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Cassava Bagasse: A Potential and Low Cost Substrate for Cellulase Production in an Economical Fermentation.

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ABSTRACT

The purpose of this work was to produce cellulase by *cellulomonas cellulans* using solid waste materials such as cassava bagasse, pine leaves, wheat bran and rice bran in solid state fermentation (SSF). According to the maximum production of cellulase, cassava bagasse was selected as solid substrate among four solid substrates and used for further studies. Various nitrogen compounds like yeast extract, beef extract, peptone, malt extract were taken. Among them, yeast extract was selected as a best nitrogen source for cellulase production. Maximum production of cellulase was obtained at an initial moisture content of 80% with an initial pH of 6.

Keywords: Cassava bagasse, cellulase and Solid State Fermentation

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INTRODUCTION

Cellulase has atleast three groups of enzymes namely endoglucanase (EC 3.2.1.4), exoglucanases called as cellobiohydrolases (EC 3.2.1.91) and b-glucosidases (EC 3.2.1.21). Cellulases are industrially important enzymes that can catalyze on cellulose material to produce fermentable sugars like mannose, glucose, xylose and arabinose [1-4]. Cellulases are obtained from various micro organisms: bacteria such as *Bacillus subtilus*, *Bacillus circulans* [5], *Bacillus sphaericus-JS1* [6], *Bacillus pumilus*[7], *Cellulomonas flavigena* [8]and fungi like *Aspergillus sp*.[9, 10]. Mainly, bacteria are more efficient for producing cellulase enzyme than fungi because of their high cell cultivation rate [11] Cellulases are widely used in processing industries such as textile, food, pulp and paper [12-15]. Besides this, cellulases act as an additive in cattle and pig feeds [16].

Cellulase enzyme production was investigated using various Solid substrates such as cellulose bagasse [17], egg shell waste [18], water hyacinth [19] ligno cellulosic waste from xylose industry [20], lignocellulosic waste from the vinegar industry [21], wheat bran [22], sugar cane bagasse [23], domestic wastewater sludge [24], sweet potato [25], Oil palm lignocellulosic biomass in the form of empty fruit bunches [26], Soyhull [27], Saw dust [28], rice bran [29], rice straw [30], potato peel [31], banana waste [32]and ground nut shell waste [33].

Cassava bagasse, renewable solid waste material generated from processing industry are disposed in surrounding lands near the processing unit. However, its higher organic content and bio degrability, it creates more pollution [34]. It contains approximately 20% cellulose fibres and does not contain cyanide content. Due to low composition of protein, it is not used as a cattle feed. Utilization of this biodegradable waste for the production of more valuable products such as organic acids, bio polymer, xylanase enzyme and antibiotics are recent trend in biochemical engineering [23, 34-37]. Because of low ash content, cassava bagasse is a potential and attractive substrate for microbial cultivation than other agricultural wastes such as rice straw and wheat straw [34].

In recent years, solid state fermentation has been preferred for enzyme production than submerged fermentation due to low water consumption, low energy requirement, low chances of contamination for producing high quality of product and easy separation of product [38, 39, 40]. Cellulase production in Submerged fermentation and Solid state fermentation was compared by Tendargy, 1998 [41]. Improved bioprocess technology under economical feasibility needed for cellulase production which could be fulfilled by Solid State Fermentation for the production of cellulase [42].

In this study, low cost substrates such as cassava bagasse, pine leaves, wheat bran and rice bran were examined for the production of cellulase in solid state fermentation. Best substrate was screened and used for cellulase production. Then, screening of nitrogen source and effects of initial pH and moisture content were studied for improving cellulase production.

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MATERIALS AND METHODS

Strain Specification

Cellulomonas cellulans isolated from Brewery sewage was procured from Microbial Type Culture Collection, Chandigarh, India, with the following specification *Cellulomonas cellulans* MTCC 1769. The medium used for cultivating *Cellulomonas cellulans* consists of yeast extract-2 g/L, beef extract-1 g/L, peptone- 5 g/L, Nacl- 5 g/L.

