International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:5, No:9, 2011

Optimization of Conditions for Xanthan Gum Production from Waste Date in Submerged Fermantation

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***Abstract*—**Xanthan gum is one of the major commercial biopolymers. Due to its excellent rheological properties xanthan gum is used in many applications, mainly in food industry. Commercial production of xanthan gum uses glucose as the carbon substrate; consequently the price of xanthan production is high. One of the ways to decrease xanthan price, is using cheaper substrate like agricultural wastes. Iran is one of the biggest date producer countries. However approximately 50% of date production is wasted annually. The goal of this study is to produce xanthan gum from waste date using *Xanthomonas campestris* PTCC1473 by submerged fermentation. In this study the effect of three variables including phosphor and nitrogen amount and agitation rate in three levels using response surface methodology (RSM) has been studied. Results achieved from statistical analysis Design Expert 7.0.0 software showed that xanthan increased with increasing level of phosphor. Low level of nitrogen leaded to higher xanthan production. Xanthan amount, increasing agitation had positive influence. The statistical model identified the optimum conditions nitrogen amount=3.15g/l, phosphor amount=5.03 g/l and agitation=394.8 rpm for xanthan. To model validation, experiments in optimum conditions for xanthan gum were carried out. The mean of result for xanthan was 6.72±0.26. The result was closed to the predicted value by using RSM.

***Keywords*—**Optimization, RSM, Waste date, Xanthan gum, Xanthomonas Campestris

I. Introduction

DATE fruit is one of the most widely cultivated crops in the south west of Asia, According to FAO official estimates; 69% of world palm date production is produced in Egypt, Iran, Soudian Arabia, Pakistan and Iraq [1]. During the last 50 years, Iranian palm date production has reached an average of 800.000 tonnes/year. Inopportunely, however, palm date harvesting is often accompanied by substantial fruit losses that occur during the picking, storage and conditioning processes [2]. Because of their inadequate texture (too soft), the lost dates, commonly named ‘‘date by-products”, are not edible and are often discarded. Currently, very little use is made of these by-products and they are, most of the time, used for limited purposes such as animal feed [3].

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Only little research has been carried out in regard to the usability of this ill-employed by-product. The currently available studies that have so far been conducted on this issue seem to have focused on the valorization and biologic transformation of this substrate for the production of biomass and various other the biologic transformation especially aiming production of biomass and a variety of other compounds, such as citric acid, ox tetracycline and ethanol [3]. Of special interest to the aims of the current study, these by-products seem particularly useful for the production of high value-added components such as xanthan gum.

Xanthan gum was discovered in late 1950s by US scientist and is the first biopolymer produced industrially [4]. In 1969, the Food and Drug Authority (FDA) authorised the use of xanthan gum in food products, marking the introduction of the first industrially produced biopolymer to the food industry. Since then the demand for xanthan gum produced from *X. campestris* has progressively increased, at an annual rate of 5–10%. Xanthan gum is widely used in a broad range of industries, such as in foods, toiletries, oil recovery, cosmetics, as water-based paints, etc., due to its superior rheological properties and is used as a rheological control agent in aqueous systems and as stabilizer for emulsions and suspensions. The xanthan gum applications have diversified its commercial value, and now become one of the widely used ingredients in food product.

Commercial production of xanthan gum uses glucose as the substrate, and generally batch production instead of continuous production due to the batch process having been proven to work successfully.

In fact, the cost of the fermentation medium has always been a major concern in the commercial production of xanthan. For this reason, recent research in the field has particularly focused on the search for cheaper natural alternatives for the currently used substrates, namely glucose or sucrose, so as to control the cost of the production process as well as of the final product. The current study is an attempt to contribute to this current search for efficient and cost-effective substrates for xanthan gum production. In this context, the authors postulate that the Allig date fruit by-products, which are abundantly available in nature as a waste of date fruit harvesting, storing and conditioning processes, can be used as a cheap substrate for xanthan gum production. Accordingly, the present work was undertaken to explore and further optimise xanthan gum production by *X. campestris* in batch experiments on date fruit by-products using response surface methodology. It evaluated three main independent variables, namely nitrogen sources, phosphor sources and

agitation rate values, in terms of their individual and combined effects on optimum xanthan.

II. Materials And Methods

A. Samples and Substrate preparation

The date samples used in the current study were second-grade dates purchased from the Jahade Keshavarzi Bushehr region (South of Iran). The physico- chemical composition of the second-grade date was previously analysed in a recent study by the authors [5].

