J Appl Biotechnol Rep. 2020 Sep;7(3):152-158

doi 10.30491/JABR.2020.109990

Journal of
Applied Biotechnology
Reports

Original Article

# Optimization of Culture Conditions and Reaction Parameters of β-Glucosidase From a New Isolate of *Bacillus subtilis* (B1)

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**Received** October 2, 2019; **Accepted** January 8, 2020; **Online Published** July 7, 2020

**Abstract**

**Introduction**: The increased need for a considerable β-glucosidase activity, especially in the enzymatic saccharification of cellulose for bioenergy, has strongly stimulated the identification of effective β-glucosidase producing microbes. This study was conducted to optimize culture condition for β-glucosidase production from the identified new isolate of *Bacillus subtilis* (B1) and to find out an ideal condition for β-glucosidase activity.

**Materials and Methods:** For β-glucosidase production, the bacterium was cultivated in a basal medium. The culture condition was optimized at several pH, different temperatures, varying cultivation periods, and various substrate concentrations. Finally, the activity of the β-glucosidase enzyme was investigated at different incubation periods, pH, temperatures, metal ions, and various percentages of methanol. The activity of β-glucosidase was measured by the capability of crude enzyme to convert pNPG (p-nitrophenyl-β-D glucopyranoside) into yellow product PNP (p-nitrophenol).

**Results:** Cellulolytic bacterial strain *B. subtilis* (B1) showed high potentiality for β-glucosidase production at a pH of 7.0 after 24 hours incubation at 40°C. The highest level of enzyme production was achieved when 3% of CMC was provided in the culture medium. Optimum reaction conditions for β-glucosidase activity were shown to be 10 minutes, 60°C and at pH 7. Salts like Magnesium Sulfate ($MgSO_4$), Calcium Chloride ($CaCl_2$), and Manganese Sulfate ($MnSO_4$) positively influenced the activity where NaCl and KCl had negative effects. The presence of methanol (80%) appreciably enhanced the activity of enzyme.

**Conclusions:** Complete saccharification of different industrial processes can be augmented by using this novel β-glucosidase produced by *B. subtilis* strain isolated from effluent of biogas plant.

**Keywords**: Saccharification, β-glucosidase, *Bacillus subtilis,* Cellobiose, Submerged Culture

**Citation:** Neesa L, Islam R, Jahan N, Zohora US, Rahman MS. Optimization of culture conditions and reaction parameters of β-glucosidase from a new isolate of *Bacillus subtilis* (B1). J Appl Biotechnol Rep. 2020;7(3):152-158. doi:10.30491/JABR.2020.109990.

## Introduction

The cellulase is the enzyme that hydrolyses the β-1,4glycosidic bonds in the cellulosic polymer to release glucose units by the synergistic action of its constituent group of enzymes which consists of endoglucanase (EC 3.2.1.4), exoglucanases (cellobiohydrolase (EC 3.2.1.91), and β-glucosidase (EC 3.2.1.21).[1,2] Complete hydrolysis of cellulose to glucose requires the synergistic action of all three enzymes in the cellulase complex.[3] β-glucosidase is an important class of cellulolytic complex that completely breaks down various lignocellulosic materials. It hydrolyzes cellobiose by cleaving the β-1,4-glycosidic bond in it to generate D-glucose. β-glucosidase is the most critical enzyme that catalyzes the final step of hydrolysis of cellulose and hemicellulose into glucose. It is generally responsible for the regulation of the whole cellulolytic process, as this enzyme not only produces glucose from cellobiose but also reduces cellobiose inhibition, allowing endocellulase and exocellulase enzymes to function more efficiently.[4]

β-Glucosidases have recently received the focus of numerous research because of their important roles in a variety of fundamental biological and biotechnological processes for their hydrolyzing and synthetic activity.[5] The β-glucosidases are mostly used in cellulose conversion process but also have broad applications such as activation of phytohormones, defense against pathogens in plants, cellular signaling and oncogenesis.[6] This enzyme has also received huge attention as it is involved in releasing aroma from wine grapes, hydrolysis of bitter compounds during juice extraction, and the formation of alkyl and aryl glycosides by trans-glycosylation from natural polysaccharides or their derivatives. This enzyme is also used in pharmaceutical,

cosmetic, and detergent industries.[7]

Most of the bioconversion processes used today do not allow complete saccharification of biomass. Hydrolysis of biomass can be enhanced by several approaches, one of which is by supplementation of cellulase complex with accessory enzymes.[8] Among all the enzymes that could be used for the supplementation of cellulases for enhancing saccharification efficiency, β-glucosidase and xylanase are the most common and have been widely studied.[9,10]

Therefore, augmenting enzymatic mixture with β-glucosidases or using microbes with a desirable proportion of β-glucosidase in their cellulases will enhance sugar yields which have been proved beyond doubt by various researchers.[11] Thus β-glucosidase producing microorganisms are potential sources that can be employed for bioconversion of cellulosic biomasses into easily employable form. For large scale production of these enzymes, it is very important to identify and improve these cellulolytic microbes.

