

## **Isolation & production of cellulase enzyme from bacteria isolated from agricultural fields in district Hardoi, Uttar Pradesh, India**

**Vipul Verma<sup>1\*</sup>, Alpika Verma<sup>1</sup> and Akhilesh Kushwaha<sup>2</sup>**

<sup>1</sup>Amity Institute of Biotechnology, Amity University, Lucknow, U.P, India

<sup>2</sup>Institute of Transgene Life Sciences, Lucknow, U.P, India

---

### **ABSTRACT**

Cellulose is one of the most abundant polymer found in nature (EC 3.2.1.4). It occurs in almost pure form in cotton fiber and in combination with other materials, such as lignin and hemicelluloses, in wood, plant leaves and stalks, etc. It has already been used in processing of coffee, in textile industry and in laundry detergents. Cellulose is a long chain polymer, made up of repeating units of glucose, a simple sugar, joined together with  $\beta$ -1,4 glycosidic linkages. Cellulases cause hydrolysis of the individual cellulose fibers to break it into smaller sugars units & finally producing glucose molecules. The soil samples were obtained (10gm) from Hardoi district, Uttar Pradesh, India. Bacterial colonies were grown over CMC-Agar medium[1]. Maximal cellulase production was obtained after 48 h of incubation at 45 °C in medium containing 1.5% carboxymethyl cellulose (CMC) as substrate. The optimum pH for the enzyme was found to be ranging between 6.5 and 7.5 at which it was found to be most stable. Bacteriological studies indicated *Bacillus subtilis* to be the most frequent cellulolytic bacteria to be found in the agricultural fields. . The purpose of the current investigation was to screen thermophilic *Bacillus* species isolated from soil in order to study its suitability with regard to waste treatment in agricultural fields (bioremediation).

**Key Words:** Cellulose, Cellulases, CMC-Agar, *Bacillus* Species, Hardoi.

---

### **INTRODUCTION**

Cellulose is the most common organic compound on Earth. It is well known that plants are the most common source of renewable carbon and energy on the earth. Cellulose has no taste, is odourless & is hydrophilic [2]. Cellulose is derived from D-glucose units, which condense through  $\beta$ (1 $\rightarrow$ 4)-glycosidic bonds [3]. Cellulose is the structural component of the primary cell wall of green plants, many forms of algae and the oomycetes. Some species of bacteria secrete it to form biofilms. Cellulolysis is the process of breaking down cellulose into smaller polysaccharides called cellodextrins or completely into glucose units, this is a hydrolysis reaction. Because cellulose molecules bind strongly to each other, cellulolysis is relatively difficult compared to the breakdown of other polysaccharides. Some ruminants like cows and sheep contain certain symbiotic anaerobic bacteria in their normal micro flora, and these bacteria produce enzymes called cellulases that help the microorganism to break down cellulose, the breakdown products are then used by the bacteria for growth. The biological degradation of cellulose has been studied for many years, and a number of cellulolytic enzymes, especially cellulases produced by fungi and bacteria, have been isolated and characterized [4]. Cellulose is the major constituent of paper, paperboard and of textiles made from cotton, linen, and other plant fibers. Thus, in our agricultural fields cellulose is found in abundance and there are great possibilities of existence of bacteria that will consume cellulose as substrate. The aim of the current study is isolation and production of cellulases, which will have applications in the treatment of agricultural waste. Optimum conditions required for the stability of the enzymes are also studied. Since the final product of hydrolysis is glucose, which a soluble sugar, bioremediation can be done.

## MATERIALS AND METHODS

The soil sample(10gm.) was obtained from the agricultural fields. Isolation and screening of the cellulase producing microorganisms was done[5]. The cellulolytic activity of the isolated *Bacillus* was determined through the amount of reducing sugar released by using dinitrosalicylic acid (DNS) method [6]. To evaluate the effect of cultivation time on cellulase production, the selected bacterial isolate was grown at 45°C in CMC broth adjusted to pH 7. After incubation for 24, 48, 72 or 96 hours, culture broths were centrifuged to obtain supernatants which were later used to measure cellulase activity. In order to determine the effect of temperature on cellulase production, the selected bacterial isolate was grown in carboxymethyl cellulose (CMC) broth and incubated at 15°C, 25°C, 35°C, 45°C, 55°C and 65° C for 48 hours. Culture broths were then centrifuged to obtain supernatants which were later used to measure cellulase activity. The effect of initial media pH on cellulase production was conducted by adjusting the CMC broth to pH 3.5, 4.5, 5.5, 6.5, 7.5, 8.5 or 9.5 before bacterial inoculation. After 48 hours of incubation at 45°C, culture broths were then centrifuged to obtain supernatants which were later used to measure cellulase activity. In order to obtain optimum substrate concentration for enzyme production the CMC broth was supplied with carboxymethyl cellulose in a range of 0.5%, 1%, 1.5% and 2% . After incubation of 48hrs culture broths were then centrifuged to obtain supernatants which were later used to measure cellulase activity.

