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Effect of alkaline and steam pre-treatment on saccharification of corn cob and production of cellullase from fungal consortium

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ABSTRACT

Lignocellulosic biomass as fossil fuel alternative comes with its challenges such as its inherent stability and recalcitrance. The use of commercial cellulase in the removal of lignin comes with high cost. This research seeks to answer questions on how alkaline and steam pre-treatment improve cellulose/glucose liberation from lignocellulosic biomass (corn cobs) using a consortium of *pichia kudriavzevii* and *cyberlindnera fabianii*.

In the current study, the effectiveness of steam and sodium hydroxide (NaOH) pre-treatment for reducing corn cob structure was examined, and the pre-treated biomass afterwards was exposed to the hydrolyzing activity of a consortium enzyme cocktail that was custom-formulated. The results of an analysis of composition showed that while alkaline pre-treated corn cob (APC) had 1.2% lignin, 75.8% cellulose, and 10.9% hemicellulose, steam pre-treated corn cob (SPC) had 2.5% lignin, 67.2% cellulose, and 25% hemicellulose. Lignin was eliminated from the biomass of corn cobs using both steam and NaOH pre-treatment. The hydrolyzing effect of the holocellulolytic enzyme cocktail, prepared with two multifunctional enzymes, was applied to the alkaline and steam pre-treated samples. This hydrolyzed SPCs more effectively than APC feedstocks, revealing that steam was a more effective pre-treatment attaining a remarkable 8.33 U/mg endoglucanase, 5.56 U/mg exoglycanase and 8.97U/mg beta-glucosidase levels (event 1) and glucose peak concentration of 0.433 mol/mL at 48 hours (event 2); according to a thorough examination of cellulase capacity and glucose levels.

Overall, the consortium enzyme cocktail effectively hydrolyzed agricultural feedstocks that had undergone alkaline pre-treatment, making it a desirable option for usage in the bioconversion procedure in the biorefinery sector. This study demonstrates an effective technique for turning agricultural waste (corn cob) into high-value products through effective and practical chemical pre-processing.

Keywords: biofuel, fermentation, fungal consortium, lignocellulose, pre-treatment

INTRODUCTION

In the last several years, the search for lignocellulosic material as an appealing substrate for bio-fuels and bioproduct production has been fueled by the worldwide shift in the direction of environmentally friendly and renewable resources alternatives (Go et al., 2019). Due to its widespread availability as agricultural and forestry leftovers, lignocellulosic biomass–which is pre-dominantly made up of cellulose, hemicellulose, and lignin–holds enormous promise. However, lignocellulose's resistant structure stands as a formidable barrier for its effective biological transformation into high-value products, demanding creative pre-treatment techniques to improve its enzymatic saccharification and further microbial digestion (Liu et al., 2022).

The production of nano-cellulose, which is important in biomedicine and public health, is a special area of interest for the possible application of the products of biodegradation. This is in drug design (drug excipients/drug delivery), tissue

bio-scaffolds for cellular culture, immobilization and protein recognition even to the level of macroscopic blood vessels and soft tissues engineering, up to skin, bones and tissues repair materials. This can be attributed to its characteristic low toxicity, biocompatibility, potential functionalization and ability to form hydrogels to mimic certain body processes (Kumar et al., 2016; Lin et al., 2014). Agricultural and allied industry biomass in recent times have been a new go-to area for novelty. Such biomass use in secondary microbial cultivation broths for generation of D-glucose useful in bioethanol industries, pharmaceutically important cellular proteins, enzymes and metabolites is being carried out (Ullah et al., 2019).

The ongoing search towards environmentally friendly substitutes for petroleum and other petroleum-based products has led to significant improvements in the lignocellulosic biomass utilization framework. Modern cutting-edge research is focused on perfecting pre-treatment techniques that increase cellulose's susceptibility to enzymatic hydrolysis. Traditional physical, chemical, and biological pre-treatment techniques have given way to cutting-edge methods that are more effective, have a smaller negative impact on the environment, and are more commercially viable (Ogechukwu et al., 2020).

The degradation of cellulose occurs through cellulase, xylanase, laccase, and peroxidases (Ogechukwu et al., 2020). Cellulose has to be broken down at different points in its chain to maximize glucose. Endoglucanases: (breaks the β-1, 4 glycosidic bonds at random internal positions in the chains, providing ends for exoglycanase to digest), exoglycanases, also known as cellobiohydrolases: (act on the reducing/non-reducing ends to produce cellobiose) and β-glucosidase: (breakdown cellobiose by hydrolyzing its β-(1-4) bond to finally produce D-glucose, an action that prevents cellobiose inhibition and favors the forward reaction leading to cellulase system efficiency (Liu et al., 2022; Singhania et al., 2012). A foremost setback relating to biomass containing lignocellulose in generation of D-glucose and subsequent bioethanol production is that commercially available cellulase enzymes are very costly (Van Dyk & Pletschke, 2012). Alternatives to the costly commercially available cellulase enzymes requires exploring microbes that possess the ability to produce these enzymes naturally. Making it important to screen for microorganisms with high cellulase production rates (Liu et al., 2022).

Alkaline pre-treatment is a technique that involves exposing biomass to alkali solutions like sodium hydroxide (NaOH). Because of the disruption of the lignin-hemicellulose matrix during this process, cellulose is more accessible and enzymatic hydrolysis is more effective. Numerous lignocellulosic feedstocks have undergone substantial alkaline pre-treatment research, which has resulted in higher glucose production and saccharification rates (Ifeanyi-Nze & Omiyale, 2022). Studies on maize cobs, in particular, have demonstrated that alkaline pre-treatment efficiently lowers lignin concentration and boosts cellulose-rich fractions, boosting ensuing bioconversion processes (Van Dyk & Pletschke, 2012).

In parallel, steam pre-treatment has become a promising substitute for delignifying lignocellulosic biomass. Using this technique, biomass is subjected to high-temperature steam under pressure, causing lignin to undergo physical and chemical changes (Olajuyigbe et al., 2019). As a result of the lignin structure being disturbed, cellulose is more easily accessible, which increases the substrate's susceptibility to enzymatic breakdown. Recent studies have shown that steam pre-treatment outperforms other methods for improving enzymatic digestibility and glucose liberation from a variety of lignocellulosic materials (Kiran et al., 2017). Additionally, steam pre-treatment benefits the environment by using fewer chemicals and producing less waste.