In this study, the cellulase producing bacteria were isolated, screened and measured in quantity. Finally, the cellulolytic bacteria with higher β-glucosidase activity were characterized by optimizing kinetic growth parameters such as different carbon sources and various concentrations, incubation period, temperature and pH. Characterization of the crude enzyme was also done by assaying β-glucosidases activity at different reaction periods, pH, temperature and observing the effects of different salt ions.

## Materials and Methods

This study was conducted at the Bio-resources Technology and Industrial Biotechnology Laboratory, Department of Biotechnology and Genetic Engineering, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.

### Isolation, Screening and Identification of Cellulolytic Bacterial Isolates

Samples for cellulolytic bacteria were collected from a dairy effluent of national dairy farm at Savar and from effluent of biogas production plant of Jahangirnagar University as well. Standard sample collection procedure was carried out using clean sterile plastic containers and samples were stored at 4°C until the analysis was performed. After screening them, purified isolated single cells were preserved and stored in 20% glycerol solution and kept at -80°C for further identification and screening for enzyme production.

### Culture Conditions for β-Glucosidase Production

Identified bacterial isolate *Bacillus subtilis* B1 with efficient β-glucosidase activity was optimized by evaluating its production at different culture conditions. For this purpose, a basal media containing 2% Tryptone, 1% CMC, 0.1% $KH_2PO_4$, 0.1% $K_2HPO_4$, 0.04% $MgSO_4$, 0.005% NaCl, and 0.000125% $FeSO_4$, pH 7.0 was used for producing β-glucosidase. For seed culture, a freshly isolated colony was inoculated in 5 mL basal media and incubated at 37°C and 120 rpm for 24 hours. Then, 5% seed culture was inoculated in 50 mL production media in a 250 mL conical flask and incubated at 37°C and 120 rpm for 24 hours in shaking water bath. For assaying β-glucosidase activity, a reaction mixture of 500 µL [375 µL of culture supernatant (enzyme), 50 µL of pNPG (10 mM) as substrate and 75 µL sodium acetate buffer (0.2 M, pH 5.0)] was incubated at 50°C for 10 minutes in hot water bath. The reaction was terminated by adding 1750 µL of 1 M $Na_2CO_3$. The developed yellow color was assayed at 410 nm using a spectrophotometer (OPTIZEN POP, Mecasys, Korea). The amount of p-nitrophenol released was quantified using the PNP standard curve previously developed in the laboratory. One unit of β-glucosidase activity was expressed as the amount of enzyme required to release 1 µM of PNP per minute under assay conditions.

### Optimization of pH for β-Glucosidase Production

In order to study the effect of pH on β-glucosidase production, the initial pH of the production medium was adjusted to different pH (5.0 to 10). After incubation for 24 h, 5% of freshly prepared seed culture was inoculated in 50 mL production media with different pH (5, 6,7,8,9,10) in a 250 mL conical flask and incubated at 37°C and 120 rpm for 24 hours in shaking water bath. Culture supernatant was collected by centrifugation and β-glucosidase activity assay was carried out.

### Optimization of Temperature for β-Glucosidase Production

To determine the effect of temperature on β-glucosidase production, cultivation temperature of the selected isolate was adjusted to 30°C, 40°C, 50°C, and 60°C. For this study, 5% of freshly prepared seed culture was inoculated in 50 mL production medium with pH 7 in 250 mL conical flask and incubated at different temperatures, 120 rpm for 24 hours in shaking water bath. Collected supernatant was centrifuged and β-glucosidase activity assay was performed.

### Time Course Study for β-Glucosidase Production

To determine the optimum cultivation period for maximum β-glucosidase production, 5% freshly prepared seed culture was inoculated into the production medium with a pH of 7 and incubated at 37°C, 120 rpm in shaking water bath. Culture samples were withdrawn at 6, 12, 24, 36, 48 hours of cultivation and the β-glucosidase activity was assayed.

### Effects of CMC Concentration on β-Glucosidase Production

To determine the optimum CMC concentration for maximum β-glucosidase production, the medium with different concentrations of CMC (1%, 1.5%, 2%, 2.5%, 3%, 4%) were prepared. Then, 5% of freshly prepared seed culture of the selected strain was inoculated in 50 mL production media with a different percentage of CMC and was incubated for 24 hours at 37°C, 120 rpm in shaking water bath. Following incubation, the culture supernatant was collected by centrifugation at 10000 rpm for 10 minutes and finally, the activity of β-glucosidase was assayed.