Solid State Fermentation

Twenty grams of solid substrate like cassava bagasse, pine leaves, rice bran and wheat bran was taken in 250 ml Erlenmeyer flasks. Production medium containing K_2HPO_4:1 g/L, KH_2PO_4: 1 g/L, KCl: 0.5 g/L, $MgSO_4$: 0.5 g/L, $FeSO_4.7H_2O$: 0.01g/L and Yeast extract: 2 g/L (43) was introduced in to the solid medium due to the moisture composition. Solid medium containing mineral salt medium was stirred well, sterilized in an autoclave at 121^oC at 15min and cooled. Then 5% (V/V) inoculmn containing optical density of 0.6 at 650 nm was added with sterile medium under aseptic condition and incubated at room temperature.

Effects of Various Parameters on Enzyme Production

Agro wastes such as cassava bagasse, pine leaves, wheat bran and rice bran were chosen as a low cost substrate for the production of enzyme. Solid substrate gained maximum production was taken and used for further studies. Influence of nitrogen sources like yeast extract, beef extract, peptone, malt extract on enzyme production was studied. Effects of initial pH (2-10) and % moisture content (10-90) on enzyme production and activity were tested.

Enzyme Extraction from Solid State Fermentation

Samples obtained from the solid fermentation were centrifuged at 10000 rpm at 4^oC for 10min.The supernatant collected was taken as crude enzyme solution. Then enzyme solution was added with 1% Carboxy Methyl Cellulose (CMC) dissolved in citrate phosphate buffer [44]. This enzymatic reaction was performed at room temperature for 10 min and enzyme activity was estimated by DNS method [45]. Cellulase activity is defined as micromoles of reducing sugar released per unit time per mg of dry solid substrate under specified condition.

RESULT AND DISCUSSION

Screening of Solid Substrate

Figure 1 shows the screening of solid substrate for maximum cellulase production in solid state fermentation with 50% moisture content. Maximum activity (2.35 U/mg) was obtained using cassava bagasse as a solid substrate. Similarly, cassava beggase was selected as a solid substrate for cellulase production by singhania et al., 2006. 130 U/g and 60 U/g enzyme

production were obtained by pre treated and untreated cassava bagasse as a solid substrate respectively [23]. Latifian et al., 2007 obtained maximum cellulase production in solid state fermentation using *Trichoderma reesei* QM9414 and *T. reesei* MCG77 mutants with rice bran as a solid substrate [29]. Maximum production of CMCase (71.526 U/g) was resulted from wheat bran in solid state fermentation [46]. Maximum cellulase activity of 2.11U/ml was resulted from rice bran with egg shell waste as a calcium content using *Neurospora crassa* [18].

