Comparative Studies for High Cell Density Fermentation

Optimization of Processes Adaption for Contract Manufacturing

Application High Cell Density Fermentation

The Challenge

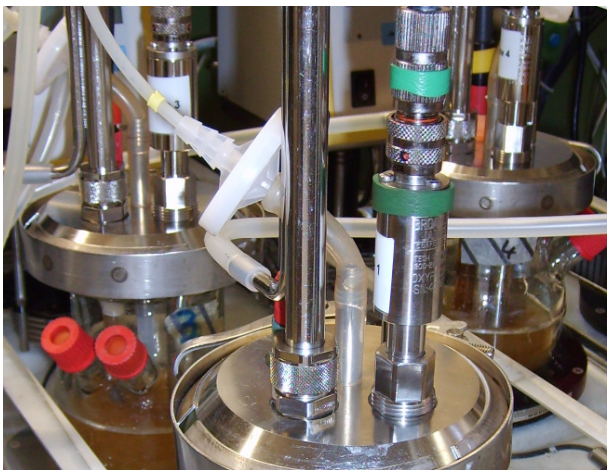
In contract manufacturing one of the most challenging needs is the adaption of proprietary production processes, their optimization as well as process improvements after a transfer from one Contract Manufacturing Organization (CMO) to another. Each single process modification has to be verified with comparable data.

The following application note gives an example for such an adapting procedure achieved by the use of the DASGIP Parallel Bioreactor System. The established process of one CMO included a high cell density fed batch process, controlled by the key process parameters pH, agitation, temperature and growth dependent glucose feeding. This process was adapted and optimised for the use with another CMO.

Biopharm GmbH, a GLP certified company offering Research and Development Services for CMO is using a derivative of the *E. coli* K-12 strain W3110 as their expression platform for prokaryotic production of recombinant, therapeutically proteins. The modified W3110BP strain is a property of Biopharm and is optimised for increased plasmid retention compared to the wild type *E. coli* W3110 as well as other conventional strains like BL21. Additionally, the Biopharm W3110BP strain is outstandingly capable of fermentation with high cell densities.

Goal

Aiming to increase efficiency parallel fermentation processes were used. The main item was the comparability of two or more parallel processes to show continuous process development by bridging results from one development cycle to another one.



The most important process parameter in the Biopharm's fermentation procedure is the dissolved oxygen (DO) since the DO level determines the setpoint from which additional feeding of the culture is needed.

Thus, the precise observation and control of the DO level is the crucial factor for efficient fermentation procedures. Scientists in the Biopharm laboratories are using the DASGIP Parallel Bioreactor System for their microbial small scale process development to get flexibility for their changing needs combined with highest precision and reliability.

Setting Up & Procedures

All experiments were carried out using a cytokine producing recombinant *E. coli* K-12 W3110BP in complex media supplemented with vitamins, trace elements and other additives.

Initial small scale experiments were done with the DASGIP Parallel Bioreactor System in 250 mL fermentation vessels which were afterwards replaced by 500 mL vessels to increase biomass production. All key process parameters like pH, agitation and temperature were controlled online as well as the critical DO. The online

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DO level was used as trigger for the automated activation of a glucose feeding profile.

To achieve comparative data from pilot scale fermentation, to proof the scalability properties of the DASGIP System additional cultivations were run in a 5 L glass reactor. Temperature, pH and DO were measured online whereas the agitation was manually adapted to the current DO levels. The used glucose feeding profile was similar to the profile in the 500 mL fermentation approaches.

The average cultivation time for all high cell density fermentations described in this application note was approximately 28 h.