After being sorted (in -18°C to prevent fermenting), the dates were pitted. The date juice was prepared by adding some water to the date flesh up to final concentration (50 and 70 g/l). The date paste–water mixture obtained was autoclaved at 121°C (15 psi) for 20 min.

B. Microorganism, growth conditions and inoculum preparation

A strain of *Xanthomonas Campestris* PTCC 1473 was used for the present study. The preparation of the inoculum was performed by the transfer of the microorganism from the stock culture to the GYC(Glucose Yeast Calcium Carbonate agar) and its subsequent incubation for 48 h at 28°C. Subculturing was carried out once in every 2 weeks and culture was stored at 4°C.

The strain was adapted to high date juice concentrations as described below. For the first adaptation passages *X. campestris* was cultured for 24 h in an rotatory shaking incubator (250 rpm) at 28°C in LB medium (in g/l: yeast extract 5, tryptone 10 and NaCl 10) containing 25 g glucose, whereas for the next four passages it was cultured under the same conditions in 50 g/l date juice. Two last passages were carried out in 70 g/l date juice. In all passages LB medium was added. Xanthan gum production cultures were subsequently inoculated with a 5% (v/v) inoculum from the last culture. At each passage OD was measured at 600 nm by the spectrophotometer to estimate biomass quantity and the best result was obtain at the above 7 passages (OD=6.8).

C. Fermentation

Experiments were carried out in 500 ml Erlenmeyer flasks with 100 ml of medium containing date juice-with 70 g/l concentration, in 250 rpm, 30°C for 2 days. Cultures were grown in duplicates and sterile additives (LB medium) were added under aseptic conditions after autoclaving. In all flasks trypton (10 g/l) and sodium chloride (10 g/l) were added.

D. Xanthan gum estimation

Xanthan gum was assayed according as previously reported [6] with the difference that the potassium chloride solution was supplemented with EDTA to achieve a final EDTA concentration of 4 mM. All assays were performed in duplicates and means were based on the values derived from the duplicate cultures [7].

E. Experimental methodology

RSM was selected for the present study to maximize xanthan production. The individual and interactive effects of the phosphor source (2–6 g/l), nitrogen source (0–15 g/l) and agitation (200–400 rpm) on xanthan production (Y) as response variable was studied.

In order to determine the significant experimental variables and develop a response surface for medium optimization, the major factors mentioned above were further investigated by Central Composite Design (CCD). The experimental range for each factor was selected on the basis of results obtained from 18 preliminary experiments carried out by CCD. A second order polynomial model was fitted for the production of xanthan gum (Y), giving an equation of the following form:

$$Y=a_0+ a_1A+ a_2B+ a_3C+ a_{12}AB+ a_{13}AC+ a_{23}BC+ a_{11}A^2 a_{33}C^2 \quad (1)$$

Where (Y) is the calculated response function, A; B; C represent the coded variables and a_0; ai; aij (i; j = 1; 2; 3) are the coefficient estimates. The obtained response values were used to estimate the model coefficients aj by the least square method using the Design Expert version 7.0.0 software.

In the case of the composite design employed in the current study, the validation of the model was carried out by subjecting the data to the analysis of variance (ANOVA). The method can be described as follows [8]. The fitted model is considered adequate if the variance due to the lack-of-fit is not significantly different (F-test at the level 95%) from the pure error variance.

III. Results and discussion

Table I presents the levels of the independent variables in coded and encoded form according to the experimental design and the response: xanthan content (Y) for all experiments. The experiments were randomized in order to maximize the effects of unexplained variability in the observed response due to extraneous factors.

A. Analysis of experimental data

The final equations for xanthan production derived from the application of the method (after eliminating non-significant terms by the forward method) are given below:

For xanthan production:

$$Y=-1.35488+0.17168A+0.018045B+0.40062C-9.6483E\text{-}4\ BC\ -0.018786\ C^2 \quad (2)$$

It can be noted that Eq (2) presented above has rather mathematical than physiological meanings particularly because they can encompass the values of the three factors (phosphor source, nitrogen source and agitation rate) and estimate the xanthan yield.

