

Development of mammalian cell culture bioreactor processes – Increasing productivity and process robustness

Jez Wayte - Lonza Biologics plc

Abstract

Increasing demand for biopharmaceuticals from mammalian cell culture coupled with capacity limitations necessitates improvement in process productivity and robustness. A strategy for the development of a high productivity animal raw material-free bioreactor process is presented. The strategy focuses on the elimination of animal derived raw materials as a means of improving process robustness in conjunction with the development of an improved fed-batch process that exhibits increased productivity.

Process development aims and strategies

The aims of process development are two-fold

1. Maximize process productivity
2. Improve process robustness

Strategies for maximizing process productivity focus on two key aspects

1. Creation and selection of highly expressing cell lines through use of an efficient expression system
2. Development of improved bioreactor processes through control of the physicochemical and nutritional environment

Improved process robustness necessitates

1. Elimination of potential sources of variability e.g. ill-defined raw materials
2. Maintenance of precise process control

Cell line selection and adaptation to protein-free culture
GS expression system facilitates rapid creation of highly productive NSO and CHO cell lines satisfying the first requirement for process development

- High specific production rate coupled with good growth characteristics
- Ease of selection using glutamine-free medium
- No requirement for amplification for NSO cell lines

Model cell line: GS-NSO transfected with GS linked genes for a B72.3 IgG₄ antibody against TAG-72 (Bebbington *et al*, 1992)

Adaptation of the cell line to growth in chemically defined protein-free medium, using a programmed adaptation procedure, improves process robustness and consistency

- Elimination of effects of lot to lot variability of ill-defined raw materials
- Improves purity prior to purification
- Satisfies regulatory issues

Adaptation can result in a loss in productivity. Losses are recoverable through development of the fermentation process.

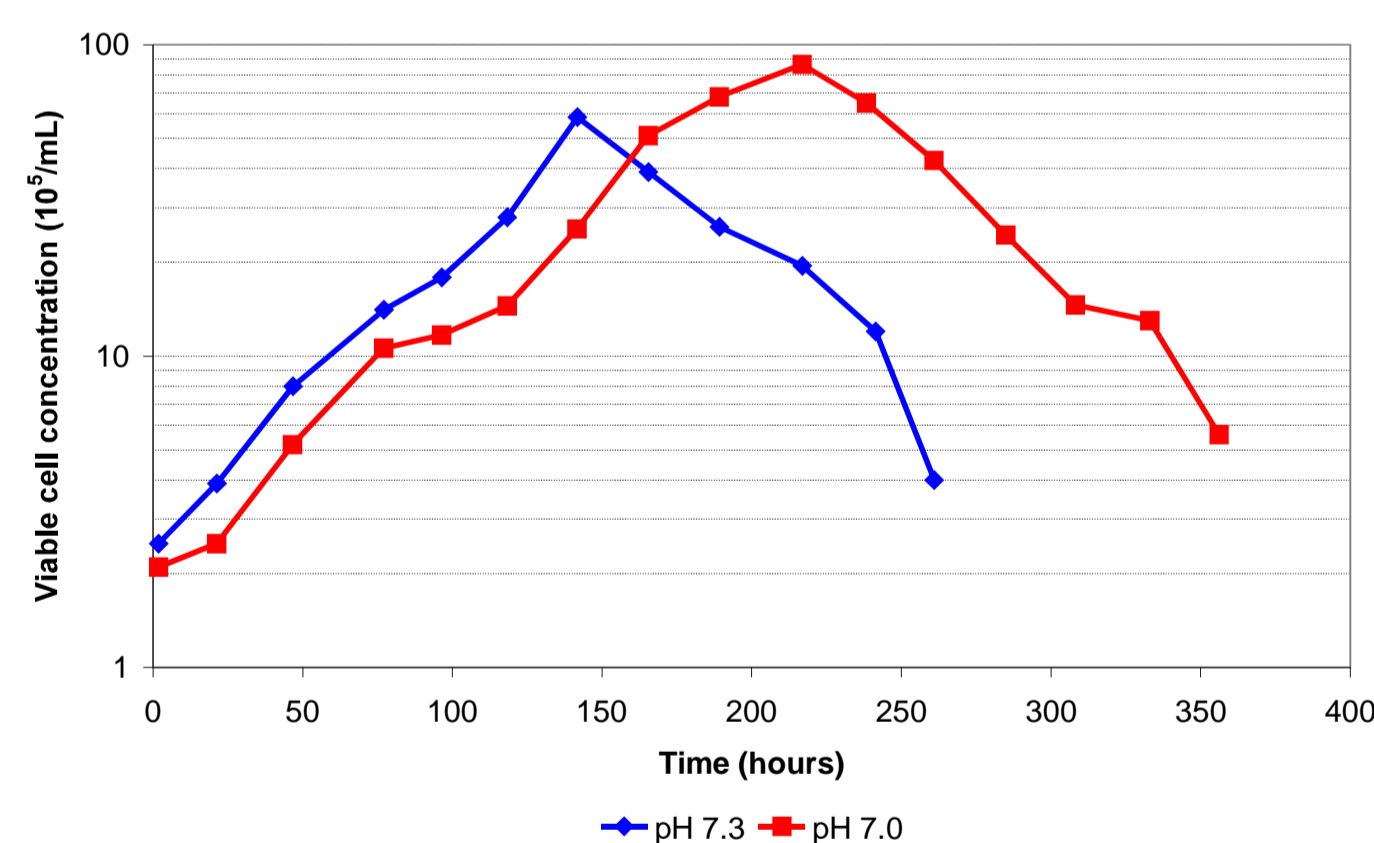
Improving the physicochemical environment