The findings of earlier studies highlight the significance of pre-treatment strategy optimization to improve enzymatic saccharification efficiency. Researchers have discovered methods that open the door for higher glucose yields and bioconversion yields by diving into the subtleties of alkaline and steam pre-treatment effects on biomass recalcitrance. Even said, further research is still required to determine the precise effects of various pre-treatment techniques on maize cobs, a widely accessible agricultural byproduct (Liu et al., 2022; Olajuyigbe et al., 2019)

This study attempts to clarify the potential of steam and alkaline pre-treatment for corn cobs (APCs) in light of previous research. And attempts to advance knowledge of lignocellulosic biomass pre-treatment techniques by investigating compositional changes, hydrolytic effectiveness, and glucose liberation. Furthermore, an understanding of the *pichia kudriavzevii* and *cyberlindnera fabianii* consortium's effectiveness in producing cellulase and saccharifying sugars will aid in the creation of sustainable bioconversion procedures, in line with the global transition to renewable energy sources and bioproducts.

Outcomes & Methodology

Results from past experiments on the pre-treatment of lignocellulosic biomass have been favorable. The results have demonstrated the effectiveness of alkaline and steam pre-treatment in lowering biomass resistance, which will increase sugar release during subsequent enzymatic hydrolysis. These findings have sparked more interest in how these pre-treatment procedures affect different lignocellulosic substrates, like corn cobs.

We examine the impact of steam and alkaline pre-treatment on the saccharification of maize cobs in this study. *pichia kudriavzevii* and *cyberlindnera fabianii* work together as a consortium to produce cellulase, which is then hydrolyzed on maize cobs that have already been treated. By pre-treating corn cobs with both alkaline and steam, we may examine how the composition of the cobs changes and how effectively enzymatic hydrolysis proceeds. Understanding lignin removal, cellulose and hemicellulose alteration by compositional analysis.

Aims

This study's main goal is to clarify the effects of alkaline and steam pre-treatment on the ability to saccharify maize cobs. Specific goals consist of:

1. Examining how the concentrations of lignin, cellulose, and hemicellulose have changed after alkaline and steam pre-treatment of maize cobs.

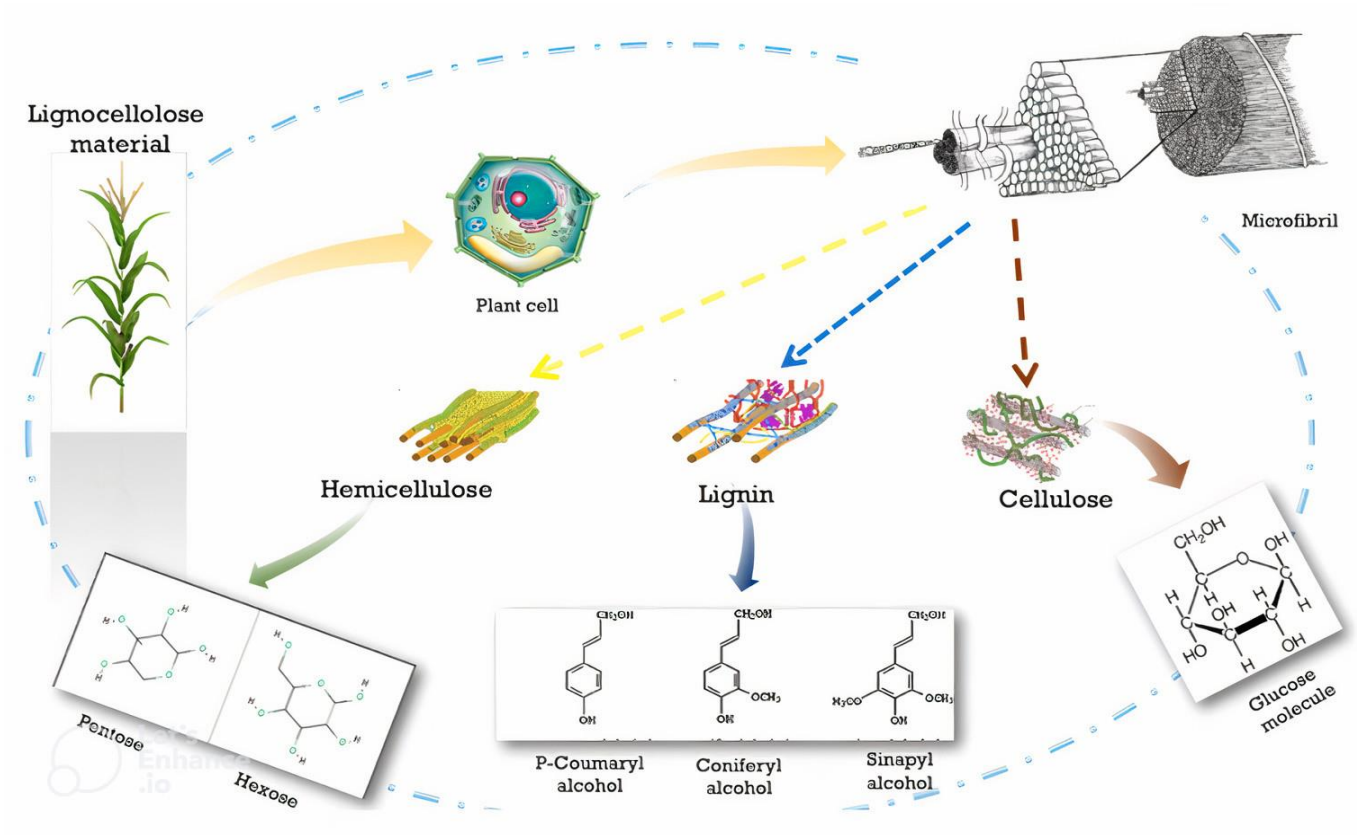

Figure 1. Lignocellulosic biomass & pre-treatment (adapted from Ifeanyi-Nze & Omiyale, 2022)

2. Measuring sugars levels as a gauge of saccharification capability on pre-treated maize cobs to assess the hydrolytic effectiveness of the fungal consortium's enzyme cocktail.
3. Evaluating how effectively steam and alkaline treatments increase glucose release and saccharification.
4. Production of efficient cellulases from the in-house microbial consortium in the enzymatic degradation process.

The results of this study will advance our understanding of lignocellulosic biomass pre-treatment techniques and offer insight on the advantages of steam and alkaline pre-treatment for maize cobs (**Figure 1**).

