Optimization of Process Conditions for High Cell Density Proliferation of DF-1 Cells in Bioreactor

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SUMMARY

The purpose of this study was to optimize agitation speed, microcarrier concentration and pO_2 levels in bioreactor for culturing DF-1cells in DMEM media supplemented with 7% fetal bovine serum. Statistical analysis has shown that the model derived from the results was proven to be significant and pO_2 level turned out as the most significant parameter in the experiment. As predicted by analysis, cells grew up to 1.190×10^6 cells/ml when moderate agitation speed, microcarrier concentration, and pO_2 level are applied.

INTRODUCTION

DF-1 cells which was discovered by Douglas N. Foster is a spontaneously immortalized continuous cell line derived from chicken embryonic fibroblast, CEF [2]. The cell line is widely used in various researches as it has rapid cell proliferation and useful as substrates for virus propagation, recombinant protein expression and recombinant virus production [3]. The cell line is anchorage dependent thus it requires surface for attachment (e.g. microcarrier beads) when cultured in stirred tank bioreactor [1]. In this study, growth of DF-1 cells in bioreactor will be optimized by manipulating several process parameters.

MATERIALS AND METHODS

Dulbecco's Modification of Eagle's Medium (DMEM) supplemented with 7% fetal bovine serum was used to culture and maintain DF-1 cells. By using STATISTICA ® software, 3**(3-1) Fractional Factorial Design was generated and was used to assist the cell growth optimization study. Three parameters that were chosen to be manipulated in nine runs of experiment are agitation speed, Cytodex 1® microcarrier concentration, and pO_2 levels. Cultures were performed in a 1.5 L bioreactor (Infors HT, Switzerland) with one liter working volume and equipped with an elephant ear impeller. Cell inoculum density for all runs was 1×10^5 cells/ml.

RESULTS

Table 1 shows the result for each run of the experiment. Maximum cell concentration of 1.210×10^6 cells/ml was achieved at Run 5. Using statistical tool, a model is derived from the results. Analysis of variance (ANOVA) proved that the derived model is significant as the p value is lower than 0.05 and the most significant parameter in the experiment is pO_2 level.

Table 1. Maximum cell concentration obtained for each run

Run	Agitation speed (rpm)	Microcarrier concentration (g/l)	pO_2 (%)	Max cell concentration (cells/ml)
1	70	5	10	1.045×10^6
2	50	5	30	0.615×10^6
3	50	3	50	0.500×10^6
4	50	1	10	0.820×10^6
5	70	3	30	1.210×10^6
6	90	5	50	0.420×10^6
7	70	1	50	0.525×10^6
8	90	3	10	0.865×10^6
9	90	1	30	0.865×10^6

For predicting the optimal values of maximum cell concentration, a linear model was developed: Y (maximum cell concentration) = -2080694 + 79167 (A) - 560 (A^2) + 177500 (B) -30417 (B^2) + 23417 (C) - 573 (C^2); where cell concentration produced as yield (Y); stirrer speed (A); microcarrier concentration (B); and pO_2 (C). Correlation coefficient or R of the model is 0.95195 indicates high degree of correlation between experimental and predicted values.

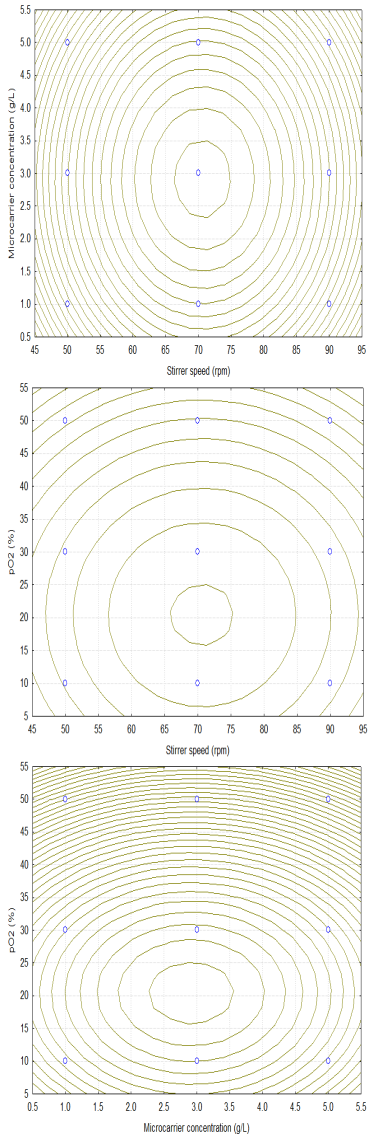


Fig 1. 2D contour plots show the effect of agitation speed, microcarrier concentration and pO_2 level on maximum cell concentration.

The linear model was validated in another experiment and maximum cell concentration of 1.190 x 10^6 cells/ml was achieved when agitation speed was 71 rpm, microcarrier concentration was 2.9 g/l and pO_2 was 20%.

DISCUSSION

Based on the results, DF-1 cells will have maximum concentration when moderate values of agitation speed, microcarrier concentration, and pO_2 level are applied. This condition is more suitable for the cells as they become more sensitive at higher hydrodynamic condition as reported by many researchers [4, 5.6]. In this case study, growth of DF-1 cells in bioreactor is most affected by pO_2 level followed by agitation speed and finally microcarrier concentration. PO_2 level has been proven to have a major role in the growth of cells in bioreactor.

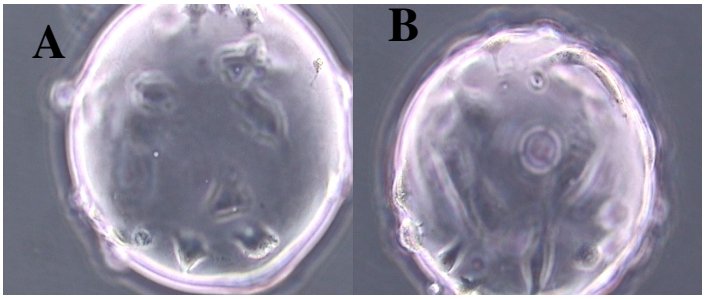
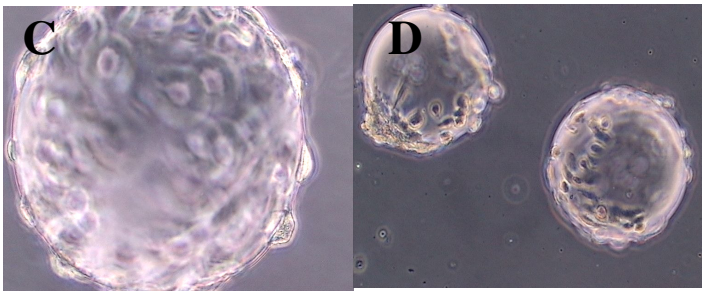


Fig 2. Attachment of DF-1 cells on Cytodex 1 microcarriers at different times. (A) 8 hours (B) 32 hours (C) 56 hours (D) 80 hours.



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