Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

European Journal of Experimental Biology, 2013, 3(5):484-490

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Combinative impact of effectors on production of celluolytic enzyme from *Brevibacillus parabrevis* (MTCC 2208)

Jagdish Singh* and Shyana Banal

Department of Biotechnology, Mata Gujri College Fatehgarh Sahib, Punjab, India

---

ABSTRACT

A quadratic central composite design (CCD) in response surface methodology was applied to explicate the combinational concentration of effectors that influence fermentative cellulase production by Brevibacillus parabrevis. It was examine that 10% inoculums ((7X10^7/ml)) concentration and 37^{0}C temperature was optimum for cellulase production after 24 h of fermentation. Concentration of effectors (g/100 ml), sodium alginate, 0.3; gum arabica, 0.476; milk whey, 4.75 and Tween 80, 0.85 and pH, 6.5 was optimized, which enhanced enzyme production (126 IU/L) by 2.26 fold on the contrary to the control (48 IU/L). Subsequently supplementation with different carbon source and metal ions also exhibited enhancement in enzyme synthesis.

Key words: Cellulase, *Brevibacillus parabrevis*, response surface methodology

---

INTRODUCTION

Cellulose is a linear polymer of glucose units linked together by β -1,4-glycosidic bonds and most abundant renewable carbon source in the world. It is hydrolyzed by enzyme cellulase to glucose, later is used for ethanol, organic acids and other chemicals production [1]. Cellulase (E.C 3.2.1.4) is mainly produced by fungi, bacteria & protozoans [2] and has broad range of commercial applications [3, 4]. Exploitation of cellulase is expensive process but cost can be minimized by enhanced production [5, 6, 7, 8, 9, 10]. Hence, cellulase production from a wide range of microorganisms has been studied extensively [11]. Considerable progress has been achieved for high cellulase production by optimization of best possible fermentation conditions by statistical and classical method. Former method is based on combinational interactions of components/conditions, in contrary to classical methods which are based on one by one variable of optimization for optimum production. A statistical technique, Response surface methodology (RSM), has been successfully used for designing experiments, building models and evaluating the effects of factors in product formation [12, 13, 14, 15, 16, 17, 18]. This study describes the statistical (response surface methodology, RSM) optimization of effectors concentration i.e. sodium alginate, gum arabica, milk whey, Tween 80 and pH, for improved cellulase production by *B. parabrevis.* The information would be useful for enhanced enzyme production which will subsequently develop cost effective hydrolysis of celluloses for broad range of application.

MATERIALS AND METHODS

Optimization of culture conditions for maximum cellulase production

B. parabrevis cell (7×10^7/ml) were grown in 250 ml Erlenmeyer flask containing 50 ml of medium (M1), composition was (g/100 ml): peptone, 0.5; sodium chloride, 0.5; beef extract, 0.5; Yeast extract, 0.5; Carboxymethyl cellulose, 2.0 and pH-7.0. The sterilized medium (15 min at 121^0C) was inoculated with *B. parabrevis* and incubated in orbital shaker (250 rev. min^{-1}) at 37^0C. Microbial biomass was harvested by centrifugation at 8000x g for 20 min in a centrifuge fitted with fixed angle rotor. The supernatant constituted the cellulase and enzyme activity was determined. In order to access the optimum conditions for enzyme production, process parameters i.e. incubation period, inoculums %, and temperature were optimized.

Cellulase assay

The cellulase (CMCase) activity was assayed according to Ghose [19], where appropriately enzyme solution (0.5 ml) was mixed to 0.5 ml CMC (0.5 % CMC in phosphate buffer 0.2M, pH 6.0) and incubated at 40^0C for 30 min. In the assay, release of reducing sugars from was determined by Miller, [20] method. One international unit (IU) of enzyme activity was defined as the amount of enzyme that catalyzed the liberation of reducing sugar equivalent to 1.0 µM glucose min^{-1} under assay conditions.

