

Biologics Manufacturing 2004, Brussels

Case Study: Strategy to improve fermentation conditions for improved yields from mammalian cell Processes

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Facilities

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U.K. – R &D
+ small scale manufacture



U.S.A. – Large Scale Manufacture

Cell Culture Reactors at Lonza

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	Capacity (Litres)
Airlift Reactors	2 x 200, 2 x 2000, 2 x 5000
Perfusion Reactors	2 x 1500
Stirred Reactors	3 x 20,000 (2004)
Wave Reactors	

Large Scale Reactor

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Mammalian Cell Products

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- Major source (approx half) of biopharmaceutical proteins
- Biopharmaceuticals represent 10-30% of all new drugs in recent years
- Several “blockbuster” products
- Biopharma sales ca. \$22bn in 2001: Mammalian cell products represent ca. 60%
- MAb market has grown from 1% of biopharma in 1995 to 14% in 2001

Polastro & Tulcinsky, SCRIP magazine Sep 2002.

Manufacturing Issues

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- Large number of proteins (hundreds) in development
- Volume requirements vary but can be as high as 100s of kg per year especially for antibodies
- Total demand for proteins > 800Kg in 2002 (J.P. Morgan Research)
- Demand for antibodies/fusion proteins has been a major driver for mammalian cell capacity

Mammalian Cell Culture

Expected Capacity Increases

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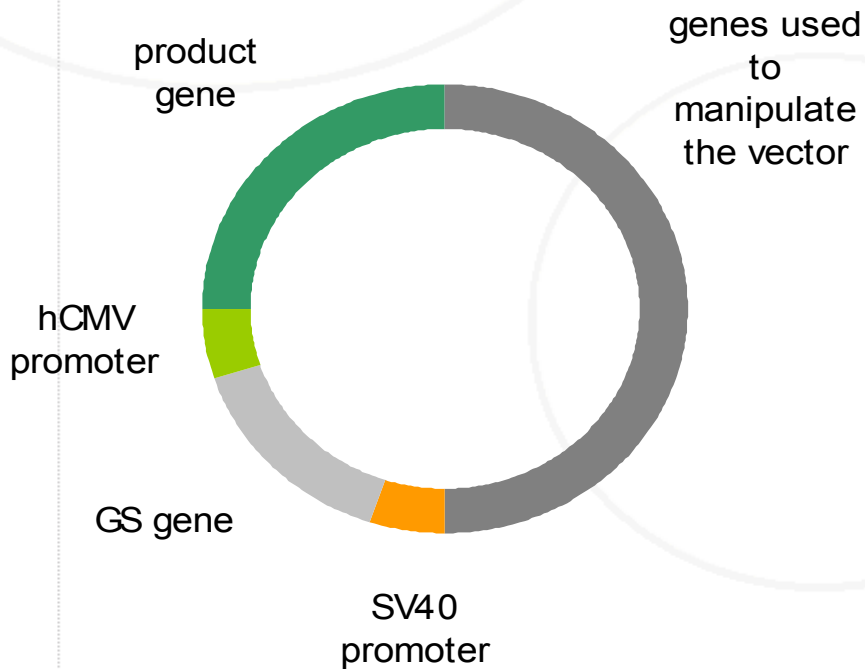
	Capacity 2002 (ca. Litres)	Expansions (ca. Litres)	Capacity 2006 (ca. Litres)
In-House	650,000	810,000	1,460,000
Contract Manufacturing Organisations (CMO)	190,000	320,000	510,000
Total Industry	840,000	1,130,000	1,970,000
% CMO	23%	28%	26%

Improved Process Efficiency

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- Efficient gene expression system
 - Gene vector
 - Host cell line
- Optimisation of culture process
- Design of cell line and process can impact growth rate, max cell numbers, culture duration at high cell viability, specific production rate
- Scalable process
- Impact of process on product quality

Glutamine Synthetase (GS) Gene Expression System



- Many mammalian cells require glutamine
- Use a gene vector which contains the product gene plus the GS gene allowing glutamine synthesis
- Only cells with GS gene (and hence product gene) survive
- Use weak promoter on GS gene and strong promoter on product gene – selects for rare integration into transcriptionally efficient sites in genome
- Allows rapid creation of highly productive cells without the need for further rounds of selection which can take many months

GS and Choice of Cell Line

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- GS widely used for antibody expression in NSO cells (which lack endogenous GS)
- GS can also be used in CHO cells using Methionine Sulphoximine to inhibit endogenous GS
- High productivity achieved for non-amplified NSO and CHO

Cell Line Screening

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- Highly productive transfectants are rare
- Various approaches to improve screening process
 - Increase transfection efficiency
 - Improve stringency of selectable marker to eliminate low producers
 - High throughput methods (FACS + cell surface product capture)
 - Target transcriptionally active sites