### Characterization of Enzymatic Activity of β-Glucosidase

#### *Activity of β-Glucosidase Enzyme on Different Incubation Period*

The effects of incubation period on β-glucosidase activity

were determined by incubating the reaction mixture 500 µL containing 375 µL of culture supernatant (enzyme), 50 µL of pNPG (10 mM) as substrate and 75 µL sodium acetate buffer (0.2 M, pH 5.0) was incubated at 50°C for different time period (5, 10, 15, 30, 45, 60, and 75 minutes) in hot water bath. The reaction was terminated by addition of 1750 µL of 1 M $Na_2CO_3$. The developed yellow color was read at 410 nm using a spectrophotometer and β-glucosidase activity was measured.

### Effect of pH on the β-Glucosidase Activity

The effect of pH on the activity of β-glucosidase was determined by assaying the enzyme activity at different pH, from pH 4.0 to 11.0 using 0.2 M of various buffers over the pH range [sodium acetate buffer (pH 4.0-5.0), phosphate buffer (7.0-8.0) and Glycine-NaOH (pH 9.0-11.0)] using the standard assay procedure described earlier. For assaying, reaction mixture 500 µL containing 100 µL of culture supernatant (enzyme), 50 µL of pNPG (10 mM) as substrate and 375 µL of the corresponding buffer with different pH were incubated at 50°C for 10 minutes in hot water bath. Following incubation, the reaction was terminated by adding 1750 µL of 1M $Na_2CO_3$. The developed yellow color was measured at 410 nm.

### Effect of Temperature on β-Glucosidase Activity

The effect of temperature on the activity of β-glucosidase enzyme was determined by incubating the reaction mixture 500 µL containing 375 µL of culture supernatant (enzyme), 50 µL of pNPG (10 mM) as substrate and 75 µL sodium acetate buffer (2 M, pH 5.0) was incubated for 10 minutes at different temperatures (20°C to 70°C) in hot water bath. The reaction was terminated by adding 1750 µL of 1M $Na_2CO_3$ and activity was measured at 410 nm.

### Effect of Different Metal Ions on β-Glucosidase Activity

The effects of different salt ions on β-glucosidase activity were determined via the addition of 10 mM of various divalent ($Cu^{2+}$, $Mg^{2+}$, $Zn^{2+}$, $Ca^{2+}$, and $Mn^{2+}$) and monovalent cations ($K^+$, $Na^+$) to the reaction mixtures. For this investigation, different salt solutions were made in 2 M sodium acetate buffer (pH 5) and then the β-glucosidase activity was measured.

### Effects of Different Percentages (%) of Methanol on β-Glucosidase Activity

For this investigation, different percentages (%) of methanol solution were prepared along with sodium acetate buffer with a pH of 5. In this experiment, the control tube contained 75 µL buffer instead of methanol, 375 µL supernatant as enzyme and 50 µL pNPG as substrate. Other test tubes contained 375 µL enzyme, 50 µL substrate and 15 µL methanol, 60 µL buffer referred as 20%; 30 µL methanol, 45 µL buffer referred as 40%; 45 µL methanol,30 µL buffer referred as 60%, 60 µL methanol, 15 µL buffer referred as 80%; 75 µL methanol referred to as 100% methanol as reaction mixtures respectively. Then, the test tubes were incubated at 50°C for 10 minutes in a hot water bath and enzyme activity was determined.

## Results

### Optimization of Initial pH for β-Glucosidase Production

Optimum initial pH in the production medium for β-glucosidase producing bacteria was 7. In this pH, enzymatic reaction of the culture supernatant with the substrate was found maximum (3.304 U/mL/min) than the rest of the conditions (Figure 1). The β-glucosidase production decreased rapidly with the increased pH and was minimum at a pH of 10.0.

### Optimization of Temperature for β-Glucosidase Production

The optimum temperature for β-glucosidase producing bacteria is 40°C. In this temperature, the activity of β-glucosidase was highest (1.164 U/mL/min) than other temperatures (Figure 2). A dramatic decrease (0.4 U/mL/min) was observed at 60°C in the production of β-glucosidase.

