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Edited by Aleš Prokop and Rakesh K. Bajpai

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High Cell Density Fermentation of Recombinant *Escherichia coli* with Computer-Controlled Optimal Growth Rate

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In recent years interest in the production of recombinant DNA products, such as enzymes and pharmaceuticals, has been growing. High cell density cultivation has been one of the most effective ways to increase cell as well as product yield. Basic work in this field was carried out by Bauer and coworkers (1974–1981) with nonrecombinant strains of *Escherichia coli*.[1,2]

A variety of process strategies have been developed in fed-batch fermentation of *E. coli*.[3] Common goals are to control the oxygen demand within the oxygen transfer capabilities of the fermentor and to avoid the accumulation of acetate and ethanol.[4] These goals can be met by feeding the carbon source to achieve C-limited growth, reducing temperature to decrease the growth rate, or increasing the oxygen transfer capability of the fermentor using oxygen as the sparging gas. Cell densities of about 110 g/L have been reported for a process-controlled fed-batch plus oxygen mode for cultivating nonrecombinant *E. coli* in a 2.5-L fermentor.[5] An impressive application of rDNA products is the production of alpha-consensus interferon by recombinant *E. coli*. Fieschko and Ritch[6] reported product concentrations of 5.5 g/L from 65 g/L cell dry mass.

ADVANTAGES OF HIGH CELL DENSITY FERMENTATION

In general, the main advantages of high-density cultivation are: reduced fermentor and closed system volume, improved space time yield (volumetric productivity), reduced medium costs, reduced volume in primary downstream processing, frequent omission of concentration steps, and reduced plant and operating costs.

HOST STRAIN *E. COLI*

For decades, *E. coli* has played an important role in molecular biological work, which explains its use as the host strain for the majority of protein productions from rDNA. For example, insulin, hGH, alpha$_2$-interferon, alpha$_{2b}$-interferon, and rennin

are available from cultivations of recombinant *E. coli.* For none of them has a high cell density process been used.

Of great interest is the control of growth rate because it strongly influences the formation of both products and inhibitory metabolites. Acetate formation is reported to increase drastically when the growth rate exceeds 0.35 h^{-1} (defined medium) and 0.2 h^{-1} (complex medium).[7]

Seo and Bailey[8] showed the existence of an optimum dilution rate (growth rate) for β-lactamase production in continuous cultivation. This finding was not confirmed in batch experiments when growth rate control was changed by altering growth medium. Despite this, Riesenberg *et al.*[9] reported an optimum growth rate in batch production of alpha$_1$-interferon.[9]

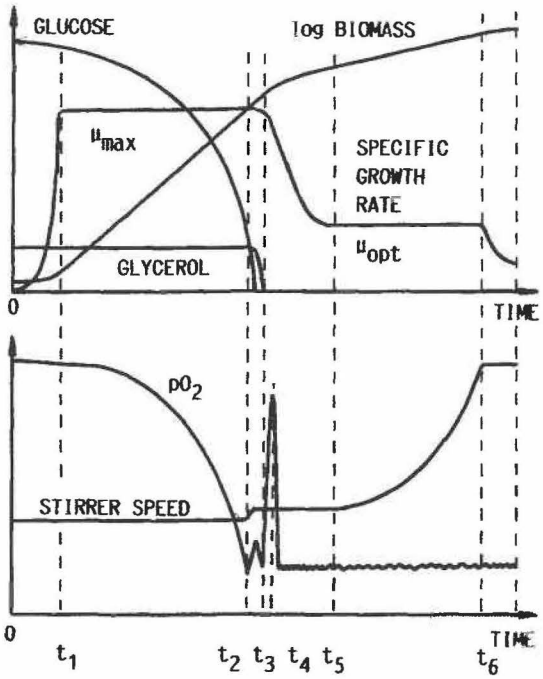

FIGURE 1. Scheme of the ZIMET HDF 30/450: t_1 = end of lag-phase; t_2 = start of pO_2-control via stirrer speed; t_3 = exhaustion of glycerol; t_4 = start of glucose feeding; t_5 = start of exponential shift in stirrer speed; and t_6 = end of exponential shift in stirrer speed.

