Cellexus

Application Note:

Testing the suitability of the CellMaker system for the growth of brewers' yeast

Introduction

Working with the International Centre for Brewing and Distilling (ICBD), Heriot Watt University, Edinburgh and supported by the Scottish Funding Council Innovation Voucher Scheme administered by Interface, we tested our CellMaker bioreactor for applications within the brewing and yeast manufacturing industries to grow yeast cells.

Ale, *Saccharomyces cerevisiae* and lager, Saccharomyces pastorianus, yeast cells were targeted in an initial set of experiments, cell count and cell viability were determined.

The precise stages of yeast propagation vary. Figure 1 shows a summary of the first few stages of standard industry practice. The Cellexus CellMaker seeks to be a single-run solution that replaces these individual stages in order to allow faster propagation without contamination issues and offering both high cell count and cell viability.

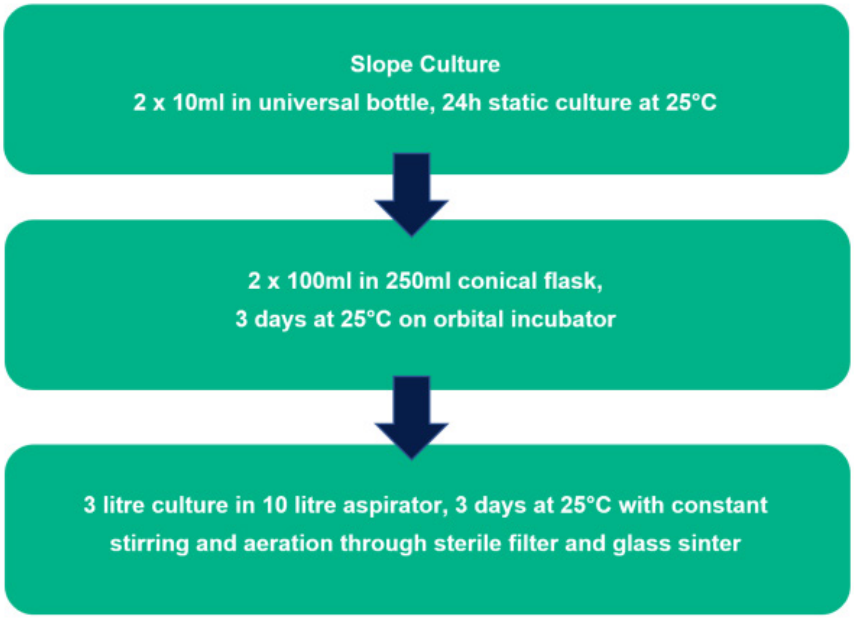

Figure 1

Flow diagram for laboratory propagation of brewing yeast – the stages prior to the 20 litre terminal culture; figure adapted from Boulton, C. and Quain, D. (2006). Brewing Yeast & Fermentation, Blackwell Science, Oxford.

Cellexus

Application Note:

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Experiment

The media used was YPD (2% and 4%). 2% w/v of agar was added to the slope used for preparing and storing yeast. All media was immediately sterilised after preparation, autoclaved at 121°C and 10 psi for 5 minutes. Anti-foam was added prior to autoclaving.

Yeast strains used were W34/70 a strain of *Saccharomyces pastorianus* typically associated with the production of lagers, and an ale strain (NCYC 1082) which is a strain of *Saccharomyces cerevisiae*. Cultures were grown on YPD agar slopes (incubated at 25°C for 48 hours), and stored in a refrigerator at 4 – 6°C until required.

A Cellexus CellMaker Plus system was set up with no in-line dissolved oxygen or pH sensors connected. The pre-sterilised CellMaker Bioreactor (8L), condenser bags and air filters (inlet and exhaust) were connected in a sterile environment. A luer lock tap connection was affixed to the upper of the side ports to enable samples to be taken without the risk of introducing sources of contamination. Media was added to the disposable, single-use bioreactor using an external peristaltic pump.

The experiments to monitor the growth performance of *S. pastorianus* were aerated at a rate of 5L/min during the day and 2L/min overnight. Experiments performed using *S. cerevisiae* were subject to a greater addition rate of antifoam and had a constant aeration rate (5L/min). The formation of foam was monitored and additional antifoam added where necessary (1 mL) using a sterile needle and syringe and injecting through one of the three ports located at the top of the CellMaker Bioreactor.