B. Xanthan production (response Y_1)

Thirty six experiments were carried out according to the conditions indicated in Table I. Xanthan yield was determined and reported. The magnitudes of coefficients presented in Table II indicate that the nitrogen source had a more positive

effect on xanthan production than the phosphor source, A p value <0.05 was obtained for the nitrogen and phosphor source and agitation rate. Conversely, nitrogen source showed a negative quadratic effect. The relationship between the response and the experimental variables could be graphically illustrated by plotting is response surface plots.

TABLE I
EXPERIMENTAL MATRIX (IN PARENTHESIS), EXPERIMENTAL CONDITIONS FOR THE CENTRAL COMPOSITE DESIGN AND THE CORRESPONDING EXPERIMENTAL YIELDS FOR XANTHAN AND BIOMASS PRODUCTION

Run	Phosphor source (g/l)	Agitation rate (rpm)	Nitrogen source (g/l)	Xanthan production (g/l)
1	6.00(+1)	400.00(+1)	15.00(+1)	2.235
2	4.00(0)	300.00(0)	7.50(0)	4.256
3	2.00(-1)	400.00(+1)	0.00(-1)	6.084
4	4.00(0)	300.00(0)	7.50(0)	4.077
5	6.00(+1)	400.00(+1)	0.00(-1)	6.618
6	2.00(-1)	200.00(-1)	15.00(+1)	1.556
7	4.00(0)	200.00(-1)	7.50(0)	2.744
8	2.00(-1)	400.00(+1)	15.00(+1)	2.023
9	6.00(+1)	300.00(0)	7.50(0)	5.107
10	4.00(0)	300.00(0)	0.00(-1)	3.72
11	4.00(0)	300.00(0)	7.50(0)	3.898
12	4.00(0)	300.00(0)	15.00(+1)	1.839
13	2.00(-1)	200.00(-1)	0.00(-1)	2.476
14	4.00(0)	300.00(0)	7.50(0)	4.666
15	4.00(0)	400.00(+1)	7.50(0)	5.58
16	6.00(+1)	200.00(-1)	0.00	3.366
17	2.00(-1)	300.00(0)	7.50(0)	3.041
18	6.00(+1)	200.00(-1)	15.00(+1)	1.683
19	2.00(-1)	200.00(-1)	0.00(-1)	2.719
20	6.00(+1)	200.00(-1)	0.00(-1)	3.983
21	2.00(-1)	400.00(+1)	0.00(-1)	6.936
22	6.00(+1)	400.00(+1)	0.00(-1)	7.157
23	2.00(-1)	200.00(-1)	15.00(+1)	1.628
24	6.00(+1)	200.00(-1)	15.00(+1)	1.989
25	2.00(-1)	400.00(+1)	15.00(+1)	2.552
26	6.00(+1)	400.00(+1)	15.00(+1)	2.719
27	2.00(-1)	300.00(0)	7.50(0)	3.6
28	6.00(+1)	300.00(0)	7.50(0)	4.625
29	4.00(0)	200.00(-1)	7.50(0)	3.197
30	4.00(0)	400.00(+1)	7.50(0)	5.055
31	4.00(0)	300.00(0)	0.00(-1)	4.395
32	4.00(0)	300.00(0)	15.00(+1)	3.636
33	4.00(0)	300.00(0)	7.50(0)	5.071
34	4.00(0)	300.00(0)	7.50(0)	5.427
35	4.00(0)	300.00(0)	7.50(0)	5.806
36	4.00(0)	300.00(0)	7.50(0)	6.209

Fig. 1 shows the effect of interaction of incubation nitrogen and agitation rate on the production of xanthan. The nitrogen source was observed to have had a significant effect on xanthan production (fig. 1). In fact, the xanthan production was noted to increase proportionally with the increase of the nitrogen source values up to 3.95 g/l. this data can be explained that during microbial fermentation, the nitrogen source is just needed to prepare growth conditions and produce enzymes used in catalyticall synthesis of biological xanthan production. In common with the synthesis of other bacterial exopolysaccharides, xanthan production is greater in media containing higher ratios of carbon to nitrogen (C/N) [9]. Owing to the fact that in this study carbon concentration in media was instant, in higher concentration of nitrogen, C/N decreases consequently xanthan production decreased.

