STATISTICAL OPTIMIZATION OF CURDLAN PRODUCTION BY LOCAL EGYPTIAN *AGROBACTERIUM* ISOLATES

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ABSTRACT

Curdlan is a linear D-glucans, belongs to the class known as beta (1, 3) - D -glucan macromolecules, Curdlan gum is a neutral polysaccharide was discovered by Dr. Harada in 1964, Curdlan gum is insoluble in water but soluble in most organic solvents. Curdlan is one of the FDA approved biopolymer used in food industries such as jelly, noodles, and edible fibers manufacturing process approved in 1996, Curdlan gum produced by many Agrobacterium species under nitrogen-limiting conditions, has many useful benefits in food, agriculture, pharmaceutical, and medicine industry.

Sequential optimization strategy, based on statistical experimental designs, was employed to enhance the production of curdlan by local *Agrobacterium* sp isolate, which include (Plackett–Burman design and Box–Behnken design)

INTRODUCTION

Curdlan is an insoluble linear polysaccharide composed of almost exclusively Beta (1, 3)-glycoside linkages, which was first produced by *Alcaligenes faecalis* var. myxogenes 10C3, a bacterial strain discovered by Harada during the screening of soil bacteria capable of metabolizing various petroleum fractions **(Harada, 1965).** However, until now only bacteria belonging to the *Agrobacterium* and *Alcaligenes species* have been reported to produce curdlan, especially under nitrogen limiting conditions **(Harada *et al.*, 1966, Lee and Lee, 2001)**. curdlan has been given its name because of its ability to "curdle" when heated **(Harada *et al.*, 1966**), such a property enable curdlan to be used as a gelling material to improve the textural quality, water-holding capacity and thermal stability of various foods; it is an useful additive for noodles, sauces, frozen foods and packaged meats (**Jezequel, 1998, Spicer, *et al.*, 1999)**.

Curdlan is biodegradable, edible and nontoxic toward humans and the environment; in addition to the application in food, its growing potential has also been found in pharmaceutical industries, and numerous other applications, such as antiviral and anticancer treatments. **(Demleitner *et al.*, 1992; McIntosh *et al.*, 2005).**

Curdlan is the third microbial exopolysaccharide approved for use in the United States by the FDA, which was approved in 1996 **(USFDA, 1996).** Over 100 tons of Curdlan are produced annually even though curdlan is relatively expensive in comparison to other food gums **(Chaplin, 2003).** So that now, it is an urgent need in Egypt to develop production fermentation technology for the production of this type of polysaccharide. recently the utilization of Curdlan as a natural source used as additive to food and food processing. Therefore, it may become important to develop the fermentation condition using low coast raw materials.

Therefore, in order to increase the economic attractiveness of curdlan, it is important to increase the productivity and minimize its production costs to allow curdlan to compete with other polysaccharides. **(Kim *et al.*, 2000; Lee and Lee, 2001).**

The conventional methods of optimization are extremely time consuming, tedious and expensive for a large number of variables **(Singh and Satyanarayana, 2006).** Optimization of all the variables by statistical experimental designs, Plackett– Burman and Box–Behnken design, can eliminate the limitations of 'one variable at a time' approach **(Stanbury *et al.*, 1997)**. In the present work, we report for the first time a sequential optimization strategy for curdlan production by *Agrobacterium sp* local isolate through statistically designed experiments as an effective tool for medium engineering. First, Plackett–Burman screening design was applied to address the most significant factors affecting curdlan production. Second, Box–Behnken design was applied to determine the optimum level of each of the significant parameters that brings maximum curdlan production.

MATERIALS AND METHODS

Microorganisms

All *Agrobacterium* isolates used in this study were isolated from different soil samples, and Crown gall tissue collected from different localities in Egypt . The strain was maintained on nutrient agar medium at 28- 30°C. The microbial isolate has been identified by microscope examination, and Biolog GN microstation.

