

Improving GS-CHO Process Performance and Process Robustness **Lonza**

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ABSTRACT

Fed batch cultivation of mammalian cells is widely utilised for the production of monoclonal antibodies. At Lonza such fed batch processes are used for cGMP production at scales up to 20,000L. In order to meet ever increasing productivity and economic targets, these processes inevitably have to operate at very high cell concentrations, in some cases in excess of 20×10^6 cells/ml. Improving control of process parameters such as pH, osmolality, dissolved oxygen, feeding strategy, all of which are tightly inter-linked, together with feed composition, become vital for improving bioreactor performance and process robustness.

Factors considered here are feed composition, feed strategy and optimizing pH.

Where highly concentrated complex nutrient feeds are used, problems can arise with the solubility and the chemical compatibility of the feed components. Also the rate of feeding is highly important. In addition to the obvious nutrient depletion resulting from insufficient feeding, excessive feeding of amino acids may result in an increase of osmolality sufficient to inhibit cell growth. These problems have been solved by reformulating the nutrient feeds and adjusting feed rates prescribed by cell concentration and a pre-determined knowledge of metabolic rates.

Carbon metabolism (but not, to a significant degree, amino acid metabolism) was found to be highly dependant on culture pH. Operating at high pH resulted in rapid cell growth but inefficient carbon metabolism with consequent high lactate accumulation. Lower pH inhibited cell growth but resulted in a lower specific lactate production rate. The best operating pH with low lactate accumulation and acceptable cell growth was found to be around 6.80.

Implementing these combined improvements led to an extension of culture duration of a GS-CHO model cell line from 15 to 23 days and an increase of harvest titre from 3.1 g/L to 6.5 g/L.

INTRODUCTION

Fed batch mammalian cell culture is a widely successful technique for recombinant protein production and monoclonal antibody production in particular. These cultures can reach cell concentrations in excess of 20×10^6 cells/mL and production concentration exceeding 5 g/L in a 15 day culture. Increasing product concentrations (without re-engineering the cell line) would then simply be a matter of increasing cell densities and/or extending culture duration. What sets the limit to this, however, is the build up of growth inhibiting metabolites and addition of potentially harmful excess feed components. Often at the end of a culture there will be high lactate and ammonium concentrations and high levels of sodium imparted by the alkali used to solubilise the nutrient feeds and alkali that is added to control pH. These chemicals all affect osmolality and pH, which in turn affect pCO_2 levels (where it is used to control pH in a bicarbonate system). Osmolalities and pCO_2 levels above a certain value together with other toxic metabolites, will lead to inhibited cell growth and ultimately premature cell death. Extending culture duration would then inevitably mainly mean preventing this from happening.

Other critical aspects in industrial cell cultures, as it is in any type of production process, are reproducibility and control of process parameters. In this case that means maintaining a favourable culture environment as tightly and for as long as possible as mammalian cells in particular are very sensitive to changes in e.g. osmolality and oxygen supply.

METHODS

Cell culture:

A CHOK1SV-derived cell line expressing the chimeric antibody cB72.3 antibody using the Glutamine Synthetase (GS) Gene Expression System (Lonza, UK) expression system was cultivated in batch and fed batch mode in 10 litre airlift bioreactors using a chemically defined animal component-free medium CD-CHO (Invitrogen) and Lonza proprietary feeds.

Shaken batch cultures in 500 mL Erlenmeyer flasks were performed using CD-CHO medium.

Protein Concentration Assay:

