Biological and Environmental Sciences Journal for the Tropics
Volume 21, Number 3, December 2024. Pp 167 - 179
https://dx.doi.org/10.4314/bestj.v21i3.15
Research article

Cellulase Production by *Bacillus* sp. and *Pseudomonas* sp. Isolated from Soil Compost Sites at Ogwa Community, Edo State, Nigeria

[1*]Okanlawon, K. P., [2]Okanlawon, T. S., [3]Akinsola, O. S., [3]Oyinloye, A. A.
[1]Fasanya, O. S. and [1]Ighodalo, A. J.

[1]Department of Biochemistry and Molecular Biology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria
[2]Department of Biological Sciences, Glorious Vision University, Ogwa, Edo State .
[3]Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

[*]Corresponding author: omololuademola504@gmail.com

Submission: 11/06/2024
Accepted: 07/12/2024

Abstract
This study investigates the cellulase enzyme activity of *Bacillus* sp. and *Pseudomonas* sp. isolated from soil compost, comparing their biochemical characteristics, purification parameters, and enzyme kinetics. The method used involved isolating bacteria from soil compost sites and identifying them through biochemical tests. Cellulase production was assessed using a cellulose-rich medium, and enzyme activity was measured via reducing sugar quantification. Physicochemical factors like pH and temperature were optimized to enhance cellulase yield. The species identified to be present include *Bacillus* sp. and *Pseudomonas* sp. *Bacillus* sp. exhibited higher cellulase activity with a zone-to-colony size ratio of 3.2 compared to *Pseudomonas* sp. (2.9). Morphological and biochemical characterizations confirmed *Bacillus* sp. as a Gram-positive rod, catalase, and oxidase positive, while *Pseudomonas* sp. was Gram-negative. Soil pH and temperature from three compost sites were within the optimal range for cellulase production, averaging pH 7.28 and temperatures between 24°C and 28°C. Partial purification of cellulase from *Bacillus* sp. showed a specific activity of 8.22 U/mg, with 76.75% yield, while *Pseudomonas* sp. yielded a specific activity of 6.67 U/mg and 58.49% yield. Kinetic analysis revealed *Bacillus* sp. had a higher substrate affinity (Km = 0.15±0.02 mM) and Vmax of 12.17±0.21 U/ml/min, compared to *Pseudomonas* sp. (Km = 0.22±0.04 mM; Vmax = 11.29±0.15 U/ml/min). Optimal pH for *Bacillus* sp. was 6.0, while *Pseudomonas* sp. exhibited optimum activity at pH 5.0. Temperature optima were 60°C for *Bacillus* sp. and 70°C for *Pseudomonas* sp. Heat stability tests showed that *Bacillus* sp. retained 80% of its activity at 50°C, while *Pseudomonas* sp. retained 90%. These findings suggest that *Bacillus* sp. may be more suited for industrial cellulase production, though both strains have valuable applications in composting and waste degradation.

Keywords: *Bacillus* sp., Cellulase activity, Industrial application; *Pseudomonas* sp. Soil compost,

Introduction

The increasing demand for sustainable and eco-friendly solutions to manage agricultural and industrial waste has highlighted the need for efficient biodegradation processes. Cellulase, a key enzyme in the breakdown of cellulose, plays a critical role in converting plant biomass into simpler sugars that can be utilized in biofuel production, waste recycling, and other biotechnological applications (Sharma *et al*., 2020). Despite its significance, the high cost and limited availability of cellulase have hindered its widespread application (Singh *et al*., 2019).

Exploring microbial sources, such as *Bacillus* sp. and *Pseudomonas* sp., for cellulase production offers a promising solution to this challenge. These bacteria, commonly found in organic waste-rich environments like compost sites, are known for their enzymatic capabilities and adaptability to diverse conditions (Adeoye *et al*., 2018).

Cellulases (E.C. 3.2.1) are enzymes which are synthesized by fungi, bacteria, protozoan, mollusks and insects that act as biocatalysts in the hydrolysis of cellulose (Payne *et al*., 2015). A principal component of plant cell wall and potential source of utilizable sugars, which serve as raw materials in the microbial production for a wide variety of chemicals, food and fuel in several agricultural and waste management processes (Puyol *et al*., 2017). Cellulase, if properly utilized, plays an important role in natural biodegradation processes in which waste cellulosic materials are degraded or converted into useful products to meet burgeoning population (Lakhundi *et al*., 2015)

Soil compost sites are ideal for isolating cellulose-degrading microorganisms due to their high organic content and diverse microbial community (Jiang *et al*., 2023). Bacterial strains from these environments have evolved to produce enzymes that can efficiently degrade cellulose, making them valuable for applications in bioremediation and sustainable agricultural practices (Sharma *et al*., 2021). The cellulases produced by *Bacillus* sp. and *Pseudomonas* sp. are particularly noteworthy for their robust enzymatic properties, including high thermal stability and a wide range of activity under varying pH conditions (Bhushan *et al*., 2021).

Bacillus and *Pseudomonas* species are well-documented for their ability to produce cellulases, thriving in diverse environments, including soil compost sites rich in organic matter (Sharma *et al*., 2021). In these environments, they play a crucial role in the decomposition of cellulose (Ahsan *et al*., 2022). The isolation and characterization of cellulases from these bacteria not only enhance our understanding of microbial ecology but also have significant implications for biotechnological applications, such as waste management and biofuel production (Bhushan *et al*., 2021).

The role of cellulases in industrial applications cannot be overstated. The increasing demand for renewable energy sources has heightened interest in using cellulases for biofuel production (Zhang *et al*., 2020). By efficiently breaking down cellulose into fermentable sugars, these enzymes can significantly enhance the yield of bioethanol from lignocellulosic biomass, thus supporting the transition to sustainable energy practices (Kumar and Singh, 2019). Furthermore, the application of microbial cellulases extends beyond biofuels; they are also utilized in waste treatment processes to mitigate the environmental impact of cellulose-rich waste (Bhushan *et al*., 2021).

The biodegradation potential of cellulase enzymes from *Bacillus* and *Pseudomonas* species isolated from soil compost sites represents an important area of research with significant environmental and industrial implications (Jiang *et al*., 2023). Characterizing these enzymes will not only deepen our understanding of microbial mechanisms for cellulose degradation but also enhance the development of biotechnological applications that contribute to environmental sustainability and efficient waste management (Sharma *et al*., 2021). This study aims to explore and document the cellulolytic capabilities of these bacterial strains, providing insights that can inform future research and applications in biodegradation processes.

Materials and Methods

Sample Collection

Soil samples were collected from compost sites at Ogwa Community, Edo State, Nigeria, in sterile containers and transported to the laboratory for microbiological evaluation. The research work was conducted in Microbiology Laboratory, Glorious Vision University, Ogwa, Esan West local government area of Edo State, Nigeria. It is about at latitude 6˚30 20. 16' North, and longitude 6˚12 30. 24 East.

