Optimized Production of Cellulase form Different Agrowaste Biomass Substrates

Abeer Zahra[1], Misbah Zafar[2], Anam Mubarak[3], Zahid Anwar[4], Taimoor Hassan[3], Khizar Hayat Bhatti[3*], Noshia Arshad[3], Muhammad Zeeshan Baqir[3], Muhammad Ishtiaq[5] and Tanveer Hussain[5]

[1]Department of Chemistry, University of Gujrat, Hafiz Hayat Campus, Gujrat-50700, Pakistan
[2]Department of Plant Sciences, Quaid-i-Azam University Islamabad-44100, Pakistan
[3]Department of Botany, University of Gujrat, Hafiz Hayat Campus, Gujrat-50700, Pakistan
[4]Department of Biochemistry and Biotechnology, University of Gujrat, H. H. Campus, Gujrat-50700, Pakistan
[5]Department of Botany, University of Bhimber, 10250, AJK, Pakistan.
[*]**Corresponding:** khizar.hayat@uog.edu.pk

Citation | Zahra. A, Zafar. M, Mubarak. A, Anwar. Z, Hassan. T, Bhatti. K. H, Arshad. N, Baqir. M, Z, Ishtiaq. M, Hussain. T, "Optimized Production of Cellulase form different Agrowaste Biomass Substrates", IJIST, Special Issue pp 590-599, June 2024

Received | June 15, 2024 **Revised |** June 22, 2024 **Accepted |** June 28, 2024 **Published |** June 30, 2024.

Cellulase is a crucial industrial enzyme, with developing countries expending significant resources on its import for various industrial and scientific applications. A major challenge in cellulase production is the lack of affordable technology and suitable substrates for cultivating enzyme-producing microbes. This study optimized a substrate mixture to enhance cellulase production using solid-state fermentation with the Aspergillus Niger strain. Five agro-industrial substrates—sugarcane bagasse, corn cobs, rice straw, orange peel, and wheat straw—were individually inoculated with A. Niger spore suspension, and their cellulase activity was compared to that of a substrate mixture. The enzyme activity from individual substrates was notably lower compared to the mixture. Response Surface Methodology (RSM) was employed to identify the optimal substrate combination, which consisted of equal amounts of sugarcane bagasse, corn cobs, orange peel, and wheat straw, with rice straw in double the amount of the other substrates. The study also optimized fermentation parameters, including temperature, pH, incubation time, substrate concentration, moisture content, urea, MgSO4, and inoculum size of A. Niger. Maximum cellulase activity was achieved at 50°C, 80% moisture content, pH 4.0, 120 hours incubation, with 6.5 g of the substrate mixture, 2% w/w urea, 0.2% w/w MgSO4, and 4 ml of A. Niger spore suspension. Optimization resulted in cellulase activity of 0.205 IU/ml, significantly higher than the 0.025 IU/ml from individual substrates. Given its key role in industries such as pulp and paper, textiles, food and beverages, detergents, and agriculture, the demand for cellulase is expected to surge, particularly with the rise in biofuel production.

Keywords: Cellulase; High Production of Cellulase; Mixture of Substrates; Optimization; RSM; Solid State Fermentation; Spectrophotometry.

Introduction:

Cellulase is recognized as the third most important industrial enzyme due to its wide range of applications in textiles, pollution treatment, biofuels, waste management, paper and pulp industries, medical and pharmaceutical fields, animal feed, genetic engineering, food processing, and various chemical industries. It represents 20% of the global value of industrial enzymes [1]. Cellulases are primarily produced through submerged fermentation (SmF) to meet commercial needs [2]. Their biotechnological potential extends to food and beverage industries, industrial waste-to-chemical feedstock conversion, animal feed, textiles and laundry, pulp and paper, agriculture, and research in single-cell protein [3].

In the pulp and paper industry, cellulase facilitates several processes. Enzymatic treatment of coarse mechanical pulp with cellulase significantly reduces energy consumption. It also enhances fiber properties, improving drainage, beatability, and runnability. Additionally, cellulase can degrade plant pathogen cell walls and manage plant diseases, and improve soil quality, thus reducing reliance on mineral fertilizers [4].

