Development of high-yielding chemically defined and animal component-free generic processes using GS cell lines.

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Introduction

- Generic processes
- Process optimisation
- GS-CHO example
- Summary

Generic processes – Why?

- Contract manufacture of biopharmaceuticals
  - Deal with many different cell lines and cell types
- Not possible or desirable to optimise every process
  - Takes time – for early phase trials need material quickly
  - Costs money
- Generic process allows rapid generation of material for the clinic

Generic processes

- Basis for generic processes is chemically defined media
- No hydrolysates are used
  - They are potential source of variability
  - Costs are associated with screening to find acceptable batches of hydrolysates
  - Effect on metabolism may differ between cell lines
  - Aids understanding of process
- Animal component-free taken as granted
  - Regulatory reasons
  - Simplifies purification

Generic processes

- Generic processes are designed to be adaptable
- Slight modifications to feeds make them suitable for other cell types
  - GS-NS0 process suitable for other selection systems with NS0 and hybridoma
    - Add glutamine, reduce glutamate concentration and changes in some other amino acid components
  - GS-CHO process suitable for dhfr-CHO with similar changes and removal of HT from inoculum process
- Reactor conditions modified depending on cell type

Process optimisation

- There are several routes to improving process productivity, falls into two main categories
  - Increase the time integral of viable cell concentration (IVC)
  - Increase the specific productivity of the cells
- Alternatively big is better approach
  - Scale-up to larger reactors

Process optimisation

- Increasing IVC can be achieved in many ways
  - Increase maximum viable cell concentration
  - Prevent cell death
  - Increase process duration
- Fed-batch processes
- Anti-apoptotic engineering
- Temperature shifts
- pH shifts
- Make each cell more productive
  - Productivity enhancers
  - Select a new cell line

Scale-down model

- Impractical to do all work at production scale
- Need a scale-down model as representative of final production bioreactor as possible
  - Ideally use small-scale fully controlled bioreactors
    - Expensive and limited number of treatments possible
  - Shake-flasks are a good compromise
    - Large numbers can be used in parallel
    - Differences in pH and DOT control
- Need to regularly test advances in a representative bioreactor

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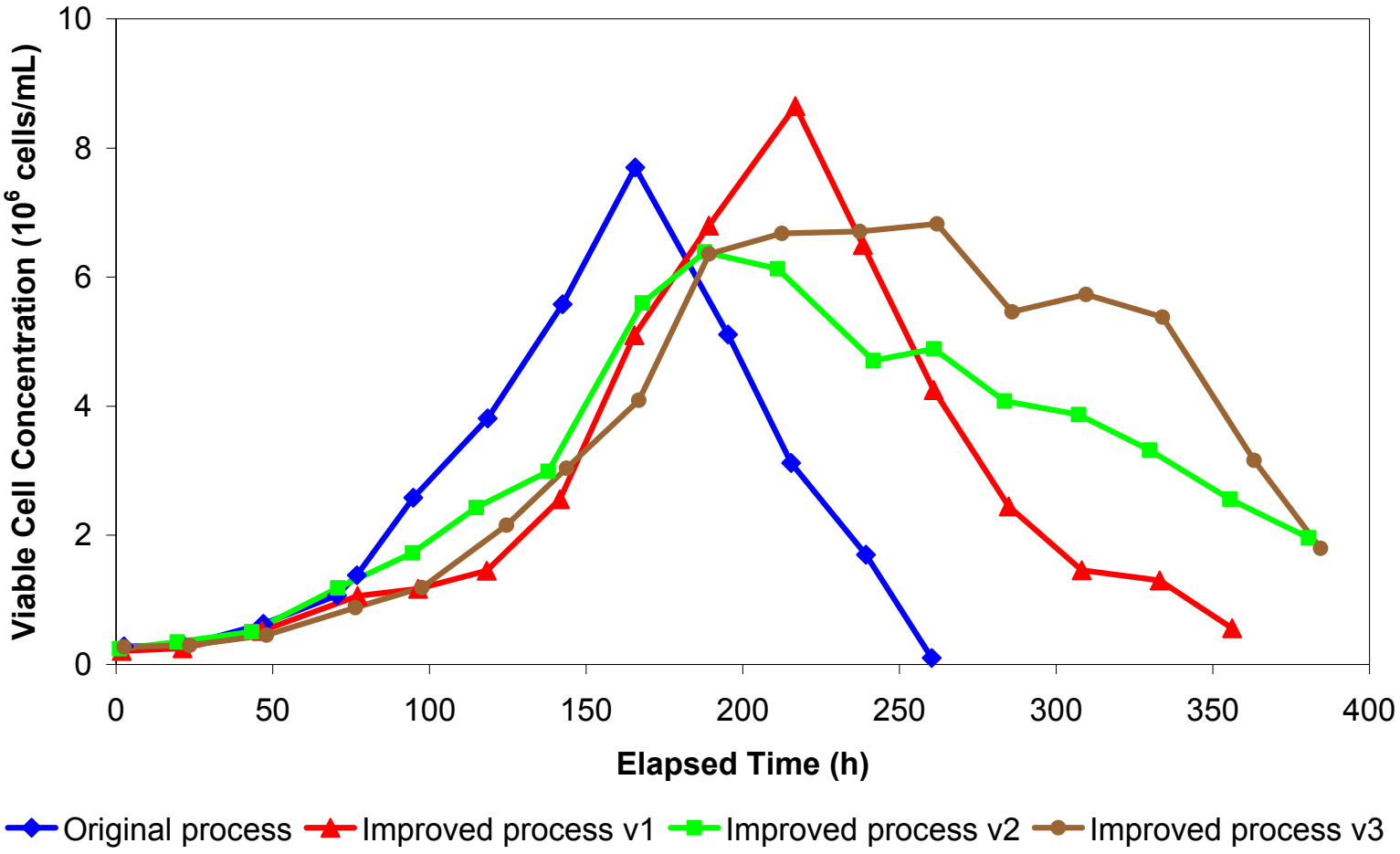
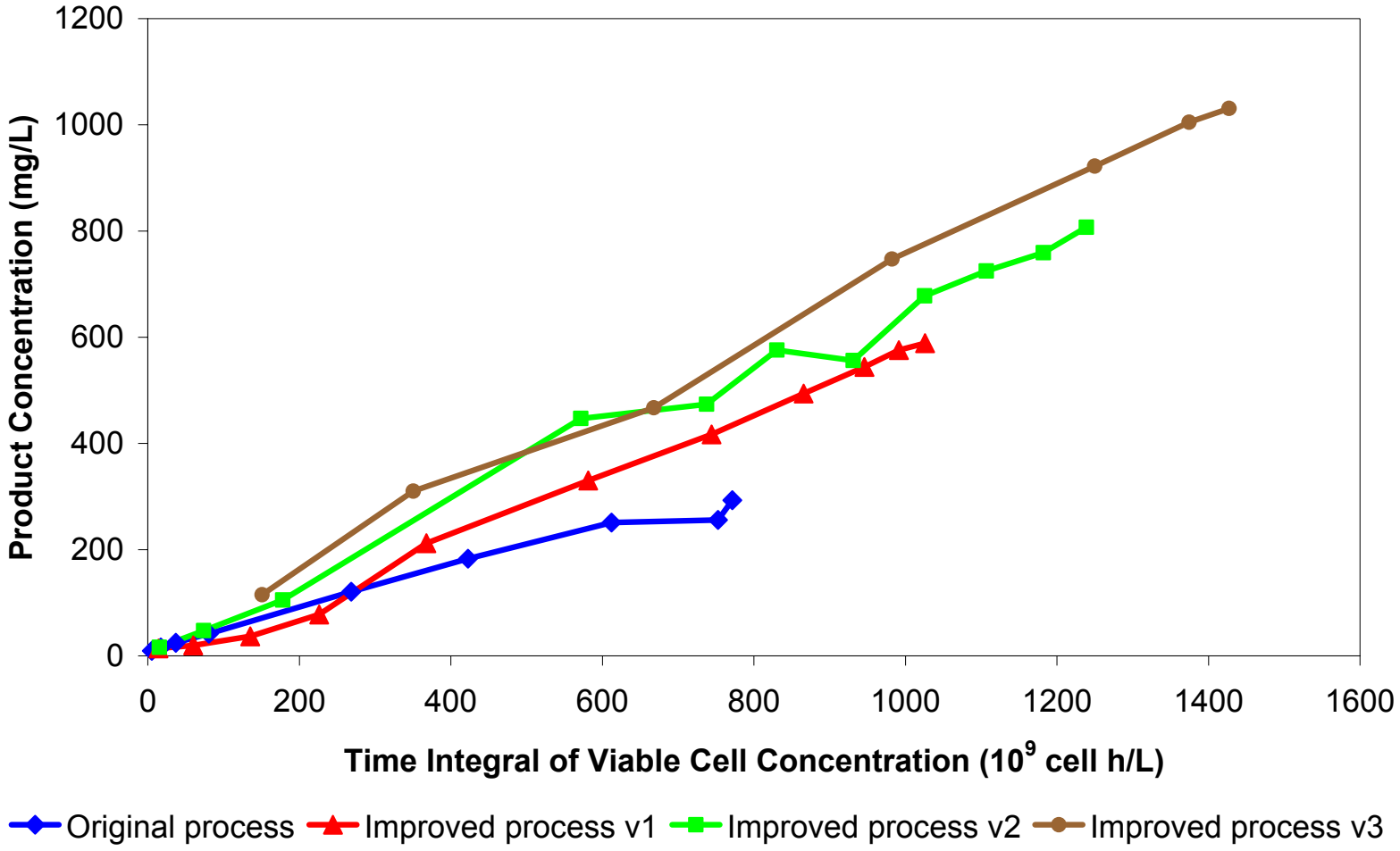
Fed-batch processes