Culture pH, temperature and dissolved oxygen tension are controlled in bioreactor processes. Precise control to defined setpoints is critical to achieving robust and consistent process performance.

Culture pH in particular can have a dramatic effect on cell growth and productivity. Responses are cell line specific.

- Changes in maximum viable cell concentration
- Changes in integral viable cells
- Changes in specific production rate
- Changes in nutrient utilization and catabolite accumulation

Example: Model cell line cultured in laboratory-scale bioreactors in chemically defined protein-free medium maintained at pH 7.3 and pH 7.0



Increased maximum viable cell concentration and culture duration resulting in increased integral viable cells

1050 x 10⁹ cell h/L at pH 7.0 compared with 548 x 10⁹ cell h/L at pH 7.3

Increased specific production rate

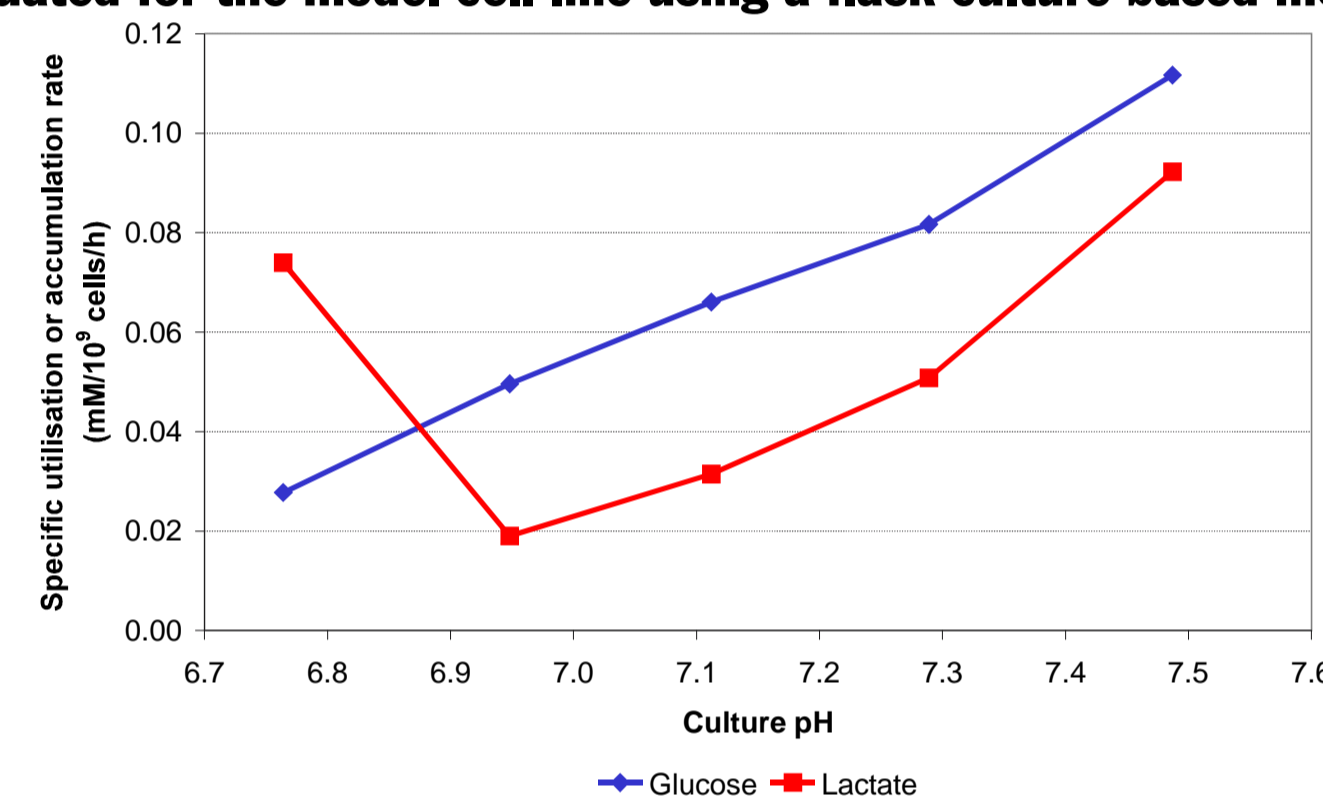
0.59 mg/10⁹ cells/h at pH 7.0 compared with 0.47 mg/10⁹ cells/h at pH 7.3

Increased productivity through combination of increased integral viable cells and increased specific production rate

590 mg/L at pH 7.0 compared with 240 mg/L at pH 7.3

Effect of culture pH on metabolism

Evaluated for the model cell line using a flask culture based methodology



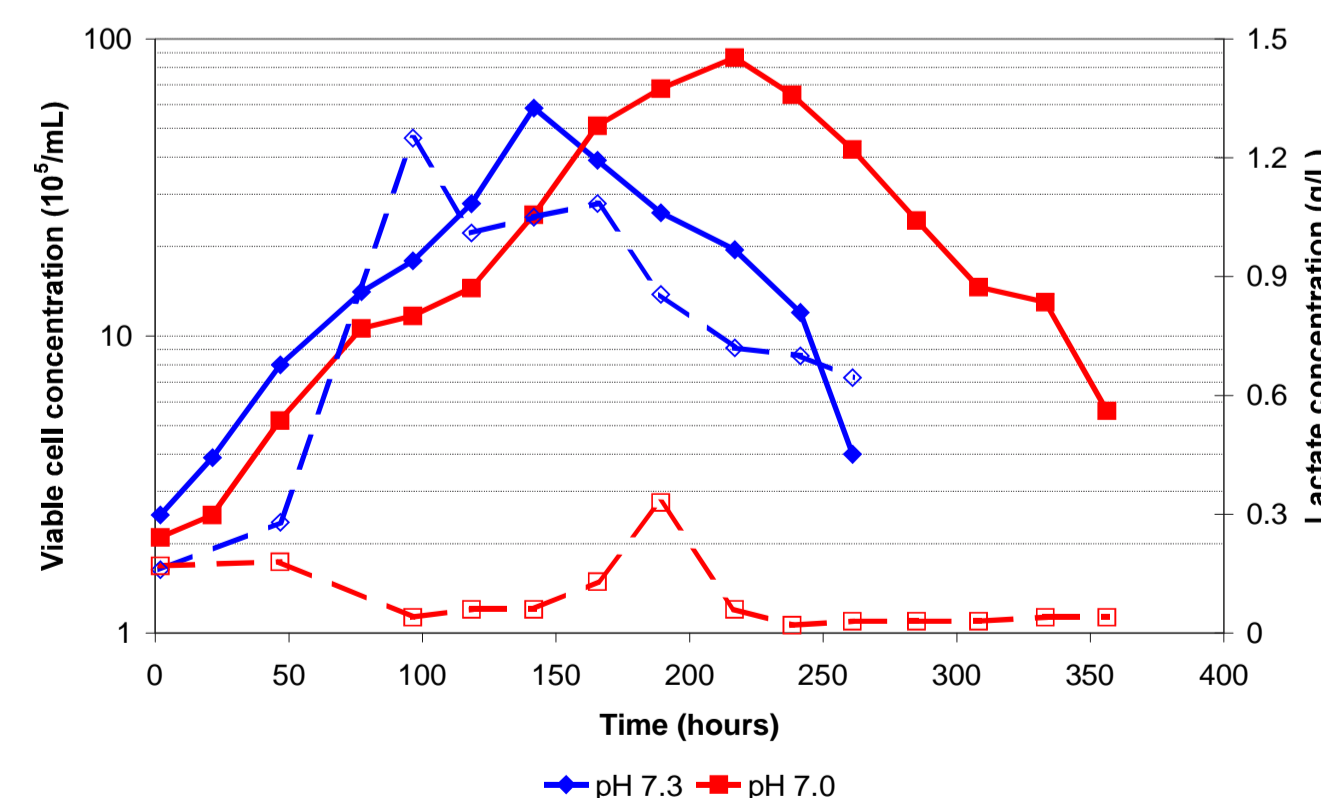
Reduction of culture pH generally results in reduced specific glucose utilization and lactate accumulation rates for the model cell line when maintained within the permissible range for cell growth.