Experimental design and optimization of process parameters for cellulase production

Design expert 8.01. software was used to construct central composite design (CCD) to investigate factors that influence cellulase production. In CCD, central values (0) and the levels of the variables investigated in experimental design were; sodium alginate (X1), 0.3 %; Gum Arabica (X2), 0.476; milk whey, (X3) 4.75; Tween 80, 0.85 ; pH (X4), 6.5 (Table 1)

Table 1. Factors involved in RSM for optimization of cellulase production

Factor	Name	units	Low actual (-1)	central values (0)	High actual (+1)
A	Sodium Alginate	%	0.1	0.3	0.5
B	Gum Arabica	%	0.2	0.47	0.75
C	Milk whey	%	2.5	4.75	7.0
D	Tween 80	%	0.2	0.85	1.5
E	pH	--	5.5	6.5	7.5

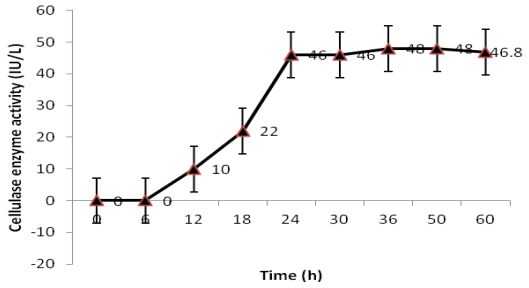
Different combination of variables was adjusted according to design (Table 2) in medium (M1) and inoculation was carried with 10% ($7x10^7$ cells/ml) inoculums and incubated at 37^0C temperature and for 24 h. Supernatant containing cellulase enzyme was separated from biomass by centrifugation and enzyme activity was determined by standard procedure. Subsequently effect of supplementation of different carbon source and metal ions on enzyme synthesis was determined for improved enzyme production.

The Design expert 8.0.1 software, was used for regression and graphical analyses of the data obtained. The optimal concentrations of critical medium components were obtained by ridge analysis and contour plots. The statistical analysis of the model was performed in the form of analysis of variance (ANOVA).

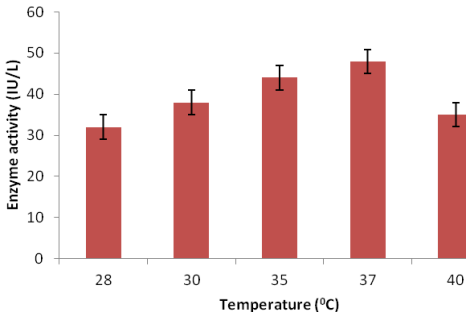
RESULTS AND DISCUSSION

Different percentage (2.5, 3, 5, 7, 10, 12, and 15%) of activated *B. parabrevis* cell (7×10^7 spores/ml) was inoculated in medium to study effect on cellulase enzyme production. Maximum cellulase enzyme activity (48 IU/L) at 10% inoculums was obtained. Increase in inoculums size (5-10 %), enhanced enzyme production but after that remains constant (Fig.1). Depletion of nutrients by the enhanced biomass, which result dwindle in metabolic activity and balance between the increasing biomass & accessible nutrient would yield an optimal enzyme production [21, 22].

The optimization of incubation temperature for cellulase production from *B. parabrevis* under submerged conditions revealed that production increased gradually from 28 to 37°C (Fig. 2), with maximal (48 IU/ml) at 37°C and pH 7 after 24 h. Beyond 37°C temperature negatively affected the production.



(a)



(b)

Fig.1 (a)Effect of inoculums conc. (%) and (b) temperature (^{0}C)on enzyme production

Combinative impact of effectors on enzyme production

Different effectors were added in fermentation medium as per CCD (Table 2), and effect of different combination on cellulase production was analyzed. The concentration of sodium alginate (0.5%), gum arabica (0.75%), milk whey (7%), Tween 80 (1.5%) and pH (6.5) in submerged fermentation were chosen as optimum for maximum production (126 IU/L) was 2.6 fold higher (Table 2) in contrary to control (48 IU/L). Friedman, [23] and Winchester et al., [24] research indicates that strains of bacteria can be induced to synthesize enzymes by gum areabica that recycle urea and other nitrogen compounds. Surfactant Tween 80 has been generally added to media where cellulolytic fungi are grown. The mechanism of yield enhancement by Tween 80 is not well understood but may be related to the increased permeability of the cell membrane, which increases release of the enzymes from cells [25]. Reese and Maguire [26] found that the addition of Tween 80 to the growth medium improved the cellulase yield in *Trichoderma*.

Regression analysis was performed to fit the response function with the experimental data. The statistical significance of the second order model equation was checked by an F-test ANOVA (Table 3).The regression model for cellulase production was highly significant ($p<0.003$) with a satisfactory value of determination coefficient ($R2=0.95$), indicating that 95 % of the variability. The Model F-value of 5.26 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. The "Pred R-Squared" of 0.7664 is as close to the "Adj R-Squared" of 0.7757 as one might normally expect (Table 4). Adeq Precision" measures the signal to noise ratio and value greater than 4 is desirable. In the analysis ratio of 9.309 indicates an adequate signal. This model can be used to navigate the design space. The canonical analysis from the 3D response surface based on dependent variables (Fig. 2) revealed that maximum cellulase activity of 126IU/L, was achieved at the point when sodium alginate (0.5%), gum arabica (0.75%), milk whey (7%), Tween 80 (1.5%) and pH (6.5) at temperature, 37^{0}C and incubation period 24 h was implied for enzyme production by submerged fermentation.

