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Research Article

OPTIMIZATION OF CELLULASE PRODUCTION FROM *MICROCOCCUS SP* USING RESPONSE SURFACE METHODOLOGY AND EVALUATION OF ENZYME STABILITY

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ABSTRACT

Cellulases are industrially important enzymes involved in the degradation of cellulose into fermentable sugars. The present study aimed to isolate and identify cellulase-producing bacteria from soil and optimize enzyme production using Response Surface Methodology (RSM). A total of ten bacterial isolates were obtained, among which five showed cellulolytic activity on CMC agar plates. Isolate S2 exhibited the highest enzyme activity (920.45 IU/mL) and was selected for further analysis. Morphological and biochemical characterization suggested that the isolate belongs to the genus *Micrococcus*. The enzyme demonstrated good stability under varying pH, metal ions, and organic solvents. Optimization using Central Composite Design significantly enhanced cellulase production, achieving a maximum activity of 1250.23 IU/mL at 72 h incubation, pH 7, and 1.5% inoculum. Statistical analysis confirmed model reliability. The findings highlight the potential of the isolate for industrial applications such as biofuel production and biomass conversion.

Keywords: Cellulase production, *Micrococcus sp,* Response Surface Methodology, Enzyme optimization, Soil bacteria.

INTRODUCTION

Enzymes are indispensable biological catalysts that regulate and accelerate biochemical reactions in living systems. Their application has expanded considerably in recent decades, particularly in industries such as food processing, pharmaceuticals, textiles, and biofuel production. Among the various industrial enzymes, cellulases have attracted significant attention due to their ability to hydrolyze cellulose into simpler sugars, which can be further utilized in diverse biotechnological processes (Lynd *et al*., 2002). Cellulose, a major structural component of plant cell walls, is the most abundant renewable organic polymer on Earth. However, its complex structure makes it resistant to degradation. This challenge is overcome by cellulase enzyme systems, which function synergistically through the coordinated action of endoglucanases, exoglucanases, and β glucosidases (Bhat, 2000). These enzymes collectively convert cellulose into glucose, thereby enabling its utilization in processes such as bioethanol production and waste biomass management. Microorganisms are considered the most efficient producers of cellulases due to their adaptability and rapid growth. Both fungi and bacteria have been extensively studied for their cellulolytic capabilities. However, bacterial systems are often preferred in industrial settings because of their shorter generation times and ability to function under a wide range of environmental conditions (Imran *et al*., 2016). Soil environments, in particular, represent a rich reservoir of diverse microbial populations capable of producing hydrolytic enzymes (Jacoby *et al*., 2017). In spite of the availability of cellulase-producing microorganisms, one of the major constraints in industrial application is the cost associated with enzyme production. The yield of cellulase is influenced by multiple factors, including pH, temperature, incubation time, and inoculum concentration. Therefore, optimizing these parameters is essential for achieving economically viable production levels (Singhania *et al*., 2010).

Traditional optimization methods often involve altering one factor at a time, which can be time-consuming and may overlook interactions between variables. In contrast, statistical approaches such as Response Surface Methodology (RSM) provide a more efficient means of optimization by evaluating multiple parameters

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simultaneously (Montgomery, 2017). Central Composite Design (CCD), a widely used RSM technique, allows for the identification of optimal conditions with fewer experimental trials while also revealing the interactions between variables.In addition to production efficiency, enzyme stability is a critical factor that determines its industrial applicability. Enzymes used in industrial processes are often exposed to harsh conditions, including extreme pH, high temperatures, and the presence of solvents or metal ions. Therefore, identifying enzymes that retain activity under such conditions is of considerable importance (Turner *et al*., 2007).The present study was undertaken to isolate cellulase-producing bacteria from soil, identify the most efficient strain, and optimize enzyme production using RSM. Furthermore, the stability of the produced cellulase under various physicochemical conditions was evaluated to assess its suitability for industrial applications.