Curdlan production conditions

Cultures were allowed to grow at 28-30°C with shaking at 200 rpm, in 250 ml conical flasks containing 50 ml aliquots pre-culture basal medium of the following components (g/l): yeast extract: 5.0; Glucose: 30.0; KH_2PO_4: 1.0 and $MgSO_4.7H_2O$: 0.5.the pH was adjusted at 6.5 Then 0.5 ml of 24 old culture was used as inoculum for the basal production medium.

Extraction of curdlan from cultural media

Extraction of curdlan was carried out adding one volume of culture media with two volumes of NaOH (1N). The mixture was kept for 1h at room temperature. To remove degraded bacterial cells the mixture was centrifuged at 4000 rpm. The Supernatant was neutralized with HCl (3N) to pH 5-7: After keeping the mixture at 28°C for overnight, curdlan was obtained by centrifugation at 4000 rpm. The residues were washed with distilled water several times to remove salts from residue and finally washed with acetone to furnish dry product and stored at room temperature until further use.

Statistical designs

Plackett–Burman design

The Plackett-Burman design based on the first order model **(Plackett and Burman, 1946)** was used to screen and evaluate the important media components that influence on the production of curdlan compounds. All the experiments were carried in triplicate according to designed matrix (Table 1) using the following equation:

$$Y= \beta_0 + \Sigma \beta_i X_i \ (i = 1, \ldots. k) \ (Equation\text{----}1)$$

Where, Y is the estimated target function, β_0 is a constant, β_i is the regression coefficient, X is independent variable and k is number of variables. **(Abdel-Fattah *et al.*, 2005)**.

Total number of experiments to be carried out according to Plackett–Burman design is n+1, where n is the number of variables. Each variable is represented at two levels, high and low denoted by (+) and (-) respectively. Each column should contain equal number of positive and negative signs. This design is practical specially when the investigator is faced with a large number of factors and is unsure which settings are likely to be nearer to optimum responses **(Strobel *et al.*, 1999).**

The main effect of each variable was determined according to the following equation:

$$Ex_i = (\Sigma M_i^+ - \Sigma M_i^-) / N \quad (Equation----2)$$

Where Ex_i is the variable main effect, M_{i+} and M_i^- are the response percentage in trials, in which the independent variable (x_i) was present in high and low concentrations, respectively and N is the half number of trials.

Standard error (SE) of the concentration effect was the square root of the variance of an effect and the significant level (p-value) of the effect of each concentration was determined using Student's t-test as given by the equation:

$$t(X_i) = E(X_i)/SE \ (Equation----3)$$

Where $E(X_i)$ is the effect of variable X_i.

Fifteen medium components were screened in 16 trials (Table 1). All experiments were carried out in triplicate and the averages of curdlan product were taken as respons.

Box–Behnken design

In order to describe the nature of the response surface in the experimental region, a Box–Behnken design **(Box and Behnken, 1960)** was applied. As presented in Table 3, factors of highest confidence levels were prescribed into three levels, coded -1, 0, and +1 for low, middle and high concentrations (or values), respectively. (Table 4) represents the design matrix of a 15 trials experiment. For predicting the optimal point, a second order polynomial function was fitted to correlate relationship between independent variables and response (curdlan product). For the three factors this equation is:

$$Y_{activity} = ß_0 + ß_1 x_1 + ß_2 x_2 + ß_3 x_3 + ß_{12} x_1 x_2 + ß_{13} x_1 x_3 + ß_{23} x_2 x_3 + ß_{11} x_1^2 + ß_{22} x_2^2 + ß_{33} x_3^2.$$

Where Y is the response represent curdlan production g/l,while β_0 model constant; X_1, X_2 and X_3 independent variables; β_1, β_2 and β_3 are linear coefficients; β_{12}, β_{13} and β_{23} are cross product coefficients and β_{11}, β_{22} and β_{33} are the quadratic coefficients. Microsoft Excel 2003 was used for the regression analysis of the experimental data obtained. The quality of fit of the polynomial model equation was expressed by the coefficient of determination R^2. Experiments were performed in triplicate and mean values are given**. (Abdel-Fattah *et al.*, 2005)**.

