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Optimization and Partial Purification of Cellulase for Enhanced Lignocellulosic Biomass Hydrolysis

Abedhusain Seliya[1], Urjitsinh Zala[1,2], Vijay Jagdish Upadhye[3], Anupama Shrivastav[1]*

[1]Department of Microbiology, Faculty of Life Health and Allied Sciences, Institute for Technology and Management-ITM Vocational University, Vadodara, Gujarat

[2]Department of Medical Laboratory Technology, Faculty of Life Health and Allied Sciences, Institute of Technology and Management-ITM Vocational University, Vadodara, Gujarat, India

[3]Research and development Cell (RDC), Parul Institute of Applied Sciences (PIAS), Parul University (DSIR-SIRO Recognized), Vadodara, Gujarat, India

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Abstract

Cellulase enzyme plays a critical role in the bioconversion of lignocellulosic biomass into fermentable sugars for bioethanol production and agricultural waste valorisation. The present study is based on the evaluation of the best carbon and nitrogen source with their optimum concentrations to maximize cellulase production followed by partial purification of the enzyme to enhance its activity. The optimum nitrogen and carbon sources were 0.4% tryptone and 3% wheat bran extract, attaining a enzyme hydrolysis zone of 0.67 cm. Enzyme activity was improved after partial purification by Ammonium sulphate precipitation (0–70%) and dialysis. Enzymatic hydrolysis using 15% (w/v) cellulase resulted in a substantial enhancement in reducing sugar yield as quantified by the DNS assay. These results highlight the potential application of cellulase in improving animal feed quality and bioethanol production to support waste utilization and renewable energy development.

Keywords: *Cellulase, Ammonium Sulphate precipitation, Dialysis*

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1. INTRODUCTION

The exponential rise in global biomass waste is largely from agricultural, forestry, and industrial activities. It is one of the natural resources that presents a lot of biomass available and that is unexploited. One of these renewable sources is the lignocellulosic waste, which can be used on multiple applications. It is made up of three closely joined components: cellulose, hemicellulose and lignin. These structure elements provide the rigidity of the plant cell wall, but also render biomass recalcitrant. Lignocellulosic residues include wheat straw, sugarcane bagasse, rice husks, and corn stover. These biomasses are usually disposed ofbyopen burning or landfilling, causing environmental concerns such as air pollution. It is a rich source of polysaccharides, but due to intractable rigidity, it is hard to process and thus limiting its utilization.

Lignocellulosic biomass can be used to produce bioethanol because of its high content in cellulose, feed for livestock, and various other industrial applications (Gupta *et al.*, 2020). However, the lignin barrier and crystalline cellulose regions limit enzyme accessibility, making its downstream processing difficult. Therefore, its structural intricacy needs to be overcome so that it can be efficiently utilized as a raw material for commercial purposes. Overcoming this problem not only requires development of new pretreatment technologies but also development of reliable enzymatic solutions that can overcome structural resistance without generating environmentally harmful by-products.

Enzymatic hydrolysis using cellulase is one of the most promising approaches for converting lignocellulosic biomass into high-value bio-based products. The process takes place through a multi-enzyme system called cellulase that consists of endoglucanases, exoglucanases, and β-glucosidases that work in synergy to convert cellulose into simple sugars such as glucose. This enzymatic pathway offers a sustainable alternative to harsh chemical methods, enabling cleaner conversion of lignocellulosic substrates and does not generate any toxic by-products. According to recent studies, cellulase treatment could greatly facilitate the release of sugar from lignocellulosic biomass for bioethanol and animal feed improvement(Singhania *et al.*, 2017).

Cellulase is primarily utilised in feeding livestock by degrading fibrous plant material into fermentable sugars, facilitating better nutrient access. Agro-residues with a high content of structural carbohydrates are hard for animals to digest. Such residues are treated with cellulase that increases its energy value and reduces theirfibre content, making it a more effective feed source.(Pandey *et al.*, 2021) Likewise, during bioethanol production, hydrolysis by cellulases is responsible for the conversion of sugar-rich residues, including sugarcane bagasse, to fermentable sugars, which can be used in subsequent microbial fermentation processes. This conversion is influenced by enzyme concentration, reaction conditions, and biomass pretreatment.