Cell Line Selection

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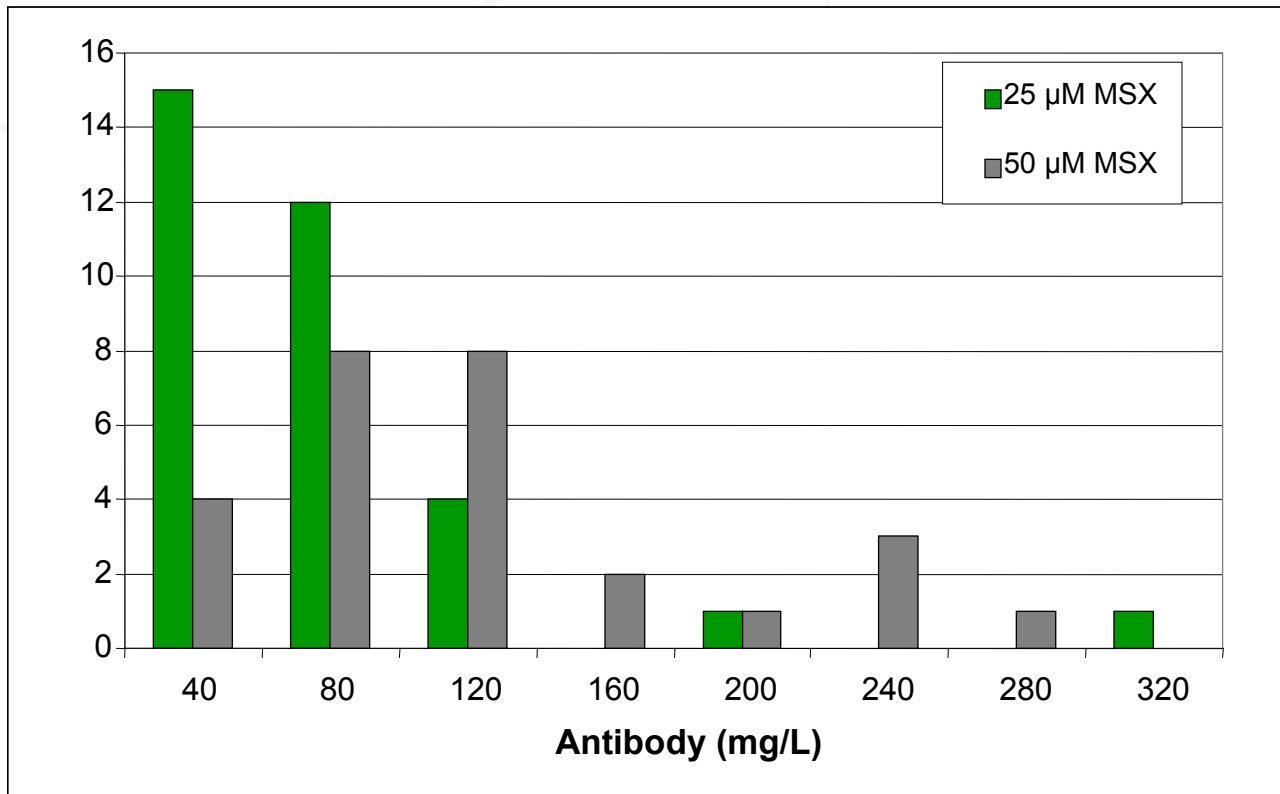
Transfection and Selection Conditions for GS-CHO Cell Lines Expressing cB72.3 Antibody

Electroporation condition	MSX (μM)	Stable transfectant numbers
1	25	68
	50	32
2	25	124
	50	57
3	25	197
	50	70

2.5 million cells electroporated for each condition



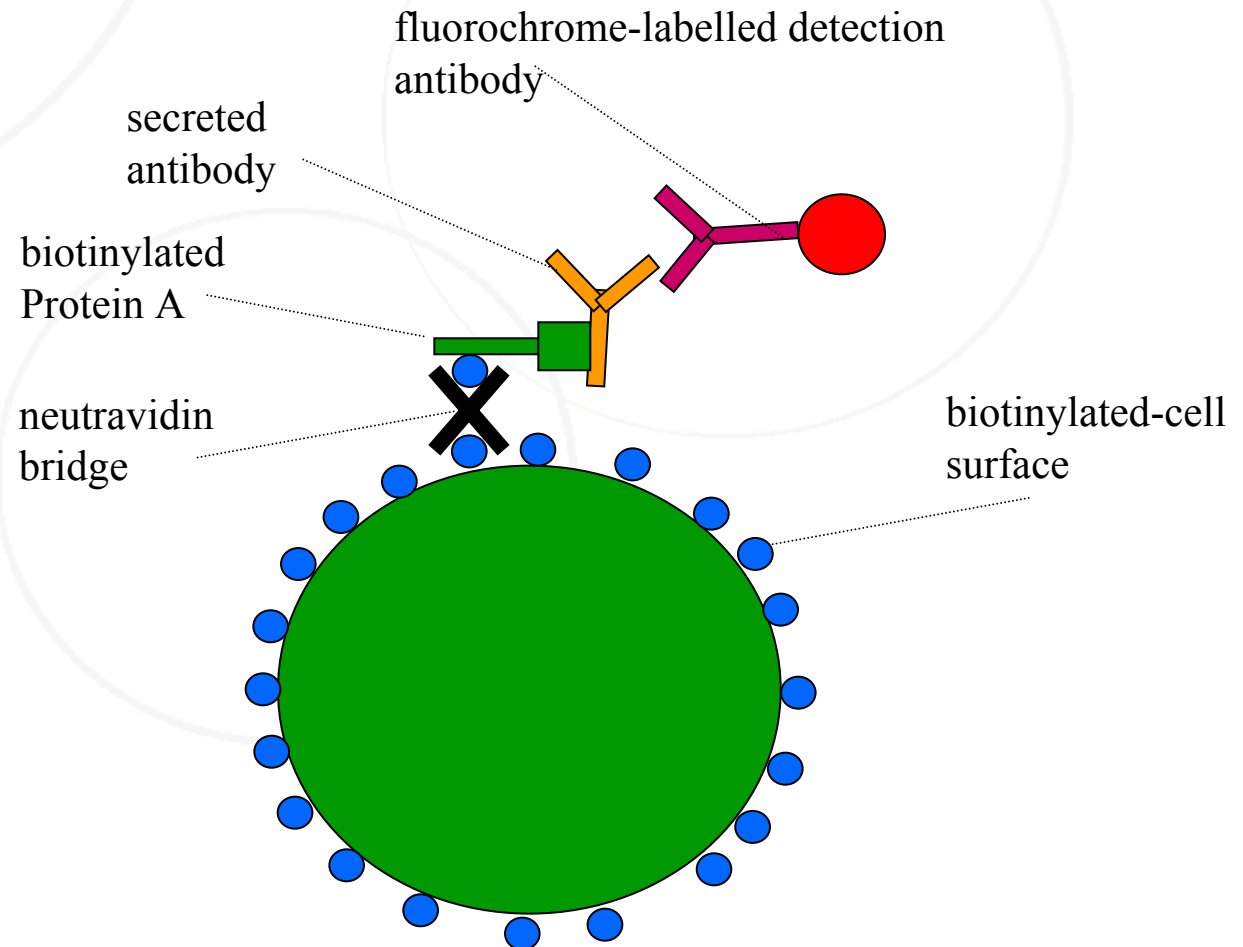
Influence of Selection Conditions for GS-CHO Cell Lines with cB72.3 Antibody



