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Research Article

MODELING AND OPTIMIZATION OF CEPHALOSPORIN C PRODUCTION USING *STREPTOMYCES GRESIOLUS*

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ABSTRACT: The objective of this study is to improve the cephalosporin C production through optimization of medium and culture conditions .The effect of agitation speed on CPC production in 3 l bioreactor was investigated with fed batch mode. The maximum CPC (0.79 g/l) , the biomass (35.4g was obtained at 400 rpm A statistical method was introduced to optimized the main culture medium constituents. Some medium constituents involving glucose, ammonium sulphate as inorganic nitrogen source and methionine were found to be the most effective factors for (CPC) production. The results showed the priority of fed batch culture than the batch one, since it increase the yield of the CPC by about 155 %.

Key words; Cephalosporin C (CPC), Production, *Streptomyces*

INTRODUCTION

Cephalosporin together with penicillin belong to the family of b. lactam antibiotics, cephalosporin are important broad spectrum antibiotic in the international market and are considerable more résistance to b lactamases than penicillin. A number of reports are available on the various aspects of CPC production such as production in batch as well as in continuous mode using free and immobilized cells of molds (Srivastava and Kundu, 1999; Cruz *et al*. ,2004 , and Kim *et al.,* 2005) . The biosynthesis of CPC is studied in bioreactor using synthetic medium, it was observed that with the progress of fermentation process increase in the viscosity of the broth occurs and the thereby decreasing oxygen mass transfer (Kundu *et al.,* 2003, Verma *e tal.,* 2006). The present investigation deals with the optimization of medium constituents to increase the CPC productivity using the strain of *Streptomyces*.

MATERIALS AND METHODS

Microorganism

The production of CPC was carried out by *Styreptomyces gresiolus* obtained from Genetic and cytology Department, National Research center, Dokki, Cairo, Egypt.

Cultivation conditions

The stock culture were maintained by transferring the organism monthly on potato dextrose agar slants. The basal production medium consists of 2.5 % glucose, 0.4% $(NH_4)_2$ SO_4, 0.3 KH_2PO_4 and 0.5DL, methionine . Sugar and ammonium sulphate were sterilized separately from other components. Erlenmeyer flasks containing 50ml medium, the culture was maintained at 300 rpm 27Con a rotary shaking incubator for inoculums preparation. The fed batch fermentation in the stirred tank ferment or (3l) was carried out at 27 C0 and the operating volume 2 liter with flow rate 1vvm and 5 % (v/v) of glucose was fed at 3 rd and 5 th day into the bioreactor culture broth according to the method described by Sirvastava *et al.,* (1999).

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Analytical methods

The dry cell weight of mycelium measured as follow: 10 ml of culture broth was centrifuged at 12000xg and filtered through a pre-weighted what man glass microfiber filter GF/C, after being dried it washed twice with distilled water. The cells were dried at 95 C to a constant weight prior to measured by using the method described by Miller , 1959 and Matsumura *et al*., 1981).

CPC was measured firstly using bioassay technique where *bacillus subtillus* used as a test microorganism . It was also measured by high liquid chromatography (HPLC) using reverse phase column of u band a pak C_{18} and 254nm UV detector . the mobile phase was acetonitrile phosphate buffer, the elution mixture was 98 % phosphate buffer and 2 % acetonitrile with a flow rate 0.9ml'min. cephalosporin C zinc salts obtained from Sigma USA was used as standard.(Cruz, *et al.,* 2004).

Experimental Design

Factional factorial design was employed to optimized the main medium constituents using analysis of variance (ANOVA) and the optimal concentration of the main medium contents which had a significant effect on CpC production was determined . the variable was coded according to the equation.

$X1= (X_1-X_0)/\Delta X$ i=1,2,3,4……

Where

Xi is the coded (dimensionless) value of the variable

X_1, X_0 the value of Xi at the center point , ΔX the step change

RESULTS AND DISCUSSION

Effect of different fermentation time on the Production of CPC using shake flask

The results in table (1) showed that time course of CPC production in shake flask using an optimized main medium. The maximum CPC production (0.46 g/l) was obtained at 5 day and then CPC production was gradually decreased. The maximum CPC production was similar to that predicted by statistical analysis .but as glucose deleted, cell mass and CPC production gradually decreased .form these results it was thought that the carbon source is necessary to maintenance of the activity and productivity process.

