Cell-tainer BIOTECH BV

Application Note

Scalable fed-batch cultivation of *Escherichia coli* in the CELL-tainer CT20 and CT200

Introduction

Low k_La values in many rocking single-use bioreactors lead to restrictions in the application for microbial cultivations. Nevertheless, these bioreactors can offer some advantageous over stirred tank reactors, like low mechanical shear forces, in-bag scalability for seed train application and low foam formation. While many reports describe k_La values between 10 and 30 h^{-1} for one-dimensional rocking motion bioreactors [1], values of up to 600 h^{-1} were measured when air was used to sparge the headspace in the two-dimensional rocking bioreactor CELL-tainer CT20 at a working volume of 12 L [2]. It was investigated how suitable the CT20 is for cultivating *Escherichia coli* to elevated cell densities.

Results

In order to prove the suitability for aerobic microbial cultivation, *E. coli* BL21 gold (Agilent Technologies Inc, USA) was cultivated with the plasmid PET-21aBH2927, which codes for a maltogenic amylase. Mineral salt media was used to perform nutrient-limited fed-batch cultivation on a 12 L scale in the CT20. Growth was carried out at 37 °C and a pH of 7.0. The rocking rate was increased stepwise to prevent oxygen depletion. The inlet gas was blended by pure oxygen with a DO-controller at elevated cell densities. Finally, less than 5 L of pure oxygen (at 155 bar) was consumed. After 11 h, a nutrient-limiting fed-batch process (growth rate 0.18 h^{-1}) followed the batch phase with non-carbon limited growth. After 32 h, a maximum dry biomass concentration of 42.8 g L^{-1} was obtained prior to an induction phase for protein expression at 30 °C (Fig. 1) [2].

Scale up to 120 L

An important aspect of a scale-up process is compliance with the geometric dimensions, mass transfer coefficients and shear forces. The latter parameter is usually not considered in stirred bioreactors as the power input requirements to achieve a certain k_La does not allow to adjust shear forces. The scale-up approach of the CELL-tainer concept was based on maintaining the geometric characteristics (angle to plate width ratio) to keep the fluid flow properties and shear forces in a similar range. The bioreactor was scaled in three dimensions (width, length, and fluid level of the bag), which may alter the mass transfer properties, however, adjusting the angle to the bag length hardly changed mass transfer properties. When comparing the ratio of the Reynolds numbers on different scales, the modified Reynolds number for the description of fluid flow velocity characteristics in rocking bioreactors [3], increased by 20 % only between 12 and 120 L. Hence, very similar conditions are achievable.

In order to proof consistency of power input-related process parameters across scales, oxygen mass transfer and mixing times were determined in the CT200 and compared to the CT20 [2]. The similar results between both scales proves that the scale-up method was able to maintain the important process parameters (Tab. 1). To achieve a high gas mass transfer, vortex formation is, however, inevitable in both systems, the angle and rocking speed have to be adjusted to filling levels.

Tab. 1: Comparison of mixing times and kLa-values at 12 L scale in the CT20 and 120 L scale in the CT200 according to [2].

Parameter	*CT20*	*CT200*
min. mixing time [s]	30	55
max. k_La value [h^{-1}]	580	420

The concentration of dry biomass increased exponentially during the first hours of the

batch and subsequently linearly in the fed-batch phase to a final value of 45 g L^{-1} during the first 29 h of the *E. coli* cultivation in 120 L scale (Fig. 1). The development of the dry biomass was very similar to the smaller scale.

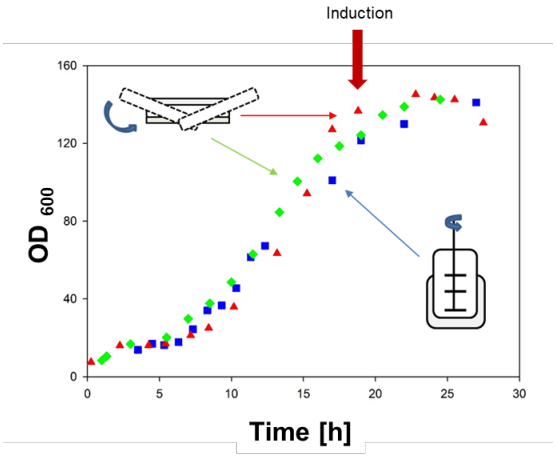

Fig.1: Comparison of growth in the 12 L E. coli CT20 cultivation (red triangles), in the 120 L CT200 cultivation (green diamonds) and a 2 L stirred tank cultivation in aerated fed-batch mode. The time of induction marks a shift in the temperature and feed rate.

E. coli accumulates acetic, succinic and formic acid, among others, whenever the oxygen supply inside the cell is insufficient during growth. In order to investigate whether oxygen limitation occurred during the cultivation, cell-free supernatant samples were analyzed with HPLC-RID for short-chain carboxylic acids. At the beginning of feeding, a slight increase in acetic, succinic and formic acid concentrations was observed with HPLC. Formic acid and succinic acid concentrations subsequently disappeared in the induction phase, most likely by gas stripping through aeration and uptake [2]. Any accumulation did not affect the process if compared to stirred tank cultivations (Fig. 1).

In 12 L cultivation, the biomass yield (g biomass per g glucose) during the growth phase reached average values of 0.45 to 0.5. The values are considered typical for a nutrient-limiting fed-batch process in *E. coli* processes compared to stirred tank reactors. Average values of Yx/s are very similar in both scales.

Summary and Outlook

Due to the larger k_La values in the CELL-tainer in comparison to other rocking-motion bioreactors, it is well-suited for microbial cultivation. As mostly air was used to provide cultures with oxygen, a four to five times higher k_La value can be achieved if pure oxygen would be used as many other studies with single-use bioreactors did. This would result in a concomitantly higher feed rate (growth rate) and the achievement of even higher biomass concentrations like in several other studies in single-use bioreactors.

Since no movable parts are used, the method is also suitable for extended cultivation times as e.g. in continuous processes. No foam formation was seen in comparison to sparged stirred tank cultivations, which allows a strong reduction of the use of antifoam. If combined with extension channels that allow an in-bag scalability (Fig. 2).

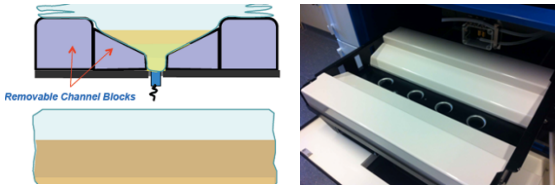

Fig.2: Removable extension channels allow an in-bag scalability down to 150 mL, while in the same bag up to 15 L or more can be cultivated without channels.

(Further information about methods and results is given in [2].)

Literature

1. Oosterhuis, N., S. Junne, and P. Neubauer, *Single-use bioreactors for microbial application.* New Biotechnology, 2014. **31**.
2. Junne, S., et al., *Cultivation of Cells and Microorganisms in Wave-Mixed Disposable Bag Bioreactors at Different Scales.* Chemie Ingenieur Technik, 2013. **85**(1-2): p. 57-66.
3. Eibl, R., S. Werner, and D. Eibl, *Bag bioreactor based on wave-induced motion: characteristics and applications.* Adv Biochem Eng Biotechnol, 2009. **115**: p. 55-87.