Statistical Modelling and Optimization of Lactic Acid Fermentation in the Presence of Anionic Clay and Ultrasonic Field

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Statistical modelling and optimization of acid lactic fermentation of milk inoculated with lactic bacteria, in the presence of anionic clay and ultrasonic field, was performed. A 3^3 factorial experiment was selected in order to correlate the lactic acid concentration in the fermentation medium (process response) to process factors, i.e., fermentation temperature (38-48 °C), clay/milk ratio (1-8 g/L), and fermentation time (2-4 hr). 27 experimental runs were performed at 3 factor levels (low, middle, and high). The factorial statistical model consisted of a second order polynomial regression correlation between process response and factors. This regression relationship was applied to optimize the fermentation process. Optimal values of factors are close to the middle level of temperature (43 °C), high level of clay/milk ratio (8 g/L), and low level of fermentation time (2 hr).

Keywords: acid lactic fermentation, statistical modelling, factorial experiment, optimization

Characteristic processes of chemical and biochemical engineering are usually described by deterministic or stochastic models [1, 2]. As the information referring to the internal phenomena associated of a process is not completely known, it is impossible to obtain an accurate deterministic or stochastic model. In this case, a statistical model, taking into account only the process inputs and outputs, can be employed. Factorial analysis of experimental data is widely used to develop statistical models consisting of regression correlations between dependent and independent process variables [3-7]. Moreover, these factorial statistical models are accessible to be applied for process optimization.

Experimental study and mathematical modelling of acid lactic fermentation, based on lactose conversion to lactic acid (LA) in the presence of lactic bacteria (LAB), have been the aims of numerous studies in the related literature [8-17]. More simple or complex models, considering LAB growth kinetics as well as property transfer/transport equations, have been used.

LAB growth is inhibited by the accumulation of LA in the fermentation broth and the corresponding drop in pH, respectively [8-10, 15, 16]. In order to avoid this inhibitory effect, which diminishes the fermentation productivity as well as has a negative impact on the quality and cost of fermented dairy product, *in situ* removal of LA from the fermentation medium by its retention on hydrotalcite-type anionic clay was experimentally investigated in previous studies [18-20]. Ultrasonic technique was applied to intensify the process of LA retention by increasing the liquid-solid contact surface and decreasing the boundary layer thickness.

This paper aims at statistical modelling and optimising of the process of lactic acid fermentation of milk inoculated with lactic bacteria, in the presence of anionic clay and under ultrasonic operation conditions. A 3^3 factorial experiment was selected in order to correlate the LA concentration in the fermentation medium to the independent process variables, *i.e.*, operation temperature, clay/milk ratio, and final fermentation time.

Experimental part

Materials and methods

A hydrotalcite-type anionic clay was prepared by co-precipitation method according to procedure described in our previous studies [18-21]. Fermentation medium consisted of pasteurized whole (4.5 % fat) milk inoculated with a mixed starter culture (YC-X11 YO-Flex, Chr-HANSEN), containing the LAB *St. thermophilus* and *Lb. bulgaricus*.

A milk volume of 0.100 L was heated at 45°C, mixed with 0.015 L starter culture, and put into the experimental fermentation setup, consisting of a stainless steel ultrasonic bath and a Thermo Haake B12 temperature controlled circulating water bath [18]. Fermentation medium *p*H values were measured by an InoLab *p*H Level 2 digital *p*H-meter. At a *p*H value of about 5, various masses of anionic clay, *i.e.*, 0.1 g, 0.45 g, and 0.8 g, respectively, were added into the fermentation medium and then the ultrasonic bath was started.

Experimental variables

27 experimental runs were conducted under ultrasonic (ν= 35 kHz) operation conditions, at three values of operation temperature (t_1 = 38 °C, t_2 = 43 °C, and t_3 = 48 °C), clay/milk ratio (R_1 = 1 g/L, R_2 = 4.5 g/L, and R_3 = 8 g/L), and final fermentation time (τ_1 = 2 h, τ_2 = 3 h, and τ_3 = 4 h).

Fermentation medium *p*H values were continuously recorded as a function of fermentation time. The values of LA concentration in the liquid phase, C_{LA}, were estimated depending on *p*H values by a linear interpolation of data reported in the literature [18].