Statistical analysis of data

The results of Plackett-Berman experiment were applied to linear multiple regression analysis using Microsoft Excel 2003 or SPSS 17 software to estimate t values, P-values and confidence levels. The linear multiple regression analysis was conducted for Curdlan product as response. From the statistical analysis the variable whose confidence levels were bigger than of equal to 90% were considered to be significant for curdlan production , variables with confidence levels were less than 90 % until 70% were considered as effective. **(Stawe and Mayer, 1966)**. The optimal value Curdlan product was estimated using the solver function of Microsoft Excel tools **(Abdel-Fattah *et al.*, 2005)**.

Table (1): Media components and test levels for Plackett–Burman experiment

Variable	*Variable code*	*Low level (-1)*	*High level (+1)*
$(Napo_4)_6$ mol /l	X1	0.024	0.048
$(CaCO_3$ (g/l	X2	1.0	2.0
(Urea (g/l	X3	1.0	2.0
Sucrose (g/l)	X4	20.0	25.0
(Glucose (g/l	X5	20.0	25.0
Pepton (g/l)	X6	1.0	1.5
(Yeast extract (g/l	X7	1.0	1.5
$(K_2HPO_4$ (g/l	X8	0.5	1.0
$(KH_2PO_4$ (g/l	X9	0.5	1.0
Fe_2SO_4 (g/l)	X10	0.01	0.05
$(MgSO_4.7H_2O$ (g/l	X11	0.25	0.5
$(NH_4Cl$ (g/l	X12	0.5	1.0
$MnSO_4.7H_2O$	X13	0.0	0.01
Culture pH	X14	6.5	7.5
Culture volume	X15	50	25

Table(2): Randomized Plackett–Burman experimental design show evaluating factors influencing curdlan production from local *Agrobacterium* sp.

Run No.	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}	*Curdlan product (g/1)*	*Final D. (g/1)w*	*Final pH*
1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	0.728	0.5252	6.2
2	-1	1	1	-1	1	-1	1	-1	1	1	1	-1	-1	-1	1	0.334	0.3687	7.1
3	1	-1	1	1	-1	1	-1	1	-1	1	1	1	-1	-1	-1	1.85	0.3936	6.0
4	-1	1	-1	1	1	-1	1	-1	1	-1	1	1	-1	-1	-1	2.81	0.3911	7.4
5	-1	-1	1	-1	1	1	-1	1	-1	1	-1	1	1	1	-1	0.684	0.4910	7.1
6	-1	-1	-1	1	-1	1	1	-1	1	-1	1	-1	1	1	1	3.046	0.2121	7.2
7	1	-1	-1	-1	1	-1	1	1	-1	1	-1	1	1	1	1	2.554	0.5510	7.0
8	1	1	-1	-1	-1	1	-1	1	1	-1	1	-1	-1	-1	1	1.082	0.3221	7.0
9	1	1	1	-1	-1	-1	1	-1	1	1	-1	1	1	1	-1	0.787	0.2111	7.1
10	-1	1	1	1	-1	-1	-1	1	-1	1	1	-1	-1	-1	1	0.2555	0.5509	5.88
11	1	-1	1	1	1	-1	-1	-1	1	-1	1	1	1	1	-1	2.342	0.6034	6.0
12	-1	1	-1	1	1	1	-1	-1	-1	1	-1	1	-1	-1	1	1.338	0.3131	6.1
13	1	-1	1	-1	1	1	1	-1	-1	-1	1	-1	1	1	-1	0.9245	0.6120	6.3
14	-1	1	-1	1	-1	1	1	1	-1	-1	-1	1	1	1	1	1.023	0.4320	7.0
15	1	-1	1	-1	1	-1	1	1	1	-1	-1	-1	-1	-1	1	1.205	0.5460	7.1
16	1	1	-1	1	-1	1	-1	1	1	1	-1	-1	1	1	-1	0.59	0.2987	6.0

Table (3): The levels of variables chosen for the Box–Behnken optimization experiment

Variable code	*Variable*	*-1*	*0*	*1*
KH_2PO_4 (g/l)	X1	0.8	1.0	1.6
$MgSO_4.7H_2O$ (g/l)	X2	0.4	0.5	0. 6
NH_4Cl (g/l)	X3	0.8	1.0	1.6

Table (4): Box–Behnken factorial experimental design, representing the response of curdlan product.