Additionally, knowledge of the *pichia kudriavzevii* and *cyberlindnera fabianii* consortium's efficacy in cellulase synthesis and saccharification will be useful for developing future the biological transformation procedures for the generation of sustainable biofuel and bioproducts.

MATERIALS & METHODS

Materials

Chemicals

Dextrose, amino acetic acid, citrucel, magnesium (II) sulphate, microcrystalline cellulose, acetic acid, NaOH, monopotassium phosphate (MKP), potassium dibasic phosphate, mercury (II) chloride, 4-nitrophenyl-β-D-glucopyranoside, manganese II chloride, calcium dichloride, ferrous chloride, ferric chloride, fraction V (bovine serum albumin), hydrochloric acid, peptone and Bradford reagent. (sigma-aldrich [Missouri, USA]). The other chemicals used in the study were of the best standard at hand.

Microbes

Fungal isolates from a rotting citrus tree located around Ijare Town, Ondo State, Nigeria served as the consortium microorganisms. Federal Institute of Industrial Research (FIIRO), Oshodi. Nigeria. Biotechnology unit identified the isolates as *pichia kudriavzevii* and *cyberlindnera fabianii* through their morphology and biochemistry described by Kloepper et al. (1991). They were cultured on potato dextrose agar slants and kept at 4 ºC.

Lignocellulosic substrate

Corn cobs were acquired from Oja-Oba in Akure, Ondo State, Nigeria, for use as substrates in this experiment. Corn cobs were dried and broken into small bits before being ground into finer particles and sieved using a 1.0 mm sieve.

Equipment

Shaking incubator (stuart), water bath (gallenkamp), UV-spectrophotometer (RAYLEIGH UV-1601), autoclave, crimson pH meter, refrigerated centrifuge (Centrikon, UK), thermo cool refrigerator, magnetic stirrer, and analytical balance were among the equipment utilized in the study.

Methods

Corn cob preparation and pre-treatment

Steam and alkaline pre-treatments were carried out on ground corn cobs (average size of 1 mm). 5% (weight/volume) corn cob in distilled water was autoclaved at 121 °C, 15 PSI for

60 minutes (steam treatment process), whereas 1% (weight/volume) corn cob in 1 normal sodium hydroxide was placed in a shaking incubator (150 rpm) for 24 hours at about 26 °C for sodium hydroxide treatment process. Both APCs and steam pre-treated corn cobs (SPCs) collection was by filtering and flooding with distilled water extensively to achieve a pH of seven. For crude enzyme synthesis and use of enzymes in biomass breakdown, treated and unprocessed corn cobs went through thorough draining. For compositional analyses, enzyme synthesis, use of enzymes in biomass breakdown, air-dried pre-processed and unprocessed substrates were utilized. Treatments were carried out in pairs.

Inoculum preparation

Pichia kudriavzevii and *cyberlindnera fabianii* seed cultures were grown in separate dishes by culturing a wire-loop measure from the mother slant into 30 mL media composed of final volume of 10.0 g/L of: ammonium nitrate 2.0 g/L, 2.0 g/L yeast extract, 0.8 g/L potassium dibasic phosphate, 0.5 g/L hydrous magnesium sulphate and 0.2 g/L dipotassium hydrogen phosphate. The media were shaken at 160 rpm for 72 hours at 30 °C in a stuart shaking incubator. By aseptically transferring the 72-hour-old seed culture of *pichia kudriavzevii* into the 72-hour-old seed culture of *cyberlindnera fabianii* at 30C, a two-member consortium *of pichia kudriavzevii* and *cyberlindnera fabianii* was formed. The consortium was brought together by incubating it for one hour at 30 °C in a shaking incubator at 160 rpm. It was then employed as an inoculum for corn cob degradation and cellulose synthesis.

Production of cellulase & saccharification of corn cob

Broths for unprocessed biomass, alkaline, steam processed biomass was done in separate 10 g/L flasks by weighing and stirring the media components listed 0.1 g/L yeast extract, 2 g/L potassium dibasic phosphate, 0.25 g/L peptone, 0.003 g/L zinc sulphate heptahydrate, 0.3 g/L urea, 0.005 g/L ferrous sulphate heptahydrate, 0.002 g/L Manganese sulphate heptahydrate, 0.3 g/L hydrous magnesium sulphate, 0.3 g/L calcium chloride, 0.002 g/L cobalt chloride, and 1.4 g/L ammonium sulphate up to 100 microliters broth adjusted to pH of six. Then 4 mL of the 4% weight/volume consortium was introduced into the sterile broth. Incubation of the media was carried out for 192 hours at 160 rpm. Sample cultures were collected at 48 hours interval and passed through Whatman filter paper filtration followed by centrifugation of the filtrate for 30 minutes at 10,000 rpm maintaining 4 °C temperature with the aid of a (eppendorf 5810R) cold centrifuge. The pellets were discarded, and the top liquid was labelled as the cellulase enzyme. Qualification of sugar released was done using (Miller, 1959) dinitro-salicylic acid (DNSA) method.

Assay for cellulase activity

The 1mL cellulase bioconversion reaction was carried with 0.15 mL cellulase enzyme, 0.40 mL of 1% standard substrates; microcrystalline cellulose, an exoglycanase, carboxyl methyl cellulose, an endo-glucans, 0.05M buffer of CH_3COONa at 4.8 pH. Process incubation was carried out for 30 minutes at 40 °C, then the process was halted by adding 0.45 mL DNSA reagent in the various reaction tubes then incubated with a water bath at a temperature of 40 °C for about five minutes with some modifications. An adapted version of the method described by Wood and Bhat, 1988 was used to determine the β-glucosidase activity. A mixture of 150 μL of enzyme solution and 6.67 mM 4-nitrophenyl-β-D-glucopyranoside (exactly 450 μL) was made and left to incubate for 30 minutes at 40 °C in a water bath. Termination of this reaction was done with 400 μL of 1 M Na_2CO_3. The stopped reaction mixture was qualified spectrophotometrically at 400 nm wavelength. An enzyme activity unit can be said to be the enzyme quantity that can generate 1 μmol of p-nitrophenol or glucose in experimental conditions (Wood & Bhat, 1988).

