INT. J. BIOL. BIOTECH., 16 (3): 601-605, 2019.

OPTIMIZATION OF FERMENTATION CONDITIONS FOR THE BIO SYNTHESIS OF CELLULASE THROUGH NEWLY ISOLATED STRAIN OF *BACILLUS*

Madiha Sattar[*] and Muhammad Noman Syed

Department of Biochemistry, University of Karachi, Karachi-75270, Pakistan
[*]Corresponding Author: Email: mdhsattar@gmail.com

ABSTRACT

Cellulose, is water insoluble, tough & fibrous material that forms plant cell wall and the major constituent of the agro waste. Cellulose can be degraded into glucose by microorganisms through cellulase. This enzyme acts on the β1→4 glycosidic linkages in cellulose thereby releasing glucose. Cellulase production can be induced in certain microorganisms, such as bacteria and fungi, by culturing them on cellulose containing medium. The aim of present investigation was to optimize cellulase production in a newly isolated *Bacillus* sp. For this, the isolated *Bacillus* sp. was grown under varying physical conditions to explore the optimum temperature, pH and incubation time for maximum enzyme induction. The medium was supplemented with various carbon and nitrogen sources to further enhance enzyme production. It was found that optimal cellulase production was achieved at temperature of 37 °C, at pH 7.0 and after 48h of incubation. The best medium for cellulase induction contained wheat bran as carbon source and ammonium chloride used as nitrogen.

Key Words: Production, Cellulose, Cellulase, *Bacillus*, induction, optimization.

INTRODUCTION

Cellulose is one of the biopolymer found on earth in abundant quantities and this polymer is used as one of the raw material in manufacturing industries. Cellulose is degraded by enzyme cellulases, which convert it into smaller subunits by acting its B-1, 4 glycosidic linkages (Sethi *et al*., 2013). Cellulases are consisting three enzymes work systematically including endo-(1, 4)-β-D-glucanase (EC 3.2.1.4) exo-(1, 4)-β-D-glucanase (EC 3.2.1.91), and β-glucosidases (EC 3.2.1.21) (Bayer *et al*., 1994, Singh, 1999). Different microorganisms secrete cellulases extracellularly, when culture in cellulose containing media (Kubicek *et al*., 1993, Sang-Mok and Koo, 2001). Fungi and bacteria are two main organisms for the production of cellulases (Sethi *et al*., 2013). Aerobic, anaerobic, mesophilic or thermophilic microorganisms can be the producer of cellulases. *Clostridium, Cellulomonas, Thermomonospora, Trichoderma*, and *Aspergillus* genera are broadly used as cellulases manufacturer (Sun and Cheng, 2002, Kuhad *et al*., 1999). It is important to introduce innovation in research for increase production of cellulases at industrial level, which may help to improve expenses in its production also manufacturing of cellulosic degradable products in new biological processes (Kuhad *et al*., 2011, Henrissat *et al*., 1998). Industries of food, animal feed, brewing and wine making, agriculture, biomass refining, pulp and paper, textile, and laundry are used cost effective biotechnological approaches for the production of this enzyme (Kuhad *et al*., 2011). Generation of biofuel is one of the noble applications of cellulases (Sukumaran *et al*., 2005, Kuhad *et al*., 2010, Gupta *et al*., 2011). Cellulase importance makes it more searchable enzyme for fast, economical procedures for cellulase production. Production of cellulase strongly influence by its method of cultivation. Submerged and solid fermentation both can be used for synthesis of cellulases (Lynd *et al*., 2002). Liquid medium of fermentation is more suitable than solid medium of fermentation because it provides homogenous cultural condition for growth of bacteria (Mussatto and Roberto, 2004). For cultivation, inoculums amount, nitrogen and carbon sources, presence of inducers and media additives are very significant. Although submerged fermentation is best for bacterial growth because it provides high moisture requirements (Bano *et al*., 2013).

MATERIAL AND METHODS

Isolation of Organism:

For isolation of *Bacillus* sp. garden soil samples were collected from Karachi Pakistan. The sample were serially diluted in deionized water and then transfer into Carboxy methyl cellulose (CMC) agar plates and incubates them in incubator at 37^0C for 24h. After 24h the colonies of plates were stained with 0.1% congo red solution and washed with 1% NaCl. Formation of clear zone around the colonies was indicative of cellulase production. Colonies

with large clear zones were chosen for cellulase production. These potential cellulase producers were stored on nutrient agar slants at 4 °C.

