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Xylanase enhanced second-generation bioethanol production through simultaneous saccharification and fermentation

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ABSTRACT

This study aimed to correlate hydrolytic enzymes with enhanced bioethanol production during the simultaneous process of saccharification and fermentation for different agricultural wastes. This study screened the activities of hydrolytic enzymes to evaluate the simultaneous process and correlated them with bioethanol production. The results of the simultaneous process showed that cantaloupe peels produced the highest amount of reducing sugars and bioethanol. Cellulase showed maximum activity in the first 24 h, indicating that yeast cells favoured glucose over xylose. The results of the simultaneous saccharification and fermentation experiments revealed a 5-fold decrease in cellulase activity after 72 h. Xylanase activity improved from 79.38 to 95.18 U g^{-1}, and bioethanol production was enhanced from 21.42 to 75.66 g L^{-1}, confirming the capability of the simultaneous process to enhance bioethanol production. Although ethanol production was lower than that obtained with xylose cultivation alone, it was higher than that observed with glucose.

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Bioethanol; simultaneous; saccharification; fermentation; xylanase

Introduction

Lignocellulosic biomass is the most abundant and reasonably priced source of sugar for bioconversion to ethanol. It comprises the carbohydrate polymers cellulose, hemicellulose, and lignin (Figure 1). Because it lowers the emissions of greenhouse gases that contribute to global warming, bioethanol is a potential and ecologically benign substitute for gasoline. Therefore, it is considered a sustainable and renewable fuel. Currently, sugar and starch crops are the sources of all bioethanol generated worldwide. However, creating commercially viable and sustainable industrial processes that use regenerative lignocellulosic materials such as cellulose and hemicellulose, which do not compete with food sources, is necessary to produce second-generation bioethanol. The most common lignocellulosic biomasses used to manufacture second-generation bioethanol are sugarcane bagasse, rice, and corn husks [1].

As part of the saccharification process, polymers from agricultural lignocellulosic complexes such as cellulose and hemicellulose are broken down into simple sugars like glucose, galactose, and mannose. Different strains of *Saccharomyces cerevisiae* then ferment these sugars to produce ethanol [2]. Cellulase is regarded as the most important enzyme for enzymatic hydrolysis in the synthesis of bioethanol because it converts cellulose into glucose [3]. The second most prevalent polysaccharide found in plant cells, xylan, can be depolymerized by xylanases. Filamentous fungi, such as *Trichoderma* and *Aspergillus* spp., are the main commercial sources of cellulases and xylanases [4].

Because *Saccharomyces* strains have difficulty using pentoses from the hydrolysis of hemicellulose, *Zymomonas mobilis*, a genetically modified strain of *Pichia stipitis*, was used for fermentation [5]. *Candida shehatae* can co-ferment pentoses and hexoses to produce ethanol and other compounds with high yields [5].The fermentation of pentose sugars in addition to hexoses is essential for the efficient use of lignocellulosic biomass. The effective microbial use of xylose, the primary pentose sugar in the hemicellulose component of lignocellulosic biomass, is particularly important because very few species can ferment this sugar [6]. Numerous economic analyses have shown that effective pentose usage is crucial for increasing the overall efficiency of wood-to-ethanol conversion [7].

The simultaneous saccharification and fermentation (SSF) method combines two steps of cellulose hydrolysis and glucose fermentation in the presence of fermentative microorganisms in one step, and it performs best at an optimum temperature. This method is promising in converting cellulose into ethanol [8,9]. Lignocellulosic biomass is processed with a weak acid as part of the SSF process to dissolve the complexes of lignin, hemicellulose, and pectin. Acid pretreatment (removal of hemicellulose) followed by alkaline pretreatment (removal of lignin) has been shown to produce relatively pure cellulose [10]. The resulting solids, which contained cellulose and lignin, were concurrently hydrolyzed and fermented using yeast and cellulase enzymes. Cellulose is hydrolyzed to glucose by the enzyme cellulase, which is then fermented to produce ethanol [11]. This combined approach enhances the kinetics of fermentation and the economics of digesting biomass by reducing the build-up of hydrolysis products (such as glucose) that inhibit cellulases.

To date, only a few studies utilized the process of simultaneous saccharification and fermentation; however, the

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This article has been corrected with minor changes. These changes do not impact the academic content of the article.