Cell lines have not been amplified.

Affinity-matrix surface capture

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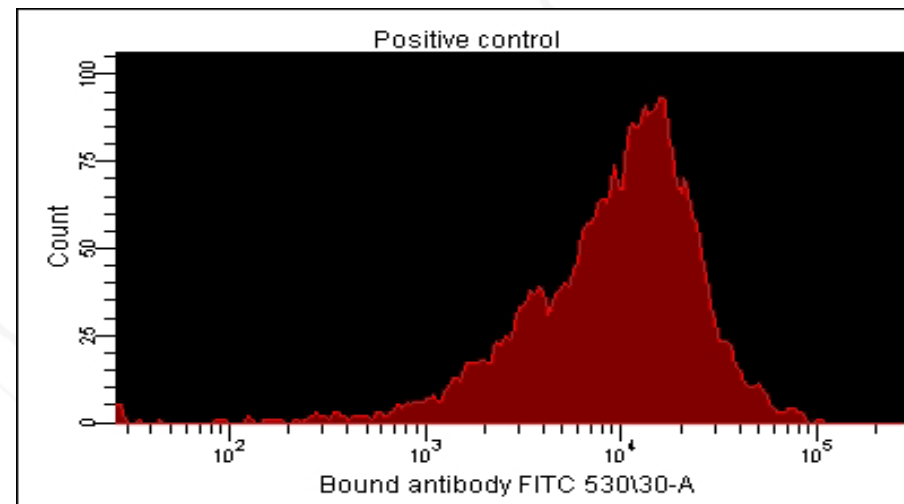
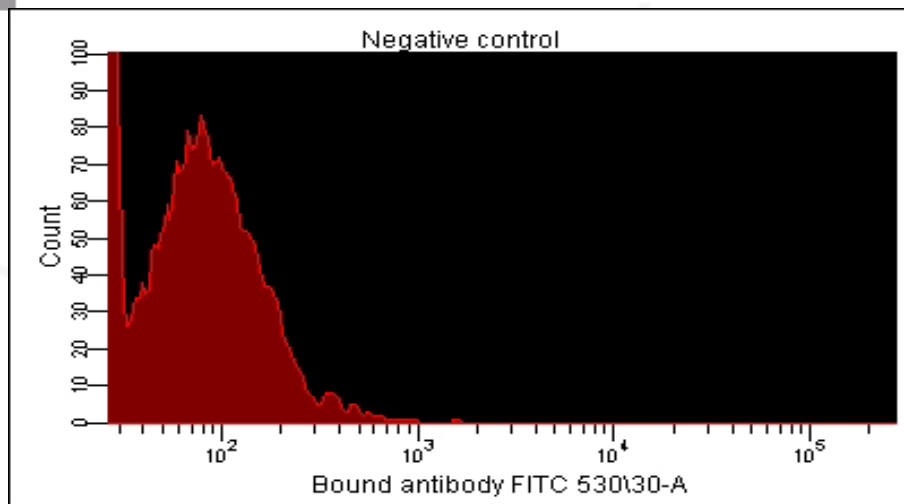


Flow cytometric analysis

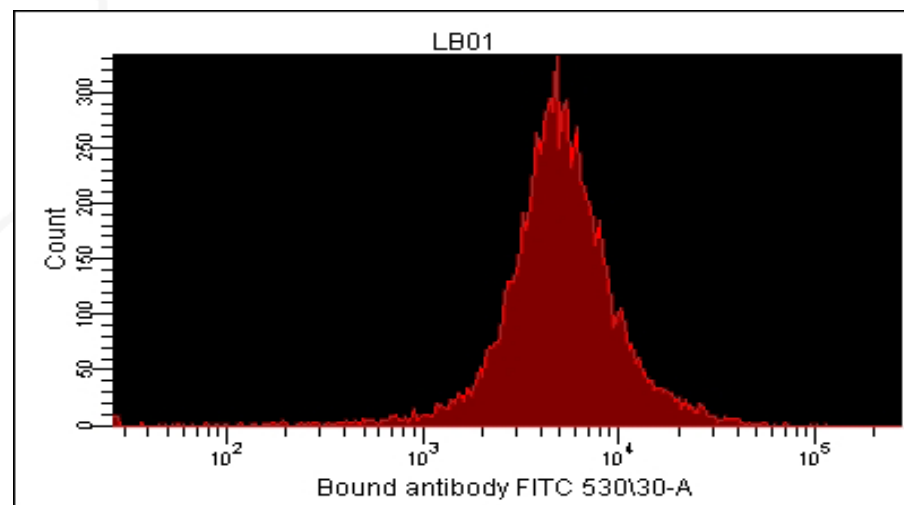
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Negative control