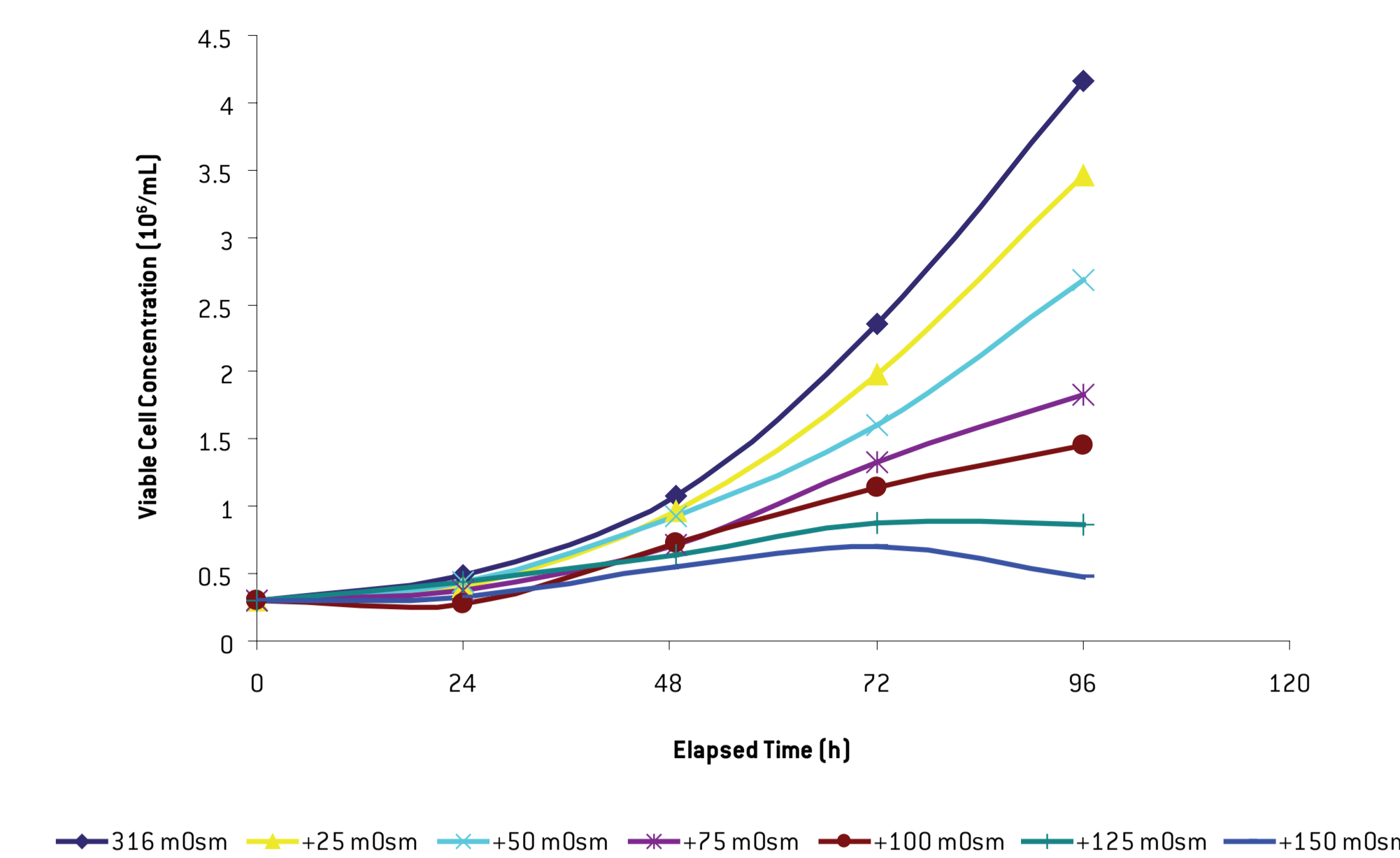
HPLC Protein A.

EFFECT OF INCREASED AMINO ACID CONCENTRATIONS ON OSMOLALITY AND CELL GROWTH.

It had previously been noted that, if a fed batch culture was accidentally overfed with nutrients, there was an increase in osmolality which was suspected to affect cell growth. To confirm these observations batch cultures in shake flasks were set up with media containing added amounts of an amino acid mix with proportions similar to that of a typical nutrient feed (and based on previous measurements of cellular uptake rates).

Cultures were set up with increased osmolalities up to ~ 470 mOsm/kg (normal medium without the amino acid supplement = 310 – 320 mOsm/Kg). Results show that even slight increases in osmolality from increased amino acid concentrations gave a significant reduction in growth rate (Figure 1). It is therefore very important that the feed rates in a fed batch culture are linked to amino acid consumption rates to avoid any unnecessary increase in osmolality that would inhibit cell growth. This can be achieved simply and reliably by adjusting feed rates based on cell concentrations and a pre-determined knowledge of amino acid consumption rates, obviating the need for complex on-line or off-line amino acid measurements.

