Carbohydrate Polymers 114 (2014) 43–47

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Short communication

Modeling and investigation of submerged fermentation process to produce extracellular polysaccharide using *Lactobacillus confusus*

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ARTICLE INFO

Article history:
Received 17 June 2014
Received in revised form 21 July 2014
Accepted 25 July 2014
Available online 10 August 2014

Keywords:
Extracellular polysaccharide
Submerged fermentation
Lactobacillus confuses
Box–Behnken design
Optimization

ABSTRACT

The main objective of the present study is to investigate and optimize the Submerged fermentation (SMF) process parameters such as addition of coconut water, NaCl dose, incubation time and temperature on the production of extracellular polysaccharide (EPS) and biomass production using *Lactobacillus confuses*. Response surface methodology (RSM) coupled with four factors three level Box–Behnken design (BBD) was employed to model the SMF process. RSM analysis indicated good correspondence between experimental and predicted values. Three dimentional (3D) response surface plots were used to study the interactive effects of process variables on SMF process. The optimum process conditions for the maximum production of EPS and biomass were found to be as follows; addition of coconut water of 40%, NaCl dose of 15%, incubation time of 24 h and temperature of 35 °C. Under these conditions, 10.57 g/L of EPS and 3.9 g/L of biomass were produced.

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1. Introduction

Nowadays, the production of food grade extracellular polysaccharides (EPS) is a topic of interest. They have high potential for applications in various fields due to its anti-tumor, anti-cancer, anti-ulcer, immune enhancing, and cholesterol lowering activities (De Vuyst, De Vin, Vaningelgem, & Degeest, 2001). Fermentation is the technique of biological conversion of complex substrates into simple compounds by various microorganisms such as bacteria and fungi. Moreover, it can be divided into two main brances as follows; Soild state fermentation (SSF) and submerged fermentation (SMF). Compared to SSF, SMF shows ease of operation and high efficiency. This SMF technique is best suited for microorganisms such as bacteria that require high humidity content. An additional advantage of this technique is that purification of final products is easier and quiker. Last few decades, SMF using lactic acid bacteria (LAB) have great attention in the field of biotechnology to produce bioactive EPS. The EPS from *Lactobacillus confusus* TISTR 1498 contains only glucose, indicating homopoly-saccharide synthesis as dextran, and it may have the potential for new applications in the pharmaceutical, health, cosmetic, and food industries (Duenas, Munduate, Perea, & Irastorza, 2003). Moreover, process conditions in SMF such as coconut water addition, sodium chloride dose, incubation time and temperature has significantly affect the process efficiency and its optimization will pay a way to produce a maximum bioactive EPS with reasonable operating cost (Thirugnanasambandham, Sivakumar, & Prakash Maran, 2014a).

RSM is essentially a particular set of mathematical and statistical methods used by researchers in order to aid in the solution of certain types of problems, which are pertinent to scientific processes. However, best of our knowledge SMF is not employed for the production of EPS using *L. confuses* via RSM. Hence, the objectives of this study is to estimate the influence of various SMF conditions such as addition of coconut water, NaCl dose, incubation time and temperature on the EPS and biomass production using *L. confusus* via RSM.

2. Materials and methods

2.1. Materials and chemicals

Bacterial strain and medium *L. confuses* TISTR 1498 was purchased from the local suppliers, Erode, Tamil Nadu.

2.2. Fermentation procedure

In SMF process, a frozen culture was poured in a 250-mL Erlenmeyer flask containing 100 mL of medium. For the inoculums preparation, 10% of the microbial seed was transferred into another

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http://dx.doi.org/10.1016/j.carbpol.2014.07.067
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Table 1
Process variables and their ranges.

Level	−1	0	1
A	15	35	55
B	5	15	25
C	8	20	32
D	25	35	45

Table 2
BBD experimental design with results.

