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Consolidated bioprocess for ethanol production from rice straw and corn cobs by using fungi

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ABSTRACT

Rice straw (Rs) and corn cobs (Cc) were pretreated with alkaline hypochlorite (1.5% NaClO) giving RS and CC of total carbohydrates 80 and 71%, respectively. The pretreatment facilitated cellulase production by *Trichoderma reesei* using 4 substrates RS, CC and their mixtures RS: CC (1:1 and 1:2). The culture broth containing cellulases on their corresponding substrates were subjected to 50°C incubation achieving saccharification efficiency of 33.20, 41.66, 39.86 and 36.87%, respectively. The pentose as xylose (C5) in CC share was bigger than in RS, while hexoses as glucose (C6) shares were nearly similar. Consolidated bioprocessing included cellulase production at 35°C, substrate saccharification at 50°C and finally cofermentation at 35°C. Cellulase produced by *Trichoderma reesei* on RS, CC, mixture 1:1 and mixture 1:2 achieved saccharification of 60.75, 81.13, 65.45 and 74.19%, while cofermentation by *Saccharomyces cerevisiae* and *Aspergillus oryzae* achieved efficiency of 42.69, 51.15, 48.08 and 47.31 %, respectively. The whole CBP best performance was positively correlated to C5 share in fermentable sugars released from each substrate.

Key words: *Trichoderma reesei, Aspergillus oryzae, Saccharomyces cerevisiae, cellulases,* rice straw, corn cobs, glucose, xylose, fermentation, ethanol.

Introduction

Rice and maize annual production in Egypt reached 6.4 and 7.1 million tons, respectively, according to FAO (2017), of which rice straw and corn cobs are post products available in local market. According to Poludasu *et al.* (2013) and Daniela *et al.* (2011), holocellulosic structures of rice straw and corn cobs include the amorphous hemicellulosic part which is rich in pentoses and more accessible to cellulolytic xylanolytic actions than the crystalline cellulosic part. Worthy to mention, the hemicellulose part in corn cob is bigger than that in rice straw, included in treated and untreated forms.

Added to that, the agricultural debris pretreatment is important for cellulose conversion processes. It is required to alter the cellulosic biomass structures to be more accessible to microorganisms for cellulase production as well as converting carbohydrate polymers into fermentable sugars (Mosier *et al.,* 2005). Mechanical, physical chemical or biological pretreatments, as well as the combination of these methods increase the digestibility of cellulose before it is exposed to enzyme or microbial conversion (Bollok, 1999).

Fungal cellulases are inducible enzymes and they are usually excreted into the environment (Bhat and Bhat, 1997) and depend on cellulose type (amorphous or crystalline) (Ortega *et al.,* 2001). The role of cellulolytic fungi exceeds its cellulolytic action to cooperative xylanolytic action too.

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Cellulolytic fungi known for their xylanolytic action belong to genera *Aspergillus, Trichoderma* and *Phanerochaete* (Collins *et al.*, 2005)

When enzymatic hydrolysis of the carbohydrates, fermentation of hexoses and pentoses are carried out separately in distinct reactors under their optimum conditions, the process is known as "Separate (or Sequential) Hydrolysis and Fermentation" (SHF). However, SHF leads to the accumulation of the glucose derived from the hydrolysis of cellulose that can inhibit cellulases, affecting the reaction rates and yields. Besides, part of glucose is adsorbed in the solid residual material, lowering the sugar conversion (Carlos *et al.,* 2010).

These disadvantages are absent when enzymatic hydrolysis and sugar fermentation are run together in a same reactor, in so called "Simultaneous Saccharification and Fermentation" (SSF). The SSF is faster, low cost process and the glucose formed is simultaneously fermented to ethanol avoiding enzymatic product inhibition. The risk of contamination is lower due to the presence of ethanol, the anaerobic conditions and the continuous withdrawal of glucose. Pentoses fermentation can be performed in a separate reactor. The major disadvantage is the difference in optimum temperatures of enzymatic hydrolysis (45–50°C) and alcoholic fermentation (28–35°C) (Carlos *et al.,* 2010).

