

DENSETEC[®], WACKER'S HIGH-CELL-DENSITY FERMENTATION – OPTIMAL SPACE-TIME YIELDS COMBINED WITH HIGH REPRODUCIBILITY

Wacker Biotech offers its clients efficient and stable high-cell-density fermentation (HCDF) processes, which are individually developed and achieve optimal space-time yields. Our proprietary DENSETEC[®] HCDF technology enables us to produce recombinant products in smaller reactor volumes than are required for regular density fermentations. DENSETEC[®] reduces the fermentation, downstream processing and equipment investment costs to the benefit of our clients.

DENSETEC[®] - WACKER's High-Cell-Density Technology
DENSETEC[®]-based processes using E.coli strains have controlled specific growth rates with stringent, balanced feeding regimes. Product yields can exceed 10 g/l, because optical densities (OD600) of more than 100 have been achieved that correspond to over 50 g/l of E.coli cell dry weight. Such high product yields are not simply the result of producing more biomass, but of optimal expression and induction strategies.

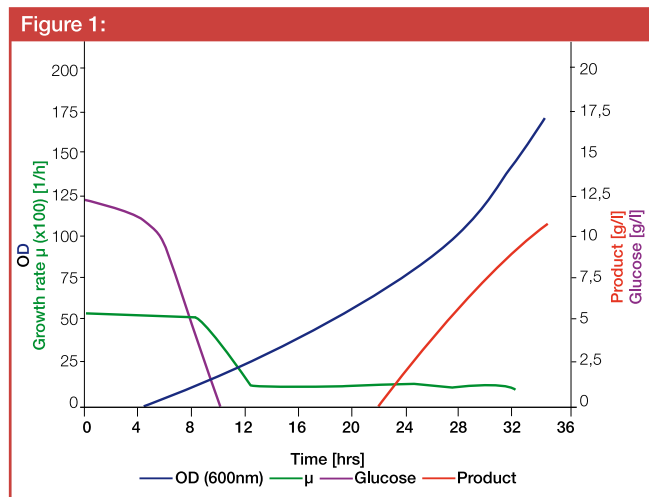


Figure 1: Typical DENSETEC[®] process with periplasmic expression of a therapeutic protein on a 300 l fermentation scale. The diagram shows the development of optical density (OD at 600 nm), growth rate μ , glucose concentration, and product content as a function of time.

Characteristics of DENSETEC[®]

A DENSETEC[®] process is characterized by a well-defined feeding strategy. Feeding strategies are based on knowledge of physiological strain parameters, growth kinetics, yield coefficients for various substrates, and product formation kinetics. DENSETEC[®] processes are characterized by:

- Chemically defined media.
- A batch phase with specific growth rate set to maximum up to a predefined time point.
- A fed batch phase in which the specific growth rate is controlled either a) by a predetermined feeding profile (open loop control) or b) by a feeding strategy dependent on the physiological state of the microbial culture (closed loop control).
- Expression of the recombinant gene at high bio mass concentrations and under optimized induction conditions depending on the respective expression system.

HCDF are stable, reproducible and efficient processes that reliably run without substrate limitations, growth inhibition, accumulation of metabolic byproducts or degradation of the recombinant product, because the kinetic parameters of the bacterial cell growth are taken into account and experimental data on fermentations are routinely analysed during process development (Fig. 2).

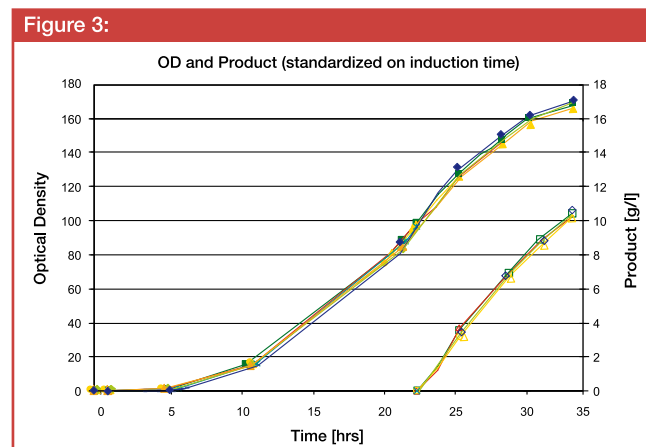


Figure 2: Graphs of the optical density (OD at 550 nm) and product concentration (g/l) as a function of fermentation time. By superimposing graphs from eight different runs, this diagram shows that DENSETEC[®] fermentations are reproducible.

Host/Vector Systems for DENSETEC®

A productive HCDF requires a well-regulated expression system that ensures consistent production on a commercial scale. We are ready to start with host/vector systems designed by our clients. We also can create customized expression plasmids for our clients at WACKER. These plasmids can encode:

- Different origins of replication that result in different copy numbers in order to fine-tune the expression level of the targeted product
- Promoter systems including the regulatory genes for chemical induction (e.g. IPTG) or for physiological induction (e.g. temperature, phosphate)
- A standard signal peptide either for secreting the product into the periplasm or into the culture broth if using an ESETEC® strain
- A standard selection marker (DENSETEC® can also be used without selection marker)

Various E. coli strains are suitable for DENSETEC® including standard expression strains, but also WACKER proprietary ESETEC® strains (see also data sheet: „ESETEC®, the Wacker Secretion System - Unique Protein Production using E. coli“).

Applicability of DENSETEC®

- When using DENSETEC®, target proteins can either be
- Soluble and located in the cell (cytoplasm or periplasm) or
 - Soluble and located in the culture broth, if secretion technology has been applied, or
 - Inclusion bodies.

What DENSETEC® offers

Our DENSETEC® technology provides our customers with well-regulated host/vector systems for efficient product expression. These systems are genetically stable during the HCDF process. We also offer efficient fermentation development based on Quality by Design (QbD) and Process Analytical Technology (PAT) principles including:

- Development of defined culture media and media optimization
- Fermentation optimization based on the physiological requirements of the microorganisms
- Optimization of product yields
- Process scales from 20 liters to 300 liters
- Manufacturing of client's product for clinical trials and for commercial production using cGMP-compliant DENSETEC®

Figure 3:



Figure 3: Production facility with 300 l fermenter at Wacker Biotech.

Availability

We offer our clients DENSETEC® high-cell-density fermentation to manufacture their product. Our stable HCDF processes boast optimal space-time yields and are cGMP-compliant. Your project will profit from our years of experience with optimized HCDF processes.

Please contact Wacker Biotech for more information:

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