Positive control



GS-CHO cell line, LB01



Improving the Host Cell Line

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- Metabolic engineering
 - Glutamine independence using GS reduces ammonia accumulation
- Variant Selection
 - Cholesterol independent NSO variant
 - Suspension variant of CHO

Improving the Fermentation Process

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- Significant potential to improve processes
 - Physicochemical environment
 - Medium design and feeding strategies (including use of chemically defined media)

Chemically Defined Media (Protein Free)

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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

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Harvest

Purity of MAb at

Optimised Protein containing culture

<30%

Optimised protein free culture

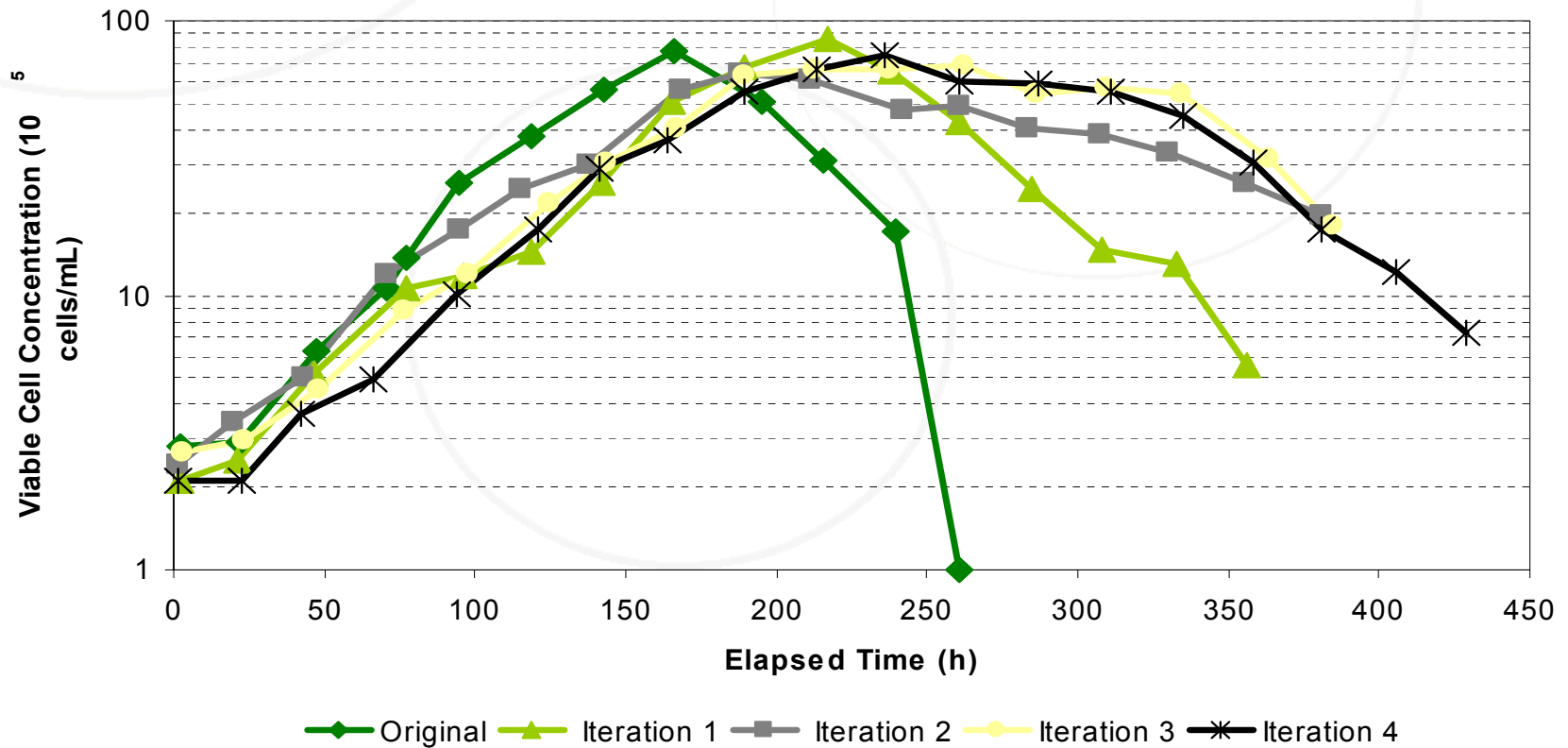
62%-76%

Medium Design and Feeding Strategies

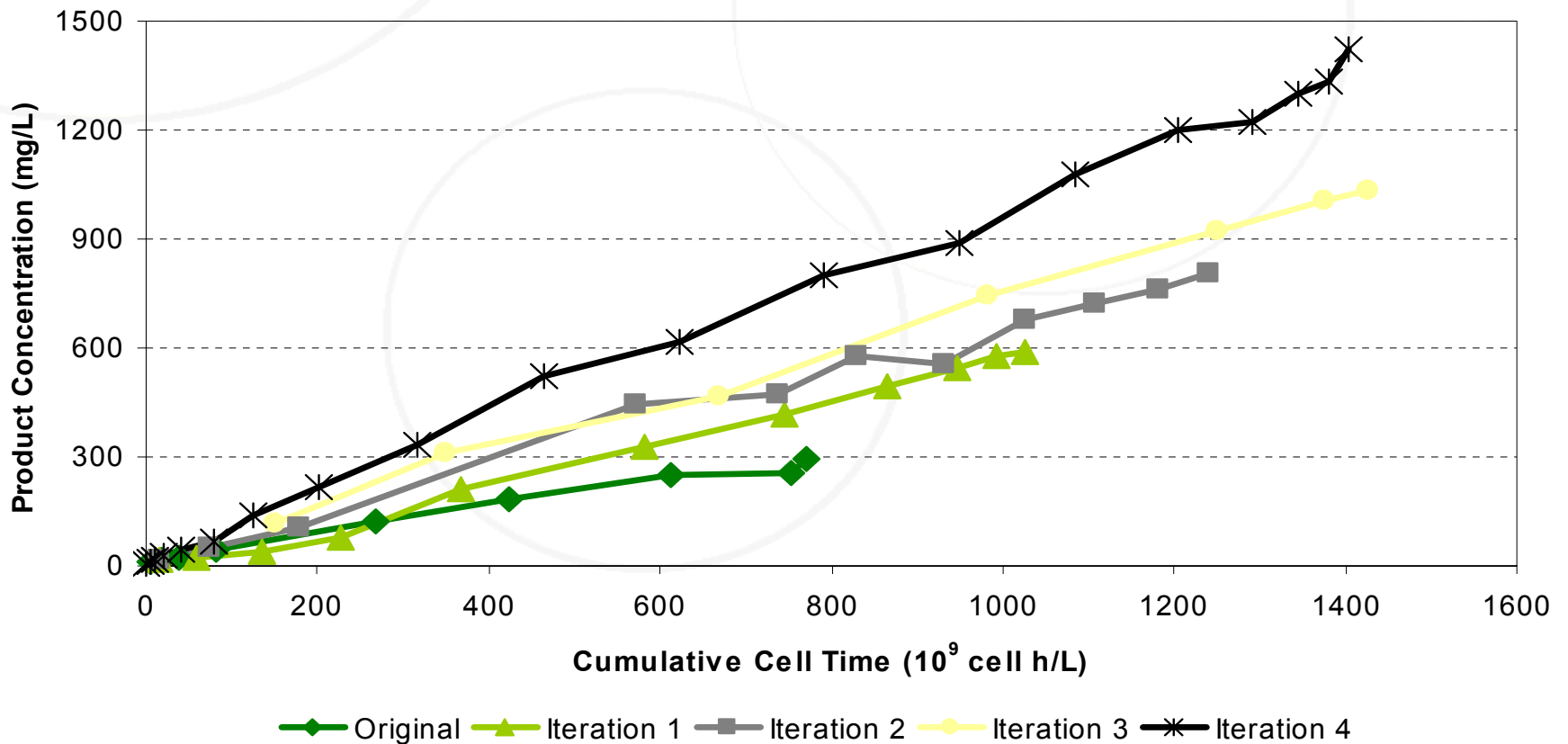
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- Optimise basal medium
- Optimise feeds
- Maintain nutrient sufficiency
- Minimise waste product formation

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

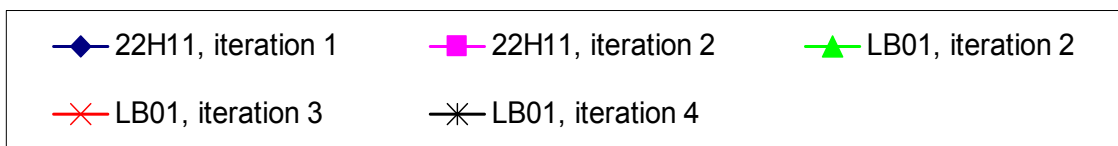
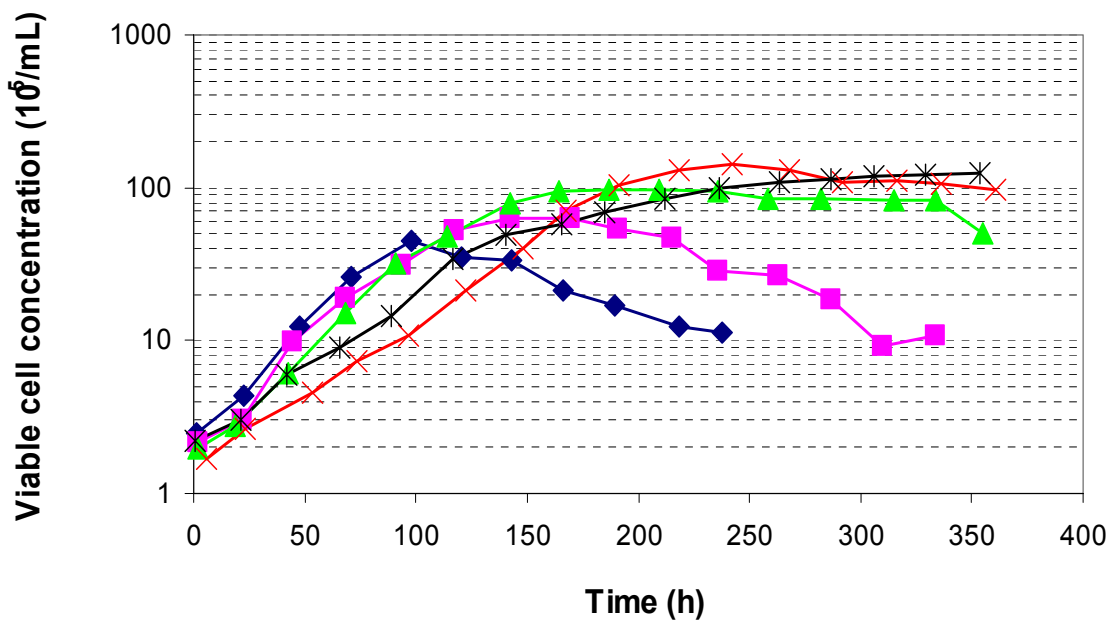
Process	Cumulative cell time (10^9 cell·h/L)	cB72.3 antibody (mg/L)	Process duration (d)
Serum-free	640	476	12
Original protein-free	772	293	12
Iteration 1	1026	589	15
Iteration 2	1239	807	16
Iteration 3	1427	1035	16
Iteration 4	1405	1422	18