- Fed-batch processes
  - Analysis of spent culture media
  - Re-supplement depleted components
- Initial rounds of optimisation lead to highest increases
  - Typically several fold increase over batch process
  - Large increases in product concentration become more difficult to achieve as process develops
- Example using GS-NS0 cell line 6A1(100)3

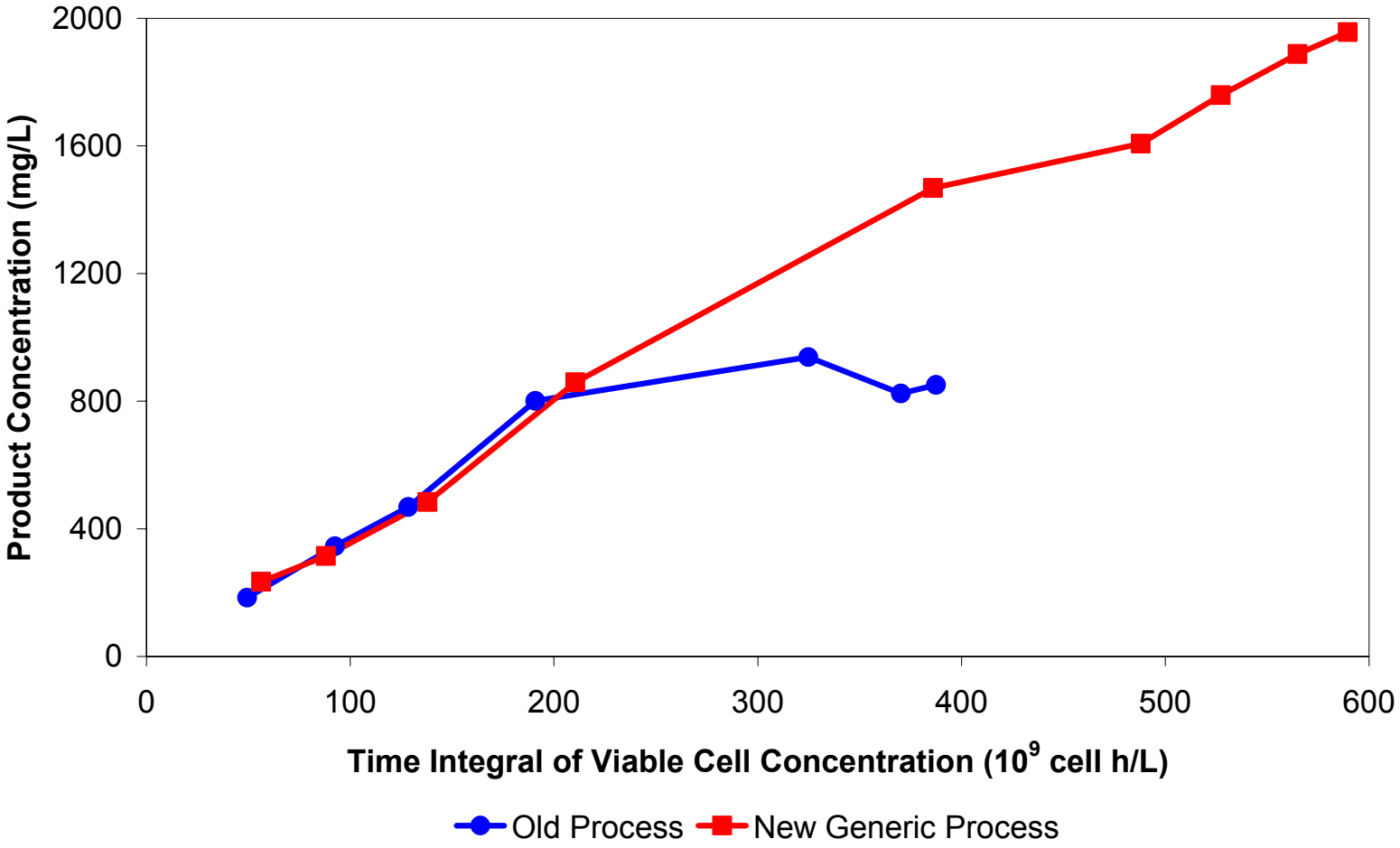
Process optimisation – GS-NS0 cell growth



Process optimisation – product concentration



Is the fed-batch process improvement generic?