Moreover, higher yields of xanthan production could be obtained with increasing agitation level from 200 to 400 rpm (Fig. 1). Agitation was then an important factor in batch fermentation of X. campestris. The beneficial effects of increased agitation was attributed by some investigators to a thinning slime layer, enhancing this way the transfer of nutrients and oxygen transfer for xanthan formation. This could explain the different values of xanthan production at various speed applied in this work [10]. These results are in agreement with other previously reported findings in the literature [11], [12] showing that low concentration of nitrogen source has a positive effect on xanthan production, which also increases upon increasing agitation level.

TABLE II
ESTIMATED COEFFICIENTS OF MULTIPLE DETERMINATIONS (R^2) FOR XANTHAN (Y) PRODUCTION USING CODED VALUES

Term	Coefficient	P value	F value
Xanthan production [R^2 = 0.8512]			
Constant	-1.35488		
Phosphor source	0.17168	0.0432	4.518
Agitation source	0.018045	0.0001>	44.776
Nitrogen source	0.40062	0.0001>	62.761
Agitation source× Nitrogen source	-0.00096	0.0005	16.054
Nitrogen source × Nitrogen source	-.018786	0.0087	8.059

The optimal conditions for xanthan yields that were selected using the Design Expert 7.0.0 software package were: 3.15 g/l of nitrogen source, 5.03 g/l of phosphor source and 394.8 rpm. Under these conditions, the expected value of the xanthan yield was 6.51 g/l. A supplementary experiment was carried out under the selected optimal conditions and led to an experimental xanthan yield value of 6.72±0.26 g/l, which was very close to the one expected by the model (6.51 g/l). D. Statistical analysis and validation of the model.

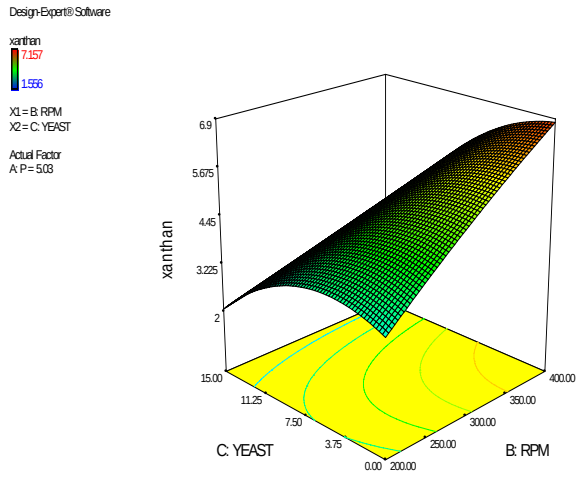

Fig. 1 Effect of nitrogen source and agitation rate values on xanthan production

The fitted model was subjected to the analysis of variance and the results showed that the regression sum of squares was statistically significant at 85.1% for xanthan production. The value of determination coefficient R^2, as a measure of the model's goodness-of-fit, was 0.8512 for xanthan production. This indicates that only about 14.88% of the total variations were not explained by the model. The lack-of-fit value can be indicative of a model's failure to represent data in the experimental domain at points, which are not included in the

regression. In fact, the lack-of-fit values for regression equation (2) was not significant ($p > 0.05$), and thus demonstrated that the model equation was adequate for predicting xanthan production under any combination of the values of the variables involved.

To model validation, experiments in optimum conditions for xanthan gum were carried out. The mean of results for xanthan was 6.72±0.26. The result was closed to the predicted value by using RSM (6.5 g/l).

IV. Conclusion

This current work investigated the possibility of using date juice by-products (Allig with hard texture) as substrate for xanthan gum production by *Xanthomonas Campestris* PTCC 1473. The influence of phosphor and nitrogen source and agitation rate were determined using response surface methodology. The selected optimal conditions for xanthan production (date juice phosphor source: 5.03 g/l, nitrogen source: 3.15 g/l and agitation rate: 394.8 rpm) have been checked and confirmed by a supplementary experiment. The experimental yield of xanthan was found to be in good agreement with the predicted one (6.72±0.26 versus 6.51 g/l, respectively). Overall, the findings of the present study indicate that the date fruit juice by-products presented in the current study seem to have strong potential and promising properties that can open new pathways for the production of efficient and cost-effective xanthan gum. They can, therefore, be considered as a strong candidate for future industrial and commercial applications related to xanthan gum. To our knowledge the present study is rare in the literature to explore and report on the direct exploitation of lost date, an abundant agro-industrial residue, for xanthan production. The results are, in fact, promising in that they suggest that xanthan gum production can be industrially extended and maximized through the use of this low cost substrate.

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