Modeling for Exopolysaccharide Production by *Alcaligenes faecalis* B14

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Abstract

Exo-polysaccharides (EPS) are environment friendly natural polymers secreted by microorganisms in the surrounding medium. EPS shows diverse applications such as food formulations, pharmaceutical and chemical industry etc. In the present study, optimization of fermentation process parameters and fermentation kinetics with respect to exopolysacharide production from the Alcaligenes faecalis B14 was done. A simple kinetic model was proposed using the logistic, the Luedeking-Piret and Luedeking-Piret-like equation for growth, EPS production and glucose consumption. The maximum Logistic specific growth rate was determined as μ_{max} = 0. 0.225 h^{-1} and the values of Luedeking-Piret constants α and β indicated that the EPS production using Alcaligenes faecalis was growth associated. A CCD (central composite Design) was used to study the influence of different parameters on the production of EPS. Optimized values of fermentation parameters include: glucose (3.15% w/v); yeast extract (0.48 % w/v); $MgSO_4$ (0.05% w/v); temperature (30.13 °C); and pH (7.20). The maximum EPS production obtained was 12.76±0.2 g/L under optimal parameter conditions stated above.

Keywords*: Exopolysaccharide, Alcaligenes sp., optimization, Kinetics, fermentation*

1. Introduction

Bacterial exopolysaccharides (biopolymer) are environment friendly natural polymers. They are either homopolysaccharide or heteropolysaccharide biosynthesized by a wide range of bacteria [1,2]. Biopolymers have potential applications in the food, pharmaceutical, and other industries due to their unique structure and physical properties. Few exopolysaccharides (EPS) like xanthan from X*anthomonas campestris*, sphingans from *Sphingomonas* sp., cellulose from *Acetobacter xylinium*, etc. have gained commercial importance [3,4,5]. In food industry natural polymers are known for their application as thickeners, stabilizers, emulsifiers, binders, gelling agents, and film former etc. Due to its wide industrial applications, it has generated much interest in microbial EPS research [6,7]. Further, production of EPS by bacteria have created great interest among researchers, because bacteria can be easily cultivated under controlled environment as well as secrete wide range of biopolymers with characteristic composition and properties [2,8,9,10,11].

The production of microbial EPS depends on many fermentation factors, which includes strain type, composition of fermentation medium and fermentation conditions [12]. Thus, the optimization of nutritional (concentration of carbon, nitrogen, and mineral salts source) and physical parameters (pH, temperature, etc) is required to get optimum production of EPS from microbial stains.

Response surface methodology (RSM) is a statistical method based on the multivariate non-linear model that has been widely used method for optimization process. It is also useful in the study of interactions of the various parameters affecting the process. A central composite design (CCD) helps to develop the matrix for optimization of the variables in cultivation medium [13]. It has been used in the optimization of EPS production by strains *Zunongwangia profunda* SM-A87 and *Frateuria aurentia* [14,15]. Also the optimization of gellan gum production by *Sphingomonas paucimobilis* ATCC 31461 using central composite design has been reported [16].

In our laboratory, exopolysaccharide containing monomer composition similar to welan gum and having good viscosity and emulsifying properties was produced by fermentation process from newly isolated strain *Alcaligenes faecalis* B14.

The development of kinetic models is crucial for understanding, controlling, and optimizing fermentation processes. The information gained using kinetic models along with designed experiments can aid bioengineers to design and control microbial processes. Gilani et al., [17] and Wang et al., [18] reported growth associated EPS (xanthan and gellan) production and glucose consumption in the fermentation kinetics studies on *Xanthomonas campestris* and *Sphingomonas paucimobilis* ATCC 31461.

Keeping in view of the industrial importance of EPS, the present investigation was carried out to study fermentation kinetics using unstructured models of cell growth, product formation and substrate consumption and optimization of fermentation parameters using RSM to obtain maximum EPS production by *A. faecalis* B14.

2. Material and methods

2.1 Materials

All the chemicals used in the experiments were purchased from HiMedia Laboratories Pvt. Ltd, Mumbai, India.

2.2 Microorganism and growth conditions

The strain used for the present study was *Alcaligenes faecalis* B14 (accession number-KF035058), isolated from sub-soil of Punjab state of India [19]. The inoculum was prepared in medium containing glucose (2%), yeast extract (0.1%), peptone (0.3%), di-hydrogen potassium phosphate (0.2%) and magnesium sulphate (0.01%) and incubated at 30 ^{o}C in a rotary shaker (100 rpm). 5% culture was inoculated in fermentation medium which was optimized using RSM. The EPS was recovered by following the method of Banik & Santhiagu (2006) [20], with slight modification. The modification included heat treatment (80^{o}C) for 15 min, cooling at room temperature and neutralization of pH of fermentation broth. The broth was centrifuged at 10,000 × g for 10 min to separate the cells. Isopropanol was added to the supernatant and kept at 4 ^{o}C for 24 hrs to precipitate the EPS. Again centrifuged to obtain the EPS, freeze dried and weighed.

