Statistical Optimization of Medium Composition for Growth of *Leuconostoc citreum*

Hyun Kim[1], Hyun-Ju Eom[1], Junsoo Lee[1], Jin-Ho Seo[2], Nam Soo Han[1*]

[1]Department of Food Science and Technology, Chungbuk National University, Research Center for Bioresource and Health, Cheongju 361-763, Korea

Tel. 82-43-261-2567, FAX 82-43-271-4412

[2]Department of Food Science and Technology, Seoul National University, Seoul 151-742, Korea

Abstract

Leuconostoc citreum is one of the representative strains of *Leuconostoc* spp. that show fast growth rates in fermented vegetables. Sequential experimental designs including the Plackett-Burman design, fractional factorial design, steepest ascent analysis, central composite design and response surface methodology were introduced tooptimize and improve the medium for *Leuconostoc citreum*. Fifteen medium ingredients were examined and glucose (20 g/l), yeast extract (12.5 g/l), sodium acetate trihydrate (6.12 g/l), potassium phosphate (42.55 g/l) and dibasic ammonium citrate (4.12 g/l)were chosen as the best components to give a critical and positive effect for cell-growth. The biomass was increased to 2.79 g/l (169%), compared to the 1.65 g/l in MRS medium.

Key words : *Leuconostoc* spp., *Leuconostoc citreum*, Plackett-Burman, fractional factorial, steepest ascent, central composite design, response surface methodology

INTRODUCTION

Leuconostoc spp. are thought to be an alternative host system to solve the demerits of *E. coli*, as their fast growth rates are similar to the *E. coli* system both in aerobic and anaerobic conditions, the external expression of proteins which enables an efficient purification, desirable recognition which can be used in industrial spheres as food-grade, or GRAS (Generally Recognized As Safe). *Leuc. citreum* HJ-P4, the major lactic bacteria

growing in the initial phase of kimchi fermentation, was isolated from lactate-fermented vegetables as a psychrophilic starter. MRS(DE MAN, ROGOSA, SHARP) medium is widely used as an optimized medium for the growth of *Lactobacillus* spp., which is generally also used for that of *Leuconostoc* spp. as well [6]. *Leuconostoc* spp., hetero-lactic acid fermenting bacteria, show quite different physicochemical properties to those of *Lactobacilli* spp. and a tailor-made medium remains to be introduced as a compromise. Statistical experimental designs can be used to optimize both fermentation processes and media.

MATERIALS AND METHODS

2.1. Microorganism and medium

Leuc. citreum HJ-P4 was grown at 26°C and 100 rpm in *Lactobacilli* MRS medium was the strain used in this study, which was stored in 50% (v/v) glycerol stock at -70°C until required. MRS medium was used as a comparatively standard medium.

2.2. Plackett-Burman design

A twenty run Plackett-Burman design [13] with three center points was selected as the screening design for the initial set of experiments.

2.3. Fractional factorial design

A 2^{6-2} fractional factorial design, leading to 16 sets of experiments, was used to verify the most significant factors affecting the biomass.

2.4. Central composite design

A response surface methodology was applied to optimize the process, using 3×3 factorial central composite design (CCD), according to the method of Box and Behnken [17].