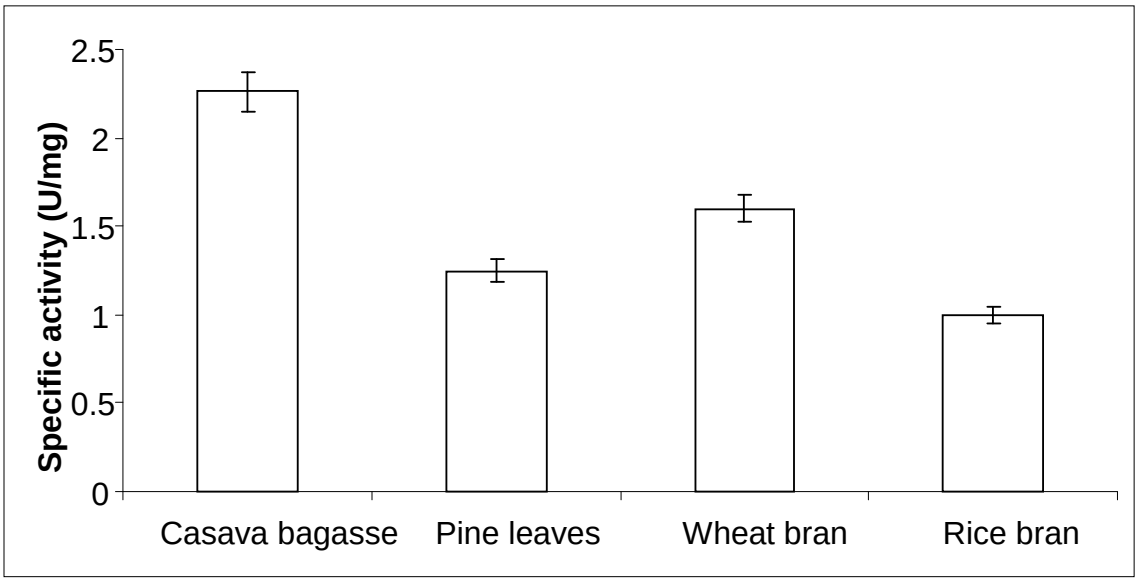

Fig 1: Screening of solid substrate

Screening of Nitrogen Source

Figure 2 shows the screening of nitrogen source for cellulase production using cassava bagasse as a solid substrate with 50% moisture content. In this present work, yeast extract was selected as the best nitrogen source for maximum enzyme production (2 U/mg). Beef extract for *Bacillus circulans* TP3 (30 U) and asparagine for *B. subtilis* CY5 (35 U) were selelcted as potential nitrogen source for cellulase production [5]. Ammonium compounds [23, 47], KNO_3 and NH_4NO_3 [8] were used as suitable nitrogen sources for cellulase production.

Effect of Initial pH

Initial pH of the medium is an important parameter for enzyme production. To narrate the influence of initial pH on cellulase enzyme production, initial pH value of medium was adjusted to 2-10 by the addition of 1N HCl or 1N NaOH. The result of specific activity in solid state fermentation is shown in figure 3. The solid state fermentation was carried out using cassava bagasse with 50% moisture content for three days. It was noted that cellulase production was so low at pH 2 because of low growth rate of cells at this initial pH. Maximum cellulase production was appeared at an initial pH of 6. Similarly, cellulase obtained from *Bacillus pumilus* EWBCM1 after 72 h and *Trichoderma koningii* AS3.4262 using lignocellulosic waste [21] were found to be maximum production at pH 6 [48]. Maximum cellulase production

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from *Bacillus* sp., *Micrococus* sp., and *Cellulomonas* sp., isolated from estuarine coir netting effluents resulted at pH 7[49]. Optimum cellulsae activity was found to be 7.5 pH from Aspergillus niger Z10 [9]. Previous reports explained that favorable pH for cellulase enzyme production was 7.0 using *Clostridiumthermocellum* and *Cellulomonas sp.*[50, 51]. Optimum cellulase activity was found to be 22 U from *Bacillus subtilus* CY5 and 27 U from *Bacillus circulans* TP3 between pH 7.0 and 7.5 in solid state fermentation [5].

