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

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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 (1.89×10^6 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.

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LIST OF ABBREVIATIONS

[GLN]	Glutamine concentration (mM)
[GLN] ₀	Initial glutamine concentration (mM)
17OHP	17 α -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
<i>d</i>	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	17 α -Hydroxypregnenolone
PREG5	5-Pregnen-3 β -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)
μ	Viscosity of the fluid (Pa.s)
τ	Shear stress (Pa)
γ	Shear rate (1/s)
ϵ	Extinction coefficient of NADH at 340 nm (L/mol.cm)