Trials	*KH_2PO_4*	*$MgSO_4.7H_2O$*	*NH_4CL*	*Curdlan product*	
				Measured	*Predicted*
1	-1	-1	0	1.986	2.272
2	1	-1	0	4.542	4.244
3	-1	1	0	3.054	3.351
4	1	1	0	2.902	2.615
5	-1	0	-1	3.096	3.085
6	1	0	-1	1.86	2.433
7	-1	0	1	1.908	1.334
8	0	0	1	3.21	3.220
9	0	-1	-1	2.265	1.989
10	0	1	-1	4.34	4.053
11	0	-1	1	3.56	3.846
12	0	1	1	0.956	1.232
13	0	0	0	3.67	3.488
14	0	0	0	3.23	3.488
15	0	0	0	3.564	3.488

RESULTS AND DISCUSSION

Several methods were studied for Curdlan production from *Agrobacterium* sp. Many researchers were studied the production of curdlan by conventional methods, one factor at time technique, physical factor such as (pH, temp, agitation, and dissolved oxygen), chemical factor such as (carbon, nitrogen source, and mineral salts).

The disadvantages of conventional methods of optimization are extremely time consuming, tedious and expensive for a large number of variables **(Singh and Satyanarayana, 2006).** So that the recent directions of optimization depend on application of statistical experimental method, this method requires the identification of the major factors that are suitable to sustain good production of Curdlan. In preliminary

experiments, we evaluated various carbon sources, nitrogen sources and pH for their suitability to sustain good production. **(Wu, 2006).**

Many physiological factors affect the production of curdlan by *Agrobacterium sp*. **(Lee *et al.,* 1997)**. In general, factors affecting curdlan production include inoculum preparation, growth medium, environmental conditions, and the formation of by-products. Increasing the rate and extent of polysaccharide synthesis, eliminating undesirable enzyme activities or transferring the genetic determinants of polysaccharide synthesis to more amenable host organisms can improve the polysaccharide production. **(Shrikant *et al.,* 2007)**.

A sequential optimization approaches were applied in the present part of the study. The first approach deals with screening for culture as well as nutritional factors affecting on curdlan production. The second approach is to optimize the factors that control curdlan production process

Evaluation of the factors affecting on Curdlan production

In the first approach, the Plackett–Burman design was applied to reflect the relative importance of various fermentation factors fifteen different factors (variables) including fermentation conditions and medium constitution were chosen to perform this optimization process. The averages of curdlan product for the different trials are given in g/l and shown in Table (2):

The main effect of each variable upon Curdlan product was estimated as the difference between both averages of measurements made at the high level (+1) and at the low level (-1) of that factor. The data in Table 2 show a wide variation from 0.334 to 3.046 g/1. This variation reflects the importance of medium optimization to attain higher productivity. The analysis of the data from the Plackett–Burman experiments involved a first order (main effects) model. The main effects of the examined factors on the curdlan product were calculated and presented graphically in Fig. 1. On the analysis of the regression coefficients of the fifteen independent variables nine factors showed positive effect on curdlan product: sucrose, yeast extract, KH_2PO_4, $FeSO_4$, $MgSO_4$, $(Na\ PO_4)_6$, NH_4Cl, $MnSO_4$, and Culture volume. While $CaCO_3$, urea, glucose, peptone, K_2HPO_4 and Culture pH volume showed negative effect on curdlan product.

Biosynthesis of curdlan affected by nutritional factors (Carbon and Nitrogen sources). Carbon sources used (glucose, sucrose, sugar cane, and molasses). Nitogen souces (ammonium, nitrate, and urea).