### Time Course Study for β-Glucosidase Production

The optimum cultivation period for maximum β-glucosidase production was determined by withdrawing the culture at 6, 12, 24, 36, 48 hours of cultivation, assaying the β-glucosidase activity. The highest level of enzyme production was found

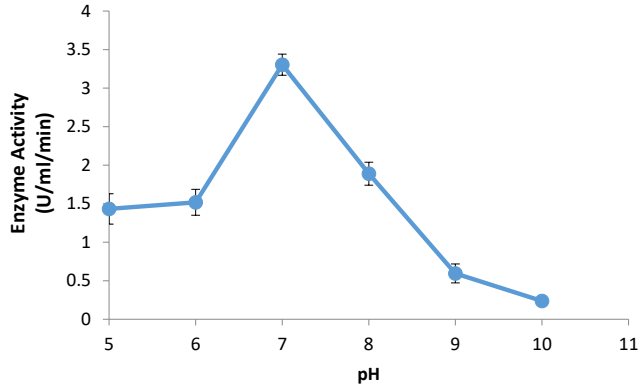


**Figure 1**. Effects of Different Initial pH of the Media on β-Glucosidase Production by Submerged Fermentation of *B. subtilis*.

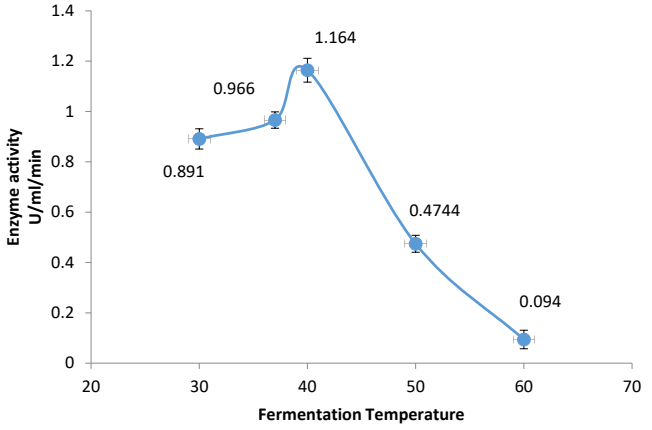


**Figure 2**. Effects of Different Cultivation Temperature on β-Glucosidase Production by *B. subtilis*.

at 24 hours of cultivation (Figure 3). After 24 hours, the production of the enzyme significantly reduced to half of its highest level. Therefore, optimum cultivation period for the production of β-glucosidase was 24 hours.

### Effects of CMC Concentration on the Production of β-Glucosidase

From the following figure, it is clear that maximum β-glucosidase activity was found when the CMC concentration was 3% in the production medium. There is no significant change even at higher substrate concentration (3.5%, and 4%). The optimum substrate concentration for the production of β-glucosidase by $B_1$ was 3% (Table 1).

### Characterization of β-Glucosidase Enzyme

#### *Effect of Reaction Time on β-Glucosidase Activity*

The effect of different reaction incubation time on β-glucosidase activity was examined over a range of 5 to 75 minutes. The highest level of enzymatic activity (2.088 U/mL/min) was observed when the reaction was incubated for 10 minutes (Figure 4). Thus the optimum incubation period of β-glucosidase activity was estimated 10 minutes.

### Effect of pH on β-Glucosidase Activity

The pH of the reaction mixture can significantly affect the activity of β-glucosidase. For this purpose, enzyme assays were carried out using buffer solution with different pH values ranging from 4 to 11 in order to determine the optimum pH for β-glucosidase activity. The ideal pH for the best β-glucosidase activity (0.56 U/mL/min) was found to be 7.0 with phosphate buffer (Figure 5).

### Effect of Temperature on β-Glucosidase Activity

Temperature has a significant effect on enzyme activity during reaction with substrate. To determine the optimum temperature for β-glucosidase activity, the reaction mixture was incubated at different temperatures ranging from 20°C to 70°C. According to Figure 6, it is clear that the optimum temperature for β-glucosidase activity was 60°C.

### Effects of Salts on β-Glucosidase Activity

Elemental ions may have either positive or negative effects on the activity of β-glucosidase enzyme. Among the studied salt ions, divalent ions $Mg^{2+}$ and $Ca^{2+}$ significantly increased the enzymatic activity while monovalent ions $Na^{+}$ and $K^{+}$ decreased the activity of β-glucosidase enzyme (Figure 7).

### Effects of Different Percentages (%) of Methanol on β-Glucosidase Activity

The results shown in Table 2 elucidate that the presence of different percentages (%) of methanol significantly increase β-glucosidase activity. There was a steady increase with the presence of 20%-60% methanol in the reaction mixture. The highest β-glucosidase activity was observed with 80%