Figure 1: Increases in osmolality through increased amino acid concentrations inhibit growth of the investigated GS-CHO cell line.



pH CONTROL AND OPTIMISATION

Standard practice in the biopharmaceutical industry is to control pH using a sodium bicarbonate - carbon dioxide system. In aqueous solutions bicarbonate forms an equilibrium with carbon dioxide following below equation:



Shifting the equilibrium by increasing CO_2 levels will thus make the solution more acid and vice versa. This however becomes problematic when metabolic byproducts such as lactate and ammonia form. As acid catabolites accumulate, more sodium bicarbonate will be added to maintain pH, which in turn raises osmolality. Later in the culture lactate can be taken up and metabolised by the cells; with the higher bicarbonate concentration and the acidic compound being stripped from the culture, higher addition of carbon dioxide will be required to maintain pH. The high partial carbon dioxide pressure may then reach toxic levels, or affect product quality. Thus reducing lactate (and ammonia, which derives from the use of amino acids as energy substrates) accumulation becomes vital for good pH control and process robustness.

One strategy to inhibit lactate metabolism is to operate at low culture pH; however lowering pH too much below physiological levels will inhibit cell growth. To find a suitable pH level, batch cultures in 10 litre bioreactors were set up with pH control setpoints ranging from 6.7 to 7.25. The results indicate that lowering pH to 6.8 will give a substantial reduction in lactate production rates yet allow a sustained acceptable growth rate. Also importantly it was found that lowering pH to 6.8 did not have any detrimental effect on specific rate of product accumulation or amino acid metabolism (Figures 2-3). Running fed batch cultures at a constant pH of 6.8 compared to 7.0 with a drift to 6.8 resulted in a significant reduction in lactate levels (Figure 5).

Figure 2: Lactate production rates of GS-CHO cells is reduced with decreasing pH but with maintained or higher production rates. Growth rates are slowed down with decreasing pH. Cells cultured at pH 6.80 were shown to have low lactate production rate with a sustained acceptable growth rate.