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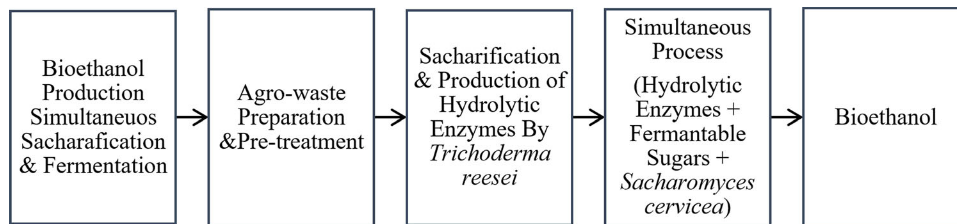

Figure 1. Flow chart for the simultaneous process of saccharification and fermentation.

correlation between hydrolytic enzymes' activities and bioethanol production had not been clarified. The current study aimed to produce hydrolytic enzymes from *Trichoderma reesei* under solid-state fermentation and utilize the enzymes produced in the SSF process to enhance bioethanol production from different agricultural wastes in the presence of *Saccharomyces cerevisiae*. The process involved monitoring the levels of hydrolytic enzymes, reducing sugars, and bioethanol in the SSF process and determining the correlation between the produced hydrolytic enzymes and the enhancement of bioethanol production.

Materials and methods

Materials

The enzymatic substrates, xylan, starch, carboxymethylcellulose, and polygalacturonic acid, were obtained from Aldrich. All other reagents and solvents were purchased from Sigma–Aldrich.

Processing of raw materials

Different agricultural waste materials (agro-waste), such as aavocado peels, cantaloupe peels, sugar bagasse peels, palm leaves, and seaweed, were collected from a local grocery market in Jeddah, Saudi Arabia. All agricultural waste materials were washed and oven-dried at −80 °C for 48 h. The dried agro-waste was then sieved into particles of approximately 1 mm in size. These powders were then processed with 1.0 M NaOH in a 1:10 (w/v) ratio for 1 h at 121 °C and 15 psi pressure [12]. The pretreated materials were rinsed with tap water until the pH of the filtrate reached 7.0. The materials were dried at 60 °C overnight to maintain a constant weight.

Inoculum preparation

Trichoderma reesei was acquired from the Microbial Toxins and Natural Products Central Laboratory (MTNPC) of King Abdulaziz University in Jeddah, Saudi Arabia. The following were present in the inoculum preparation medium (g/L): $(NH_4)_2SO_4$, 19.6; KH_2PO_4, 28; urea, 4.2; yeast extract, 7; glucose, 15; $CaC1_2$, 0.028; $ZnSO_4$, 0.019; $FeSO_4{\cdot}7H_2O$, 0.021; $CoCl_2$, 0.07; $MgSO_4{\cdot}7H_2O$, 4.2; and the pH was set to 5.0. The medium was autoclaved for 15 min at 121 °C and 15 psi for sterilization. Before being transferred to the production medium, the previous culture was incubated and shaken in an orbital shaking incubator at 30 °C for 48 h at 150 rpm [13]. *Saccharomyces cerevisiae* was obtained from the MTNPC and was cultured in a fermentation medium containing the following components (g/L) Santos, Lucena [14]: glucose, 100; yeast extract, 4; $(NH_4)_2SO_4$, 2; KH_2PO_4, 2; and $MgSO_4{\cdot}7H_2O$, 0.75, and incubated at 30 °C for 24 h. *T. reesei* uses pretreated agricultural waste materials as substrates to manufacture hydrolytic enzymes using solid-state fermentation as feedstock for the concurrent fermentation of bioethanol.

Hydrolytic enzyme production

T. reesei was subjected to solid-state fermentation to produce hydrolytic enzymes. The agricultural waste materials were sterilized in an autoclave for 20 min at 121 °C and 15 psi before inoculation. Then, 5 g of sterilized agricultural waste, 5×10^5 fungal spores/g, and an appropriate volume of water (10% moisture) were added to 50 mL Erlenmeyer flasks. Three sets of experiments were performed. After incubating 5 g of fermented material with 50 mL of distilled water overnight on a rotary shaker (180 rpm), crude hydrolytic enzymes were recovered. After centrifuging the suspension for 10 min at 12000 rpm, the supernatant obtained was referred to as the crude extract.

Simultaneous production of bioethanol

S. cerevisiae was incubated with crude hydrolytic enzymes produced in the presence of pretreated agricultural waste at 30 °C under anaerobic conditions. At regular intervals, samples were collected and centrifuged for 15 min at 6000 rpm. The reducing sugar content, hydrolytic enzyme activity, and percentage of ethanol were examined as described in the biochemical analysis section.