(Lee *et al.*, 1997). found that Nitrogen depletion is essential for a higher production of curdlan by *Agrobacterium sp.* ATCC 317.

Biosynthesis of curdlan occurs in the post stationary growth phase. Optimal production to it at (28-30°C) the cell growth rate is optimal at pH 7.0, whereas curdlan production is optimal at pH 5.5.

Fig. (3) Showed the ranking of factor estimates in a Pareto chart. The Pareto chart displays the magnitude of each factor estimate and it is a convenient way to view the results of a Plackett–Burman design.

The polynomial model describing the correlation between the 15 factors and the Curdlan product could be presented as follows:

$$Y_{activity} = 1.347 + 0.135\ X_1 - 0.627\ X_2 - 0.552\ X_3 + 0.241\ X_4 - 0.055\ X_5 - 0.151\ X_6 + 0.217\ X_7 - 0.055\ X8 + 0.266\ X9 + 0.147\ X10 + 0.327\ X11 + 0.468\ X12 + 0.202\ X_{13} - 0.024\ X_{14} + 0.080\ X_{15}.$$

Table (5): Statistical analysis of Plackett- Burman design showing coefficient values for each variable on curdlan production.

Variable	*Variable code*	*Coefficients*	*Main effect*	*Standard Error*	*t-Stat*	*P-value*	*Confidence level (%)*
Intercept	1.347						
$(NapO_4)_6$	*X1*	0.135	0.27	0.078	1.542	0.366	63.4
$(CaCO_3$ (g/l	*X2*	- 0.627	- 1.254	0.079	- 7.785	0.0813	91.87
(Urea (g/l	*X3*	- 0.552	- 1.104	0.077	- 7.423	0.085	91.5
(Sucrose (g/l	*X4*	0.241	0.482	0.079	3.092	0.199	80.1
(Glucose (g/l	*X5*	- 0.055	- 0.11	0.079	- 0.558	0.675	32.5
(Pepton (g/l	*X6*	- 0.151	- 0.302	0.080	- 1.933	0.303	69.7
(Yeast extract (g/l	*X7*	0.217	0.434	0.072	3.434	0.180	82
$(K_2HPO_4$ (g/l	*X8*	- 0.055	- 0.11	0.072	- 0.305	0.811	18.9
$(KH_2PO_4$ (g/l	*X9*	0.266	**0.532**	0.080	3.294	0.187	81.3
Fe_2SO_4	*X10*	0.147	0.294	0.079	1.998	0.295	70.5
$(MgSO_4.7H_2O$ (g/l	*X11*	0.327	**0.654**	0.079	4.166	0.149	85.1
$(NH_4Cl$ (g/l	*X12*	0.468	**0.936**	0.077	5.790	0.108	89.2
$MnSO_4$.7H2O	*X13*	0.202	0.404	0.079	2.630	0.231	76.9
Culture pH	*X14*	- 0.024	- 0.048	0.078	- 0.494	0.707	29.3
Culture volume	*X15*	0.080	0.16	0.133	0.422	0.745	25.5

