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Statistical optimization of L-ascorbic acid production by *Xanthomonas campestris* MTCC 2286 using sucrose as a low cost carbon source

Dnyaneshwar V. Bhand, Anand V. Patwardhan*

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Abstract

The objective of this work was to statistically optimize the cultural and nutritional parameters for L-ascorbic acid by the submerged fermentation of *Xanthomonas campestris* MTCC 2286 using sucrose as the sole carbon source. After studying the potential parameters that influence the bioprocess by "one variable at a time" approach, we found that the sucrose, yeast extract, and dipotassium phosphate (K_2HPO_4) were the most critical parameter for the production of L-ascorbic acid. Sucrose, a low cost carbon source supports the biomass, yeast extract, an enriched nitrogen source, and K_2HPO_4 acts as a stimulator for growth and production. The central composite design (CCD) of the RSM was employed to evaluate the interactive effects of these three variables in the production L-ascorbic acid from *Xanthomonas campestris* MTCC 2286 in fully aerobic batch fermentation (180 rpm). The L-ascorbic acid production was enhanced to 135.5mg/Las compared to the fermentation using non-optimized medium (95 mg/L).

Key words: L-ascorbic acid (L-AA), optimization, Response Surface Methodology (RSM), Central Composite Design (CCD), productivity improvement, fermentation.

Introduction

L-AA (vitamin C) plays key roles in m any biological processes, such as collagen formation, carnitine synthesis, and iron absorption (Naidu 2003). AA is widely used as a nutrient in the pharmaceutical and cosmetic industries, as well as a preservative by virtue of its excellent antioxidant activity (Yamane et al. 1997). It is used in large scale as an antioxidant in food, animal feed, beverages, pharmaceutical formulations, and cosmetic applications. The current world market of ascorbic acid is approximately 60,000-70,000 metric tonnes per year and generates annual revenues in excess of US$ 500 million.

Dnyaneshwar V. Bhand, Anand V. Patwardhan*

Department of Chemical Engineering, Institute of Chemical Technology, Nathalal M. Parekh Marg, Matunga (East), Mumbai 400019, Maharashtra, India.

*Tel: +91-22-3361 2019; Fax: +91-22-33611020
Email: av.patwardhan@ictmumbai.edu.in

In the global context of increased concerns for our environment, use of bioprocess as a replacement for existing chemical-based Reichstein process (Reichstein 1942; Roland et al. 1986) for the production of L-AA is an important challenge (Hancock et al. 2000).

L-AA or Vitamin C is required in human diet because human being is unable to produce this vitamin. It is an important cofactor for many biochemical reactions occurring within the body. It is required in iron incorporation into heme and collagen synthesis. Deficiency of L-AA is the root cause of Scurvy, which is characterized by poor wound healing and bleeding (Hancock and Viola 2001).

L-AA market is expanding rapidly as it is finding more and more applications into cosmetic preparations, vitamin supplements, and beverage industry (Hancock and Viola 2002). It also finds use in animal fodder. These applications are generally attributed to its antioxidant property. L-AA has also been explored in the treatment of cancer and AIDS, although controversially. Being an antioxidant, it reacts with many oxidizing species such as reactive oxygen species, singlet oxygen, and hydroxyl radicals etc., and therefore many times used as a preservative. It is also added into aquaculture since some fish species such as Salmon are unable to synthesize vitamin C.

Because of these widespread applications, demand for L-AA is increasing. Current production of L-AA is estimated to be 110,000 tonnes per year. Current production of L-AA is dominated by the chemical synthesis popularly known as Reichstein process. This process comprised of six chemical synthesis steps and one microbial biotransformation process. The overall yield of the Reichstein process is around 50% (Hancock and Viola 2001). This process is in use for more than 50 years, and further improvement in the productivity is difficult to achieve. The process is highly energy intensive, requires hazardous conditions, and creates waste disposal problem (Hancock and Viola 2002). In addition, current regulatory concerns and environmental control put constraints over the chemical process. Therefore, processes involving microbial biotransformation, which provide attractive

alternative for chemical synthesis, are most sought after. Microbiologically produced L-AA may be regarded as natural. There exists a separate demand for natural L-AA, which is compensated for by supplying L-AA isolated from natural sources like Citrus fruits. This may act as another incentive for microbial L-AA production.