MATERIALS AND METHODS

Collection and Preparation of Soil Samples

Soil samples were collected from different locations within the campus environment, particularly from areas rich in organic matter such as garden soil and decomposed plant litter zones. Approximately 10–15 g of soil was collected from a depth of 5–10 cm using sterile spatulas and transferred into sterile, airtight containers. The samples were transported immediately to the laboratory and stored at 4°C until further processing to minimize microbial alteration. Prior to analysis, the soil samples were homogenized thoroughly to ensure uniform distribution of microorganisms.

Isolation of Bacterial Strains

Serial dilution technique was employed for the isolation of bacterial strains. One gram of soil sample was suspended in 9 mL of sterile distilled water and mixed vigorously to obtain the initial suspension. This was followed by successive dilutions up to 10^{-6}. Aliquots of 0.1 mL from appropriate dilutions were spread evenly onto nutrient agar plates supplemented with 1% carboxymethyl cellulose (CMC) as the sole carbon source. The plates were incubated at 37°C for 24–48 hours. Distinct colonies exhibiting varied morphological characteristics such as size, shape, and pigmentation were selected and sub-cultured repeatedly to obtain pure isolates. The purified cultures were maintained on nutrient agar slants at 4°C for further experimentation.

Screening for cellulase-producing bacteria

Qualitative Screening (CMC agar plate assay)

Preliminary screening for cellulolytic activity was carried out using the CMC agar plate method. Isolated bacterial cultures were spot-inoculated onto CMC agar plates and incubated at 37°C for 24 hours. Following incubation, the plates were flooded with 0.1% Congo red solution and allowed to stand for 15 minutes. Excess stain was removed by washing with 1 M NaCl solution. The formation of clear zones around bacterial colonies indicated the hydrolysis of cellulose. The diameter of the hydrolysis zone was measured to compare cellulolytic efficiency among isolates (Teather and Wood, 1982).

Quantitative estimation of cellulase activity (DNS Method)

Cellulase activity was quantified by measuring the release of reducing sugars using the dinitrosalicylic acid (DNS) method (Miller, 1959). Bacterial isolates were cultured in CMC broth and incubated under standard conditions. After incubation, the culture was centrifuged at 10,000 rpm for 10 minutes to obtain the crude enzyme extract. The reaction mixture consisted of 0.5 mL of enzyme extract and 0.5 mL of 1% CMC prepared in phosphate buffer (pH 7.0). The mixture was incubated at 50°C for 30 minutes. The reaction was terminated by adding 1 mL of DNS reagent, followed by boiling for 5 minutes. Absorbance was measured at 540 nm using a UV–Vis spectrophotometer. One unit (IU) of cellulase activity was defined as the amount of enzyme required to release 1 µmol of glucose per minute under assay conditions.

Protein estimation and specific activity

The protein concentration of the crude enzyme extract was determined using the Bradford assay (Bradford, 1976), with bovine serum albumin (BSA) as the standard. Absorbance was recorded at 595 nm. The specific activity of cellulase was calculated by dividing enzyme activity (IU/mL) by the protein concentration (mg/mL), providing an estimate of enzyme efficiency and purity.

Morphological and biochemical characterization

The selected high-performing isolate was subjected to detailed morphological and biochemical characterization. Gram staining was performed to determine cell wall characteristics, while colony morphology was observed on nutrient agar plates. Biochemical tests, including catalase, oxidase, urease, nitrate reductase and oxidative fermentative tests were conducted following standard microbiological protocols (Cappuccino and Sherman, 2014). These tests provided preliminary identification of the bacterial isolate.

Effect of organic solvents

The stability of cellulase in the presence of various organic solvents such as ethanol, methanol, chloroform, and isopropanol was evaluated. The enzyme extract was incubated with each solvent at a specific concentration for a fixed duration. Residual enzyme activity was measured using the DNS method and compared with the control to determine the effect of solvents on enzyme stability (Gupta *et al*., 2002).