However, large-scale implementation of cellulase technology is hindered by the economic burden of enzyme production. High costs associated with microbial fermentation, suboptimal nutrient utilisation, and variability in enzyme yield remain major bottlenecks. Therefore, optimising culture conditions with carbon and nitrogen source selection, their optimised concentrations, and fermentation parameters while enhancing enzyme activity and purification strategies, is imperative to advance cost-effective bioconversion processes.

This study aims to address these gaps by investigating the role of optimised cellulase treatment in the valorization of lignocellulosic waste. This study investigates the potential role of cellulase treatment in the valorization of lignocellulosic waste.By integrating enzyme production enhancement with application-specific evaluations for livestock feed improvement and bioethanol generation, this work aims to identify the most effective carbon and nitrogen sources, determine their optimal concentrations, and improve both the purification process and catalytic efficiency of the target enzymes. This work contributes to the development of scalable, sustainable bioprocesses aligned with circular bioeconomy principles.

2. MATERIALS AND METHODS

2.1 Change in Carbon and Nitrogen Source

The cellulase producing microorganism used in this work was isolated from soil sample and preserved in Nutrient agar. Inoculum was prepared by inoculating one colony into nutrient broth and incubating at 37 ° C for 24 hours. Various carbon sources (Sugarcane Bagasse Extract, Wheat Bran Extract and Coconut shell extract) at 1% concentration and nitrogen sources (Tryptone, Peptone, Urea) at 0.2% concentration were supplemented in the basal medium(g/l: CMC - 10g, Glucose – 5g, Tryptone - 2g, Yeast Extract -10g, KH_2PO_4 - 4g, Na_2HPO_4 - 4g, $MgSO_47H_2O$ – 0.2g, $FeSO_47H_2O$ – 0.004g, $CaCl_22H_2O$ – 0.001g, pH 7.0) to optimize cellulase production. Fermentation was performed in 250 ml flask with 50 mL of optimized medium and 96Hours at 37 ° C and 120 RPM.Crude enzyme was extracted by centrifuging the media at 10,000 RPM at 4°C and collecting the supernatant. 100 µL of supernatant was pipetted in CMC agar plates by well diffusion method and assessed for cellulase activity.

2.2 Change in Carbon and Nitrogen Source Concentration

To optimize cellulase production, the effect of carbon and nitrogen source concentration was investigated. Best Carbon source (3%, 5%, and 7%) and nitrogen source (0.4%,0.6%, and 0.8%) was supplemented in the production media respectively. Isolate V4 was used for further production based on maximum zone of clearance. Production was carried out for 96 Hours followed by centrifugation of samples at 10,000g for 10 minutes. The supernatant was used as the crude enzyme for screening.

2.3 Media Standardization

Media standardization was carried out based on the best carbon and nitrogen sources from the optimization experiments, including their optimum concentrations. Fermentation was carried out in duplicates in 2L Erlenmeyer flask containing 400mL of the standardized medium, and incubated at 37 ° C under 120 rpm for 96 Hours.

2.4 Partial Purification of Cellulase

Crude cellulase was partly purifiedby ammonium sulphate precipitation. Ammonium sulphate was gradually added to crude enzyme to achieve saturation up to 80% at 20°C. Precipitates of enzymeswere formed overnight at 4°C. The enzyme precipitates were redissolved in 50mM Phosphate Buffer pH 7.0.After ammonium sulphate precipitation, this precipitate solution was dialysed against phosphate buffer pH 7.0 solution at 4°C.

2.5 SDS PAGE for molecular weight determination

Molecular weight determination by Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in a 3-mm slab gel with 12% Polyacrylamide separating gel and 4% stacking gel. Samples have been treated with loading dye and directly loaded on the Gel. 40 µl sample volume is loaded on the gel. The gel was stained with Coomassie brilliant blue G-250 and destained. The low range molecular weight marker was used for the molecular weight determination. Molecular weight of cellulase was find out from MW marker standard curve.