Biochemical analyses

Estimation of biomass concentration

Throughout the culture period, samples were collected in centrifuged Falcon tubes at various intervals. Following appropriate dilution, the optical density of the cultures at 600 nm was determined using a spectrophotometer immediately after sampling. To improve accuracy, the culture broth was diluted for all samples to obtain OD_{600} readings that were less than 1. Using a linear correlation standard curve, the ODs of the cultures were converted into the dry cell mass. According to the standard curve generated, an OD_{600} of 1.0 was comparable to a dry cell mass of 0.3 g/L.

Enzyme assays

The activities of four different enzymes (xylanase, amylase, cellulase, and pectinase) were measured using glucose, galacturonic acid, and xylose as standards [15]. The substrates used for xylanase, amylase, cellulase, and pectinase were xylan, starch, carboxymethylcellulose, and polygalacturonic

acid, respectively. Each 0.5 mL reaction mixture contained 0.1 mL of crude extract, 0.05 M sodium acetate buffer, and 1% substrate. Assays were run for one hour at 37 °C. Afterward, 0.5 mL of a dinitrosalicylic acid reagent was added to each tube. After thoroughly mixing, the reaction mixture was boiled in a water bath for 10 min. Absorbance was measured at 560 nm after cooling to room temperature. The amount of enzyme that released one mole of reducing sugar per minute under standard test conditions was considered one unit of enzyme activity. Three trials were conducted in each experiment.

Determination of total reducing sugar content

The Miller method [15] was used to calculate the total amount of reducing sugar in the solutions. Briefly, 0.5 ml of the crude extract and 0.5 ml dinitrosalicylic acid reagent were mixed. The tubes were then heated in a bath of boiling water. Afterward, the absorbance of each was measured at 560 nm after cooling to room temperature. Glucose was used as a calibration standard to determine the total reducing sugar content.

Colorimetric estimation of bioethanol content using the dichromate method

In a 50 mL volumetric flask, 10 mL of a 1.6 g L^{-1} of an ethanol standard stock solution or a microbial extract aliquot, 5 mL of a 40 mg mL^{-1} sodium dichromate solution, 5 mL of acetate buffer (pH 4.3), and 25 mL of 1 N sulfuric acid were added. The mixture was gently shaken for one minute and then incubated for 120 min at room temperature until a green product was formed. A UV/visible spectrophotometer (Jenway 6305) was used to determine the absorbance at 578 nm after the incubation period. Each sample was prepared in triplicate. A standard curve from an ethanol stock solution at gradient concentrations of 1.6–12.8 mg mL^{-1} was used to calculate the concentration of bioethanol utilizing the Beer-Lambert law [16].

Statistical analysis

Statistical analyses were performed using GraphPad Prism version 8.4.2 software. Descriptive and two-way analysis of variance, including interaction effects (two-tailed; $p \leq 0.05$), were performed to compare differences among groups. The results are expressed as the mean ± standard deviation of triplicates measures.

Results and discussion

Production of hydrolytic enzymes

Figure 2 shows the production of hydrolytic enzymes by *T. reesei* where no significant difference was found among the utilized agricultural wastes. Sugar bagasse peels, palm leaves, and seaweeds revealed minimal reductions in the activities of the analyzed hydrolytic enzymes. In contrast, the agricultural waste materials hay grass, cantaloupe peels, and avocado peels increased xylanase, cellulase, and amylase activities. High xylanase activities were observed using hay grass and cantaloupe peels, at 83.9 ± 1.82 and 79.92 ± 1.21 U g^{-1} waste material, respectively. However, the maximum cellulase activity was observed using cantaloupe peels, with an activity of 84.91 ± 2.12 U g^{-1} waste material. These results reveal that cantaloupe peels are the most suitable waste media for producing the saccharification enzymes cellulase and xylanase. Hence, it was utilized as the sole nutrient source in this study to observe the SSF effect on bioethanol production. Moreover, pectinase showed the highest activity 4.78 U g^{-1} waste material of cantaloupe peels.