Table-1: Effect of different fermentation time on the production of CPC using shake flask

Fermentation time (day)	Biomass (g/l)	CPC production(g/l)
2	1.72	0.22
3	2.31	0.31
4	2.50	0.36
5	2.41	0.46
6	2.62	0.30
7	2.60	0.25

Effect of different agitation speed on CPC production using laboratory stirred ferment or

In the present investigation the effect of different agitation speed (200, 300, 400, and 500 rpm) were tested for the production of CPC process. The results presented in t able (2) and showed that the best CPC out put (0.79mg/L) was obtained at 400 rpm .while the other speeds showed reduced levels of CPC .

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Table (2): Effect of different agitation speed on the production of CPC

Agitation speed (rpm)	Biomass (g/L)	CPC production (g /L
200	24.64	0.54
300	31.52	0.62
400	35.41	0.79
500	29.2	0.55

Optimization of the main medium constituents using statistical methods

From preliminary experiments made in order to examine the effects of different components on CPC production. Three factors which play the most important role in the synthesis process. These were glucose, ammonium sulphate and DL methionine . Tables (3,4) showed the value of these variables at different levels . The CPC production varied in a range of 0.047 g/l . on the basis of thesis experimental values statistical testing was carried out using analysis of variance (ANOVA) as shown in Table (5) , when the test was applied an each factor for CPC production with three variable .

Table 3: Level of the variable tested in functional factorial design

Medium constituents	Symbol	Coded value		
		-1	0	1
glucose	A	1.2	2.9	4.2
Ammonium sulphate	B	0.4	0.8	1.1
DL methionine	D	0.3	0.5	0.8

The behavior of the system was explained by the following second degree polynomial equation

$Y = Bo + S\ B_i\ x_i + S\ B_{ii} + i_2 + S\ B_{iii} + i_3 + \ldots\ldots..$

Where, y is the predicted response. Bo the offset best term
Bi the liner effect, B_{ii} the square effect and B_i the interaction effect

Table (4) Experimental design and results for analysis of variance (ANOVA)

Run	A	B	C	CPC production
1	-	-	-	0.420
2	-	-	-	0.449
3	+	-	-	0.350
4	+	-	-	0.375
5	-	+	-	0.185
6	-	+	-	0
7	+-	+	-	0
8	-	+	+	0.478
9	-	-	+	0.349
10	+	-	+	0.378
11	+-	-	+	0
12	-	-	+	0
13	-	-	+	0.41
14	+	+	+	0.372
15	+	+	+	0.310

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Table (5) Satirical analysis of factors through analysis of variance

Factor	CPC production	
	F value	P value
A	867.60	0.0001
B	713.01	0.0001
C	41.05	0.1114
AB	121.5	0.2227
AC	187.83	0.0006
BC	15.48	0.0300

Table (6): Experimental design and results for determination of optimal concentration of glucose and ammonium sulphate

Run	Glucose	Ammonium sulphate	CPC production
1	-1	-1	0.270
2	1	-1	0
3	-1	1	0.185
4		1	0.315
5	1.314	0	0
6	0	1.414	0
7	1.414	0	0
8	0	1.414	0.334
9	0	0	0.340
10	0	0	0.408

Glucose and ammonium sulphate were statistically at 1% level of significant, so the effect of each component on CPC production was investigated. Especially, in the case of glucose the F and P value foe CPC were 867.60 and 0.0001 respectively. From these results, it was conducted that glucose was the most effective components on CPC production.

The basis of the experimental design given in table (6) using these results, the following second order polynomial equation relating to the CPC production explain the experimental data.

CPC production = $0.478- 0.005X- 0.188X^2+0.101y- 0.14Y^2 +0.076XY$

Where X = glucose concentration

Y= ammonium sulphate concentration

CONCLUSION

The results obtained showed that the productivity of CPC was affected by different factors (glucose, ammonium sulphate, agitation speed), it revealed that glucose concentration was highly significant on the production process than the other parameters tested.

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