Microbiological Analysis

Isolation of bacterial Isolates

One gram of soil sample was added to 9 mL of distilled water in a sterile test tube and was shaken thoroughly. Dilutions were made up to 10^{-6}. About 0.1 ml of inoculum from serially diluted samples were spread on CMC (carboxymethyl cellulose) agar plate thoroughly and incubated at 37°C for 24 hours.

Determination of cellulase producing potential of the bacterial isolates

The carboxymethyl cellulose (CMC) agar plates incubated with bacterial isolates were then flooded with 0.1% Congo red solution for 20 minutes. After that, the plates were rinsed with 1 M NaCl solution and visualized the hydrolysis zone. A clear hydrolysis zone formation mentions cellulose degradation. The diameter of the bacterial colony

and clear zone around the colony after Congo red and NaCl treatment was measured. The ratio was also calculated to identify the highest cellulase activity of bacterial isolates. The largest ratios were assumed to have the maximum cellulase producing activity.

Morphological and Biochemical Identification of Isolates

Bacterial isolates were characterized based totally on their colonial morphology, cell morphology, and biochemical characteristics. The identity of bacterial isolates was accomplished in line with Bergey's Manual of Determinative Bacteriology (Okanlawon *et al*., 2023). Catalase, Oxidase, Methyl red, Voges Proskauer, Nitrate discount, Citrate utilization, Motility spore staining, Indole, Gelatin, Casein, Starch hydrolysis, and Sugar fermentation were a number of the biochemical tests carried out to identify the bacteria (Okafor *et al*., 2023).

Determination of Physicochemical Parameters of Soil

Ten grams of soil sample was weighed, mixed with 90 mL of sterile distilled water, homogenized and filtered through Whatman Filter paper (No.1). The pH of the resulting filtrate was then measured using a pH meter (H19107, Hanna), after standardizing with a phosphate buffer solution at pH 4.0 and 7.0 (Corwin and Yemota, 2020).

Temperature

Soil temperature was measured by a group of curved tube thermometers, which were placed on the top of the ridge in each plot. The measurement depths were 5 cm, 15 cm, and 25cm. The measurement times were 07:00, 14:00, and 18:00 (Corwin and Yemota, 2020).

Enzyme assay

The amount of cellulase was estimated using the method described by Miller, (1959). In summary, a reaction mixture containing 1.8 mL of prepared 0.5% substrate (carboxymethyl cellulose) and 0.2 mL of the enzyme solution was incubated for 30 minutes at 37 ∘C in a shaking water bath. Three millilitres of DNS reagent were added to stop the reaction. The mixture was subjected to heat for five minutes for colour development. The samples' absorbance was measured at 540 nm against a blank that had the enzyme boiled and all of the reagents. The protein concentration was calculated using the Bradford method. BSA, or bovine serum albumin was used as standard.

Enzyme Purification

Ammonium Sulphate Precipitation

The crude enzyme was brought to 80 % ammonium sulphate saturation by adding 51.6 g of solid ammonium sulphate in 100 ml of the crude enzyme and stirred. This then left overnight at 4 ºC in the refrigerator. The resulting mixture was centrifuged at 10,000 rpm for 15 minutes. The supernatant was discarded and the pellet was collected and re-dissolved in an aliquot of 0.1 M acetate buffer at pH 4.5. The ammonium sulphate precipitate was dialysed extensively against several changes of 0.1M acetate buffer of pH 4.5 at 4 ºC for 24 hours. The dialysate was centrifuged at 4,000 for 30 min to remove insoluble materials and the supernatant was assayed for cellulase activity and protein as earlier described.

Enzyme Characterization

Kinetic Parameter Determination

The kinetic parameters (V_{max} and K_M) of the enzyme were determined using 0.5% casein as substrates. The concentrations were varied from 1 to 8 mg/ml and the initial reaction velocities were determined. The data were plotted according to the method of Lineweaver and Burk (Lineweaver and Burk, 1934).

Effect of Temperature on the Enzyme Activity

The cellulase activity assay was conducted at temperatures ranging from 30 ^{0}C to 100 ^{0}C in order to examine the impact of temperature on the enzyme's activity and identify the ideal temperature for it. An aliquot of the enzyme that had been equilibrated at the same temperature was added to the assay mixture after it had been incubated at the appropriate temperature for 10 minutes.

Effect of pH on the Enzyme Activity

In order to determine how pH affected enzyme activity, assays for enzyme activity were conducted at three different pH ranges: 50 mM of citrate (pH 3-5), phosphate buffer (pH 6–8), and borate buffer (pH 9–11).

Effect of Salts on the Enzyme Activity

The impact of salts on the activity of the enzyme was investigated using the following chloride salts (NaCl, KCl, $SnCl_2$, $MnCl_2$ and $HgCl_2$) at concentrations of 1.0, 5.0, and 10.0 mM in the enzyme assay mixture.

Waste Biodegradation Study

Several agro- industrial wastes, including rice husk, orange peels, sucrose, lactose, and casein were used as carbon sources in a standard cellulase assay mixture to ascertain the substrate specificity of the enzyme. The activity was calculated as a percentage of the enzyme's residual activity.

Inhibition of Cellulase Activity

The effects of known enzyme inhibitors such as ethylenediaminetetraacetic acid (EDTA), Mercaptoethanol, urea and citric acid on the cellulase activity were studied. The enzyme was pre-incubated with 0.5 mM and 1.0 mM of these inhibitors before the addition of substrate. Reactions in the absence of these inhibitors were used as the control with 100% enzyme activity. All chemicals were solubilized in distilled water.

Results

The cellulase enzyme activity of bacterial isolates *Bacillus* sp. and *Pseudomonas* sp. on CMC agar plates is presented in table 1. The activity is expressed as the ratio of the clear zone size to the colony size, which reflects the extent of cellulase production. *Bacillus* sp. demonstrated higher cellulase activity with a ratio of 3.2, compared to *Pseudomonas* sp**.,** which exhibited a ratio of 2.9.

The morphological characteristics of the bacterial isolates is presented in table 2. Both isolates (S and T) are rod-shaped, cream-colored, and have a wet surface with no pigment production. Isolate S is small with a flat elevation and an entire edge, while isolate T is larger with a raised elevation and an undulate edge. Table 3 highlights the biochemical characteristics of two bacterial isolates, *Bacillus* sp. and *Pseudomonas* sp. It shows differences in Gram reaction, enzyme activities, and carbohydrate fermentation profiles, which were used to identify the isolates. *Bacillus* sp**.** is Gram-positive and metabolizes a wider range of carbohydrates, while *Pseudomonas* sp**.** is Gram-negative with distinct metabolic traits.