Table 2. Values of effectors concentration for CCD along with the experimental values of cellulase enzyme production from *B. parabrevis*

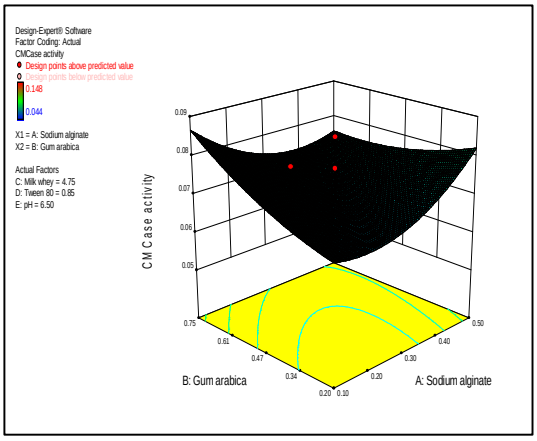
Run	Factor under investigation for cellulase production					CMCase activity (IU/L)
	A (%)	B (%)	C (%)	D (%)	E	
1	0.10	0.75	7.00	0.20	7.50	81
2	0.10	0.20	7.00	1.50	5.50	111
3	0.10	0.20	7.00	1.50	7.50	74
4	0.50	0.20	2.50	0.20	7.50	96
5	0.10	0.20	2.50	0.20	7.50	96
6	0.30	0.47	4.75	2.40	6.50	93
7	0.50	0.75	7.00	1.50	6.50	126
8	0.50	0.20	2.50	1.50	5.50	97
9	0.50	0.20	7.00	0.20	7.50	107
10	0.30	0.47	0.60	0.85	6.50	77
11	0.30	0.47	4.75	0.85	6.50	85
12	0.50	0.75	2.50	0.20	5.50	112
13	0.30	0.47	4.75	0.70	6.50	44
14	0.50	0.75	2.50	1.50	5.50	85
15	0.50	0.75	7.00	0.20	7.50	63
16	0.30	0.47	4.75	0.85	6.50	77
17	0.30	0.47	4.75	0.85	8.88	77
18	0.30	0.47	4.75	0.85	6.50	55
19	0.10	0.20	2.50	1.50	7.50	88
20	0.78	0.47	4.75	0.85	6.50	81
21	0.30	0.18	4.75	0.85	6.50	74
22	0.30	1.13	4.75	0.85	6.50	63
23	0.10	0.20	7.00	0.20	7.50	55
24	0.50	0.20	2.50	1.50	7.50	74
25	0.30	0.47	4.75	0.85	6.50	55
26	0.30	0.47	4.75	0.85	6.50	77
27	0.18	0.47	4.75	0.85	6.50	81
28	0.30	0.47	4.75	0.85	6.50	55
29	0.50	0.75	2.50	0.20	7.50	81
30	0.50	0.20	2.50	0.20	5.50	111
31	0.10	0.75	2.50	1.50	7.50	96
32	0.50	0.20	7.00	0.20	5.50	112
33	0.10	0.20	2.50	1.50	5.50	70
34	0.30	0.47	4.75	0.85	6.50	77
35	0.50	0.75	2.50	1.50	7.50	114
36	0.10	0.75	2.50	0.20	7.50	112
37	0.10	0.75	7.00	1.50	7.50	120
38	0.50	0.75	7.00	0.20	5.50	96
39	0.50	0.75	7.00	1.50	7.50	122
40	0.30	0.47	10.10	0.85	6.50	96
41	0.10	0.20	7.00	0.20	5.50	107
42	0.30	0.47	4.75	0.85	6.50	59
43	0.10	0.75	7.00	1.50	5.50	126
44	0.10	0.75	7.00	0.20	5.50	115
45	0.50	0.20	7.00	1.50	5.50	133
46	0.30	0.47	4.75	0.85	4.50	112
47	0.50	0.20	7.00	1.50	7.50	98
48	0.10	0.75	2.50	0.20	5.50	114
49	0.10	0.20	2.50	0.20	5.50	112
50	0.10	0.75	2.50	1.50	5.50	94

