Strategies to Improve Process Operability and Shorten Process Duration for GS-CHO cell lines.

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ABSTRACT

Current GS-CHO processes run at Lonza Biologics have achieved product concentrations exceeding 4.5g/L at 200L cGMP manufacturing scale. Process improvement is currently focused on increasing product concentration, maintaining product quality and shortening bioprocess duration.

Previously, we showed that pH strongly influences growth and productivity kinetics of the current GS-CHO process operated at Lonza. Using an antibody producing GS-CHO cell line grown in 10L airlift bioreactors, we investigated the effect of varying dissolved oxygen tension (DOT) from 15% to 50% upon the kinetics of the current GS-CHO process. No major differences were seen in the kinetic profiles, indicating that the process is insensitive to changes in DOT.

Modification and reformulation of the feed to remove caustic components was evaluated. This improved process control by making pH control easier. A further benefit of these changes, most pertinent to large-scale operation, was improved safety since the caustic components needed to prepare this nutrient feed were eliminated.

INTRODUCTION AND AIMS

- Monoclonal antibodies are becoming increasingly important for the treatment of human disease. Currently at least 18 monoclonal antibodies are approved for therapeutic use. These antibodies are produced using mammalian cell culture, with the majority (83%) produced by recombinant DNA technology using either CHO or murine lymphoid cell lines.
- Over the past few years, we have continued developing the Glutamine Synthetase Gene Expression System (Lonza Biologics plc) for the rapid creation of highly productive cell lines for antibody production.
- The production of monoclonal antibody in CHO cells has been maximised through the use of an improved host cell line (CHOK1SV), better selection protocols to generate high yielding cells lines and improved bioreactor processes all contribute to the high (up to 5.5g/L) product concentrations achieved with GS-CHO cell lines.
- Improvement of the current GS-CHO processes is focused upon increasing product concentration, maintaining product quality, whilst shortening production bioreactor occupancy and improving process robustness. Process control strategies are a key part of the improved bioreactor processes improving monoclonal antibody production in CHO cells. Our previous process development studies showed that culture pH is a critical parameter for achieving high cell and product concentrations. As part of our on-going process development, we evaluated the effects of varying dissolved oxygen tension (DOT) upon the current GS-CHO process. The feeds currently used with the GS-CHO process contain a number of highly alkaline components. Replacement of these components with less alkaline ones could improve pH control and improve safety during large-scale manufacturing operation.

MATERIALS AND METHODS

Cell lines
A model CHO cell line (LB01) was used, which had been transfected with a GS vector encoding the genes for an IgG_4 antibody.

Medium and feed components
Medium: CD-CHO (Invitrogen).
Feeds: Proprietary chemically defined animal component free.

Procedure for Operating Laboratory-Scale Fermentations
10 litre airlift bioreactors were operated with on-line control of dissolved oxygen, temperature and pH.

Assays
Product concentration of the supernatant samples was quantified using Protein A affinity high performance liquid chromatography (HPLC). Product quality of Protein A purified samples was profiled by reduced and non-reduced SDS electrophoresis by LabCHIP using an Agilent 2100 Bioanalyser. Nutrient and metabolite concentrations were determined using a Nova Bioprofile 400 analyser.

RESULTS AND DISCUSSIONS

Physiochemical modifications

- The effect of varying DOT from 15% to 50% on cell culture performance and product concentration was investigated. Cell culture performance was assessed by comparing cell growth profiles and culture viability, the time integral of viable cell concentration (IVC), the specific antibody production rate and product concentration at harvest.
- A pilot study suggested that increasing the DOT from 15% to 40% resulted in a substantial increase in both maximum viable cell concentration and IVC: this was not seen with either 30% or 50% DOT. Additional runs were done at 15% and 40% DOT.
- The growth and productivity data (Table 1, Figures 1 & 2) do not show substantial differences in maximum viable cell concentration, IVC or antibody concentration at harvest. Minor differences are seen in some parameters e.g. time to reach maximum viable cell concentration.
- Overall, the data indicate that the current GS-CHO process is insensitive to changes in DOT over the range 15% to 50%. This is in agreement with the literature where other studies have shown that large changes in DOT have not substantially altered the growth or productivity kinetics of CHO cells (Subczynski *et al.*, 1992; Trummer *et al.*, 2006; Restelli *et al.*, 2006).
- Examination of the antibody-accumulation profiles for cultures grown at 15% and 40% DOT, show marked differences in the profiles between 200 and 360 h (Figure 2A). For the 40% DOT culture, 85 to 90% of the final antibody concentration is achieved between days 12 and 13, whilst for the 15% DOT culture this level is achieved between days 14 and 15.