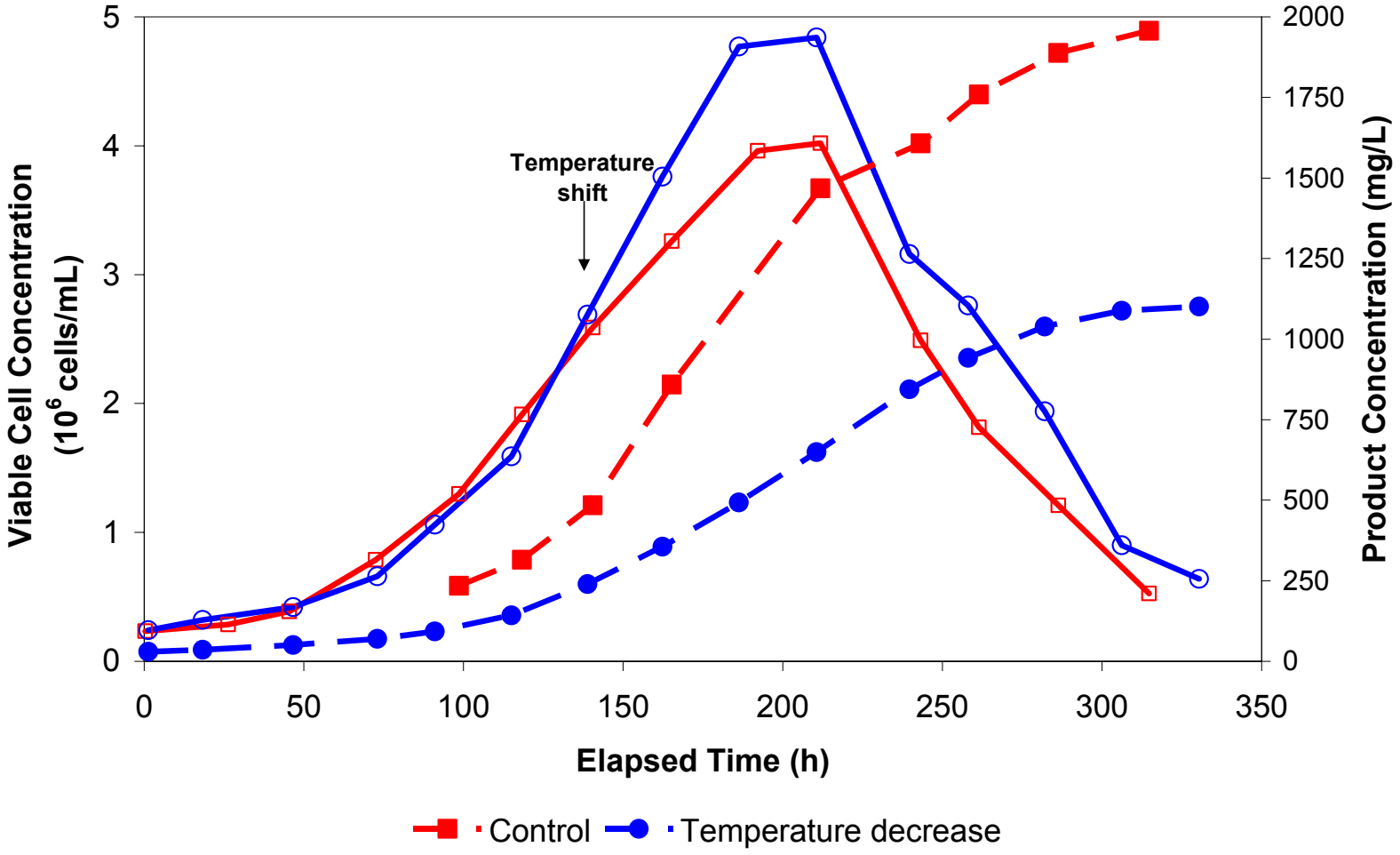


Further process optimisation

- Fed-batch process is applicable to multiple cell lines
  - Generic
  - Similar results with GS-CHO process

- Other factors are considered during optimisation
  - Temperature shifts
    - GS-NS0 example
  - Productivity enhancers
    - Effect on two different GS-NS0 cell lines
  - pH shifts
    - GS-CHO

