Single-use bioreactor for high density bacterial fermentation: evaluation of the CELL-tainer® bioreactor

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ABSTRACT

Single-use bioreactors have many advantages for R&D fermentation process development. Rapid system implementation, increased flexibility in size and turn-around, reduced infrastructure, reduced cross contamination risk and minimized maintenance requirement are main drivers. However, most of applications are restricted to animal cell culture, little if no equipments are available for high density bacterial fermentation as a result of oxygen and heat transfer limitations.

A new system, the CELL-tainer® bioreactor, has been evaluated within our standard recombinant *E.coli* fermentation process and achieving similar biomass and protein productions as in stainless steel reactor.

Due to its flexibility (1L to 10L volume, batch or fed- batch culture mode with on-line glucose measurement) this equipment is well adapted for R&D process development and could further pave the way for seed train and small scale cGMP production as required for early development clinical lot.

GOAL OF THE STUDY

Single-use bioreactor are well adapted for fast track supply of material for pre-clinical studies or early development cGMP lot production. However because of oxygen and heat transfer limitations these equipments are often not adapted to bacterial fermentation.

A new equipment, the CELL-tainer®, has been recently launched on the market by CELLON with claimed kLa above 300h^{-1}. The goal of the study was:

-to perform a recombinant *E.coli* batch fermentation in this bioreactor and compare the results to what is obtained in a conventional stainless steel fermentor (biomass reached and recombinant protein production).

-to assess the possibility to perform a fed batch culture in the CELL-tainer® bioreactor

- to measure the kla value in our operating conditions

METHODS

Strain
Recombinant *E.coli* BL21(DE3) overproducing target protein under the control of the T7 promoter was used as model.

Fermentation
The cultures are performed in:

-CELL-tainer® single-use bioreactor (with a 12L bag) characterized by a two-dimensional rocking motion (expressed as rpm) allowing improved gas-liquid mass transfer. DO regulation is based on a cascade with increased rpm and capability to inject pure oxygen instead of air. pH regulation is performed with ammoniac or phosphoric acid addition. Temperature is controlled within the chamber where the bag is incubated.

-Conventional 20L stirred tank fermentor. The DO regulation is obtained with a cascade of agitation, air flow and finally enrichment of air with oxygen. The pH correctors are ammoniac and phosphoric acid.

The set point for pO2 is 30% in both culture models. The culture is run at 37°C and pH 7. IPTG induction is performed at OD600nm between 25-35 and the culture is harvested 3 hours post induction.

kLa was measured in CELL-tainer® at different operating conditions for gas transfer performance assessment.
Preliminary batch cultures were performed in the CELL-tainer® to adjust parameters during the run according to pO_2 value (parameters assessed: rocking speed, angle setting, ratio of working volume vs disposable bag volume, pure oxygen addition).

A batch culture conducted with the optimized CELL-tainer® parameters was performed and compared with a batch culture within our standard stirred tank fermentor.

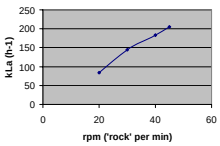
Glucose measurement is performed on-line (TRACE® analyser). This allows to follow-up glucose uptake rate and monitor fed batch culture with retro-control on the glucose pump such as maintain glucose level at predefined set point.
Cultures were regularly sampled to monitor biomass (OD at 600 nm and CDW (g/l)), glucose and acetate concentrations (Daytona analyser®) and protein production (SDS PAGE).

RESULTS

kLa measurement

kLa was measured in the CELL-tainer® increasing the rocking speed, the other parameters fixed at optimum operating setting. At 45 rpm (with the medium culture) a value of about 200 h^{-1} was obtained

Operating conditions	
Gas flow	2 vvm
Volume	7L
Antifoam	0,5mL/L
Temperature	37°C
Maximum angle setting	



Preliminary runs

Preliminary cultures in the CELL tainer® (results not shown) were run to optimize the parameters in order to maintain a pO2 value at least > 0% (set point 30%). The final settings are:
- the angle setting was fixed to the maximum
- the working volume was decreased from 10L to 7L
- the rocking speed was increased from 20 rpm to 45 rpm during the culture
- pure oxygen was added after rocking speed was at maximum rpm
- the air flow was fixed to 2 vvm

CONCLUSION

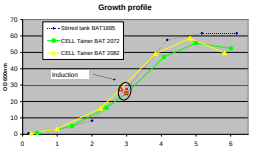
It's the first time efficient recombinant *E.coli* batch process has been successfully transferred in a single-use bioreactor.
-Comparable biomass profile and protein production, versus conventional stirred fermentor, can be achieved in a CELL-tainer® with a working volume of 7L (in a 12L bag) and optimized culture parameters.
-Fed batch culture can be operated under control of glucose TRACE® analyser and can sustain a biomass production as high as OD600nm=90, although additional optimization will be required to limit the acetate production.

The conclusion of this study is that the CELL-tainer® can advantageously replace the conventional stirred fermentor for process development, small scale batches as seed train, representative batch for preclinical study or cGMP lot for early phase clinical study.
Main advantages are the high turn-around, flexibility, no cleaning, lower maintenance and less safety risks (no steam).
However the scale-ability of the system is limited.

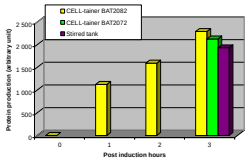
RESULTS

Batch fermentation

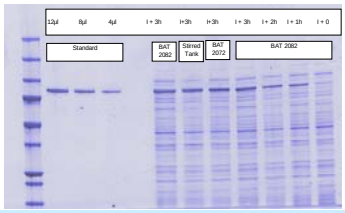
A culture with the optimum setting was performed in a batch mode and compared to the results obtained in our conventional stirred fermentor



The biomass achieved is similar to the stirred fermentor



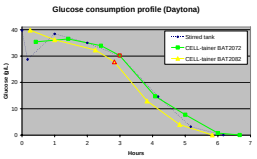
Protein production: SDS PAGE

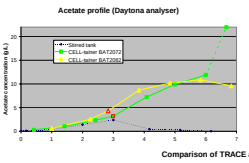


The protein production is reaching same high level as in the conventional fermentor.

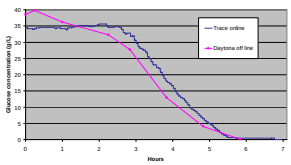
The pO_2 is > 0% however set-point (30%) can't be maintained all along the culture.

Comparative glucose profiles analysis show that the glucose uptake is comparable in both culture model. In contrast the acetate production is higher in the CELL-tainer® reactor. Further investigation is required for explaining this different behaviour.



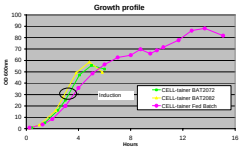


Moreover the glucose concentration profile obtained with the TRACE® system is comparable to the off-line measurement although all the values are higher. This is attributed to the glucose consumption in the 'off-line' sample before the sample is processed for analysis.



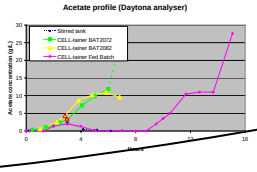
Fed batch fermentation

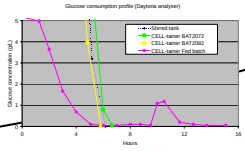
A fed-batch culture was developed with glucose addition and based on continuous residual glucose monitoring at target set point. When glucose concentration is below 1 g/L, feed pump is activated.



Although the conditions were not optimized a OD at 600 nm closed to 90 was achieved.

The glucose concentration is maintained below 1.5 g/L but the acetate concentration increases all along the culture until 28 g/L





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