Optimisation of a GS-CHO Process

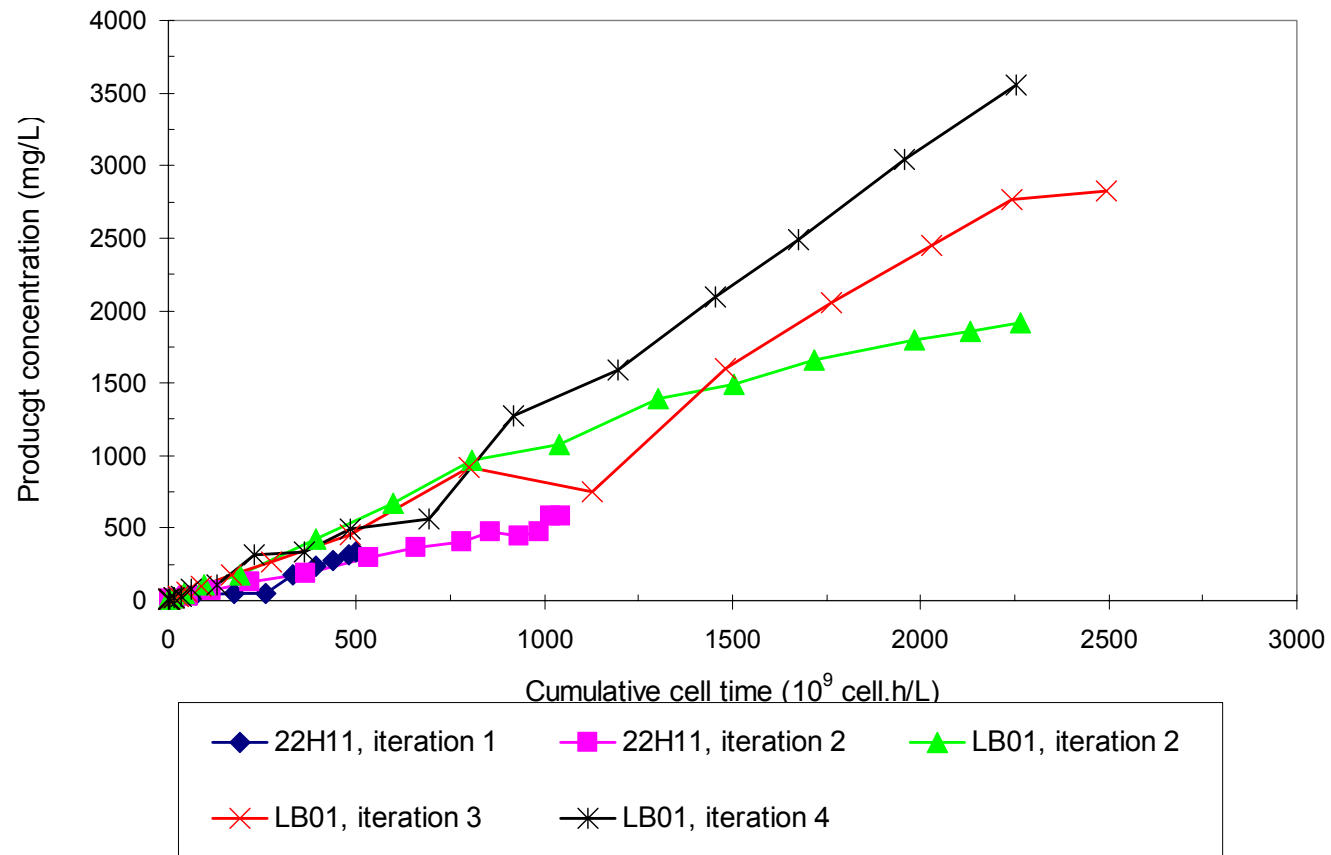
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- Similar approach taken as with GS-NS0 cell lines
 - Fed-batch culture, initially using the same feed as the GS-NS0 process
- Suspension variant of CHO-K1 which grows in chemically defined medium without need for adaptation (can take several months)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