2.3 Experimental design and statistical analysis

A Central Composite Rotatable Design (CCRD) with five variables at five levels each were used to investigate the effects of concentration of glucose, yeast extract, $MgSO_4$ and process parameters (pH, temperature) on EPS production. Based on preliminary experiment, low and high levels were selected for each five independent variable to get maximum EPS production. The design was generated by Design Expert, Trial version 6.0.10 statistical software (Stat-Ease INC., Minneapolis, MN, USA). In this design, the experiments were conducted randomly. The matrix designs for the levels of variables in coded and un-coded form for EPS production are given in Table 1 and Table 2, respectively. A second order polynomial equation (Eq. 1) was fitted to study the effect of variable as given below

$$Y_i = \beta_0 + \sum_{i=1}^{n} \beta_i x_i + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \beta_{ij} x_i x_j + \sum_{i=1}^{n} \beta_{ii} x_i^2 \qquad (1)$$

Where Y_i = EPS production (g/L)

x_i & x_j= Independent variables, (x_1 = glucose concentration, x_2 = yeast extract concentration, x_3 = $MgSO_4$ concentration, x_4 = Temperature (°C), x_5 = pH), β_0 is the value of coefficient of fitted response at the central point of design, β_i, β_{ij}, β_{ii} are the linear, quadratic and cross product regression coefficients, respectively.

Total number of experiments = $2^{\text{No. of Variables}}$ + 2* No. of variables + Central Points

For five variables, total no. of experiments = 2^5 +2*5 + 8 = 50

Five different levels for each experiment in coded form are

- α, -1, 0, +1, + α

Where, $\alpha = 2^{\text{No. of variables} / 4} = 2^{5/4} = 2.378$

$$Coded\ value = x_i = \frac{2\left(X_i - \bar{X}_i\right)}{R_i} \qquad (2)$$

Where X_i is the actual setting in the un-coded units of the i^{th} factor $\bar{X}_i$ is the average of the low and high settings for the i^{th} factor and R_i is the range between the low and high settings. Analysis of variance (ANOVA) and regression analysis were carried out.

2.4 Kinetic models

The logistic equation: In many biopolymer fermentation systems, the microbial processes do not follow the classical kinetic model of substrate-limited cell growth and product formation proposed by Monod in 1949. Alternative to this, the logistic equation, a substrate-independent model, is used [21]. Therefore, in fermentation process, the cell growth has been characterized by the logistic equation as shown in equation (3)

$$\frac{dX}{dt} = \mu_m \left(1 - \frac{X}{X_m}\right) X \qquad \text{..... (3)}$$

Where, μ_m is the maximum specific growth rate (h^{-1}) and X_m is the maximum attainable biomass concentration (g/L). The integrated form of equation (3) using $X = X_0$ ($t = 0$) gives a sigmoid variation of X as a function of t, which may represent both an exponential and a stationary phase (equation 4).

$$X(t) = \frac{X_0 e^{\mu_m t}}{1-(X_0/X_m)(1-e^{\mu_m t})} \qquad \text{..... (4)}$$

The Luedeking-Piret equation: The production of microbial metabolites was based on the Luedeking-Piret equation [21]. Where, the product formation rate (r_p) depends on both the instantaneous biomass concentration and the growth rate in a linear manner.

$$r_p = \frac{dp}{dt} = \alpha \frac{dX}{dt} + \beta \qquad \text{..... (5)}$$

where α and β are the product formation constants and may differ under different fermentation conditions. The integration of equation (5) using $P = P_0$ at $t = 0$ with equation (6) provides:

$$P(t) = P_0 + \alpha X_0 \left\{ \frac{e^{\mu_m t}}{1-(X_0/X_m)(1-e^{\mu_m t})} - 1 \right\} \qquad \text{..... (6)}$$

The modified Luedeking-Piret equation: Glucose is used in the present fermentation process, which is required by the microbe to form cell components and metabolic products as well as for the maintenance of cells. The modified Luedeking-Piret equation (equation 7) was used to explain the glucose consumption.

$$-\frac{dS}{dt} = \left(1/Y_{X/S}\right)\frac{dX}{dt} + \left(1/Y_{X/S}\right)\frac{dP}{dt} + m_s X \qquad \text{..... (7)}$$

where m_S is the maintenance coefficient (g substrate · g cell/h), $Y_{X/S}$ and $Y_{P/S}$ are the is the cell yield coefficient and product yield coefficient, respectively for glucose

Combining equations 5 and 7 gives:

$$-\frac{dS}{dt} = \left(1/Y_{X/S} + \alpha/Y_{p/S}\right)\frac{dX}{dt} + \left(\beta/Y_{P/S} + m_s\right)X \qquad \text{..... (8)}$$

Equation 8 can be simplified to express glucose consumption by the following model:

$$-\frac{dS}{dt} = \gamma \frac{dX}{dt} + \delta X \qquad \text{..... (9)}$$

where, γ is the sum of $1/Y_{X/S}$ and $\alpha/Y_{P/S}$ and δ is the sum of $\beta/Y_{P/S}$ and m_S. The integration of equation 9 using $S=S_0$ at t=0 gives:

$$S(t) = S_0 - \gamma X_0 \left\{ \frac{e^{\mu_m t}}{1-(X_0/X_m)(1-e^{\mu_m t})} - 1 \right\} - \delta \frac{X_m}{\mu_m} \ln \left[1 - \frac{X_0}{X_m} (1 - e^{\mu_m t}) \right] \quad \ldots\ldots\ldots (10)$$