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i	X_i	Low level $X_{i,min}$	Middle level $X_{i,0}$	High level $X_{i,max}$	Variation step value ΔX_i
1	t_f (°C)	38	43	48	5
2	R (g/L)	1	4.5	8	3.5
3	τ_f (hr)	2	3	4	1

Table 1
LEVELS AND VARIATION STEP VALUE OF X_i FACTORS

Results and discussion

Statistical model

A 3^3 factorial statistical model was used to correlate the final values of dependent variable (response), *i.e.*, LA concentration in the fermentation medium, $C_{LA} = Y_1$ (g/L), with the values of independent variables (factors) for the process of lactic acid fermentation in the presence of anionic clay under ultrasonic operation conditions. Fermentation temperature, $t_f = X_1$ (°C), clay/milk ratio, $R = X_2$ (g/L), and fermentation time, $\tau_f = X_3$ (hr), were selected as process factors, X_i (i = 1, 2, 3). Values (levels) of natural factors, *i.e.*, minimum (low), $X_{i,min}$, plan centre (middle), $X_{i,mn}$, and maximum (high), $X_{i,max}$, as well as variation step value, ΔX_i, are summarized in table 1. Table 2 contains characteristic data of 3^3 experimental matrix, where dimensionless factors, x_i (i = 1, 2, 3), were calculated depending on natural factors, X_i, using equation (1).

$$x_i = \frac{X_i - X_{i,0}}{\Delta X_i} \tag{1}$$

The statistical model consists of second order polynomial regression relationship (2), where regression coefficients, a_j (j = 0, 1, 2, 3, 11, 22, 33, 12, 13, 23, 123), were calculated based on experimental data (table 2) by means of eqs. (3)-(6), according to characteristic procedure of a 3^3 factorial experiment [1]. By substituting the values of a_j in eq. (2), the correlation (7) was obtained between process response and factors.

$$Y_1 = a_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_{11}x_1^2 + a_{22}x_2^2 + a_{33}x_3^2 + a_{12}x_1x_2 + a_{13}x_1x_3 + a_{23}x_2x_3 + a_{123}x_1x_2x_3 \tag{2}$$

$$a_1 = \frac{\sum_{k=1}^{27} x_{1k}Y_{1k}}{\sum_{k=1}^{27} x_{1k}^2} \quad a_2 = \frac{\sum_{k=1}^{27} x_{2k}Y_{1k}}{\sum_{k=1}^{27} x_{2k}^2} \quad a_3 = \frac{\sum_{k=1}^{27} x_{3k}Y_{1k}}{\sum_{k=1}^{27} x_{3k}^2} \tag{3}$$

$$a_{11} = \frac{\sum_{k=1}^{27}(x_1^2 - 2/3)_k Y_{1k}}{\sum_{k=1}^{27}(x_1^2 - 2/3)_k^2} \quad a_{22} = \frac{\sum_{k=1}^{27}(x_2^2 - 2/3)_k Y_{1k}}{\sum_{k=1}^{27}(x_2^2 - 2/3)_k^2}$$

$$a_{33} = \frac{\sum_{k=1}^{27}(x_3^2 - 2/3)_k Y_{1k}}{\sum_{k=1}^{27}(x_3^2 - 2/3)_k^2} \tag{4}$$

$$a_{12} = \frac{\sum_{k=1}^{27}(x_1x_2)_k Y_{1k}}{\sum_{k=1}^{27}(x_1x_2)_k^2} \quad a_{13} = \frac{\sum_{k=1}^{27}(x_1x_3)_k Y_{1k}}{\sum_{k=1}^{27}(x_1x_3)_k^2} \quad a_{23} = \frac{\sum_{k=1}^{27}(x_2x_3)_k Y_{1k}}{\sum_{k=1}^{27}(x_2x_3)_k^2}$$

$$a_{123} = \frac{\sum_{k=1}^{27}(x_1x_2x_3)_k Y_{1k}}{\sum_{k=1}^{27}(x_1x_2x_3)_k^2} \tag{5}$$

$$a_0 = \frac{\sum_{k=1}^{27} Y_{1k}}{27} - \frac{2}{3}(a_{11} + a_{22} + a_{33}) \tag{6}$$

$$Y_1 = 4.50 + 0.2599x_1 - 0.1488x_2 + 0.9799x_3 + 0.1792x_1^2 + 0.0792x_2^2 + 0.4833x_3^2 - 0.1911x_1x_2 + 0.0733x_1x_3 - 0.1088x_2x_3 + 0.0955x_1x_2x_3 \tag{7}$$

Regression coefficients significance

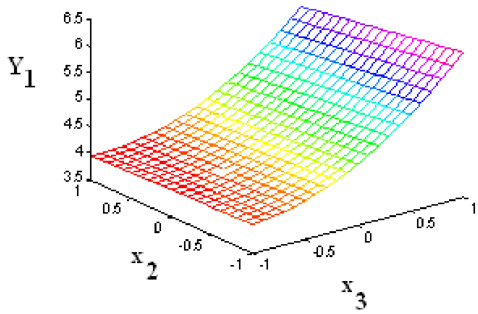
In order to test the significance of regression coefficients using the Student random variable, N = 3 additional measurements were performed within the experimental plan centre, as follows: $Y^1_{1,0}$ = 4.64 g/L, $Y^2_{1,0}$ = 4.61 g/L, and $Y^3_{1,0}$ = 4.94 g/L. The mean value of response, $Y^{mn}_{1,0}$, reproducibility standard dispersion, σ_{rp}, standard dispersion associated to regression coefficients, σ_a, and Student

