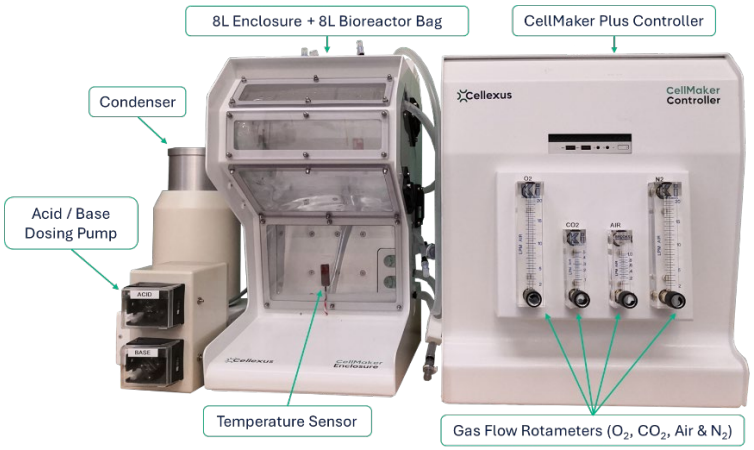
Quick Guide:

Recombinant Protein Production with CellMaker

Introduction

The CellMaker Plus bioreactor system is an easy-to-use, scalable solution for microbial cultures such as *E. coli*, offering flexible control of bioprocess parameters including temperature, dissolved oxygen (DO), pH, and pressure. The system employs sterile, single-use bioreactor bags with integrated ports for sampling, inoculation, and media filling.



Bioreactor Assembly

To preserve sterility of the bioreactor, remove the four components parts from the sterile packaging within a laminar flow hood using good aseptic technique. Within the flow hood connect the bioreactor bag gas exhaust line to the inlet of the Condenser Liner. Next, fit the capsule filters to the Condenser Liner exhaust line and the Gas Inlet Line to create a closed system.

Then install any immersion probes (e.g. pH) that are being used and the media fill/harvest tubing.

Once assembled the bioreactor can be removed from the flow hood/chamber and fitted into the CellMaker Enclosure.

Gas Connections

Gasses are supplied to the CellMaker Controller via standard 8 mm push-fit nylon gas lines from any supply source which has been regulated to a 2.5-5 bar pressure.

		Integrated compressor	Gas Inputs		
			Aux	2	3
CellMaker Regular	**Typical Gas**	Atmospheric air	O_2	N/A	N/A
	Control	Software Set-point (lpm)	Software Set-point (lpm)	N/A	N/A
CellMaker Plus	**Typical Gas**	Atmospheric air	O_2	N_2	CO_2
	Control	Software Set-point (lpm)	Software Set-point (lpm) & Automatic (DO%)	Manual Set-point (lpm)	Manual Set-point (lpm)

NB: Whilst the above indicates the typical gasses used for the Gas Inputs these can be interchanged and exchanged as required by the protocol being run. CellMaker is compatible with any non-corrosive gasses.

Air is supplied via an integrated compressor within the CellMaker Controller unit.

The gasses are blended within the CellMaker controller and supplied to the CellMaker Bioreactor Gas Inlet Line via a 0.22 µm capsule filter to maintain sterility of the culture.

The Bioreactor's exhaust gas passes through a cooled condenser unit to remove moisture and ultimately through a 0.22 µm capsule filter before venting to the environment, into a fume cabinet or extractor system. In the case of anaerobic experiments which are venting into an environment containing normal atmosphere it is recommended that a one-way valve be incorporated within the exhaust line to preventing atmospheric air from re-entering the system.

Cell Culture Procedure

Step 1 – Media Filling and Initial Setup

Fill the bioreactor bag with sterile media, ideally pre-heated to desired temperature, via the media fill/harvest tubing using a peristaltic pump. Set the desired temperature and gas flow rate. Press "Run" to initiate air supply and allow the system conditions to stabilise (~5-10minutes)

Step 2 – Dissolved Oxygen (DO) Probe Calibration

Once gas flow and internal pressure has stabilised (0.1 mbar fluctuation normal and expected), perform a 3-point DO calibration using the CellMaker software.