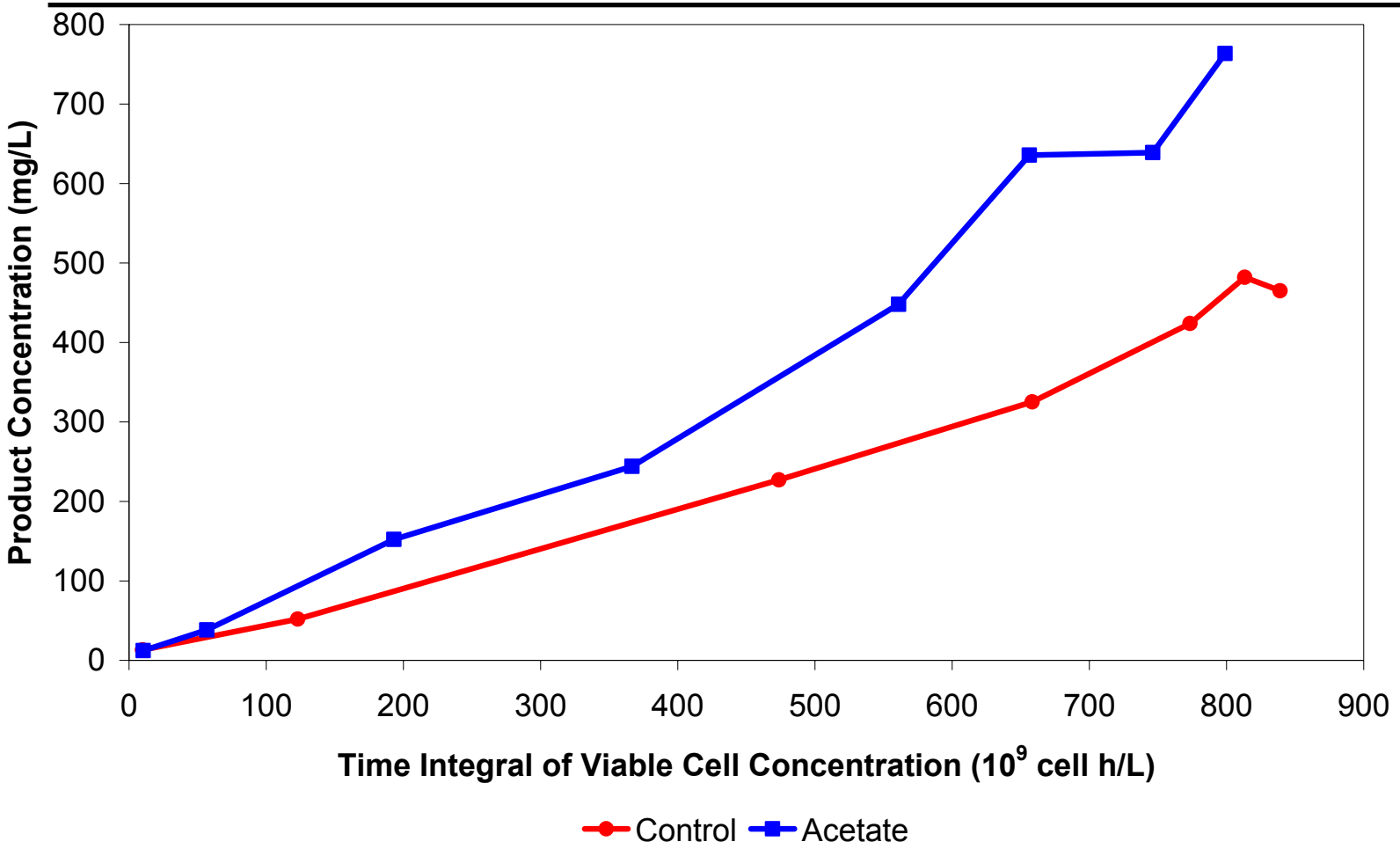
Temperature shifts – GS-NS0



Increasing specific productivity

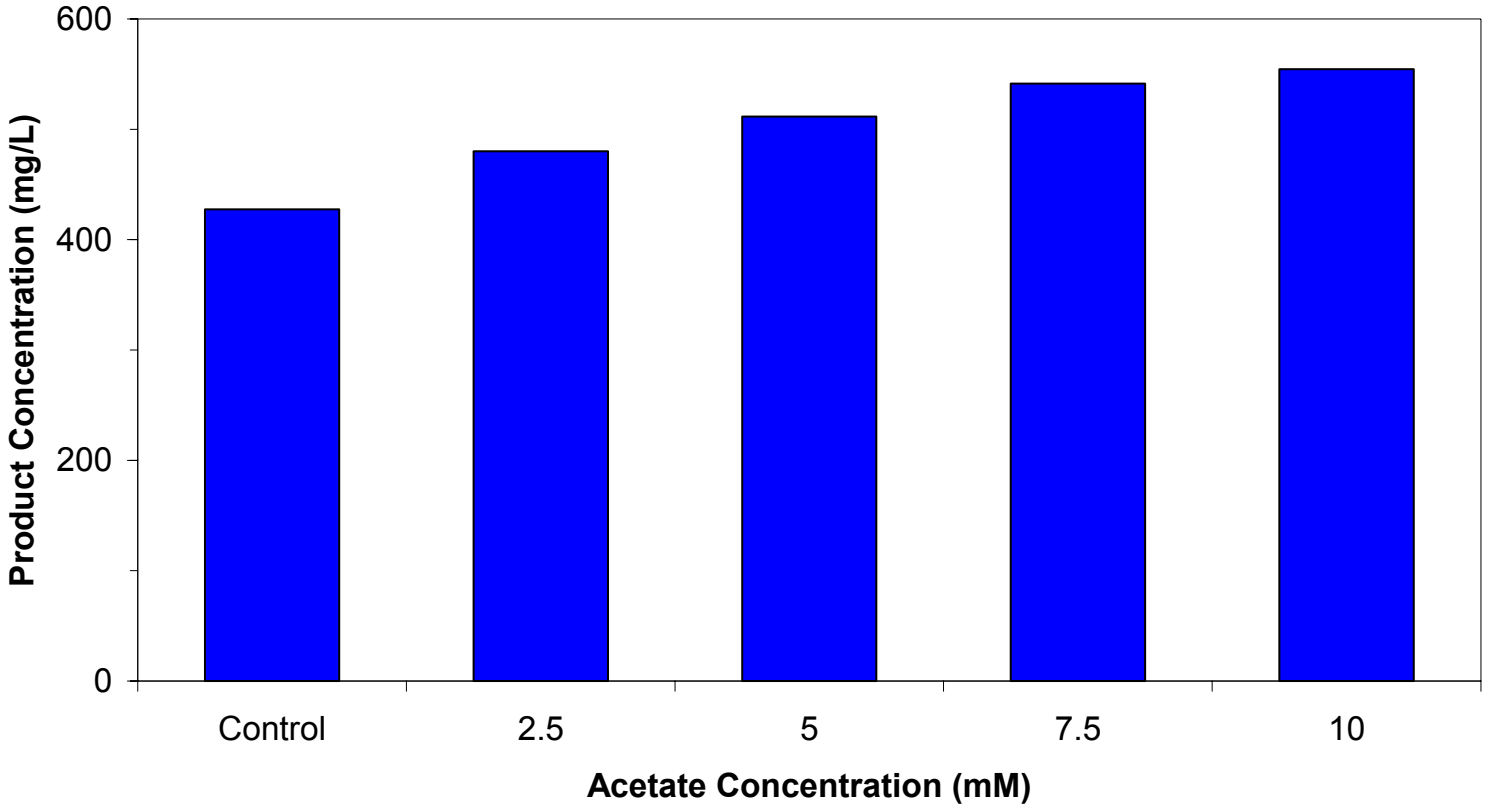
- Ideally should be selecting high producers during cell line selection
- Can modify culture conditions to increase specific productivity
  - pH, temperature
- Productivity enhancers
  - Rely on increasing the cell specific rate of product synthesis
- Often do not know how they function
- Are they generic?