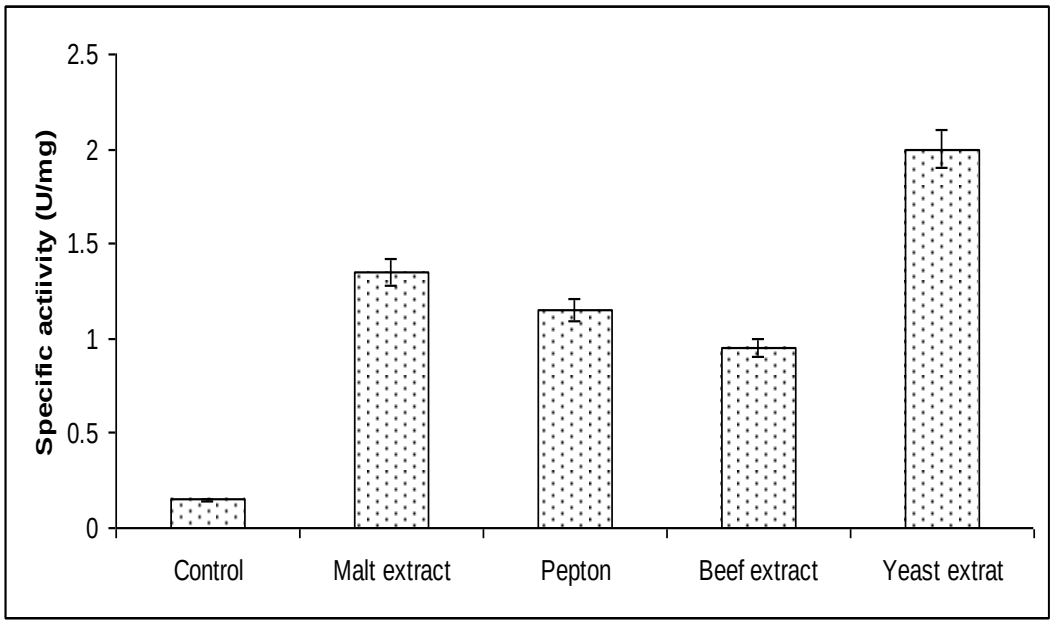

Fig 2: Screening of Nitrogen source

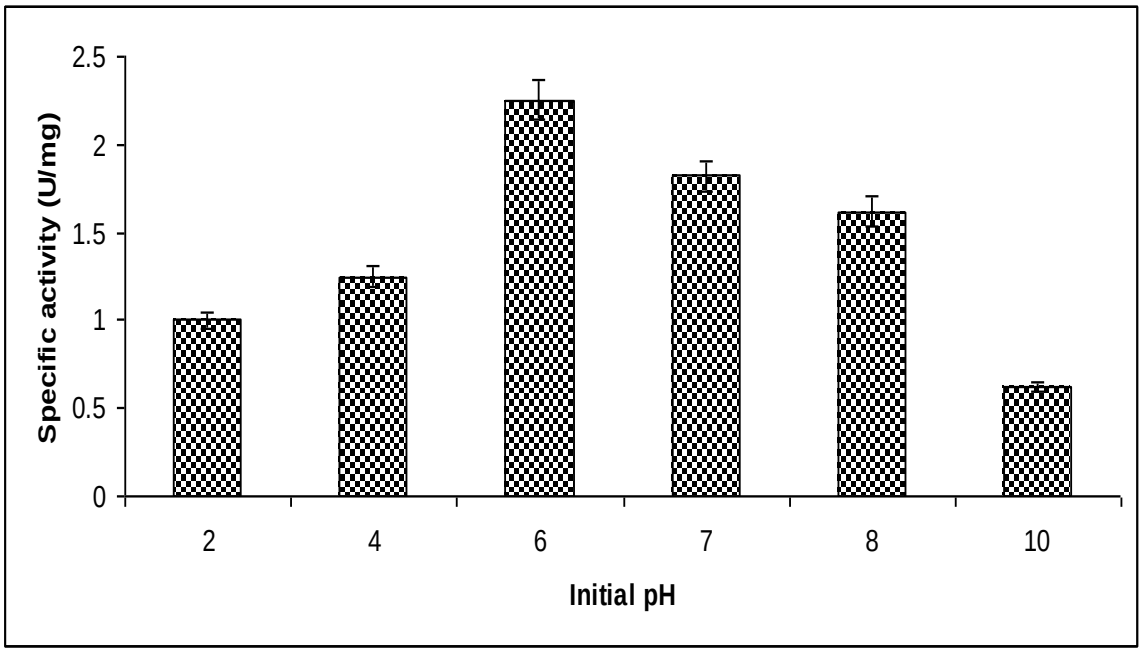

Fig 3: Effect of initial pH on specific activity of cellulase.

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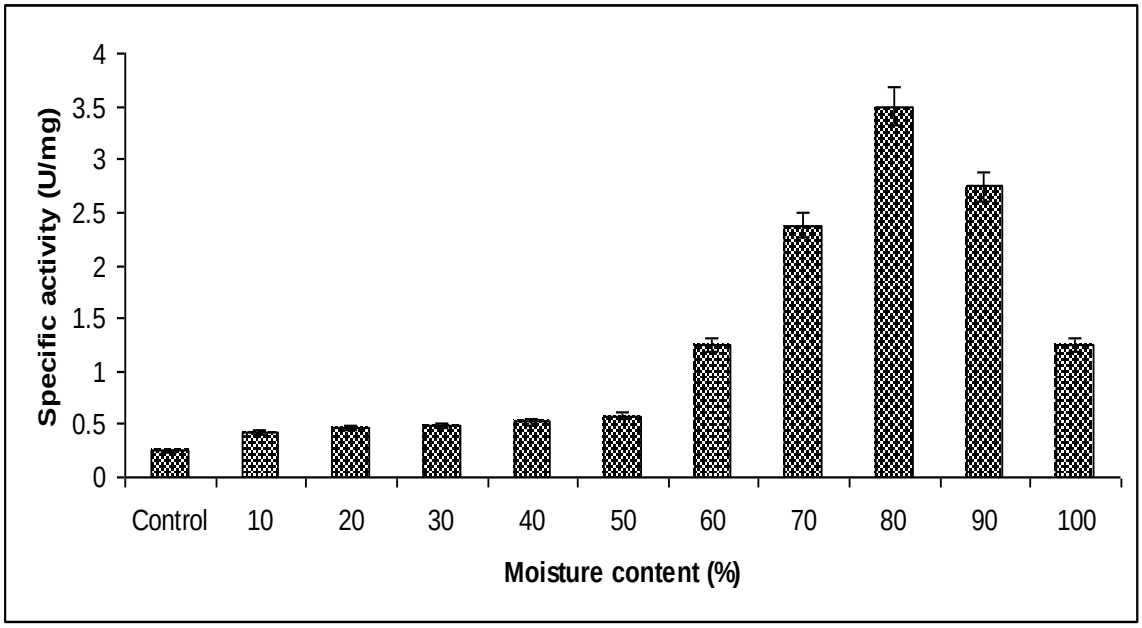