S. no.	*A*	*B*	*C*	*D*	Y_1	Y_2
1	15	15	20	25	8	1.4
2	35	15	32	25	8.6	1.88
3	35	5	8	35	7.55	0.83
4	15	15	8	35	7.71	0.99
5	55	15	8	35	7.61	1.21
6	35	15	20	35	10.4	3.68
7	35	25	20	45	9.14	2.42
8	35	15	8	25	7.71	0.99
9	55	15	32	35	10	3.56
10	55	15	20	45	9.4	2.68
11	55	25	20	35	8.49	1.77
12	35	5	20	25	8.97	2.25
13	35	15	20	35	10.4	3.87
14	35	15	20	35	10.4	3.68
15	35	15	8	45	8.27	1.55
16	35	5	32	35	8.16	1.44
17	15	15	20	45	8.97	2.31
18	35	15	32	45	9.73	3.01
19	35	15	20	35	10.4	3.68
20	35	15	20	35	10.4	3.68
21	55	15	20	25	9.27	2.48
22	15	25	20	35	7.5	0.78
23	35	25	8	35	7	0.28
24	55	5	20	35	8.71	1.99
25	15	5	20	35	7.88	1.16
26	15	15	32	35	8.24	1.52
27	35	25	32	35	8.49	1.77
28	35	25	20	25	7.7	1.02
29	35	5	20	45	7.9	1.25

250-mL Erlenmeyer flask containing 100 mL of medium, and it was incubated at 35 °C for 24 h until an optical density (OD) 650 nm of 0.8 was reached. The 10%(v/v) inoculum was transferred into a 3-L fermenter with a working volume of 2 L of media, and incubated at 35 °C for 24 h with 50 rpm of agitation.

2.3. Analytical methods

The concentration of EPS and biomass was determined by the standard procedure (Phisit, 2012). All samples were evaluated in triplicate.

Table 3
Sequential model sum of squares for SMF process.

Source	Sum of squares	*df*	Mean square	*F* Value	Prob > *F*	Remarks
Sequential model sum of squares for EPS production (g/L)						
Mean	2207.21	1.00	2207.21			Suggested alised
Linear	7.65	4.00	1.91	1.99	0.1282	
2FI	2.90	6.00	0.48	0.43	0.8488	
Quadratic	19.81	4.00	4.95	189.26	< 0.0001	
Cubic	0.35	8.00	0.04	19.12	0.0010	
Residual	0.01	6.00	0.00			
Total	2237.93	29.00	77.17			
Sequential model sum of squares for biomass production (g/L)						
Mean	120.56	1.00	120.56			Suggested alised
Linear	7.94	4.00	1.99	2.05	0.1188	
2FI	2.68	6.00	0.45	0.39	0.8753	
Quadratic	20.01	4.00	5.00	131.77	< 0.0001	
Cubic	0.41	8.00	0.05	2.50	0.1405	
Residual	0.12	6.00	0.02			
Total	151.72	29.00	5.23			

2.4. Experimental design

In this present study, RSM coupled with (BBD) was employed to investigate the individual and interactive effects of process variables such as addition of coconut water (*A*), NaCl dose (*B*), incubation time (*C*) and temperature (*D*) on the submerged fermentation process (Table 1). Whereas EPS production (Y_1) and biomass production (Y_2) are selected as responses. The obtained results were fitted into the empirical second order polynomial model, as shown in the following equation (Thirugnanasambandham, Sivakumar, Prakash Maran, & Kandasmay, 2014b)

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_j X_j + \sum_{j=1}^{k} \beta_{jj} X_j^2 \sum_{i} \sum_{<j=2}^{k} \beta_{ij} X_i X_j + e_i \quad (1)$$

where Y is the response; X_i and X_j are variables (i and j range from 1 to k); β_0 is the model intercept coefficient; β_j, β_{jj} and β_{ij} are interaction coefficients of linear, quadratic and the second-order terms, respectively; k is the number of independent parameters ($k = 4$ in this study); and e_i is the error (Prakash Maran, Sivakumar, Thirugnanasambandham, & Sridhar, 2014). Then the adequacy of the model was examined using ANOVA and actual versus predicted plots. Finally, optimization of process variables were done according to numerical optimization method. All the statistical analyses were done with the help of Stat ease Design Expert 8.0.7.1 statistical software package (Stat-Ease Inc., Minneapolis, USA) ((Prakash Maran, Sivakumar, Thirugnanasambandham, & Sridhar, 2013a).