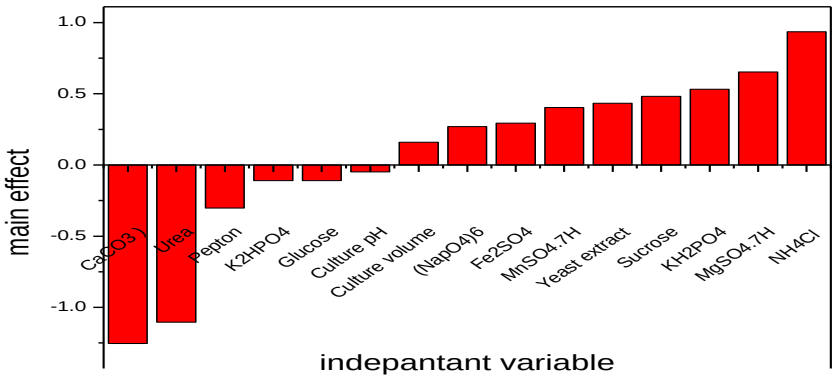
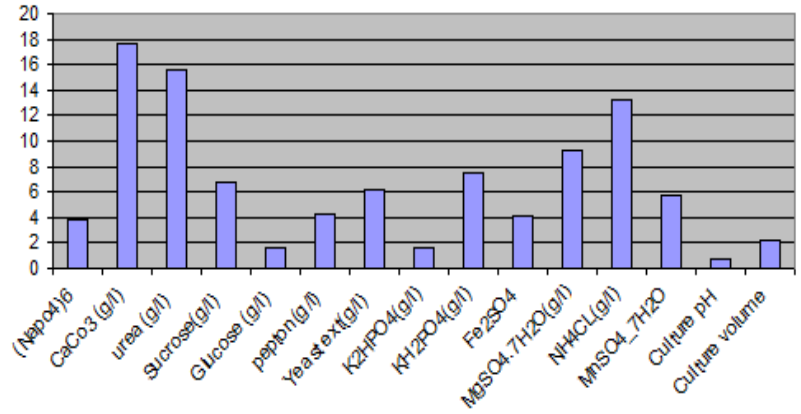


Fig. (1): Main effect of each variable using Plackett–Burman experimental design for evaluating factors influencing Curdlan production from local *Agrobacterium* sp.



(Medium components for each independent variable)

Fig. (2): Pareto chart rationalizing the effect of each variable on curdlan production from local *Agrobacterium* sp.

According to these results, a medium of the following composition is expected to be near optimum: (g/l): sucrose: 25.0, KH_2PO_4: 1.0, Fe_2SO_4: 0.05, $MgSO_4.7H_2O$: 0.5, NH_4Cl: 1.0, and ($NapO_4$)6: 0.048 mol/l, in culture volume: 25 ml, the initial pH was adjusted to 6.5. This result presented about 3-folds increase on Curdlan production, when compared to results obtained in basal production medium.

Many physiological factors affect the production of curdlan by *Agrobacterium sp*. **(Lee *et al.*, 1997)**. In general, factors affecting curdlan production include inoculum preparation, growth medium, environmental conditions, and the formation of by-products. Increasing the rate and extent of polysaccharide synthesis, eliminating undesirable enzyme activities or transferring the genetic determinants of polysaccharide synthesis to more amenable host organisms can improve the polysaccharide production. **(Shrikant *et al.*, 2007)**.

Biosynthesis of curdlan affected by nutritional factors (Carbon and Nitrogen sources). Carbon sources used (glucose, sucrose, sugar cane, and molasses). Nitogen souces (ammonium, nitrate, and urea).

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Biosynthesis of curdlan occurs in the post stationary growth phase. Optimal production to it at (28-30°C) the cell growth rate is optimal at pH 7.0, whereas curdlan production is optimal at pH 5.5.

Optimization of the culture conditions by Box–Behnken design

In order to approach the optimum response region of the Curdlan production, significant independent variables (KH_2PO_4, X_1; $MgSO_4.7H_2O$, X_2 and NH_4Cl, X_3) were further explored, each at three levels. Table 4 represents the design matrix of the coded variables together with the experimental results of the curdlan production. All cultures were performed in triplicate and the average of the observations was used.

For predicting the optimal point, within experimental constrains, a second-order polynomial function was fitted to the experimental results (linear optimization algorithm) of curdlan production.

$$Y_{activity} = 3.488 + 0.3085\,X_1 - 0.138\,X_2 - 0.241\,X_3 - 0.677\,X_1X_2 + 0.635\,X_1X_3 - 1.169\,X_2X_3 - 0.314\,X_1^2 - 0.052\,X_2^2 - 0.655\,X_3^2.$$

Where $Y_{activity}$ is the response (curdlan production) and X_1, X_2 and X_3 are the coded values of the test variables (KH_2PO_4, $MgSO_4$and NH_4Cl) respectively. The three-dimensional response surface are the graphical representations of the regression equation. They are helpful in understanding both the main and the interaction effects of the factors on the response value. Figure 3.(A–C) showed the response surface and contour plots of $KH_2PO_4$and $MgSO_4$, KH_2PO_4 and NH_4Cl and $MgSO_4$and NH_4Cl on curdlan production respectively, keeping the other component at the fixed zero level.