Acetate in bioreactors – GS-NS0



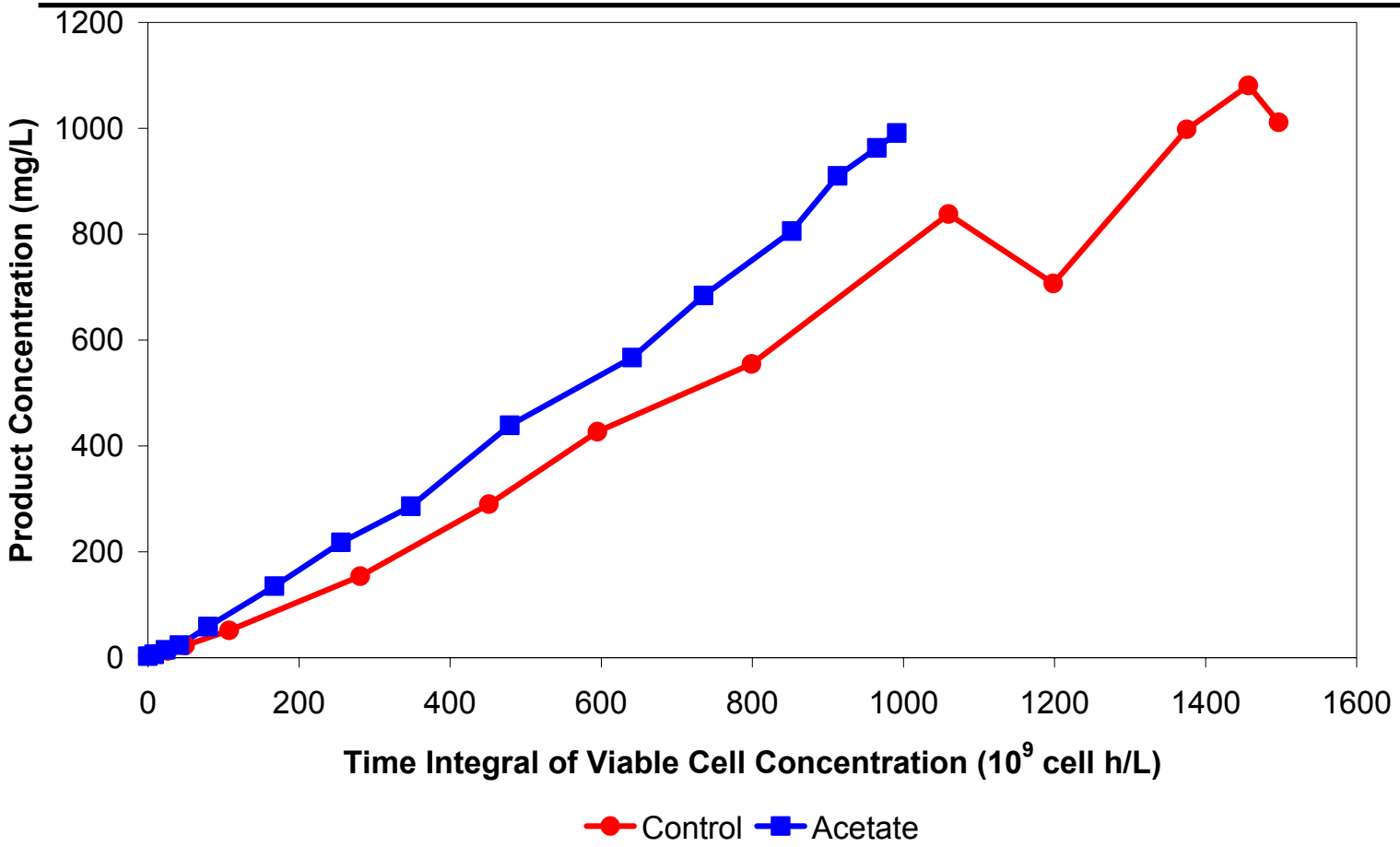
- 1.6-fold increase in product concentration
- No change in IVCC, direct result of increased q_P

Productivity enhancers – flask



- Product concentration increases with acetate concentration, a result of increased q_P

Productivity enhancers - bioreactor



- 1.4 fold increase in q_P, concomitant decrease in IVC of similar magnitude
- No increase in product concentration

Productivity enhancers

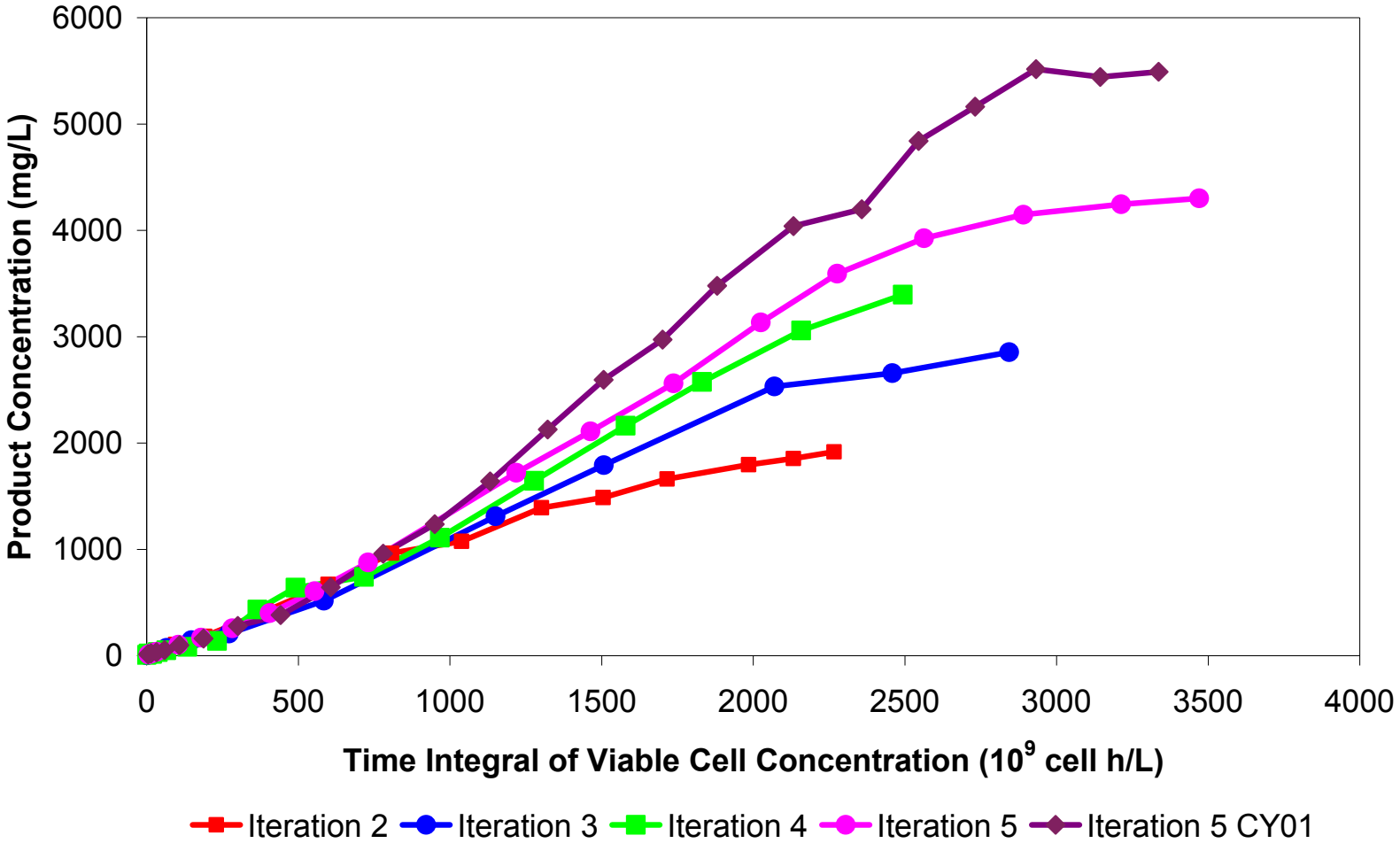
- Productivity enhancers can be highly effective at increasing product concentration