Previous studies of hydrolytic enzymes production showed that a co-culture of *T. reesei* and *Aspergillus niger* GS1 was grown on a mixture of Bermuda grass and corn cob to produce fermented fodder rich in hydrolytic enzymes as value-added components in animal feed. The productivities of this co-culture for cellulase, amylase, and xylanase were 8.8, 181.4, and 42.1 U g^{-1}, respectively [17]. Another study confirmed that the maximum production of cellulase and xylanase were obtained by *Trichoderma virens* (123.26 and 348 U g^{-1} solid, respectively) under solid state fermentation containing alkali pre-treated wheat bran [18]. The maximum production of pectinase and xylanase were obtained by *Trichoderma harzianum* (90 and 50 U g^{-1} solid, respectively) and *Trichoderma virnes* (110 and 45 U g^{-1} solid, respectively) in solid state fermentation containing cantaloupe and watermelon rinds, respectively [19]. Investigating the enzymatic interactions necessary for this process is vital because it is still unclear how lignocellulolytic enzymes degrade lignocellulosic biomass. To increase bioethanol production, a study screened hydrolytic enzymes during the SSF processes. The cellulase and xylanase yields show that they perform their initial roles in the biodegradation of lignocellulosic biomass [20].

According to other studies, agricultural waste has a diverse composition, with high concentrations of proteins, carbohydrates, and minerals [21]. One of the by-products that are eliminated is cantaloupe rind. Several studies have indicated that the peel is abundant in bioactive compounds that have a beneficial influence on health [22]. A study reported that cantaloupe rind contained carbohydrates (69.77%), ash (3.67%), total dietary fibers (41.69%), and antioxidants such as polyphenols and flavonoids (332 mg/100 g extract and 95.46 mg/100 g extract, respectively) [23].These residues are not considered trash because of their high nutritional content; they are viewed as raw materials for creating and developing new products. The presence of these nutrients as raw materials provides conditions conducive to the growth of microbes and their use as substrates for hydrolytic enzymes. Through fermentation, these microorganisms can reuse some of these source materials. Developing solid-state fermentation platforms for manufacturing various useful products could use agro-industrial leftovers as a strong foundation. Reducing the production costs based on food crops also aids in producing fermentable sugar. Numerous studies have been conducted to understand how bacteria can convert agricultural waste into sugar [21].

Simultaneous production of bioethanol

The resulting hydrolytic enzymes were pooled and incubated with the pretreated agricultural waste media utilized in the current study in the presence of *S. cerevisiae* under

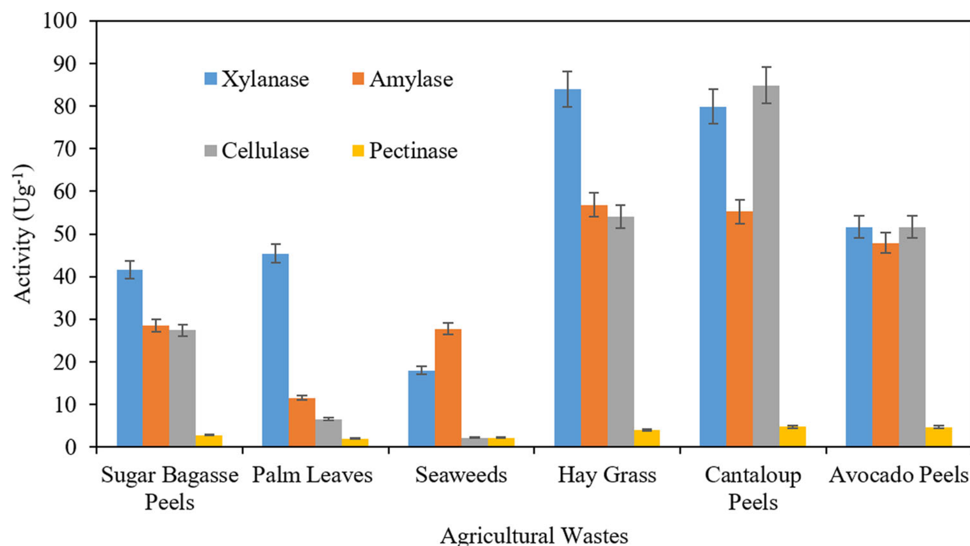

Figure 2. Comparison of hydrolytic enzymes cellulase, pectinase, amylase and xylanase production mean activities by *Trichoderma reesei* using different agricultural wastes under solid state fermentation. Process was conducted in triplicate measures for 5 days at 30 °C.

