

# TECHNOLOGY

## UPSTREAM PROCESSING

# Protein production in human cells

Ruth Essers, CEVEC Pharmaceuticals GmbH, Cologne, Claudia M. Huether, DASGIP AG, Juelich, Germany

➤ A pH-based biphasic batch process has now been established to evaluate physical parameters for the batch cultivation of stable human CAP cell lines. In an important new development, the biphasic culture process resulted in a significant increase of time-dependent volumetric productivity and final product concentrations of a highly complex and fully-glycosylated and sialylated protein.



Fig. 1: DASGIP 4-fold parallel bioreactor system for cell culture

CEVEC's proprietary expression system based on human amniocytes offers significant advantages over existing production technologies. CEVEC's amniocyte cell line is derived from amniotic fluid cells obtained by amniocentesis. The generation of stable

cell lines from amniocytes is fully documented, and an ethically approved and accepted procedure.

CAP (CEVEC's Amniocytes Production) cells are adapted to serum-free suspension medium, and allow rapid development of

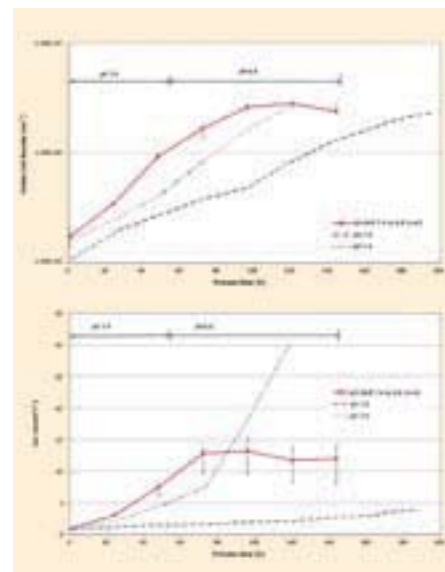


Fig. 2: Influence on pH and pH-shifting on cell growth and lactate production (in medium A)

stable cell lines with high product yields. Using CAP-cells for stable protein production or CAP-T cells for transient protein production is resulting in proteins with authentic human glycosylation.

To achieve high product yields of recombinant protein in stable cell lines, there is a need for increased growth rate and high productivity. Low levels of inhibitory metabolic products and sufficient substrate supply are known to lead to extended process runtime, increased product yields and optimised product quality.

A DASGIP parallel bioreactor system was used for the precisely-controlled parallel cultivation of four 1L vessels to investigate the influence of pH and other process parameters on the cell culture. The DASGIP system for cell culture is designed for easy but comprehensive parallel cultivation of human and animal cells, increasing process understanding and accelerating process development.

The CAP cells used in the experiments were cultivated at different temperatures, pH levels, DO levels and stirring rates.

For the parallel fermentations, exponentially growing cells were taken from the seed spinner at a cell density of  $1 \times 10^6$  to  $2 \times 10^6$  cells  $\text{mL}^{-1}$  to inoculate each DASGIP bioreactor using a working volume of 600mL with an initial cell concentration of

## TECHNOLOGY

$1 \times 10^5$  to  $2 \times 10^5$  cells  $\text{mL}^{-1}$  under standard conditions ( $T=37^\circ\text{C}$ ,  $\text{DO}=40\%$ ,  $N=120\text{rpm}$ ).

Growth rates, productivity and metabolic rates of the culture are mainly affected by the pH level. pH levels in the range of pH 6.8 to pH 7.4 were investigated. The longevity of the culture and total product concentration at pH 6.8 were 25% higher than at pH 7.4. In contrast, cell growth was enhanced at increased pH levels. Due to the decreased growth rate, the time-dependent volumetric productivity at low pH was reduced by 20%.

In addition to the differences in protein yields, the cell-specific metabolic rates significantly changed with the pH of the culture medium. Lactate production rate in particular fell with decreasing pH levels of the cultures.

To combine improved growth with higher productivity and decreased lactate production at different pH-conditions, the pH was shifted during fermentation and a biphasic culture process with a pH-shift during the exponential growth phase was developed.