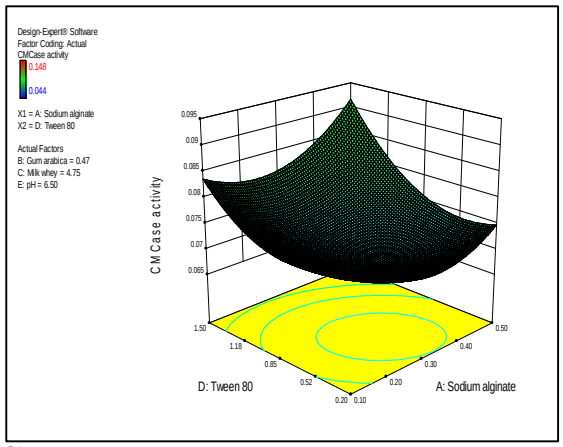
Table 3: Analysis of variance table (ANOVA) for Response Surface Model

Source	Sum of Squares	Df	Mean Square	F value	p-value Prob > F
Model	0.013	5	2.505E-003	5.28	0.0007
A-Sod. Alginate	3.251E-005	1	*3.251E-005*	*0.068*	0.7948
B-Gum arabica	3.796E-004	1	*3.796E-004*	*0.80*	0.3761
C-Milk Whey	4.770E-003	1	*4.770E-003*	*10.05*	0.0028
D-Tween 80	2.055E-033	1	*2.055E-003*	*4.33*	0.0433
E-pH	5.132E-003	1	*5.132E-003*	*10.81*	0.0020
Residual	0.021	44	4.747E-004	*1.66*	0.2507
Lack of Fit	0.09	37	*5.067E-004*	----	---
Pure Error	2.140E-003	7	3.056E-004	---	---
Cor Total	0.033	49		---	---

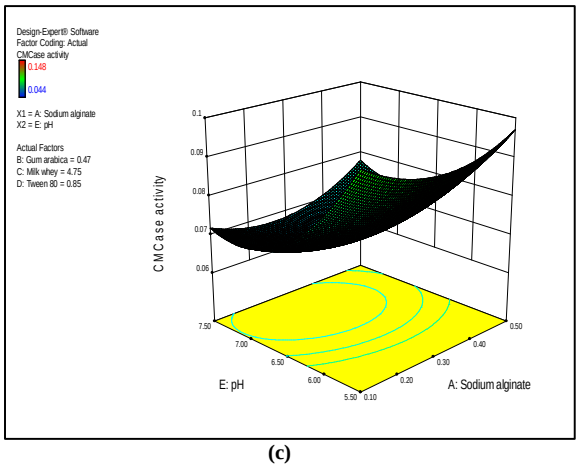
Table 4. ANOVA results for CCD to determine the impact of factors for cellulase production

Parameter	Value
Std. Dev	271.22
Mean	817.00
Adj R-Squared	0.7757
C.V. %	33.20
R-Squared	0.8840
Pred R-Squared	0.7664
Adeq Precision	9.309