- Several downsides
  - Can be cytotoxic
  - Cell type *and* cell line specific
    - Need optimising for each cell line and process – time consuming
  - Not suited to generic processes

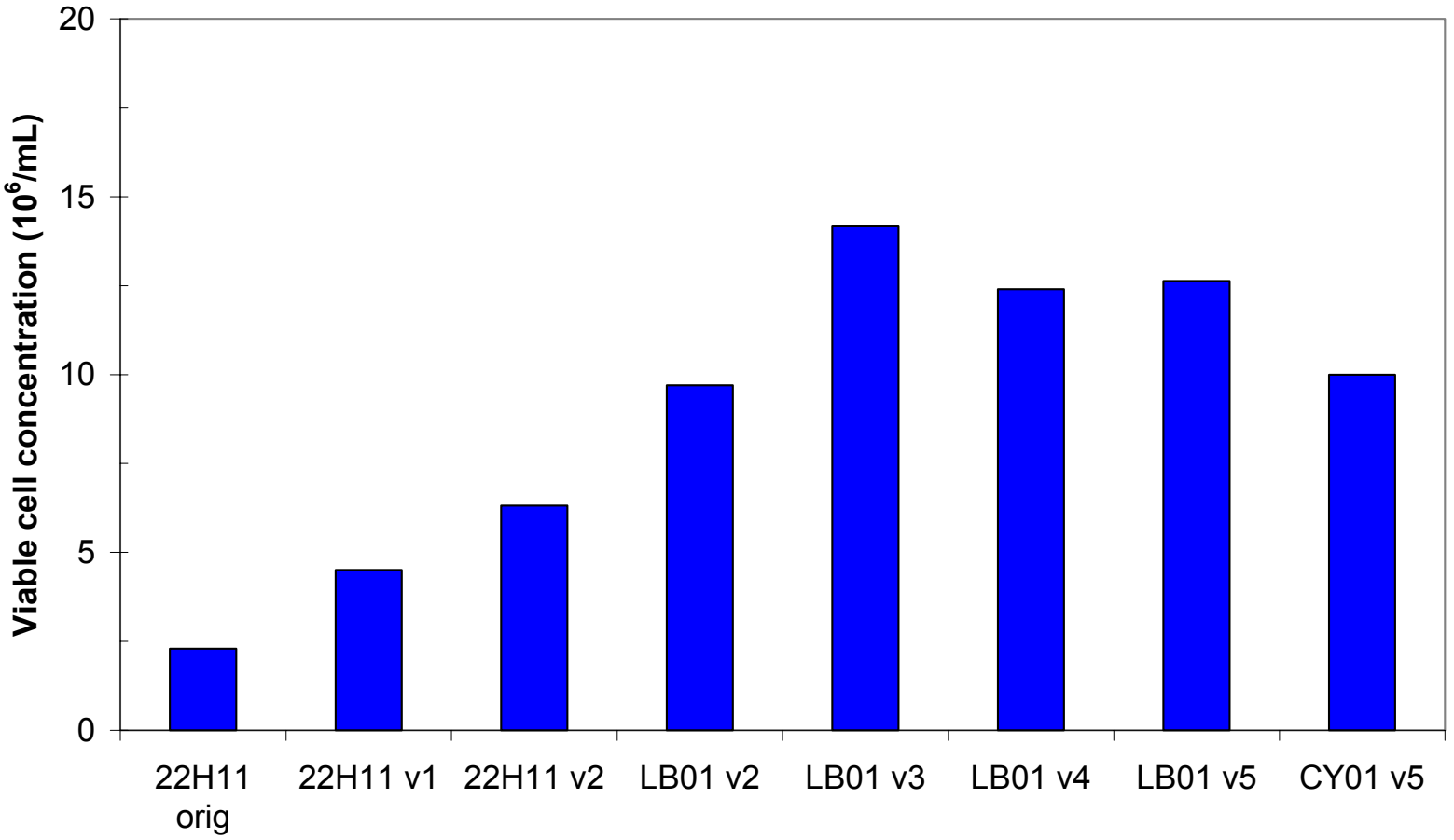
GS-CHO process optimisation

- Principles used for development and optimisation of the GS-NS0 process applied to GS-CHO
- Multiple modifications to process
  - Change of cell line from CHOK1 to CHOK1SV host
  - Iterative feed modifications
  - Basal medium change
  - Increased feed volume
  - Extended culture duration
  - Modification of pH control strategy
  - Cloning of cell line