2.6 Treatment of Agriculture Waste for its use as Animal Feed

- Agriculture waste from wheat farm (WFAW) was taken and grinded into fine powder using a grinder.
- Precisely weighed amount of 5g WFAW was dissolved in 50ml distilled water in a 250ml flask.
- 5ml dialysed enzyme solution was added to the flask containing WFAW, maintaining a solid-liquid ratio of 1:10 (w/v). The flasks were incubated in an incubator shaker for 2 hours at 50°C.
- After the incubation 1 ml hydrolysate sample was taken from the flask to estimate the concentration of simple sugar. 1mL of sample was taken for DNS analysis before carrying out the enzymatic hydrolysis as control.
- 1.5mL of DNS reagent was added in the test tube and kept in water bath at 100 ° C for 10 minutes.
- After 10 min, tubes were cooled and the absorbanceof the sample was read at 540 nm. 1mL of solution was taken from test tubes and diluted with 9ml distilled water to measure the absorbance.

Calculation:
Glucose concentration was measured and theglucose released was calculated by:
Glucose (mg/g WFAW) = (Glucose Concentration (mg/ml) * Volume of Hydrolysate (ml))/Weight of WFAW

2.7 Treatment of Sugarcane Bagasse for its use as raw material in Bioethanol production

- Precisely weighed amount of 10g Sugarcane Bagasse (SGB) was taken and dissolved in 100mL distilled water in a 500mL flask.
- Different concentrationsof 5mL, 10mL, and 15mL dialysed enzyme solution was added to the flask containing Sugarcane Bagasse. The flasks were incubated in an incubator shaker at 50 °C for 24 h.
- A 1-mL volume of hydrolysate sample was removed from the flask after incubation for determination of the concentration of simple sugar. A sample of 1 mL was taken for DNS analysis before the enzymatic hydrolysis to serve as control.
- In the test tube, 1.5 mL of DNS reagent was added and boiled in the water bath for 10 min at 100 ° C.
- The tubes were cooled and after 10 min the absorbance was measured at 540 nm. From the test tubes, 1 mL aliquots of the solution were taken and diluted to 9 mL with the distilled water for absorbance reading.

Calculation:
Glucose concentration was measured and theglucose released was calculated by:
Glucose (mg/g SGB) = (Glucose Concentration (mg/ml) * Volume of Hydrolysate (ml))/Weight of SGB

3. RESULT

3.1 Change in Carbon and Nitrogen Source

The findings show that of all nitrogen sources tested tryptone produced the highest cellulase enzyme with a zone of hydrolysis of 0.66 cm (graph 3.1). Among, all the carbon sources tested, wheat bran extract showed the highest activity of cellulase with a hydrolysis zone of 0.6 cm, suggesting its potential as the most optimal carbon substrate(graph 3.2).