Effect of pH on Enzyme Stability

The effect of pH on enzyme stability was evaluated by incubating the enzyme in buffers of varying pH ranging from 5.0 to 10.0. After incubation, residual activity was measured using the standard assay. This study provided insights into the pH tolerance range of the enzyme and its suitability for industrial applications.

Effect of Metal ions

To assess the influence of metal ions on enzyme activity, the crude enzyme was incubated with different metal salts such as Ca^{2+}, Mg^{2+}, Zn^{2+}, and Fe^{3+}. After incubation, cellulase activity was measured and expressed as a percentage relative to the control without metal ions. This experiment helped in identifying activators or inhibitors of enzyme activity.

Optimization of cellulase production using response surface methodology (RSM)

Response Surface Methodology (RSM) was employed to optimize fermentation parameters for enhanced cellulase production by the selected bacterial strain. A Central composite design (CCD) was adopted to evaluate the combined effects of key process variables and to determine optimal conditions for maximum enzyme yield (Khusro *et al*., 2016). Three independent variables, i.e., incubation period (A), pH (B), and inoculum size (C) were selected based on preliminary screening experiments. Each variable was studied at three coded levels (−1, 0, +1), representing low, central, and high values, respectively. The experimental design consisted of 17 runs, including factorial points, axial points, and center points, allowing efficient estimation of linear, interaction, and quadratic effects. All experiments were performed in triplicate, and the average cellulase activity (IU/mL) was considered as the response variable.

Experimental Design and Response Data

The experimental matrix generated by CCD, along with the observed cellulase activity, is presented in Table 1.

Table 1. Central Composite Design matrix and observed cellulase activity.

Run	A (Incubation)	B (pH)	C (Inoculum)	Cellulase Activity (IU/mL)
1	0	-1	1	1216.23
2	0	-1	-1	810.23
3	0	0	0	945.23
4	-1	1	0	750.45
5	0	0	0	940.15
6	1	0	1	1250.23
7	1	-1	0	1135.76
8	-1	0	-1	610.21
9	0	1	1	1030.23
10	1	0	-1	890.35
11	-1	-1	0	875.31
12	-1	0	1	1010.14
13	0	0	0	930.23
14	0	1	-1	660.23
15	0	0	0	925.32
16	1	1	0	960.23
17	0	0	1	1135.23

Model Development and Statistical Analysis

The experimental data were fitted to a second-order polynomial equation to describe the relationship between the independent variables and cellulase activity. The final regression equation in terms of coded factors is given below:

$$R1 = 934.28 + 123.81A + 79.55B + 192.97C - 12.67AB - 10.01AC - 9.00BC$$

This equation was used to predict cellulase production at different combinations of variables. The coded coefficients represent the relative contribution of each factor and their interactions. Among the variables, inoculum size (C) exhibited the highest positive effect, followed by incubation period (A) and pH (B), indicating their significant influence on enzyme production. Statistical validation of the model was carried out using analysis of variance (ANOVA). The significance of the model and individual coefficients was determined based on p-values, F-values, and the coefficient of determination (R^2). A high

R^2 value indicated a good fit between experimental and predicted data. Response surface and contour plots were generated to visualize the interaction effects among variables and to identify optimal conditions. Design-Expert software (version 10.0.0, Stat-Ease Inc., Minneapolis, MN, USA) was used for experimental design, statistical analysis, and graphical representation of the data. ANOVA was applied to evaluate the significance and validity of the model.

RESULTS AND DISCUSSION

A total of ten morphologically distinct bacterial isolates were successfully obtained from soil samples collected from different locations. The colonies exhibited noticeable variation in size, shape, elevation, margin, and pigmentation, suggesting microbial diversity within the sampled environment. All isolates were subjected to primary screening for cellulolytic activity using CMC agar plates. Out of the ten isolates, five demonstrated visible zones of hydrolysis after Congo red staining, indicating their ability to degrade cellulose. The diameter of the clearance zones varied among the isolates, reflecting differences in enzymatic efficiency. Among these, isolate S2 showed the largest hydrolysis zone, indicating superior cellulase production potential.

Table 1. Qualitative screening of cellulase-producing isolates.