Simultaneous Saccharification and Co Fermentation (SSCF) is where pentoses and hexoses conversion are carried out in the same reactor (Castro and Pereira, 2010), that is considered as consolidated bioprocessing (CBP). The process can even involve advances in using a single microbial community that can produce the required cellulolytic enzymes, saccharify cellulosic materials and convert resulting fermentable sugars into ethanol in a single reactor lowering overall costs (Lynd *et al.*, 2002).

Brethauer and Studer (2014) developed consolidated bioprocess containing cellulolytic fungi *T. reesei* and two ethanol producing yeasts including *S. cerevisiae* and *Pichia stipitis* in multi-species single biofilm membrane reactor. The *T. reesei* was grown directly on the membrane in an aerobic condition, producing cellulases where carbohydrate fraction was saccharified to soluble sugars that were further fermented anaerobically by both *S. cerevisiae* and *P. stipitis* to ethanol in the anaerobic parts of the reactor.

Maehara *et al.* (2018) stated that in solid state application the co cultivation of *T. reesei* with *A. oryzae* on sugar cane bagasse increased saccharification around 1.5 and 3 fold, compared to their individual cultivations and their enzyme extracts, respectively. Further solid state alcoholic fermentation using *S. cerevisiae* in the presence of those filamentous fungi achieved 83.5% of the theoretical yield. This demonstrated the feasibility of the proposed one-pot integrated strategy using the whole medium of mixed filamentous fungi with yeast for on-site enzymes production, biomass hydrolysis and ethanol production.

Xu *et al.* (2009) confirmed that *T. reesei* had metabolic pathways for converting sugars to ethanol (i.e. glycolysis, pentose phosphate pathway, assimilation pathways of xylose and arabinose leading to ethanolic fermentation) and was able to survive up to 13 days. Another advantage of using *T. reesei* in CBP is that cellobiase does not accumulate during fermentation owing to the cellobiase activity of the fungus.

In the present work, it is intended to study some individuals involved in CBP research contributing both cellulolytic fungus *Trichoderma reesei* and yeast *Saccharomyces cerevisiae* known to ferment hexoses with the aid of fungus *Aspergillus oryzae* known to ferment pentoses (Maheswari and Kalaikodi, 2011).

Materials and Methods

Rice straw and corn cobs pretreatment and analysis:

Rice straw (Rs) and corn cobs (Cc) were collected from the Egyptian local fields. They were dried, chopped and pretreated with alkaline hypochlorite (sodium hypochlorite solution 1.5% w/v, at pH=11.5) as mentioned by Wise *et al.* (1946). Both pretreated rice straw (RS) and corn cobs (CC) were used as substrates for cellulase production and in saccharification processes, as they were applied individually (RS and CC) as well as in mixtures RS:CC (1:1 and 1:2). The substrates total carbohydrate contents were determined by the new method of sulphuric acid-UV technique measured

at 315 nm, as described by Ammar *et al.*, (2013) and were found to be 80, 71, 77 and 74 % (w/w), respectively.

Fungal and yeast strains:

Trichoderma reesei, Aspergillus oryzae and *Saccharomyces cerevisiae* were generously supplied by Microbiology Department in Soils, Water and Environmental Research Institute (SWERI) at Agricultural Research Center (ARC), Giza, Egypt. *T. reesei* was used for cellulases production while *S. cerevisiae* and *A. oryzae* were used in fermentation process.

Enrichment media and conditions:

T. reesei and *A. oryzae* were enriched for 5 days on Mandels medium (Mandels and Reese, 1957) containing (g/L): yeast extract (Difco), 0.1g; $(NH_4)_2SO_4$, 1.4 g; urea, 0.3 g; KH_2PO_4, 2.0 g; $MgSO_4.7H_2O$, 0.3 g; $CaCl_2$, 0.3 g and glucose 5.0 g. Trace elements were added as $FeSO_4$ $7H_20$, 1.0 mg; $ZnCI_2$, 0.8mg; $MnSO_4.H_20$, 0.5mg; $CoCl_2.6H_20$, 0.5mg. *S. cerevisiae* was enriched on potatoes dextrose broth for 24 hours. All media were adjusted at pH 5 and growth proceeded on shaker incubator (125 rpm) at 35°C. The enriched cultures were used as starter for inoculation at 10% (V/V).