However, cellulase production via SmF is costly due to excess water usage, energy-intensive downstream processes like purification, and stringent aseptic conditions. Solid-state fermentation (SSF) offers a cost-effective alternative, utilizing solid agro-waste biomass as a substrate. SSF requires fewer resources and has a projected production cost of approximately $0.20/kg, about ten times less than SmF, which costs $20/kg [5]. SSF's benefits include lower energy consumption, significant productivity, and a simpler fermentation medium, making it a promising option for biofuel or enzyme production.

Despite its advantages, SSF has primarily been tested at the laboratory scale, facing challenges such as bioreactor design, heat and mass transfer issues, and the scarcity of low-cost substrates that could reduce overall enzyme production costs [6]. Agro-waste, which consists mainly of cellulosic or lignocellulosic materials, is ideal for microbial growth [7]. Using such low-cost carbon sources can make enzyme or biofuel production more sustainable and economical. Research indicates that mixing substrates can enhance production compared to using a single substrate. Therefore, this study focuses on optimizing cellulase production using a mixture of agro-wastes [8].

Objective and Novelty:

The aim of this study is to develop a cost-effective method for cellulase enzyme production using a mixture of agro-industrial substrates through solid-state fermentation with Aspergillus niger. This study focuses on optimizing the substrate combination and fermentation parameters to enhance cellulase yield and reduce dependency on imported enzymes in developing countries. The novelty of this research lies in introducing a new and economical approach for industrial cellulase production. By optimizing a blend of five agro-industrial substrates with Aspergillus niger using response surface methodology (RSM), the study identified an optimal substrate mix that significantly improves cellulase activity compared to individual substrates. The optimized mixture and refined fermentation conditions achieved a notable increase in enzyme activity (0.205 IU/ml) over traditional methods (0.025 IU/ml), offering a viable solution for decreasing reliance on imported cellulase in developing countries.

Materials and Methods:

Selection and Preservation of Strain:

Aspergillus niger was chosen for enzyme production. Pure cultures of A. niger (FCBP 787) were sourced from the First Fungal Culture Bank of Pakistan, Institute of Plant Pathology, Punjab University Lahore, Pakistan. For preparing Potato Dextrose Agar (PDA):

- Peel and dice 200 grams of potatoes into small pieces, then boil them in 500 milliliters of distilled water for approximately 30 minutes.
- After boiling, filter the potato infusion through cheesecloth or a strainer to remove solid potato pieces and adjust the volume to one liter with distilled water.

- Dissolve 20 grams of dextrose (glucose) and 15 grams of agar into the potato infusion.
- Heat the mixture while stirring until the agar fully dissolves, typically bringing the solution to a boil.
- Once the agar is dissolved, pour the PDA solution into flasks or bottles suitable for autoclaving.
- Sterilize the medium by autoclaving at 121°C (15 psi) for 15-20 minutes.
- After autoclaving, cool the PDA to approximately 45-50°C without allowing it to solidify.
- Pour the cooled PDA into sterile Petri dishes or culture tubes in a sterile environment, such as a laminar flow hood, to prevent contamination.
- Allow the agar to solidify at room temperature.

The prepared PDA plates or slants were stored at 4°C. This medium provides all necessary nutrients for A. niger growth. The PDA media was solidified in Petri plates, and the fungus was introduced using the streaking method, incubated at 35°C for 3 days. After 5 days of incubation, adequate growth of A. niger was observed [6]. The flow diagram of the proposed methodology is shown in Figure 1.