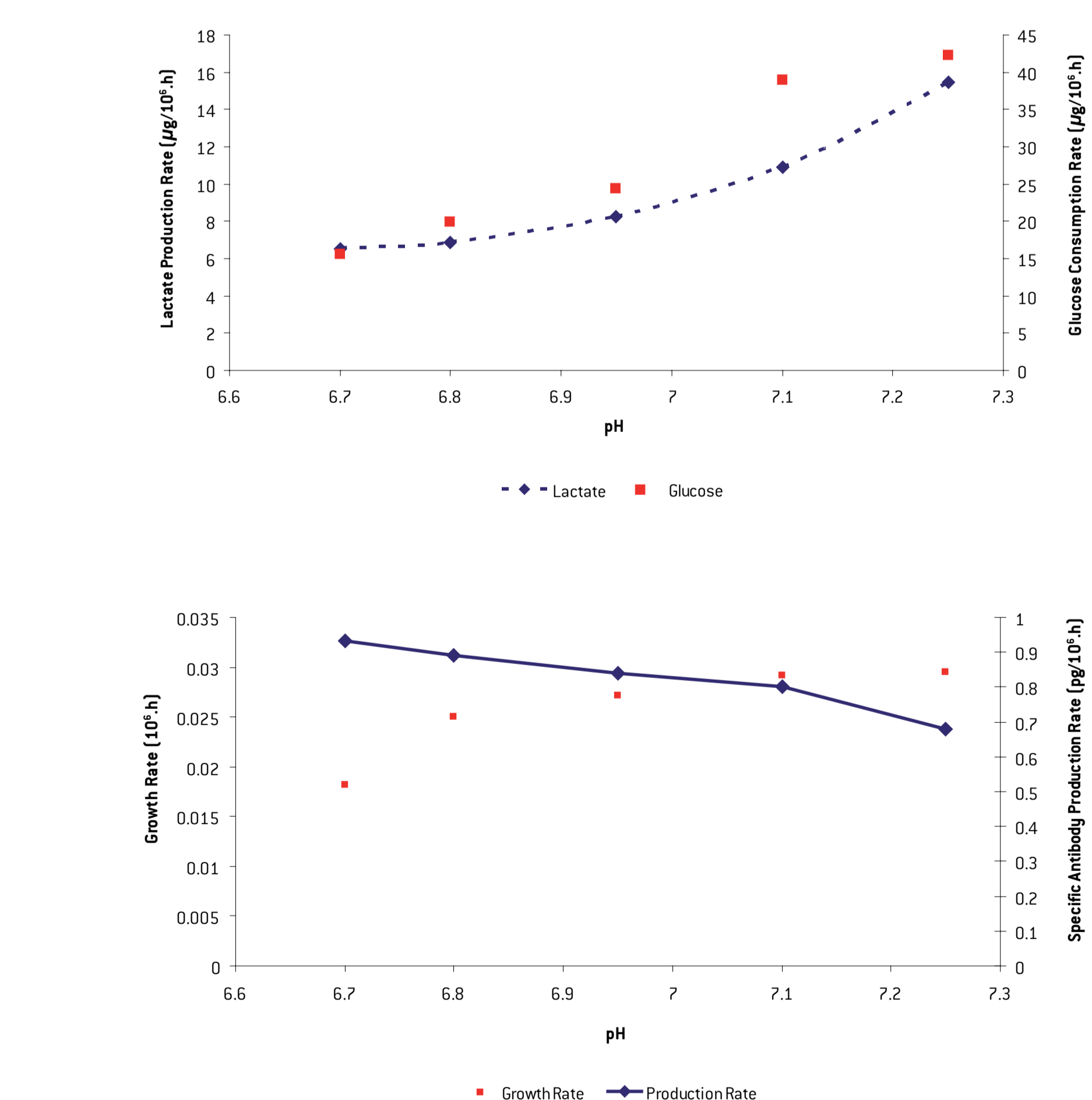
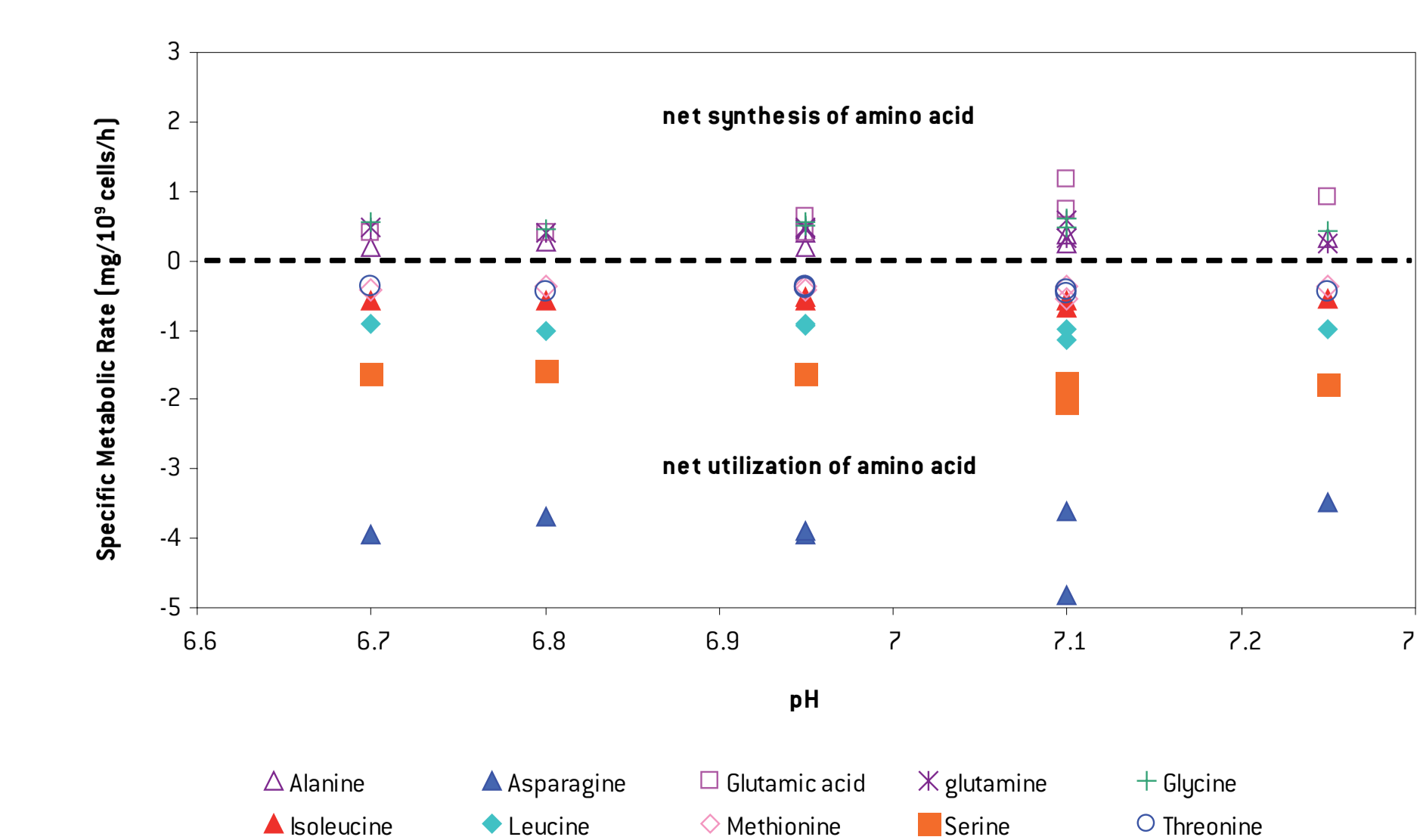