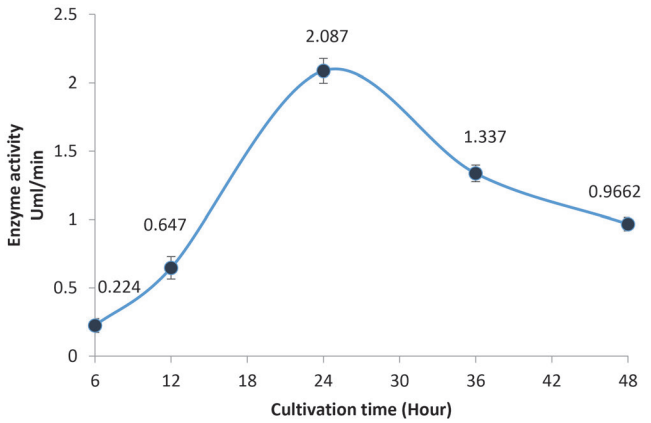


**Figure 3.** Time Course Study for maximum β-Glucosidase Production by *B. subtilis*.

**Table 1.** Effect of Different Concentration of CMC (%) on β-Glucosidase Production

| CMC (%) | Enzyme activity (U/mL/min) |
|---|---|
| 1 | 0.215 ± 0.01272 |
| 1.5 | 0.405 ± 0.002828 |
| 2 | 0.56 ± 0.03535 |
| 2.5 | 0.793 ± 0.02757 |
| 3 | 1.337 ± 0.03394 |
| 3.5 | 1.25 ± 0.04949 |
| 4 | 1.233 ± 0.0586 |

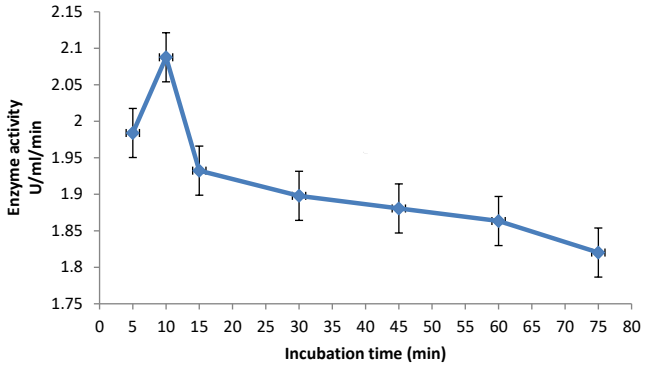


**Figure 4.** Determination Optimum Reaction Time for β-Glucosidase Activity.

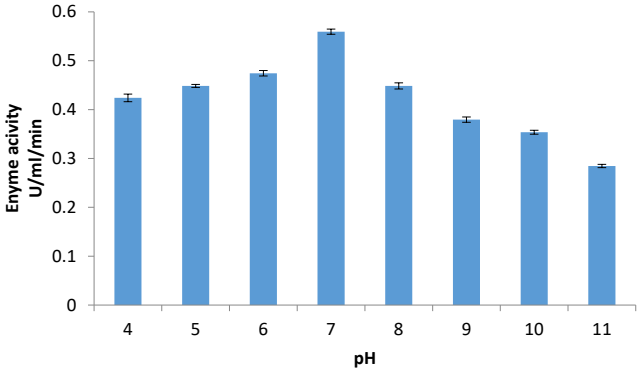


**Figure 5.** Determination Optimum pH for β-Glucosidase Activity.

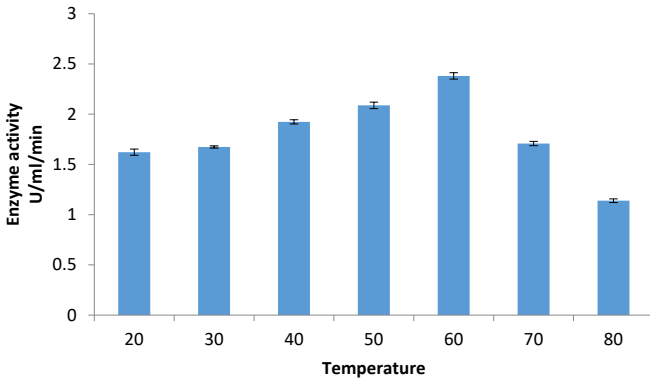


**Figure 6.** Determination Optimum Temperature for β-Glucosidase Activity.

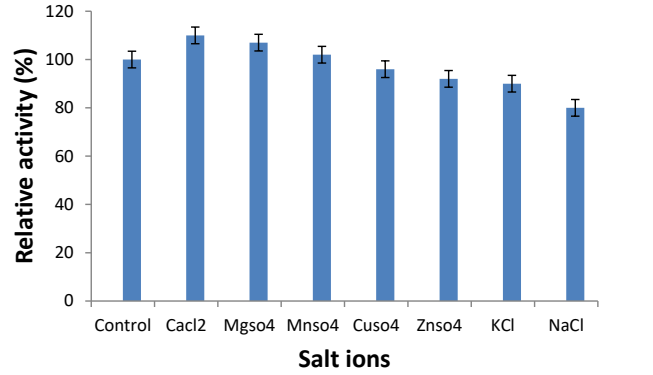


**Figure 7.** Effects of Metal Ions on β-Glucosidase Activity.

methanol and no significant level of activity was observed with further increase in methanol concentration.