3. Results and Discussion

A Central Composite Rotatable Design was used to select the concentration of various nutritional and physical parameters to get conditions responsible for maximum exopolysaccharide production. The preliminary experiments were followed to choose the low and high levels for the five independent variables i.e. pH (6.5-8), incubation temperature (25-35^{o}C), glucose concentration (%, w/v; 1-5), yeast extract concentration (%, w/v; 0.3-0.7) and $MgSO_4$ (%, w/v; 0.04-0.06).

Table 1. Coded levels of different variables in the central composite design variables (CCD)

Factor	Variable	Coded Levels				
		-2.378	**-1**	**0**	**+1**	**+2.378**
X_1	**Glucose (%, w/v)**	0.62	1.0	3.0	5.0	5.38
X_2	**Yeast extract (%, w/v)**	0.02	0.3	0.5	0.7	0.98
X_3	**$MgSO_4$**	0.03	0.04	0.05	0.06	0.07
X_4	**Temperature** (°C)	18.11	25	30	35	41.89
X_5	**pH**	5.47	6.5	7.25	8	9.03

3.1 Statistical analysis for EPS production

A total of 50 trials of CCD were carried out randomly to optimize the production of EPS, the results of these trails were presented in Table 2. The change of concentration of variables affects the EPS production ranging from 1.23 to 12.76 g/L. A second-order polynomial regression model (equation 11) was established from the experimental data:

$$Y = +12.17 + 0.43x_1 - 0.31\, x_2 - 0.34\, x_3 + 0.21\, x_4 - 0.22\, x_5 - 0.28\, x_1 x_2 - 0.25\, x_1 x_3 - 0.13\, x_1 x_4 + 0.11\, x_1 x_5 + 9.375E\text{-}003\, x_2 x_3 - 0.14\, x_2 x_4 + 0.049\, x_2 x_5 - 0.11\, x_3 x_4 + 0.061\, x_3 x_5 - 0.15\, x_4 x_5 - 1.63\, x_1^2 - 1.59\, x_2^2 - 1.61\, x_3^2 - 1.81\, x_4^2 - 1.65\, x_5^2 \quad \ldots\ldots\ldots (11)$$

where Y was the EPS production, x_1 to x_5 are independent variables.

ANOVA analysis was performed (Table 3) which indicated that the quadratic regression to produce the second order model was significant ($p<0.001$; F value 65.83). The value of the coefficient of determination near to 1 indicates better correlation of quadratic model to the experimental data [15]. The coefficient of determination R^2 was 0.9784 for EPS production, indicating that only 2.16% of the total variation was not explained by the model.

The p-value was used to ensure the significance of coefficients and to determine the interaction strength between each independent variable [22]. The smaller *p*-value points to the bigger significance of the corresponding coefficient [23]. The coefficients of quadratic polynomial equation along with p-values are given in Table 3. It indicated that linear and quadratic terms of glucose, yeast extract, $MgSO_4$, temperature, and pH have significant effect on EPS production at 5% level of significance ($p<0.05$).

The relative magnitude of β coefficients revealed that the linear term of glucose had maximum positive effect (β= 0.43) followed by temperature (β= 0.21), whereas, yeast extract (β= -0.31), $MgSO_4$ (β= -0.34), pH (β= -0.22) had negative effect on EPS production. The interactive terms 'glucose and pH' (β = 0.11), had the maximum positive effect followed by '$MgSO_4$ and pH' (β= 0.061), 'yeast extract and pH' (β= 0.049), 'yeast extract and $MgSO_4$' (β= 9.375×10^{-3}) on EPS production. The interaction of 'glucose and yeast extract' (β = -0.28), glucose and $MgSO_4$' (β = -0.25), glucose and temperature' (β = -0.13), 'yeast extract and temperature' (β= -0.14) '$MgSO_4$ temperature' (β= -0.11), 'temperature and pH' (β= -0.015), had negative effect on EPS production at 5% level of signifince ($p< 0.05$). The interactive effects of these parameters are also shown in the Fig. 1A-1C.