The results obtained by (ANOVA) analysis showed a significant *F*-value (4.74) which implied the model to be significant. Model terms having values of Prob > F (0.005) less than 0.05, considered significant. The determination of coefficient (R^2) was calculated as 0.895 for curdlan activity (a value of $R^2 > 0.75$ indicated the aptness of the model) which indicates the statistical model can explain 89.5% of variability in the response. The goodness of the model can be checked by the determination of coefficient (R^2) and correlation coefficient (R). The R^2 value is always between 0 and 1. The closer the R^2 to 1, the stronger the model and the better in predicted response Munk *et al.* (1963). The value of R (0.946) for (Eq. 6) being close to 1 indicated a close agreement between the experimental results and the theoretical values predicted

by the model equation. An overall 4.5 - fold increase in curdlan was being achieved after application of RSM. This reflects the necessity and value of optimization process

Validation of the model

The validation was carried out under optimum conditions of the media predicted by the polynomial model. The experimental curdlan production of 4.542 g/l was obtained which is closer to the predicted curdlan production of 4.42 g/l after 4 days of fermentation validating the proposed model. A second order polynomial model was established using Box–Behnken design to identify the relationship between the three factors and the curdlan yield. The final concentrations of the medium components optimized with RSM were (g/l): sucrose: 25.0, KH_2PO_4: 1.6, Fe_2SO_4: 0.05, $MgSO_4.7H_2O$: 0.4, NH_4Cl: 1.0, and $(NapO_4)6$: 0.048 mol/l, in culture volume: 25 ml, the initial pH was adjusted to 6 .5 as a plane medium for further production.

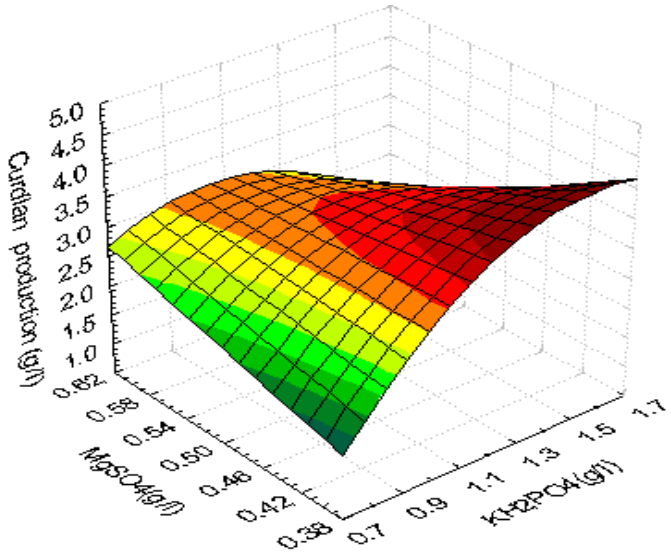

Fig. 3. A. Response surface plot of curdlan production by *Agrobacterium* showing the Interactive effects of different concentrations of $MgSO_4$t and KH_2PO_4 at X_3= 0

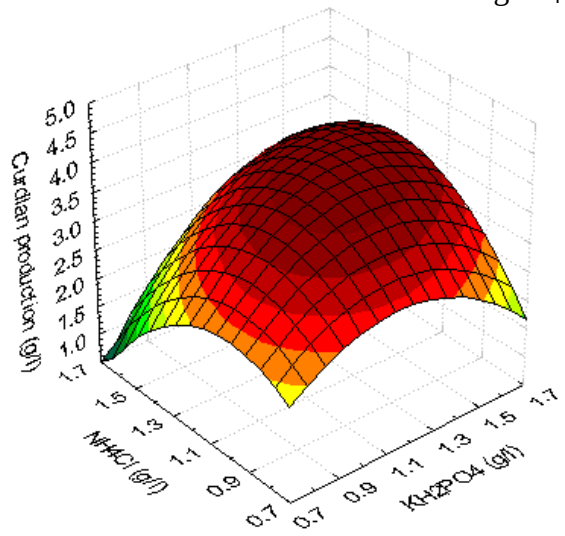

Fig. 3. B. Response surface plot of curdlan production by *Agrobacterium* showing the Interactive effects of different concentrations of NH_4 Cl and KH_2PO_4 at X_2= 0