Table 1: Table showing the effects of DOT on cell growth kinetics and antibody concentration. Values shown in brackets represent the standard deviation of multiple fermentations operated under each condition.

DOT (%)	15 (n=5)	30 (n=2)	40 (n=5)	50 (n=2)
[Max Viable Cell] (10^6 viable cells/mL)	14.7 (± 1.21)	14.7 (± 2.19)	19.7 (± 2.09)	14.6 (± 4.7)
Elapsed time to reach Max Viable Cell Conc. (day)	15	11	12	12
IVC at harvest (10^6 cells/h/mL)	3902 (± 1259)	3505 (± 1133)	5535 (± 2178)	3232 (± 1677)
Product at harvest (g/L)	3.5 (± 0.89)	3.8 (± 0.09)	3.6 (± 0.22)	3.7 (± 0.25)
Specific antibody production rate (mg/ 10^9 cells/h)	0.9 (± 0.59)	1.1 (± 0.39)	0.7 (± 0.26)	0.8 (± 0.51)
Volumetric productivity (g/L/day)	0.22	0.24	0.27	0.24

Figure 1: Figure showing the effects of increasing DOT from 15% (blue line) to 40% (red line) on viable cell concentration in a 15 day fermentation process. The error bars on the graphs represent the standard deviation of the average of 5 fermentations run under each condition.

