Single-use bioreactors for bacterial fermentation : evaluation of different technologies for cultures of aerobic and anaerobic strains from 3L to 200L scale

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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 minimal maintenance requirement are the main drivers. However, most applications are restricted to cell culture, little if no equipments are available for high density bacterial fermentation due to oxygen and heat transfer limitations.

Here we describe the evaluation of different single-use bioreactors :

- for anaerobic strain : process was first evaluated at 3L scale in a Cell-ready bioreactor, showing same results as classical glass bioreactor. Then scale-up was successfully implemented at 200L scale in a STR 200 single-use bioreactor.

- For aerobic strain : The Cell-tainer bioreactor has been evaluated with our standard recombinant *E.coli* process achieving similar biomass and protein production as in stainless steel reactor.

Single-use bioreactors are well adapted for R&D. The main issues (oxygen and heat transfer) linked to aerobic strains such as *E.coli* were even overcome for the first time in a single-use equipment.

GOAL OF THE STUDY

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

Aerobic strains: 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 asses the possibility to perform a fed-batch culture in the CELL-tainer® bioreactor

-To measure the kLa value in our operating conditions

-Anaerobic strains: Most bioreactors available on the market should be suitable for culture of anaerobic bacteria which do not require high transfer rates. The goal of the study was :

-To assess the Cell-ready® from Millipore as an alternative to glass fermentor for early development work

-To perform a scale-up of our process in a STR-200® from Sartorius

MATERIAL & METHOD

Aerobic strain culture with CELL-tainer® single-use bioreactor

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

-Cultures were performed in the following equipments:

-**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 injection of pure oxygen instead of air. pH regulation is performed with ammoniac or phosphoric acid addition. The bag is incubated in the temperature controlled chamber of the equipement.

- **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).

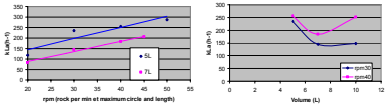
A batch culture conducted with the optimized CELL-tainer® parameters was performed and compared with a batch culture within our standard stirred tank fermentor.

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).

Set-up and preliminary runs with CELL-tainer® single-use bioreactor

kLa measurements

kLa was measured in the CELL-tainer® increasing the rocking speed, the other parameters fixed at optimum operating setting. At 45 rpm (with 7L of medium culture) a value of about 200 h^{-1} was obtained



kLa also depends on the working volume with higher kLa at low volumes.

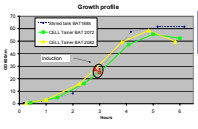
Preliminary runs

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**

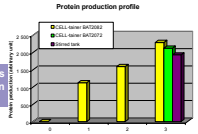
RESULTS

Batch fermentation

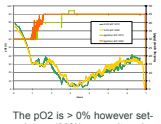
A culture with the optimum setting was performed in a batch mode and compared to the results obtained in our conventional stirred fermentor



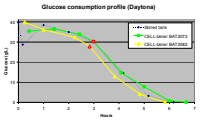
The biomass achieved is similar to the stirred fermentor

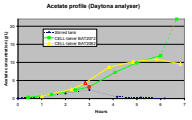


The protein production is reaching same high level as in the conventional fermentor.

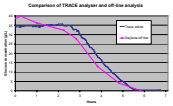


The pO2 is > 0% however set-point (30%) can't be maintained all along the culture.





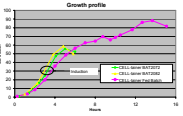
Moreover the glucose concentration profile obtained with the TRACE® system is comparable to the off-line measurement although all the values are higher. This is attributed to the glucose consumption in the 'off-line' sample before the sample is processed for analysis.

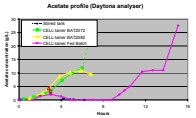


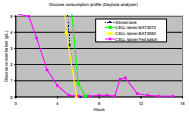
Comparative glucose profiles analysis show that the glucose uptake is comparable in both culture model. In contrast the acetate production is higher in the CELL-tainer® reactor. Further investigation is required for explaining this different behaviour.

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.



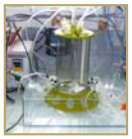




Although the conditions were not optimized a OD at 600 nm closed to 90 was achieved.

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

Anaerobic strain culture with Cell-ready® and STR-200® single-use bioreactors

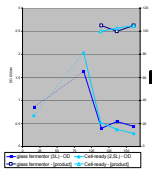


Cell-ready is a single-use 3L (2,5L working volume) fermentor, equiped with one marine impeller. pH and temperature are monitored with probes which are heat sterilized before introduction into the fermentor. A heating blanket system is wrapped around the fermentor to ensure temperature regulation at setpoint.
-Conventional glass reactor (3L working volume) equiped with one 45° three blade impeller.

STR-200 is a single use bioreactor with a working volume of 200L.. pH, temperature and DO sensors are included in the single-use bag (optical detection). Bags can be provided with Rushton or marine impeller. Temperature is controlled by a jacket which can ensure cooling if needed.

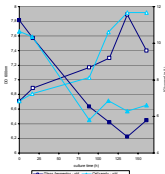
Batch cultures were performed with temperature regulation, and nitrogen flow rate in headspace to maintain an anaerobic environment. Nitrogen flow rate to culture volume ratio was kept constant for scale-up. Stirring speed was adjusted in order to have the same desorption of oxygen from the medium as at small scale.

The results obtained at 200L scale were predictive of the behaviour of the culture at 1000L scale (data not shown).

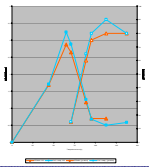


Growth and product production are similar in Cell-ready and glass fermentor

pH profile and glucose consumption are comparable in Cell-ready and glass fermentor

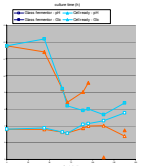


Cell-ready can be used as replacement of glass fermentor for anaerobic strain growth and production of product of interest



Growth and product production are very close between both models (bench scale: Cell-ready vs scale-up : STR200)

pH profile and glucose consumption are close



First results are very encouraging. Disposable technology such as STR200 can be used for rapid and easy scale-up of anaerobic processes from bench- to pilot-scale.

CONCLUSION

Aerobic bacterial culture : It is 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.
The system was even able to sustain a biomass production as high as OD_{600nm}=90, with a glucose feeding under control of glucose TRACE® analyser.
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.
However the scale-ability of the system is limited.

Anaerobic bacterial culture : as there is no oxygen transfer issue, and heat production is very low in our case, anaerobic bacterial culture is less challenging with respect to single-use technology use. Equipment conventionally proposed for cell-culture can rapidly be implemented. Cell-ready is easy to use for bench-scale early development work. In our case a scale-up was achieved up to 200L scale.

Overall, for both aerobic and anaerobic bacterial cultures, these studies demonstrate that comparable results can be achieved while combining advantages of single use equipments.
Still for aerobic cultures, scale-up is limited but based on this study can be opened for further developments.

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