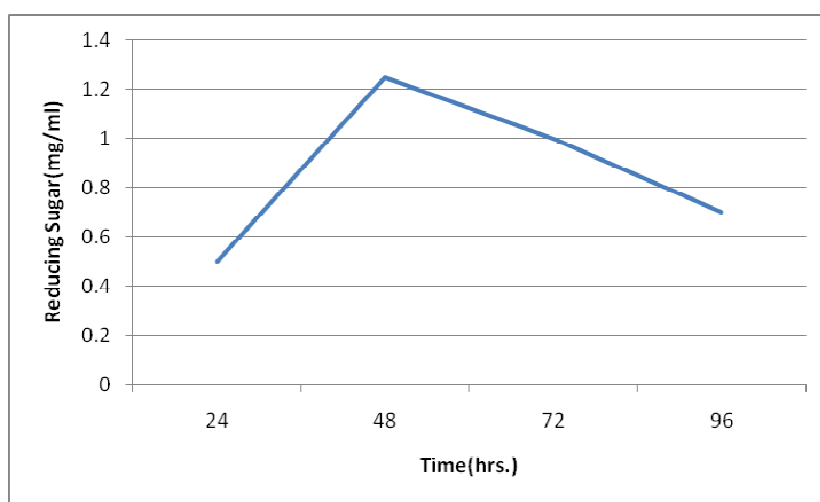


Fig. 1 Changes in reducing sugar content

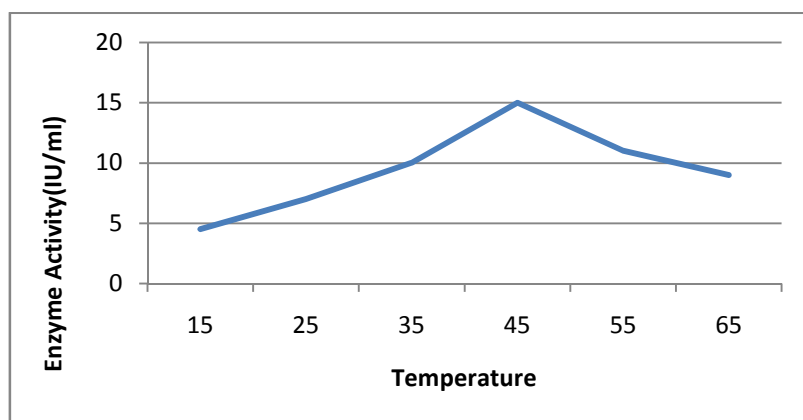


Fig. 2 Effect of Temperature

## RESULTS AND DISCUSSION

Strains of *Bacillus* species were isolated and identified from agricultural fields from the district Hardoi, Uttar Pradesh. The morphological and biochemical characterization revealed the presence of *Bacillus subtilis* in the samples. All the *Bacillus* isolates were Gram-positive, rod-shaped, spore formers and hydrolyzers of cellulose. In this study, the activity of cellulase produced increased from 0 to 48 h with a reducing sugar content of 1.25 mg/ml (Figure 1). The temperature stability result of cellulase obtained from *Bacillus subtilis* is shown in (Figure 2).

The figure revealed that the enzyme remained stable at 45°C. The enzyme stability declined at temperatures above 50°C. The maximum activity was displayed at 45°C [7] with enzymatic activity 15 IU/ml. The optimum pH of the enzyme comes out to be ranging between 6.5 & 7.5 [8] with enzyme activities of 11.5 IU/ml & 12 IU/ml (Figure 3). The optimum substrate concentration for enzyme production came out to be 1.5% with enzyme activity of 20 IU/ml (Figure 4).