## Discussion

The activity of different cellulase enzymes depends on the sources and amounts of biowaste in natural environments.[5] Soil is the big reservoir of different kinds of microorganisms with different potential enzymes. Earlier studies revealed that *Bacillus* sp. producing β-glucosidases are abundant in soil.[12]

Cellulolytic bacterial isolates from the Persian Gulf showed their optimal production of cellulase after 48 hours of cultivation.[14] However, optimum cultivation period varies from strain to strain with their characteristics and culture conditions.[15] In an earlier study by Bagudo et al[16] it was observed that the maximum yield of β-glucosidase activity produced by *Bacillus* sp. was recorded 1.750 U/mL after 10 h of fermentation using dinitrosalicylic (DNS) method, after that it began to decrease. In this study, the effect of incubation period on the growth and β-glucosidases production by *B. subtilis* revealed that production begins to increase from 6 hours to peak at 24 hours with enzyme activity of 0.647 to 2.087 U/mL/min. After 24 hours, the production of enzyme significantly reduced (Figure 3). Increase in incubation period resulted in a decrease in the production of β-glucosidase by *B. subtilis*. This may be due to the fact that after maximum production of β-glucosidase enzyme (maximum incubation time), there was a production of other byproducts and a

**Table 2.** Effect of Different Percentage (%) of Methanol on β-Glucosidase Activity

| Methanol (%) | Relative Activity (%) |
|---|---|
| Control | 68 ± 2.5166 |
| 20% | 82 ± 2.6457 |
| 40% | 87 ± 1 |
| 60% | 91±1 |
| 80% | 103 ± 3.2145 |
| 100% | 99 ± 1 |

depletion of nutrients. Due to depletion of nutrients, the cells may use their secreted enzyme as nutrient for their survival. In addition, the by-products may inhibit the growth of the organisms and hence, enzyme formation.[1]

Media optimization is an important aspect to be considered in the development of fermentation technology. The isolated *Bacillus* species was inoculated in culture medium and production of β-glucosidase was assayed. Temperature is one of the most important variables controlling bacterial growth and enzyme production. In Figure 2, it is clearly indicated that the highest enzyme activity of $B_1$ was found to be at 40°C. There was a gradual increase in the enzyme activity from 30°C-40°C. After on, the enzyme activity decreased with an increase in the temperature. Similar to culture temperature, pH is also an important factor that influences the yield of β-glucosidase. As shown in Figure 1, it is clear that the β-glucosidase activity gradually increased as the pH increased from 6 to 7 and reached its maximum at 7.

The concentration of substrate (CMC) in the production medium also greatly influences enzyme production and catalytic activities. There was a gradual increase with the rise of CMC % in the medium from 1% to 3% (Table 1). There is no significant change even at higher substrate concentrations greater than 3%. Therefore, the optimum substrate concentration for the production of β-glucosidase was 3%.

In addition, some bacterial strains have been reported as potential β-glucosidase producers, such as *B. subtilis*[16] and *Acidothermus cellulolyticus*,[17] which produce more thermostable β-glucosidase compared to fungi, but are slow producers. In this study, the bacterium showed high levels of cell growth and β-glucosidase production when grown at 40°C, pH 7.0 in the presence of 3% CMC for 24 hours.

There was a significant change in β-glucosidase activity with a change in the pH, temperature and incubation period. The incubation time for reaction affected the β-glucosidase activity. It was found that the isolated cellulolytic strain exerts best β-glucosidase activity when the reaction time was 10 min (Figure 4).

Temperature and pH are the most important factors, which markedly influence enzymatic activity. Each enzyme has its own optimum pH and if the pH increases or decreases beyond the optimum, the ionization groups at the active site may change, slowing or preventing the formation of an enzyme substrate complex.[18] Optimum pH values of 4.5-8.0 have been reported for different microbial cellulases.[19-21] Highest β-glucosidase activity was recorded at a pH of 7 (Figure 5) implying that the enzyme is best activated at neutral pH

condition. Although optimal pH was 7.0, but during the entire study all the experiments were done with a pH of 5 to maintain the uniformity of all the data analysis. There was no significant difference in enzyme activity at pH 5, 6 and pH 8, 9. This finding suggests that these pH pairs have more or less the same effect on the enzyme activity. Similar observations were made by Olajuyigbe and Ajele[22] who recorded optimum pH of 8.0 for *Bacillus* species.