Table 4
Model summary statistics for SMF process.

Model	Model summary statistics					
	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	Remarks
	EPS production (g/L)					
Linear	0.9805	0.2491	0.1240	0.0493	29.2	Suggested aliased
2FI	1.0587	0.3434	0.0214	0.1528	35.4	
Quadratic	0.1618	0.9881	0.9762	0.9313	2.1101	
Cubic	0.0480	0.9996	0.9979	0.9352	1.9908	
	Biomass production (g/L)					
Linear	0.9835	0.2549	0.1308	0.0566	29.4	Suggested aliased
2FI	1.0682	0.3408	0.0254	0.1698	36.4	
Quadratic	0.1948	0.9829	0.9659	0.9056	2.9400	
Cubic	0.1430	0.9961	0.9816	0.5648	13.5607	

Table 5
ANOVA results for SMF process.

Source	EPS production (g/L)					Biomass production (g/L)				
	Sum of Squares	*df*	Mean square	*F* value	*p*-Value	Sum of squares	*df*	Mean square	*F* value	*p*-value
Model	30.36	14.0000	2.16868	82.8777	<0.0001	30.63	14.0000	2.1877	57.6301	<0.0001
A	2.24	1.0000	2.23603	85.4516	<0.0001	2.55	1.0000	2.54841	67.1321	<0.0001
C	4.53	1.0000	4.52641	172.98	<0.0001	4.48	1.0000	4.47741	117.947	<0.0001
D	0.83	1.0000	0.83213	31.8006	<0.0001	0.85	1.0000	0.85333	22.4792	0.0003
AC	0.86	1.0000	0.8649	33.0528	<0.0001	0.83	1.0000	0.8281	21.8145	0.0004
BD	1.58	1.0000	1.57503	60.1907	<0.0001	1.44	1.0000	1.44	37.9336	<0.0001
CD	0.08	1.00	0.08	3.10	0.0999	0.08	1.00	0.08	2.14	0.1656
A^2	4.71	1.00	4.71	180.15	<0.0001	4.33	1.00	4.33	114.03	<0.0001
B^2	12.51	1	12.51	478.079	<0.0001	13.2302	1	13.2302	348.521	<0.0001
C^2	9.20482	1	9.20482	351.769	<0.0001	8.8705	1	8.8705	233.674	<0.0001
D^2	2.47334	1	2.47334	94.5203	<0.0001	2.68322	1	2.68322	70.6835	<0.0001
CV			2.14					2.38		
AP			28.35					31.02		

3. Results and discussions

3.1. Development and analysis of mathematical model

BBD experimental data (Table 2) are investigated using multi regression analysis namely the sequential model sum of squares (Table 3) and model summary statistics (Table 4). From the results, second order polynomial equation is selected to represent the SMF process, due to higher *F*-value, higher correlation coefficient (R^2) value with lower *p*-values. The obtained equations with coded factors are given below,

$$Y_1 = 10.40 + 0.43A + 0.71C + 0.26D + 0.040AB + 0.46AC + 0.63BD + 0.14CD - 0.85A^2 - 1.39B^2 - 1.19C^2 - 0.61D^2 \quad (2)$$

$$Y^2 = 3.72 + 0.46A + 0.48C + 0.27D + 0.235AB + 0.16AC + 0.60BD + 0.18CD - 0.76A^2 - 1.95B^2 - 2.15C^2 - 0.74D^2 \quad (3)$$

Then, adequcy of the developed mathematical models are examined using analysis of variance (ANOVA) and results are shown in Table 5. As can be seen in Table 5, higher *F*-values for both EPS and biomass production implied that the developed quadratic model is highly significant. A *p*-value smaller than 0.05 implies for both the developed models shows that the corresponding model term is significant. The obtained correlation variance (CV%) and adequate precision (AP) values of this study, demonstrate a significant and intense correlation between the observed and predicted values. Meanwhile, the actual and predicted production of EPS and biomass production are plotted in Fig. 1. The data points on this plots lie reasonably close to the straight line and indicate that an adequate agreement between real data and the data obtained from the models (Thirugnanasambandham et al., 2014c).