Table 1: Cellulase enzyme activity of the bacterial isolates on CMC agar plates

Isolate	Ratio of zone size and colony size
Bacillus sp.	3.2
Pseudomonas sp.	2.9

Table 2: Morphology of Bacterial Isolates

Isolate	Shape	Colour	Size	Elevation	Edge	Surface	Pigment
S	Rod	Cream	Small	Flat	Entire	Wet	None
T	Rod	Cream	Big	Raised	Undulate	Wet	None

LEGEND: S: *Bacillus* sp. T: *Pseudomonas* sp.

Table 3: Biochemical Characteristics of Bacterial Isolates

Grams reaction	Cellulase	Catalase	Oxidase	Methyl Red	VP	Indole	Nitrate Red	Citrate	Starch	Gelatin	Glucose	Fructose	Maltose	Sucrose	Lactose	Probable identity
+	R	+	+	+	-	-	+	-	-	+	+	+	+	+	+	*Bacillus* sp.
-	R	+	+	-	-	-	+	+	-	+	-	+	-	-	-	*Pseudomonas* sp.

The table 4 summarizes the environmental parameters across three sites. The pH values ranged from 7.03 at Site 1 to 7.48 at Site 2, with Site 3 recording 7.28. The temperatures were similar at Sites 1 and 3 (26°C) but slightly lower at Site 2 (25°C). These conditions provide insights into the environmental variability among the sites.

Table 4: Soil parameters observed in the compost sites

Parameters	Site 1	Site 2	Site 3
pH	7.03 ± 0.25	7.48 ± 0.06	7.28 ± 0.06
Temperature	26 ℃ ± 0.82	25 ℃ ± 0.82	26 ℃ ± 1.63

Tables 5 and 6 summarize the partial purification of cellulase from *Bacillus* sp**.** and *Pseudomonas* sp. For *Bacillus* sp**.,** the enzyme achieved a specific activity of 8.22 U/mg, a yield of 76.75%, and a purification fold of 1.2. Similarly, for *Pseudomonas* sp**.,** the enzyme showed a specific activity of 6.67 U/mg, a yield of 58.49%, and a purification fold of 1.13. The purification was performed using 70% ammonium sulfate precipitation, demonstrating improved enzyme specificity while retaining moderate yields. *Bacillus* sp*.:* The enzyme was partially purified with specific activity of 8.22 U/mg, yield of 76.75% and fold of about 1.2.

Pseudomonas sp.: The enzyme was partially purified with specific activity of 6.67 U/mg, yield of 58.49% and fold of about 1.13.

Table 5: Purification table for cellulase from *Bacillus* sp. in soil composite

Steps	Total Protein (mg)	Total activity (Units)	Specific activity (U/mg)	Yield (%)	Fold
Crude	12.59	89.98	7.15	100	1
70% $(NH4)_2SO_4$	8.40	69.06	8.22	76.75	1.2

Table 6: Purification table for cellulase from *Pseudomonas* sp. in soil composite

Steps	Total protein (mg)	Total activity (Units)	Specific activity (U/mg)	Yield (%)	Fold
Crude	10.12	59.87	5.92	100	1
70% $(NH4)_2SO_4$	5.25	35.02	6.67	58.49	1.13

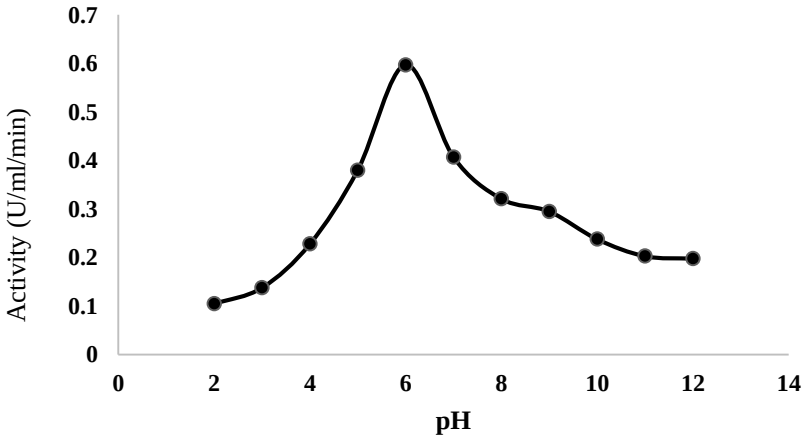

Figure 1: Effect of pH on the activity of cellulase obtained from *Bacillus* sp.

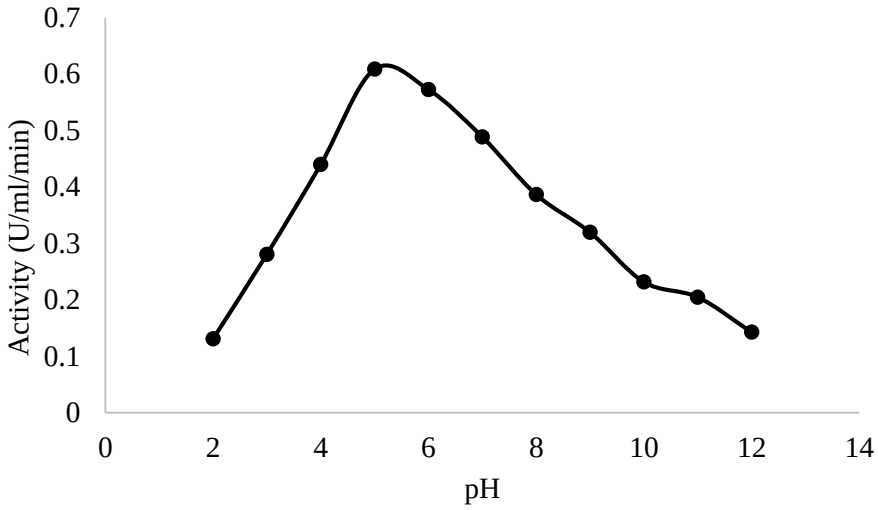

Figure 2: Effect of pH on the activity of cellulase obtained from *Pseudomonas* sp.