Cellulases production medium and conditions:

The RS, CC and their mixtures (1:1 and 1:2) were added individually as 5 g supplementation in 100ml Mandels medium (pH=5) within conical flasks (250ml) and sterilized by autoclaving. The sterilized media were inoculated with 10 ml *T. reesei* starter and incubated at room temperature under static conditions for 4 days. The cultures were examined periodically for Fp-ase, CMC-ase, β-glucosidase and xylanase activities.

Saccharification conditions:

After achieving maximum cellulases production, temperature was raised to 50°C for another 4 days. Samples were collected daily, examined for released sugars (measured as glucose and xylose) and saccharification % was estimated.

$$\text{Saccharification (\%)} = \frac{\text{Total reducing sugar}}{\text{Total carbohydrate}} \times 0.9 \times 100$$

Fermentation medium and conditions:

The fermentation medium (FM) contained (g/L) yeast extract, 5; $(NH_4)_2SO_4$, 7.5; K_2HPO_4, 3.5; $CaCl_2.2H_2O$, 1; $MgSO_4.7H2O$, 0.75 (pH=5) was used with the saccharified substrate as the sole carbon source (Reihaneh *et al.,*2011). Fermentation preceded by the saccharification step was carried out at 35°C. Fermentation medium was added to the saccharified substrates (submerged fermentation), as the flasks were filled to 2/3 of their volume to ensure minor aeration conditions. At first it was inoculated by the enriched culture of *S. cerevisiae* (10% v/v), then after 48 hours it was inoculated by the enriched culture of *A. oryzae* (10% v/v), to examine their capacity to ferment both fermentable hexoses and pentoses available in the saccharified substrates. Inoculated triplicate flasks were arranged in a completely randomized design at room temperature for 5^{th} days through which samples were daily collected for determination of ethanol, reducing sugars as glucose and xylose.

Enzyme assay:

The cellulase activity was estimated in terms of filter paper cellulase activity (FP-ase) using Whatman No.1 according to the standard procedure recommended by the commission of Biotechnology IUPAC (Ghose, 1987), carboxy methyl-cellulose (CMC-ase activity) using CMC according to Mandel *et al.* (1976) and β-glucosidase activity, using salicin as the substrate, according to Chahal (1985). Xylanase activity was determined in terms of xylan, according to Mahamud and Gomes (2012). One enzyme activity unit (U) is defined as the quantity of enzyme that can release 1µg reducing sugar in 1ml of reaction mixture per recommended time under assay conditions.

Sugar estimation:

Sugars liberated by enzyme actions included glucose from FP-ase, CMC-ase, β-glucosidase and xylose from xylanase were measured according to Chi *et al.* (2013) who based his research on the "Phloroglucinol Glacial Acetic Acid" method described by Browning (1967). The procedure was as follows:

1- The color reagent solution was prepared by dissolving 2 grams of phloroglucinol in 110 ml glacial acetic acid, followed by adding 10 ml of anhydrous alcohol and 2 ml of concentrated hydrochloric acid.
2- One ml of sample was mixed with 10 ml of color reagent in a test tube, which was then placed in a boiling water bath for exactly 10 minutes and cooled down with tap water.
3- Spectral measurement was conducted in the wavelength range of 410 nm for hexoses and 553 nm for pentoses. The reference was the resultant solution after reacting distilled water with color reagent under the same conditions as above. The resulting measured concentrations are in mmole/L.

4- Calculations:

$$\text{Xylose concentration} = \frac{A_{x553}}{\varepsilon_{x553}} \quad \text{Glucose concentration} = \frac{A_{g410}}{\varepsilon_{g410}}$$

Where: ε_{x553} and ε_{g410} are the absorption coefficients of xylose and glucose, to be 0.662 and 0.176, respectively.