3.2. Effect of process conditions on the SMF process

From the developed mathematical models, three dimentional plots were constructed in order to study the interactive effect of the process variables on the SMF and it is shown in Figs. 2 and 3 (Prakash Maran, Sivakumar, Thirugnanasambandham, & Sridhar, 2013b). In order to investigate the effect of coconut water addition on the SMF, experiments were carried out in various coconut water addition and the results are shown in Fig. 2 (a–b). From the results, it is observed that, the EPS and biomass production is increased linearly with increasing coconut water addition upto 45%. However, coconut water addition beyond 45% shows the negative impact on process of SMF. Experiments were carried out to study the effect of NaCl on the SMF. From the results, it is observed that, the EPS and biomass production is increased linearly with increasing NaCl from 5 to 20% (Fig. 2a–b). However, NaCl dose beyond 20%, resulted in lower EPS and biomass production (Svensson, Waak, Svensson, & Radstrom, 2005).

To examine the effect of incubation time on SMF process, experiments were carried out in various incubation time and the results are shown in Fig. 3 (a–b). From the results, it is found that, the EPS and biomass production are increased rapidly with increasing the incubation time upto 25 h. Beyond 25 h shows the negligible EPS

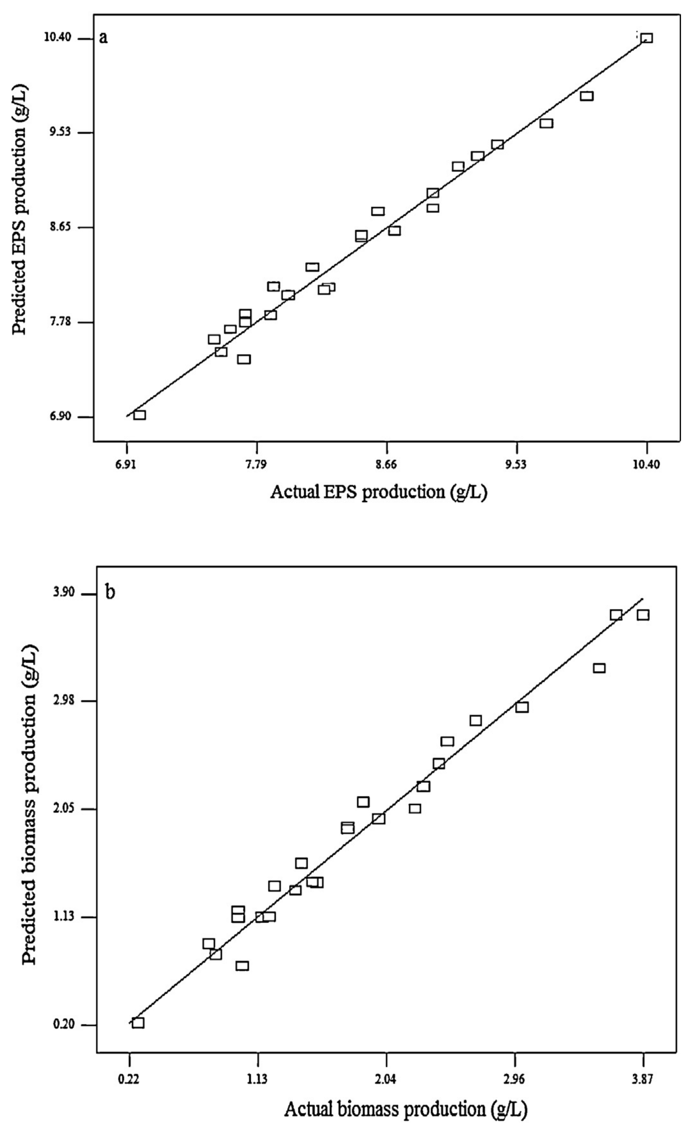

Fig. 1. Predicted versus actual plot for SSF process.