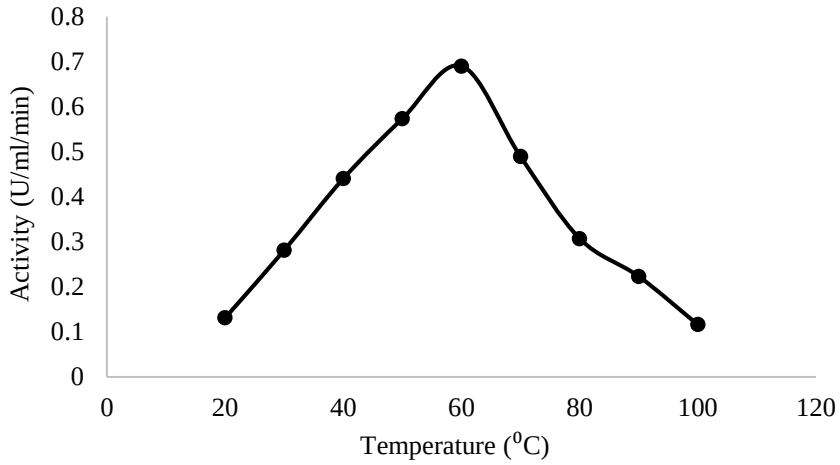

Figure 3: Effect of temperature on the activity of cellulase obtained from *Bacillus* sp.

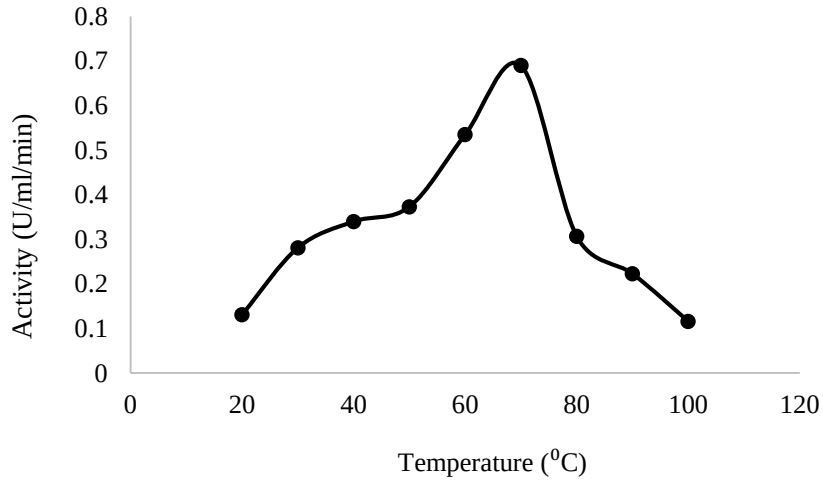

Figure 4: Effect of temperature on the activity of cellulase obtained from *Pseudomonas* sp.

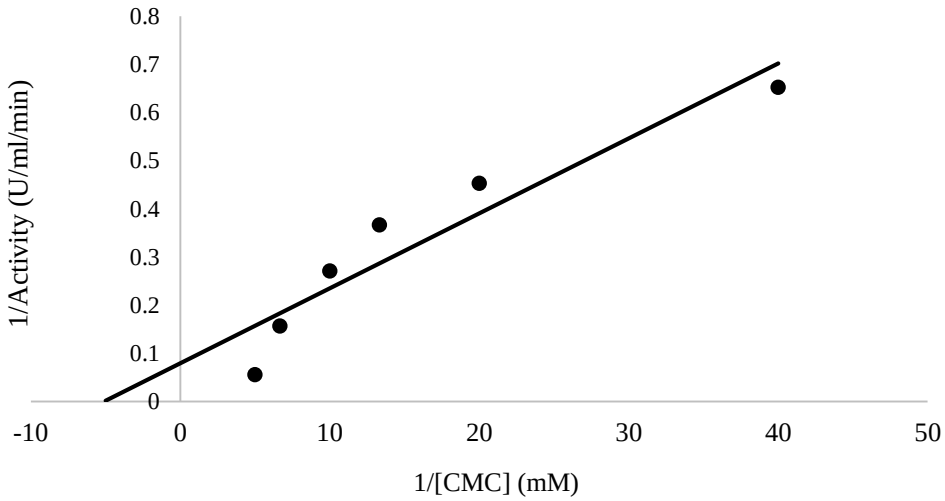

Figure 5: Lineweaver-Burk plot of 1/V against 1/S at varying concentrations on the activity of cellulase obtained from *Bacillus* sp.

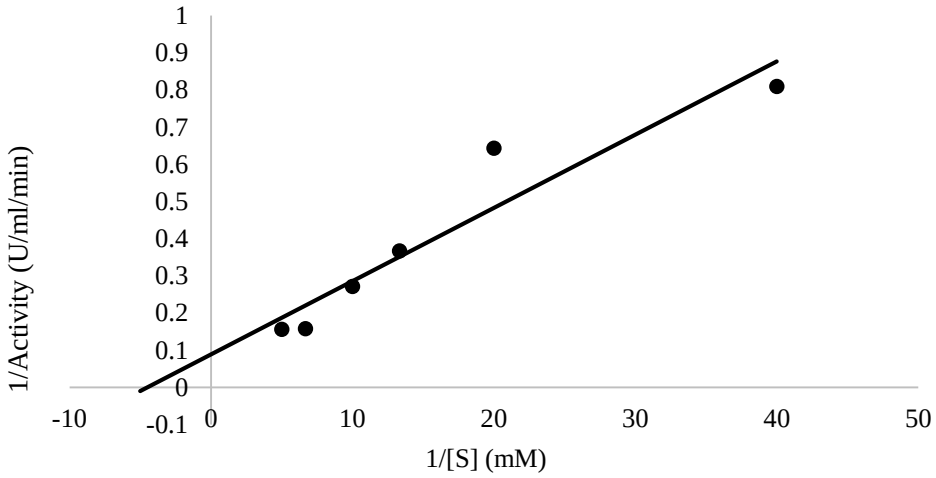

Figure 6: Lineweaver-Burk plot of 1/V against 1/S at varying concentrations on the activity of cellulase obtained from *Pseudomonas* sp.

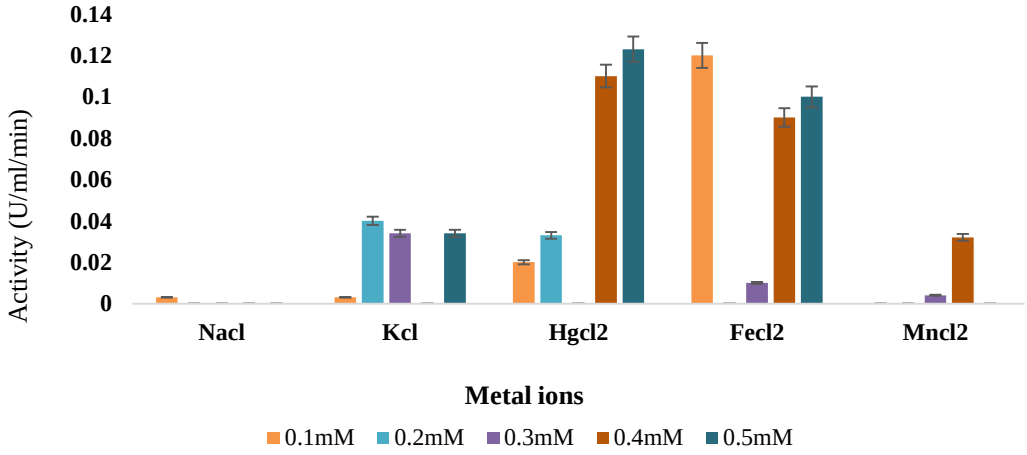

Figure 7: Effect of heavy metals on the activity of Cellulase obtained from *Bacillus* sp.