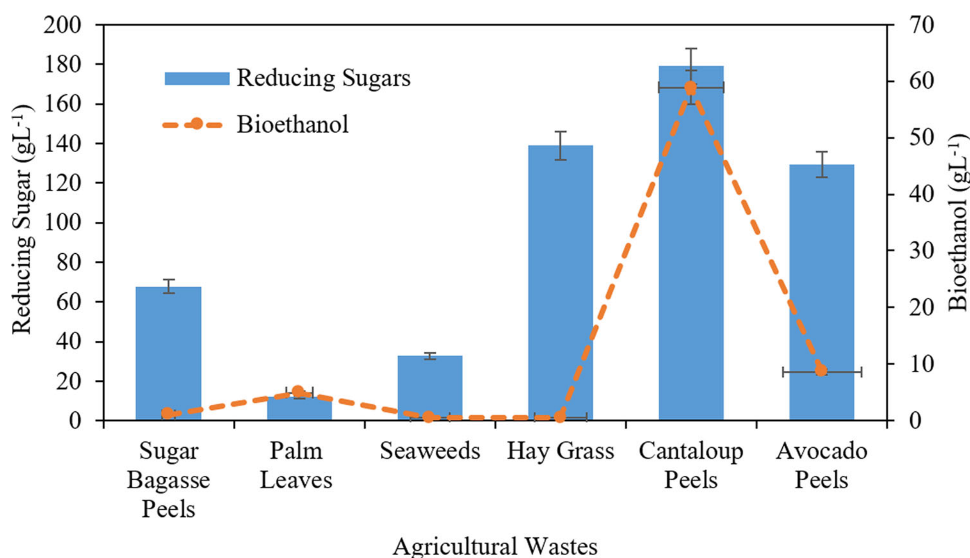

Figure 3. Comparison among different agricultural wastes as substrates for the production of reducing sugars and bioethanol in the simultaneous process of saccharification and fermentation by *Saccharomyces cerevisiae* under anaerobic condition. Process was conducted in triplicate for 24 h at 30 °C.

anaerobic conditions. This simultaneous process utilized the hydrolyzed and pretreated waste to produce sugars that could be simultaneously fermented by *S. cerevisiae* cells into bioethanol. As previously reported, *S. cerevisiae* relays on hexoses to produce bioethanol [11]. The amount of bioethanol produced from cantaloupe peels (Figure 3) was higher than that from hay grass, which may be due to a high hexose content in the substrate. These results corroborate the results of producing hydrolytic enzymes shown in Figure 2, where there was no significant difference ($p > 0.05$) between the utilized substrates, and the levels of reducing sugars and bioethanol produced. The results revealed that cantaloupe peels produced the highest reducing sugar content and bioethanol, at 179.08 ± 1.02 and 58.88 ± 2.33 g L^{-1}, respectively. Even though they did not produce a significant amount of bioethanol, the hay grass and avocado peels released a high amount of reducing sugars, at 138.79 ± 0.08 and 129.35 ± 2.6 g L^{-1}, respectively. Elevated yields of reducing sugars and bioethanol are associated with high cellulase and xylanase activity, as shown in Figure 2 with cantaloupe peels. Therefore, the current study revealed cantaloupe peels as the best agricultural waste to be utilized in the simultaneous fermentation process.

Differences in the sugar compositions of agricultural waste produce varying bioethanol content. For example, the galacturonic acid content in cantaloupe peel was the highest (88.32%) among the materials analyzed in a previous study [24]. Banana peel and guava pulp both contain rhamnose, glucose, and galactose, in addition to galacturonic acid, while the pectin of cantaloupe peel is made up of xylose and glucose [24]. Xylan is the main hemicellulosic carbohydrate found in grass cell walls. Typically, 20–30% of the total cell wall of grass is composed of xylan, while non-cellulosic polysaccharides comprise 10–15% of the cell wall [25]. The type of cultivar and growth conditions affect the chemical composition of avocado peels [26]. Their major component is lignin (41.91%), followed by glucan (19.43%), and hemicelluloses (26.51%), which include xylan, acetyl groups, arabinan, and galacturonic acid, the content of which was directly related to the amount of pectin in this type of feedstock [27]. Seaweed, palm leaves, and sugar bagasse peels, on the other hand, have relatively lower concentrations of reducing sugars and bioethanol.

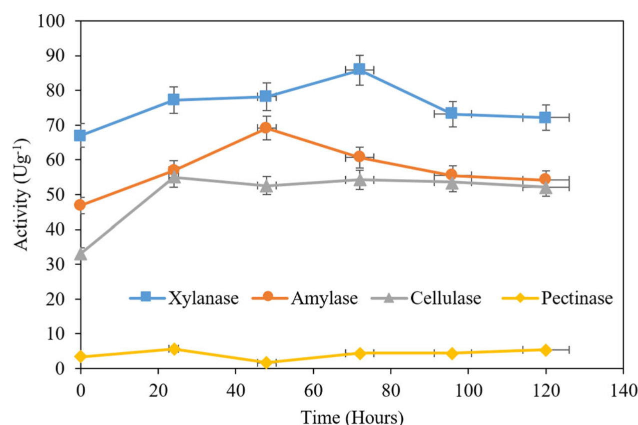

Figure 4. Effect of time for the simultaneous process of saccharification and fermentation by *Sacharomyces cerevisiae* utilizing cantaloupe peels as a substrate. Process was conducted at sequential time intervals in triplicate measures at 30 °C.