Yeasts are known to produce L-AA. In addition, algae and bacteria can produce L-AA intra or extracellular (Takeyama et al. 1997; Running et al. 2002). Many bacteria are known to produce 2-keto-L-gulonic acid (2-KLG) – the immediate precursor (both in chemical and microbial processes) of L-AA, which is then subsequently converted to L-AA chemically (Saito et al. 1998; Sugisawa et al. 1990). In addition, in almost all microbial processes the final product is 2-KLG and not L-AA. Thus, the problem of 2-KLG conversion to L-AA remains to be tackled. This puts serious limitations on microbial processes for L-AA acid production.

Till date, the only bacterium known to produce L-AA directly is Ketogulonicigenium vulgare DSM 4025. It produces L-AA from D-sorbitol, L-sorbose, and L-gulose, but not from common and cheaper sources like glucose or sucrose. Rao and Sureshkumar (2000) have reported that *Xanthomonas campestris* MTCC 2286 produces L-AA directly from glucose under oxidative stress condition.

RSM is a useful statistical technique for the investigation and optimization of complex processes. It uses quantitative data from an appropriate experimental design to determine and simultaneously solve multivariate equation (Rastogi et al. 2010). There is an abundant literature on the use of RSM for process development and optimization (Bankar et al. 2008; Mamatha et al. 2008; Shankar et al. 2008).

In the present study, it has been found that L-AA production from *Xanthomonas campestris* is possible from sucrose using hydrogen peroxide as an inducer. Further, the production and optimization of L-ascorbic acid from *Xanthomonas campestris* MTCC 2286 was investigated using optimized nutritional and cultural conditions, an optimal statistical model was established by the full factorial central composite design (CCD) (Belur et al. 2012 and then validated through a few fermentation experiments.

Material and methods

Materials

All chemical reagents used were of analytical grade standard reagents and with more than 99.5% purity. Media components viz. sucrose, yeast extract, tryptone, glucose, was purchased from Hi-Media Laboratory, Mumbai, Maharashtra, India. Acetonitrile (HPLC grade) was also procured from Hi-Media Laboratory, Mumbai, Maharashtra, India. Potassium dihydrogen orthophosphate, dipotassium hydrogen orthophosphate, magnesium sulphate heptahydrate, urea, hydrogen peroxide were purchased from S. D. Fine Chemicals Limited., Mumbai, Maharashtra, India. An L-ascorbic acid standard for HPLC analysis was purchased from Sigma-Aldrich (Bangalore, India). All solvents used in this experiment were HPLC-grade, with the exception of ethanol.

Microorganism and growth medium

Xanthomonas campestris MTCC 2286 was procured from Microbial Type Culture Collection, Institute of Microbial Technology (IMTECH), and Chandigarh, India. *Xanthomonas campestris* was grown on tryptone, glucose, yeast extract (TGY) agar slants maintained at 30 ºC for 48 hrs, stored at 40 C and sub cultured every month.

Seed medium as proposed by Rao and Sureshkumar (2000) was used. Media consisted of 40 g glucose/L, 3 g yeast extract/L, 5 g/L K_2HPO_4, 0.6 g MgSO4.7H2O/L and 0.4 g urea/L. Glucose was separately sterilized, and then added into the media. The same media was used for the production of L-AA.

Seed culture inoculation

5 mL of saline solution was added in to 48-hour-old slants and scrabbled to prepare a uniform suspension. A 1 mL slant suspension were transferred to 50 mL of sterile seed medium in 250 mL Erlenmeyer flask and incubated at 30 ± 2 ˚C for 24 h at 180 rpm.

Production media inoculation

From 24 hrs-old seed culture, 3 mL were transferred into each production media contained in 250 mL Erlenmeyer flask and incubated at 30 ± 2 ˚C for 72 hrs at 180 rpm.