A lower specific glucose utilization rate is beneficial as it reduces the quantity of glucose to be added as part of the fed-batch process, simplifying feed formulation and addition.

A lower specific lactate accumulation rate reduces the overall lactate concentration and thereby limits the amount of alkali required to control culture pH, simplifying process operation and improving process robustness.

Effects observed in flask culture were translated into bioreactor process performance benefits

- Maximum lactate concentration reduced from 1.25 g/L for a culture maintained at pH 7.3 to 0.33 g/L at pH 7.0
- Utilization of lactate characteristic of GS-NSO processes



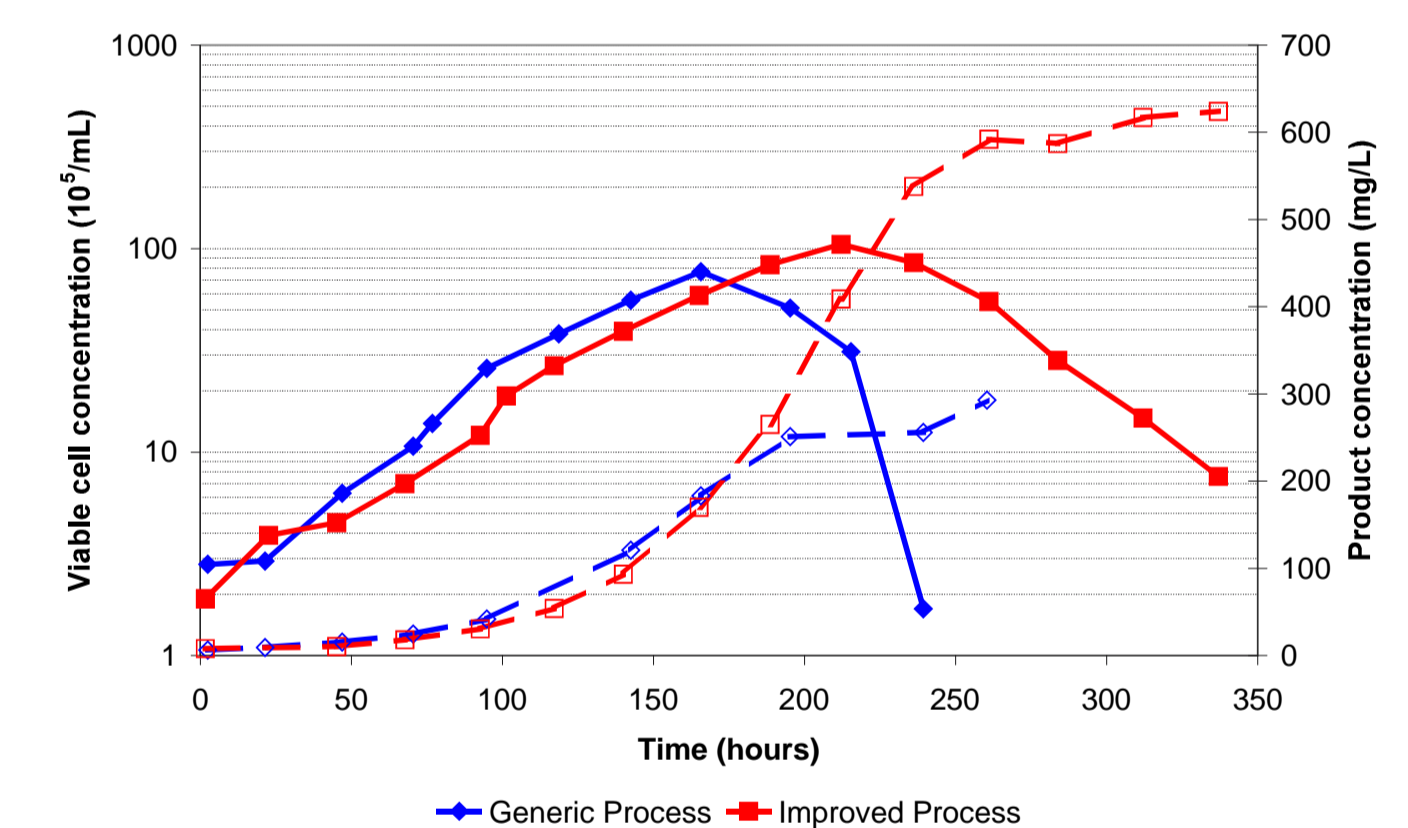
Maintenance of the nutritional environment

Processes are typically operated in fed-batch mode. Nutrient supplementation results in higher productivity than achieved in batch culture.

Development of improved basal medium, feed formulations and feed addition strategies improves process performance

- Changes in maximum viable cell concentration
- Changes in integral viable cells
- Changes in specific production rate

Combination of improved physicochemical environment and development of strategies to maintain the nutritional environment results in significantly improved process performance



Increased maximum viable cell concentration and culture duration resulting in increased integral viable cells

1280 x 10⁹ cell h/L for the improved process compared with 737 x 10⁹ cell h/L for the generic process