Table 2. The matrix of CCD and values of experimental data

Glucose Conc. (%, w/v)	Yeast extract Conc. (%, w/v)	$MgSO_4$	Incubation temp. (^oC)	pH	Yield (g/L)
2.00	0.30	0.04	25.00	6.50	2.97
4.00	0.30	0.04	25.00	6.50	4.25
2.00	0.70	0.04	25.00	6.50	3.29
4.00	0.70	0.04	25.00	6.50	4.02
2.00	0.30	0.06	25.00	6.50	3.24
4.00	0.30	0.06	25.00	6.50	4.21
2.00	0.70	0.06	25.00	6.50	3.54
4.00	0.70	0.06	25.00	6.50	2.25
2.00	0.30	0.04	35.00	6.50	4.65
4.00	0.30	0.04	35.00	6.50	5.79
2.00	0.70	0.04	35.00	6.50	3.92
4.00	0.70	0.04	35.00	6.50	4.37
2.00	0.30	0.06	35.00	6.50	3.98
4.00	0.30	0.06	35.00	6.50	4.68
2.00	0.70	0.06	35.00	6.50	4.21
4.00	0.70	0.06	35.00	6.50	2.94
2.00	0.30	0.04	25.00	8.00	2.33
4.00	0.30	0.04	25.00	8.00	4.89
2.00	0.70	0.04	25.00	8.00	3.16
4.00	0.70	0.04	25.00	8.00	3.78
2.00	0.30	0.06	25.00	8.00	2.98
4.00	0.30	0.06	25.00	8.00	4.11
2.00	0.70	0.06	25.00	8.00	3.15
4.00	0.70	0.06	25.00	8.00	3.65
2.00	0.30	0.04	35.00	8.00	4.23
4.00	0.30	0.04	35.00	8.00	4.87
2.00	0.70	0.04	35.00	8.00	2.97
4.00	0.70	0.04	35.00	8.00	3.88
2.0	0.3	0.06	35.00	8.00	3.43
4.0	0.3	0.06	35.00	8.00	3.96
2.0	0.7	0.06	35.00	8.00	3.67
4.0	0.7	0.06	35.00	8.00	2.89
0.62	0.5	0.05	30.00	7.25	1.23
5.38	0.50	0.05	30.00	7.25	5.34
3.00	0.02	0.05	30.00	7.25	4.45
3.0	0.98	0.05	30.00	7.25	2.62
3.0	0.5	0.03	30.00	7.25	5.12
3.0	0.5	0.07	30.00	7.25	1.67
3.00	0.50	0.05	25.24	7.25	2.16
3.00	0.50	0.05	34.76	7.25	2.43
3.00	0.50	0.05	30.00	5.47	4.33
3.00	0.50	0.05	30.00	9.03	2.09
3.00	0.50	0.05	30.00	7.25	11.69
3.00	0.50	0.05	30.00	7.25	12.11
3.00	0.50	0.05	30.00	7.25	12.11
3.00	0.50	0.05	30.00	7.25	12.1
3.00	0.50	0.05	30.00	7.25	12.63
3.00	0.50	0.05	30.00	7.25	11.87
3.00	0.50	0.05	30.00	7.25	12.54
3.00	0.50	0.05	30.00	7.25	12.76

From the present study, the 3D response surfaces (Figure 1) were generated to investigate the interactions between any two variables for EPS production. Figure 1A shows the effect of glucose and yeast extract on EPS production and their interaction when the other variables were kept constant. Appropriate carbon and nitrogen concentration is required for microbial growth and to obtain high production. The maximum EPS production was obtained with glucose 3%, yeast extract 0.05%. Further increase in concentration of glucose and yeast extract in the media, the production was decreased. The decrease is probably due to osmotic stress in the fermentation medium for cell

growth thus affecting the EPS production [24]. In comparison to carbon concentration, low nitrogen concentration is essential parameter for EPS production [7]. The maximum EPS production from *Alternaria alternate* using yeast extract as organic nitrogen source has been reported [25].

It was observed that there was an increases in the EPS production, with the increase in glucose (up to 3%) and $MgSO_4$ (up to 0.05%) concentration and beyond these concentrations, EPS production was decreased (Figure 1B). This may be due to influence of mineral ions on the catalytic activities of enzymes involved in growth and EPS production [26].

The effect of pH and temperature on EPS production and their interaction is shown in Figure 1C. There was an increase in EPS production with increase in pH (6 to 7) and temperature (25 to 30 °C) and then decreased. The incubation temperature of 30 °C for EPS production was found optimum and beyond 30 °C temperature an inhibitory effect on EPS production was seen. Also the initial pH increase found to be favorable for growth and EPS production. The variation in pH causes changes in hydrogen ion concentration in the fermentation medium that effects the cell growth and EPS production [27].

Table 3. Regression summery and ANOVA table for enzyme activity for uncoded value of process variable