Vegetative inoculum preparation:

Stored culture of *Bacillus* was revived in the nutrient broth and incubated at 37^0C for 24h.

Submerged fermentation

For enzyme production, submerged fermentation was carried out (Acharya and Chaudhary, 2012). Briefly, fermentation medium (nutrient broth supplemented with 0.05% carboxy methyl cellulose) was inoculated with 24 hours old culture grown in nutrient broth. After 48h of incubation, contents of the flasks were centrifuged at 10,000 rpm at 0^0C for 10 min. After centrifugation, the supernatant was used for the enzyme estimation.

Optimization of physical parameters

Submerged fermentation was carried out at different temperatures, pH with varying period of incubation to optimized physical conditions for maximum cellulase production,

Optimization of chemical parameters

To further enhance cellulase production, the fermentation medium was supplemented with various different carbon and nitrogen sources. The experiment was designed to work out best carbon source and nitrogen source that can induce maximum cellulase induction. Carboxy methyl cellulose, wheat bran, isphagol, bagasse were used as carbon source. Urea, glycine, peptone, ammonium sulphate and ammonium chloride were used as nitrogen source.

Analytical methods

Assay of Cellulase using 4-nitrophenyl β-D glucopyranoside

The cellulase assay was carried out as described by earlier (Keshri and Magan, 2000) using 4-nitrophenyl β-D glucopyranoside as substrates. One unit of enzyme activity is defined as the 1.0µM of 4-nitrophenol released under assay condition in unit time.

Lowry Protein Assay

Total protein was assayed using Lowry's method (Lowry *et al*., 1951).

RESULTS AND DISCUSSION

Enzyme production was influenced with change in temperature and pH. Results showed higher enzymes yield at 37°C (Fig.**1**) when pH is adjusted to 7.5 pH (Fig**.2)**.Temperature and pH greatly influences the bacterial enzyme production. It has been reported earlier that maximum bacterial cellulase production is recorded in the pH range of 7.0- 8.0. Some exceptions do occur, but most notable enzyme induction by *Bacillus* spp. was observed at pH 7.5. As far as temperature is concerned, it is widely reported that bacterial cellulase are produced over a broad range of temperature i.e. between 30-80°C (Gaur and Tiwari, 2015). Maximum cellulase induction was observed after 48 hours of incubation (Fig.**3**) that declines afterwards. Cellulose is not the preferred substrate of bacteria. Its utilization as substrate begins when primary nutrient which is glucose in this case was completely exhausted. This triggers the cellulase production in the bacteria. As the process of fermentation went on, bacteria entered into lag phase of growth. In this phase depletion of nutrient in fermentation media, accumulation of toxic wastes that affect the cell growth. Most notably in this phase rate of death overtake the rate of multiplication and overall number of viable cells thus enzyme production decreased with increase in time (Haq *et al*., 2005).

The medium that contained wheat bran as carbon source (Fig.**4**) and Ammonium chloride as nitrogen source (Fig.**5**) supported the cellulase production. Enzyme induction in bacteria is always dependent on availability of preferred substrate which induces the genes of enzyme production. The nature of cellulose from one source to another varies in terms of their coiled structure. This may result in different level of enzyme induction by different types of cellulose. Nitrogen source is a key factor that determines the rate of bacterial growth which ultimately affects the enzyme production. It has been reported earlier that Extracellular cellulase production is very susceptible to suppression with different nutritional inducers like nitrogen, carbon and different agro waste (Sethi *et al*., 2013).

Present study showed that newly isolated strain of *Bacillus* has great potential for production of extracellular cellulase using cheap raw materials.