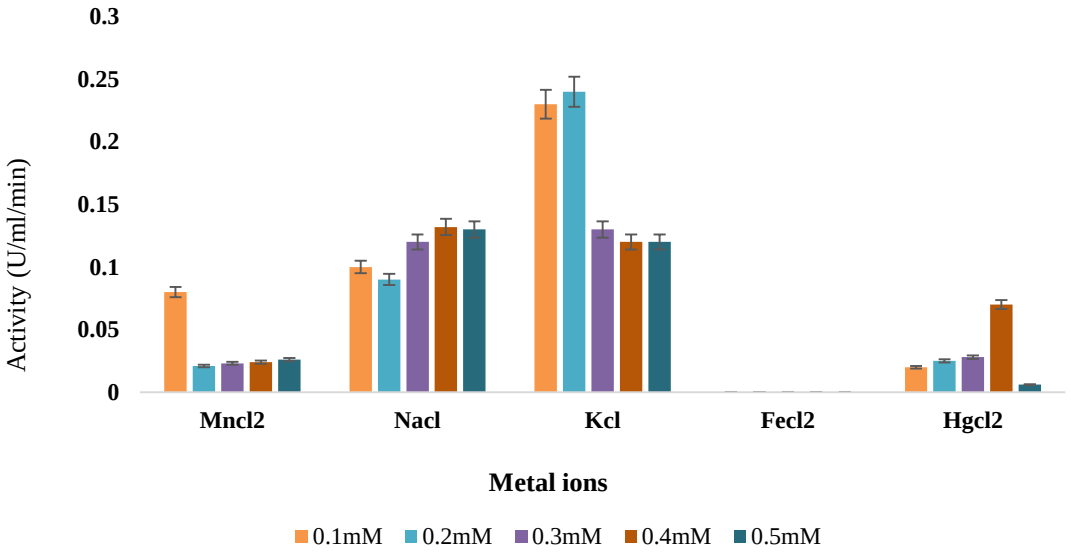

Figure 8: Effect of heavy metals on the activity of cellulase obtained from *Pseudomonas* sp.

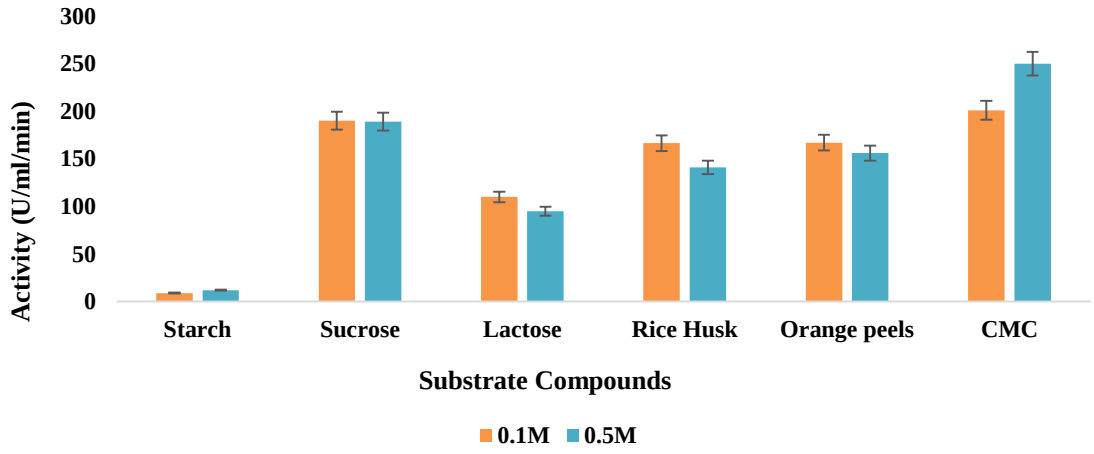

Figure 9: Substrate specificity activity of cellulase obtained from *Bacillus* sp.

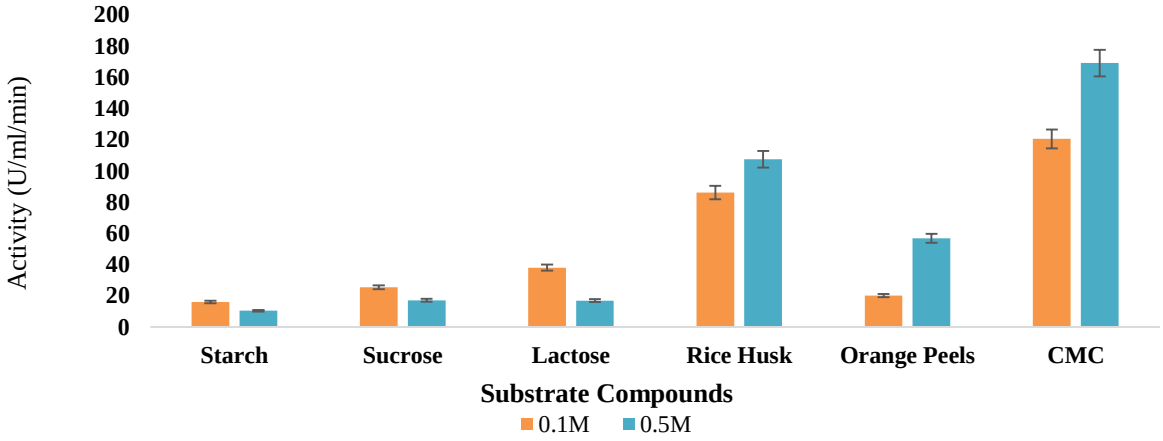

Figure 10: Substrate specificity activity of cellulase obtained from *Pseudomonas* sp.