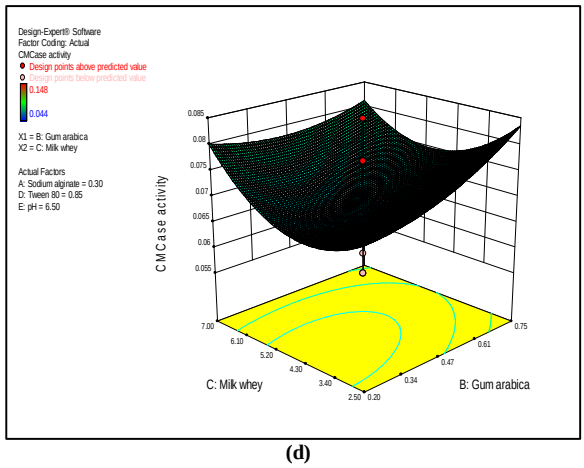




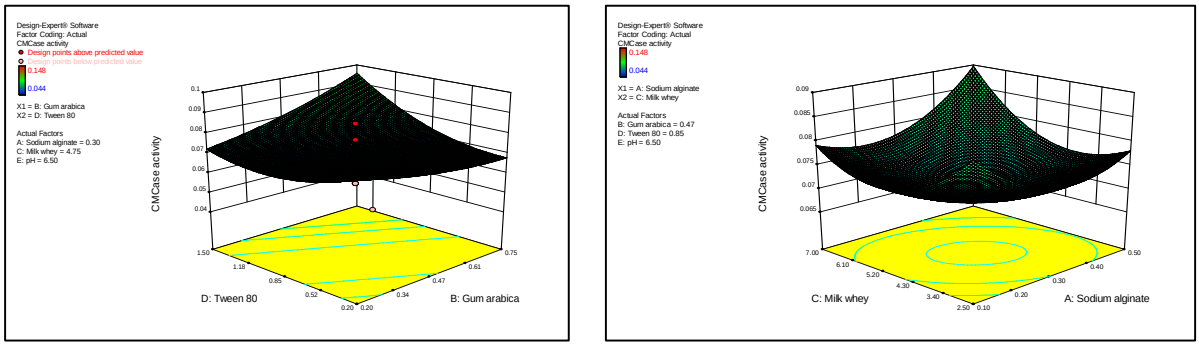
(b)



(c)



(d)

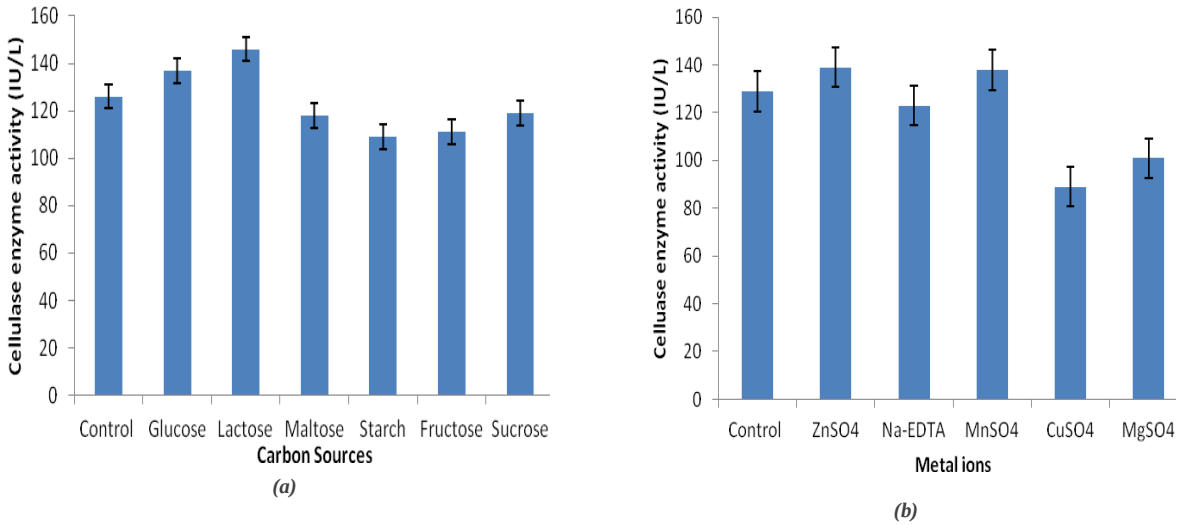


(e) **(f)**

Fig.2 Surface plot for the effect of (a) sodium alginate and gum Arabica (b)sodium alginate and Tween 80 (c)pH and sodium alginate (d)milk way and gum Arabica(e)Tween 80 and Gum Arabica and(f)milk way and sodium alginate conc. on CMCase activity

Effect of different supplement carbon sources and metal ions on cellulase production

Although CMC supports the growth of *B. parabrevis* for cellulase production, but it may not supply sufficient nutrients needed by the organism for maximum enzyme production. Hence, the addition of different carbon sources glucose, lactose, maltose, starch, fructose and sucrose to the medium was conceded to improve enzyme production. The supplement of glucose and lactose had little effect on cellulase production, while maltose, starch, fructose and sucrose were not effective for enhanced cellulose production. Among them, lactose and glucose improved the cellulase production by 1.15, 1.05 fold respectively as compared to the control (Fig. 3a). Addition of chemical compounds in fermentation medium can increase or decrease enzyme production and are called inducer or inhibitor respectively. Cellulase production was enhanced with $ZnSO_4$ and $MnSO_4$ (10mM), while Na-EDTA, $MgSO_4$, and $CuSO_4$ reduced cellulase production. But the effect was in contrary to enhancement with NaCl and $MgSO_4$ (30mM) in *Pseudomonas fluorescens* [27].