Protein content determination

The method reported by Bradford (1976) was employed in this protein assay. 200 microliters of Bradford (1976) dye reagent was pipetted into sample solution of 10 μL. The reaction mixture was left to sit for 15 minutes at 26 °C. Then the absorbance recorded at a wavelength of 595 nm. Cellulase enzymes system efficiency was calculated and documented in U/mg protein. Standard of serum albumin (BSA) was used.

Concentration of sugar yielded at corn cob bioconversion

Quantification of sugar released in the saccharification media was determined by passing through spectrophotometer analysis every 48 hours intervals through the whole fermentation period as Miller (1959). 700 microliters of DNSA solution was added to 300 µL of the broth supernatant, boiled for five minutes at about 100 °C. The cooled mixture was analyzed spectrophotometrically at a wavelength of 575 nm. Sugar concentration released at corn cob bio-conversion process was measured with the standard graph approach (**Appendix A**).

Enzymatic hydrolysis of pre-treated & unprocessed corn cob

Chukwuma et al. (2020) described this method of breakdown of pre-processed and unprocessed biomass with enzymes. It was performed in triplicates in 250 mL conical flasks. 75 microliters of 0.05 molar CH_3COONa adjusted to 4.8 pH and 2% (weight/volume) biomass, was autoclaved for 15 minutes at 121 °C. Then, exactly 75 mL of the cellulases was introduced. Pre-processed and unprocessed biomass without the enzyme cocktail was employed as a control, with the final reaction volume completed with the CH_3COONa solution. Incubation was then carried out at 50 °C in a shaking incubator at 150 revolutions per minute. Then at 24 hours interval, 2 mL aliquots of reaction solutions were taken with a pipette and centrifuged at 4 °C for 30 minutes at 10,000 rpm.

RESULTS & DISCUSSION

A thorough investigation was done into how alkaline and steam pre-treatments affected the synthesis of cellulase and saccharification of maize cobs by the *pichia kudriavzevii* and *cyberlindnera fabianii* consortium. The goal of the research was to evaluate how these pre-treatment techniques affected the breakdown of lignocellulose, the production of cellulase, and the resulting release of glucose.

Corn Cob Pre-Treatment & Composition

The compositional alterations of maize cobs after alkaline and steam pre-treatments were examined (**Table 1**).

Table 1. Compositional analysis of UPC, SPC, & APC biomass

	Lignin	Cellulose	Hemicellulose
UPC	2.2%	75.0%	13.0%
SPC	2.0%	67.2%	25.0%
APC	1.2%	75.8%	10.9%

The lignin, cellulose, and hemicellulose contents of the steam pre-treated maize cob (SPC) were 2%, 67.2%, and 25%, respectively. In comparison, the alkaline processed maize cob (APC) had lignin levels reduced to 1.2%, cellulose levels raised to 75.8%, and hemicellulose levels dropped to 10.9%. These findings indicate that the lignocellulosic composition was substantially changed by both pre-treatments, with the alkaline pre-treatment resulting in an increased improvements of cellulose and a larger decrease of lignin. Due to the pre-treatments, the above materials had considerable quantity of lignin eliminated, further indicating that the carbohydrates. received greater exposure for hydrolytic reactivity (Mafa et al., 2020).

Cellulase Production & Activity

Cellulase was produced using the collaboration of *pichia kudriavzevii* and *cyberlindnera fabianii*, and its saccharification efficiency was assessed. The enzymatic capacity of the fungal consortium was shown by measuring the cellulase activity of the consortium using the β-glucosidase method. In addition, the Bradford assay's measurement of protein content revealed information about the effectiveness of the enzyme system, which helped characterize the consortium's capacity to produce cellulase (Wood & Bhat, 1988).

More significantly, the outcomes generated suggests a positive relationship between production of glucose and *β*glucosidase, as the amount of glucose released within the cultivation period depends on the final concentration of β-glucosidase (specific activity at 96 hours of culture [peak] yielded the maximum glucose concentration in all cases). However, Quiroz-Castaneda et al. (2009) noted greatest yield of *β*glucosidase, whereas Garcia et al. (2015) reported an optimum output of β-glucosidase from *lichtheimia ramosa* (27.2 U/mL) (Quiroz-Castaneda et al., 2009). Some more trends in this first major event is, as follows: SPC (0.03 specific activity) at 96 h (peak) has a better β-glucosidase activity over APC (0.05 specific activity) and unpre-treated corn cob (UPC) (0.02 specific activity) corn cobs.

Saccharification of Corn Cob

The cellulase cocktail created by the microbial consortium was used to carry out the hydrolysis process of pre-treated and untreated maize cobs. The total amount of liberated sugars was measured every 48 hours to keep track of the saccharification process. The performance of saccharification of the steam pre-treated maize cob (SPC) was found to be greater compared to that of the alkaline pre-treated maize cob (APC), demonstrating the better efficacy of steam pre-treatment in promoting glucose release.

Production of glucose during enzymatic breakdown of unprocessed, alkaline pre-processed and steam pre-processed biomass corn cob. With evidence confirming its outstanding efficiency in terms of cellulase generation and glucose liberation, the steam pre-treatment method seems to be the preferable pre-treatment approach for maize cob biomass based on the study's results.