Addition of inducer

In the present study, hydrogen peroxide was used as an inducer. Since L-AA is produced in stationary phase, H_2O_2 (5 mL/L) addition was performed after 48 hrs of incubation. After 24 hrs of peroxide addition batch was harvested. Fermentation broth was centrifuged at 9000 rpm. Supernatant was then taken for L-AA analysis. Pellet obtained after centrifugation was suspended in potassium phosphate buffer at pH 7.0 and then it was taken for cell disruption.

Cell disruption

Before the cell disruption, the pellet was dispersed in potassium phosphate buffer at pH 7.0. Cell suspension was then probe-sonicated for 20 min with intervals of 30 seconds at a frequency of 240 KHz. Cell suspensions were then centrifuged at 9000 rpm to remove the cell debris. Enzyme solution thus obtained was stored at 40C until required.

Analysis of L-AA

The HPLC system of Agilent 1200 infinity series with a UVW detector and a C18 column (4.6 mm × 250 mm, 5 µm, Agilent ZORBAX Eclipse XDB-C18) was used for the qualitative and quantitative analysis of L-ascorbic acid in culture supernatant. Each injected sample (20 µL) was eluted with a mobile phase comprising 50% acetonitrile and 50% ultrapure water (HPLC grade). The elution flow rate and detection wavelength were set at 1.0 mL/min and 254 nm, respectively. The authentic L-ascorbic acid standard from Sigma Aldrich was used to construct a calibration curve by HPLC analysis.

Optimization of medium and time using one-factor-at-a-time classical method

The optimization of medium components for maximization of L-AA production by *Xanthomonas campestris* MTCC 2286 was performed in two stages. The first step was to identify the medium components that have significant effect on L-AA production. For this reason, medium components were identified by one variable at a time approach. The second step

was to determine the concentrations of the selected medium components using RSM. All experiments in shake flask were conducted in triplicates and mean of the results were taken as response.

Effect of carbon source

In the production medium, sucrose was substituted with five different carbon sources viz. glucose, fructose, maltose, xylose, and lactose. All carbon sources were used at 7%concentration (w/v).

Effect of nitrogen source

To study the effect of different nitrogen sources on L-AA production, different organic and inorganic nitrogen sources were used. Organic sources such as proteose peptone, soya peptone, meat peptone, mycological peptone, yeast extract, beef extract, and corn steep liquor were used at 1%concentration. Inorganic nitrogen sources such as diammonium hydrogen orthophosphate, sodium nitrate, and ammonium nitrate were used at 0.002%.

Effect of initial pH

Based on preliminary experimental results, the effects of initial pH have been explored within the range of 5.0 and 9. It is well known that the initial pH value of the solution plays an effective role on the system behaviour (Figure 1).

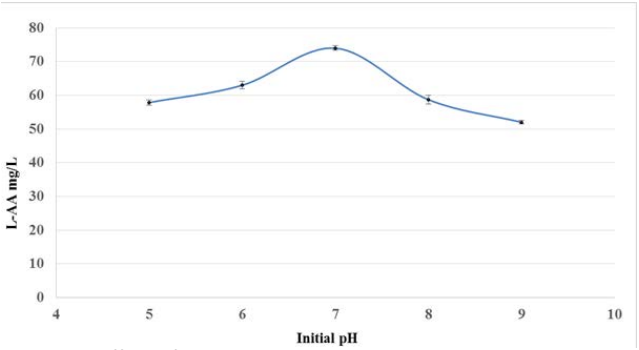

Figure 1: Effect of Initial pH on L-AA production by *Xanthomonas campestris* MTCC 2286. Results were means of three independent and standard deviation.