Yeast strains were suspended through the aseptic addition of 10 mL YPD to the vial containing the slope and yeast lawn. Gentle agitation of the vial removed the yeast from the slope, this cell suspension was added to the CellMaker Bioreactor using a sterile syringe and needle.

A sample of this suspension was taken to determine the cell count of the inoculum and the viability of the population. Cell viability were assessed using methylene blue dye; a viable population is of those cells capable of excluding the dye. An Aber Instruments make Countstar system was used for performing cell counts and determination of cell viability.

Discussion

A) Increasing the media components appeared to aid lager yeast cell growth and higher level of cell viability (Figure 2). After 48 hours of growth the total cell count increased by over 16,000 times and viability was >96%. The volume of media was up to 8L (the maximum volume), with the result that the initial cell concentration was lower on a cell/ml basis.

Cellexus

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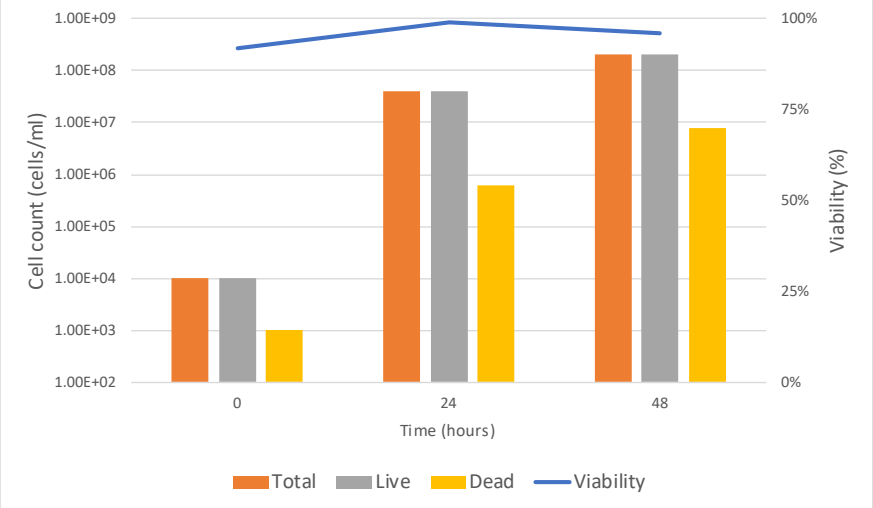

Figure 2

Growth data, 4% (w/v) YPD (*Saccharomcyes pastorianus*)

B) The next experiment used strain NCYC 1082. In this first ale experiment (Figure 3) it was found that after 44 hours the cell growth appeared to represent a 20,000 fold increase in cell number. The viability was >95% after 24 hours and increased further. This experiment was repeated with the media volume increased to 8L.

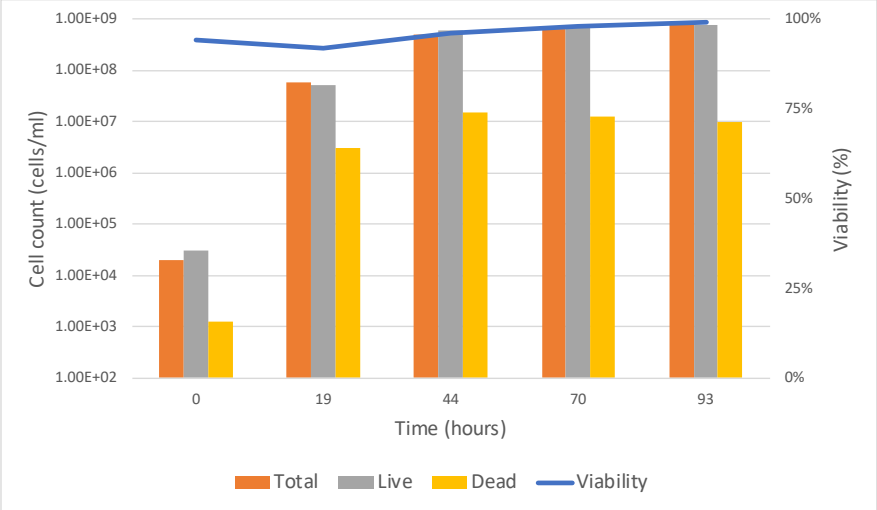

Figure 3

Growth data, 4% (w/v) YPD (*Saccharomyces cerevisiae*)