Source	*β*-value	Sum of Squares	DF	Mean Square	F value	Prob.
Model	12.17	530.24	1	26.51	65.83	< 0.0001
Glucose	0.43	7.98	1	7.98	19.82	0.0001
Yeast extract	-0.31	4.04	1	4.04	10.04	0.0036
$MgSO_4$	-0.34	4.98	1	4.98	12.36	0.0015
Temp	0.21	1.98	1	1.98	4.92	0.0346
pH	-0.22	2.17	1	2.17	5.38	0.0276
$Glucose^2$	-1.63	148.08	1	148.08	367.68	< 0.0001
$Yeast\ extract^2$	-1.59	140.17	1	140.17	348.05	< 0.0001
$MgSO_4^2$	-1.61	144.57	1	144.57	358.98	< 0.0001
$temp^2$	-1.81	181.53	1	181.53	450.75	< 0.0001
pH^2	-1.65	150.49	1	150.49	373.68	< 0.0001
Glucose * Yeast extract	-0.28	2.58	1	2.58	6.40	0.0171
Glucose * $MgSO_4$	-0.25	1.92	1	1.92	4.77	0.0372
Glucose * temp	-0.13	0.55	1	0.55	1.36	0.2538
Glucose * pH	0.11	0.36	1	0.36	0.90	0.3514
Yeast extract * $MgSO_4$	9.375×10^{-3}	2.813×10^{-3}	1	2.813×10^{-3}	6.984×10^{-3}	0.9340
Yeast extract * temp	-0.14	0.66	1	0.66	1.64	0.2102
Yeast extract * pH	0.049	0.078	1	0.078	0.19	0.6631
$MgSO_4$ * temp	-0.11	0.35	1	0.35	0.88	0.3570
$MgSO_4$ * pH	0.061	0.12	1	0.12	0.29	0.5930
temp * pH	-0.015	0.76	1	0.76	1.88	0.1810
Residual		11.68	29	0.40		
Lack of Fit		10.68	22	0.49		
Pure Error		1.00	7	0.14	3.38	0.0516
Cor Total		541.92	49			
R-Squared		0.9784				
Adj R-Squared		0.9636				

3.1.1Optimization of EPS production

A numerical optimization technique was used to optimize the process variables for EPS production. The optimum values of the variables to get maximum EPS production were: glucose concentration 3.15% (w/v); yeast extract concentration 0.48 % (w/v); $MgSO_4$ concentration 0.05% (w/v); temperature 30.13 °C; pH 7.20. Under these optimal conditions of variables the predicted value for EPS production was found to be 12.76 g/L. The experiments were carried out in triplicate under the optimal conditions as given above to confirm the result. The mean value of EPS production obtained was 12.76± 0.2 g/L which were in accordance with the predicted value. From the results, it was observed that optimum concentration of glucose, nitrogen and mineral ion along with optimum physical parameters (temperature and pH) is critical factor for higher EPS production.