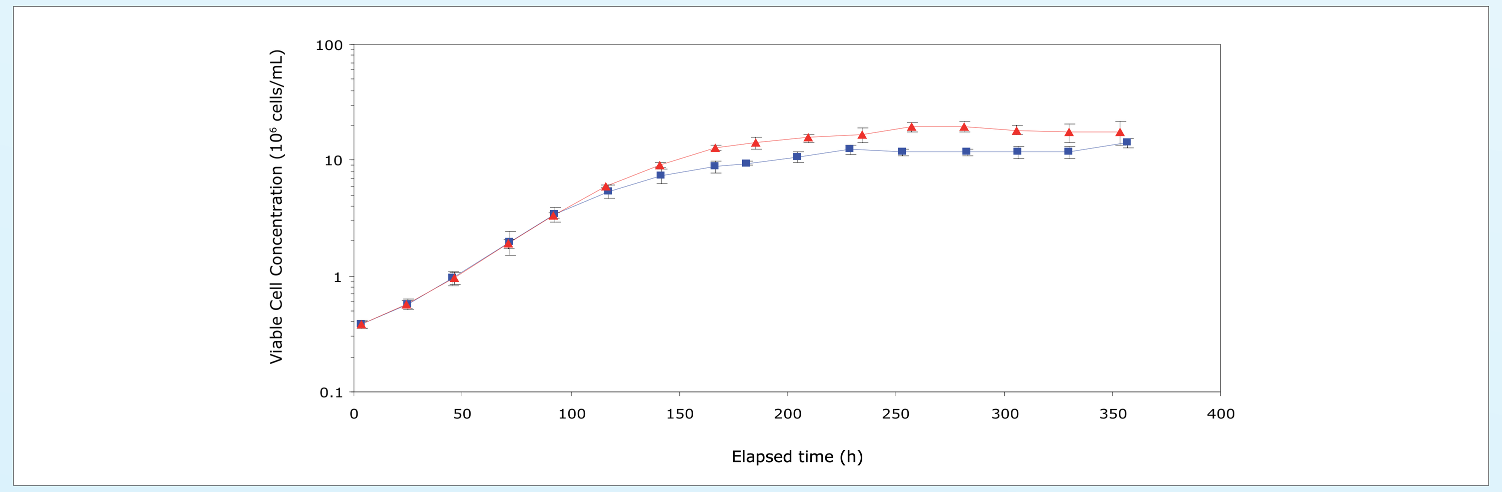
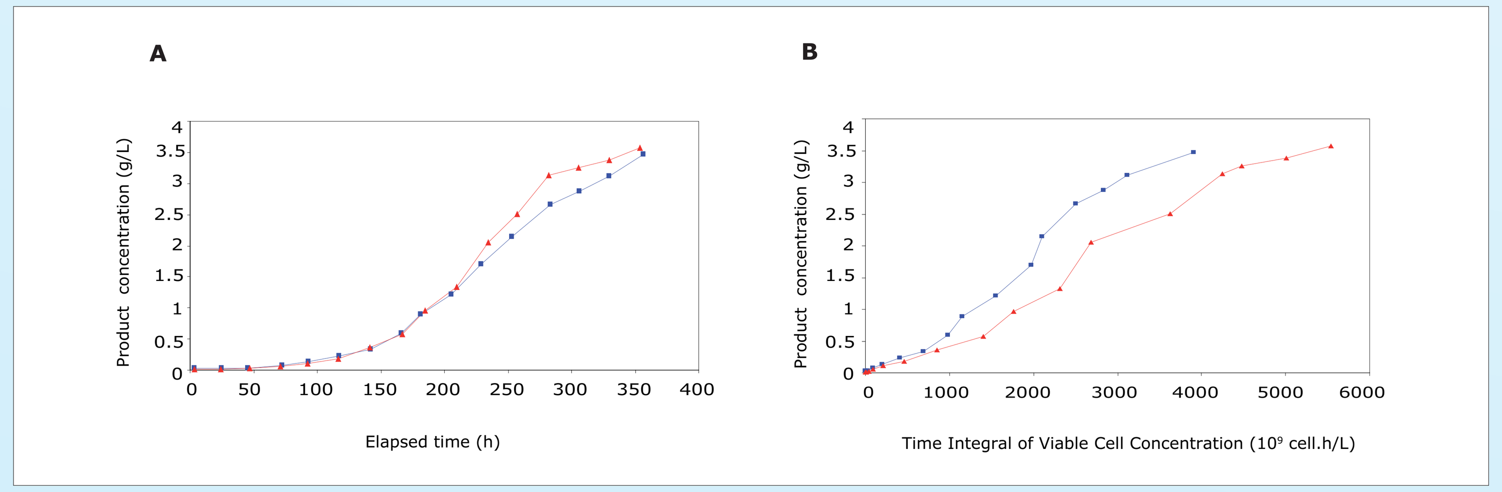
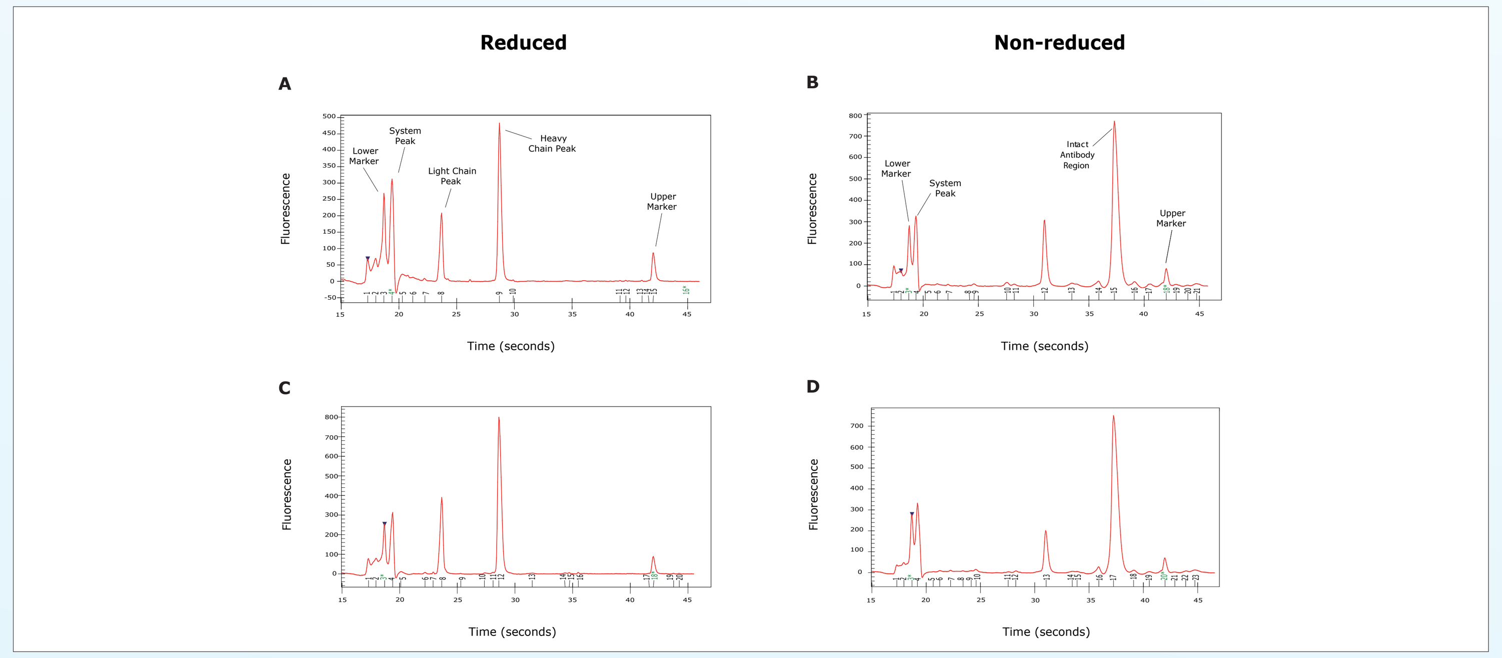