RESULTS AND DISCUSSION

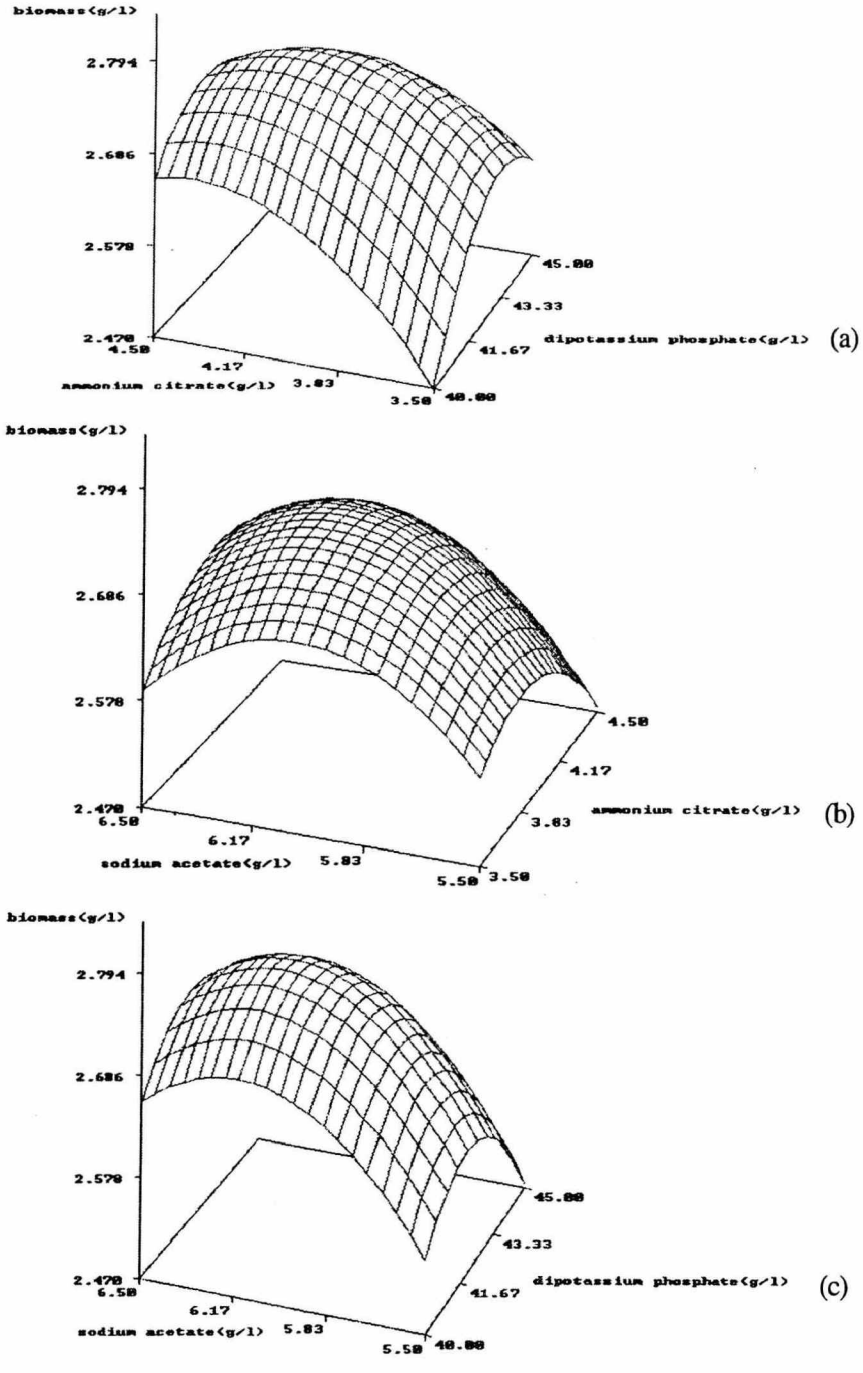

Fig. 1. The response surface plots of biomass on dipotassium phosphate, sodium acetate and ammonium citrate. (a) : ammonium citrate and dipotassium phosphate, (b) : sodium acetate and ammonium citrate, (c) : sodium acetate and dipotassium phosphate.

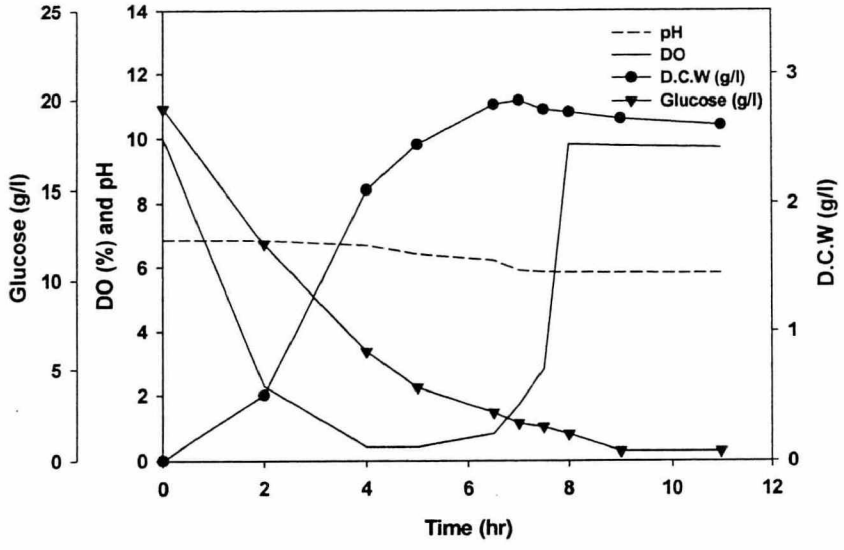

Fig. 2. Batch culture of Leuc. citreum in a newly optimized medium. *Leuc. citreum* HJ-P4 was cultivated at 26°C and pH 6.5 in medium containing glucose (20 g/l), yeast extract (12.5 g/l), K_2HPO_4 (42.55 g/l), sodium acetate (6.12 g/l), and ammonium citrate (4.12 g/l).