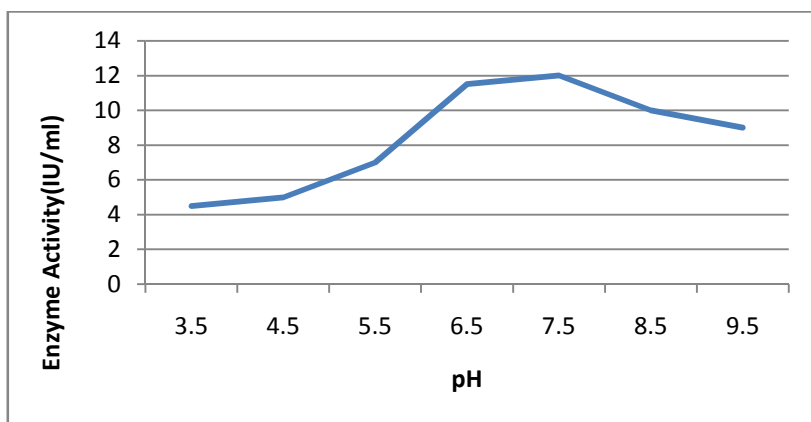


Fig. 3 Effect of pH

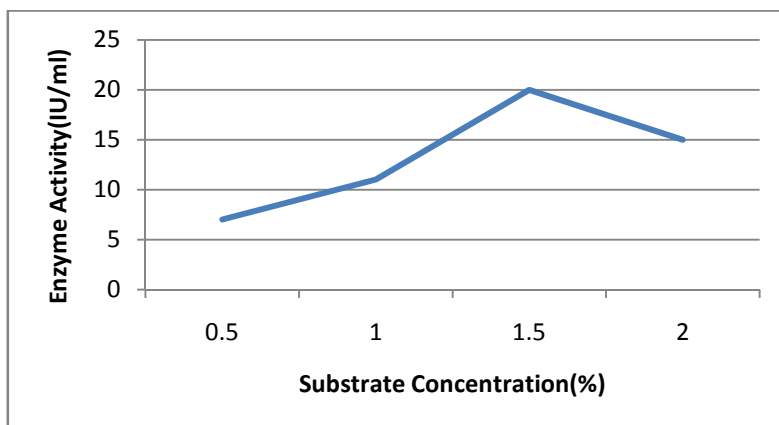


Fig.4 Effect of Substrate Concentration

### CONCLUSION

In the current study cellulase enzyme is isolated and its optimum conditions for production are determined. It is found that maximum production of enzyme took place at the incubation period of 48hrs. The optimum temperature of the enzyme was 45°C. The optimum pH at which the enzyme was stable was between 6.5&7.5. The optimum substrate concentration for enzyme production was 1.5%.

Future work of the present work includes purification of enzyme in order to gain higher specific activity of cellulase by the help of sophisticated purification procedures including Salt precipitation, Dialysis & Ion Exchange chromatography.

The present study thus, reveals that agricultural waste harbours cellulolytic bacteria[9] that can be produced commercially for its industrial applications and for the treatment of agricultural waste in the process of bioremediation[10].

### Acknowledgement

Vipul Verma & Alpika Verma express special thanks to Er. Akhilesh Kushwaha Director of Transgene Life Sciences for his kind support and encouragement.

## REFERENCES

- [1] Jahir Alam Khan *et al.*, *European Journal of Experimental Biology*, **2011**, 1 (1):139-147.
- [2] Ghosal *et al*, *Der Pharmacia Sinica*, **2011**, 2 (2): 152-168.
- [3] Yakubu *et al*, *Advances in Applied Science Research*, **2011**, 2 (6):532-540.
- [4] Tomme, P, Warren, R. A, and Gilkes, N. R, *Advances in Microbial Physiology*, **1995**, Vol. 37, No. 1, pp. 1-81.
- [5] Sasithon Krairitthichai and Narumol Thongwai, "ISOLATION AND SCREENING FOR CELLULASE PRODUCING BACTERIA", 34th Congress on Science and Technology of Thailand.
- [6] Miller, G.L, *Anal. Chem.*, **1959**, 31, 426.
- [7] Saheb *et. al*, *World Applied Science Journal*, **2010**, 8(1): 35-42.
- [8] Li-Jung Yin, Hsin-Hung Lin, and Zheng-Rong Xiao, *Journal of Marine Science and Technology*, **2010**, Vol. 18, No. 3, pp. 466-471.
- [9] Lalit K. Singh *et al*, *Advances in Applied Science Research*, **2011**, 2 (5):508-521.
- [10] J. Raja Brindha *et al*, *European Journal of Experimental Biology*, **2011**, 1 (3):90-96.