Increased specific production rate

0.50 mg/10⁹ cells/h for the improved process compared with 0.37 mg/10⁹ cells/h for the generic process

Increased productivity through combination of increased integral viable cells and increased specific production rate

620 mg/L for the improved process compared with 293 mg/L for the generic process

Summary

1. Increased productivity from mammalian cell culture requires a combination of highly productive cell lines and improved fermentation processes
2. Selection of an efficient expression system, e.g. GS, is essential for the creation of highly productive cell lines
3. Fermentation process improvements can be achieved through modification of the physicochemical environment and maintenance of the nutritional environment
4. Culture pH can have a significant effect on process performance and process robustness
5. Development of chemically defined protein-free medium formulations improves process robustness

Lonza group Correspondence Address

Jez Wayte, Lonza Biologics plc, 228 Bath Road, Slough, Berkshire, SL1 4DY
Tel: +44 (1753) 777000
Fax: +44 (1753) 777001
E-mail: jwayte@lonza.co.uk
Website: www.lonzabiologics.com

References

Bebbington, C.R., Renner, G., Thomson, S., King, D., Abrams, D. & Yarrington, G.T. (1992) *Biotechnology* 10, 169
'High-level expression of a recombinant antibody from myeloma cells using a glutamine synthetase gene as an amplifiable selectable marker'