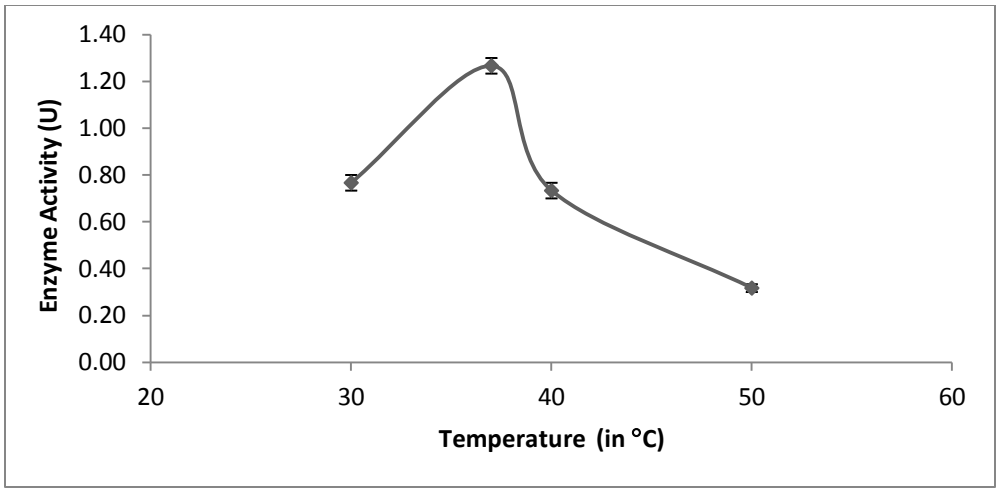

Fig.**1.** Effect of Incubation Temperature on cellulase production.

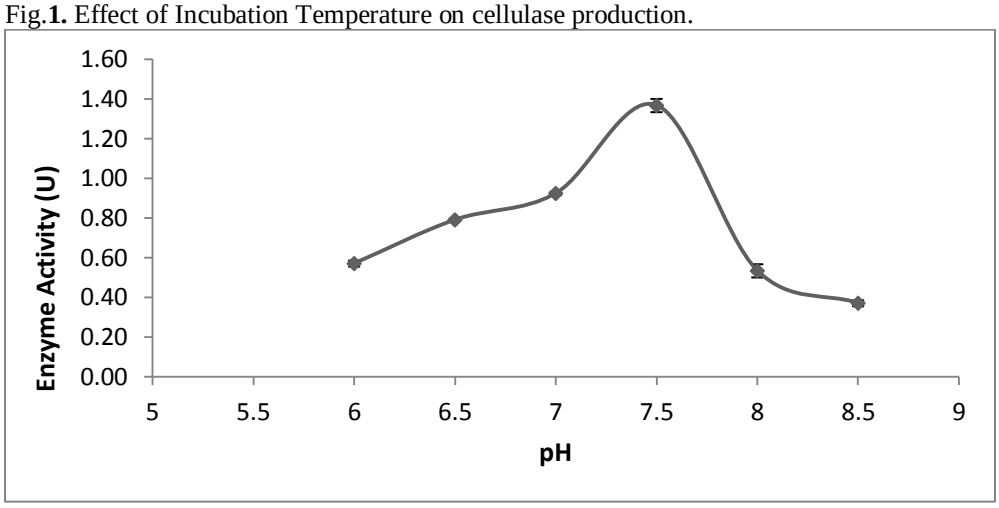

Fig.**2.** Effect of pH on cellulase production.

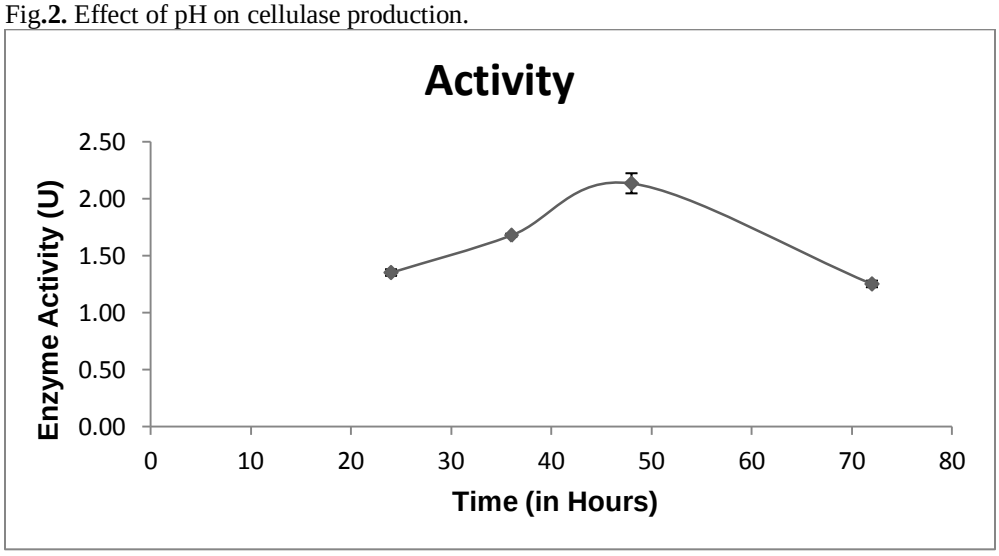

Fig.**3.** Time Course for cellulase production.