Figure 3: Metabolic rates of a selection of amino acids. These were not significantly affected by culture pH at investigated levels.



FEED RATES AND FORMULATION

In mammalian fed batch cell cultures, the feed(s) will have a highly complex formulation due to cellular nutrient requirements, and must be in a very concentrated form due to volume limitation in the bioreactor. The feed components have varying chemical properties and aqueous solubilities. They may also be chemically reactive, forming unwanted chemical compounds with other feed components. Solubilising some feed components requires adding acid or alkali, which makes an undesirable contribution to culture osmolality. Acidic and alkaline feeds also interact with control of culture pH; alkaline feeds in particular elicit addition of carbon dioxide to counteract increases in culture pH, and this carbon dioxide can reach toxic concentrations. It is therefore important to find feed formulations that are chemically stable and as much as possible reduce any non essential components such as alkalis. In addition, the feed components must be balanced to cellular consumption rates to prevent nutrient depletion or overfeeding of one or several feed components, which may be inhibitory to cell growth through their contribution to osmolality.

RESULTS

A combination of feed reformulation and feed rate adjustment, together with a lower culture pH of 6.80 (previously operated with a pH 7.0 to 6.8 dead band) resulted in an improved process compared to Lonza's original generic GS-CHO process. Lactate levels were significantly reduced, osmolality was maintained at a low level for longer (Figure 5) and high culture viability was maintained for longer (Figure 4). Growth was slightly reduced, but due to a longer culture duration, from 15 to 23 days, and higher specific production rates, the product concentration at harvest was increased more than two-fold from 3.1 g/L to 6.8 g/L (Figure 6).

Figure 4: An initial slower growth rate was seen when operating at a constant pH of 6.8, compared to the previous process operating with a pH 7.0 to 6.8 dead band, but combined with reformulation of feeds and feed strategy, this led to a much extended culture duration and higher viability.