Fig 4: Effect of moisture content on specific activity of cellulase

Effect of Moisture Content

Solid state fermentation is different from submerged fermentation, because cell growth as well as metabolite appeared on or near the solid substrate. The suitable water composition in solid state fermentation needs the growth of cells as well as metabolic activity [52]. Therefore, moisture content is a crucial parameter for enzyme production since it appreciably affects the performance of solid state medium [41]. In this study, effect of moisture content on enzyme production was carried out at an initial pH 6 using cassava bagasse as a solid substrate shown in Figure 4. At 80% moisture content, maximum enzyme production (3.5 U/mg) was appeared in solid state fermentation. However, maximum cellulase production was carried out with only 10% moisture content and resulted activity as 26 U for *B. subtilis* and 20 U for *B. circulans* [5].Cellulase produced from pre treated sugarcane bagasse by *Trichoderma koningii* AS3.4262 was found to be 11 U/ g ds with 66.4% moisture content [21].

CONCLUSION

Low cost solid state materials such as cassava bagasse, pine leaves, wheat bran and rice bran were tried owing to cellulase production. Cassava bagasse was emerged as a potential substrate among four solid materials and used for further studies. Screening of nitrogen sources was examined and yeast extract was obtained as a best source. Effect of initial pH and moisture composition on cellulase production was investigated. In this study, it was found that suitable conditions for cellulase production were: solid substrate- cassava bagasse; initial pH -6; nitrogen source - yeast extract and moisture content - 80%.

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REFERENCES

[1] Fujimoto S, Inoue H, Yano S, Sakaki T, Minowa T, Endo T, Sawayama S, Sakanishi K. J Japan Petrol Inst 2008; 51: 264–73.
[2] Yanagida T, Fujimoto S, Hideno A, Inoue H, Tsukahara K, Sawayama S, Minowa T. J Japan Soc Ene Res 2009; 30:8–14.
[3] Lee SM, Koo YM. J Microbiol Biotechnol 2001, 11: 229-233.
[4] Cai YJ, Chapman SJ, Buswell JA, Chang ST. Appl Environm Microbiol 1999; 65: 553-559.
[5] Ray AK, Bairagi A, Sarkar Ghosh K, Sen SK. Acta Ichthyol et Piscat 2007; 37 (1): 47–53.
[6] Singh J, Batra N, Sobti RC. J Ind Microbiol Biotechnol 2004; 31: 51–56.
[7] Christakopoulos P, Hatzinikolaou DG, Fountoukidis G, Kekos D, Claeyssens M, Macris BJ. Arch Biochem Biophy 1999; 364: 61–66.
[8] Rajoka MI. Electronic J Biotechnol 2004; 7: 259–266.
[9] Coral G, Arikan B, Ünaldi MN, Güvenmez H. Turkish J Biol 2002, 26: 209–213.
[10] Stewart JC, Parry JB. J Gen Microbiol 1981; 125: 33–39.
[11] Ariffin H, Abdullah N, Umikalsom MS, ShiraiY, Hassan MA. J Biosci Bioeng 2008; 106: 231-236.
[12] Bajpai P. Biotechnol Prog 1999; 15: 147–157.
[13] Beguin P. Anal Biochem 1983; 131: 333–336.
[14] Bhat MK. Biotechnol Adv 2000; 18: 355–383.
[15] Endo K, Hakamada Y, Takizawa S, Kubota H. Appl Microbiol Biotechnol 2001; 57:109–116.
[16] Dvorak, R.A. (2000). Proc American Soc Animal Sci, Midwestern Section, pp. 82.
[17] Long C, Ou Y, Guo P, Li Y, Cui J, Long M, Hu Z. Ann Microbiol 2012; 59(3): 517-523.
[18] Verma N, Kumar V, Bansal MC. Polish J Environm Studies 2012; 21(2): 491-497,
[19] Zhao SH, Liang XH, Hua DL, Ma TS, Zhang HB. African J Biotechnol 2011; 10(50): 10178-10187.
[20] Xia L, Cen P. Proc Biochem 1999; 34:909–912.
[21] Liu J, Yang J. Food Technol Biotechnol 2007; 45 (4): 420–425.
[22] Maurya DP, Dhananjay Singh, Pratap D, Maurya JP. J Environm Biol 2012; 33: 5-8.
[23] Singhania RR, Sukumaran KR, Pillai A, Prema P, szakacs G, Pandey A. J Biotechnol 2006; 5: 332-336.
[24] Alam MZ, Muyibi SA, Wahid R. Biores Technol 2008; 99: 4709–4716.
[25] Zhang C, Xing XH, Liu MS. Biochem Eng J 2004; 19: 181–187.
[26] Alam MZ, Mamun AA, Qudsieh IY, Muyibi SA, Salleh HM, Omar NM. Biochem Eng J 2009; 46: 61–64.
[27] Krishna Jha, Khare SK, Gandhi AP. Biores Technol 1995; 54: 321-322.
[28] Guruchandran V, Sasikumar C. J Cell Tissue Res 2011; 10(1): 2115-2117.
[29] Latifian M, Hamidi-Esfahani Z, Barzegar M. Biores Technol 2007; 98, 3634-3637.
[30] Hideno A, Inoue H, Tsukahara K, Yano S, Fang X, Endo T, Sawayama S. Enz Microbial Technol 2011; 48: 162–168.
[31] Dos Santos TC, Gomes DPP, Bonomo RCF, Franco M. Food Chem 2012; 133 (4): 1299-1304.
[32] Krishna C. Biores Technol 1999; 69: 231-239.