Figure 2: Figure showing the effects of increasing the DOT from 15% (blue line) to 40% (red line) on product concentration (A) and the specific antibody production rate (B) in a 15 day fermentation process.



- These results suggest that switching from 15% to 40% DOT could potentially reduce the occupancy of the production bioreactor with minimal reduction in product concentration. Further work is needed to confirm this.
- Preliminary product quality analysis by SDS electrophoresis shows no gross differences when the DOT set point was increased from 15% to 40% (Figure 3). More detailed analyses (e.g. oligosaccharide profiling) are needed to confirm this (in progress).
- The electropherogram profiles of reduced samples were comparable with the reference and between each other. The heavy and light chain peaks have molecular weights of approximately 60 kDa and 25 kDa respectively.
- The electropherogram profiles of non-reduced samples were also comparable with the reference and between each other. The intact antibody had a molecular weight of approximately 157 kDa. An additional fragment was also observed in both conditions which had a molecular weight of approximately 87 kDa. This half-antibody structure is commonly observed with IgG_4 molecules.
- In summary, the data show that the current GS-CHO process is insensitive to changes in DOT over the range of 15% to 50%. However, there appears to be the potential to reduce production bioreactor occupancy, by using 40% compared to 15% DOT.

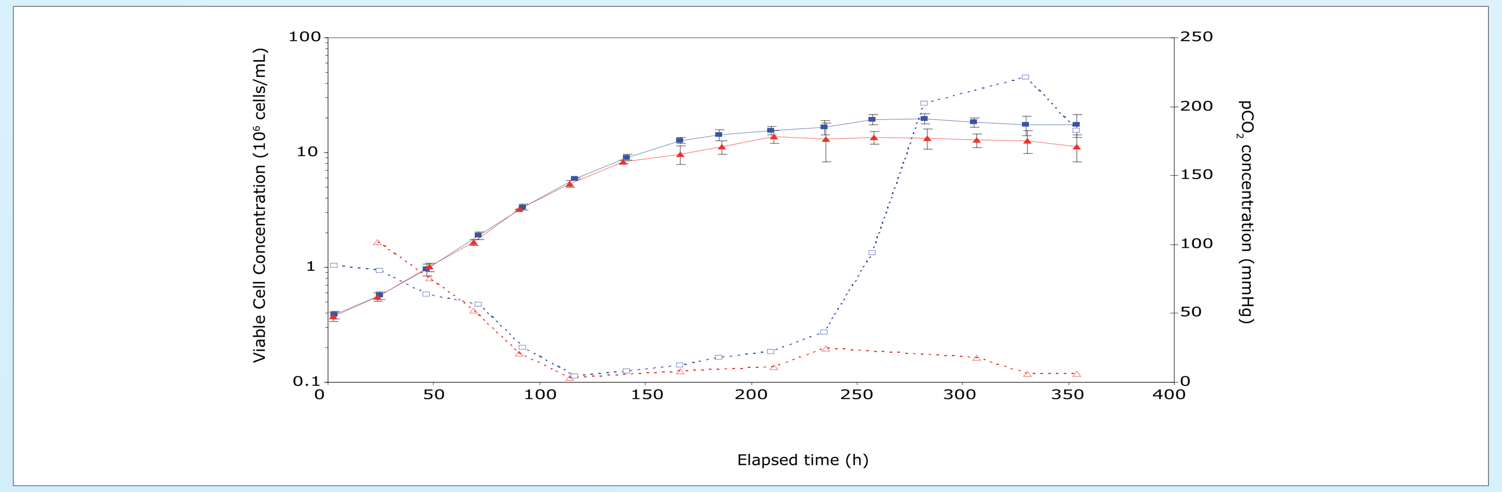
Figure 3: Figure showing reduced and non-reduced electropherogram images of harvest samples taken from fermentation processes in which the DOT was set at 15% (A and B) and 40% (C and D).



