Optimization of Medium Composition for Growth of *Leuconostoc mesenteroides*

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Abtract

The MRS medium is widely used as an optimized medium for the growth of *Lactobacillus* spp. and also used for the growth of *Leuconostoc* spp. *Leuconostoc mesenteroides* shows quite different physicochemical properties compared to *Lactobacilli* spp. and it is one of the major strain of kimchi fermenting microorganisms with its usefulness in our traditional foods and availability in biotechnology in the future, specifically tailor-made medium is necessary for the growth of *Leuconostoc mesenteroides.* Sequential experimental designs (Plackett-Burman, fractional factorial, steepest ascent, central composite design and response surface methodology) were introduced to optimize and improve the *Leuconostoc* medium. Fifteen medium ingredients were investigated and fructose, sodium acetate and ammonium citrate were determined to give a critical and positive effect for cell-growth. The yield of biomass using the optimal medium was improved more than that of the MRS medium and the result of fed-batch culture showed the capability of the improvement in cell mass similar to the *E. coli* system.

Introduction

The use of statistical experimental design in the optimization of fermentation processes and media is well documented. Many factors such as carbon and nitrogen sources, inorganic salts are important variables affecting the growth and, thus, it is difficult to find the most important factors and to optimize them. Screening should be done when the investigator is faced with a large number of factors and is unsure which setting are likely to produce optimal or nearly optimal responses. Then, a large number of continuous factors are screened and insignificant factors are eliminated in order to obtain a smaller set of factor. The remaining factors are optimized by response surface modeling. Finally, after model builing and optimization, the predicted optimum is verified.

Material and Method

Bacterial strains

Leuconostoc mesenteroides isolated from dongchimi was used in this experiment. The strain was kept frozen in an stock solution containing 50% (w/v) glycerol and propagated at 26℃ before use.

Analytical methods

Growth measured as absorbance readings at 600nm, was converted to dry cell mass using a calibration graph. An absorbance of 1.0 was equilvalent to 0.55gdry cells ℓ^{-1}.
D.C.W (g/l) = Absorbance(600nm) × 0.55

Culture conditions

In the medium optimization, the concentration of culture medium was changed according to the required. Each test tube containing 10ml of fermentation medium was inoculated with 10%(v/v) of the seed culture, then cultivated for 24h at 26℃. The optimal temperature was at 30℃. For fed-batch culture a nutrient feeding solution was added by an constant-rate feeding strategy. The feeding solution contained 500g of glucose and 375g of yeast extract per liter of distilled water. A pH was adjusted by 1N NaOH. Constant-rate feeding was done at 10g glucose h^{-1}, temperature at 37℃, with agitation speed of 150 rpm, and aeration at rate of 1vvm.

Results and discussion

The media components having the most significance (P-value < 0.05) at this stage include KH_2PO_4, sodium acetate and ammonium citrate. Of secondary importance (P-value < 0.10) are the factors fructose and yeast extract. The R^2 value was 0.97 indicating the model explained over 97% of the total variability in the data. The curvature term is signifcant, indicating the optimal conditions are within the current experimental region.

The experimetal results also showed that fructose, sodium acetate and ammonium citrate had a significant effect on the growth. But the curvature term is marginal, thus a directional search method, like steepest ascent, can be used to determine the next set of experiments. Between sodium acetate and ammonium citrate, they had no interactions on the effect of growth.

Table 1. Parameter estimates of first order model including curvature from Plackett-Burman experiment

Term	Estimate	Standard Error	Prob > |t|
Intercept	1.3200	0.21483	0.0016
C_1	0.00100	0.00403	0.8140
C_2	0.00920	0.00403	**0.0714**
C_3	0.00240	0.00403	0.5576
N_1	0.02133	0.01075	**0.1041**
N_2	-0.00053	0.01075	0.9624
N_3	0.00680	0.00806	0.4376
N_4	-0.00520	0.00806	0.5475
N_5	-0.00680	0.00806	0.4376
T_1	0.14700	0.02016	**0.0008**
T_2	-0.02700	0.02016	0.2382
T_3	0.56000	0.40324	0.2236
T_4	-0.0200	0.40324	0.9624
T_5	-0.0400	0.04032	0.3250
T_6	0.03920	0.00806	0.0046
T_7	0.02880	0.00806	**0.0160**
Curvature	-0.16600	0.05074	**0.0222**

R^2 = 0.9651 Adj R^2 = 0.8536 F = 8.65 Pr>F = 0.0129
Root MSE 0.08065 Coeff Var 4.42234

C_1: glucose, C_2: fructose, C_3: maltose, N_1: yeast extract, N_2: peptone, N_3: beef extract, N_4: tyrptone, N_5: $(NH_4)_2SO_4$, T_1: KH_2PO_4, T_2: $MgSO_4 \cdot 7H_2O$ T_3: $MnSO_4 \cdot 7H_2O$, T_4: $CaCl_2 \cdot 2H_2O$, T_5: tween 80, T_6: sodium acetate T_7: ammonium citrate

Table 2. Results of the fractional factorial design regression anaylsis for cell mass

Term	Parameter estimate	Regression analysis		
		Standard error	T for H0	Significant level
Intercept	1.68180	0.25418	6.62	<.0001
Curvature	-0.13740	0.08197	-1.68	0.1218
X_1	0.15217	0.03371	4.51	**0.0009**
X_2	0.21644	0.16285	1.33	0.2107
X_3	-0.11688	0.16285	-0.72	0.4879
X_4	0.05819	0.06743	0.86	0.4066
X_5	0.58186	0.06964	8.36	**<.0001**
X_6	0.41735	0.06514	6.41	**<.0001**

R^2 = 0.9183 Adj R^2 = 0.8664 F = 17.67 Pr>F = <.0001
Root MSE 0.13028 Coeff Var 5.55208

X_1: fructose, X_2: yeast extract, X_3: dummy variable, X_4: KH_2PO_4, X_5: Sodium acetate
X_6: Ammonium citrate

Table 3. Experimental designs of steepest ascent and corresponding responses

Run	Sodium acetate	Ammonium citrate	Biomass (g/l)
1	**0.25%**	1.75%	**1.66**
2	**0.5%**	1.5%	1.60
3	0.75%	1.25%	1.53
4	1.0%	1.0%	1.60
5	1.25%	0.75%	1.66
6	1.5	**0.5%**	**1.70**
7	1.75%	**0.25%**	**1.71**
8	0.75%	0.5%	1.70
M			1.64

M: MRS medium

The result of steepest ascent showed that the optimal region of two factors can be wihtin 0.25% ~0.5%.

References

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