### Influence of pH and pH-shifting on cell growth and production

Cells were either cultivated at constant pH or with a shift in pH from 7.4 to 6.8. Initial cell densities were  $1\text{-}2 \times 10^5$   $\text{mL}^{-1}$  and pH-shift was carried out at a viable cell density of  $1\text{-}1.5 \times 10^6$   $\text{mL}^{-1}$ . Cell densities, viability, glucose, lactate, glutamine and product concentration were monitored continuously. While longevity was prolonged at low pH levels, the growth rate decreased compared to the cultures started with pH 7.4. The shift in pH from 7.4 to 6.8 resulted in extended viabilities of over 90% for 2-3 days. In addition, shifting of pH resulted in steady lactate concentrations.

### Influence of pH-shift on cultures using different media

Cultures in two different serum-free, chemically defined media were started at pH 7.4 and a cell density of  $1\text{-}2 \times 10^5$   $\text{mL}^{-1}$ . The pH was shifted when cell densities reached  $2 \times 10^6$   $\text{mL}^{-1}$ . Product and metabolite concentrations were monitored continuously, along with cell densities and viabilities.

Medium B is beneficial compared to medium A, and resulted in prolonged culture times and improved lactate consumption even before depletion of glucose.

### Product quality

We also studied the effect of pH and pH-shift on product quality. The accompanying SDS-PAGE and western blots showed one distinct product band throughout the whole cultivation process.

For investigating the N-glycosylation and sialylation of the protein, we used glycosidases to study the carbohydrate groups attached to glycoproteins. PNGase F hydrolyzes nearly all types of N-glycan chains from glycopeptides, and neuraminidase catalyzes the hydrolysis of N-acetyl-neuraminic acid residues from glycoproteins.

Supernatant from cultures in both media were digested with PNGase F and neuraminidase. Subsequent western blot analysis revealed a clear shift in molecular weight, indicating fully N-glycosylation and sialylation of the product.

### Influence of pH-shift and medium on productivity

The effect of pH and pH-shift on production and time-dependent volumetric produc-

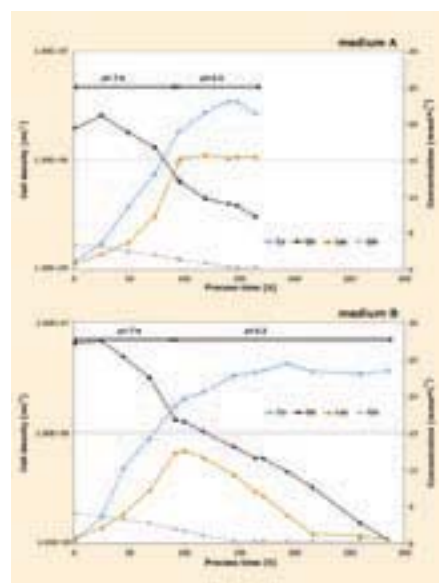


Fig. 3: Influence of pH-shift on cultures using different serum-free, chemically defined media

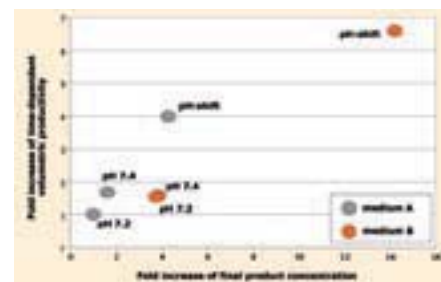


Fig. 4: Influence on pH-shift and medium on productivity and space-time yield

ductivity was evaluated by measuring the product concentration by ELISA (Enzyme linked Immunosorbent Assay). The specific and time-dependent volumetric productivity was calculated for each culture.

Based on the final product concentration and the time-dependent volumetric productivity in medium A at constant pH 7.2, it was possible to achieve a fourfold increase in the final product concentration and time-dependent volumetric productivity in the same medium.

By changing the medium and shifting the pH, a 6.5-fold increase in time-dependent volumetric productivity and a 14-fold increase in final product concentration were achieved.

### Conclusion

In order to evaluate physical parameters for batch cultivation of stable CAP cell lines, a biphasic batch process was established based on a pH-shift in the culture medium. This shift optimised metabolite consumption, cell density and viability and could be applied to different culture media. The biphasic culture process resulted in a significant increase in time-dependent volumetric productivity and final product concentrations of a highly complex and fully N-glycosylated and sialylated protein. ▼

#### Contact

Ruth Essers  
Senior scientist at CEVEC Pharmaceuticals GmbH  
essers@cevec-pharmaceuticals.com  
www.cevec-pharmaceuticals.com  
Claudia M. Huether  
Marketing and Communication at DASGIP AG  
c.huether@dasgip.de  
www.dasgip.com