[Insert Journal Logo Here]
Al-Qadisiyah Journal of Biotechnology

Al-Qadisiyah Journal of Biotechnology | Vol. __, No. __ (20__)

TEMPLATE / TRIAL PAPER — FOR DEMONSTRATION PURPOSES ONLY

This is not a real submission. All author names, affiliations, email addresses, data, results, and references in this document are fictitious and have been created solely to illustrate how a completed manuscript should look for AL-Qadisiyah Journal of Biotechnology (QJBT). Do not cite, distribute, or treat any content below as genuine research.

ORIGINAL RESEARCH ARTICLE

Optimization of Extracellular Cellulase Production from a Locally Isolated Bacillus subtilis Strain Using Response Surface Methodology

Rana Trial Author [1], **Mustafa J. Trial Author**[2], **Sara Trial Author**[1*]

1 Department of Biotechnology, College of Science, University of Al-Qadisiyah, Al-Diwaniyah, Iraq (template affiliation)

2 Department of Agricultural Biotechnology, College of Biotechnology, University of Al-Qadisiyah, Al-Diwaniyah, Iraq (template affiliation)

*** Corresponding author (template email):** sara.hassani.trial@example-qjbt.edu.iq

Received: DD Month 20__ | Revised: DD Month 20__ | Accepted: DD Month 20__ | Published: DD Month 20__

DOI: 10.XXXXX/qjbt.trial.0001 (template placeholder)

ABSTRACT

Agricultural residues such as rice straw represent an abundant, low-cost substrate for microbial cellulase production, yet locally isolated strains from Al-Qadisiyah Governorate remain poorly characterized. In this template study, a cellulolytic bacterium (designated Bacillus subtilis QU-27) was isolated from decomposing rice straw and its extracellular cellulase activity optimized using a Box–Behnken response surface design across three factors: pH (5.5–7.5), incubation temperature (30–50 °C), and substrate concentration (0.5–2.5% w/v carboxymethyl cellulose). Enzyme activity was quantified using the dinitrosalicylic acid (DNS) method and expressed in U/mL. The model predicted maximum cellulase activity of 18.4 ± 0.6 U/mL at pH 6.5, 45 °C, and 1.5% substrate concentration, a result confirmed experimentally within 5% of the predicted value. Analysis of variance indicated the model was statistically significant ($p < 0.05$) with temperature exerting the largest individual effect on yield. These illustrative findings suggest that regionally isolated Bacillus strains can be optimized into efficient cellulase producers suitable for downstream applications in biofuel production and agricultural waste valorization. As a template document, all data presented are fictitious and intended only to demonstrate the expected structure, tone, and level of detail of a QJBT original research article.

Keywords: *cellulase; Bacillus subtilis; response surface methodology; agricultural waste; enzyme optimization*

1. INTRODUCTION

Lignocellulosic agricultural residues, including rice straw, wheat straw, and sugarcane bagasse, are generated in large quantities across Iraq's agricultural governorates and are frequently burned or left to decompose without valorization. Microbial cellulases capable of hydrolyzing cellulose into fermentable sugars offer a route to convert these residues into biofuels, animal feed additives, and industrial feedstocks, supporting both waste management and the bioeconomy.

Members of the genus Bacillus are widely reported as robust extracellular cellulase producers due to their tolerance of variable pH and temperature conditions and their amenability to large-scale fermentation. However, enzyme yield is highly strain- and condition-dependent, and locally isolated strains from Iraqi agricultural soils and residues remain largely uncharacterized in the literature, representing a gap this (template) study addresses.

The specific aims of this study were to (i) isolate and identify a cellulolytic bacterial strain from decomposing rice straw collected in Al-Diwaniyah, (ii) statistically optimize pH, temperature, and substrate concentration for maximal extracellular

cellulase activity using response surface methodology (RSM), and (iii) evaluate the model's predictive accuracy against experimental validation. This template introduction is illustrative and intentionally concise; an actual submission should expand this section to 500–700 words with full literature citations as required by the QJBT Author Guidelines.

2. MATERIALS AND METHODS

2.1 Experimental Design and Scope

This was an in vitro laboratory optimization study. A three-factor, three-level Box–Behnken design was used to model the combined effects of pH, temperature, and substrate concentration on cellulase activity, generating 15 experimental runs including three center-point replicates.

2.2 Isolation and Identification of the Bacterial Strain

Soil and decomposing rice straw samples were collected from an agricultural field in Al-Diwaniyah, Al-Qadisiyah Governorate (template sampling location). Serial dilutions were plated on carboxymethyl cellulose (CMC) agar, and colonies producing clear zones after Congo red staining were selected as candidate cellulase producers. The selected isolate (designated QU-27) was identified by 16S rRNA gene sequencing (template placeholder — GenBank accession number XXXXXXXX) as Bacillus subtilis.

2.3 Enzyme Production and Assay

The isolate was cultured in CMC-supplemented minimal salt broth under the pH, temperature, and substrate conditions specified by the experimental design. Crude enzyme extracts were obtained by centrifugation (10,000 × g, 10 min, 4 °C) of the culture supernatant. Cellulase activity was determined using the dinitrosalicylic acid (DNS) method, measuring the release of reducing sugars at 540 nm against a glucose standard curve, and expressed as U/mL (one unit defined as the amount of enzyme releasing 1 µmol of glucose equivalent per minute).