Step 3 – Culture

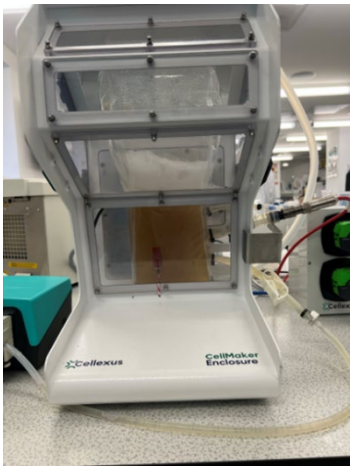
Prior to microbe inoculation add any supplementary nutrients or antibiotics via the sampling port. Inoculate the bioreactor with the desired microbe via the sampling port. The cells can then be left to cultivate at the defined parameters and their growth monitored via either real-time monitoring (Optura Spy) or off-line optical density (OD_{600}) measurements. Once the culture reaches the optimal point during log growth phase induce protein expression by injecting the required inducer via the sample port.

Case study Results Snapshot

An unoptimised comparative trial was conducted to evaluate the growth of *E.coli* and GFP expression using the CellMaker vs. traditional flask culture.

Table 1: Experimental parameters

	3L Flask	CellMaker (4L Bag)
Media	LB Broth	LB Broth
Media volume	1.5 L	1.5 L
Cells	E. coli	E.coli
Inoculum	6.00 x 10^9 cells	6.00 x 10^9 cells
Mixing	Shaker @ 150 rpm	Airlift @ 1 L/min
Temperature	37°C	37°C



E.coli Growth

It was observed that the growth rate in the shake flask was slower and plateaued sooner than in CellMaker suggesting metabolic burden from protein expression due to oxygen or nutrient limitations (Figure 1). Cultures in the CellMaker continued steadily growth indicating better oxygen transfer and nutrient mixing. The bioreactor maintained a consistently higher Final Yield (OD_{600}) after 10h.

This experiment revealed that the generation time of the E. coli grown in CellMaker was 43.3 minutes, compared to 52.4 minutes in the shake flask, enabling CellMaker to achieve a specific cell density the bioreactor would reach it 17.6% faster, equivalent to a 4.9 hour culture time compared to 6 hours in a flask, allowing earlier harvesting, reducing fermentation time and increasing throughput. After 6 hours, the CellMaker yielded, on average, **2.05x** more *E.coli* biomass than could be produced in the flask (Figure 2)

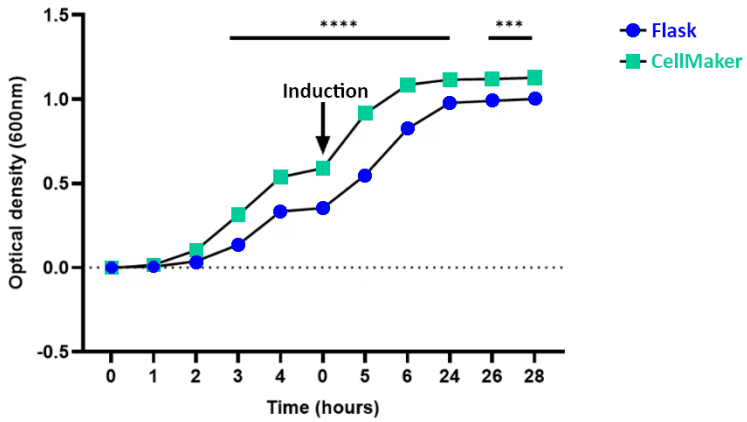


Figure 1: Cell Growth via OD_{600} readings within the CellMaker vs shake flask over 24h. Data represented as mean ±SEM (n=3), significant difference via Two-way ANOVA (****p≤0.0001), and Šídák's multiple