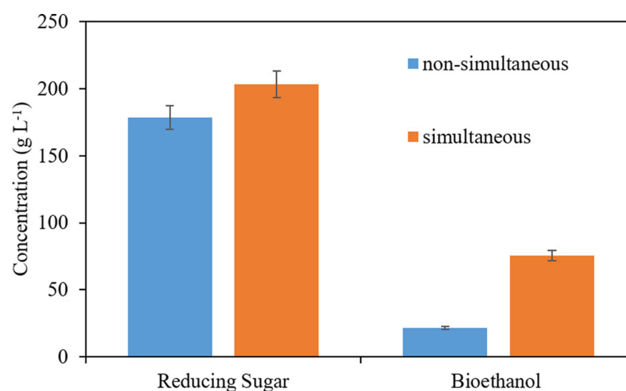

Figure 6. Comparison between the mean concentrations of produced reducing sugars and bioethanol in simultaneous 'sacharification and fermentation' and non-simultaneous 'fermentation' processes utilizing cantaloup peels. Process was conducted for 72 h in triplicate measures at 30 °C.

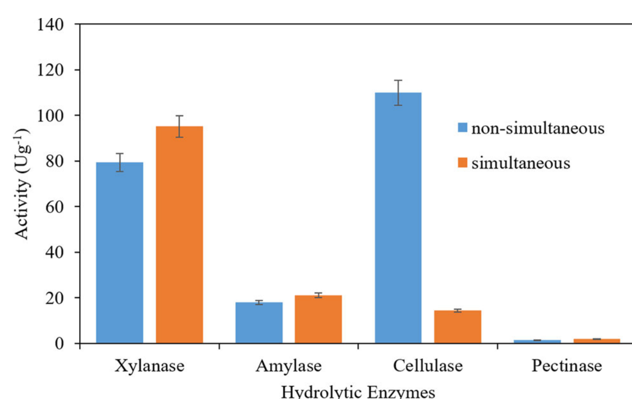

Figure 5. Comparison among the mean activities of hydrolytic enzymes xylanase, amylase, cellulase and pectinase in simultaneous 'sacharification and fermentation' and non-simultaneous 'fermentation' processes utilizing cantaloup peels. Process was conducted for 72 h in triplicate measures at 30 °C.

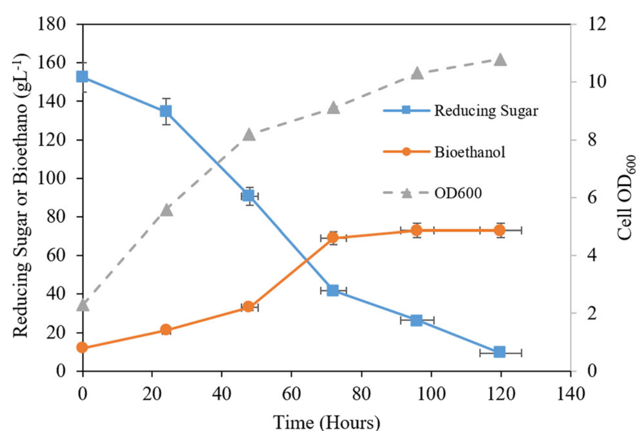

Figure 7. Effect of time for the simultaneous process of saccharifiction and fermentation utilizing cantaloupe peels. Process was conducted in triplicate measures at 30 °C.

The effect of time was also assessed to examine the nature of the pool of hydrolytic enzymes during concurrent saccharification and fermentation. As shown in Figure 4, the ideal incubation times for the hydrolytic enzymes in the current experiment were 24 h for pectinase and cellulase, 48 h for amylase, and 72 h for xylanase. Figure 4 demonstrated increased cellulase activity in the first 24 h, showing that yeast cells prefer glucose over xylose. Its peak activity was observed after 72 h. According to other studies, glucose is absorbed first, but the two sugars, glucose and xylose are co-utilized when they are present in a 50:50 ratio [28]. The initial rate of xylose utilization was low; however, when the glucose concentration decreased, it increased progressively [26].