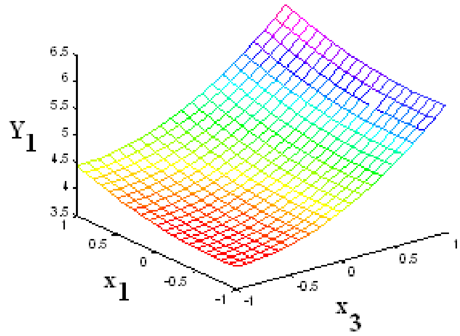
Run	Factors natural values			Factors dimensionless values			Response values
	$X_1 = t_f$ (°C)	$X_2 = R$ (g/L)	$X_3 = \tau_f$ (hr)	x_1	x_2	x_3	Y_1 (g/L)
1			2			-1	3.70
2		1	3		-1	0	4.70
3			4			+1	5.80
4			2			-1	4.00
5	38	4.5	3	-1	0	0	4.70
6			4			+1	5.90
7			2			-1	4.50
8		8	3		+1	0	4.45
9			4			+1	5.40
10			2			-1	3.90
11		1	3		-1	0	4.80
12			4			+1	6.48
13			2			-1	3.96
14	43	4.5	3	0	0	0	4.35
15			4			+1	5.88
16			2			-1	3.90
17		8	3		+1	0	4.25
18			4			+1	6.35
19			2			-1	4.94
20		1	3		-1	0	5.40
21			4			+1	6.80
22			2			-1	4.48
23	48	4.5	3	+1	0	0	4.70
24			4			+1	6.50
25			2			-1	4.20
26		8	3		+1	0	4.70
27			4			+1	6.10

Table 2
CHARACTERISTIC 3^3 EXPERIMENTATION MATRIX OF FERMENTATION PROCESS

Table 3
VALUES OF STUDENT RANDOM VARIABLE

t_0	t_1	t_2	t_3	t_{11}	t_{22}	t_{33}	t_{12}	t_{13}	t_{23}	t_{123}	$t_{\alpha,\nu}$
128.57	7.40	4.23	27.97	5.11	2.26	13.80	5.46	2.09	3.09	2.71	2.92





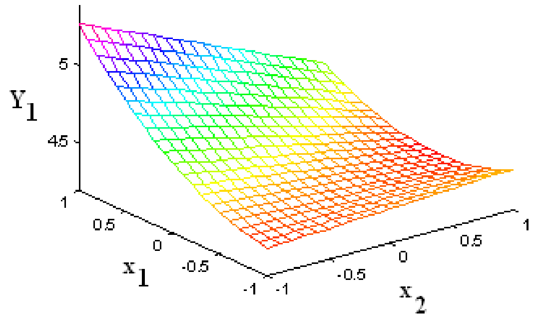


Fig. 1. Lactic acid concentration, $Y_1 = C_{LA}$ (g/L), depending on dimensionless factors, x_1, x_2, and x_3:

a. $Y_1 = 4.50 - 0.148x_2 + 0.979x_3 + 0.483x_3^2 - 0.108x_2x_3$ ($x_1 = 0$, $X_{1.0} = t_{f.0} = 43$ °C);

b. $Y_1 = 4.50 + 0.259x_1 + 0.979x_3 + 0.179x_1^2 + 0.483x_3^2$ ($x_2 = 0$, $X_{2.0} = R_0 = 4.5$ g/L);

c. $Y_1 = 4.50 + 0.259x_1 - 0.148x_2 + 0.179x_1^2 - 0.191x_1x_2$ ($x_3 = 0$, $X_{3.0} = \tau_{f.0} = 3$ hr).

random variable, t_j (j = 0, 1, 2, 3, 11, 22, 33, 12, 13, 23, 123), were estimated by eqs. (8) - (11). Values of t_j, which are listed in table 3, were compared with the Student variable theoretical value, $t_{\alpha,\nu} = 2.92$, estimated depending on the significance level $\alpha = 0.1$ and the number degrees of freedom $\nu = N - 1 = 2$ [1]. The regression coefficients satisfying the condition $tj < t_{\alpha,\nu}$, i.e. t_{22}, t_{13} and t_{123}, were considered as insignificant and were neglected, consequently relationship (7) can be written in the simplified form (12).

