

John Birch, Lonza Group Ltd

**Lonza**



# Progress in the Large Scale Production of Monoclonal Antibodies

2nd International Congress on Recombinant Antibodies

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A horizontal strip of microscopic images showing various cell cultures and laboratory equipment, located below the title bar.

## Manufacturing Issues

- Large number of antibodies (ca. 200) in development
- Volume requirements vary but can be as high as 100s of kg per year
- Demand for antibodies/fusion proteins has been a major driver for mammalian cell capacity

## **Manufacturing Systems**

- Mammalian cell culture
  - Batch and fed batch culture (10'000+ litre reactors)
  - Continuous (perfused) culture
- Microbial culture
  - Especially for antibody fragments
- Transgenic animals and plants

**Currently mammalian cell culture is the predominant system for products in development – what can it deliver ?**

- Sufficient capacity
- Speed in development (especially for early phase material)
  - Rapid cell line creation
  - Use of generic technology
  - Predictive scale-up systems
- Cost efficiency
  - Productive cell lines
  - Process optimisation (fermentation and purification)

## Estimated Capacity (Thousands of Litres)

	<b>Total</b>	<b>Contract Manufacturers</b>
2002	500	130
2006	1500	330

- Most of current capacity filled with small number of products
- Long lead times (3-5 years) and high costs (\$200m - \$500m)  
(\$3+ Million / m<sup>3</sup>)

# Lonza Biologics – Expansion Project

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- Lonza Biologics  
Portsmouth, NH (US)





- Production of biopharmaceuticals by mammalian cell culture fermentation
- Four-fold expansion of the existing fermentation volume by
  - 3 x 20'000L
  - with downstream processing
- Start up mid 2004
- Total investment of CHF 330 million

# Maximising Productivity/Cost Efficiency in Mammalian Cell Systems

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- Efficient gene expression technology
  - The Gene Vector
  - The Cell Line
- Process optimisation
  - Cell culture conditions
  - Product recovery – minimise losses
- Maintain product quality

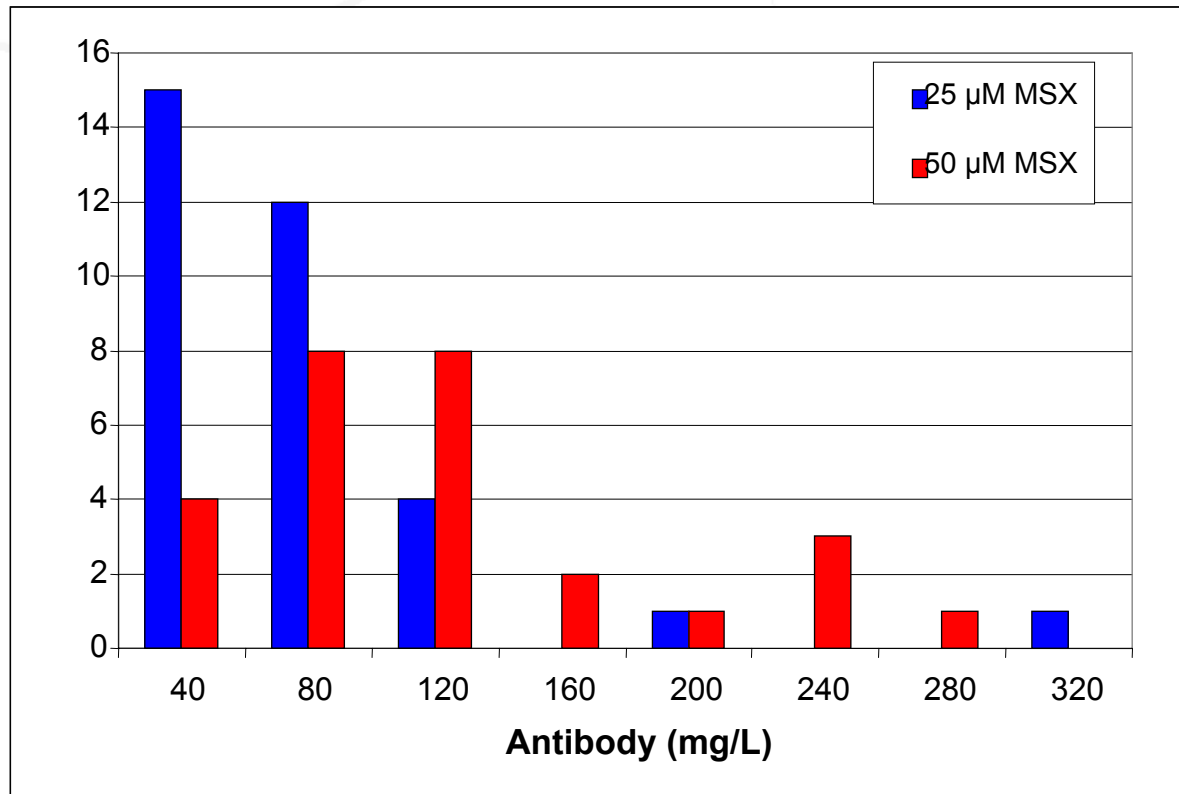
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- The GS (Glutamine Synthetase) expression system enables the rapid creation of highly productive NS0 and CHO cell lines
  - Straightforward and stringent selection using glutamine free medium
  - High specific production rates and good growth characteristics
  - No requirement for amplification for NS0 cell lines (Time/stability benefits)
  - High yields also achieved with non-amplified CHO cell lines

