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Research Article

An Improved Cell Culture Process for Production of Omalizumab

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Abstract

The demand for biopharmaceutical products such as monoclonal antibodies (MAbs), fusion proteins, viral vaccines, and hormones is rapidly increasing. Mammalian cell cultures with ease of process operations are adopted to get the desired product. The challenges of low productivity and impurities in the desired product can be eliminated by optimizing the process parameters. The present study focuses on the optimization of temperature with other parameters to achieve improved titer and reduced heterogeneity in Omalizumab. An anti-IgE antibody expressed in mammalian cell culture. It is noticed that maintaining a specific temperature of 36.5° C ± 0.5 and pH 6.8± 0.1 during fed-batch cell culture provides improved quality (reduced galactosylation and reduced acidic variants) and quantity (improved titer) of the Omalizumab.

Keywords: antibody, omalizumab, mammalian cells, acidic variants

INTRODUCTION

Mammalian cell cultures such as Chinese hamster ovary (CHO) cells are widely used for the commercial production of therapeutic biomolecules like monoclonal antibodies and proteins[1]. Several process parameters like pH, temperature, pO2, CO2, air flow, feeding, agitation rate etc. are essential to maintain cells in a culture environment[2]. Temperature and pH being one of the critical parameters requires optimization for enhanced cell growth, viability, productivity for the generation of therapeutic biomolecules. The optimization of fed-batch culture for the production of monoclonal antibodies with desired quantity with desired quality is a challenging process and usually it depends on particular biotherapeutic molecule as well[3]. Omalizumab is a recombinant DNA-derived humanized IgGlK monoclonal antibody with a molecular weight of approximately 149 kD that selectively binds to human immunoglobulin (IgE). Omalizumab inhibits the interaction of IgE with high affinity IgE receptor (FceRI)[4]. Acidic species or acidic variants produced during the production process are defined as the antibody variants that elute earlier than the main peak during CEX or later than the main peak during AEX analysis. Sialic acid or deamidation has been commonly reported to contribute to the formation of acidic species. These charge variants substantially affect the in vitro and in vivo properties of antibodies[5]. Galactose a unit for the glycosylation chain reaction by linking it next to a N-acetylglucosamine sugar via galactosyltransferase[6]. Galactosylation is known to affect the process of complement dependent (CDC), as the highest modes of action (MOA) of monoclonal antibodies. Furthermore, the low amount of terminal galactosylation required to increase serum half-life of antibody [7,8]. The present study follows the production of omalizumab antibody at temperature 36.5° C ± 0.5 and maintaining pH 6.8± 0.1 during production phase to get enhanced titer and desired characteristics likely reduced galactosylation and/or reduced acidic variants in omalizumab antibody.

MATERIAL AND METHODS

The monoclonal antibody cell bank vial was thawed in Shake flask 125 (SF125) (corning make) into the seed media supplemented with growth nutrient and cell counts were checked. The incubation was continued for 3-4 days during which cells reach the optimum cell density for subculture. cells were subsequently expanded to Shake flask 250 (SF250) and Shake flask 500 (SF500) to generate sufficient inoculum for 5L glass bioreactors (working volume 3500 mL) and Shake flask 125 (SF125), Shake flask 500 (SF500) and Shake flask 3000 (SF3000) to N-1 50 L (seed bioreactor) to generate sufficient inoculum for 200L bioreactors (working volume 185 L) and 500L bioreactor (working volume 360 L). The production culture starts with production media supplemented with growth nutrient. Additions are made to the bioreactor over the 12-day run including scheduled feeding of Feed 1 and Feed 2, with glucose solution and antifoam added as needed. Production fed-batch bioreactor run was executed at 5L, 200L and 500L bioreactor (Sartorius make) using process parameters as mentioned in Table 1-3. The overall batch cycle was for 12 days and was harvested depending upon the culture viability.

Table 1: Process parameters for 5L bioreactor

Control Process			
Sr. No	**Process Parameter**	**Unit**	**Setpoint/parameter details**
1	Post Inoculation Initial volume	mL	3500
2	DO%	% Saturation	40
3	pH	N/A	7.1 ± 0.3 day 0-3, 6.7 ± 0.1 day 3-harvest
4	Temperature	˚C	37.5 or 35.5
5	Agitation rate	RPM	250
6	Initial seeding cell density	x 10^6 cells/mL	0.5
7	Feed medium	% v/v	3.3% (Day 3-11)
8	Sodium bicarbonate	mL	As per requirement for pH maintenance (Auto mode)
9	Antifoam	%	Up to 10% addition (as required)
10	% DO maintenance by	mL/min	Air +O2
11	Air flow.	mL/min	0-200
12	O_2 flow	mL/min	0-500
13	Air overlay flow rate	mL/min	0-100
14	Harvest Criteria	N/A	Day 12 or Viability ≤ 70 % which comes first