In the simultaneous production of bioethanol, yeast cells were incubated with cantaloupe peels in the presence of hydrolytic enzymes at 30 °C under anaerobic condition for 72 h. The activities of the hydrolytic enzymes were measured in in the simultaneous process of saccharification and fermentation than the fermentation process utilizing pretreated cantaloup peels as substrate media. The results of the SSF experiments revealed a sharp drop in cellulase activity after 72 h, whereas amylase activity slightly improved in the presence of yeast cells. On the other hand, xylanase showed improved activity from 79.38 to 95.18 U g^{-1} waste in the simultaneous process, while pectinase showed no change in activity, as shown in Figure 5.

In the fermentation medium, yeast cells favoured glucose utilization in the first 24–48 h; however, xylose was utilized after the levels of these hexoses diminished. Therefore, the yeast isolate did not utilize xylose at high levels until glucose was exhausted.

Figure 6 shows the overall simultaneous production of bioethanol and reducing sugars. The results indicated a 3-fold increase from 21.42 to 75.66 g L^{-1} in bioethanol production in the simultaneous process of saccharification and fermentation than the fermentation process. Similarly, yeast cells can convert xylose or cellobiose into ethanol compared, where at least one cultivation condition increases the fermentation rate or yield from xylose, cellobiose, or a mixture of at least one of these sugars [28]. For example, the presence of glucose may induce the fermentation of xylose or cellobiose. Compared to bioreactors containing glucose, those containing xylose had higher rates of ethanol generation. Moreover, total cell yields were lower in the bioreactors containing xylose [23]. When producing ethanol from xylose, as opposed to glucose, increased ethanol accumulation and reduced cell accumulation have been shown to lead to higher ethanol yields [28].

Figure 7 shows that bioethanol production increased over time. After 72 h, the sugar concentration drastically decreased, whereas the percentage of bioethanol production increased, revealing that the optimum bioethanol production occurred after 72 h. In the meantime, reducing sugars were also depleted more rapidly, while more

ethanol accumulated in the simultaneous process for which the cell yield was significantly increased. After 72 h, glucose started to decrease, possibly due to diminished glucose concentration and dependence on xylose.

Another study during the fermentation of maple hydrolysate found that ethanol production started within 60 h of switching the sparging gas to 2.1% oxygen (90% nitrogen in air) [28]. As soon as the oxygen concentration decreased, both glucose and xylose were simultaneously consumed; they were completely consumed after 48 and 60 h, respectively. Between 23 and 57 h of culture, the average output of mixed ethanol sugars was 0.40 g/g [28].

Conclusion

The simultaneous process revealed improved bioethanol production, as indicated by the activity of xylanase upon diminishing hexose levels. Cellulase had maximum activity in the first 24 h, revealing that yeast cells favoured glucose over xylose and showed maximum activity after 72 h. In the SSF processes, wherein yeast cells were combined with the produced hydrolytic enzymes, xylanase activity was improved from 79.38 to 95.18 U g^{-1}, and bioethanol production was enhanced from 21.42 to 75.66 g L^{-1}. These results confirm that the simultaneous process could enhance bioethanol production. Therefore, second-generation feedstock cultivation could produce more ethanol during the simultaneous process. Although ethanol production was lower than that obtained when fermentation utilized xylose alone, another study revealed that it was higher than that observed with glucose alone. This work is novel as it describes the patent filed by Jeffries et al. (2013) [27] entitled 'Co-fermentation of glucose, xylose and/or cellobiose by yeast' in terms of hydrolytic enzyme correlation. Further evaluations should be conducted using purified hydrolytic enzymes to enhance bioethanol production.