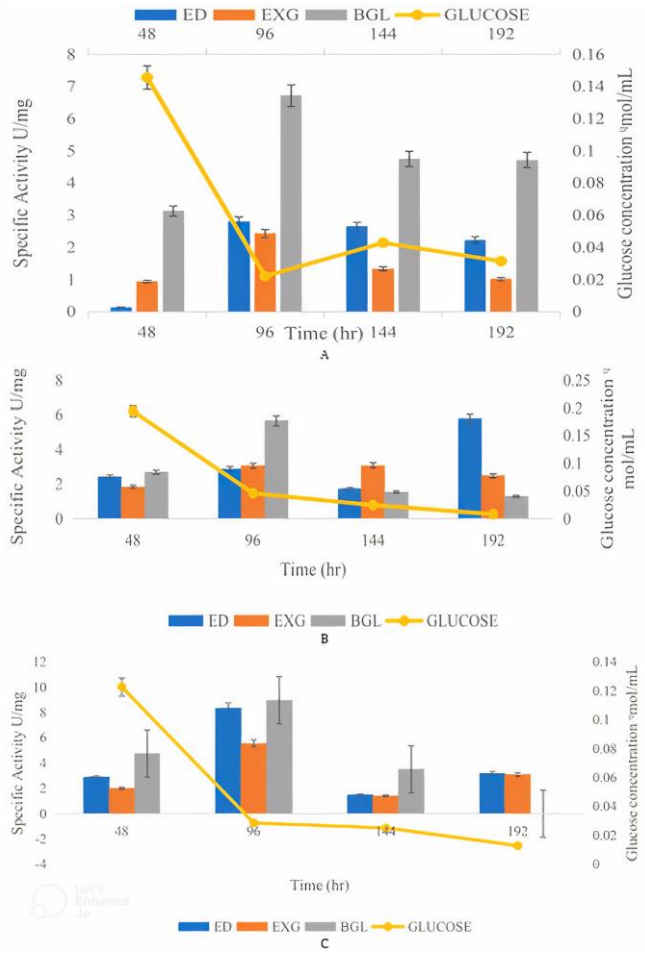

Figure 2. Production of cellulase & glucose during bioconversion of UPC by consortium of *cyberlindnera fabianii* & *pichia kudriavzevii* (error bars represents mean±standard deviation) (A); production of cellulase & glucose during fermentation of APC by consortium of *cyberlindnera fabianii* & *pichia kudriavzevii* (error bars represents mean±standard deviation) (B); & production of cellulase & glucose during fermentation of SPC by consortium of *cyberlindnera fabianii* & *pichia kudriavzevii* (error bars represents mean±standard deviation) (C) (Source: Authors' own elaboration)

SPC biomass consistently displayed greater specific activity units for cellulase enzymes (endoglucanase, exoglycanase, and glucosidase) throughout the enzymatic breakdown of UPC, APC, and SPC biomass as compared to the other pre-treatment techniques. Additionally, the glucose production profiles for SPC biomass showed a higher peak concentration of glucose (0.433 µmol/mL) at 48 hours, compared to APC (0.366 µmol/mL) and UPC (0.42 µmol/mL) biomass (**Figure 2**).

Also, steam pre-treatment led to optimal cellulase production at 96 hours, with significantly higher specific activity units for all cellulase enzymes (8.33 U/mg for endoglucanase, 5.56 U/mg for exoglycanase, and 8.97 Uk/mg for β-glucosidase) (**Table 2**) compared to other pre-treatment methods. This suggests that SPC method effectively enhanced the accessibility of cellulose to enzymatic hydrolysis, resulting in improved cellulase activity and glucose liberation.

Table 2. Production of cellulase and glucose during bioconversion of UPC, APC, & SPC by consortium of *cyberlindnera fabianii* & *pichia kudriavzevii*

	48 hrs activity	96 hrs activity	144 hrs activity	192 hrs activity
UPC				
Endoglucanase (U/mL)	0.14	2.81		
Exoglycanase (U/mL)	0.94	2.43		
β-glucosidase (U/mL)	3.13	6.71		
Glucose (µmol/mL)	0.15	0.02	0.043	0.03
APC				
Endoglucanase (U/mL)	2.43	2.87		
Exoglycanase (U/mL)	1.86	3.06		
β-glucosidase (U/mL)	2.70	5.67		
Glucose (µmol/mL)	0.2	0.05	0.025	0.001
SPC				
Endoglucanase (U/mL)	2.89	8.33		
Exoglycanase(U/mL)	1.99	5.56		
β-glucosidase (U/mL)	2.70	8.97		
Glucose (µmol/mL)	0.12	0.03	0.02	0.01

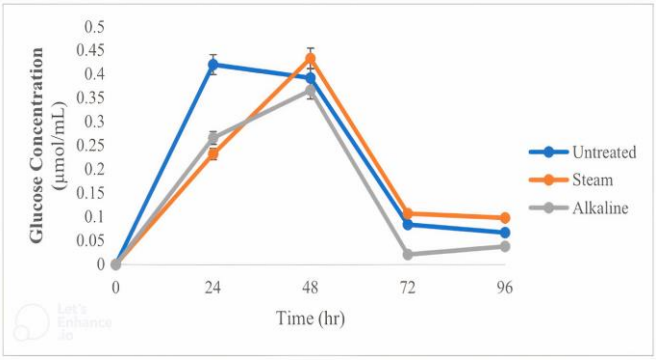

Figure 3. Production of glucose during enzymatic hydrolysis of UPC, APC, & SPC biomass corn cob (error bars represents mean±standard deviation) (Source: Authors' own elaboration)

In general, the analysis's findings show conclusively that the steam pre-treatment approach is superior to the alkaline pre-treatment technique in boosting cellulase synthesis and glucose release from maize cob biomass. The steady and increased levels of cellulase enzyme specific activity units and glucose concentrations seen in the steam-preprocessed biomass over the course of the experiment corroborate this conclusion (**Figure 3**).

Comparison With Previous Studies

The findings of this investigation are consistent with earlier studies showing how alkaline and steam pre-treatments might increase saccharification potential. Following alkaline pre-treatment, the observed rise in cellulose content and decrease in lignin content are compatible with research by Mafa et al. (2020) and Venkatesh et al. (2014) for steam. Additionally, results by Chukwuma et al. (2020) are supported by the increased cellulase production and saccharification efficiency attained by the *pichia kudriavzevii* and *cyberlindnera fabianii* consortia. Findings obtained by Mafa et al. (2020) from the use of a consortium formed from the termite hindgut constituted of multifunctional enzymes and exoglycanase to ferment corn cob as a carbon source (Mafa et al., 2020). Thota et al. (2018) proceeded to degrade groundnut shell biomass with an innovative consortia of *aspergillus oryzae* (AO, NCIM1212, micro fungi), *pycnoporous sanguineus* (PS, wood decaying macro fungi) and *trichoderma harzianum* (TH, endophytic fungi), maximum β-glucosidase and cellulase enzymes was reported with evident synergistic balance of consortium in saccharification activity. Moya and Torres (2012) degraded empty palm fruit bunch with a consortium of four fungi *emericella nidulla* and *aspergillus fumigatus*, which increased efficiency by over 400% (Thota et al., 2018). These previous studies collectively underscore the pivotal role of microbial consortia in enzymatic degradation processes and emphasize their potential for bioprocess optimization.

Additionally, by particularly examining the effects of alkaline and steam pre-treatment on the saccharification procedure and cellulase synthesis utilising a fungal consortium, the current study expands on these earlier findings. A greater comprehension of the complex interaction between pre-treatment techniques and cellulose breakdown strategies is provided by the thorough examination of compositional changes, enzyme activity, and glucose liberation.

