# Process optimization and scale-up for the production of a diagnostic monoclonal antibody against congenital adrenal hyperplasia (CAH)

A thesis presented in partial fulfillment of the requirements for the degree of

Doctor of Philosophy
in
Bioprocess Engineering

at Massey University, Manawatu Campus,
New Zealand

Gek Kee, Chua @ Yeo

2010

## ABSTRACT

The objective of this work was to optimize and scale up the suspension culture of the hybridoma 192 cell line for producing a diagnostic monoclonal antibody (MAb) against Congenital Adrenal Hyperplasia (CAH), a rare human genetic disorder. An inexpensive and simple culture method was desired.

The hybridoma 192 had previously been grown only in serum-containing media, mostly in static flasks. Baseline data were obtained in tissue culture flasks (T-flask) with the cells grown in Dulbecco Modified Eagle's Medium (DMEM) supplemented with 5% fetal bovine serum (FBS). Cells were first adapted to grow in DMEM with a reduced serum content of 2%. These adapted cells were grown in T-flasks, spinner flasks and a 2 L stirred tank bioreactor, to assess process scalability. Compared to T-flasks and spinner flasks, a high specific MAb production rate could be achieved in the highly controlled conditions of the 2 L bioreactor.

In the next step, the serum content of the media was further reduced to 0.4% by adding inexpensive components (ferric citrate, sodium selenite, zinc sulfate, 2-mercaptoethanol, essential amino acids and ethanolamine) to the medium to replace the functionality of serum. Media design experiments were conducted in T-flasks using a design of experiment methodology to screen components for their effectiveness in substituting serum and supporting the growth of the hybridoma cells. The significant components were then optimized through a central composite design (CCD). The optimized medium was verified after adapting the cells to this new formulation. Of the screened components, only sodium selenite, zinc sulfate and ferric citrate were found to substitute for some of the functions of serum. Serum requirement of the cells was successfully reduced to 0.4% by supplementing the medium with 311.8 μM ferric citrate, 17 nM sodium selenite and 4.5 μM zinc sulfate. With this new formulation, the total cost of the medium was reduced by nearly 80% compared to DMEM supplemented with 5% FBS. The specific growth rate of the cells in this new formulation was comparable to that in the DMEM medium supplemented with 2% FBS. Similarly, the specific MAb production rate in the new medium was comparable to that of DMEM + 2% FBS. The antibody produced in the new formulation could specifically detect its antigen, 17-OHP.

In a further step, amino acids, lysozyme, dimethyl sulfoxide (DMSO) and lipopolysaccharide (LPS) were screened as potential inducers of MAb production in this cell line. An effective inducer (i.e. LPS) was identified, but the level of enhancement of the average specific MAb production rate could not compensate for the high cost of the inducer. Therefore, the inducer was not used in further work.

Using the new low-serum medium, the cell culture was scaled-up to a 2 L bioreactor and the operating parameters were screened with a fractional factorial design. A total of 5 parameters were screened. They were pH, temperature, dissolved oxygen, stirring speed and gas sparging rate. The significant parameters were then optimized with a central composite design. The resulting optimized parameters were verified and used to scale up to a 20 L bioreactor using constant impeller tip speed as the scale up criterion. The performance of cells in the 20 L bioreactor was then evaluated. All 5 operating parameters screened and optimized in the 2 L bioreactor significantly affected the culture performance. The optimum operational condition to maximize the MAb production rate were a sparging rate of 0.09 vvm, a stirring speed of 100 rpm, a pH of 7.4, a temperature of 36.8°C and a dissolved oxygen level of 30% of air saturation. The specific Mab production rate at this optimum point was 0.2730 pg/cell.h and the specific growth rate was 0.0363/h. This was equivalent to a production of 11.941 µg/mL (or 11.9 mg/L) of MAb per batch. By using the constant impeller tip speed scale up criterion, the stirring speed in the 20 L bioreactor was calculated to be 37 rpm. The other operating settings remained the same as in the 2 L bioreactor. There were improvements in the specific growth rate (0.0401/h) and the maximum viable cell density ($1.89 \times 10^6$ cells/mL) attained by the hybridoma 192 in the 20 L bioreactor. Nevertheless, both the maximum MAb titer (9.782µg/mL) and the average specific MAb production rate (0.1695 pg/cell.h) were slightly reduced relative to the 2 L bioreactor. This was due to the negatively-growth associated production characteristics of the MAb. The product quality was examined through specificity tests and SDS-PAGE. The MAb produced in the 20 L bioreactor was found to be specific to its antigen, 17-OHP.

In conclusion, the production of the diagnostic MAb against CAH was successfully optimized in a newly developed medium that greatly reduced the cost of culture. A successful scale up of the production process to a 20 L bioreactor was demonstrated.

## ACKNOWLEDGEMENT

I wish to extend my special thanks and appreciation to my supervisors, Prof. Yusuf Chisti, Dr. Badarulhisam bin Abdul Rahman and Prof. Badhrulhisham bin Abdul Aziz for their supervision, suggestions and guidance throughout this work. Special thanks go to Dr. Badarul for his invaluable advice, support and encouragement.

Special appreciation is due to Prof. Cheah Swee Hung, University of Malaya, Malaysia, and Dr. Heilly Chong of InnoBiologics Sdn Bhd for their technical support, time and effort in helping troubleshoot the analytical problems that I faced.

I also wish to gratefully acknowledge the financial support from Universiti Malaysia Pahang and Science Fund from the Ministry of Science, Technology and Innovation, Malaysia, in making this study a success.