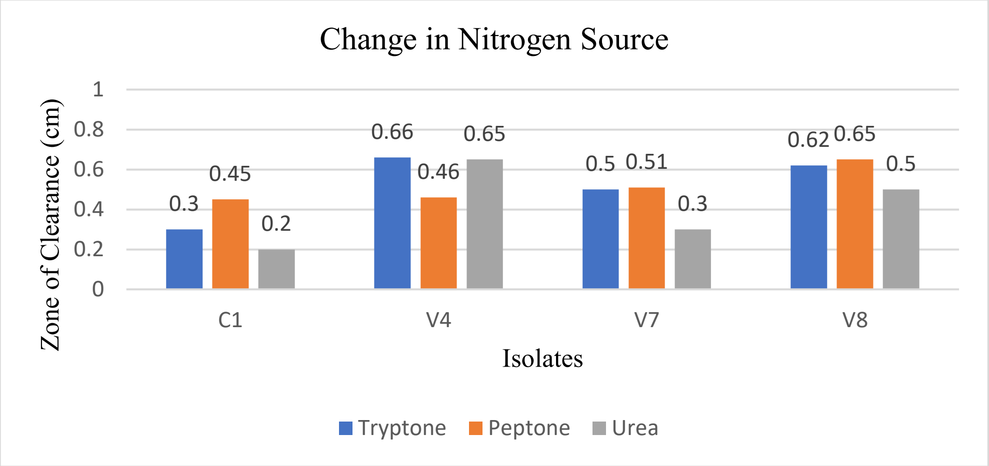

Figure 1. Cellulase production by isolates with change in Nitrogen source

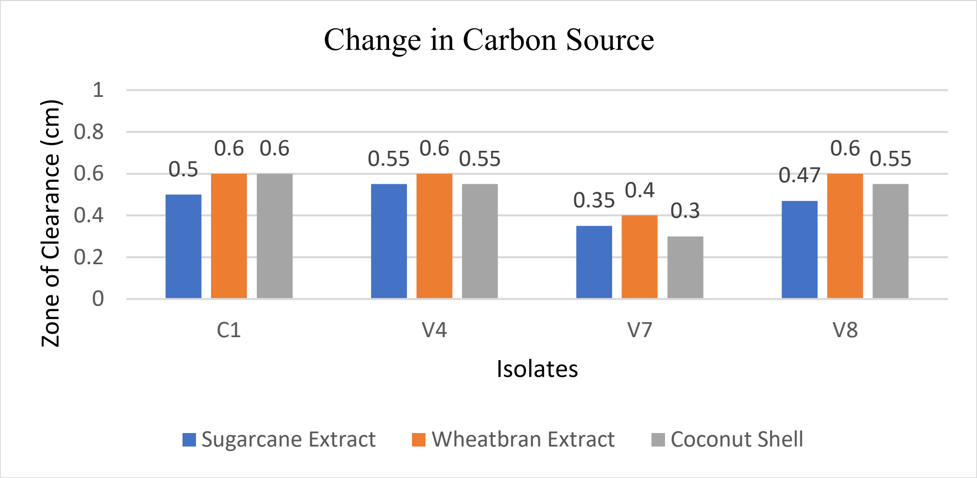

Figure 2. Cellulase production by isolates with change in carbon source

3.2 Change in Carbon and Nitrogen Source Concentration

The results showed that 0.4% nitrogen source concentration was optimal for cellulase production and resulted in the highest hydrolysis zone of 0.67 cm, (graph 3.3) implying its effectiveness for promoting microbial enzyme secretion. Similarly,carbon source concentration of 3% resulted in a maximum hydrolysis zone of 0.57 cm (graph 3.4) suggesting that this concentration is suitable for improving cellulase production. The higher enzyme yields at these concentrations can be attributed to the balanced nutrient supply, where sufficient nitrogen favours protein synthesis and enzyme formation while an adequate carbon source provides energy for microbial growth and cellulase production.

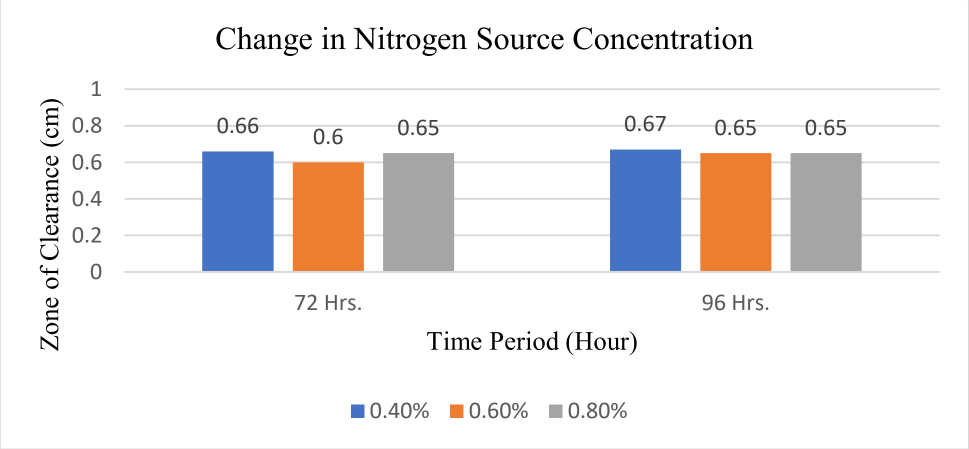

Figure 3. Cellulase production with change in nitrogen source concentration.