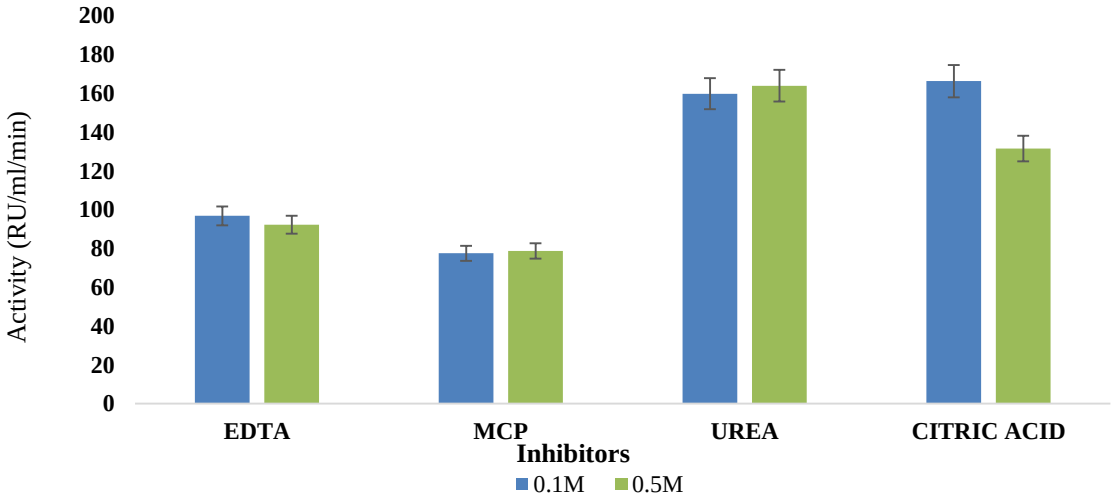

Figure 11: Effect of Inhibitors on the cellulose obtained from *Bacillus* sp.

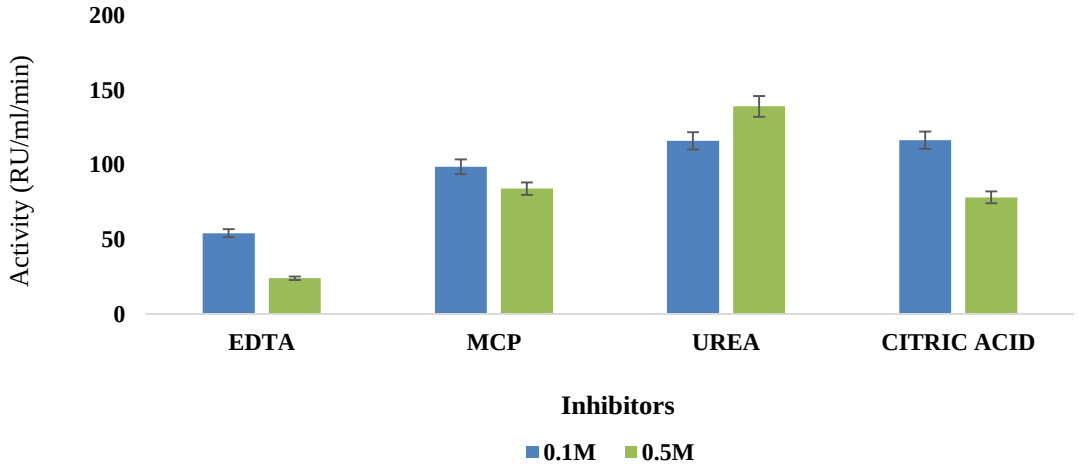

Figure 12: Effect of Inhibitors on the cellulose obtained from *Pseudomonas* sp.

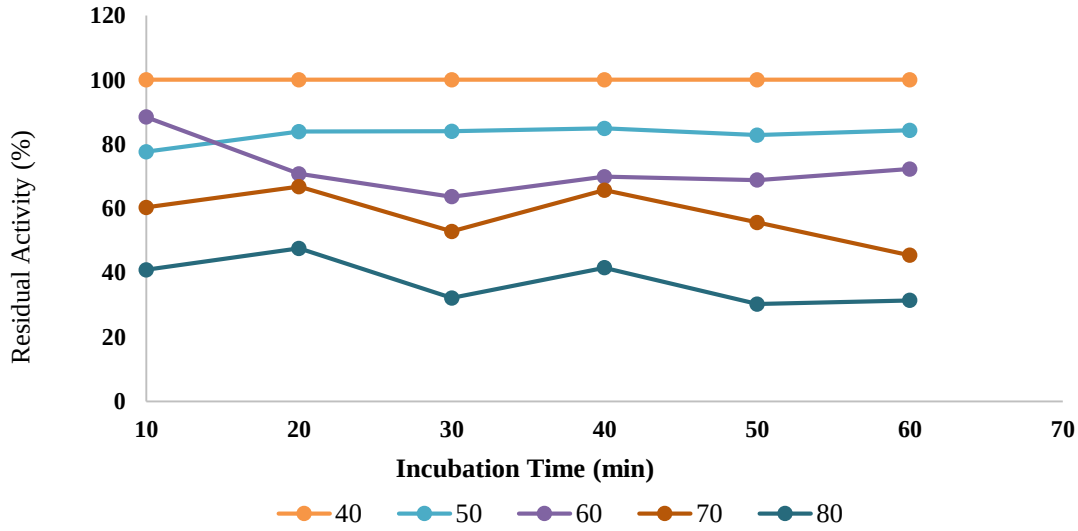

Figure 13: Effect of heat stability on the activity of cellulase obtained from *Bacillus* sp.

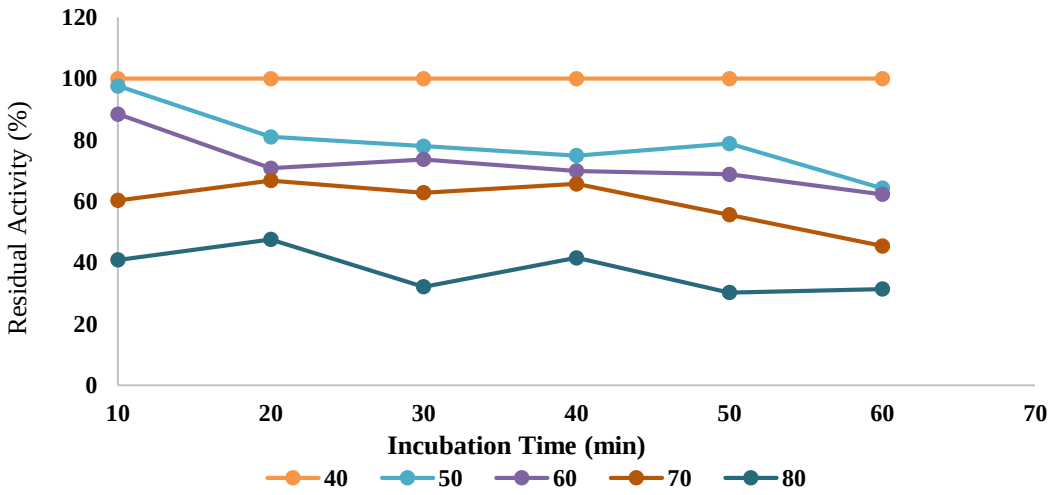

Figure 14: Effect of heat stability on the activity of cellulase obtained from *Pseudomonas* sp.