Cellexus

Application Note:

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C) Data summarised in Figures 4 and 5, for total cell count and cell viability, demonstrates that both ale and lager yeast cells grew well in the CellMaker. Further experiments are planned to understand the yields.

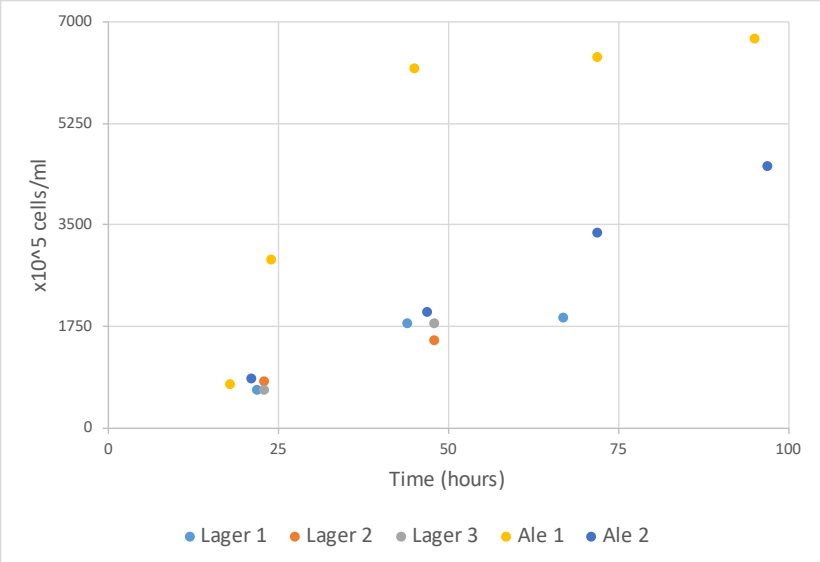

Figure 4

Summary of mean total cell count data

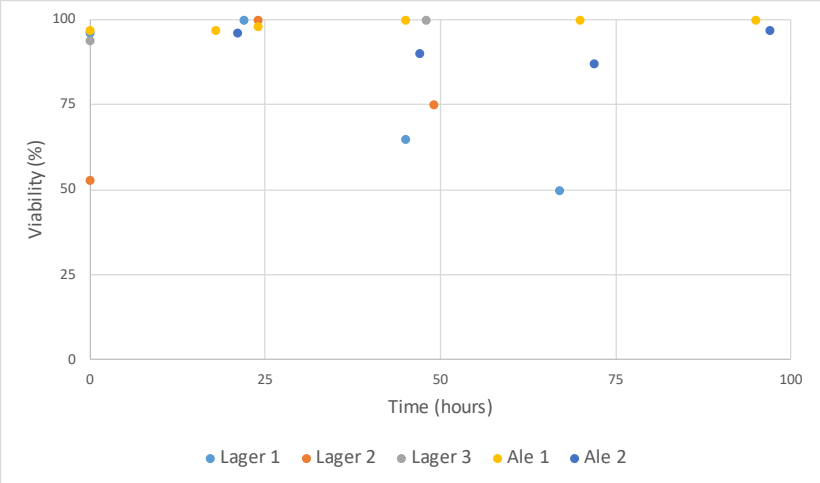

Figure 5

Summary of mean viability data

Cellexus

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Conclusion

The CellMaker was able to successfully grow the yeast cells. The CellMaker can perform the majority of individual laboratory steps in a single run and was proven to be effective to growing yeast cells rapidly. There was an encouraging increase in cell count. Both Ale and Lager yeast grew and replicated well. The work occurred without any noticeable issues of contamination with cells appearing very healthy.

References

Boulton, C. and Quain, D. (2006). Brewing Yeast & Fermentation, Blackwell Science, Oxford.

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