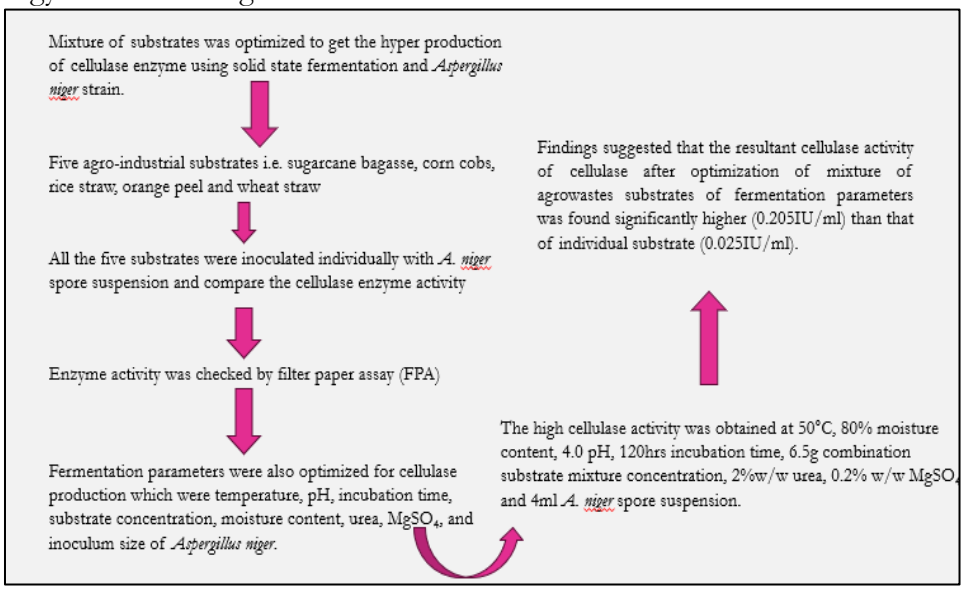

Figure 1: Flow Diagram of the proposed methodology

Collection of Substrates:

Five substrates—sugarcane bagasse, corn cobs, wheat straw, rice straw, and orange peel powder—were collected. Each substrate was washed, dried, ground into a powder, and stored in plastic bags for future use.

Preparation of Fungal Spore Suspension:

A PDA broth medium was prepared in a conical flask using the same ingredients as the PDA medium, excluding agar. The medium was sterilized in an autoclave at 121°C for 20 minutes after being sealed with a cotton plug and aluminum foil. Following sterilization, the medium was inoculated with A. niger from Petri plates. It was then placed in an orbital shaker at 28°C, and after 3 days, a spore suspension was obtained. This spore suspension was stored in a refrigerator and refreshed every 15 days to maintain strain viability [7].

Optimization of Mixture of Substrates:

Each of the five selected substrates was inoculated separately with 5g of each to compare the enzyme activity of individual substrates against combinations. Optimization was performed using 32 combinations of the substrate mixture, designed through Response Surface Methodology (RSM). These trials are detailed in Table 1.

Table 1: Combination of different substrates to optimize suitable mixture for hyper production of cellulase

Sr. no.	Factor 1 Rice straw(g)	Factor 2 Corn cobs(g)	Factor 3 Sugarcane bagasse(g)	Factor 4 Wheat straw(g)	Factor 5 Orange peels(g)
1	1	1	1	1	4
2	4	1	1	1	1
3	1	4	1	1	1
4	4	4	1	1	4
5	1	1	4	1	1
6	4	1	4	1	4
7	1	4	4	1	4
8	4	4	4	1	1
9	1	1	1	4	1
10	4	1	1	4	4
11	1	4	1	4	4
12	4	4	1	4	1
13	1	1	4	4	4
14	4	1	4	4	1
15	1	4	4	4	1
16	4	4	4	4	4
17	0	2.5	2.5	2.5	2.5
18	5.5	2.5	2.5	2.5	2.5
19	2.5	0	2.5	2.5	2.5
20	2.5	5.5	2.5	2.5	2.5
21	2.5	2.5	0	2.5	2.5
22	2.5	2.5	5.5	2.5	2.5
23	2.5	2.5	2.5	0	2.5
24	2.5	2.5	2.5	5.5	2.5
25	2.5	2.5	2.5	2.5	0
26	2.5	2.5	2.5	2.5	5.5
27	2.5	2.5	2.5	2.5	2.5
28	2.5	2.5	2.5	2.5	2.5
29	2.5	2.5	2.5	2.5	2.5
30	2.5	2.5	2.5	2.5	2.5
31	2.5	2.5	2.5	2.5	2.5
32	2.5	2.5	2.5	2.5	2.5

Sterilization and Inoculation of Substrates:

A mixture of substrates in the proportions specified in Table 2 was placed into 32 flasks. Each flask was moistened with distilled water equivalent to the substrate weight and then autoclaved for 2 hours. After sterilization, all 32 flasks were inoculated with 3 ml of A. Niger spore suspension and incubated at 35°C for 5 days [6].

Extraction of Cellulase:

Following 5 days of fermentation, the enzyme was extracted by adding 50 ml of distilled water to each flask and shaking the mixture for 30 minutes on an orbital shaker. The biomass was then filtered through filter paper into falcon tubes. These tubes were centrifuged at 10,000 rpm for 20 minutes. The supernatant was collected for further experiments, while the pellet was discarded [8]. Cellulase enzyme activity was measured using Whatman filter paper No. 1 as the substrate. In a test tube, a reaction mixture consisting of 0.5 ml enzyme, 1 ml citrate buffer (pH 4.8), and a filter paper strip was prepared. The test tube was incubated at 50°C in a water bath.