Nutritional modifications

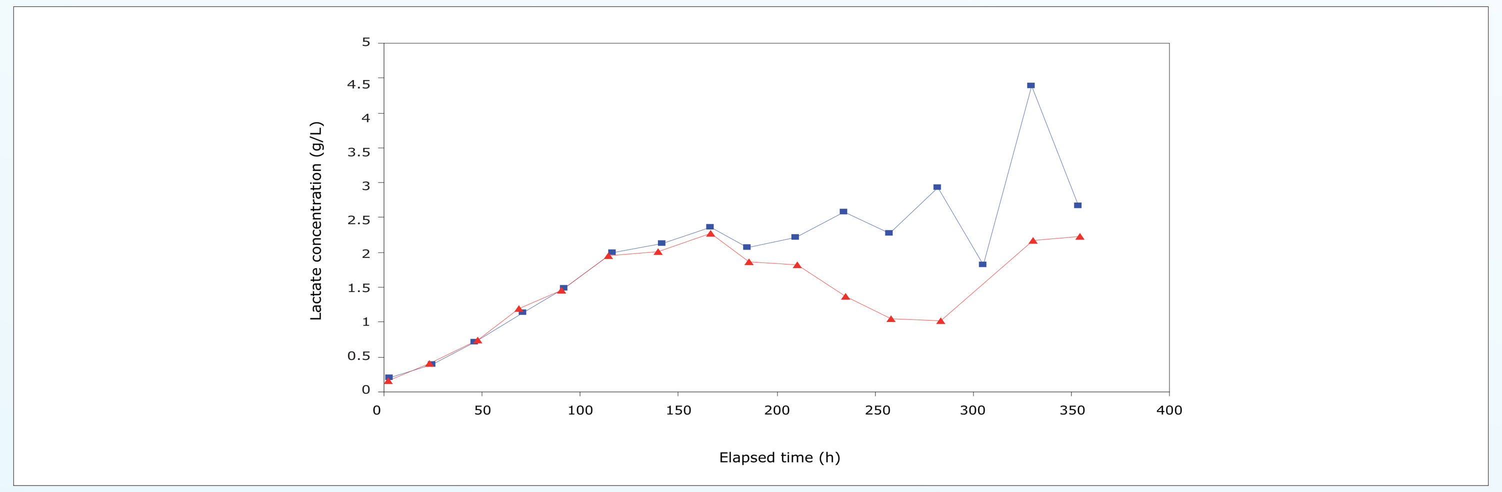
- Solubility issues with nutrients currently used in feeds often requires large amounts of NaOH to be added to aid dissolution of the solutes.
- The use of alkaline feeds causes problematic osmolality increases or CO_2 partial pressure (pCO_2) accumulation that increases the need for base addition. Also, the use of NaOH to aid solute dissolution poses safety issues when preparing multiple 100L batches of feed.
- To improve process operability and robustness, nutrient feeds were modified and reformulated in order to remove caustic components.
- Removal of caustic components had no impact on maximum viable cell concentration when compared to results obtained using the original feed (Figure 4). In addition no substantial difference in antibody concentration was observed at harvest (3.6g/L using the original feed versus 3.4g/L using the reformulated feed).
- Typically, elevated pCO_2 levels were observed towards the end of the fermentation process when using the original feed (reaching values of 210 mmHg) (Figure 4). Such levels have been shown to result in a reduction in specific growth rate (Zhu *et al.*, 2005).
- Utilizing the reformulated feed, it was possible to restrict the pCO_2 levels to a maximum of 102 mmHg (Figure 4).

Figure 4: Figure showing the effects of using either the original (blue line) or modified (red line) feed on viable cell (solid lines) and pCO_2 concentrations (dotted lines).