$$Y_{1.0}^{mn} = \frac{\sum_{r=1}^{3} Y_{1.0}^{r}}{3} = 4.73 \text{ g/L} \tag{8}$$

$$\sigma_{rp} = \sqrt{\frac{\sum_{r=1}^{3}\left(Y_{1.0}^{r} - Y_{1.0}^{mn}\right)^2}{N-1}} = 0.182 \text{ g/L} \tag{9}$$

$$\sigma_a = \frac{\sigma_{rp}}{\sqrt{27}} = 0.035 \text{ g/L} \tag{10}$$

$$t_j = \frac{|a_j|}{\sigma_a} \tag{11}$$

$$Y_1 = 4.50 + 0.259x_1 - 0.148x_2 + 0.979x_3 + 0.179x_1^2 + 0.483x_3^2 - 0.191x_1x_2 - 0.108x_2x_3 \tag{12}$$

Factors effect on process response

Relationship (12) reveals that x_1 and x_3 individual factors have a favourable effect, whereas x_2 factor as well as x_1x_2 and x_2x_3 double interactions have an unfavourable one. These effects are also emphasized by the plots in Figure 1, representing the response surface associated to Y_1 function (12) depending on two dimensionless factors as the third factor is equal to zero (centre-point).

Model optimization

The statistical model described by correlation (12) can be optimized applying Lagrange method [1]. Considering first order partial derivates of Y_1 function (12) equal to zero and solving the system of equations (13), the optimal solution (14) was obtained. Based on eq. (1) and data listed in table 1, the dimensionless solution (14) can be written in dimensional form (15). It is observed that $t_{f,opt} \approx t_{f,0} = 43$ °C, $R_{opt} \approx R_{max} = 8$ g/L, and $\tau_{f,opt} \approx \tau_{,min} = 2$ hr.

$$\begin{cases} \dfrac{\partial Y_1}{\partial x_1} = 0.259 + 2 \cdot 0.179x_1 - 0.191x_2 = 0 \\ \dfrac{\partial Y_1}{\partial x_2} = -0.148 - 0.191x_1 - 0.108x_3 = 0 \\ \dfrac{\partial Y_1}{\partial x_3} = 0.979 + 2 \cdot 0.483x_3 - 0.108x_2 = 0 \end{cases} \tag{13}$$

$$\begin{cases} x_{1.opt} = -0.257 \\ x_{2.opt} = 0.874 \\ x_{3.opt} = -0.916 \end{cases} \tag{14}$$

$$\begin{cases} X_{1.opt} = t_{f.opt} = -0.257 \cdot 5 + 43 = 41.7 \text{ °C} \\ X_{2.opt} = R_{opt} = 0.874 \cdot 3.5 + 4.5 = 7.6 \text{ g/L} \\ X_{3.opt} = \tau_{f.opt} = -0.916 \cdot 1 + 3 = 2.1 \text{ hr} \end{cases} \tag{15}$$

Conclusions

Factorial design was applied aiming at statistical modelling and optimising of LA fermentation process. Experimental study of LA fermentation was conducted in the presence of anionic clay and under ultrasonic operation conditions. Fermentation medium consisted of whole milk inoculated with a mixed starter culture of *St. thermophilus* and *Lb. bulgaricus*. LA concentration in the fermentation medium, C_{LAI} (g/L), was selected as dependent variable (process response), whereas fermentation temperature, t_f

= X_1 (38-48 °C), clay/milk ratio, $R = X_2$ (1-8 g/L), and fermentation time, $\tau_f = X_3$ (2-4 hr), were chosen as independent variables (process factors), X_i (i = 1, 2, 3). 27 experimental runs were performed at 3 levels of factors, *i.e.*, low ($X_{i,min}$), middle ($X_{i,0}$), and high ($X_{i,max}$), according to a 3^3 factorial experiment. The statistical model consisted of a second order polynomial regression relationship between process response and factors. Characteristic coefficients of regression correlation were estimated based on experimental data and their significance was evaluated using the Student test. The insignificant coefficients were removed from the statistical model, resulted a simplified form of correlation between process response and factors, which was used in order to evaluate the factors effect and also to optimize the fermentation process. It was observed a favourable effect of t_f and τ_f factors and an unfavourable one of R factor as well as $t_f R$ and $\tau_f R$ double interactions. Process optimization was performed based on Lagrange method and the optimal values of factors were as follows: $t_{f,opt} \approx t_{f,0}$ = 43 °C, $R_{opt} \approx R_{max}$ = 8 g/L, and $\tau_{f,opt} \approx \tau_{,min}$ = 2 hr.

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