After 1 hour, the reaction was halted by adding 3 ml of DNS reagent and boiling for 5 minutes. Absorbance was measured at 560 nm after diluting with 16 ml of water, and the results were expressed in IU/ml. The substrate mixture with the highest enzyme activity was selected for further experiments [8].

Optimization of Fermentation Parameters:

Various factors affecting enzyme production were optimized using Response Surface Methodology (RSM). The parameters studied included incubation temperature, pH, inoculum size, fermentation duration, moisture content, magnesium source, nitrogen source, and substrate concentration. Trials were conducted over different parameter ranges, and enzyme activity was assessed using the filter paper assay (FPA).

Statistical Analysis:

Minitab-19 was utilized for response surface methodology to optimize substrate combinations and fermentation parameters, as well as to generate contour and 3-D surface plots. Analysis of Variance (ANOVA) was performed using Minitab-19, while Excel 2010 was used to create graphs for optimizing fermentation parameters [9].

Results and Discussion:

Aspergillus Niger Growth on Petri Plates and Broth Media:

Aspergillus Niger was cultured on Petri plates, where significant growth was observed after 5 days of incubation (Figure 2). The A. Niger strain was then transferred from the Petri plates into broth PDA medium in shaking flasks. Yellow spores were visible after 3 days (Figure 2), indicating successful fungal growth on the PDA medium.

Figure 2: Growth of A. Niger on solidified PDA medium

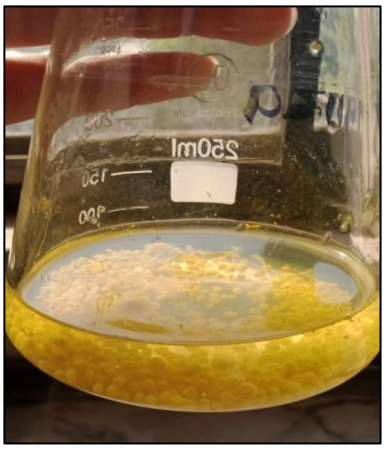

Figure 3: Spore suspension of A. Niger after 3 days of incubation

Collection of Substrates and Screening of Best Combination:

The enzyme activity of individual substrates was notably lower compared to the mixture of substrates. Among the individual substrates, rice straw exhibited the highest enzyme activity at 0.025 IU/ml, followed by sugarcane bagasse at 0.023 IU/ml (Figure 3). In contrast, the substrate mixture achieved an enzyme activity of 0.0921 IU/ml, surpassing the activities of individual substrates such as orange peels, rice straw, sugarcane bagasse, wheat straw, and corn cobs. The optimal substrate combination consisted of equal amounts of four substrates (2.5 g each), with rice straw in a higher amount of 5.5 g. Minitab-19 was employed to analyze cellulase activity using response surface design. Statistical analysis, including Analysis of Variance (ANOVA) and significance tests for each regression coefficient, was performed to validate the model equation and results, as detailed in Table 2. The model's R^2 value of 91.71% indicated a strong influence of substrate combinations on cellulase production.

Effect of Temperature on Cellulase Production:

The highest cellulase activity was 0.125 IU/ml at 50°C (Figure 4), while the lowest was 0.032 IU/ml at 20°C. Temperature significantly impacts cellulase production, as microorganisms

have specific temperature ranges for optimal growth. Enzyme activity increased with temperature from 20°C to 50°C but declined beyond 50°C.

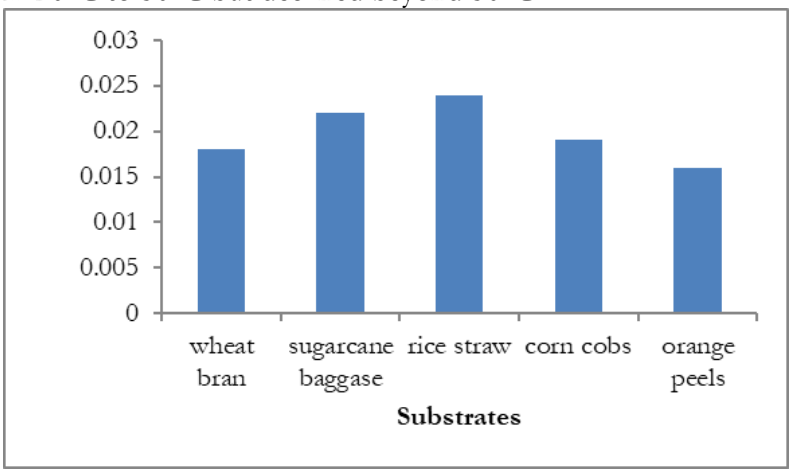

Figure 4: Graphical representation of cellulase activity obtained by rice straw, corn cobs, orange peels, wheat straw and sugarcane bagasse