Special thanks are due to Mr. Zainal bin Giman for his technical assistance in operating the bench scale and the pilot scale bioreactors. I also wish to thank Miss Siti Fatimah binti Mohd Yunus and Mr. Liu Meng for their help in taking care of the cell line when I was away from the laboratory. Special appreciation is expressed to Mr. Abd. Razak bin Abd. Hamid for his kindness in helping me to change the gas tanks during weekends and public holidays. My sincere thanks to all of them.

Likewise I specially thank my friends and colleagues at Faculty of Chemical Engineering and Natural Resources, Universiti Malaysia Pahang, Malaysia, especially Chin, Jolius, Chong and SMS for their ever ready willingness to assist me when I encountered problems. Thanks also go to Chin and Chong for always accompanying me for a short and fast lunch. Thank you all for the assistance and friendship.

My special thanks are also due to my friends, Shawn and Zaki for their willingness to listen when I was depressed and grumble about my work. Thanks to their caring support and blessings in helping me complete this thesis.

Finally, I express my gratitude to my husband for his years of dedicated patience and love, which gave me strength to go through those good days and bad days. To my girls, I would like to apologize for my negligence in participating in their life these few years. I appreciate the understanding and the touch of love from them and thanks for working together with me in the lab during the weekends.

## TABLE OF CONTENTS

## LIST OF FIGURES

## LIST OF TABLES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| [GLN] | Glutamine concentration (mM) |
| $[GLN]_0$ | Initial glutamine concentration (mM) |
| 17OHP | 17α-Hydroxyprogesterone |
| ABTS | 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) |
| ACTH | Adrenocorticotropic hormone |
| AMM | Ammonia |
| ANDRD | 4-Androstene-3,17-dione |
| ANOVA | Analysis of variance |
| BESTR | β-Estradiol |
| BSA | Bovine serum albumin |
| CAH | Congenital adrenal hyperplasia |
| CCD | Central composite design |
| CHO | Chinese hamster ovary cell line |
| CV | Coefficient of variation |
| CYP21 | 21-Hydroxylase |
| *d* | Length of the light path (cm) |
| DF | DMEM/Ham's F12 medium in a ratio of 1:1 by volume |
| DHEA | Dehydroepiandrosterone |
| $D_i$ | Impeller diameter (cm) |
| DMEM | Dulbecco Modified Eagle's Medium |
| DMSO | Dimethyl sulfoxide |
| DO | Dissolved oxygen level (% of air saturation) |
| $D_T$ | Bioreactor diameter (cm) |
| ELISA | Enzyme Linked Immunosorbent Assay |
| eRDF | Enhanced RPMI/DMEM/F12 medium in volum ratio of 2:1:1 |
| F12 | Ham's F12 |
| FBS | Fetal bovine serum |
| FCS | Fetal calf serum |
| FDA | United States Food and Drug Administration |
| FMOC | 9-Fluorenylmethylchloroformate |
| GC/MS | Gas chromatography tandem mass spectrometry |

| | |
|---|---|
| GIDH | Glutamate dehydrogenase |
| GLN | Glutamine |
| GLU | Glucose |
| HPLC | High Performance Liquid Chromatography |
| IgG | Immunoglobulin G |
| IMDM | Iscove's Modified Dulbecco medium |
| *k* | Glutamine degradation rate constant (1/h) |
| $k_La$ | Volumetric oxygen transfer coefficient (1/s) |
| LAC | Lactate |
| LC/MS/MS | High-performance liquid chromatography tandem mass spectrometry |
| LPS | Lipopolysaccharide |
| LSD | Low serum DMEM |
| MAb | Monoclonal antibody |
| MEM | Minimum Eagle's Medium |
| MW | Molecular weight (g/mol) |
| MWCO | Molecular weight cut off (Da) |
| N | Rotational speed (rpm) |
| NADH | Nicotin-amide-adenine dinucleotide |
| NM | New medium |
| OD | Optical density |
| OPA | Ortho-phthalaldehyde |
| OUR | Oxygen uptake rate (mmol/(L.h)) |
| PBS | Phosphate buffer saline, pH 7 |
| PEG | Polyethylene glycol |
| PREG17 | 17α-Hydroxypregnenolone |
| PREG5 | 5-Pregnen-3β-ol-20-one |
| q | Specific consumption/production rate (mmol/$10^9$ cells.h) |
| Q | Volumetric gas flow rate (vvm) |
| $Q^2$ | Goodness of prediction |
| r | Average specific MAb production rate (pg/cell.h) |
| $R^2$ | Goodness of fit |
| RIA | Radioimmunoassay |

| | |
|---|---|
| RPMI 1640 | Roswell Park Memorial Institute 1640 medium |
| SV | Simple virilising |
| SW | Salt-wasting |
| t | Culture period (h) |
| TEA | Triethylamine |
| THF | Tetrahydrofuran |
| TRFS | Time-resolved fluorescence spectroscopy |
| UP | Ultrapure water |
| $V$ | Final volume of solution mixture in ammonia assay |
| $v$ | Sample volume in ammonia assay |
| $\int X_v.dt$ | Viability index (cells.h) |
| $X_v$ | Viable cell density (cells/mL) |
| Y | Yield |
| μ | Specific growth rate (1/h) |
| $\mu$ | Viscosity of the fluid (Pa.s) |
| $\tau$ | Shear stress (Pa) |
| γ | Shear rate (1/s) |
| ε | Extinction coefficient of NADH at 340 nm (L/mol.cm) |