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- A decorative horizontal bar is located below the title. It features a series of small, colorful images (microscopy, lab equipment) in shades of blue, green, and yellow.
- By definition, the transfectants with potentially the highest specific productivities are rare
  - To find these rare events, it is necessary to have:
    - High efficiency of transfection
    - Selection method that eliminates the vast majority of low producers from the transfectant pool i.e. increase stringency of selection


## Transfection and Selection Conditions for GS-CHO Cell Lines Expressing cB72.3 Antibody


Electroporation condition	MSX ( $\mu\text{M}$ )	Stable transfectant numbers
1	25	68
	50	32
2	25	124
	50	57
3	25	197
	50	70

## Influence of Selection Conditions for GS-CHO Cell Lines with cB72.3 Antibody

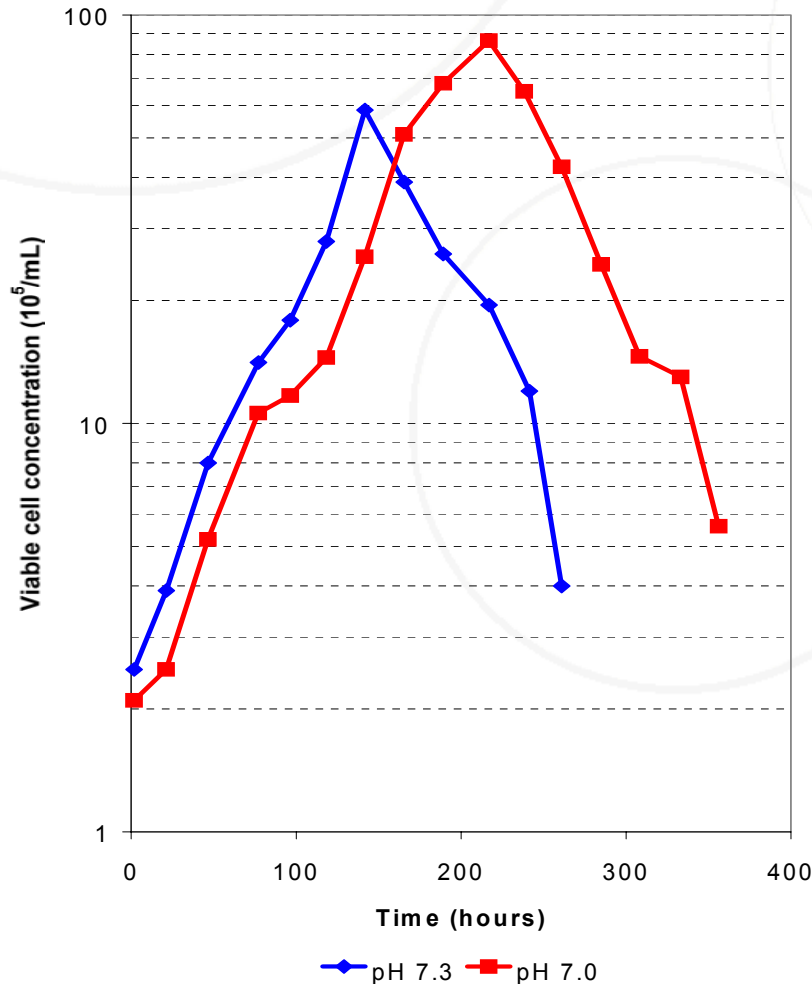


Cell lines have not been amplified.


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- A decorative banner is located below the title, featuring a horizontal strip of various scientific and industrial images in shades of blue, green, and yellow.
- Typically operate fed batch processes
  - Significant potential to improve process
    - Physicochemical environment (especially pH)
    - Medium design (including the use of chemically defined media)
    - Feeding strategies

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- Control pH, temperature, dissolved oxygen concentration
  - Small changes in pH can have profound effect on cell growth and productivity
    - Responses are cell line specific and can impact:
      - Maximum cell concentration
      - Integral viable cell hours
      - Specific production rate

# Effect of Culture pH



- Reduction of culture pH for a protein-free (chemically defined medium) GS-NS0 process
  - Increased maximum viable cell concentration
  - Increased culture duration
  - Increased integral viable cell hours
  - Increased productivity
    - 590 mg/L compared with 240 mg/L

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- Increasing use of chemically defined media free of animal derived raw materials
    - Reduced risk of introducing adventitious agents
    - Improved process consistency and robustness (avoids potential variability of raw materials such as serum proteins and hydrolysates)
    - Chemical definition assists process optimisation
    - Benefits purification (reduced contaminant load)