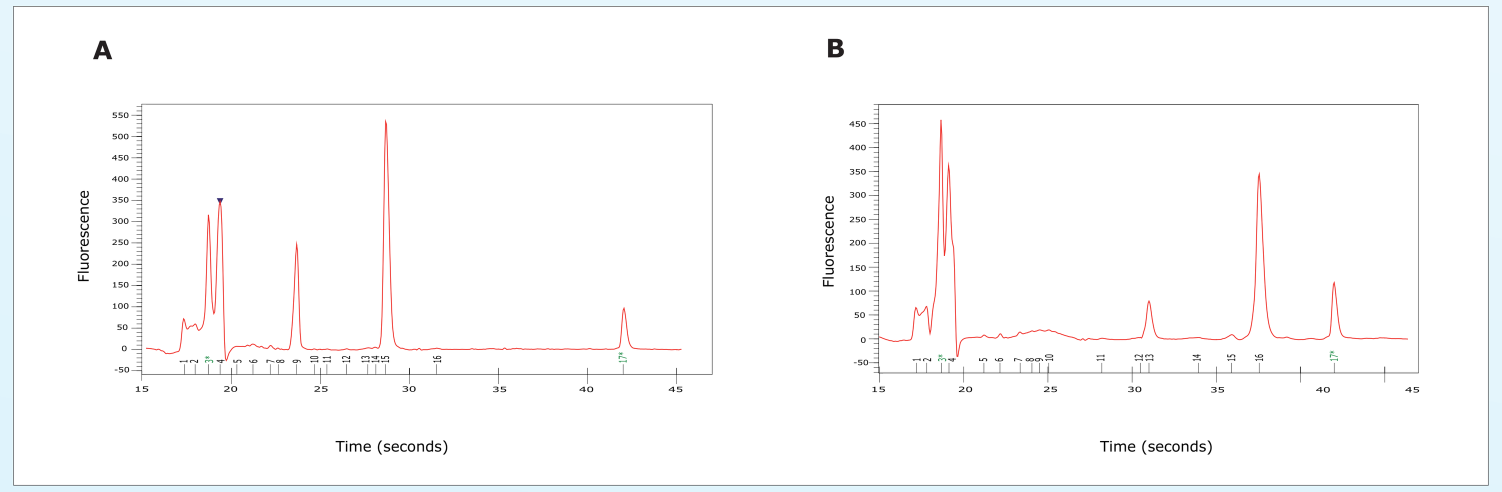
- Furthermore, osmolality levels reached at harvest when using the modified feed were reduced by 24% compared to levels reached using the original feed.
- A reduction in lactate production was also apparent in fermentations using the reformulated feed, suggesting a shift in cell metabolism (Figure 5).
- This reduction in lactate concentration simplifies process operability and improves process robustness as there is a significant reduction in the requirement for alkali addition, making pH control easier.

Figure 5: Figure showing the effects of using either the original (blue line) or modified (red line) feed on lactate concentrations.



- Initial data show no gross differences in product quality by SDS electrophoresis when either the original or the modified feed was used (Figure 6).
- The electropherogram profiles of reduced samples were comparable with the reference and between each other. The heavy and light chain peaks have molecular weights of approximately 60 kDa and 25 kDa respectively.
- The electropherogram profiles of non-reduced samples were also comparable with the reference and between each other. The intact antibody had a molecular weight of approximately 157 kDa. An additional half-antibody fragment, molecular weight of approximately 87 kDa, was also observed in all samples. This was expected as the molecule is an IgG_4 antibody.

Figure 6: Figure showing reduced (A) and non-reduced (B) electropherogram images of harvest samples taken from fermentation processes in which a modified, reformulated feed was used. Control reduced (C) and non-reduced (D) electropherogram images are displayed in Figure 3.



- In summary, modification and reformulation of the original feeds resulted in a significant decrease in pCO_2 and osmolality levels at harvest whilst having no impact on product concentration or specific antibody production rate.
- Furthermore, use of this feed would eliminate the requirement for caustic components in feeds. This safety issue is most pertinent to large scale operation when making multiple 100L batches of feed.

SUMMARY

- The current GS-CHO process operated at Lonza Biologics is robust to changes in DOT over the range 15% to 50%.
- The data indicate that production bioreactor occupancy can be reduced with minimum impact upon product concentration by operating the process at 40% DOT compared to 15% DOT.
- Reformulation of the feeds to remove strongly alkaline components improved process control and process safety.

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