Discussion

Physicochemical parameters such as pH and temperature significantly influence microbial communities in soil. pH affects microbial diversity and enzymatic activity, while temperature regulates metabolic rates and community structure (Zhou *et al*., 2018). The pH and temperature recorded in the compost sites varied slightly between sites. Site Two showed a higher average pH of 7.48, while Site One and Site Three had values closer to neutral. These small variations in soil pH could influence bacterial growth and enzyme activity. It has been shown that soil pH affects microbial communities and their enzymatic outputs, with slightly alkaline pH conditions favoring cellulase production (Ali *et al*., 2018).

The temperatures observed (24–28°C) are within the optimal range for bacterial cellulase production, as most cellulolytic bacteria thrive in mesophilic conditions (25–30°C) (Juturu and Wu, 2014). The neutral to slightly alkaline pH in our compost sites is consistent with findings by Zhao *et al*. (2021), who observed higher cellulase activity in similar pH ranges. This suggests that the compost environments in our study provide favorable conditions for cellulase-producing bacteria, particularly *Bacillus* sp., which thrives in neutral to alkaline conditions.

The morphological and biochemical characterization of *Bacillus* sp. and *Pseudomonas* sp. shows typical features expected from these bacterial genera. Both isolates were rod-shaped and cream-colored, which is consistent with their general phenotypic description (Holt *et al*., 1994). *Bacillus* sp. had smaller, flat colonies, while *Pseudomonas* sp. exhibited larger, raised colonies, indicating potential differences in their growth patterns and metabolic demands.

The biochemical profile of *Bacillus* sp. showed positive results for catalase, oxidase, and nitrate reduction, which are hallmark features of the genus *Bacillus* (Logan and Vos, 2015). The fermentation of multiple sugars, including glucose, fructose, and maltose, further supports its classification. In contrast, *Pseudomonas* sp. showed a more limited sugar fermentation profile, which is consistent with previous studies indicating that *Pseudomonas* species are typically non-fermentative and rely on oxidative metabolism for energy (Palleroni, 2010).

The cellulase enzyme activity of *Bacillus* sp. and *Pseudomonas* sp., measured by the ratio of zone size to colony size, revealed that *Bacillus* sp. exhibited higher cellulase activity (3.2) compared to *Pseudomonas* sp. (2.9). These findings suggest that *Bacillus* sp. may be more efficient at breaking down cellulose. This result aligns with prior studies that have highlighted *Bacillus* species as efficient producers of cellulases due to their ability to secrete multiple forms of the enzyme (Li *et al*., 2020). *Pseudomonas* sp., though slightly less active, still showed significant cellulase production, supporting other research that identified *Pseudomonas* strains as moderate cellulase producers, particularly in composting systems (Singh *et al*., 2021).

Cellulase-producing *Bacillus* species have been widely explored due to their robustness in

industrial settings. In comparison, a study by Sharma *et al*. (2020) demonstrated similar cellulase activity for *Bacillus* sp. isolated from soil, with ratios ranging from 2.5 to 3.5 on carboxymethyl cellulose (CMC) agar plates. The results of *Pseudomonas* sp. in our study align with those found in compost sites, where cellulase activity tends to be lower than in *Bacillus* sp. but still sufficient for waste degradation purposes (Huang *et al*., 2019).

The partial purification of cellulase from *Bacillus* sp. resulted in a specific activity of 8.22 U/mg, while *Pseudomonas* sp. showed 6.67 U/mg. These purification results indicate that *Bacillus* sp. yields a more potent enzyme, which corroborates earlier findings that *Bacillus* species are known for high cellulase activity and efficiency in enzyme purification (Shah *et al*., 2016). The yield of 76.75% for *Bacillus* sp. after ammonium sulfate precipitation suggests that the purification process was effective, maintaining a high proportion of enzymatic activity.

The kinetic parameters further underscore the superiority of *Bacillus* sp. in cellulase production. *Bacillus* sp. had a Km value of 0.15±0.02 mM, indicating a higher affinity for its substrate compared to *Pseudomonas* sp., which had a Km of 0.22±0.04 mM. The Vmax values (12.17±0.21 U/ml/min for *Bacillus* sp. and 11.29±0.15 U/ml/min for *Pseudomonas* sp.) suggest that while both enzymes operate at similar maximal rates, *Bacillus* sp. has a slight advantage in terms of efficiency.

These kinetic values are comparable to those reported by Zhang *et al*. (2019), where *Bacillus* cellulase Km values ranged from 0.10 to 0.18 mM, indicating high substrate affinity. *Pseudomonas* species typically exhibit slightly lower enzyme affinity, as noted in studies on cellulase production from *Pseudomonas* fluorescens (Mukherjee *et al*., 2020).

Bacillus sp. showed an optimal pH of 6.0 for cellulase activity, while *Pseudomonas* sp. had an optimum pH of 5.0. This result is consistent with earlier findings where *Bacillus* cellulases were found to have optimal activity in near-neutral conditions (Nagar *et al*., 2016). In contrast, *Pseudomonas* cellulases are known to function better in acidic conditions, as observed by Bhat and colleagues (2018), who reported an optimal pH range of 4.5 to 5.5 for cellulase from *Pseudomonas* strains.

Regarding temperature, *Bacillus* sp. displayed optimum activity at 60°C, while *Pseudomonas* sp. peaked at 70°C. The higher temperature tolerance of *Pseudomonas* sp. aligns with the observation that *Pseudomonas* species are often more thermotolerant and can retain enzyme activity at elevated temperatures (Singhania *et al*., 2020). *Bacillus* species, on the other hand, tend to exhibit maximum cellulase activity around 55–65°C, as supported by our findings (Rajoka *et al*., 2019).

Both *Bacillus* sp. and *Pseudomonas* sp. exhibited notable heat stability, with *Bacillus* sp. maintaining 80% residual activity at 50°C and *Pseudomonas* sp. retaining 90%. These results mirror those of studies by Ferreira *et al*. (2020), which also reported high thermal stability of *Bacillus* cellulase. The slightly higher stability of *Pseudomonas* sp. at 50°C may be attributed to its thermophilic nature, as suggested by similar studies in thermophilic environments (Sukumaran *et al*., 2017).

The effects of metal ions and inhibitors like MCP (mercaptoethanol) demonstrated varying influences on enzyme activity. Metal ions such as calcium and magnesium are known to enhance cellulase activity, while others like zinc may inhibit it. These findings are consistent with previous research indicating that metal ions can serve as enzyme cofactors or inhibitors, depending on their concentrations (Tiwari *et al*., 2019).