2.4 Statistical Analysis

Response surface analysis and Box–Behnken design generation were performed using Design-Expert software (v.13, template placeholder). Analysis of variance (ANOVA) was used to assess model significance and lack-of-fit, with $p < 0.05$ considered statistically significant. All experiments were performed in triplicate; results are reported as mean ± standard deviation.

3. RESULTS

3.1 Model Fitting and Optimum Conditions

The Box–Behnken design produced a quadratic model with a coefficient of determination (R^2) of 0.96 (template value), indicating a good fit between predicted and observed cellulase activities. Temperature had the largest individual effect on enzyme yield ($p < 0.01$), followed by pH ($p < 0.05$); substrate concentration showed a significant but smaller quadratic effect. The model predicted maximum cellulase activity of 18.4 ± 0.6 U/mL at pH 6.5, 45 °C, and 1.5% (w/v) CMC, which was confirmed experimentally at 17.9 ± 0.5 U/mL, within 5% of the predicted value.

Table 1. *Predicted versus experimentally validated cellulase activity under optimized conditions (template data; mean ± SD, n = 3).*

Condition	pH	Temperature (°C)	Substrate (% w/v)	Activity (U/mL)
Model-predicted optimum	6.5	45	1.5	18.4 ± 0.6
Experimental validation	6.5	45	1.5	17.9 ± 0.5
Baseline (unoptimized)	7.0	37	1.0	9.2 ± 0.4

3.2 Supporting Findings

Activity declined sharply above 50 °C, consistent with thermal denaturation of the enzyme, and below pH 5.5, consistent with reduced enzyme stability under strongly acidic conditions. No significant interaction effect was observed between substrate concentration and pH ($p > 0.05$).

[Figure — insert image here, min. 300 DPI]

Figure 1. Response surface plot (template placeholder) showing the combined effect of pH and temperature on predicted cellulase activity (U/mL), holding substrate concentration at its optimal level (1.5% w/v).

4. DISCUSSION

The optimized cellulase activity obtained in this template study (18.4 U/mL) is comparable to, or exceeds, values reported for other locally isolated Bacillus strains in the regional literature (template comparison), suggesting that Bacillus subtilis QU-27 is a promising candidate for further scale-up. The dominant effect of temperature on yield is consistent with the known thermal sensitivity of bacterial cellulase active-site conformation.

Limitations of this template study include its reliance on a single isolate, use of crude rather than purified enzyme, and shake-flask rather than bioreactor-scale validation. An actual submission should discuss these limitations honestly and propose specific follow-up experiments, such as scale-up fermentation trials and enzyme purification/characterization.

5. CONCLUSIONS

This template study demonstrates that response surface methodology can be used to efficiently optimize extracellular cellulase production from a locally isolated Bacillus subtilis strain, achieving a predicted maximum activity of 18.4 U/mL under mild, industrially feasible conditions. These illustrative findings support the broader potential of regionally isolated microorganisms for agricultural waste valorization and point toward future bioreactor-scale validation and enzyme characterization work.

ACKNOWLEDGEMENTS

The authors thank [Template Name, Template Institution] for assistance with 16S rRNA sequencing, and the College of Biotechnology, University of Al-Qadisiyah, for access to fermentation facilities. (Template text.)

FUNDING STATEMENT

This template research received no external funding.

AUTHOR CONTRIBUTIONS

R.K.A.: Conceptualization, Methodology, Writing – Original Draft. M.J.A.: Investigation, Formal Analysis, Data Curation. S.T.A.: Resources, Supervision, Writing – Review & Editing. All authors have read and agreed to the published version of the (template) manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ETHICS STATEMENT

Not applicable — this template study involved only environmental bacterial isolates and no human participants or vertebrate animals.

DATA AVAILABILITY STATEMENT

As this is a template document, no real dataset exists. In an actual submission, state where the supporting data can be accessed.

AI & GENERATIVE TOOLS DISCLOSURE

This template manuscript was generated with the assistance of an AI language model (Claude, Anthropic) to illustrate the expected structure and content of a QJBT submission. It does not represent real research and must not be cited.

REFERENCES

1. Al-Hassan, M.T., & Karim, R.A. (2022). Cellulase production by locally isolated Bacillus spp. from agricultural soils. Iraqi Journal of Science, 63(7), 2901-2915. (template reference)

2. Zhang, W., Li, F., & Nie, L. (2023). Response surface methodology in microbial enzyme optimization: a review. Applied Microbiology and Biotechnology, 107(4), 1123-1138. (template reference)

3. Ibrahim, H.K., Saleh, N.M., & Al-Jubouri, T.A. (2023). Valorization of rice straw through microbial bioconversion in Iraq. Al-Qadisiyah Journal of Biotechnology, 1(1), 1-14. (template reference)

4. Kumar, S., & Fernandez, L.A. (2021). Bacterial cellulases: sources, production, and industrial applications. Biotechnology Advances, 43, 107589. (template reference)

5. Taylor, R.J., Moore, A.L., & Davis, K.P. (2022). Thermal stability of microbial cellulolytic enzymes. Molecular Biotechnology, 64(6), 512-524. (template reference)

6. Chen, X., Nguyen, T., & Okonkwo, A. (2023). Lignocellulosic waste-to-energy: microbial strategies. Proceedings of the National Academy of Sciences, 120(15), e2300000120. (template reference)