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Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1] Hayes DJ. An examination of biorefining processes, catalysts and challenges. Catal Today. 2009;145(1-2):138–151.
[2] Sun Y, Cheng J. Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresour Technol. 2002;83(1):1–11.
[3] Mahalakshmi N, Jayalakshmi S. Cellulase production by *Aspergillus Niger* under solid state fermentation using agro industrial wastes. Int J Adv Multidiscip Res. 2016;3(2):78–83.
[4] Sridevi A, Narasimha G, Reddy BR. Production of cellulase by *Aspergillus Niger* on natural and pretreated lignocellulosic wastes. Internet J Microbiol. 2009;7(1):580–592.
[5] Dinita B, Malla SJJR, Sreerama L. Lignocellulosic ethanol production: current practices and recent developments. Biotechnol Mol Biol Rev. 2011;6(8):172–182.
[6] Barnett JA. The utilization of sugars by yeasts. Adv Carbohydr Chem Biochem. 1976;32:125–234.
[7] Lee J. Biological conversion of lignocellulosic biomass to ethanol. J Biotechnol. 1997;56(1):1–24.
[8] Lynd LR. Overview and evaluation of fuel ethanol from cellulosic biomass: technology, economics, the environment, and policy. Annu Rev Energy Environ. 1996;21(1):403–465.
[9] Demain AL, Newcomb M, Wu JD. Cellulase, clostridia, and ethanol. Microbiol Mol Biol Rev. 2005;69(1):124–154.
[10] de Jong E, Gosselink RJA. Chapter 17, Lignocellulose-Based chemical products. In: Gupta VK, Tuohy MG, Kubicek CP, Saddler J, Xu F, editors. Bioenergy research: advances and applications. Amsterdam: Elsevier; 2014. p. 277–313.
[11] Krishna SH, Reddy TJ, Chowdary G. Simultaneous saccharification and fermentation of lignocellulosic wastes to ethanol using a thermotolerant yeast. Bioresour Technol. 2001;77(2):193–196.
[12] Lynd LR, Grethlein HE, Wolkin RH. Fermentation of cellulosic substrates in batch and continuous culture by Clostridium thermocellum. Appl Environ Microbiol. 1989;55(12):3131–3139. English.
[13] Duenas R, Tengerdy R, Gutierrez-Correa M. Cellulase production by mixed fungi in solid-substrate fermentation of bagasse. World J Microbiol Biotechnol. 1995;11(3):333–337.
[14] Santos JRA, Lucena MS, Gusmão NB, et al. Optimization of ethanol production by *Saccharomyces cerevisiae* UFPEDA 1238 in simultaneous saccharification and fermentation of delignified sugarcane bagasse. Ind Crops Prod. 2012;36(1):584–588.
[15] Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem. 1959;31(3):426–428.
[16] Sumbhate S, Nayak S, Goupale D, et al. Colorimetric method for the estimation of ethanol in alcoholic-drinks. J Anal Tech. 2012;1:1–6.
[17] Amaro-Reyes A, Gracida J, Huizache-Peña N, et al. On-site hydrolytic enzymes production from fungal co-cultivation of Bermuda grass and corn cob. Bioresour Technol. 2016;212:334–337.
[18] El-Shishtawy RM, Mohamed SA, Asiri AM, et al. Saccharification and hydrolytic enzyme production of alkali pre-treated wheat bran by *trichoderma virens* under solid state fermentation. BMC Biotechnol. 2015;15(1):37.
[19] Mohamed SA, Al-Malki AL, Khan JA, et al. Solid state production of polygalacturonase and xylanase by trichoderma species using cantaloupe and watermelon rinds. J Microbiol. 2013;51(5):605–611.
[20] Ogunyewo OA, Olajuyigbe FM. Unravelling the interactions between hydrolytic and oxidative enzymes in degradation of lignocellulosic biomass by *sporothrix carnis* under various fermentation conditions. Biochem Res Int. 2016;2016:1–8.
[21] Nguyen T-AD, Kim K-R, Han SJ, et al. Pretreatment of rice straw with ammonia and ionic liquid for lignocellulose conversion to fermentable sugars. Bioresour Technol. 2010;101(19):7432–7438.
[22] Moon J-K, Shibamoto T. Antioxidant assays for plant and food components. J Agric Food Chem. 2009;57(5):1655–1666.
[23] Mallek-Ayadi S, Bahloul N, Kechaou N. Characterization, phenolic compounds and functional properties of cucumis melo L. peels. Food Chem. 2017;221:1691–1697.
[24] Kamal AM, El-Tantawy ME, Haggag EG, et al. Chemical and biological analysis of essential oils and pectins of banana, cantaloupe peels, guava pulp and formulation of banana pectin gel. J Pharmacogn Phytochem. 2019;8(4):1808–1816.
[25] Hatfield RD, Rancour DM, Marita JM. Grass cell walls: a story of cross-linking. Front Plant Sci. 2016;7:2056.
[26] Araújo RG, Rodriguez-Jasso RM, Ruiz HA, et al. Avocado by-products: nutritional and functional properties. Trends Food Sci Technol. 2018;80:51–60.
[27] Del Castillo-Llamosas A, Rodríguez-Martínez B, Pablo G, et al. Hydrothermal treatment of avocado peel waste for the simultaneous recovery of oligosaccharides and antioxidant phenolics. Bioresour Technol. 2021;342:125981.
[28] Jeffries TW, Willis LB, Long TM, et al. Co-fermentation of glucose, xylose and/or cellobiose by yeast. Google Patents; 2013.