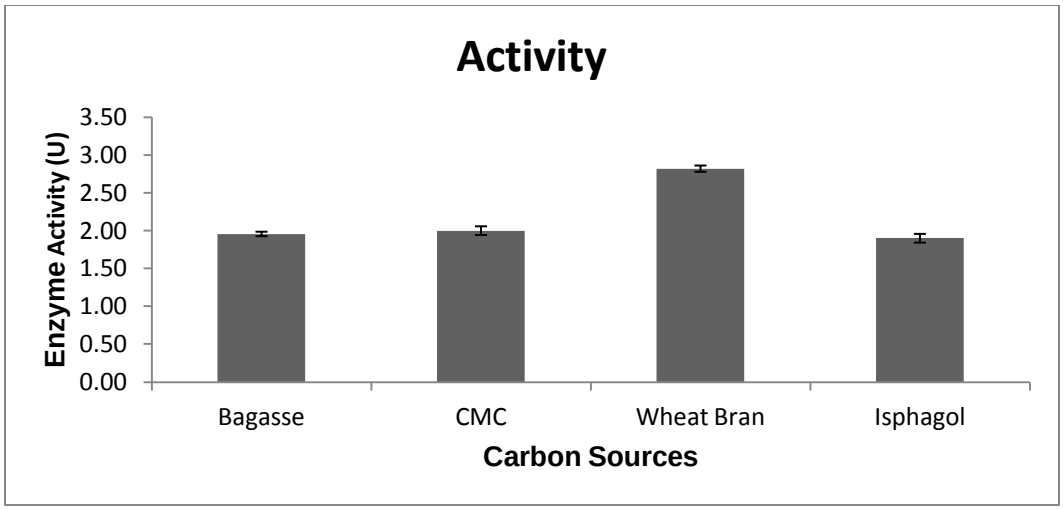

Fig.**4.** Effect of various carbonsources on cellulase production.

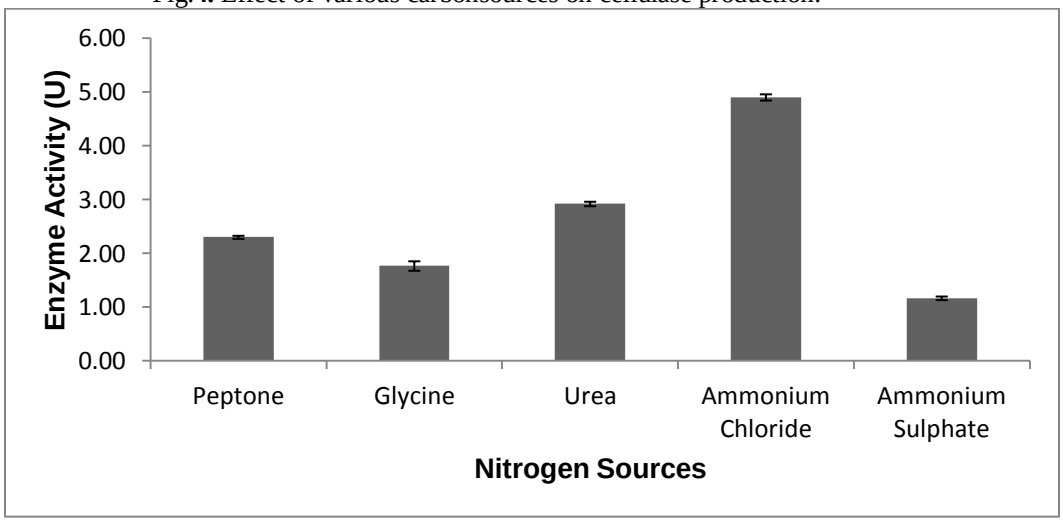

Fig.**5.** Effect of different nitrogen sources on cellulase production.

REFERENCES

Bano S, S.A.U. Qader, A. Aman, M.N. Syed and K. Durrani (2013). High production of cellulose degrading endo-1, 4-b-D-glucanase using bagasse as a substrate from *Bacillus subtilis*, *Carbohydrate Polymer*, 91: 300–304.