ZIMET HIGH CELL DENSITY FERMENTATION 30/450

In the ZIMET a high cell density fermentation (HDF) process for a glucose/mineral salt medium allows growth of a recombinant *E. coli* strain (TG 1, pBB 210) up to a cell density of 60 g/L in a 30- and 450-L Chemap fermentor.[9] Except for the feeding of glucose as a carbon source and of aqueous ammonia for pH control, there was no need for the feeding of other nutrients and for the supply of oxygen-enriched air. FIGURE 1 schematically illustrates this process with a batch phase with glucose

consumption and a feed-batch phase with glucose feeding. In the fed-batch phase the pO_2 was kept at 20% of saturation via closed-loop controls with two variables, namely, stirrer speed and feeding rate of glucose. The fed-batch mode prevented significant accumulation of acetate and other metabolic byproducts. The recombinant *E. coli* expressed alpha$_1$-interferon constitutively with a higher efficiency at a lower specific growth rate ($\mu_{opt} = 0.17\ h^{-1}$) than at the maximal specific growth rate ($\mu_{max} = 0.45\ h^{-1}$) (FIG. 2). Therefore, after reaching a suitable cell density with growth at μ_{max}, the culture was forced to grow at the optimal specific growth rate, μ_{opt}, by open-loop control for agitation directing the input of oxygen and hence the supply of glucose. The stirrer speed was increased according to an e-function profile.

ZIMET/GBF-HIGH CELL DENSITY FERMENTATION 70/1500

To overcome the well-known disadvantages of high density processes, GBF and ZIMET in 1989 developed a special HDF process for *E. coli* that produces more than

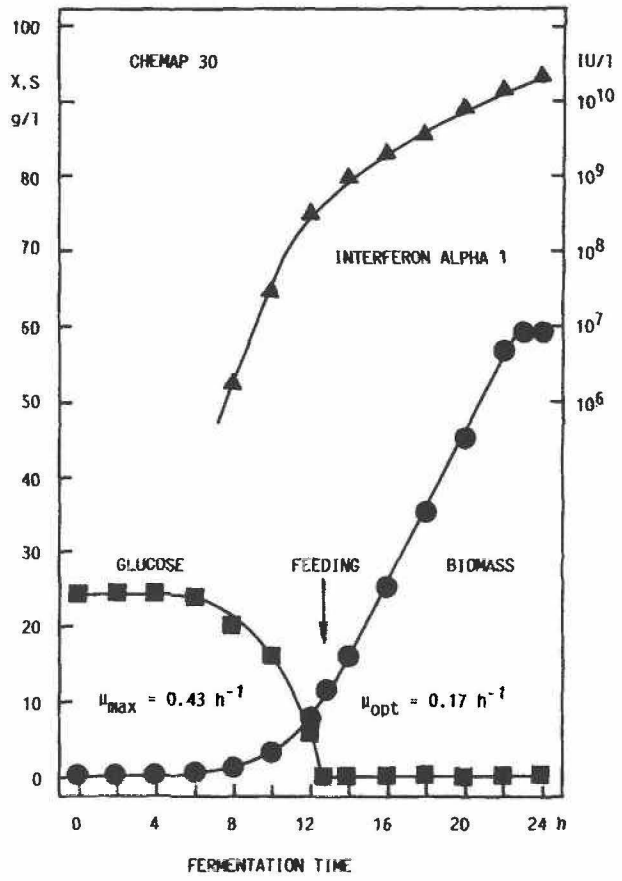

FIGURE 2. ZIMET HDF 30 process: Kinetics of glucose, biomass, and alpha$_1$-interferon.

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