Table 2: Response surface analysis showing interactions among enzyme activity vs. rice straw, corn cobs, sugarcane bagasse, wheat straw and orange peels

Source	DF	Adj SS	Adj MS	F- Value	P-Value
Model	20	0.002524	0.000126	5.68	0.003
Linear	5	0.000543	0.000109	4.89	0.013
rice straw	1	0.000131	0.000131	5.89	0.034
corn cobs	1	0.000075	0.000075	3.36	0.094
sugarcane bagasse	1	0.000065	0.000065	2.92	0.115
wheat straw	1	0.000239	0.000239	10.77	0.007
orange peels	1	0.000016	0.000016	0.73	0.410
Square	5	0.001492	0.000298	13.43	0.000
rice straw*rice straw	1	0.000780	0.000780	35.10	0.000
corn cobs*corn cobs	1	0.000334	0.000334	15.01	0.003
sugarcane bagasse*sugarcane bagasse	1	0.000004	0.000004	0.17	0.686
wheat straw*wheat straw	1	0.000427	0.000427	19.24	0.001
orange peels*orange peels	1	0.000028	0.000028	1.25	0.287
2-Way Interaction	10	0.000419	0.000042	1.89	0.156
rice straw*corn cobs	1	0.000093	0.000093	4.19	0.065
rice straw*sugarcane bagasse	1	0.000017	0.000017	0.76	0.403
rice straw*wheat straw	1	0.000024	0.000024	1.08	0.321
rice straw*orange peels	1	0.000000	0.000000	0.01	0.918
corn cobs*sugarcane bagasse	1	0.000042	0.000042	1.90	0.195
corn cobs*wheat straw	1	0.000010	0.000010	0.43	0.525
corn cobs*orange peels	1	0.000132	0.000132	5.95	0.033
sugarcane bagasse*wheat straw	1	0.000001	0.000001	0.05	0.827
sugarcane bagasse*orange peels	1	0.000095	0.000095	4.28	0.063
wheat straw*orange peels	1	0.000005	0.000005	0.23	0.642
Error	11	0.000244	0.000022		
Lack-of-Fit	6	0.000144	0.000024	1.20	0.430
Pure Error	5	0.000100	0.000020		
Total	**31**	**0.002769**			

R2 = 91.71% Adjusted R2= 75.12%

Effect of Inoculum Size on Cellulase Production:

Cellulase production peaked at 0.125 IU/ml with a 4 ml spore suspension (Figure 4). The enzyme activity was 0.0426 IU/ml with a 2 ml spore suspension and 0.07 IU/ml with a 6 ml spore suspension. Activity declined beyond 4 ml due to excess biomass consuming more nutrients, which reduces enzyme production efficiency.

Effect of Incubation Time on Cellulase Production:

The incubation time with substrates significantly influenced cellulase production. The highest enzyme activity was 0.125 IU/ml at 120 hours (Figure 4), while the lowest was 0.035 IU/ml at 48 hours.

Effect of Substrate Concentration on Cellulase Enzyme:

The optimal substrate concentration for maximum cellulase production was 6.5 g, with a maximum enzyme activity of 0.125 IU/ml. The minimum production was observed at 3 g (0.04 IU/ml). Production declined above 6.5 g due to insufficient biomass to utilize all available energy sources.

Effect of pH on Cellulase Yield:

The highest cellulase production was achieved at pH 4 (Figure 5), with enzyme activity reaching 0.205 IU/ml. The lowest activity was 0.042 IU/ml at other pH levels.

Effect of Moisture Content on Cellulase Production:

The maximum cellulase production occurred at 80% moisture content, yielding 0.205 IU/ml, while the minimum was 0.038 IU/ml at 20% moisture content (Figure 5). Optimal moisture content depends on the substrate concentration and microorganism used in fermentation.