Bayer, E. A., E. Morag, and R. Lamed (1994). "The cellulosome— a treasure-trove for biotechnology," *Trends in Biotechnology*, 12(9): 379–386.

Gaur R., and S. Tiwari (2015). Isolation, production, purification and characterization of an organic-solvent-thermostable alkalophilic cellulase from *Bacillus vallismortis* RG-07. *BMC Biotechnology*, doi:10.1186/s12896-015-0129-9.

Gupta, R., Y. P. Khasa, and R. C. Kuhad (2011). Evaluation of pretreatment methods in improving the enzymatic saccharification of cellulosic materials. *Carbohydrate Polymers*, 84: 1103–1109.

Haq, I.U., K. Hameed, M.M. Shahzadi, S.A. Javed and M.A Qadeer (2005). Cotton Saccharifying activity of cellulase *Trichoderma harzianum* UM-11 in shake flask. *Int J Bot*., 1: 19-22.

Henrissat, B., T. T. Teeri and R. A. J. Warren (1998). A scheme for designating enzymes that hydrolyse the polysaccharides in the cell walls of plants. *FEBS Letters*, 425(2): 352– 354.

Keshri, G., and N. Magan (2000). Detection and differentiation between mycotoxigenic and non-mycotoxigenic strains of two *Fusarium* spp. using volatile production profiles and hydrolytic enzymes. *J Appl Microbiol.*, 89: 825–833.

Kubicek, C. P., P. Suominen and T. Reinikainen (1993). From cellulose to cellulase inducers: facts and fiction. *Enzyme Research*, 8: 181–188.

Kuhad, R. C., M. Manchanda and A. Singh (1999). Hydrolytic potential of extracellular enzymes from a mutant strain of *Fusarium oxysporum*. *Bioprocess Engineering*, 20(2): 133–135.

Kuhad, R. C., R. Gupta and Y.P. Khasa (2010). Bioethanol production from lignocellulosic biomass: an overview. In: *Wealth from Waste* (B. Lal, Ed.), Teri Press, New Delhi, India.

Legler, G., C.M. Muller-Platz, M. Mentges-Hettkamp, G. Pflieger and E. Julich (1985). On the Chemical Basis of the Lowry Protein Determination. *Anal. Biochem.*, 150: 278–287.

Lowry, O. H., N.J. Rosebrough, A. L. Farr and R.J. Randall (1951). Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem.*, 193: 265–275.

Lynd, L.R., P.J. Weimer W.H. Van Zyl and I.S. Pretorius (2002). Microbial cellulose utilization: fundamentals and biotechnology. *Microbiol MolBiol Rev*., 66: 506–577.

Mussatto S.T. and I.C. Roberto (2004). Alternatives for detoxification of diluted acid lignocellulose hydrolysates for use in fermentative process: A review. *Bioresource Technology*, 93: 1–10.

Kuhad, R. C., R. Gupta and A. Singh (2011). Microbial Cellulases and Their Industrial Applications. *Enzyme Research*, 2011: 1-10.

Sang-Mok, L., and Y. M. Koo (2001). Pilot-scale production of cellulase using *Trichoderma reesei Rut* C-30 in fed-batch mode. *Journal of Microbiology and Biotechnology*, 11(2): 229–233.

Singh, A. (1999). Engineering enzyme properties. *Indian Journal of Microbiology*, 39(2): 65–77.

Sethi S., A. Datta, B.L. Gupta and S. Gupta (2013). Optimization of Cellulase Production from Bacteria Isolated from Soil. *ISRN Biotechnology*, 3: 7-14.

Sukumaran, R. K., R.R. Singhania and A. Pandey (2005). Microbial cellulases—production, applications and challenges. *Journal of Scientific and Industrial Research*, 64(11): 832– 844.

Sun, V and J. Cheng (2002). Hydrolysis of lignocellulosic materials for ethanol production: A review. *Bioresource Technology*, 83(1): 1–11.

Acharya, S. and A. Chaudhary (2012). Optimization of Fermentation Conditions for Cellulases Production by *Bacillus licheniformis* MVS1 and *Bacillus* sp.MVS3 Isolated from Indian Hot Spring. *Brazilian Archives of Biology and Technology*, 55(4): 497-503.

(*Accepted for publication June 2019*)