Temperature 36.5° C Process			
Sr. No	**Process Parameter**	**Unit**	**Setpoint/parameter details**
1-14	All parameters as per Control Process except below parameters		
15	Temperature	˚C	36.5
16	pH	N/A	7.1 ± 0.3 day 0-3, 6.8 ± 0.1 day 3-harvest

Table 2: Process parameters for 200L bioreactor

Sr. No	Process Parameter	Unit	Setpoint/parameter details
1	Post Inoculation Initial volume	L	185
2	DO%	% Saturation	40%
3	pH	N/A	7.1±0.3 Day 0-3 6.8±0.1 Day 3-12
4	Temperature	°C	36.5°C ± 0.5°C
5	Agitation rate	RPM	108
6	Initial seeding cell density	x 106 cells/mL	0.5
7	Feed medium	% v/v	Feed 3.3% Days 3 to 11 inclusive
8	Sodium bicarbonate	mL	As per requirement for pH maintenance (Auto mode)
9	Antifoam	%	Up to 10% addition (as required)
10	% DO maintenance by	L/min	Air +O2
11	Air flow.	L/min	3.2 lpm
12	O2 flow	L/min	On demand (cascade)
13	Air overlay flow rate	L/min	1.0 lpm
14	Harvest Criteria	N/A	Day 12 or within 24 hours when the cell culture viability is < 70%

Table 3: Process parameters for 500L bioreactor

S.No	Process Parameter	Set point/parameter details
1	Post Inoculation Initial volume (L)	360L
2	Initial VCD (× 10^6 cells/mL)	0.50
3	Temperature (°C)	36.5°C ±0.5°C
4	pH (Day 0 - Day 2)	7.1/ dead band 0.3
5	pH (Day 2 - Day 3)	7.0/ dead band 0.3
6	pH (Day 3 – Harvest)	6.8 / dead band 0.1
7	Dissolved Oxygen % (% Saturation)	40
8	Agitation speed (RPM)	74
9	Overlay Air (L/min)	3.0
10	Air flow rate (L/min)	Day 0 - 3.0, Day 1 - 6.0, Day 2 - 9.0, Day 3 - 12.0
11	Oxygen flow rate (L/min)	On demand (cascade)
12	CO_2 flow rate (L/min)	On demand (cascade)
13	Antifoam Strategy (%)	Add 10% antifoam “as needed basis”
14	Feeding Strategy (% v/v)	3.3% Days 3 to 11
15	Sodium Bicarbonate (mL)	7.5% Sodium Bicarbonate As per need to maintain pH set point
16	Harvest Criteria	Day 12 or within 24 hours when the cell culture viability is < 70%

RESULT AND DISCUSSION

The study was performed for the production of omalizumab without modulating the temperature with setpoint 36.5°C±0.5 and pH 6.8± 0.1 to obtain increased omalizumab titer with reduced percentage of acidic variants and reduced percentage of total galactosylation in omalizumab.

1) Process run at 36.5 °C and pH 6.8± 0.1 vs Process with 37.5 ° C and pH 6.7

In 5L bioreactor, the fed-batch process parameters were kept at 36.5°C temperature and pH set point of 7.1 ± 0.3 day 0-3, 6.8 ± 0.1 day 3 till harvest. Along with it control process with 37.5°C and pH 6.7 in 5L bioreactor fed-batch run was conducted. Harvest was performed in both the batches by d) centrifugation of cells at followed by 0.2 um filtration. NPEL generated from day 12 harvests and samples were analyzed for titer by protein A HPLC, %Acidic charge variants by CEX-HPLC method and N-Glycosylation (% Total galactosylation) was checked by HILIC-UPLC method. Following observations were made pertaining to Titer and product quality as below.

a) Titer was increased by 135.6% (Batch 1), 128.2% (Batch 2), 162.8% (Batch 3).

b) Total % Galactosylation was decreased by 16.1 % (Batch 1), 31.8% (Batch 2), 15.7% (Batch 3).

c) %Acidic charge variants were decreased by 29.1% (Batch 1), 30.6% (Batch 2), 28.8% (Batch 3).