(a) *(b)*

Fig.3 Effect of different supplement (a) carbon sources and (b) metal ions on the cellulase production from *B. parabrevis*

CONCLUSION

In this study, CCD model using RSM was successfully developed for analysis of concentration of effectors for cellulase production from *B. Parabrevis* in submerged fermentation. CMCase activity 126 IU/L was achieved when microorganism was cultivated at 37°C in the CMC medium containing effectors sodium alginate (0.5%), gum arabica (0.75%), milk whey (7%), Tween 80 (1.5%) at pH 6.5 with incubation period for 24 h. Supplementation of medium with carbon sources and metals ions favored the enzyme production. So findings indicated that the model is reliable for maximize cellulase production.

REFERENCES

[1] Beguin, P. and Aubert, J. P, *FEMS. Microbiol. Rev*., **1994**, 25.
[2] Bhat, M.K, *Biotechnol. Adv*., **2000**, 18, 355.
[3] Adsul, M.G, Bastawde, K.B, Varma, A.J. and Gokhale, D.V, *Bioresour. Technol*., **2007** 98, 1467.
[4] Kaur, J, Chadha, B. S, Kumar, B.A. and Saini, H. S, *Bioresour. Technol*., **2007**, 98, 74.
[5] Duff, S. J. B. and Murray, W. D, *Bioresour. Technol*., **1996**, 55, 1.
[6] Nieves, R.A. Ehrman, C.L. Adney, W. S. Elander, R.T. and Himmel, M. E, *W. J. Microbiol. Biotechnol*., **1998**, 14, 301.
[7] Chahal, P. S. Chahal, D. S. and Andre, G, *J. Ferment. Bioeng.*, **1992**, 74, 126.
[8] Reczey, K. Szengyel, Z. Eklund, R. and Zacchi, G, *Bioresour. Technol.*, **1996**, 57, 25.
[9] Kristo, E. Biliaderis, C. G. and Tzanetakis, N, *Food Chem.*, **2003**, 83, 437.
[10] Chen, F. Cai, T.Y. Zhao, G.H. Liao, X.J. Guo, L.Y. and Hu, X.S, *J. Food. Eng.*, **2005**, 70, 47.
[11] Ariffin, H.N. Abdullah, M.S. Umikalsom, Y. Shirai, A. and Hassan, M. A, *J. Biosci. Bioeng.*, **2008**, 106, 231.
[12] Wejse, P.L. Ingvorsen, K. and Mortensen, K. K, *Enzy. Microb. Technol.*, **2003** 32, 721.
[13] Kristo, E. Biliaderis, C.G. and Tzanetakis, N, *Food Chem.*, **2003**, *83*, 437.
[14] Chen, F. Cai, T. Y. Zhao, G.H. Liao, XJ. Guo, L.Y. and Hu, X. S, *J. Food. Eng.*, **2005**, 70, 47.
[15] Dey, G. Mitra, A. Banerjee, R. and Maiti, B. R, *Biochem. Eng. J.*, **2001**, 7, 227.
[16] Teruel, M. L. A. Gontier, E. Bienaime, C. Saucedo, J. E. N. and Barbotin, J. N, *Enz. Microb. Technol*., **1997**, 21, 314.
[17] Lee, S.L. and Chen, W.C, *Enzy. Microb. Technol.*, **1997**, 21, 436.
[18] Silva, C. J. S. M. and Roberto, C, *Process. Biochem*., **2001**, 36, 1119.
[19] Ghose, T. K, *Pure. App. Chem.*, **1987**, 59, 257.
[20] Miller, G. L, *Anal Chem*, **1959**, 31, 426.
[21] Kashyap, P. Sabu, A. Pandey, A. Szakacs, G, *Process Biochem.*, **2002**, 38, 307.
[22] Ramachandran, S. Patel, A. K. Nampoothiri, K. M. Francis, F. Nagy, V. Szakacs, G. Pandey, *Bioresour. Technol*., **2004**, 93, 169.
[23] Friedman, E. A, *Am. J. Kidney Dis*., **1996**, 28, 943.
[24] Winchester, J. F. Brady, J. A. Salsberg, J. A, Mok, W. Quartararo P. J, *Nephrol*., **2003**, 140, 305.
[25] Domingues, F. C. Queiroz, J. A. Cabral, J. M. S. Fonseca L. P, *Enzy, Microbial. Technol*., **2000**, 26, 394.
[26] Reese, E.T. Maguire, A, *Appl. Microbiol.*, **1969**, 17, 242.
[27] Bakare, M. K. Adewale, I. O. Ajayi, A. and Shonukan, O, O, *African. J. Biotechnol*., **2005**, 9, 898.