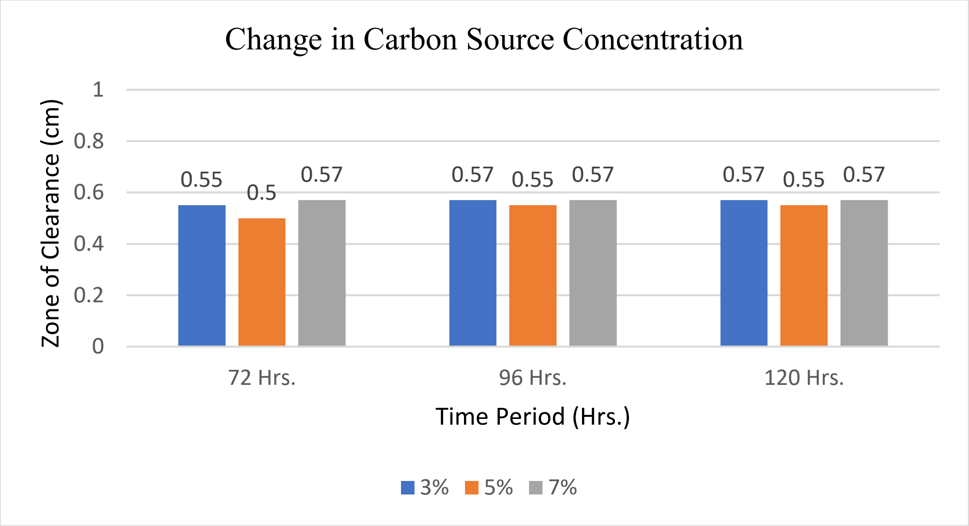

Figure 4. Cellulase production with change in carbon source concentration

3.3 Media Standardization

Tryptone and wheat bran extract were found to be the nitrogen and carbon source of choice among the test sources and in optimized media for maximum cellulase production and was standardized at 0.4% and 3% respectively. The cellulase activity of 0.67 cm hydrolysis zone (graph 3.5) was observed for this combination which is the highest amongst all the combinations studied. The amount of enzymeproduced in this optimized medium is because of the protein laced with various essential amino acids and peptides that are necessities for microbial growth, derived from tryptone, whereas the wheat bran extract functions as an efficient carbon source for enzyme production. A standardized formulation represents a consistent nutrition profile conducive to optimal cellulose secretion and activity.

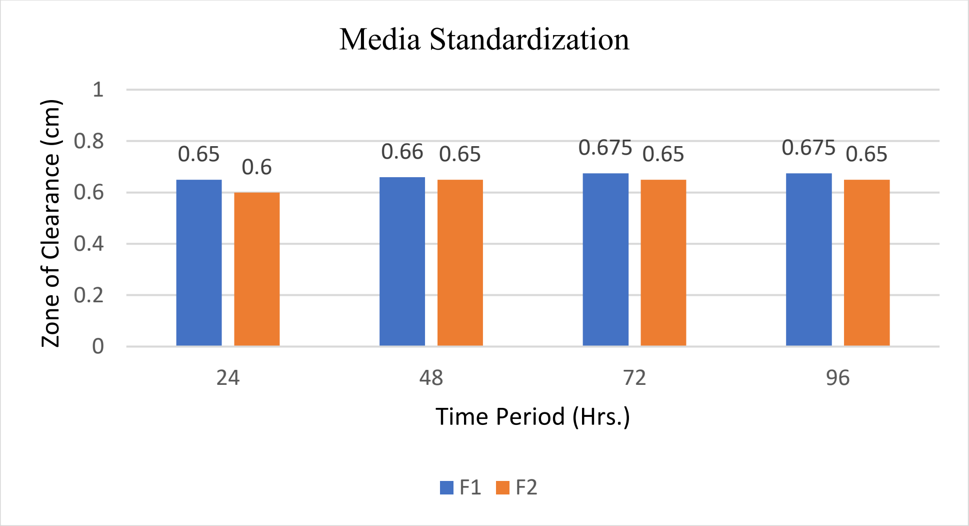

Figure 5. Cellulase production with media standardization.

3.4 Partial Purification of Crude Cellulase

The enzyme activity of the partial purification of crude cellulase by ammonium sulphate precipitation and dialysis gradually increased. The hydrolysis zone of the 0–70% ammonium sulphate fraction was 0.72 cm, indicating that effective cellulase precipitation occurred. However, in a 70–80% fraction, the enzyme activity decreased and produced a smaller zone of 0.57 cm indicating that in more concentrated salt solution, the enzyme may aggregate or denature. Cellulase activity was increased highly after the dialysis with a hydrolysis zone of 0.8 cm, which demonstrated that excess salts and low-molecular-weight impurities were completely removed, which would lead to greater stability of the enzyme and higher reacting activity.