Experimental Design and Statistical analysis

Statistically based optimization is a proven tool for overcoming the limitations of the "one-factor-at-a-time" method. It is a more efficient technique since it can provide statistical data with a relatively small number of experiments. Moreover, it is a valuable tool for measuring interactions among factors and for the prediction of optimal fermentation conditions (Wen and Chen 2001). Another strong point of using statistically based optimization is that no complex calculations are required to analyze the resulting data (Berthouex and Brown 1994). Design-Expert (Trial Version 8.0.7, State-Ease, Inc., Minneapolis, MN, and Copyright @ 2012) was used for the design of the experiments and regression analysis of experimental data. A central composite design (CCD) under RSM was employed in order to illustrate the nature of response surface in the experimental design and to elucidate the optimal concentration of most significant independent variables as screened by the one-factor-at-a-time (OFAT) method. RSM with CCD design were used to optimize the process, identifying the interactions among the significant factors obtained from OFAT. A total number of 20 experiments were employed (Table 2).In developing the regression equation; factors were coded according to the equation:

$$X_1 = (X_i - X_{0i}) / X_i$$

Where X_1 is coded value of i^{th} independent variable, X_i is the natural value of i^{th} independent variable; X_{0i} is the natural value of the ith independent variable at the centre point and is the difference in effect.

Response surface methodology (RSM)

A Box-Wilson CCD was used to estimate the optimum levels of each variable. A second-order polynomial model was developed to predict the optimum conditions for EPA production:

$$Y = \beta_0 + \Sigma\beta_i X_i + \Sigma\beta_i X_{ii}^2 + \Sigma\beta_{ij} X_i X_j$$

Where Y is the predicted response, β_0 is the intercept term, β_i the linear effect, X_{ii} the squared effect and X_{ij} the interaction effect, β_i are the regression coefficients for each factor, βii is the regression coefficients for square effects and β_{ij} are the regression coefficients for interactions. Analysis of variance (ANOVA) was carried out using Design Expert 8.0.7 statistical package (StatEase, Inc, Minneapolis, MN, USA). All the regression variables were entered into the model and were significant at a 95% confidence level. The optimization process involves three major steps: (i) performing statistically designed experiments, (ii) estimating the coefficients in a mathematical model, and (iii) predicting the response and checking the adequacy of the model (Cao et al. 2010).

Table 1: Actual values and coded values of the independent variables for design of experiment

Factor Name	Unit	Min	Max	Coded Values		Mean	SD
				–1	1		
X_1	g/L	6.36	73.6	20	60	40	16.5
X_2	g/L	1.32	4.68	2	4	3	0.83
X_3	g/L	3.32	6.68	4	6	5	0.83

*X_1: Sucrose; X_2: Yeast Extract; X_3: Dipotassium phosphate; Max: maximum; Min: minimum; SD: standard deviation

Validation of the experimental model

The model was validated for all three variables within the design space. A random set of five combinations of variables were prepared and tested for L-AA production. The experimentally determined production values were in close agreement with the statistically predicted ones, confirming the model's authenticity and applicability of the statistical model (RSM) for the optimization of process variables. Each experiment was carried out in triplicate and repeated to observe the reproducibility.

Results and discussion

Fermentation Optimization Using One-Factor-at-a-Time

Figures 1 and 2 show the influence of different types of carbon and nitrogen sources on the production of L-AA by X. campestris grown in mineral salts medium with continuous shaking, which showed that sucrose, yeast extract, and the ion source K_2HPO_4, resulted in the highest enzyme activity. Statistical analysis showed that sucrose, yeast extract, and ion

source K_2HPO_4 were the most significant factors and their interactions are shown in Figures 4a-c.

Effect of Carbon Source

During bacterial fermentation, the carbon source acts as a building block for cellular structure as well as energy source (Stanbury et al. 1997). Figure 2 shows the effect of different carbon sources on L-AA production. The medium was supplemented with carbohydrates as carbon sources. Different carbohydrates such as sucrose, glucose, sorbitol, lactose, and galactose were used as carbon sources, but only glucose and sucrose were found to be promising.