Process optimisation - productivity



Maximum viable cell concentration



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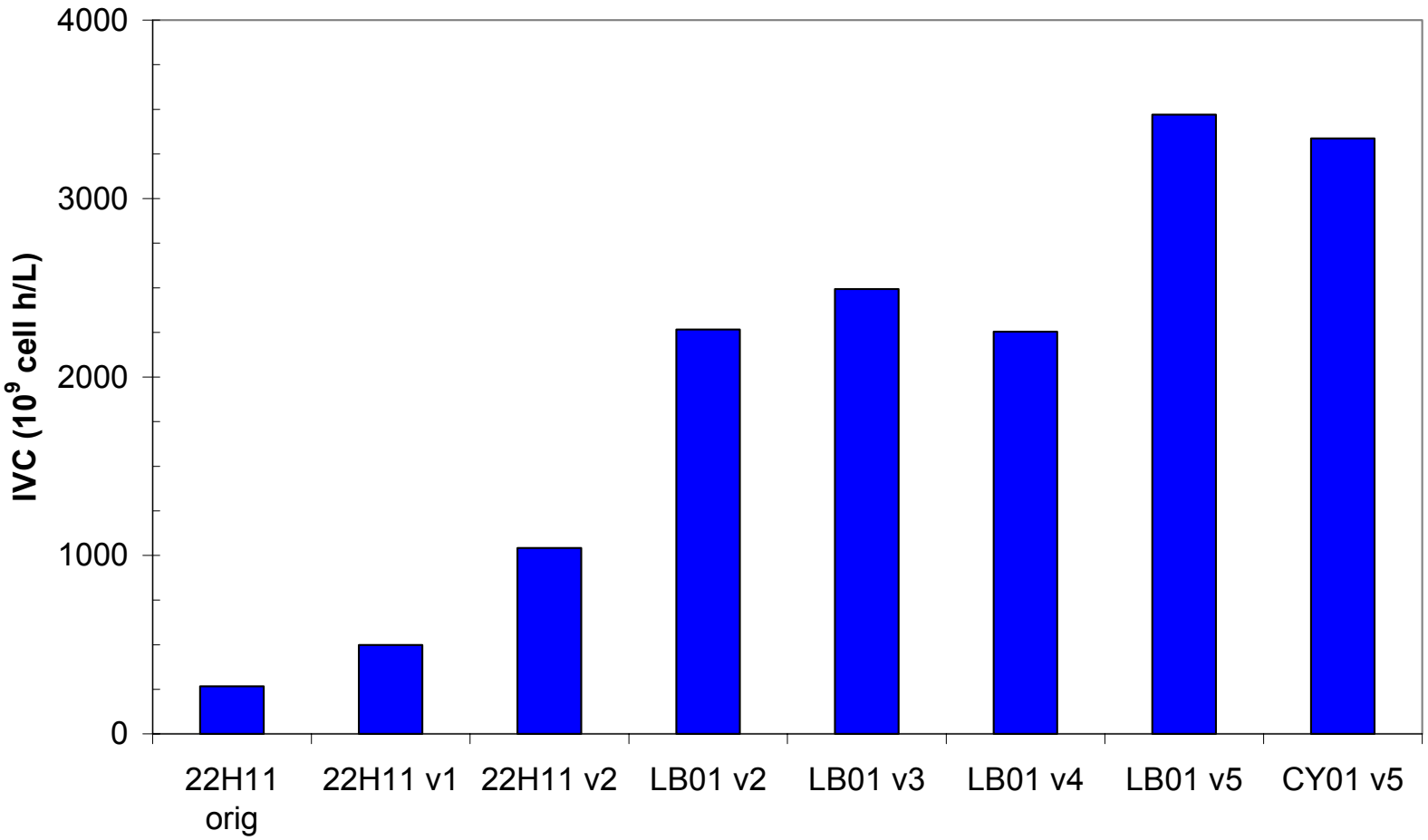
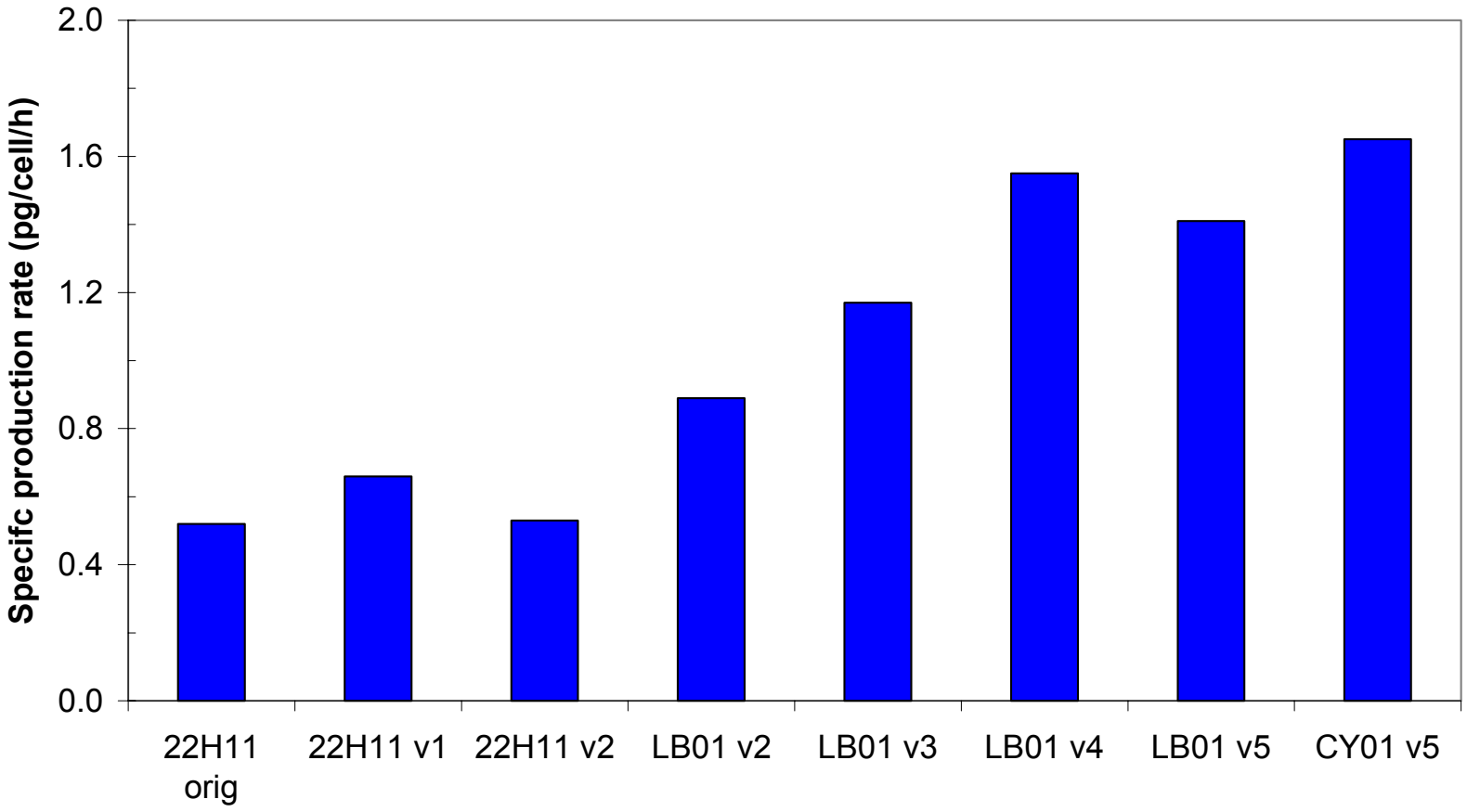
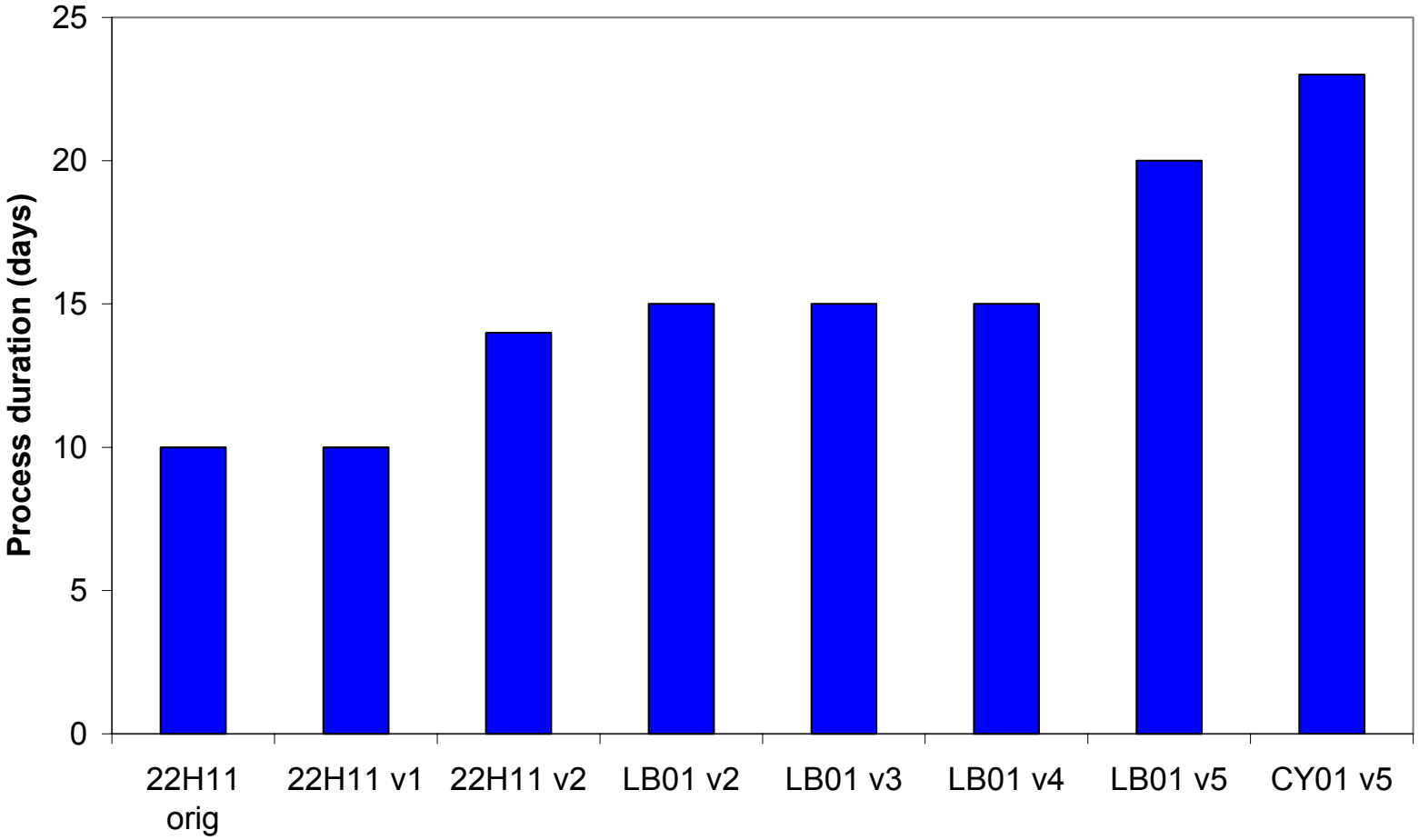
Time integral of viable cell concentration