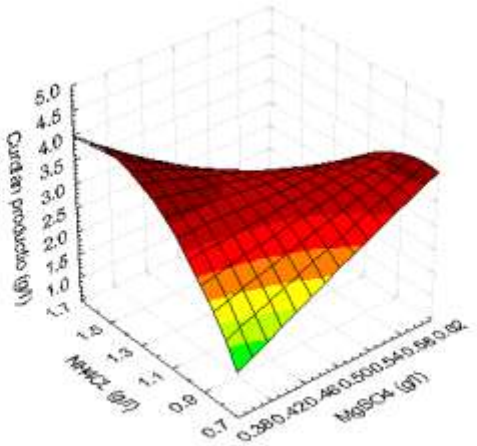

Fig. 3. C. Response surface plot of curdlan production by *Agrobacterium* showing the Interactive effects of different concentrations of NH_4Cl and Mg_2SO_4 at $X_1= 0$

CONCLUSION

Plackett–Burman and Box–Behnken designs have been proved to be effective in the optimization of Curdlan production by local Egyptian *Agrobacterium* Isolates.

In screening the factors affecting production of certain secondary metabolite it is very important to test as many factors as possible and to identify the significance of each of them. Plackett–Burman design offers good and fast screening procedure and mathematically computes the significance of large number of factors in one experiment, which is time saving and maintain convincing information on each component. Although, otherwise, interaction is not included in this design, it is not of first priority in the screening program to examine the interaction between these large numbers of variables. of these, only the most effective factors with positive significance would be selected for further optimization, while those showing high negative effect on the bioprocess may be dropped in all further experiments. This indicates the effectiveness of the Plackett–Burman design as a tool for elucidating the most important variables affecting the response. This design is recommended when more than five factors are under investigation. Applying Box–Behnken design to optimize the selected factors for maximal production is an efficient method that tests the effect of factors interaction. Besides, it converts the bioprocess factor correlations into a mathematical model that predicts where the optimum is likely to be located. It is worth while to advise the microbial industry sponsors to apply such experimental designs to maintain high efficiency and profit bioprocesses.

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ضبط البيئه المغذيه لانتاج الكيردلان من عزلات اجروبكتيريم مصريه باستخدام التحاليل الاحصائيه

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الكوردلان وهو اسم عام للمركب ١ - ٣ بيتا جلوكان تم اكتشافه سنه ١٩٦٤ بواسطه العالم Harada و هو من الالياف الغير قابلة للذوبان والتى تم الموافقة على استخدامة كأضافات غذائية فى يناير ١٩٩٦.وقد وافقت علية هيئة أدارة الاغذية والعقاقير الامريكية ، حيث يستخدم فى العديد من المكونات التى تدخل فى تصنيع الغذاء ومثال ذلك اللحوم المصنعة والمربات وايضا الاضافات الغذائية قليلة الدهون والصلصات والمرق . ويتم أنتاج الكوردلان بأستخدام الكائن الحى Agrobacterium *biovar* وله استخدامات كثيره فى مجالات الاغذيه والزراعه والدواء والطب ونظرا لاهميه مادة الكوردلان لابد من ايجاد سلالات ذات انتاجيه عاليه وكذلك ضبط مكونات الميديا بالبحث عن مصادر كربونيه ونتيروجنيه رخيصه الثمن ولاختيار افضل الظروف لتنمية البكتريا وانتاج الكيردلان تم ضبط الميديا من خلال تحاليل احصائيه بطريقه Plackett–Burman design وكذلك طريقه Box–Behnken design