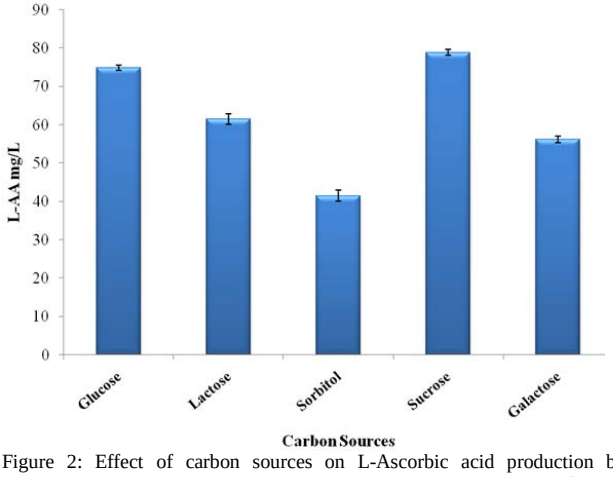

Figure 2: Effect of carbon sources on L-Ascorbic acid production by *Xanthomonas campestris* MTCC 2286. Results were means of three independent and standard deviation.

Effect of Nitrogen Source

Different organic and inorganic nitrogen sources were tested for their effect on L-AA productivity (Figure 3).Urea was the best inorganic nitrogen source (44.2 mg/L), while yeast extract was found to be the best organic nitrogen source for the production of L-AA. Out of the selected organic nitrogen sources, yeast extract gave the maximum yield of (78mg/L) of L-AA.

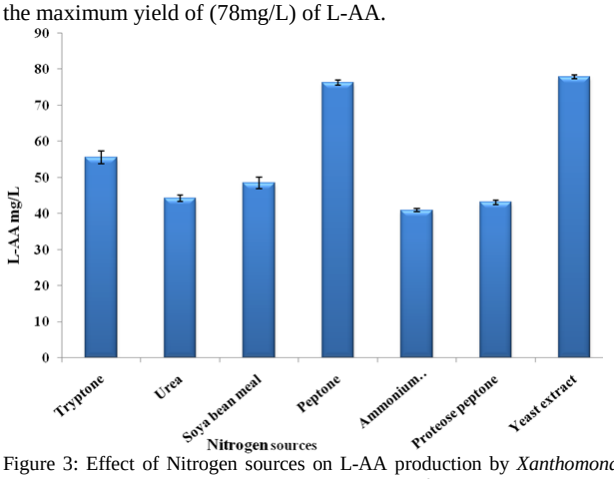

Figure 3: Effect of Nitrogen sources on L-AA production by *Xanthomonas campestris* MTCC 2286**.** Results were means of three independent and standard deviation.

Effect of phosphorus sources

The concentration of exogenous phosphorus in medium had significant effect on cell multiplication and metabolite production. K_2HPO_4 as an effective ion source for the growth X. campestris (Umashankar et al. 1996).

Optimization of Fermentation Using Experimental Design

The coefficients of the response surface model were evaluated by regression analysis and tested for their significance. The lack of fit was observed to be insignificant, indicating the model to be adequate to represent the experimental data. The coefficient of determination (R^2) of the model is 0.9894, and the model F value is 93.67, which indicates the model to be suitable to represent adequately the real relationship among the parameters used. The final predictive equation obtained is given by Eq.1. Using CCD, the experiments with different combinations of sucrose, yeast extract and ion source K_2HPO_4 were performed (Table 1). The maximum L-AA was taken from the time course data of fermentation run, in which, different medium formulations gave different fermentation times to reach a maximum productivity. From the present study, regression equation as shown in Eq. 1 was obtained after analysis of variance using Design Expert Software (Table 2).

The experimental outcomes of the CCD were fitted with a second-order polynomial function that could predict the L-AA productivity and had the following form:

$$Y = 128.67 - 1.12X_1 + 3.48X_2 + 4.23X_3 - 5.95X_1X_2 + 11.78X_1X_3 - 0.55X_2X_3 - 7.06X_1^2 - 7.64X_2^2 - 6.17X_3^2$$

This equation is based on the production of L-AA from X. campestris MTCC 2286 as a function of the three different variables, concentration of sucrose (X_1; g/L), concentration of yeast extract (X_2; g/L), and concentration of phosphate source K_2HPO_4 (X_3; g/L). The F-test with a very low probability value ($P_{model} > F \leq 0.0001$) and high F value (F = 93.67) demonstrates that the model is statistically significant. The "lack of fit" value of 0.2097implies that the model obtained was valid for the present work as it was more than 0.05. The goodness of fit of the model was again checked by the value of determination coefficient (R^2). In this case, the value of R^2 implies that the sample variation of 98.94% for L-AA production is attributed to the independent variables tested. The R^2 value also indicates that only 1.06% of the total variation was not explained by the model. Higher value of |R| (0.9503) also indicates good agreement between experimental and predicted values. Coefficient of variation (CV) shows the degree of precision with which the treatments are compared. In this study, a relatively low value of CV (1.83) was obtained, which indicates improved precision and reliability of the conducted experiments. The adequate precision measures the signal (response) to noise ratio. A ratio greater than 4 is desirable (Azila et al. 2008; Deepak et al. 2008). Here, a ratio of 37.25 was obtained, which indicated an adequate signal, and thus, the model can be applied for the process under study.

Design-Expert (Trial Version 8.0.7, State-Ease, Inc., Minneapolis, MN, Copyright @2012) Software was also used to calculate the coefficient values of Eq. 1. The significance of each coefficient was determined by p-values. The smaller the p-value, the more significant is the corresponding coefficient. Furthermore, p-values of less than 0.05 indicate that model terms are significant.

The variable with the largest effect was the linear term of K_2HPO_4 (X_3) with a p-value of<0.0001. This was followed by the linear term of yeast extract (X_2; p= 0.0002), cubic term of yeast extract concentration (X_{23}; p≤0.0001), squared term of sucrose (X_{12}; p=0.0001), squared term of yeast extract concentration(X_{22}; p=0.0002), interaction term of sucrose and yeast extract concentration (X_1X_2; p=0.0007), and squared term of K_2HPO_4 (X_{32}; p=0.0083). The linear term of sucrose (X_1) was not significant (p=0.0764); however, it is required to support the hierarchy of the model (Azila et al. 2008). These values suggest that the concentration of sucrose and yeast extract has a direct relationship on the production of L-AA. When testing the significance of the regression model, it was found that p-values obtained were very small (p<0.0001; Table 1) compared with the desired significance level, 0.05. This indicates that the regression model was accurate in describing or predicting the pattern of significant to the production of L-AA from X. campestris MTCC 2286.

Table 2: Experimental design and results of CCD of response surface methodology for the optimization of L-ascorbic acid production from *X. campestris* MTCC 2286 using Sucrose as a carbon source

Run	Actual factor level at coded variable level			L-AA	
	X_1 gm/L	X_2 gm/L	X_3 gm/L	Observed value[a] (mg/L)	Predicted value (mg/L)
1	20	2	6	83.8	83.97
2	40	3	5	120.5	120.15
3	60	4	4	79.3	80.23
4	20	2	4	96.5	97.96
5	60	4	6	111.5	111.14
6	40	3	5	121.5	120.15
7	40	3	5	119.5	120.15
8	20	4	6	101.2	102.72
9	40	3	5	123.5	120.15
10	60	2	6	115.5	117.18
11	60	2	4	83.5	84.08
12	20	4	4	118.5	117.92
13	6.36	3	5	119.5	119.10
14	40	1.3	5	111.5	109.72
15	73.63	3	5	116.5	115.34
16	40	3	3.3	113.5	112.61
17	40	3	5	134.2	137.19
18	40	3	5	135.5	137.19
19	40	3	6.68	127.5	126.83
20	40	4.68	5	121.2	121.42

*X_1: Sucrose; X_2: Yeast Extract; X_3: Dipotassium phosphate;

The optimal levels of variables were determined by constructing contour plots according to Eq. 1. Figures 4a–c show the contour plots for the variation in the yields of L-AA, as a function of concentrations of two variables, with the other one being kept constant at the centre point values. These contour plots clearly brought out the trend of L-AA production under the experimental conditions employed, and thus, the optimum levels of variables can be easily understood and located.