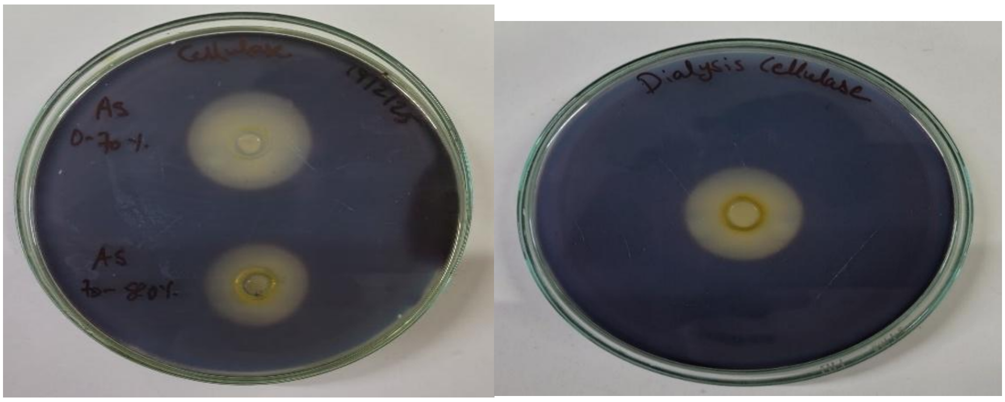

Figure 6. (A) Enzyme Screening of Ammonium sulphate purification fractions, (B) Enzyme screening of dialysed cellulase

3.5 SDS PAGE for molecular weight determination

The molecular weight of the partially purified cellulase enzyme was estimated using SDS-PAGE. The electrophoresis profile showed a distinct protein band corresponding to the cellulase sample. The relative mobility (Rf value) of the cellulase band was calculated to be 0.45. By comparing its migration with that of standard protein markers, the molecular weight of the cellulase was determined to be approximately 53.7 kDa.

Table 1: molecular weight determination

Enzyme	Distance Travelled by Band (cm)	Rf Value	Log MW	Molecular Weight (KDa)
Dialysed Cellulase	3.4	0.45	4.73	53.7
Crude Cellulase	3.4	0.45	4.73	53.7
AS 0-70% Cellulase	3.4	0.45	4.73	53.7
AS 70-80% Cellullase	3.4	0.45	4.73	53.7

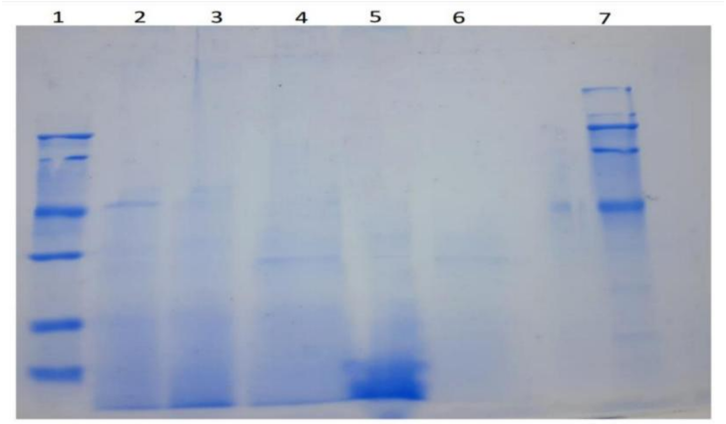

Figure 7. SDS PAGE of Crude and Partially Purified Cellulase (1 – Protein MW Marker (Low), 2 – Dialysed Cellulase, 3 – Crude Cellulase, 4 – AS 0-70% Cellulase, 5 – Standard Cellulase, 6 – AS 70-80% Cellulase, 7 – Protein MW Marker (High))

3.5 Treatment of Agriculture Waste for its use as Animal Feed

The treatment of wheat farm agricultural waste (WFAW) with cellulase at different concentrations showed significant improvement in sugar release for enhancing nutritional properties to use as feedstock for animals. The sugar concentrations of WFAW increased by using 5% cellulase, 10% cellulase, and 15% cellulase, respectively, 90% 100%, and 109% indicating a dose-dependent enzymatic hydrolysis effect (Table 3.1). A maximum sugar yield of 15% cellulase maycorrelate to the breakdown of complex polysaccharides into fermentable sugars, thereby improving the digestion and energy content of the easily digestible form of waste. The process to enzymatically pretreat agricultural residues could ideally turn waste into an effective livestock feed, and sustainable feed source, thus reducing waste.