# Downstream Benefits of Chemically Defined Medium for GS-NSO Cell Line



## Harvest


## Purity of MAb at

Optimised Protein containing culture

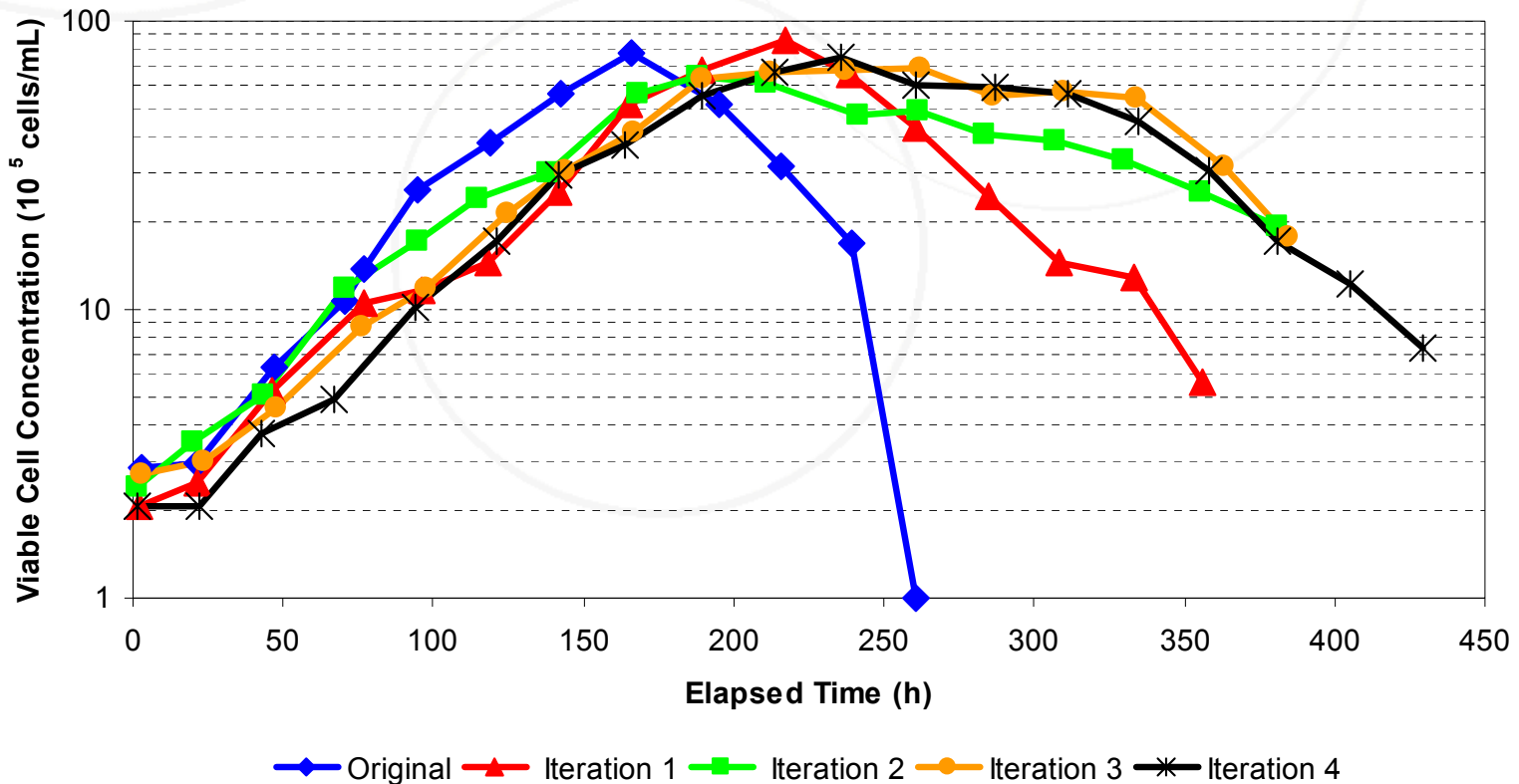
<30%

Optimised protein free culture

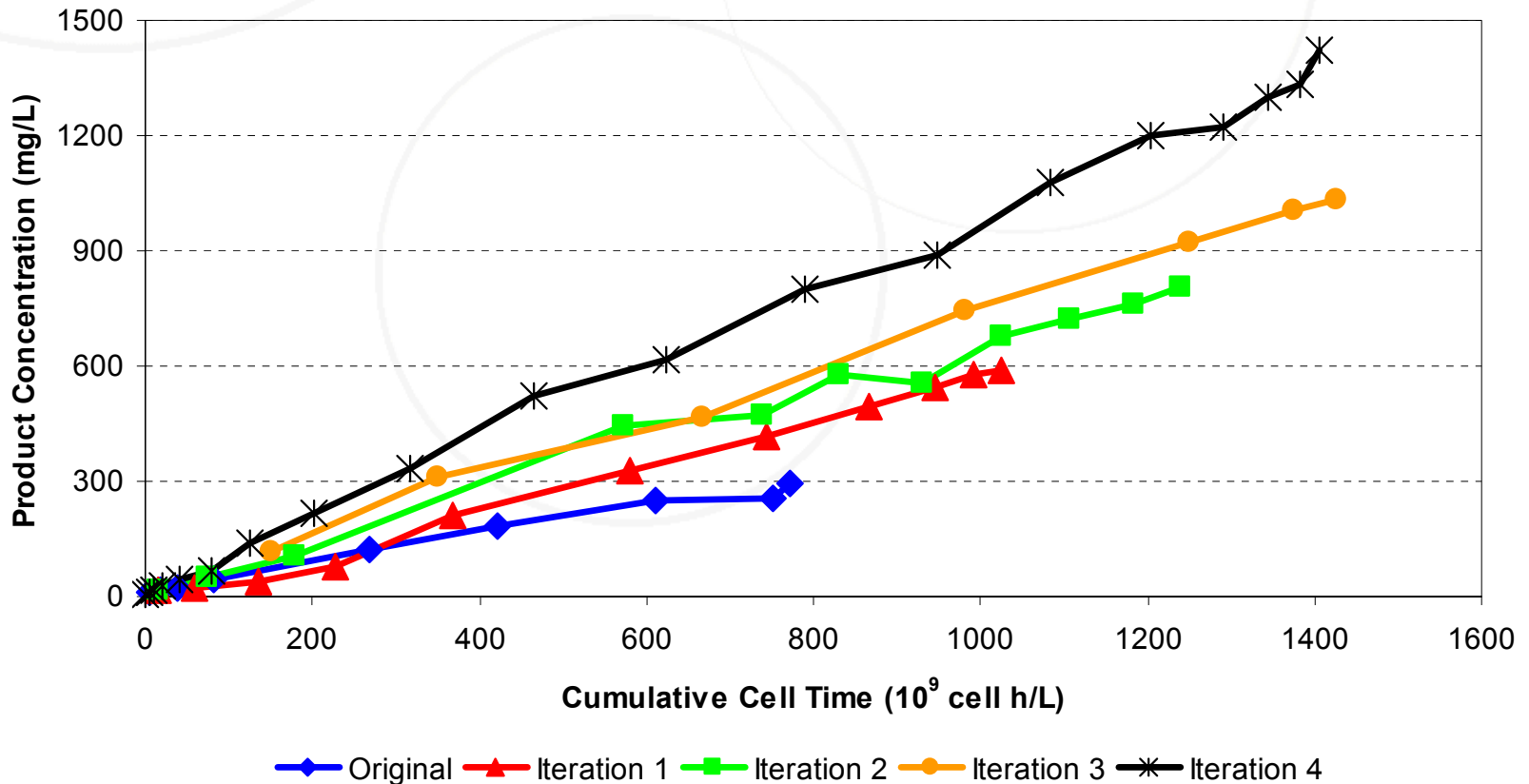
62%-76%

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- A decorative banner is located below the title, featuring a horizontal strip of various scientific and industrial images, including laboratory equipment and green plants.
- Optimise basal medium
  - Optimise feeds
  - Maintain nutrient sufficiency
  - Minimise waste metabolite formation
    - Use of GS system avoids accumulation of ammonium ions from metabolism of glutamine