There was a significant change in the activity of β-glucosidase with a change in the temperature. Optimum temperature for activity was recorded as 2.381 U/mL/min at 60°C (Figure 6), however there was a gradual increase in enzyme activity as the temperature decreased from 20°C to 60°C. Actually, activity declines as the temperature increases beyond 60°C. Microbial cellulases from different sources have been found to have optimum temperature of approximately 35°C-50°C.[19,23] At elevated temperatures, many enzymes become partly unfolded and inactivated thus rendering them unable to perform their desired tasks.[18] Industrial application of the enzyme from $B_1$ isolate will provide an advantage over other cellulases due to their stability at high temperature.

Moreover, there were significant effects of various salt ions (Figure 7) on the activity of β-glucosidase. It was observed that $MgSO_4$, $CaCl_2$, and $MnSO_4$ greatly increased the activity of β-glucosidase which means that these metal ions probably worked as co-factors during enzymatic reaction whereas NaCl and KCl influenced negatively.

An increased enzyme activity was observed in the presence of different percentages of methanol. The presence of methanol (80%) had a positive influence on the hydrolytic activity of β-glucosidase where pNPG was used as donor and methanol as an acceptor. Activation by alcohol has been earlier observed for β-glucosidase from fungal species *Thermoascus aurantiacus*,[1] *Aspergillus oryzea*,[24] *Fusarium oxysporum*.[1] In the present study, the highest β-glucosidase activity was observed with 80% methanol, so no significant level of activity was observed with further enhancement of methanol concentration (Table 2).

It is a common observation that members of *Bacillus* lack a complete cellulase system with all the three enzymes required for the cellulase complex for efficiently converting cellulose to fermentable sugars or glucose. In contrast to these observations, a crude enzyme extract form $B_1$ isolate was found to produce higher amounts of β-glucosidase that can significantly increase the rate of cellulose hydrolysis as well as allowing endocellulase and exocellulase enzymes to function more efficiently. In addition, for its thermo stability and higher yield this new isolate might be used in industrial applications enabling the liquefaction and saccharification steps to be performed simultaneously under the same conditions.

## Conclusions

The development of rapid and reliable methods for the screening of microbial cellulases within inhospitable environments will allow a greater number of novel bacterial cellulases to be isolated with a purpose for industrial use. Moreover, most of the bioconversion processes used today do not allow complete saccharification of the biomass. Supplementation of cellulase complex with accessory enzymes like β-glucosidase can enhance the hydrolysis of cellulosic biomass. The successful exploitation of these isolated bacterial strains producing β-glucosidase with proper biotechnology will be beneficial for efficient hydrolysis of cellulosic biomass as they relieve the inhibition of the cellobiohydrolases and endoglucanases by reducing cellobiose accumulation. In addition, different approaches of genetic engineering might help for a deeper understanding of these bacterial strains to improve their enzymatic yield, activity and purification for proper industrial application of the enzyme.

### Authors' Contributions

All authors contributed equally to this work.

### Conflict of Interest Disclosures

The authors declare they have no conflicts of interest.

### Acknowledgments

This research was partially supported by the Ministry of Science and Technology, Bangladesh and INSPIRE program, British Council, Bangladesh.

### References

1. Parry NJ, Beever DE, Owen E, Vandenberghe I, Van Beeumen J, Bhat MK. Biochemical characterization and mechanism of action of a thermostable beta-glucosidase purified from *Thermoascus aurantiacus*. Biochem J. 2001;353(Pt 1):117-127. doi:10.1042/bj3530117.
2. Pérez J, Muñoz-Dorado J, de la Rubia T, Martínez J. Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview. Int Microbiol. 2002;5(2):53-63. doi:10.1007/s10123-002-0062-3.
3. Bhat MK, Bhat S. Cellulose degrading enzymes and their potential industrial applications. Biotechnol Adv. 1997;15(3-4):583-620. doi:10.1016/s0734-9750(97)00006-2.
4. Kaur J, Chadha BS, Kumar BA, Kaur G, Saini HS. Purification and characterization of beta-glucosidase from *Melanocarpus* sp. MTCC 3922. Electron J Biotechnol. 2007;10(2):260-270. doi:10.2225/vol10-issue2-fulltext-4.
5. Czjzek M, Cicek M, Zamboni V, Bevan DR, Henrissat B, Esen A. The mechanism of substrate (aglycone) specificity in beta -glucosidases is revealed by crystal structures of mutant maize beta -glucosidase-DIMBOA, -DIMBOAGlc, and -dhurrin complexes. Proc Natl Acad Sci U S A. 2000;97(25):13555-13560. doi:10.1073/pnas.97.25.13555.
6. Bhatia Y, Mishra S, Bisaria VS. Microbial beta-glucosidases: cloning, properties, and applications. Crit Rev Biotechnol. 2002; 22(4):375-407. doi:10.1080/07388550290789568.
7. Bhat MK. Cellulases and related enzymes in biotechnology. Biotechnol Adv. 2000;18(5):355-383. doi:10.1016/s0734-9750(00)00041-0.
8. Kumar R, Wyman CE. Effect of enzyme supplementation at moderate cellulase loadings on initial glucose and xylose release from corn stover solids pretreated by leading technologies. Biotechnol Bioeng. 2009;102(2):457-467. doi:10.1002/bit.22068.
9. Rani V, Mohanram S, Tiwari R, Nain L, Arora A. Beta-glucosidase: key enzyme in determining efficiency of cellulase and biomass hydrolysis. J Bioprocess Biotech. 2014;5(1):197. doi:10.4172/2155-9821.1000197.
10. Zimbardi AL, Sehn C, Meleiro LP, et al. Optimization of β-glucosidase, β-xylosidase and xylanase production by *Colletotrichum graminicola* under solid-state fermentation and application in raw sugarcane trash saccharification. Int J Mol Sci. 2013;14(2):2875-2902. doi:10.3390/ijms14022875.
11. Del Pozo MV, Fernández-Arrojo L, Gil-Martínez J, et al.