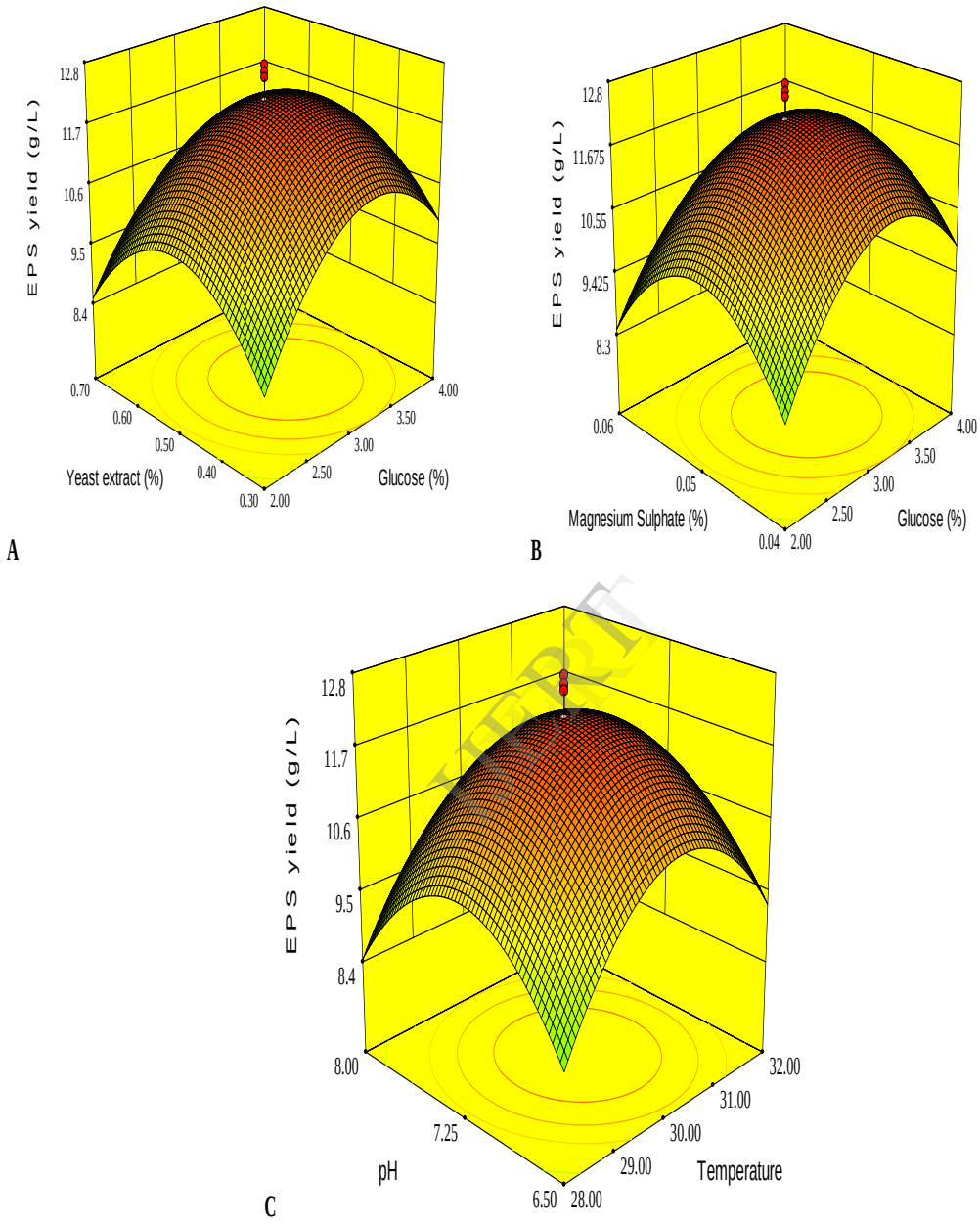


Figure 1: 3D plots showing effect of variables: glucose and YE (**A**); glucose and $MgSO_4$ (**B**); pH and temperature (**C**) on EPS yield (g/L)

3.2 Fermentation kinetics

The batch fermentation was carried out under the optimized conditions in one liter production medium and observed the production of EPS.

The EPS production in batch cultivation was rapid during the exponential phase and continued slowly during the stationary phase. Similar trends in the EPS production such as gellan gum and xanthan gum using *Sphingomonas paucimobilis* ATCC 31461 and *Xanthomonas campestris* [17,18] and also *Pediococcus damnosus* strain 2.6 [28] were reported. The maximum exopolysaccharide obtained were (12.76 g /L) with the residual glucose concentration of 11.1 g/L (Figure 2).

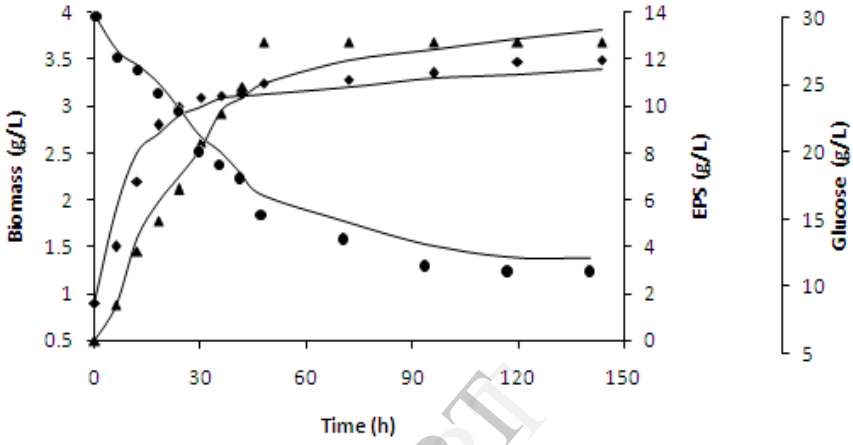
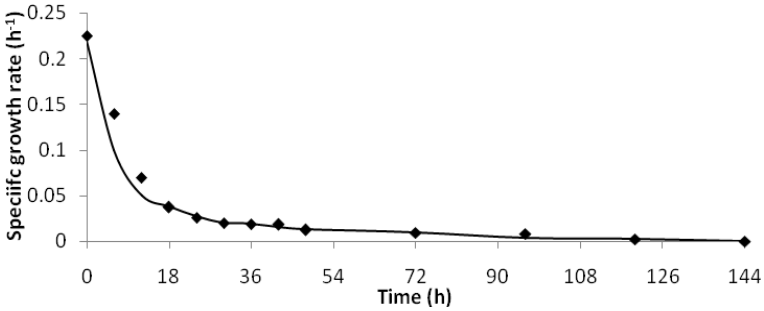


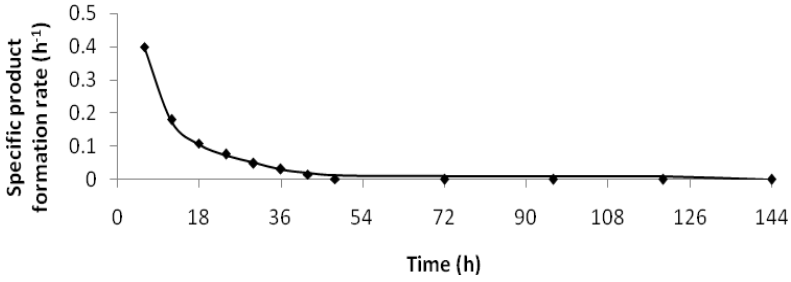
Figure 2. Batch fermentation (solid symbols-experimental; solid lines- model prediction) for formation of biomass (♦), EPS (▲) and glucose consumption (●) using *Alcaligenes faecalis* B14

Microbial growth: Logistic equation: In batch fermentation using *A. faecalis* B14, maximum cell growth obtained were 3.5 g/L at 144 h. A logistic equation was employed to estimate the cell growth and specific growth rate as shown in Figure 2 and 3a. The calculated parameters for *A. faecalis* B14 were: X_m= 3.5 g/L; X_0 was 0.9 g/L and μ_m was 0.225 (h^{-1}). The correlation coefficient for cell growth and specific growth rate were 0.939 and 0.886 respectively. The fitting of experimental values in logistic equation was found to be satisfactory.