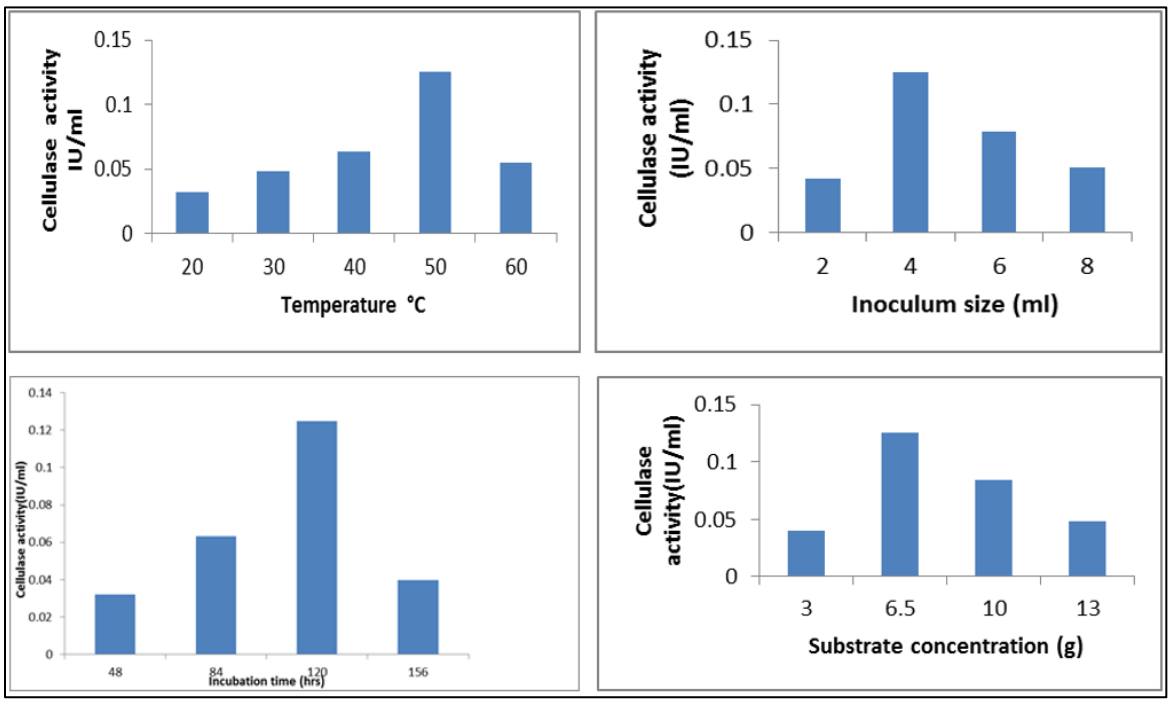

Figure 5: Effect of Temperature, Inoculum size, Incubation time and substrate concentration on Cellulase production from mixture of agrowastes

Effect of Urea on Cellulase Production:

The highest cellulase concentration (Figure 5) was achieved with 2% w/w urea, but production decreased at 2.5% due to pH fluctuations. Urea, as a nitrogen source, enhanced fungal cell growth, leading to increased enzyme activity from 1% to 2%. The maximum cellulase activity recorded was 0.205 IU/ml, while the minimum was 0.065 IU/ml.

Effect of MgSO4 on Cellulase Production:

The optimal concentration of MgSO4 was 0.2%, yielding the highest cellulase activity of 0.25 IU/ml (Figure 6). MgSO4 acts as an electron donor and a structural regulator, even in small amounts, contributing significantly to enzyme production.