Isolate Code	Colony Characteristics	Zone of Clearance (mm)	Cellulase Activity
S1	Small, circular, white	8 ± 0.5	+
S2	Large, creamy, smooth	18 ± 0.8	+++
S3	Irregular, yellow	12 ± 0.6	++
S4	Round, opaque	10 ± 0.4	++
S5	Mucoid, off-white	15 ± 0.7	+++
S6–S10	Various	No zone	–

(Values represent mean ± standard deviation; + indicates cellulolytic activity)

Table 3. Quantitative cellulase activity of selected isolates.

Isolate Code	Cellulase Activity (IU/mL)	Protein Content (mg/mL)	Specific Activity (IU/mg)
S1	450.21 ± 12.4	1.25 ± 0.05	360.16
S2	920.45 ± 18.7	1.10 ± 0.04	836.77
S3	610.33 ± 15.2	1.30 ± 0.06	469.49
S4	580.12 ± 10.9	1.28 ± 0.05	453.21
S5	810.32 ± 17.6	1.15 ± 0.03	704.62

The significantly higher specific activity observed in isolate S2 confirmed its superior enzymatic efficiency. Based on these findings, isolate S2 was selected for further characterization and optimization studies.

The five positive isolates were further evaluated quantitatively using the DNS assay to measure reducing sugar release. The results revealed considerable variation in enzyme production among the isolates. Isolate S2 exhibited the highest cellulase activity, reaching 920.45 IU/mL under initial unoptimized conditions, followed by isolate S5 (810.32 IU/mL). The remaining isolates showed moderate activity levels. The selected isolate (S2), identified based on its high cellulolytic activity, was subjected to morphological and biochemical characterization. The colonies were circular, smooth, and creamy-white with a convex, glossy surface. Gram staining revealed Gram-positive cocci arranged predominantly in clusters. Biochemical analysis showed positive results for catalase, oxidase, and urease. In O-F test, the isolate is strictly oxidative (produces acid only in open tubes). The isolate tested negative for nitrate reductase. The morphological and biochemical profile of isolate S2 strongly suggested its affiliation with the genus *Micrococcus*. The cellulase enzyme demonstrated considerable tolerance to various organic solvents. Among the solvents tested, ethanol and methanol showed minimal inhibitory effects, retaining more than 80% of enzyme activity. In contrast, chloroform resulted in a moderate reduction in activity.

Table 4. Effect of organic solvents on cellulase activity.

Solvent	Residual Activity (%)
Control	100
Ethanol	88.5 ± 2.1
Methanol	85.3 ± 1.8
Isopropanol	78.6 ± 2.4
Chloroform	65.2 ± 2.7

The presence of metal ions influenced enzyme activity to varying degrees. Calcium and magnesium ions slightly enhanced enzyme activity, whereas zinc and iron ions showed inhibitory effects.

Table 5. Effect of metal ions on cellulase activity.

Metal Ion	Residual Activity (%)
Control	100
Ca^{2+}	110.2 ± 3.0
Mg^{2+}	105.6 ± 2.5
Zn^{2+}	72.4 ± 2.2
Fe^{3+}	68.9 ± 2.8

The enzyme exhibited stability over a broad pH range, with maximum activity observed at neutral to slightly alkaline conditions (pH 7–8). A gradual decline in activity was noted at higher pH levels.

Table 6. Effect of pH on cellulase activity.

pH	Relative Activity (%)
5.0	62.4 ± 2.1
6.0	78.6 ± 1.8
7.0	100 ± 2.5
8.0	95.3 ± 2.0
9.0	81.7 ± 2.3
10.0	68.9 ± 2.6

Table 7. Experimental and predicted values for cellulase activity using RSM.