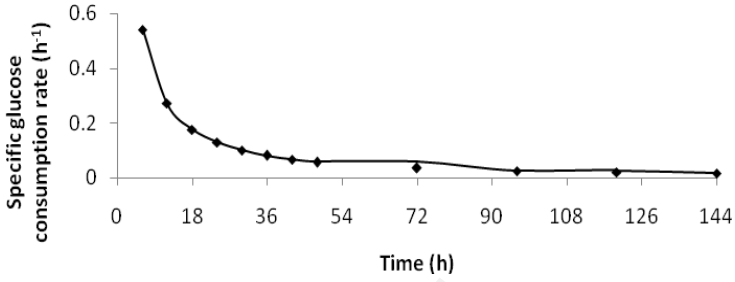
Product formation: Luedeking-Piret equation: In batch fermentation, the values for the kinetic parameters α and β were 2.058 g · g cell^{-1} and 0.067 g · g cell h^{-1}, respectively. Luedeking-Piret equation (Equation 5) was used to estimate the product formation. A comparison between kinetic model predictions and the experimental data were found to be consistent as given in Figure 2 and 3b. The correlation coefficient EPS production and specific product formation rate were 0.987 and 0.941, respectively. In comparing α (growth-associated) and β (non-growth-associated) constants, the value of α is higher than β, which showed that the rate of exopolysaccharide formation is high during the exponential growth phase. The small value of β indicated that product formed at low rate during the stationary growth phase.



(a)



(b)



(c)

Figure 3. Comparison between experimental results (solid symbols) and the model prediction (solid lines) for specific growth rate (a), specific product formation rate (b), and specific substrate consumption rate (c); in batch fermentation.

Substrate consumption: Modified Luedeking-Piret equation: Glucose is required by the cells to form cell material and metabolic products as well as the maintenance of cells. As the cell concentration increased, there was decrease in residual glucose concentration. Modified Luedeking-Piret equation was used for glucose consumption. At the beginning the glucose concentration was 30 g/L and at the end of fermentation the residual glucose concentration was 11.5 g/L. The comparisons between model prediction data and experimental results are shown in Figure 2 and 3c. The correlation coefficients for glucose consumption and specific glucose consumption rate were 0.898 and 0.869, respectively. The parameters γ and δ, provided by the model, were 2.013 g · g cell^{-1} and 0.057 g · g cell h^{-1}, respectively.

On examination of various fermentation kinetics models studied above (Logistic equation, Luedeking-Piret equation, Modified Luedeking-Piret equation), it was observed that the exopolysaccharide production was growth associated. In comparing growth-associated (α, γ) and non-growth-associated (β, δ) constants, the values of former are higher than later, which showed that the rate of exopolysaccharide formation and glucose consumption is high during the exponential growth phase and at low rate during the stationary growth phase. Similar pattern of growth associated exopolysaccharide (xanthan and gellan) production and glucose consumption was reported in the fermentation kinetics studies using *Xanthomonas campestris* and *Sphingomonas paucimobilis* ATCC 31461 [17,18].

4. Conclusions

Response surface methodology proved to be effective and reliable tool in selecting optimum process conditions for EPS production. The optimized values obtained for the production of EPS production were glucose 3.15% (w/v); yeast extract 0.48 % (w/v); $MgSO_4$ 0.05% (w/v); temperature 30.13 °C; and pH 7.20. The maximum production obtained was 12.76±0.2 g/L under optimal parameter conditions. Using the optimized conditions, the kinetic model for the exopolysaccharide production with *Alcaligenes faecalis* B14 has been analyzed. The calculated data revealed that the exopolysaccharide production was growth associated. Further research on other functional properties and food toxicology of welan gum like exopolysaccharide is needed to predict its possible use in food industry.

5. References

[1] P. Vijayabaskar, S. Babinastarlin, T. Shankar, T. Sivakumar, and K.T.K. Anandapandian, Quantification and characterization of exopolysaccharides from *Bacillus subtilis* (MTCC 121), Advances in Biological Research, 2011, 5: 71-76.

[2] U.U. Nwodo, E. Green, and A.I. Okoh, Bacterial exopolysaccharides: functionality and prospects, Internatioal Journal of Molecular Sciences, 2012, 13: 14002-14015.

[3] R. Minoshima, and O. Yamada, Method of producing xanthan gum, 1988, US Patent 4717663.

[4] Y.P. Chao, Y. Sugano, T. Kouda, F. Yoshinaga, and M. Shoda, Production of bacterial cellulose by *Acetobacter xylinum* with an air-lift reactor, Biotechnology Techniques, 1997, 11: 829-832.

[5] M.A. Fialho, M.L. Moreira, and O.A. Popescu, Occurrence, production, and applications of gellan: current state and perspectives, Applied Microbiology and Biotechnology, 2008, 79: 889-900.

[6] B. Degeest and L. de Vuyst, Indication that the nitrogen source influences both amount and size of exopolysacchaides produced by *Streptococcus thermophilus* LY03 and modeling of the bacterial growth and exopolysaccharide production in a complex medium, 1999, Applied and Environmental Microbiology, 65: 2863-70.