Parameters	Control Process with 37.5 ° C and pH 6.7	Process with 36.5°C and pH 6.8± 0.1 (Batch 1)	Process with 36.5°C and pH 6.8± 0.1 (Batch 2)	Process with 36.5°C and pH 6.8± 0.1 (Batch 3)
Titer (g/L)	1.91	4.50	4.36	5.02
Total Galactosylation (%) by N-Glycan Analysis	15.15	12.7	10.32	12.76
% Acidic charge variants (%)	17.51	12.4	12.14	12.45

2) Process run at 36.5 °C and pH 6.8± 0.1 vs Process with 35.5 °C and pH 6.8

The process with 35.5°C temperature and pH set point of 7.1 ± 0.3 day 0-3, 6.8 day 3 – day 12 (harvest) in 5L bioreactor fed-batch run was conducted. Harvest is performed by centrifugation of cells at followed by 0.2 um filtration. NPEL generated from day 12 harvest and samples were analyzed for titer by Protein A HPLC, %Acidic charge variants by CEX-HPLC method and N-Glycosylation (% Total galactosylation) was checked by HILIC-UPLC method. Following observations are made pertaining to Titer and product quality shown below when compared with 36.5 °C and pH 6.8± 0.1.

a) Titer was increased by 64.23.%.

b) Total % Galactosylation was decreased by 2.38 %.

Parameter	Process with 35.5°C and pH 6.8	Process with 36.5°C and pH 6.8± 0.1
Titer (g/L)	2.74	4.50
Total Galactosylation (%) by N-Glycan Analysis	13.01	12.7

3) 200L bioreactor fed-batch process run at 36.5 °C and pH 6.8± 0.1

The process with 36.5°C temperature and pH set point of 7.1 ± 0.3 day 0-3, 6.8 ± 0.1 day 3-harvest in 200L bioreactor fed-batch run was conducted. Harvest was performed by two-stage depth filtration followed by 0.2 um filtration. NPEL generated from Day 12 harvest and sample ware analyzed for titer by protein A HPLC, %Acidic charge variants by CEX-HPLC method and N-Glycosylation (% Total galactosylation) was checked by HILIC-UPLC method. Following observations were made pertaining to Titer and product quality shown below when compared with control process results at 37.5°C and pH 6.7.

a) Titer was increased by 105.75%.

b) Total Galactosylation was decreased by 41.98 %.

c) %Acidic charge variants were decreased by 11.9%.

Parameters	Scale 200 L
Day 12 Titer (mg/mL)	3.93
Day 12 Total Galactosylated (%) by N-Glycan Analysis	8.79
Day 12 Acidic charge variants (%)	15.41

4) 500L bioreactor fed-batch process run at 36.5 °C and pH 6.8± 0.1

The process with 36.5°C temperature and pH set point of 7.1 ± 0.3 day 0-2, 7.0 ± 0.3 day 2-3, 6.8 ± 0.1 day 3-harvest in 500L bioreactor fed-batch run was conducted. Harvest was performed by two-stage depth filtration followed by 0.2 um filtration. NPEL generated from Day 12 harvest and sample ware analyzed for Titer by protein A HPLC, %Acidic charge variants by CEX-HPLC method and N-Glycosylation (% Total galactosylation) was checked by HILIC-UPLC method.

Following observations were made pertaining to Titer and product quality shown below when compared with control process results at 37.5°C and pH 6.7.

a) Titer was increased by 150.75%.

b) Total % Galactosylation was decreased by 40.59%.

c) %Acidic charge variants were decreased by 6.91 %.

Parameters	Scale 500 L
Day 12 Titer (mg/mL)	4.79
Day 12 Total Galactosylated (%) by N-Glycan Analysis	9
Day 12 Acidic Variants (%)	16.30

CONCLUSION

The mammalian cell culture process is very complex and significantly affects the quality and quantity of protein. The selection of pH, temperature, duration, media selection, and other parameters make the cell culture complex and scientists need to optimize culture conditions to obtain the desired product. In the present study, we investigated the effect of specific temperature and pH on the quality and quantity of Omalizumab which is produced at a large scale through a fed-batch cell culture process. It is found that a specific temperature of 36.5° C ± 0.5 and pH 6.8± 0.1 maintained during fed-batch cell culture is most suitable for the production of omalizumab as it provides high titer with reduced undesired product such as reduced galactosylation and reduced acidic variant of Omalizumab. Further, this study avoids the requirement of temperature shift during cell culture, and thereby cell culture can be performed for long duration without shifting the temperature.

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Conflict of interests

Authors declare that there is no conflict of interest.

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