Run	Actual (IU/mL)	Predicted (IU/mL)	Residual	Leverage	Std. Residual (Internal)	Std. Residual (External)	Cook's Distance	DFFITS
1	1216.23	1215.80	0.43	0.533	0.059	0.056	0.001	0.060
2	810.23	811.86	-1.63	0.559	-0.231	-0.219	0.010	-0.247
3	945.23	934.28	10.95	0.059	1.062	1.070	0.010	0.268
4	750.45	743.59	6.86	0.559	0.972	0.969	0.171	1.091
5	940.15	934.28	5.87	0.059	0.569	0.549	0.003	0.138
6	1250.23	1241.05	9.18	0.533	1.264	1.308	0.260	1.397
7	1135.76	1150.31	-14.55	0.559	-2.062	-2.579	0.770	-2.905*
8	610.21	607.49	2.72	0.559	0.386	0.369	0.027	0.415
9	1030.23	1038.71	-8.48	0.533	-1.167	-1.191	0.222	-1.272
10	890.35	875.13	15.22	0.559	2.158	2.800	0.844	3.153*
11	875.31	877.36	-2.05	0.559	-0.290	-0.276	0.015	-0.311

12	1010.14	1013.46	-3.32	0.533	-0.457	-0.438	0.034	-0.468
13	930.23	934.28	-4.05	0.059	-0.393	-0.376	0.001	-0.094
14	660.23	670.76	-10.53	0.559	-1.493	-1.606	0.404	-1.809
15	925.32	934.28	-8.96	0.059	-0.869	-0.858	0.007	-0.215
16	960.23	965.87	-5.64	0.559	-0.800	-0.784	0.116	-0.883
17	1135.23	1127.25	7.98	0.158	0.818	0.803	0.018	0.348

*Values exceeding acceptable influence limits

The adequacy of the developed model was evaluated using analysis of variance (ANOVA), and the results are summarized in Table 2. The model F-value (749.17) with a p-value < 0.0001 indicates that the model is highly significant and reliable.

Table 8. ANOVA for cellulase production (quadratic model).

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	5.076E+05	6	84595.75	749.17	<0.0001
A (Incubation)	1.226E+05	1	1.226E+05	1085.96	<0.0001
B (pH)	50624.03	1	50624.03	448.32	<0.0001
C (Inoculum)	3.330E+05	1	3.330E+05	2948.62	<0.0001
AB	641.86	1	641.86	5.68	0.0383
AC	401.00	1	401.00	3.55	0.0889
BC	324.00	1	324.00	2.87	0.1211
Residual	1129.20	10	112.92	—	—
Lack of Fit	881.78	7	125.97	1.53	0.3949
Pure Error	247.41	3	82.47	—	—
Total	5.087E+05	16	—	—	—

The optimization of cellulase production was performed using Response Surface Methodology (RSM), and the experimental design along with factor ranges has been described in the Materials and Methods section. The experimental results obtained from the Central Composite Design (CCD) are presented in Table 1. Among the experimental runs, the highest cellulase activity (1250.23 IU/mL) was observed in Run 6, corresponding to an incubation period of 72 h, pH 7, and inoculum size of 1.5%. This indicates that a balanced combination of neutral pH and higher inoculum level plays a crucial role in maximizing enzyme production. The statistical analysis revealed that the linear terms A (incubation period), B (pH), and C (inoculum size) significantly influenced cellulase production ($p < 0.05$). Among these, inoculum size (C) exhibited the highest F-value, indicating its dominant role. The interaction term AB was also significant, whereas AC and BC showed non-significant effects. The lack of fit F-value (1.53) was not significant ($p > 0.05$), confirming that the model adequately fits the experimental data.

Table 9. Fit Statistics of the Model.