Together, the contrasts support the validity of the study's findings and add to the body of information about techniques for using lignocellulosic biomass for bioconversion. The reproducible nature and dependability of the patterns seen are validated by the consistency of these findings spanning numerous studies, confirming the possible uses of alkaline pre-treatment, steam pre-treatment, and fungal consortia in the generation of environmentally safe biofuel and bioproducts.

CONCLUSIONS

1. This study employed a consortia of *pichia kudriavzevii* and *cyberlindnera fabianii* to investigate the impact of alkaline and steam pre-treatment on cellulose liberation and glucose synthesis from maize cob biomass.
2. Steam pre-treatment surpassed alkaline pre-treatment, leading to enhanced cellulase synthesis and sugar release.

3. Alkaline and steam pre-treatment both resulted in lignin removal from UPC biomass, with alkaline pre-treatment enhancing cellulose richness and lignin clearance.
4. Steam pre-processed maize cob biomass exhibited consistently higher specific activity units for cellulase enzymes, reaching notable levels of 8.33 U/mg endoglucanase, 5.56 U/mg exoglycanase, and 8.97 U/mg beta-glucosidase. This was accompanied by a glucose peak concentration of 0.433 mol/mL at 48 hours, indicating enhanced cellulase production and glucose release.
5. The findings underscore the superior effectiveness of steam pre-treatment in eliminating lignocellulosic barriers, thereby improving cellulose accessibility for enzymatic hydrolysis.
6. Steam pre-treatment offers advantages such as improved biomass permeation, lignin removal, and increased porosity through heat, organic acid production, and shearing forces interaction. These factors collectively elevate cellulase activity and glucose production in steam-pre-treated biomass.
7. This study highlights the potential of steam pre-treatment as a valuable method for enhancing the bioconversion potential of lignocellulosic materials. The insights gained emphasize the crucial role of pre-treatment techniques in optimizing biomass conversion processes.
8. The outcomes hold significant implications for the bioenergy and biorefinery sectors, providing an effective and environmentally sustainable approach for harnessing lignocellulosic biomass.

Implications and Future Directions

Further research into improving steam pre-treatment conditions and investigating possible integration with other pre-treatment techniques are necessary, moving forward. Further understanding of the processes behind the increases in enzymatic hydrolysis brought about by steam can also be very helpful in enhancing lignocellulosic biomass conversion technology. Novel approaches like scanning electron microscopy (SEM) and Fourier-transform infrared spectroscopy (FT-IR) have contributed to developing the study of lignocellulosic biomass characterization. By analyzing distinctive peak levels of absorption in FT-IR spectra, scientists may determine the respective makeup of these constituents, thereby providing information regarding the alterations generated by various pre-treatment techniques. These methods of analysis have substantially facilitated an improved knowledge of the chemical and physical attributes of biomass sources. SEM, on the contrary, provides an optical depiction of the pre-treatment-induced alteration of the morphology of the plant matter. The effect of pre-treatment on feedstock surface environment, permeability, and dimension of particles can be studied by scientists due to the superior quality scanning abilities of SEM. The comparison of the SEM images shows the impacts of pre-treatment clearly, emphasizing changes in biomass structure and porosity that affect enzyme accessibility and hydrolysis effectiveness.

In summary, this study offers convincing indications that steam pre-treatment sticks out as a potential successful strategy for enhancing cellulase yield and glucose release, providing an option regarding long-term and useful lignin-rich biomass utilization in a variety of bio-based purposes.

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Declaration of interest: No conflict of interest is declared by the authors.

Ethical statement: The authors stated that ethics committee approval was not required for the work, therefore it was exempted. The study involves data collection using online resources involving information freely available in the public domain that does not collect or store identifiable data. All related laws, rules, and regulations required for the study's implementation have been followed. The authors further stated that the article is the original study of the authors, and it has not been published elsewhere.

Data sharing statement: Data supporting the findings and conclusions are available upon request from corresponding author.

REFERENCES

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*(1-2), 248-254. https://doi.org/10.1006/abio.1976.9999

Chukwuma, O. B., Rafatullah, M., Tajarudin, H. A., & Ismail, N. (2020). Lignocellulolytic enzymes in biotechnological and industrial processes: A review. *Sustainability, 12*(18), 7282. https://doi.org/10.3390/ijerph18116001

Go, A. W., Conag, A. T., Igdon, R. M. B., Toledo, A. S., & Malila, J. S. (2019). Potentials of agricultural and agro-industrial crop residues for the displacement of fossil fuels: A Philippine context. *Energy Strategy Reviews, 23*, 100-113. https://doi.org/10.1016/J.ESR.2018.12.010

Ifeanyi-Nze, F. O., & Omiyale, C. O. (2023). Insights into the recent advances in the pre-treatment of biomass for sustainable bioenergy and bio-products synthesis: Challenges and future directions. *European Journal of Sustainable Development Research, 7*(2), em0209. https://doi.org/10.29333/ejosdr/12722

Kiran, R., Riaz, A., Irfan, M., & Shakir, H. A. (2017). An overview of pre-treatment methods used for bioconversion of lignocellulosic biomasses into valuable products. *Punjab University Journal of Zoology, 32*(2), 283-294.

Kloepper, J. W., Rodríguez-Kábana, R., McInroy, J. A., & Collins, D. J. (1991). Analysis of populations and physiological characterization of microorganisms in rhizospheres of plants with antagonistic properties to phytopathogenic nematodes. *Plant and Soil, 136*, 95-102. https://doi.org/10.1007/BF02465224

Kumar, A., Gautam, A., & Dutt, D. (2016). Biotechnological transformation of lignocellulosic biomass into industrial products: An overview. *Advances in Bioscience and Biotechnology, 7*(3), 149-168. https://doi.org/10.4236/abb.2016.73014

Lin, N., & Dufresne, A. (2014). Nanocellulose in biomedicine: Current status and future prospect. *European Polymer Journal, 59*, 302-325. https://doi.org/10.1016/j.eurpolymj.2014.07.025

Liu, S., Yu, Y., Xu, Z., Chen, S., Shen, G., Yuan, X., Deng, Q., Shen, W., Yang, S., Zhang, C., Chen, X., & Jin, M. (2022). Efficient corn cob biorefinery for ethanol initiated by a novel pre-treatment of densifying lignocellulosic biomass with sulfuric acid. *Fermentation, 8*(11), 661. https://doi.org/10.3390/fermentation8110661

Mafa, M., Malgas, S., Bhattacharya, A., Rashamuse, K., & Pletschke, B. I. (2020). The effects of alkaline pre-treatment on agricultural biomasses (corn cob and sweet sorghum bagasse) and their hydrolysis by a termite-derived enzyme cocktail. *Agronomy, 10*(8), 1211. https://doi.org/10.3390/agronomy10081211

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry, 31*(3), 426-428. https://doi.org/10.1021/ac60147a030

Moya, L. A., & Torres, E. (2012). Hydrolysis of cellulose and oil palm empty fruit bunches by using consortia of fungi isolated from the soil of Colombian high andean forest. *Agronomía Colombiana* [*Colombian Agronomy*], *30*(3), 411-418.