Ethanol estimation:

Ethanol spectrophotometric determination was done by Potassium dichromate ($K_2Cr_2O_7$) method at 600 nm according to Caputi *et al.,* (1968). Ethanol production was expressed in gram per gram substrate. The basics of the following calculations were clarified by Hatzis *et al.,* (1996).

$$\text{Ethanol yield (\%)} = \frac{\text{ethanol produced}}{\text{sugar consumed}} \times 100$$

$$\text{Fermentation efficiency (\%)} = \frac{\text{ethanol yield}}{\text{theoretical yield}} \times 100$$

Theoretical yield for hexoses and pentoses = 51.14%, as one mole hexose when fermented gives two moles ethanol while three moles pentose give five moles ethanol, as mentioned by McMillan (1993) and Krishnan *et al.,* (1999).

Statistical analysis:

Data in triplicates were statistically analyzed for the least significant difference (LSD) in completely randomized analysis of variance (ANOVA) at $P \leq 0.05$, where capital superscript letters indicate the rank, calculated using CoStat (6.311) software (Maruthai *et al.,* 2012).. Standard deviation bars calculated and applied on graphs through Excell v.10 software, Microsoft cooperation.

Results and discussion

I- Cellulases production magnitude by *T. reesei*

T. reesei ability to produce different cellulolytic and xylanolytic enzymes on RS, CC and their mixtures (1:1 and 1:2, respectively) as four substrates was the main target under investigation for this experiment. Cellulases (Fp-ase, CMC-ase and β-glucosidase) and xylanase activities are shown in Fig (1).