[33] Vyas A, Vyas D. (2005). J Sci Ind Res 2005; 64: 767-770.
[34] Pandey A, Soccol CR, Nigam P, Soccol VT, Vandenberghe LPS, Mohan R. Biores Technol 2000; 74: 81-87.
[35] Ray RC, Moorthy SN. J Sci Ind Res 2007; 66: 252-255.
[36] Asagbra AE, Sanni AI, Oyewole OB. World J Microbiol Biotechnol 2005; 21: 107–114.
[37] Sugumaran KR, Kumar BK, Mahalakshmi M, Ponnusami V. Intl J ChemTech Res 2013; 5(1): 394-400.
[38] Genckal H, Tari C. Enz Microbial Technol 2006; 39: 703-710.
[39] Kumar PKR, Lonsane BK. Biotechnol Lett 1987; 9(3): 179-182.
[40] Durand A, Almanza S, Renaud R, Maratray J. Agro-Food Industry Hi-Tech. 1997; 8(3): 39-42.
[41] Tengerdy RP. 1998. Solid substrate fermentation for enzyme production. In: Pandey A. (ed.) Advances in biotechnology. Educational Publishers and Distributors, New Delhi. pp. 103-105.
[42] Pandey A, Selvakumar P, Soccol CR, Nigam P. Curr Sci 1999; 77: 149–162.
[43] Agarwal R, Mahanty B, Venkata Dasu V. Chem Biochem Eng 2009; 23 (2): 213–218.
[44] Song J, Wei D. Biomass Bioenergy. 34(12); 1930-1934.
[45] Miller GL. Ana Chem 1959; 31: 426–428.
[46] Deswal D, Khasa YP, Kuhad RC. Biores Technol 2011; 102(10): 6065–6072, 2011.
[47] Spiridonov NA, Wilson DB. J Bacteriol 1998; 180: 3529–3532.
[48] Shankar T, Isaiarasu. Middle-East J Sci Res 2011; 8 (1): 40-45.
[49] Immanuel G, Dhanusha R, Prema P, Palavesam A. Intl J Environm Sci Technol 2006; 3: 25–34.
[50] Garcia-Martinez DV, Shinmyo A, Madia A, Deman AL. European J App Microbiol Biotechnol 1980; 9: 189–197.
[51] Prasetsan P. and Doelle, H.W. Mircen J 1987; 3: 33–44.
[52] Hahn-Hägerdal, B. Enz Microbial Technol 1986; 8: 322–327.