Olajuyigbe, F. M., Nlekerem, C. M., & Ogunyewo, O. A. (2016). Production and characterization of highly thermostable β-glucosidase during the biodegradation of methyl cellulose by fusarium oxysporum. *Biochemistry Research International, 2016*, 3978124. https://doi.org/10.1155/2016/3978124

Quiroz-Castaneda, R. E., Balcazar-Lopez, E., Dantan-Gonzalez, E., Martinez, A., Folch-Mallol, J., & Martinez-Anaya, C. (2009). Characterization of cellulolytic activities of bjerkandera adusta and pycnoporus sanguineus on solid wheat straw medium. *Electronic Journal of Biotechnology, 12*(4), 5-6. https://doi.org/10.2225/vol12-issue4-fulltext-3

Singhania, R. R., Patel, A. K., Sukumaran, R. K., Larroche, C., & Pandey, A. (2013). Role and significance of β-glucosidases in the hydrolysis of cellulose for bioethanol production. *Bioresource Technology, 127*, 500-507. https://doi.org/10.1016/j.biortech.2012.09.012

Thota, S. , Badiya, P., Guragain, Y., Vadlani, P., Pandey, M., Dandamudi, R., Ramamurthy, S.,& Belliraj, S. (2018). Innovative consortia of micro and macro fungal systems: Cellulolytic enzyme production from groundnut shell biomass and supportive structural analysis. *Journal of Sustainable Bioenergy Systems, 8*, 47-66. https://doi.org/10.4236/jsbs.2018.83004

Ullah, S., Ai, C., Huang, S., Zhang, J., Jia, L., Ma, J., Zhou, W., & & He, P. (2019). The responses of extracellular enzyme activities and microbial community composition under nitrogen addition in an upland soil. *PloS ONE, 14*(9), e0223026. https://doi.org/10.1371/journal.pone.0223026

Van Dyk, J. S., & Pletschke, B. I. (2012). A review of lignocellulose bioconversion using enzymatic hydrolysis and synergistic cooperation between enzymes-factors affecting enzymes, conversion and synergy. *Biotechnology Advances, 30*(6), 1458-1480. https://doi.org/10.1016/j.biotechadv.2012.03.002

Wood, T. M., & Bhat, K. M. (1988). Methods for measuring cellulase activities. *Methods in Enzymology, 160*, 87-112. https://doi.org/10.1016/0076-6879(88)60109-1

Zhang, F., Bunterngsook, B., Li, J. X., Zhao, X. Q., Champreda, V., Liu, C. G., & Bai, F. W. (2019). Regulation and production of lignocellulolytic enzymes from trichoderma reesei for biofuels production. In *Advances in bioenergy* (pp. 79-119). Elsevier. https://doi.org/10.1016/bs.aibe.2019.03.001

APPENDIX A

Glucose Standard Curve (Concentration of Stock Solution=2 mg/mL)

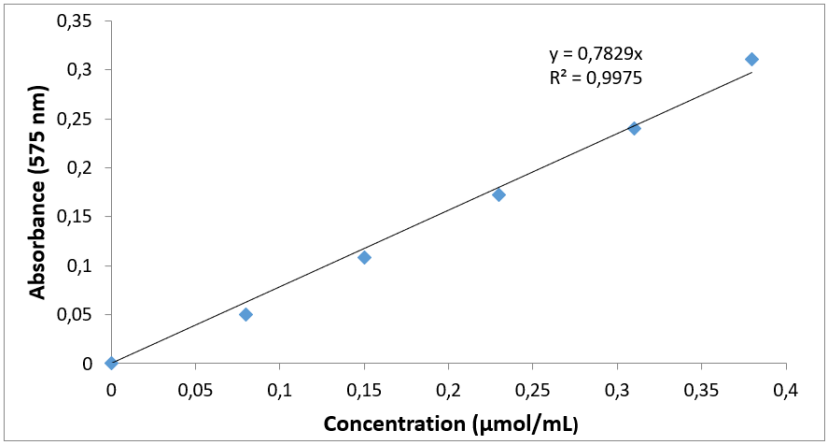

Figure A1. Glucose standard curve (concentration of stock solution=2 mg/mL) (Source: Authors' own elaboration)

Para-Nitrophenol Standard Curve (Stock Solution=0.02 mg/mL)

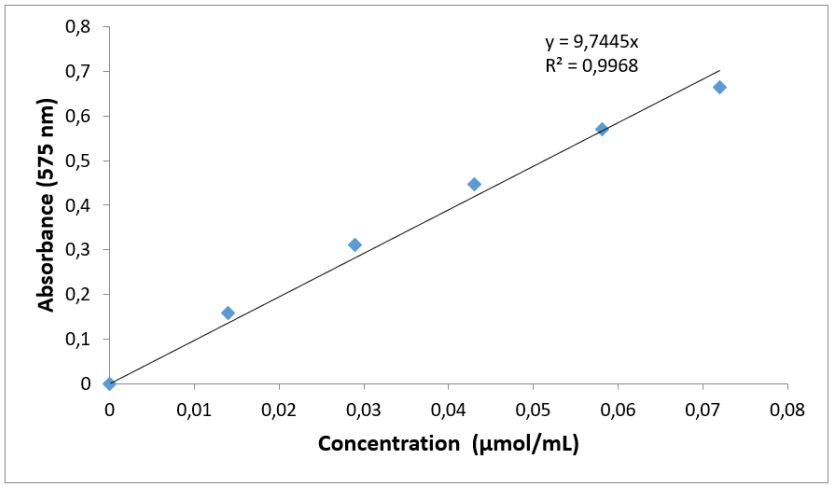

Figure A2. Para-nitrophenol standard curve (stock solution=0.02 mg/mL) (Source: Authors' own elaboration)