### Growth Kinetics in a Chemically-Defined, Protein Free Bioreactor Process




### Product Kinetics in a Chemically-Defined, Protein-Free Bioreactor Process

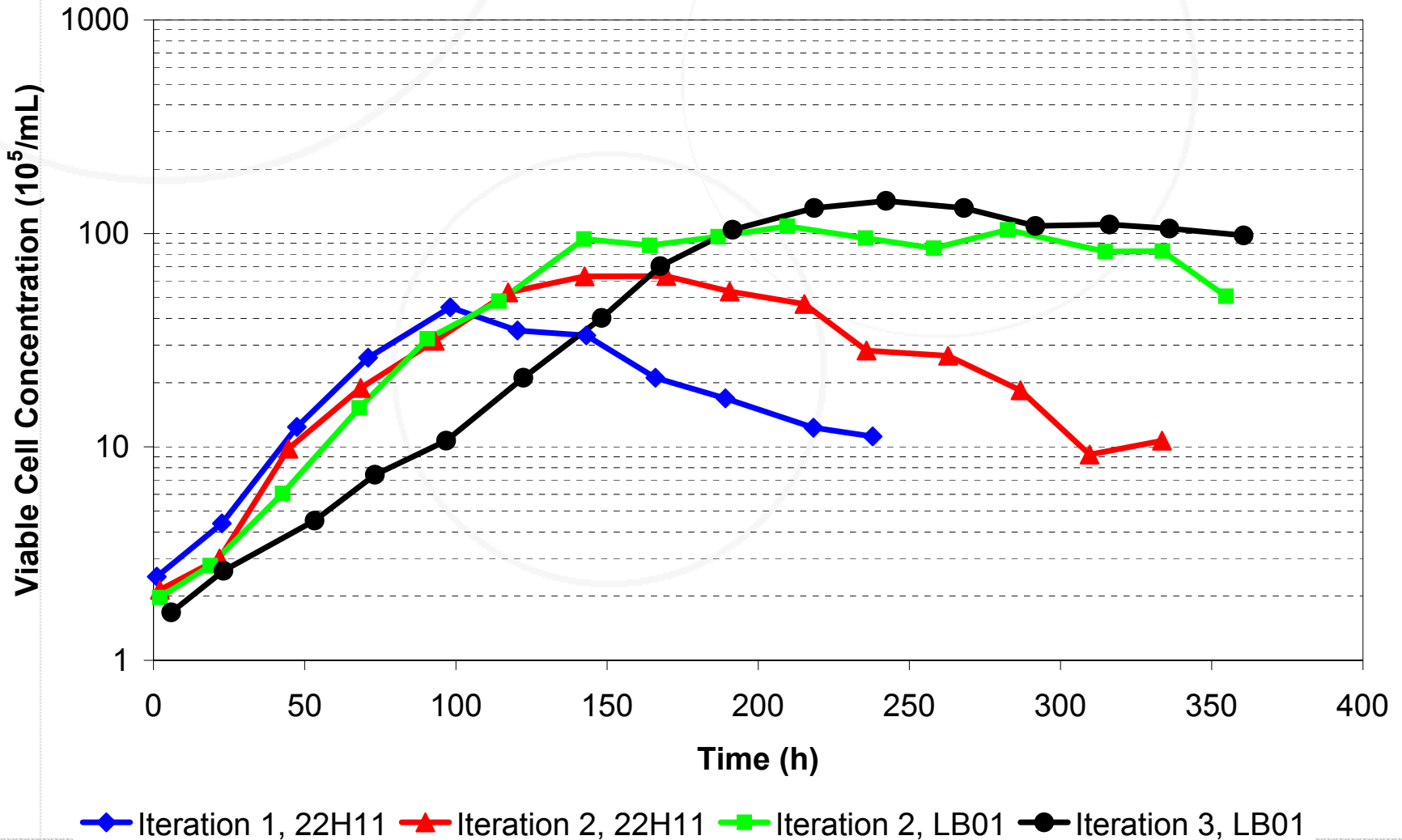


## Chemically Defined, Protein-Free Bioreactor Process

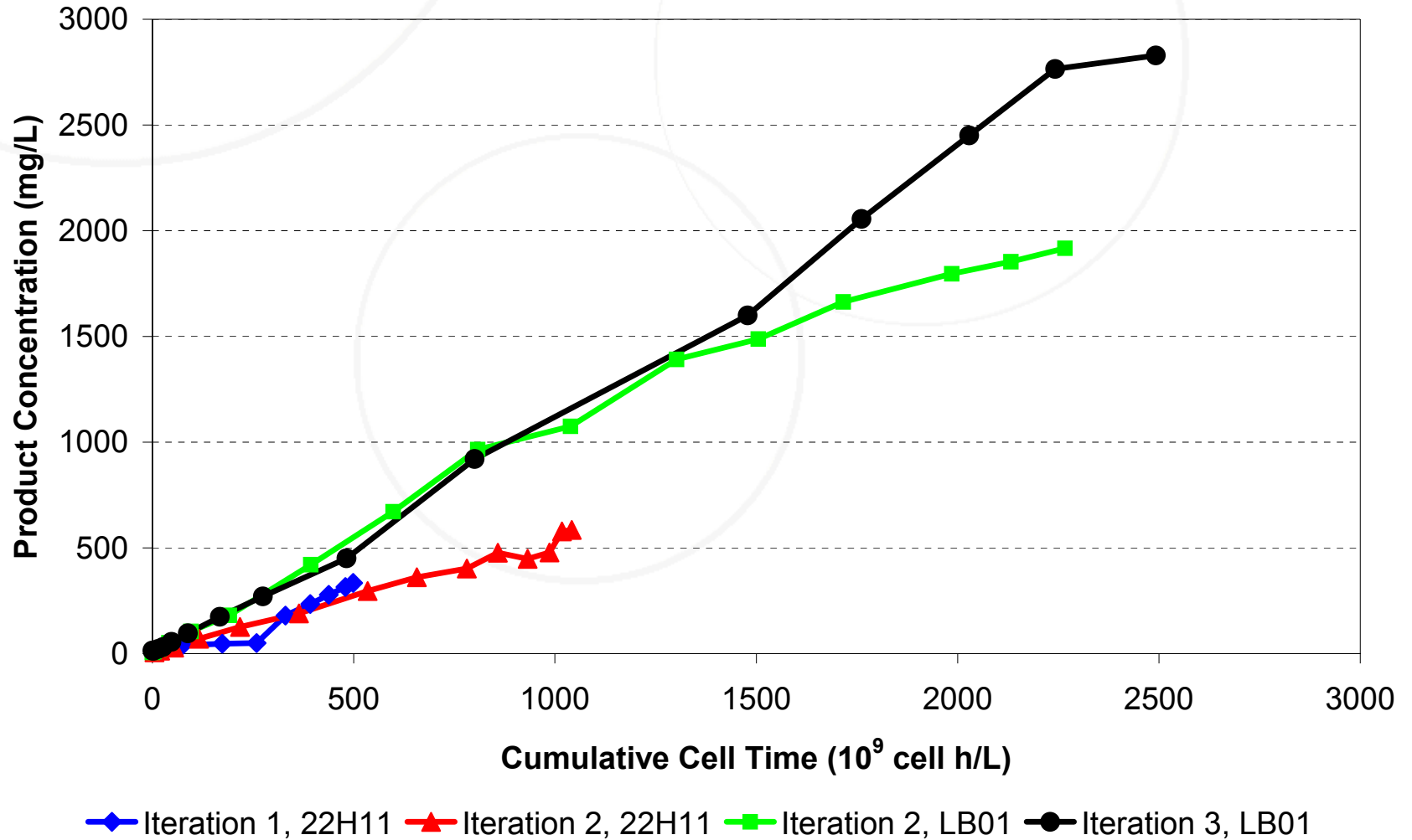
Process	Cumulative cell time ( $10^9$ cell·h/L)	cB72.3 antibody (mg/L)	Process duration (d)
Serum-free	640	476	12
Original protein-free	772	293	12
Iteration 1	1026	589	15
Iteration 2	1239	807	16
Iteration 3	1427	1035	16
Iteration 4	1405	1422	18