[7] A. Mishra and B. Jha, Microbial exopolysaccharides, The prokaryotes, 2013, 179-192.

[8] C.J. Lawson, and I.W. Sutherland, Polysaccharides, In: Rose JH (ed) Economic Microbiology, Academic Press, London, pp 327-389, 1978.

[9] P.A. Sandford, I.W. Cottrell, and D.J., Pettit Microbial Polysaccharides: New products and their commercial applications, Pure and Applied Chemistry**,** 1984, 56: 879-892.

[10] I.W. Sutherland, Novel and established applications of microbial polysaccharides, Trends in Biotechhology, 1998, 16:41-46.

[11] R.M. Banik, B. Kanari, and S.N. Upadhyay, Exopolysaccharide of the gellan family: prospects and potential, World Journal of Microbiology and Biotechnology, 2000, 16: 407-414.

[12] B. Rabha, R.S. Nadra, and B. Ahmed, Effect of some fermentation substrates and growth temperature on exopolysaccharide production by *Streptococcus thermophilus* BN1, International Journal of Bioscience, Biochemistry and Bioinformatics, 2012, 2: 44-47.

[13] A. Dean, and D. Voss, Response surface methodology, In: Design and analysis of experiments, New York: Springer-Verlag, pp. 547-586, 1999.

[14] S.B. Liu., L.P. Qiao, H.L. He, Q. Zhang, X.L. Chen, W.Z. Zhou, B.C. Zhou, and Y.Z. Zhang Optimization of Fermentation Conditions and Rheological Properties of Exopolysaccharide Produced by Deep-Sea Bacterium *Zunongwangia profunda* SM-A87, 2011, PLOS ONE, www.plosone.org.

[15] T. Sivakumar, S.S. Narayani, T. Shankar, and P. Vijayabaskar, Statistical optimization of exopolysaccharide production by *Frateuria aurentia*, International Journal of Biological & Pharmaceutical Research, 2012, 3: 457-462.

[16] S. Arockiasamy and B. Banik, Optimization of gellan gum production by *Sphingomonas paucimibilis* ATCC 31461 with non-ionic surfactants using central composite design, Journal of Bioscience and Bioengineering, 2008, 105: 204-210.

[17] S.L. Gilani, G.D. Najafpour, H.D. Heydarzadeh, and H. Zare, Kinetic models for xanthan gum production using *xanthomonas campestris* from molasses, Chemical Industry & Chemical Engineering Quarterly, 2011, 17 (2): 179-187.

[18] X. Wang, P. Xu, Y. Yuan, C. Liu, D. Zhang, Z. Yang, C. Yang, , and M. Cuiqing, Modeling for gellan gum production by *Sphingomonas paucimobilis* ATCC 31461 in a Simplified Medium, Applied and Environmental Microbiology, 2006, 72 (5) 3367-3374.

[19] V. Kaur, M.B. Bera, and P.S. Panesar, Production and characterization of exopolysaccharide produced by *Alcaligenes faecalis* B14 isolated from indigenous soil, International Journal of Biotechnology and Bioengineering Research, 2013, 4(4): 365-374.

[20] R.M. Banik and A. Santhiagu, Improvement in production and quality of gellan gum by *Sphingomonas paucimobilis* under high dissolved oxygen tension levels, Biotechnology Letters, 2006, 28: 1347-1350.

[21] Luedeking, R., and E. L. Piret. 1959. A kinetic study of the lactic acid fermentation: batch process at controlled pH. Journal of Biochemical and Microbiological Technology and Engineering, 1: 393-431.

[22] C. Liu, Y. Liu, and W. Liao, Application of statistically based experimental designs for the optimization of nisin production from whey, Biotechnology Letters, 2003, 25: 877-882.

[23] R.V. Muralidhar, R.R. Chirumamila, R. Marchant, and P. Nigam, A response surface approach for the comparison of lipase production by *Candida cylindracea* using two different carbon sources, Biochemical Engineering Journal, 2001, 17-23.

[24] R. Xu, S. Ma, Y. Wang, L. Liu, and P. Li, Screening, identification and statistic optimization of a novel exopolysaccharide producing *Lactobacillus paracasei* HCT, African Journal of Microbiology Research, 2010, 4(9): 783-795.

[25] E.A. Nehad and A.R. El-Shamy, Physiological studies on the production of exopolysaccharide by *Fungi*, Agriculture and Biology Journal of North America, 2010, 1(6): 1303-1308.

[26] F.P. Duta, F.P. de França, L.M. de A. Lopes, Optimization of culture conditions for exopolysaccharides production in *Rhizobium* sp. using the response surface method. Electronic Journal of Biotechnology, 2006, 9: 391-399.

[27] S. Shivakumar, and S.V.N. Vijayendra, Production of exopolysaccharides by *Agrobacterium* sp. CFR-24 using coconut water-a byproduct of food industry, Letters in Applied Microbiology, 2005, 477-482.
[28] M. Duenas, A. Munduate, A. Perea, and A. Irastorza, Exopolysaccharide production by *Pediococcus damnosus* 2.6 in a semidefined medium under different growth conditions, International Journal of Food Microbiology, 87, 2003, 113-120.