Protein Standard Curve (Stock Solution=0.1 mg/mL)

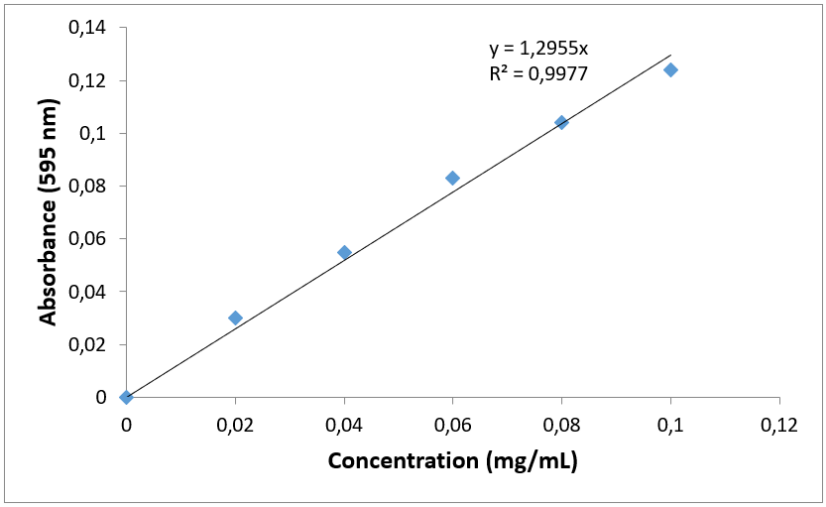

Figure A3. Protein standard curve (stock solution=0.1 mg/mL) (Source: Authors' own elaboration)

Production of Glucose During Enzymatic Hydrolysis of Unprocessed Corn Cob

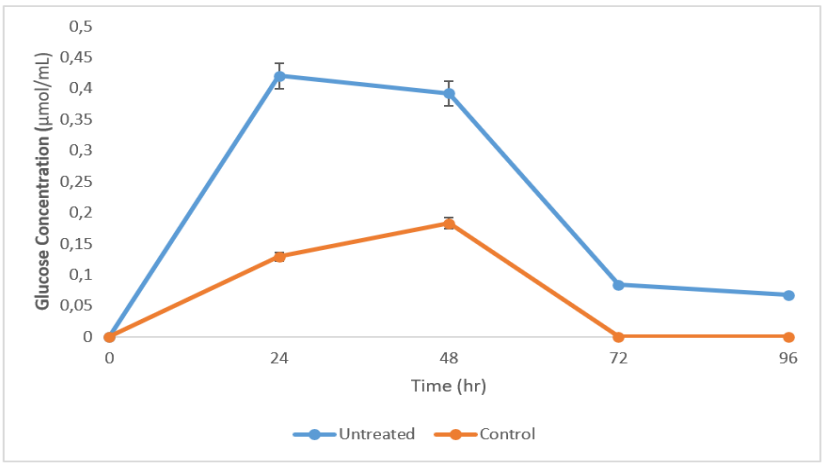

Figure A4. Production of glucose during enzymatic hydrolysis of unprocessed corn cob (error bars represents mean±standard deviation) (Source: Authors' own elaboration)

Production of Glucose During Enzymatic Hydrolysis of Alkaline Pre-Processed Corn Cob

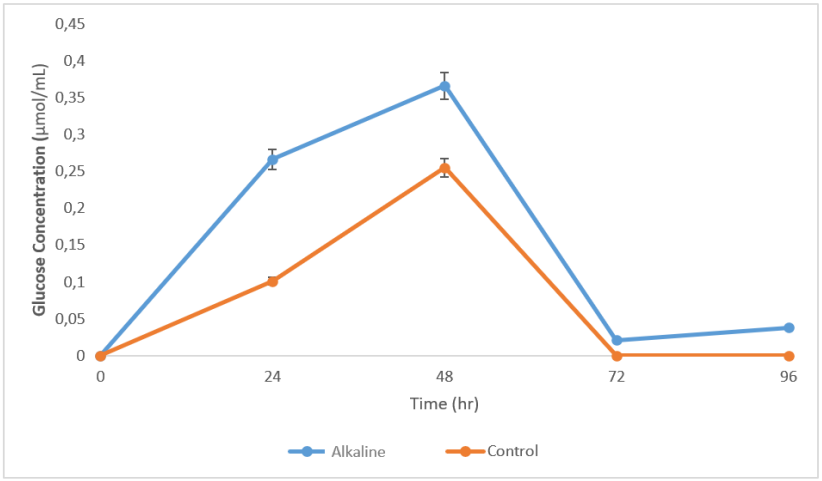

Figure A5. Production of glucose during enzymatic hydrolysis of alkaline pre-processed corn cob (error bars represents mean±standard deviation) (Source: Authors' own elaboration)

Production of Glucose During Enzymatic Hydrolysis of Steam Pre-Processed Corn Cob

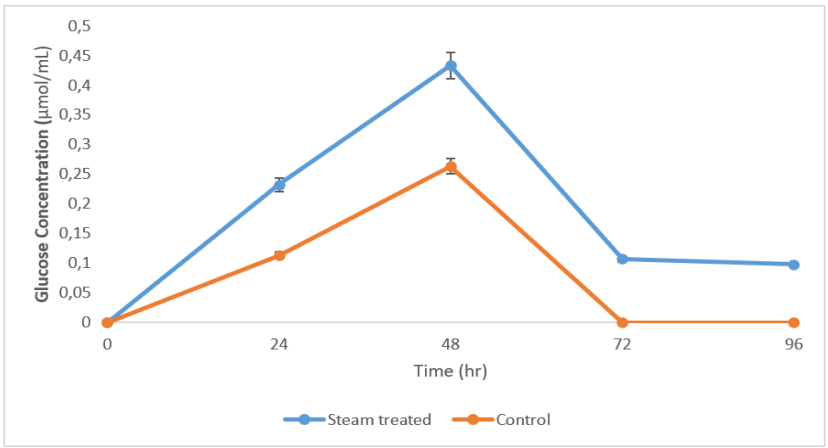

Figure A6. Production of glucose during enzymatic hydrolysis of steam pre-processed corn cob (error bars represents mean±standard deviation) (Source: Authors' own elaboration)