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- Similar approach taken as with GS-NS0 cell lines
    - Fed-batch culture, initially using the same feed as the GS-NS0 process
  - Suspension variant of CHO-K1 which grows in chemically defined medium without need for adaptation
  - Efficiency and stringency of transfection conditions increased to improve selection of highly productive clones

# GS-CHO Growth Characteristics



# GS-CHO Product Accumulation




## Chemically Defined, Protein-Free Bioreactor Process

Cell line	Process	Cumulative cell time ( $10^9$ cell·h/L)	Antibody (mg/L)	Process duration (d)
22H11	Original protein-free	267	139	10
22H11	Iteration 1	498	334	10
22H11	Iteration 2	1041	585	14
LB01	Iteration 2	2266	1917	13
LB01	Iteration 3	2493	2829	15

A horizontal strip of microscopic images showing various cell cultures and fermentation processes, located below the title bar.

## Summary

- Process development coupled with selection of improved cell lines gives substantial improvements in productivity
  - GS-NS0 process: product concentration increased by 385%
  - GS-CHO process: product concentration increased by 1935%

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- Continued process improvements
  - Continued improvements in selection procedures for finding highly productive clones
  - Improved cell lines e.g. engineered for improved metabolism or apoptosis resistance

- GS confers glutamine independence
  - GS transfected hybridoma
  - Increased growth and productivity without glutamine
    - Increased integral viable cells
    - Increased product concentration

<b>Medium glutamine (mM)</b>	<b>Specific growth rate (h<sup>-1</sup>)</b>	<b>Integral viable cells (10<sup>9</sup> cell h L<sup>-1</sup>)</b>	<b>Product concentration (mg L<sup>-1</sup>)</b>	<b>Specific production rate (mg/10<sup>9</sup> cell h)</b>
0	0.034	510	517	1.01
6	0.058	430	325	0.76

Birch J.R. *et al* Cytotechnology (1994) 15 p11-16

- Combination of efficient expression system (GS) and process optimisation gives high productivity for both NSO and CHO cell lines
- Use of chemically defined media simplifies process optimisation and product purification
- Significant potential for further improvements based on process optimisation and cell line improvements e.g. apoptosis resistance, modified metabolism