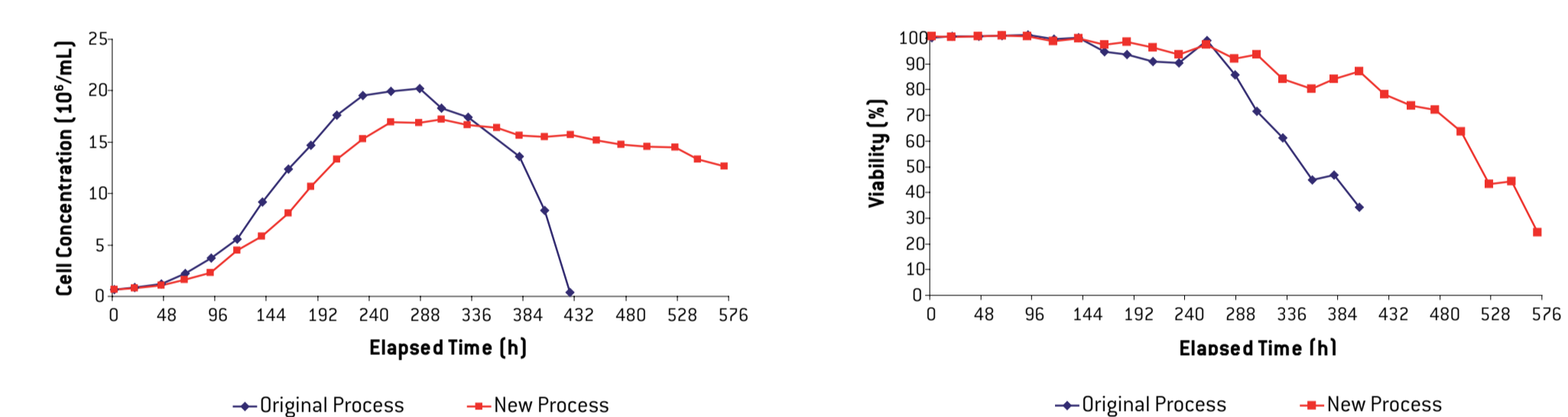


Figure 5: Lactate accumulation is substantially reduced at the constant pH 6.8. Due to the reduced lactate accumulation, feed reformulation and adjusted feed rates, osmolality was maintained lower for longer. Both cultures exhibited high lactate accumulation in the decline phase.