Parameter	Value
R^2	0.9978
Adjusted R^2	0.9964
Predicted R^2	0.9912
Std. Deviation	10.63
Mean	945.63
C.V. (%)	1.12
Adeq Precision	92.91

The high R^2 value (0.9978) indicates excellent agreement between experimental and predicted values. The predicted R^2 (0.9912) is in close agreement with the adjusted R^2 (0.9964), demonstrating strong model predictability. The Adeq Precision value (>4) confirms a high signal-to-noise ratio, indicating that the model can effectively navigate the design space. Distribution of experimental and predicted values for cellulase activity was shown by Parity plot (Figure 14) where data points are localized close to the diagonal line, suggesting that the model is accurate and satisfactory. Figure 2a depicts that maximum cellulase activity was observed with lower tomiddle level of pH (6-7) and middle to high level of incubation period (48-72 h). The interaction effect between inoculum level (C/X_3) and incubation period (A/X_1) on cellulase activity is illustrated in Figure 2b**.** An increase in cellulase production was observed when both variables were maintained at higher levels, indicating a synergistic effect between extended incubation time and increased inoculum concentration. Similarly, Figure 2c demonstrates the combined influence of pH and inoculum level on enzyme production. Cellulase activity showed a marked increase when pH was maintained at a slightly acidic to neutral range (pH 6–7) along with a higher inoculum level (1.5%). The normality assumption Vs externally studentized residuals were found to be satisfactory due to the plot obtained in straight line (Figure 3).

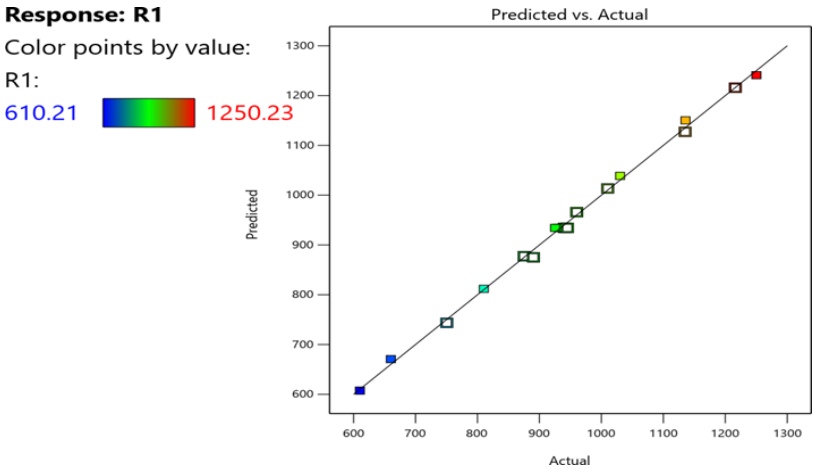

Figure1. Actualvaluesandpredictedvaluesfor cellulose activity residing close to the diagonal line.

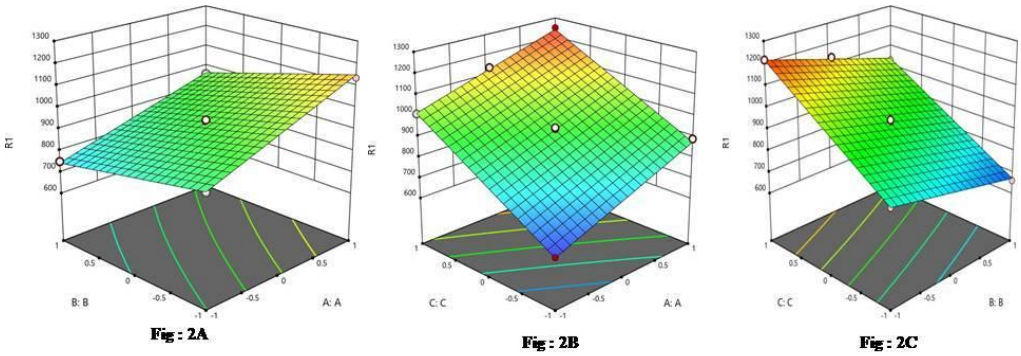

Fig : 2A Fig : 2B Fig : 2C

Figure 2a. Response surface plot showing interaction between incubation period (A:A) and pH (B:B)

Figure 2b. Response surface plot showing interaction between incubation period (A:A) and inoculum level (C:C)

Figure 2c.Response surface plot showing interaction between pH (B:B) and inoculum level (C:C)