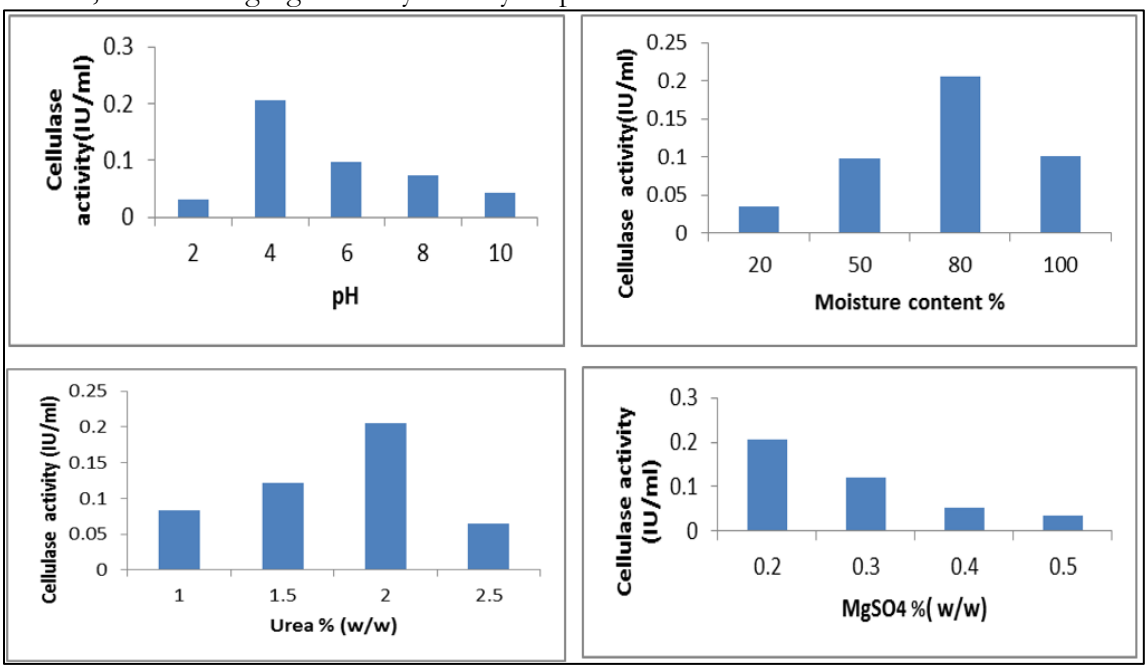

Figure 6: Effect of pH, Moisture content, Urea and MgSO4 on Cellulase production from mixture of agrowastes

Discussion:

Cellulase Production from Agro-Industrial Substrates:

Cellulase, crucial for industrial processes, can be produced from cellulosic waste, offering a sustainable and cost-effective alternative to expensive imports. Maximizing enzyme activity through cost-effective methods is essential. Research indicates that the optimal pH range for maximum cellulase activity is between 4.2 and 5.2. The highest cellulase activity occurs at 28°C, with pH 4.0 showing the best results when using rice straw and orange peel substrates with *Aspergillus niger*. Maximum β-glucosidase activity is also observed at pH 4.0, but it decreases sharply at pH 5.0. Studies using pineapple peel and orange peel with *Aspergillus niger* at pH 4 reported peak cellulase activities of 0.270, 0.200, and 0.173 mg/ml, respectively. Additionally, rice straw achieved the highest cellulase activity at 45°C, with a value of 20.35 μmol/min [10].

Cellulase and hemicellulase production by *Aspergillus niger* from lignocellulosic biomass peaked after 96 hours, with the highest enzyme secretion occurring after 7 days. Notably, *Aspergillus niger* achieved the greatest CMCase activity using fruit peel waste, consistent with earlier findings where wheat straw yielded the highest β-glucosidase (0.1320 IU/ml) and CMCase (0.225 IU/ml) after 24 hours. Under submerged fermentation conditions, *Aspergillus niger* produced 0.72 IU/ml CMCase and 0.43 U/ml FPase on wheat bran. The maximum CMCase activity of 0.499 IU/ml was recorded with a 4% wheat bran concentration. The optimal titers of FPase, CMCase, and BGL obtained from a rice bran and wheat straw combination were 2.632, 2.478, and 2.984 IU/ml, respectively. Furthermore, *A. awamori* demonstrated activity levels of 14.88 IU/ml on wheat bran.

The optimal agro-industrial substrate combination for cellulase production by Aspergillus niger NCIM 548 consisted of equal amounts of sugarcane bagasse, corn cobs, orange peels, and wheat straw, with rice straw being twice the amount of the others. This highlights the critical role of substrate composition in enhancing cellulase production [11]. Response Surface

Methodology (RSM) further optimized these substrate combinations, significantly increasing cellulase activity compared to using individual substrates [12].