GS-CHO Growth Characteristics



GS-CHO Product Accumulation

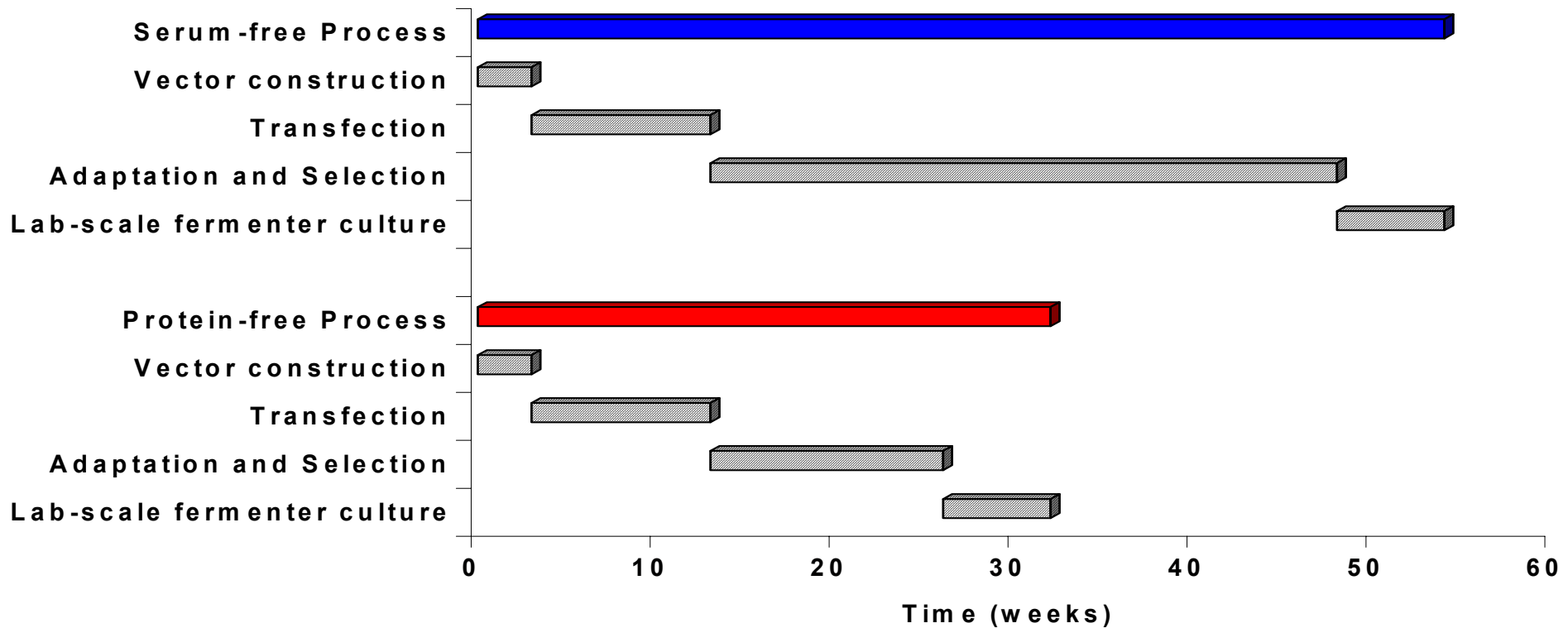


Chemically Defined, Protein-Free Bioreactor Process

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
LB01	Iteration 4	2254	3560	15