Figure 4a shows that the response is expected to increase with increasing yeast extract concentration. At low sucrose concentration, supplementation of yeast extract enhanced the production of L-AA. The maximum production of L-AA was obtained, with the concentration of yeast extract expected to be between 2 and 4 g/L. The consumption of sucrose at low initial concentration resulted in higher L-AA production as obtained by the most of the experiments.

Figure 4a also shows that lower concentration of sucrose was needed for the enhancement of L-AA production by X. campestris MTCC 2286. The use of high initial concentration of sucrose tends to inhibit growth of X. campestris MTCC 2286and L-AA production.

In order to search for the optimum conditions for maximum production of L-AA, the function of desirability was applied using the software. The experimental conditions with the highest desirability were selected to be verified. The validation experiments were conducted under the experimental conditions, together with the predicted and experimental values for the maximum L-AA production.

Table 3: Analysis of variance (ANOVA) for the Quadratic polynomial model of L-ascorbic acid production from *Xanthomonas campestris* MTCC 2286 reduced cubic response surface fitting

Source	SS	DF	MS	F Value	p-value	
Block	1258	1	1258			
Model	3581	9	397	93.67	< 0.0001	
X_1	17	1	17.02	4.01	0.0764	
X_2	165	1	165.3	38.9	0.0002	
X_3	244	1	244	57.4	< 0.0001	S
$X_1 X_2$	283	1	283	66.6	< 0.0001	
$X_1 X_3$	1109	1	1109	261	< 0.0001	
$X_2 X_3$	2.4	1	2.42	0.57	0.4697	
X_1^2	717	1	717	168	< 0.0001	
X_2^2	840	1	840	197	< 0.0001	
X_3^2	548	1	548	129	< 0.0001	
Residual	38.2	9	4.25			
Lack of Fit	28.6	5	5.73	2.39	0.2097	
Pure Error	9.6	4	2.4			
Correlation Total	4878	19				NS

SS: Sum of Squares; DF: Degree of freedom; MS: Mean Squares; S: Significant; NS: Not Significant; R^2 = 0.9894; Adj R^2= 0.9789; Pred R^2 = 0.9503; Std. Dev. = 2.06; Adequate Precision = 37.26

Validation of the optimization model

Statistical validity of the polynomials was established on the basis of ANOVA provision in the Design Expert Software. Subsequently, the feasibility and grid searches were performed to locate the composition of optimum formulations. The 3-D response surface plots were drawn using this software. The formulations corresponding to these check points were prepared and evaluated for various response properties. The resultant experimental data of response properties were compared with that of the predicted values. Linear regression plots between the observed and predicted values of the response properties were drawn. Linearity correlation plots between the observed experimental values and the predicted values are shown in Figure 5. High R^2 values of 0.9894 explain the linearity between the observed and the predicted values.

Optimal conditions realized from the optimization experiments were verified experimentally and compared with the calculated data from the model. The analysis of the design indicated that optimal conditions for the highest production of L-AA were sucrose of 40 g/L, yeast extracts of 3 g/L, and K_2HPO_4 of 5 g/L The maximum production L-AA experimentally was found to be 135.5mg/L, which was clearly very close to the predicted value (137.19mg/L). This verification revealed a high degree of accuracy of the model of more than 97.3 %, which is evidence for the model validation under the investigated conditions. Response surface methodology has been broadly discussed in the literature for optimizing different processes (Chopra et al. 2009; Tripathi and Mishra, 2009).

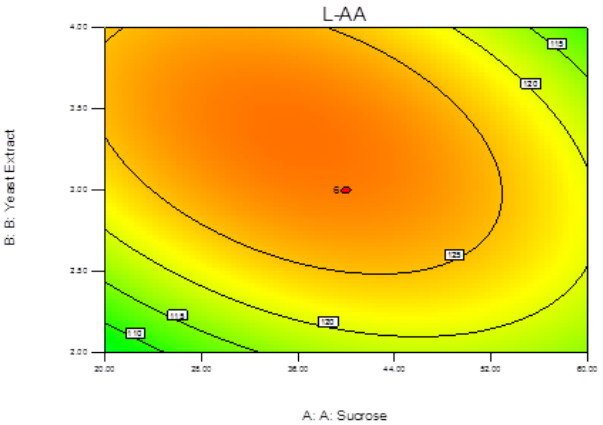

Figure 4a: 2D contour plot showing the relative effect of Sucrose concentration (g/L) and Yeast extract concentration (g/L) on L-AA production.