Moreover, the fermentation parameters for cellulase production were optimized. Key factors such as temperature, pH, incubation time, substrate concentration, moisture content, urea, $MgSO_4$, and inoculum size were adjusted for optimal results [11]. The highest cellulase activity was recorded at 50°C, 80% moisture content, pH 4.0, 120 hours of incubation, 6.5 g of substrate mixture, 2% w/w urea, 0.2% w/w $MgSO_4$, and 4 ml of A. niger spore suspension. These findings underscore the importance of fine-tuning both substrate combinations and fermentation parameters to enhance cellulase production [13].

Conclusion:

The results demonstrate that using a mixture of agro-industrial wastes, such as rice straw, orange peels, corn cobs, and wheat straw, for cellulase production is more efficient than using a single substrate. Additionally, optimizing various fermentation parameters, including temperature, pH, inoculum size, substrate concentration, moisture content, incubation time, urea, and $MgSO_4$, significantly boosts cellulase production, proving the method's suitability for scalable, efficient cellulase production.

Recommendations:

This study provides valuable insights for industrial applications, advocating the use of agro-industrial substrate mixtures instead of expensive artificial carbon and nitrogen sources. Recommended actions include:

- Utilizing agro-waste mixtures for hyper-production of cellulase.
- Enhancing commercial cellulase production through optimized fermentation conditions.
- Addressing the high costs and limited availability of cellulase by employing selected agro-wastes, which can also benefit bioethanol production.

References:

[1] Srivastava, N., Srivastava, M, Alhazmi, A, Kausar, T, Haque, S, Singh, R, Ramteke, P. W, Mishra, P. K, Tuohy, M, Leitgeb, M. 2021. Technological advances for improving fungal cellulase production from fruit wastes for bioenergy application: A review. Environmental Pollution, 287, 117370.

[2] Kuhad, RC., Gupta, R., Singh, A. 2011. Microbial cellulases and their industrial applications. Enzyme.

[3] Han, W., He, M. 2010. The application of exogenous cellulase to improve soil fertility and plant growth due to acceleration of straw decomposition. Bioresour Technol 101:3724–3731

[4] Caldeira, C., Vlysidis, A., Fiore, G., De Laurentiis, V., Vignali, G, Sala, S. 2020. Sustainability of food waste biorefinery: A review on valorisation pathways, techno-economic constraints, and environmental assessment. Bioresource Technology, 312, 123575.

[5] Ogbonna, A. I., Sila, M. D., Onyimba, I. A, Ogbonna, C. I. C. 2017. Extracelullar Cellulase Production by Solid State Bioprocesses of Artemisia annua L. Agro Waste. Journal of Academia and Industrial Research (JAIR), 6(4), 54-61.

[6] Shrestha, S., Chio, C., Khatiwada, J. R., Kognou, A. L. M, Qin, W. 2022. Formulation of the agro-waste mixture for multi-enzyme (pectinase, xylanase, and cellulase) production by mixture design method exploiting Streptomyces sp. Bioresource Technology Reports, 19, 101142.

[7] Usman, A., Mohammed, S, Mamo, J. 2021. Production, optimization, and characterization of an acid protease from a filamentous fungus by solid-state fermentation. International Journal of Microbiology, Vol. 2021, 1-12.

[8] Rashid, M., 2016. Characterization and immobilization of cellulases by screening agro-industrial wastes, MPhil thesis of University of Gujrat, Pakistan.

[9] Luo, X., Xia, J, Jiang, X, Yang, M., Liu, S. 2019. Cellulose-based strips designed based on a sensitive enzyme colorimetric assay for the low concentration of glucose detection. Analytical Chemistry, 91(24), 15461-15468.

[10] Islam, F., Roy, N. 2018. Screening, purification and characterization of cellulase from cellulase producing bacteria in molx`asses. BMC Research Notes, 11(1), 1-6.

[11] Prasanna HN, Ramanjaneyulu G, Rajasekhar Reddy B. 2016. Optimization of cellulase production by Penicillium, Biotech.6, 162.

[12] Kumar, Sanjay, Sharma, H, Sarkar, B. C. 2011. Effect of substrate and fermentation conditions on pectinase and cellulase production by Aspergillus niger NCIM 548 in submerged (SmF) and solidstate fermentation (SSF). Food Science and Biotechnology ,20, 1289-1298.

[13] Abdullah, J, Greetham, D, Pensupa, Nattha, Tucker, G, Du, C. 2016. Optimizing Cellulase Production from Municipal Solid Waste (MSW) using Solid State Fermentation (SSF). Journal of Fundamentals of Renewable Energy and Applications, 10-12.