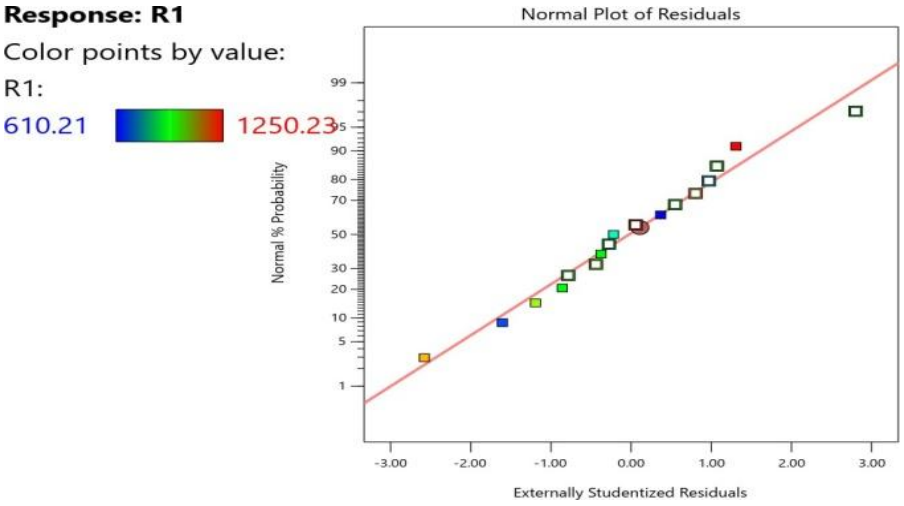

Figure 3. Plot showing normal assumption Vs externally studentized residuals.

The central point representing the maximum cellulase production had the following values: incubation period (72 h), pH 7 and inoculum level (1.5%). The validation of the optimization was carried out under above mentioned optimized conditions of the variables. It showed the dependent response of 1250.23 IU/ml which is very much close to the predicted response. The RSM optimization showed64%enhancementintheresponse, compared to the lower response of 610.21 IU/ml. The optimized conditions revealed the responses values close to the predicted one, indicating the validation strategy towards biomass yield and protein production. The reasonable accepted values of R^2define the true behavior of the statistical system that can be used for interpolation in the experimental domain. The present study provides a detailed evaluation of cellulose producing bacterial isolates, their biochemical characteristics, enzyme stability, and optimization of production parameters using Response Surface Methodology (RSM). The isolation and screening results demonstrated considerable microbial diversity in soil samples, as reflected by variations in colony morphology and cellulolytic potential. Among the ten isolates obtained, only five exhibited cellulase activity, indicating that cellulolytic capability is restricted to specific microbial groups. The prominent hydrolysis zone observed in isolate S2 suggests its superior ability to degrade cellulose, which is consistent with earlier reports that larger clearance zones correlate with higher extracellular enzyme secretion (Teather and Wood, 1982; Kasana *et al*., 2008).

The quantitative estimation of cellulase activity further confirmed the superiority of isolate S2, which showed the highest enzyme activity and specific activity among all tested isolates. High specific activity indicates efficient catalytic performance per unit protein, suggesting that S2 possesses a highly active enzyme system. Similar observations have been reported by Singh *et al*. (2010), where selected bacterial strains exhibited significantly higher cellulase activity due to enhanced enzyme expression and secretion mechanisms. The variation in enzyme production among isolates may be attributed to differences in genetic makeup, regulatory pathways, and environmental adaptation (Lynd *et al*., 2002). Comparable enhancement of enzyme production under optimized conditions has also been reported in *Bacillus* species (Arun Sasi *et al*., 2008) and fungal systems such as *Penicillium chrysogenum*, where environmental factors significantly influenced enzyme yield (Devi *et al*., 2022).