Microbial β-glucosidases from cow rumen metagenome enhance the saccharification of lignocellulose in combination with commercial cellulase cocktail. Biotechnol Biofuels. 2012;5(1):73. doi:10.1186/1754-6834-5-73.
12. Rehana F, Venkatasubbaiah P, Nand K. Preliminary studies on the production of thermostable alpha-amylase by a mesophilic strain of *Bacillus licheniformis*. Chemie, Mikrobiologie, Technologie der Lebensmittel; 1989.
13. Veena V, Poornima P, Parvatham R, Sivapriyadharsini, Kalaiselvi K. Isolation and characterization of β-glucosidase producing bacteria from different sources. Afr J Biotechnol. 2011;10(66):14907-14912. doi:10.5897/ajb09.314.
14. Majidi S, Roayaei M, Ghezelbash G. Carboxymethyl-cellulase and filter-paperase activity of new strains isolated from Persian Gulf. Microbiol J. 2011;1(1):8-16. doi:10.3923/mj.2011.8.16.
15. Chawla PR, Bajaj IB, Survase SA, Singhal RS. Microbial cellulose: fermentative production and applications. Food Technol Biotechnol. 2009;47(2):107-124.
16. Bagudo AI, Argungu AU, Aliero AA, Suleiman N, Kalpana S. *Bacillus subtilis* as an alternative source of beta-glucosidase. Int J Mod Cell Mol Biol. 2014;3(1):1-9.
17. Li Y, Bu M, Chen P, et al. Characterization of a thermophilic monosaccharide stimulated β-glucosidase from *Acidothermus cellulolyticus*. Chem Res Chin Univ. 2018;34(2):212-220. doi:10.1007/s40242-018-7408-7.
18. Sørbotten A, Horn SJ, Eijsink VG, Vårum KM. Degradation of chitosans with chitinase B from *Serratia marcescens*. Production of chito-oligosaccharides and insight into enzyme processivity. FEBS J. 2005;272(2):538-549. doi:10.1111/j.1742-4658.2004.04495.x.
19. Bakare MK, Adewale IO, Ajayi A, Shonukan OO. Purification and characterization of cellulase from the wild-type and two improved mutants of *Pseudomonas fluorescens*. Afr J Biotechnol. 2005;4(9):898-904.
20. Immanuel G, Dhanusha R, Prema P, Palavesam A. Effect of different growth parameters on endoglucanase enzyme activity by bacteria isolated from coir retting effluents of estuarine environment. Int J Environ Sci Technol. 2006;3(1):25-34. doi:10.1007/bf03325904.
21. Dutta T, Sahoo R, Sengupta R, Ray SS, Bhattacharjee A, Ghosh S. Novel cellulases from an extremophilic filamentous fungi *Penicillium citrinum*: production and characterization. J Ind Microbiol Biotechnol. 2008;35(4):275-282. doi:10.1007/s10295-008-0304-2.
22. Olajuyigbe FM, Ajele JO. Production dynamics of extracellular protease from *Bacillus species*. Afr J Biotechnol. 2005;4(8):776-779.
23. Aygan A, Karcioglu L, Arikan B. Alkaline thermostable and halophilic endoglucanase from *Bacillus licheniformis* C108. Afr J Biotechnol. 2011;10(5):789-796.
24. Riou C, Salmon JM, Vallier MJ, Günata Z, Barre P. Purification, characterization, and substrate specificity of a novel highly glucose-tolerant beta-glucosidase from *Aspergillus oryzae*. Appl Environ Microbiol. 1998;64(10):3607-3614. doi:10.1128/aem.64.10.3607-3614.1998.