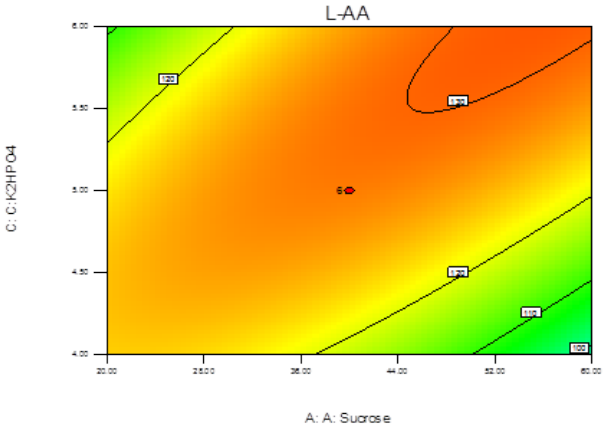

Figure 4b: 2D contour plot showing the relative effect of Sucrose concentration (g/L) and Dipotassium phosphate concentration (g/L) on L-AA production.

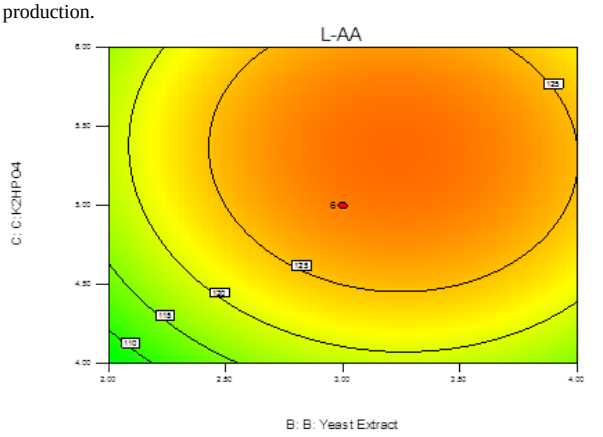

Figure 4c: 2D contour plot showing the relative effect of Yeast extract concentration (g/L) and Dipotassium phosphate concentration (g/L) on L-AA production.

In order to validate the statistical model, experiments were carried out at optimal levels of most significant variables. The special features of the RSM tool, "contour plot generation" was analyzed for determining the optimized value of the factors, but it was difficult to analyze all these simultaneously. Hence, point prediction of software was used to determine optimum values of the factors for maximum L-AA production. Finally, the optimum combination of sucrose 40 g/L, yeast extracts 3 g/L, and K_2HPO_4 5 g/L was determined. This combination predicted 139.19 mg/L of L-AA production. Finally, a verification experiment was performed under the optimum operational conditions. The highest L-AA productivity achieved in the verification experiment was 136.5 mg/L, nearby the value predicted by model (139.19 mg/L).

Conclusion

Response surface methodology was found to be effective in the modelling and optimization of the parameters. The CCDs and response surface analysis were found to be useful in localizing the optimum level of the most significant factors that contribute to the highest L-AA production by X. campestris MTCC 2286. The statistical approach showed significant results for optimizing the process parameters for maximal production of L-AA under SmF. The CCD design was used to check interactions and concentration of significant media components. The highest L-AA productivity was achieved at 40 g/L sucrose; 3 g/L yeast extracts, and 5 g/L K_2HPO_4 and found to be 135.5mg/L. RSM and CCD, the selected methods in this study, help to maximize the amount of information that can be obtained, while limiting the number of individual experiments required. Both methods are simple, efficient, time and material saving. Thus, with increase yield and productivity, the industrial L-AA production can be possible and economically feasible. Further studies are required to maintain the production conditions by controlling aeration, agitation, and other physical parameters such as pH and temperature in the fermenter.

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