CellMaker Application Note:

Culturing E.Coli using high optical density media in a Cellmaker

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Summary

In this application note, an *Escherichia coli* (*E. coli*) fermentation run was conducted using the CellMaker system. High cell density was achieved at 17hr as determined by an average optical density (OD600) measurement of 35.

Materials and methods

Cell line and culture media

A plasmid containing a His(6)-tagged protease was transformed into *E. coli* cell line BL21 (DE3) pLysS by heat shock method prior to being plated onto LB agar plates supplemented with 50 µg/mL carbenicillin. The plate was incubated at 37˚C degrees overnight.

Medium

The initial fermentation medium was prepared as follows: 350ml 10x phosphate/citric acid buffer [133g/L KH_2PO_4, 40g/L $(NH_4)_2HPO_4$, 17g/L citric acid] and 3.15L MilliQ water was autoclaved at 121 ˚C in 5L Duran bottle for 20 min. After the solution was cooled to room temperature, the following sterile components were added to make the complete fermentation medium: 148ml of 70% glucose solution, 8.4ml of 500g/L $MgSO_4$ solution, 0.8ml of 20g/L Thiamine solution, 1.75ml of 50 µg/mL carbenicillin and 35ml of 100x Trace metal solution [1]. The 100 X trace element solution contained: 10.0g/L Iron (III) citrate, 0.25g/L Cobalt (II) chloride, 1.50g/L Manganese (II) chloride, 0.15g/L Copper (II) chloride, 0.30g/L Boric acid, 0.25g/L Sodium molybdate, 1.30g/L Zinc acetate, 0.84g/L EDTA.

Bioreactor preparation

Prior to inoculation, the CellMaker bag was set up in a laminar hood. Bag was inserted into enclosure, connected to O_2 optical sensor and Electro Lab FerMac 280 Foam Control Module, and 3.5L media was pumped into the bag before being heated to 37˚C.

pH Calibration and Control

pH calibration was done outside the vessel using a two-point calibration in buffers pH 4 and 7. The pH sensor was calibrated prior to sterilisation. During the run, pH was automatically maintained at 6.8 with NH_4OH (Sigma).

Dissolved oxygen (DO) sensor calibration

pO2 sensors were calibrated using one-point calibration of 100% air obtained by running 4 SLPM air flow until the DO value stabilised at maximum (10min).

Antifoam System

Two probes are linked to an Electro Lab FerMac 280 Foam Control Module, which detects foam when an electrical connection is made between the two probes. This in turn then pumps in a small volume of 10% Antifoam C (Sigma) through one of the top luer locks on the CellMaker bag.

Inoculum preparation

A cell scraping was used to create a starter culture in 100ml LB supplemented with 50 µg/mL carbenicillin, which was incubated at 37˚C, 200 rpm shaking overnight.
A 20ml volume of the starter culture was used to inoculate each litre of citrate/phosphate media, which was supplemented with 50 µg/mL carbenicillin. Cells were subsequently grown at 37˚C degrees in either the CellMaker or 1L shaker flask. Shaker flasks were maintained at 200rpm.

Sampling and analysis

Samples were taken as required from 3-way tap using 10ml syringe. 10ml dead space volume was discarded and sample tested using OD600 on spectrometer (Eppendorf BioPhotometer).

Experiment 1

A 17hr procedure was set up to determine *E. coli* cell growth in the bioreactor using optical density. The machine was set up and the process started using the following process parameters:

Culture volume	3.5L
pH value	6.8
Temperature	37˚C
Dissolved oxygen s.p	40%
Air flow rate	4 SLPM
Max. O2 flow rate	1 SLPM
Start cell concentration	20ml/L
Growth time	17hrs

After 17hrs optical density and pH were recorded.

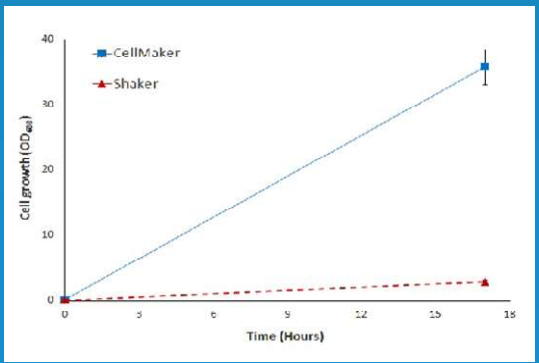

Figure 1
Results showed an average OD600 of 35 after 17hrs compared to 3 in the shaker flask (n=3).

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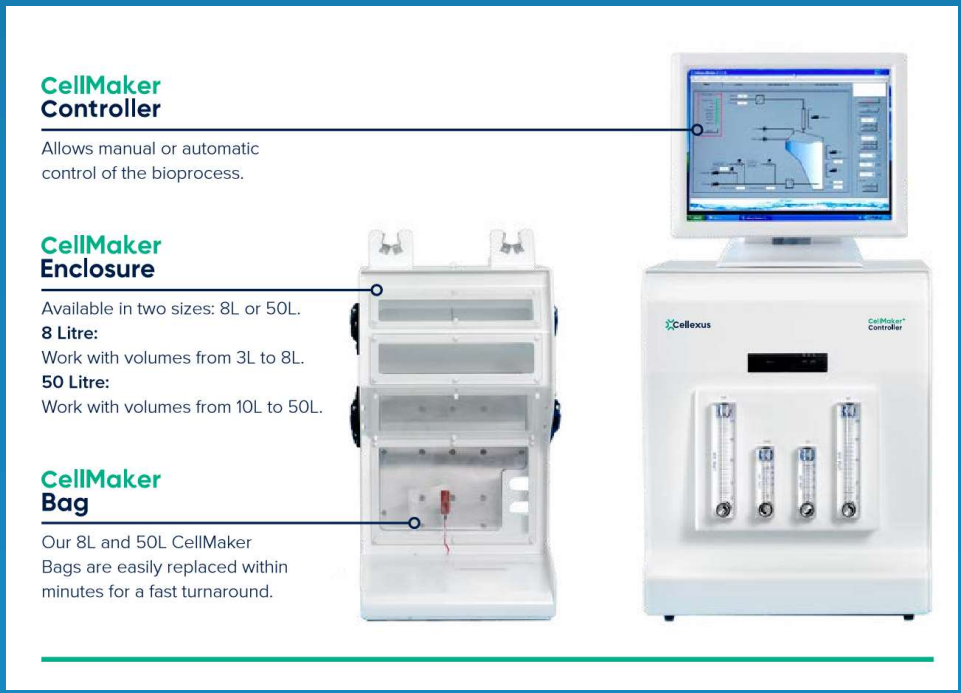
Conclusion

High density *E. coli* growth in the CellMaker was achieved using citrate/phosphate media. Three repeats of the experiment reached an average optical density of 35 after 17hrs compared to 3 in the shaker flask.

References

[1] Korz DJ, Rinas U, Hellmuth K, Sanders EA, Deckwer WD. Simple fedbatch technique for high cell-density cultivation of *Escherichia coli*. J. Biotechnol. 1995;39:59-65.



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