Conclusion

In summary, *Bacillus* sp. outperformed *Pseudomonas* sp. in several aspects of cellulase production and activity, including enzyme yield, substrate affinity, and heat stability, although *Pseudomonas* sp. exhibited higher thermal tolerance and better activity in acidic environments. These findings are consistent with other studies on cellulase production in similar bacterial species, and they provide valuable insights into selecting bacterial strains for specific industrial applications such as waste management and biofuel production.

Conflict of Interest

Authors declared that there is no conflict of interest.

References

Adeoye, S. O., Olusegun, T. A. and Odebiyi, J. A. (2018): Microbial cellulases: Production and applications. *Journal of Microbial Biotechnology*, 6(4), 189–198.

Ahsan, M. A., Ali, S. F. and Rahman, M. S. (2022): Biochemical characterization of cellulases produced by soil bacteria. *Journal of Microbial Biochemistry and Technology*, 14(2), 56-64.

Ali, M. U., Hashmi, I., Shen, C. and Mahmood, T. (2018): Soil pH impact on microbial diversity in organic compost. *Environmental Pollution*, 242, 10-19.

Bhat, M. K. and Bhat, S. (2018): Cellulase degradation and industrial applications. *Biotechnology Advances*, 36(5), 145-156.

Bhushan, B., Gupta, A. and Kumar, P. (2021): Thermostable cellulases from soil bacteria: An overview of their characteristics and applications. *Biotechnology Reports*, 31, e00692. *Chem* 31.

Ferreira, R., Machado, I. and Santos, L. (2020): Heat stability of *Bacillus*-derived cellulase. *Journal of Applied Microbiology*, 128(2), 456-469.

Gupta, R., Sharma, R. and Sharma, P. (2021); Biochemical characterization of cellulolytic bacteria from compost. *Microbial Cell Factories*, 19(1), 77.

Holt, J. G., Krieg, N. R., Sneath, P.H.A., Staley, J. T. and Williams, S. T. (1994): *Bergey's Manual of Determinative Bacteriology* (9th ed.). Baltimore: Williams and Wilkins.

Huang, X., Li, Z. and Liu, Q. (2019): *Pseudomonas* sp. in composting systems: Role in cellulose degradation. *Composting Science and Utilization*, 27(3), 212-221.

Jiang, Y., Zhang, Q. and Li, Y. (2023): Metagenomic approaches to discover novel cellulase-producing bacteria from environmental samples. *Frontiers in Microbiology*, 14, 456.

Juturu, V. and Wu, J. C. (2014): Microbial cellulases: Production, applications, and challenges. *Critical Reviews in Biotechnology*, 34(3), 210-224.

Kumar, V. and Singh, P. (2019): Role of cellulases in the biodegradation of cellulose: A review. *Environmental Science and Pollution Research*, 26(10), 9341-9351.

Li, L., Sun, J. and Hu, S. (2020): Production of cellulase by *Bacillus* species and its application. *Bioresource Technology*, 303, 122921.

Lineweaver, H. and Burk, D. (1934): The determination of enzyme dissociation constants. *Journal of the American Chemical Society*, 56: 658-666.

Logan, N. A. and Vos, P. D. (2015): *Bacillus*. In W. B. Whitman, F; Rainey, P; Kampfer, M Goodfellow (Eds.), *Bergey's Manual of Systematics of Archaea and Bacteria* (pp. 1-163). New York: Springer.

Miller, G. L. (1959): Use of dinitrosalicylic acid reagent for detection of reducing sugars. *Anal*

Mukherjee, S., Roy, P. and Ray, A. (2020): Cellulase production from *Pseudomonas fluorescens* and its application. *Process Biochemistry*, 88, 1-10.

Nagar, S., Gupta, V. K., Kumar, D., Kumar, L. and Kuhad, R. C. (2016): Production and optimization of cellulase from *Bacillus* sp. and its application. *Bioresource Technology*, 213, 237-244.

Palleroni, N. J. (2010): *The Pseudomonas Genus: Molecular Biology and Biotechnology*. Berlin: Springer.

Rajoka, M. I., Mehwish, H. M. and Haque, S. (2019): Thermophilic cellulases from *Bacillus* species for biofuel production. *Renewable Energy*, 138, 1423-1432.

Shah, P., Jain, A. and Patel, D. (2016): Purification and characterization of cellulase from *Bacillus* sp. *International Journal of Biological Macromolecules*, 87, 236-244.

Sharma, A., Gupta, R. and Yadav, N. (2021): Microbial cellulases: A review on their characteristics and applications in bioremediation. *Journal of Environmental Management*, 292, 112727.

Sharma, P., Bhattacharya, A. and Singh, R. (2020): Advances in cellulase production and its applications. *Biotechnology Reports*, 25, e00485.

Sharma, R., Tewari, R. and Ramani, R. (2020): Isolation and characterization of cellulase-producing *Bacillus* sp. from soil. *Biotechnology Reports*, 25, e00417.

Singh, A., Singh, P. and Verma, P. (2021): Composting microbial diversity and cellulase activity in composting systems. *Environmental Technology*, 42(5), 723-733.

Singh, J., Kumar, D. and Tiwari, S. (2019): Cost-effective cellulase production strategies for industrial applications. *Bioresource Technology*, 290, 121788.

Singh, S., Kaur, J. and Sharma, A. (2020): Recent advances in microbial cellulases and their applications in biofuels production. *Renewable and Sustainable Energy Reviews*, 123, 109746.

Singhania, R. R., Patel, A. K. and Sukumaran, R. K. (2020): Cellulase production by thermophilic *Pseudomonas* species for industrial applications. *Industrial Crops and Products*, 147, 112252.

Sukumaran, R. K., Patel, A. K. and Singhania, R. R. (2017): Cellulase production by thermophilic microbes for bioethanol. *BioEnergy Research*, 10(3), 846-854.

Tiwari, R., Rana, P. and Sharma, R. (2019): Metal ions as activators or inhibitors in cellulase activity from *Bacillus* species. *Enzyme and Microbial Technology*, 123, 103401.

Zhang, J., Chen, H. and Wang, L. (2020): The role of microbial enzymes in bioremediation: A review of cellulose-degrading bacteria. *Bioremediation Journal*, 24(1), 1-15.

Zhang, X., Wang, L. and Huang, C. (2019): Kinetic parameters of cellulase from *Bacillus* sp. isolated from composting. *Journal of Chemical Technology and Biotechnology*, 94(5), 1307-1313.

Zhao, J., Liu, J. and Xu, Z. (2021): pH and cellulase activity in composting systems. *Composting Science and Utilization*, 29(2), 158-166.

Zhou, J., Deng, Y. and Shen, L. (2018): Soil pH and microbial community structure. *Applied Soil Ecology*, 116, 42-50.