Table 2. Amount of Sugar released by enzymatic treatment on WFAW

Treatment	**Concentration (mg/g WFAW)**	**Sugar Conc. Increase %**
Control	0.43	
5% Cellulase	0.82	90%
10% Cellulase	0.86	100%
15% Cellulase	0.9	109%

3.6 Treatment of Sugarcane Bagasse for its use as raw material in Bioethanol production

Enzymatic hydrolysis of sugarcane bagasse with cellulase (at various concentrations) resulted in great levels of sugar in solution therefore they may be used as raw material for bioethanol production. The concentration of sugar, determined by the DNS method, increased by 100% at 5% cellulase, 125% at 10% cellulase, and 250% at 15% cellulase, (Table 3.2) which demonstrates that the elevation of the enzyme concentration led to an enhanced reduction of cellulose to fermentable sugars. The maximum sugar yield of 15% cellulase indicates that lignocellulosic biomass enzymatic degradation is optimal, thus improving the suitability of the hydrolysate for subsequent ethanol fermentation. These findings confirm the potential of sugarcane bagasse as an economical and sustainable feedstock for bioethanol production, promoting advancements in renewable energy sources.

Table 3. Amount of Sugar released by enzymatic treatment on SGB

Treatment	**Concentration (mg/g SGB)**	**Sugar Conc. Increase %**
Control	0.16	
5% Cellulase	0.32	100%
10% Cellulase	0.36	125%
15% Cellulase	0.56	250%

4. CONCLUSION

The study reported successful optimization of cellulase production and application by determining 0.4% tryptone and 3% wheat bran extract as the best nitrogen and carbon source respectively, with achieving maximum hydrolysis zone of 0.67 cm. Ammonium sulphate precipitation (0–70%) and dialysis both increased the enzyme purity further, and the highest hydrolysis zone of 0.8 cm was achieved through dialysis.Cellulase had significant sugar release in agricultural waste treatment and, WFAW had 109% increase with 15% cellulase concentration and sugarcane bagasse with 250% increase. These discoveries underscore cellulase's efficacy in biowaste valorisation, advocating its application in animal feed improvement and eco-friendly biofuel creation.

REFERENCES

[1.] Gupta, A., Verma, J. P., & Kumar, D. (2020). Sustainable bioethanol production from lignocellulosic biomass: Challenges and future prospects. *Renewable and Sustainable Energy Reviews, 134*, 110177.

[2.] Singhania, R. R., Patel, A. K., Sukumaran, R. K., Larroche, C., & Pandey, A. (2017). Role and significance of β-glucosidases in the hydrolysis of cellulose for bioethanol production. *Bioresource Technology, 245*, 1246–1253.

[3.] Lynd, L. R., Beckham, G. T., Guss, A. M., Himmel, M. E., Bomble, Y. J., &Elander, R. T. (2017). Advances in consolidated bioprocessing for lignocellulosic biofuel production. *Current Opinion in Biotechnology, 45*, 202–211.

[4.] Bansal, N., Tewari, R., Soni, R., & Soni, S. K. (2012). Production of cellulases from *Aspergillus niger* under submerged and solid-state fermentation using agricultural residues. *Journal of Scientific & Industrial Research, 71(1)*, 51–56.

[5.] Kumar, R., Singh, S., & Singh, O. V. (2018). Bioconversion of lignocellulosic biomass: Biochemical and molecular perspectives. *Journal of Industrial Microbiology & Biotechnology, 45(4)*, 527–546.

[6.] Pandey, A., Soccol, C. R., Nigam, P., &Soccol, V. T. (2021). Biotechnological potential of agro-industrial residues: Sugarcane bagasse and wheat bran in fermentation processes. *Bioresource Technology, 302*, 122871