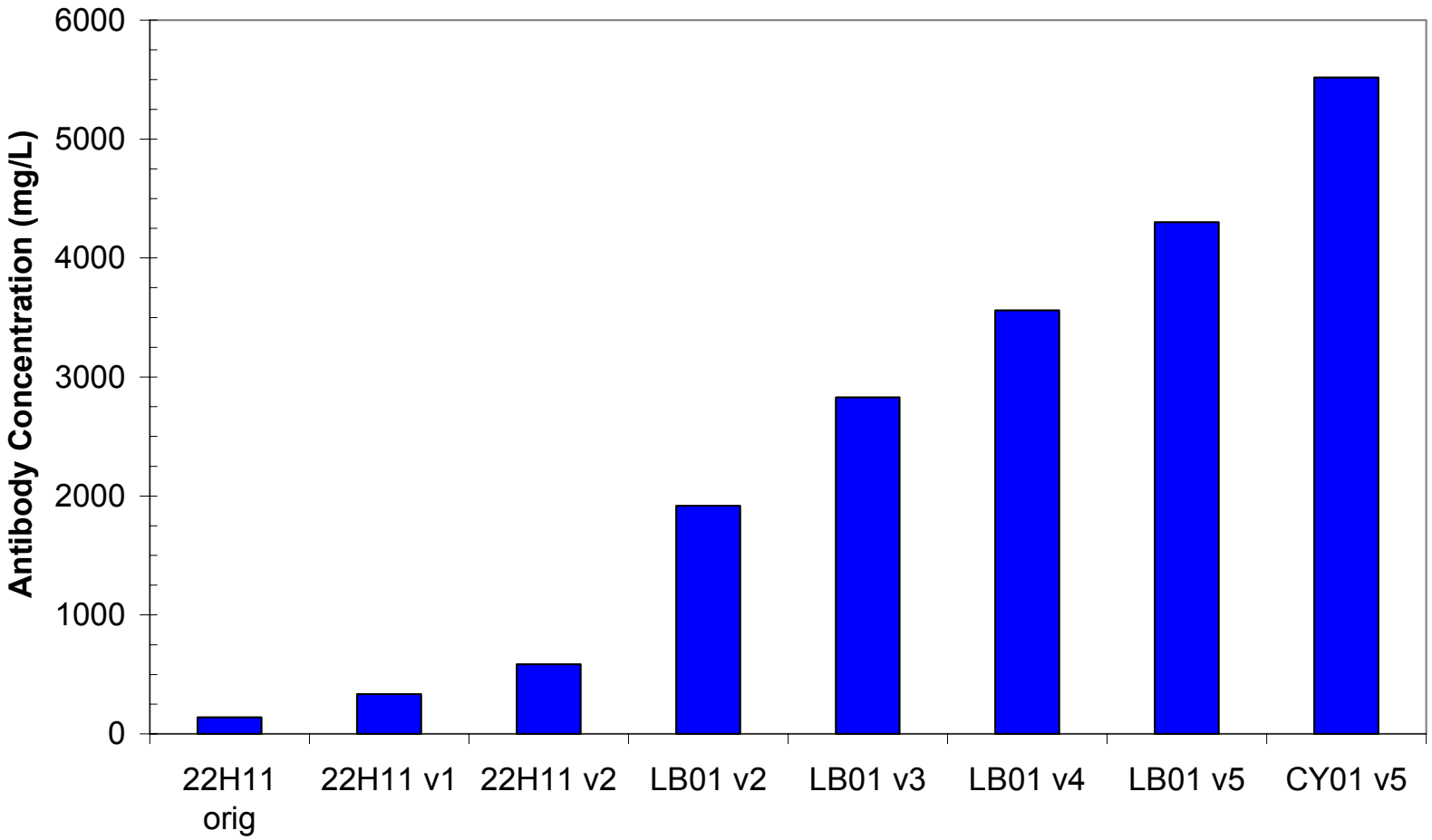
Specific productivity



Process duration



Product concentration



Summary

- Generic processes enable rapid generation of clinical material
  - Typically yield 1–2 g/L
- Processes can be optimised if more material is required
- Process optimisation can achieved by increases in IVC and cell specific productivity
- Chemically defined, optimised processes yielding over 5 g/L can be achieved

Acknowledgements

- Cell culture process development

- Assay development group for support