Morphological and biochemical characterization identified isolate S2 as belonging to the genus *Micrococcus*. Although *Micrococcus* species are not widely recognized as primary cellulase producers, studies have indicated their potential in enzyme production under specific environmental conditions (Dworkin *et al*., 2006). The positive catalase and oxidase reactions observed in this study are characteristic of aerobic bacteria, suggesting that oxygen availability may influence enzyme synthesis. The ability of *Micrococcus* sp. to produce cellulase highlights its metabolic versatility and ecological adaptability. The enzyme stability studies revealed that the cellulase retained significant activity in the presence of organic solvents such as ethanol and methanol. This solvent tolerance is advantageous for industrial processes, particularly in biofuel production and bioconversion systems. Similar findings have been reported by Gupta *et al*. (2016), indicating that solvent-tolerant enzymes possess enhanced structural stability and industrial relevance. The moderate inhibition observed with chloroform may be attributed to its disruptive effects on enzyme conformation. The influence of metal ions on enzyme activity showed that Ca^{2+} and Mg^{2+} enhanced cellulase activity, while Zn^{2+} and Fe^{3+} exhibited inhibitory effects. Metal ions play a crucial role

in enzyme stabilization and catalytic activity. Calcium ions, for instance, are known to improve enzyme stability and functionality (Beg *et al*., 2001), whereas heavy metals may interfere with enzyme active sites, leading to reduced activity (Nielsen and Borchert, 2000). The pH stability profile indicated that the enzyme exhibited maximum activity at neutral to slightly alkaline conditions (pH 7–8), which is typical for bacterial cellulases. The decline in activity at extreme pH levels can be attributed to enzyme denaturation and disruption of ionic interactions essential for catalysis. Similar observations have been reported in earlier studies (Immanuel *et al*., 2006), confirming the importance of pH in enzyme performance. Optimization of cellulase production using RSM significantly enhanced enzyme yield, demonstrating the effectiveness of statistical tools in process optimization. The maximum cellulase activity (1250.23 IU/mL) obtained under optimized conditions represents a substantial improvement over initial value. This enhancement can be attributed to the combined effects of incubation period, pH, and inoculum size. Among these, inoculum size had the most significant impact, as indicated by the highest F-value in ANOVA. The interaction effects between variables revealed synergistic relationships that enhanced cellulase production. The combined effect of incubation period and inoculum size showed increased enzyme activity at higher levels of both factors, likely due to increased biomass and prolonged enzyme secretion. Similarly, the interaction between pH and inoculum size indicated that optimal production occurs at neutral pH with higher inoculum concentration. These findings emphasize the importance of multi-variable optimization approaches, as supported by Bezerra *et al*. (2008).

The statistical validation of the model confirmed its reliability and predictive accuracy. The high coefficient of determination ($R^2 = 0.9978$) indicates excellent agreement between experimental and predicted values. The non-significant lack of fit further confirms that the model adequately represents the experimental data. The parity plot and residual analysis also supported the normal distribution and consistency of the data, validating the model assumptions. Similar levels of accuracy have been reported in RSM-based optimization studies (Montgomery, 2017). Finally, the validation experiment confirmed the effectiveness of the optimized conditions, with experimental values closely matching predicted results. The observed 64% increase in cellulase production highlights the importance of optimization strategies in enhancing enzyme yield. Such improvements are crucial for industrial applications including biomass conversion, bioethanol production, and environmental waste management (Lynd *et al*., 2002).

CONCLUSION

The present study successfully isolated and identified a potent cellulase-producing bacterial strain from soil samples, with isolate S2 showing the highest enzymatic activity. Morphological and biochemical characterization suggested its affiliation with the genus *Micrococcus*, indicating its potential as an efficient enzyme producer. The cellulase enzyme exhibited good stability across a range of environmental conditions, including tolerance to organic solvents, metal ions, and varying pH levels, highlighting its industrial applicability. Optimization using Response Surface Methodology (RSM) significantly enhanced cellulase production, with maximum activity achieved at 72 hours incubation, pH 7, and 1.5% inoculum size. The statistical model was found to be highly reliable and predictive, confirming the effectiveness of the optimization approach. Overall, the study demonstrates the importance of process optimization in improving enzyme yield and suggests that the selected isolate could be a promising candidate for industrial applications such as biomass conversion and biofuel production.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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