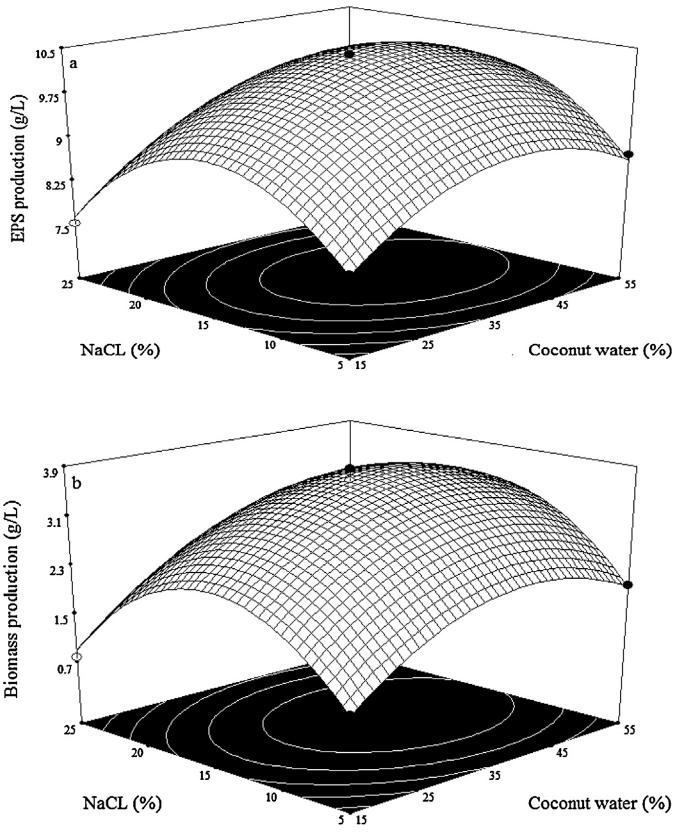

Fig. 2. Response surface plots representing the effect of process variables (*A* and *B*) on SSF.

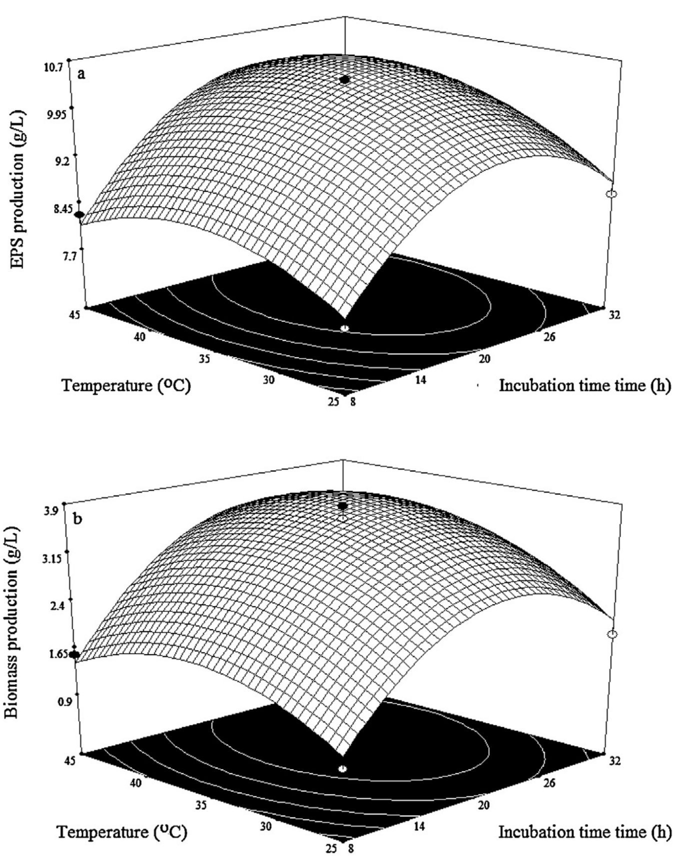

Fig. 3. Response surface plots representing the effect of process variables (*C* and *D*) on SSF.

