻¹.

**Key words:** *Escherichia coli*; Recombinant protein; Fed batch; High cell density; Maintenance energy; Viability

**Introduction**

In order to achieve high cell densities in cultivations of bacteria it is usually necessary to restrict the specific growth rate in order to avoid conditions of oxygen starvation or excessive heat evolution. Formation of inhibiting by-products from high consumption rates of substrate may also be a problem.

The fed batch technique is suitable to overcome the mentioned problems [1]. However, one important factor limiting the final cell density in a fed batch culture with limiting glucose feed is the requirement for maintenance energy. Running a fed batch cultivation with a constant feed rate of limiting glucose will have the consequence that more and more of the incoming substrate will, with time, be used for maintenance purposes. This means that less and less of the cellular resources can be used for the synthesis of the product.

We have compared the production of a fused protein in batch and fed batch cultivations [2]. The fused protein, SpA-βgal, consisted of the immunoglobulin-binding regions of staphylococcal protein A fused to β-galactosidase from *Escherichia coli* [3]. The intracellular expression in *E. coli* of the fused protein was placed under the control of the temperature-inducible $p_R$ promoter.

**Materials and Methods**

Cultivations were performed on a 5-l scale with a defined medium [2]. The bacterial produc-
tion system consisted of *E. coli* KA197 expressing the fusion protein SpA-βgal under the control of the temperature-inducible *p*R promoter [2]. β-Galactosidase assay with ONPG as substrate [4] was used to measure the amount of SpA-βgal. SpA-βgal has a specific activity of 400 U (mg protein)⁻¹ [5]. Determination of viable count [2], cell dry weight [6], and glucose concentration [6] has been described elsewhere. The oxygen concentration in the off-gas was measured with a paramagnetic analyser.

An exponential feed profile for growth on glucose can be applied by using the following equation:

\[ F = \frac{1}{s} \times \left( \frac{\mu}{Y_{x,s}} + m \right) \times X_0 \times e^{\mu t} \]

where: \( F \), feed rate (l h⁻¹); \( s \), substrate concentration in feed solution (g l⁻¹); \( \mu \), desired specific growth rate (h⁻¹); \( Y_{x,s} \), true cell yield (g g⁻¹) on limiting substrate; \( m \), maintenance coefficient (g substrate (g cell, h)⁻¹); \( X_0 \), total amount of cells (g) in the reactor at the start of the feeding; and \( t \), time after feed start (h).

**Results and Discussion**

The use of fed batch technique made it possible to increase final cell densities as compared to batch cultivation (Fig. 1). The final cell dry weight was 77.0 g l⁻¹ in the fed batch culture and 16.4 g l⁻¹ in the batch culture. A high total product concentration was also achieved in the fed batch culture, (Fig. 1). A cultivation temperature of 30°C and an induction temperature of 40°C were used. Temperature induction took place at a cell dry weight of 5.2 g l⁻¹ in batch culture and at 61.6 g l⁻¹ in the fed batch culture.

The fed batch culture started as a batch culture (the specific growth rate was 0.45 h⁻¹). When the cell dry weight reached 11.6 g l⁻¹, the feeding of glucose (60% solution) started. At this time almost all of the initial glucose (25 g l⁻¹) was consumed. The use of a feed rate that increases with time in a logarithmic fashion allows the specific growth rate, \( \mu \) (h⁻¹), to be maintained at a constant value. The exponential feed profile can be maintained as long as the oxygen demand of the cells can be met. Thereafter, the feeding can continue at a constant rate during which the specific growth rate will decrease continuously. During the first 3 h of feeding, the specific growth rate was maintained at approximately 0.3 h⁻¹ by increasing the feed rate exponentially from 0.044 l h⁻¹ to 0.10 l h⁻¹. In the following phase, where the feed rate was maintained constant (0.10 l h⁻¹), \( \mu \) declined from 0.3 h⁻¹ to 0.02 h⁻¹ at the end of the cultivation. The production of SpA-βgal was induced at a cell dry weight of 61.6 g l⁻¹ (Figs. 1 and 2). At this time \( \mu \) was 0.08 h⁻¹. This can be compared to the batch culture, where the

![Fig. 1. Cell dry weight (CDW: g l⁻¹) and total activity of SpA-βgal (U ml⁻¹). The activity of SpA-βgal was measured as β-galactosidase activity.](image)

![Fig. 2. Specific activity (U (mg cell dry weight)⁻¹) and specific production rate (U (mg cell dry weight, h)⁻¹) of SpA-βgal. The activity of SpA-βgal was measured as β-galactosidase activity.](image)
cell dry weight was 5.2 g l⁻¹ and \( \mu \) was 0.45 h⁻¹ at the time of induction.

In the batch culture, the final concentration of SpA-β-gal corresponded to 38% of the cell dry weight or 6.2 g SpA-β-gal l⁻¹. In the fed batch culture, the corresponding figures were 25% of the cell dry weight and 19.2 g l⁻¹, respectively. This means that even though the glucose feeding only allowed a \( \mu \) of 0.08 h⁻¹ (18% of \( \mu_{\text{max}} \)) in the fed batch at the time for induction, the supply of glucose was enough to support production of recombinant protein.

Fig. 3 shows that during the period with constant feed rate the amount of consumed glucose that was used for energy purposes increased with time. This can be seen from the fact that the amount of consumed oxygen per consumed glucose (OCR/GLU; mol, h (mol, h⁻¹)) increased with time. This was also true for the amount of produced carbon dioxide per consumed glucose. Theoretically, the quotient OCR/GLU is 6 for the complete oxidation of glucose. The quotient increased from 2.1 to 3.7 during the period with constant feed. This means that more and more of the consumed glucose was used for maintenance energy. The increase in OCR/GLU at the time of temperature induction also shows that the demand for maintenance energy increased as the temperature was increased. The extensive production of recombinant protein may also influence the requirement for maintenance energy. The feed rate of glucose and the maintenance energy are two important parameters determining the maximum cell mass that can be achieved in a fed batch culture. This problem is addressed in the accompanying paper in this issue [7]. Fig. 3 also shows that even if the feed rate is kept constant the oxygen consumption rate will increase with time due to the demand for maintenance energy.

Plasmid instability presented no problem during batch and fed batch cultivations. The viability according to viable count on agar plates showed a decrease during the induction period in the fed batch cultivation. This decline was found both on plates with and without antibiotics. Fig. 4 shows viable count on plates without antibiotics in batch and fed batch cultivations. The decline in viability in the fed batch was probably due to the conditions of glucose limitation. The specific rate of glucose consumption, \( q_{GLU} \) (g (g cell dry weight)⁻¹h⁻¹), was 0.16 g g⁻¹ h⁻¹ or 14% of the maximum value (in the batch phase) at the end of the cultivation. Another possible explanation could also be the high intracellular concentration

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**Fig. 3.** Total oxygen consumption rate (OCR; mmol h⁻¹), specific oxygen consumption rate (mmol (g cell dry weight, h⁻¹)), and the amount of consumed oxygen divided by the amount of consumed glucose (OCR/GLU; mol, h (mol, h⁻¹)). Data from fed batch culture.

**Fig. 4.** Viable count (colony forming units × 10⁻⁹ ml⁻¹) in batch and fed batch culture and specific consumption rate of glucose in fed batch culture. Viable count was performed on tryptic soy broth plates without the addition of antibiotics.
of recombinant protein. However, the cells from the batch culture had a higher intracellular concentration of recombinant protein without a concomitant reduction in viability.

Conclusion

High intracellular concentrations of recombinant protein can be achieved despite severe energy limitation at high cell densities in fed batch cultures.

References