CONCLUSION

The final composition of the newly optimized medium was: 20 g/l glucose, 12.5 g/l yeast extract, 42.55 g/l K_2HPO_4, 6.12 g/l sodium acetate trihydrate and 4.12 g/l dibasic ammonium citrate. The biomass production was increased to 2.79 g/l compared with the 1.65 g/l in MRS medium, showing a 169% relative productivity. This microorganism seems to be sensitive to the pH, so the high amount of K_2HPO_4 may be considered to be related to any buffer effect in concomitant with other physiological requirements. Additional studies on the oxygen effect including the variable pH conditions will be essential to accomplish high cell-density cultures.

REFERENCES

[1] Lee, S. Y., and Blanch H.,Recombinant protein expression in high cell density cultures of *Escherichia coli*, 1992, *Biotechnology*. 10:1550-1556.

[2] Lee, S. Y., High cell-density culture of *Escherichia coli*, 1996, *Trends Biotechnol.* 14: 98-105.

[3] Riesenberg, D., at al., High-cell-density cultivation of microorganisms, 1999, *Appl. Microbiol. Biotechnol.* 51(4): 422-430.

[4] U. S. Food and Drug Administration (2003) List of the substances that are the subject of each GRAS Notice and the file number that FDA has assigned to the notice (GRAN No.). Center for Food Safety & Applied Nutrition Office of Food Additive Safety.

[5] Eom, H. J., Isolation of psychrotrophic *Leuconostoc mesenteroides* producing highly active dextransucrase and application to lactate-fermented foods, M. S. Thesis, 2002, Chungbuk National University, Cheongju, Chungbuk, Korea.

[6] Lee, M., at al., Selective media for isolation and enumeration of lactic acid bacteria form Kimchi. 1996, *J. Korean Soc. Food Sci. Nutr.* 25(5): 754-760

[7] Strobel, R. J., and Nakatsukasa W. M., Response surface methods for optimizing *Saccharopolyspora spinosa*, anovel macrolide producer, 1993, *J. Ind. Micorbiol.* 11: 121-127.

[8] Swanson, T. R., et al., Development and field confirmation of a mathematical model for amylogucosidase/ pullanase saccharification, 1986, *Starch.* 38: 382-387.

[9] Maddox, I. S., et al.,Use of response surface methodology for the rapid optimization of microbiological media, 1977, *J. Appl. Bacteriol.* 43: 197-204.

[10] Goulas, P., et al., Optimization of microbial transglutarninase production using experimental designs, 1997, *Appl. Microbiol. Biotechnol.* 48(6): 730-734.

[11] Akay B., and Ertunc S., Application of a statistical technique to the production of *Saccharomyces cerevisiae* (baker's yeast), 1997, *Biotechnol. Appl. Biochem.* 26: 91-96.

[12] Bloor S., and England R. R., Elucidation and optimization of the medium constituents controlling antibiotic production by the cyanobacterium *Nostoc muscorum*, 1981, *Enzyme Microb. Technol.* 13: 76-81.

[13] Plackett R. L., and Burman J. P., The design of optimum multifactorial experiments, 1946, *Biometrika.* 33: 305-25.

[14] Khuri A. I., and Cornell J. A., Response surface methodology, 1987, ASQC Quality press, New York.

[15] Haaland P. D., Experimental Design in Biotechnology, 1989, Marcel Dekker, Inc., New York.

[16] Myers R. H., and Montgomery D. C., Response Surface Methodology: Process and Product Optimization Using Designed Experiments, 1995, John Wiley & Sons, Inc., New York.

[17] Box G. E. P., and Behnken D. W., Some new three level design for the study of quantitative variables, 1960, Technometrics. 2: 456-476.

[18] Chen Q. H., at al.,Optimization of medium composition for the production of elastase by *Bacillus* sp. EL31410 with response surface methodology, 2002, *Enzyme Microbial. Technol.* 30: 667-672.