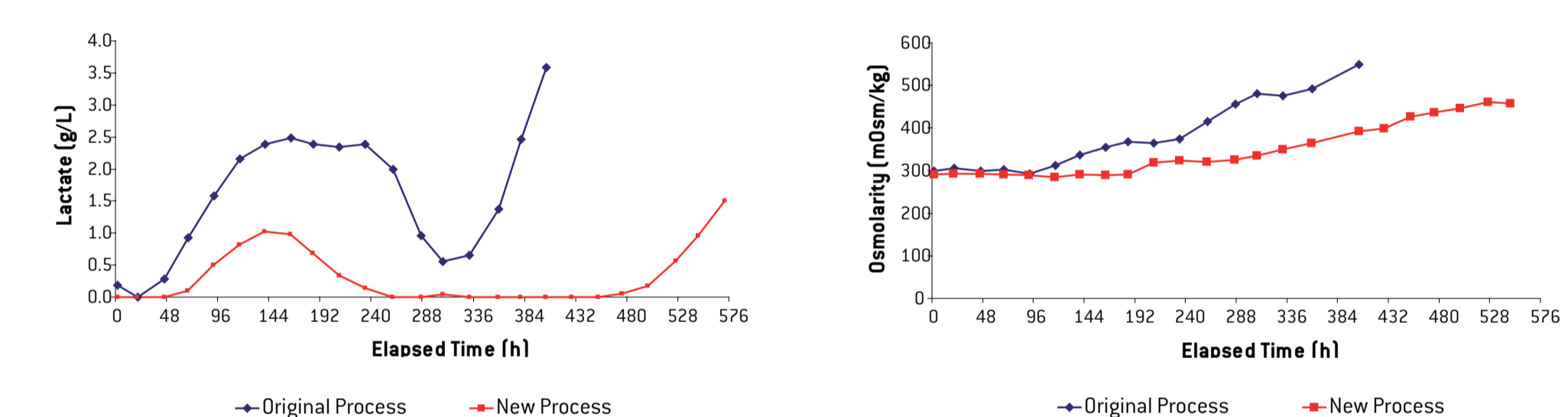
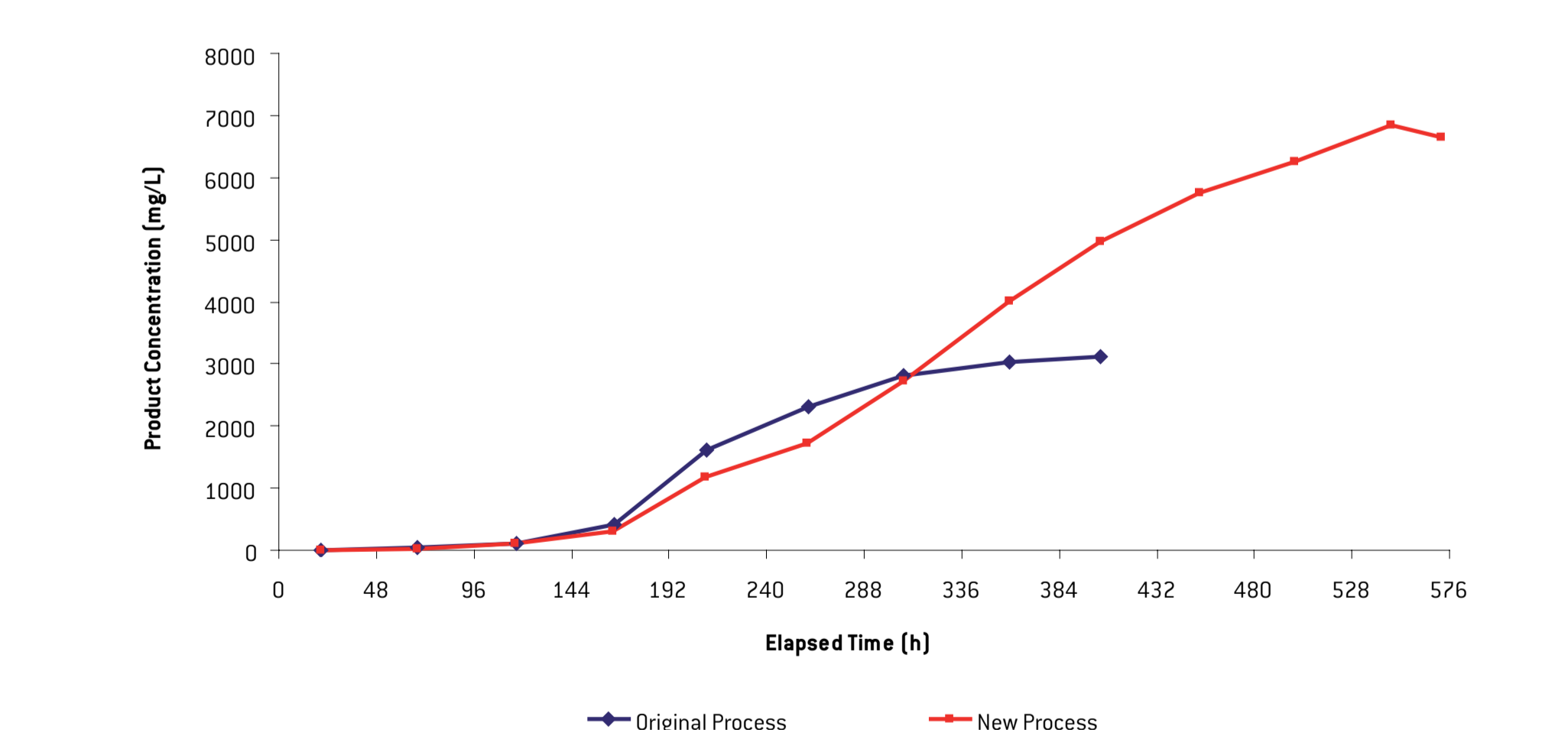


Figure 6: Harvest product concentration of the modified process, with reformulated feeds and constant low pH, was increased more than two fold to the original process to 6.8 g/L.



CONCLUSIONS

Culture performance and robustness was shown to be improved by:

- Reformulating nutrient feed to avoid chemical instability and reducing the need of potentially harmful components.
- Adjusting feed rates by pre-determined knowledge of metabolic rates thus avoiding detrimental osmolality increases caused by excess amino acid concentrations.
- Adjusting pH down to achieve a more effective carbon metabolism and lower lactate accumulation reducing the need for alkali additions for pH control and subsequent later stage high pCO_2 .

These combined modifications increased culture duration from 15 to 23 days and increased harvest product concentration from 3.1 g/L to 6.8 g/L.

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