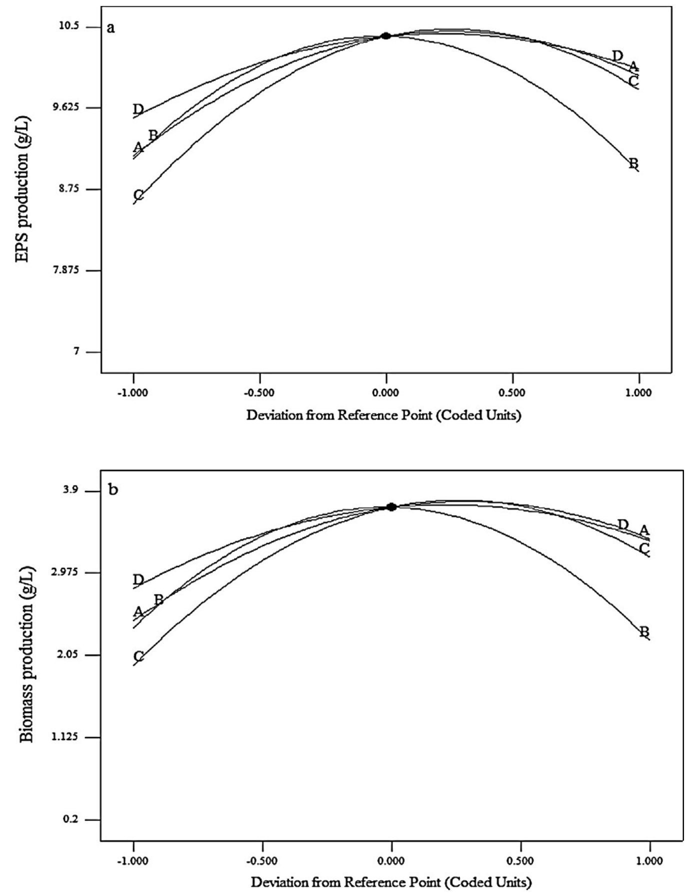

Fig. 4. Perturbation plot for SSF process.

and biomass production. In order to investigate the effect of temperature, the experiments were carried out at various temperature and the results are illustrated in Fig. 3(a–b), which shows that EPS and biomass production are increased with increasing temperature upto 40 °C (Looijesteijn & Hugenholtz, 1999). There after there is a drastic decrease in EPS and biomass production. Perturbation plot for SMF process is shown in Fig. 4. and it also confirms the significant effect of process variables on the SMF.

3.3. Multi response optimization

Optimum conditions of present SMF is found to be as follows; addition of coconut water of 40%, NaCl dose of 15%, incubation time of 24 h and temperature of 35 °C. Under these conditions, 10.57 g/L of EPS and 3.9 g/L of biomass are produced (Thirugnanasambandham, Sivakumar, & Prakash Maran, 2014d).

4. Conclusions

In this present study, production of EPS and biomass concentration using *L. confusus* in submerged fermentation is examined. RSM coupled BBD with four factors at three levels was used to develop the second order polynomial models. ANOVA was used to analysis the adequacy of developed mathematical models. Addition of coconut water of 40%, NaCl dose of 15%, incubation time of 24 h and temperature of 35 °C were found to be optimal conditions. Under these conditions, 10.57 g/L of EPS and 3.9 g/L of biomass were produced. Results exhibited that, SMF is found to be a suitable technique to produce the maximum EPS using *L. confusus*.

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