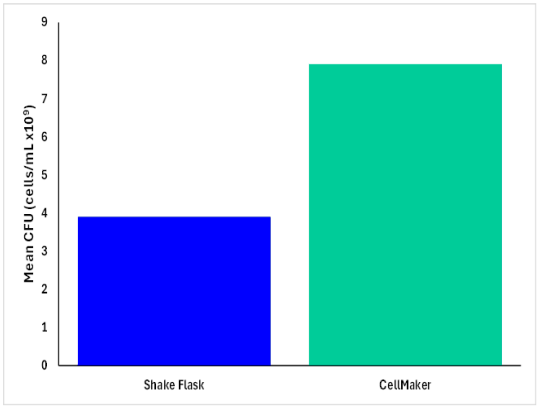


Figure 2: Mean CFU (cells/mL) comparison in shake flask vs CellMaker at 6hours.

Expression of Green Fluorescent Protein

Cultures were cultured for 5 hours prior to induction with arabinose with identical volume sample being removed for off-line fluorescence assay. Significantly higher fluorescence intensity was observed in the CellMaker culture compared to the flask culture possibly due to higher cell density therefore higher GFP expression. (Figure 3).

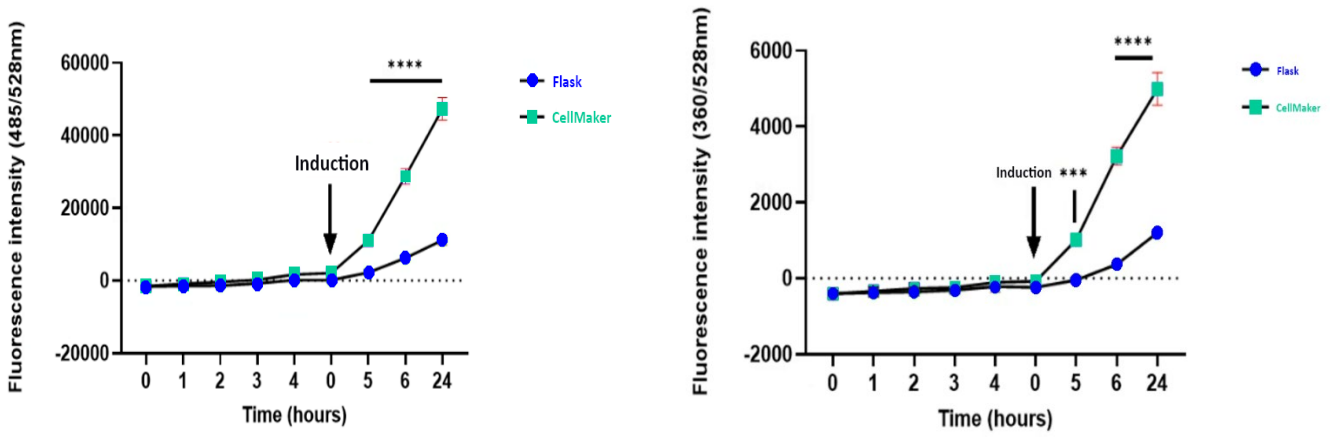


Figure 3: Fluorecence intensity measured from the CellMaker sampling and shaker flask throughout the 24h with the inducer at the basepoint between 4-5hours. Data presented as mean ±SEM (n=3), significant difference via Two-way ANOVA (****p≤0.0001), and Šídák's multiple comparisons tests compared to the traditional method.

Summary

The CellMaker system offers a highly effective and efficient platform to increase biomass production and recombinant protein production in *E. coli*, streamlining upstream processes with precise control of environmental conditions and simple setup. In this case study, the CellMaker demonstrated superior performance compared to traditional shake flask methods, producing significantly higher cell densities and protein expression which could have significant implications for poorly expressed proteins or where culture space is limited.

These results highlight CellMaker as a robust bioreactor for high-throughput protein production and process development, with the potential to increase yields, accelerate timelines and support scalable biomanufacturing.

Further work will be undertaken to optimise and further improve performance in this model, specifically, utilising CellMaker's ability to monitor and maintain dissolved oxygen to maximise available oxygen to cells during log growth and protein expression. Additional biophysical characterisation will be performed on the proteins produced – watch this space or contact us if you would like to explore what CellMaker could do for you.

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