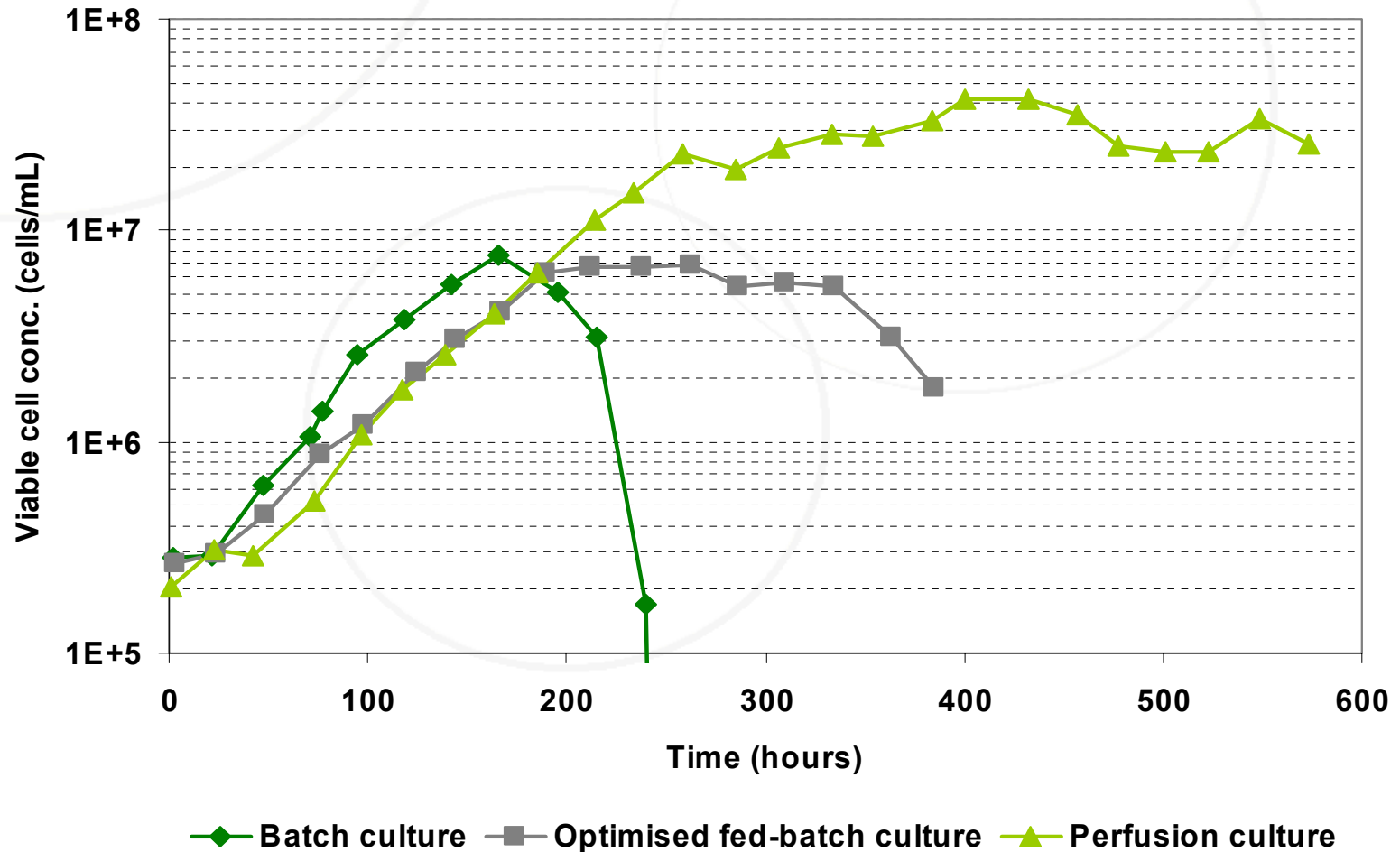
GS-CHO Process Development Timelines

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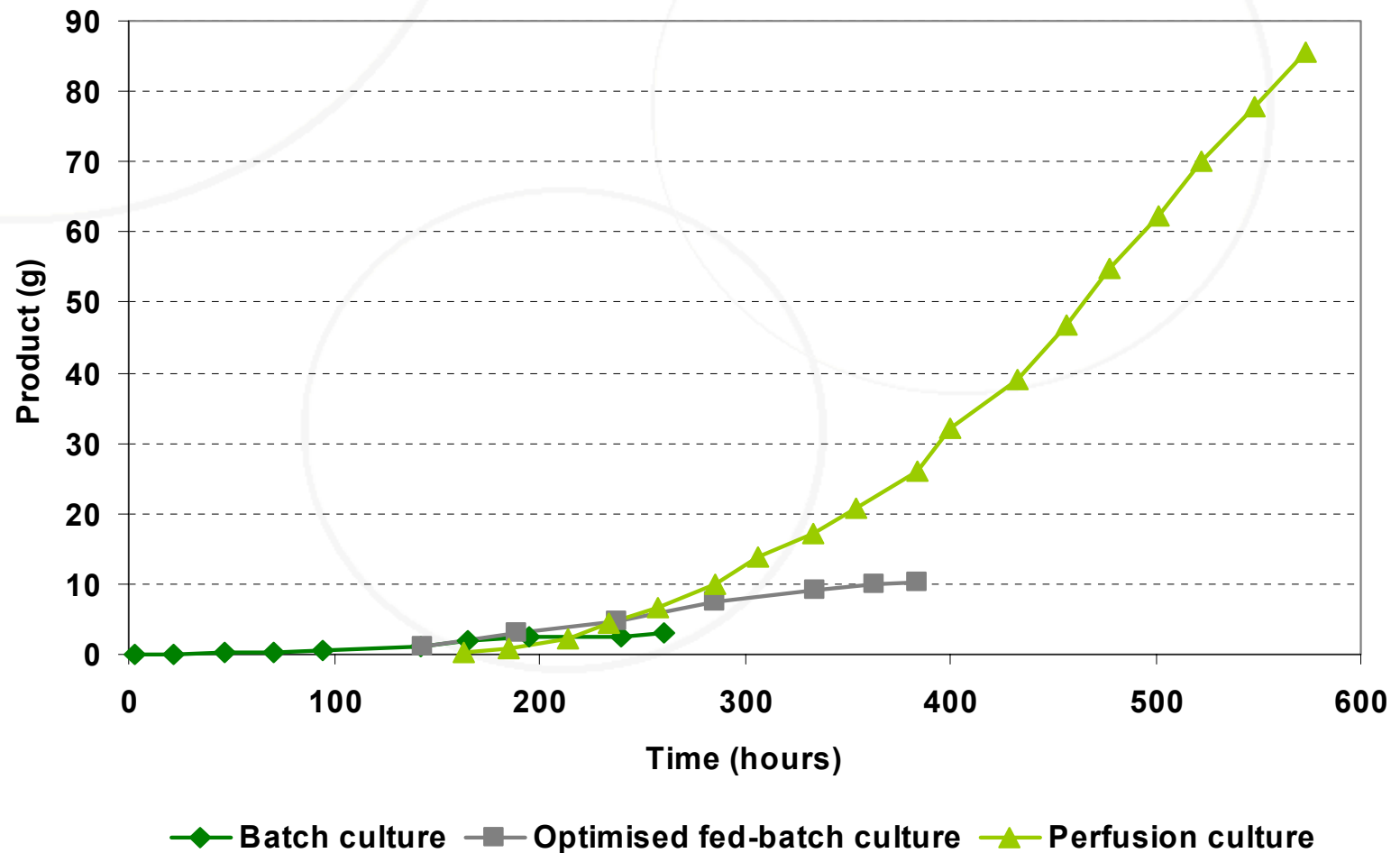
Comparison of Batch and Perfusion Culture for a GS-NSO Cell Line Making a Recombinant Antibody

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Comparison of Batch and Perfusion Culture for a GS-NSO Cell Line Making a Recombinant Antibody

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- Combination of efficient expression system (GS) and process optimisation gives high productivity for non-amplified 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