Results

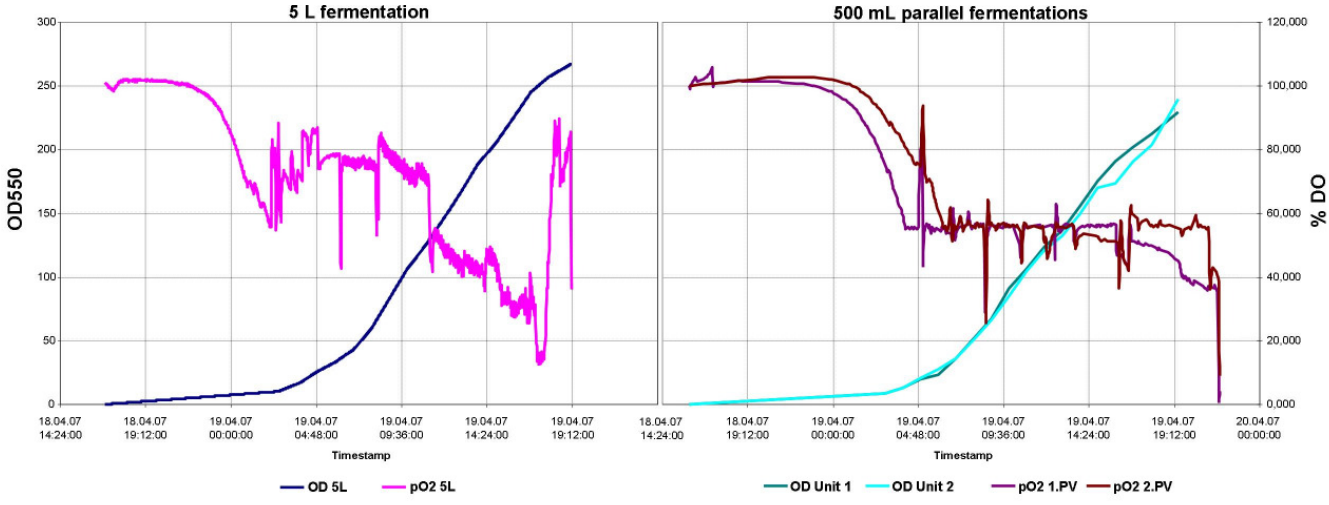
High cell density fermentation was performed successfully. As shown in the table all fermentation results were similar in regard to the final biomass production and product formation. The different working volumes of 500 mL in contrast to 5 L did not influence the course of the process, demonstrating the easy scalability of test results gained with the DASGIP Parallel Bioreactor System.

	Unit 1	Unit 2	5 L
Final optical density	230	240	268
Final Bio dry mass [g/L]	56	54	65
Final Bio wet mass [g/L]	260	242	275
Finall cell number [cells/mL]	9.0×10^{10}	7.0×10^{10}	8.8×10^{10}
Final product level [g/L]	2.8	3.0	2.5

Taking the online measured DO levels into account the vantages of an online controlled agitation as offered by the DASGIP System are displayed: Constant and precise DO control by automatically adapted variable stirring.

All recent improvements which were achieved for the described fermentation processes were successfully implemented into a large scale manufacturing process by a CMO (confidential data, not shown).

Two parallel fermentations in DASGIP units compared to a 5 L fermentation



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Benefits of a DASGIP

The parallel set up and control of independent fermentations guarantees an easy comparability of different approaches. "When comparing e.g. different bacterial host/plasmid combinations for protein production it is advantageous to use parallel approaches to avoid environmental influences. Thus, the experimental outcomes can be compared directly." points out Ute Ehringer, Head of Development at Biopharm.

The DASGIP Parallel Bioreactor System for microbiology was also used for several other projects at Biopharm to accelerate the process development. When searching for suitable host/plasmid combinations for new products, advanced fermentation processes could be established with short development cycles by the timesaving parallel fermentation approaches.

Structural Data

Ute Ehringer, operator of the DASGIP System:

Company	Biopharm GmbH; Heidelberg, Germany
Business function	Head of Development
Educational background	Biotechnologist
Bioreactor experience	For over 7 years

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Quality System certified by DQS ■ DIN EN ISO 9001 ■ Reg.-No. 63431