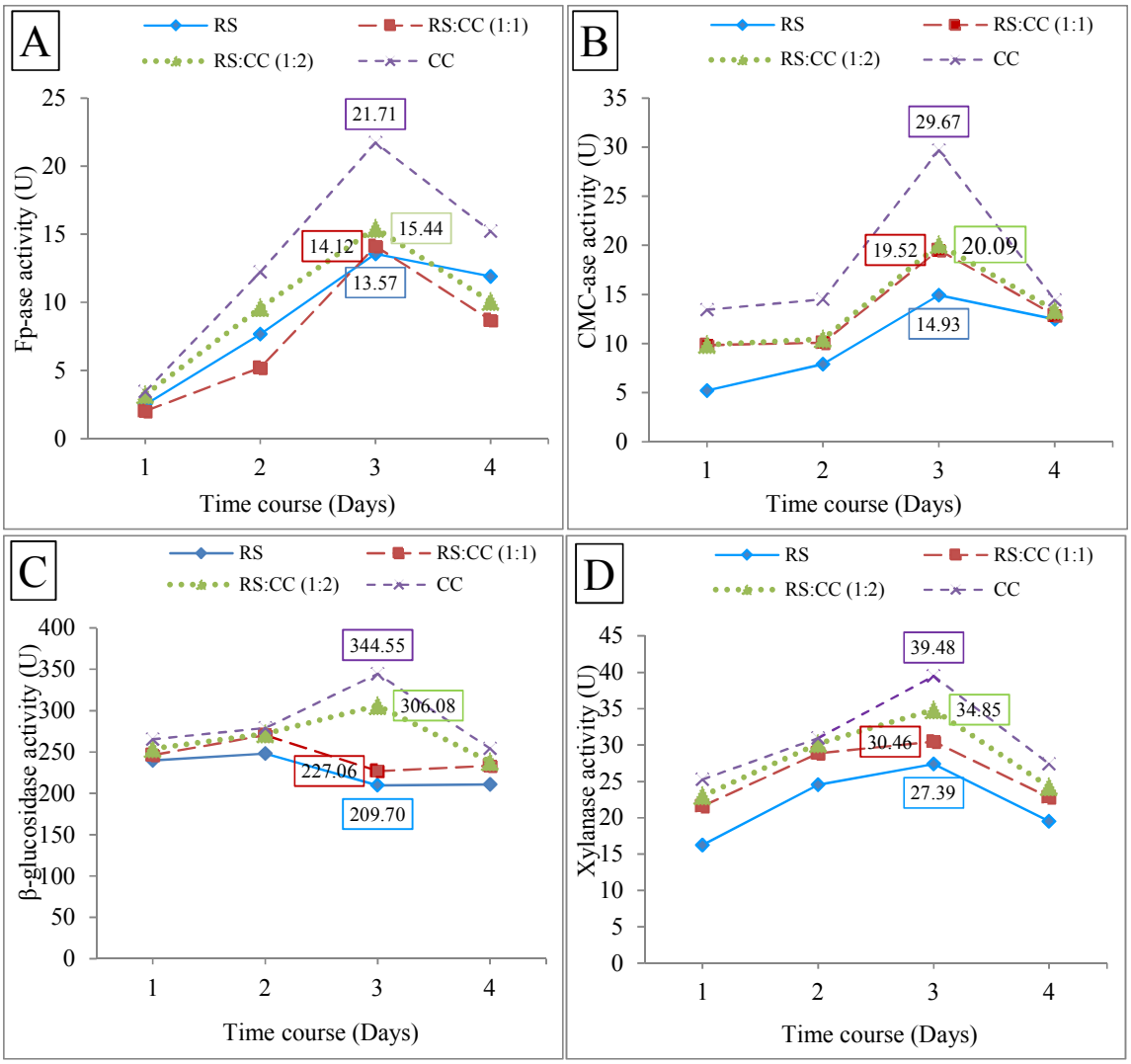

Fig. 1: *T. reesei* enzymatic activities; A) Fp-ase, B) CMC-ase C) β-glucosidase and D) Xylanase, all detected during growth on RS, CC and their mixtures (1:1 and 1:2) at 35°C. ($LSD_{0.05}$ for A= 0.454, B= 1.122, C= 24.567 and D= 2.207).

Fpase, CMCase, β-Glucosidase and Xylanase activity results were ranked according to their statistical $LSD_{0.05}$ of 0.454, 1.122, 24.567 and 2.207, respectively, proving that their highest values mostly were achieved significantly after 3 days of growth.

On the same trend, cellulases produced on CC were maximum on the 3rd day of growth when *T. viride* (Gautam *et al.*, 2010) and *Alternaria alternate* (Amir *et al.,* 2011) were used. On the other hand, Fatma and Fadel (2010) and Dashtban *et al.,* (2011) revealed that the maximum cellulases produced by *T. reesei* on RS and CC were obtained on the 5th day of incubation, respectively. Also, Choudhary *et al.,* (2014) recorded high xylanase, FP-ase and β-glucosidase production by *Aspergillus awamori* F18 under submerged culture on RS after 5 days of incubation, while maximum CMC-ase was after 7 days.

It was remarkable that the enzyme activity decreased after the 3rd day of incubation. In this regard, the decreases might be due to the inhibitory effect of possible accumulated cellobiose on cellulolytic activities (Singh *et al.,* 2009).

Worthy to notice, that the highest activities for Fp-ase (Fig. 1-A), CMC-ase (Fig. 1-B) and β-glucosidase (Fig. 1-C) were achieved on CC to be 22, 30 and 345U, followed by RS/CC mixture (1:2) recording 15, 20 and 306 U, respectively. Fp-ase produced on CC in present study surpassed that produced by *Alternaria alternate* (15U) and *A. awamori* F18 (8.64U) in the work demonstrated by

Amir *et al.*, (2011) and Choudhary *et al.*, (2014), respectively. On the contrary, that maximum activity of CMC-ase (30U) was less than that produced by *Aspergillus awamori* F18 (44U), as mentioned by Choudhary *et al.,* (2014). Accordingly, the time of the highest cellulase activity depends upon the type of substrate and fungus strain (Poludasu *et al.,* 2013). Pandey *et al.,* (2015) reported that *Trichoderma* produced higher cellulases significantly on CC, depending on the organism genotype and carbon sources.

As shown in Fig. (1-D), the maximum xylanase produced on RS (27.39U), is considered higher than that produced on RS by *A. awamori* F18 (25.50U) as done by Choudhary *et al.,* (2014). On the other hand, xylanase produced on CC (39.48U) was superior by >30% than both former mentioned cases on RS, which might be correlated to xylan availability in CC than in RS.

Enzymatic saccharification of rice straw and corn cobs

The cellulolytic production by *T. reesei* on same substrate types under investigation was carried out at 35°C, as done before. Maximum cellulolytic activities of 15, 24, 17 and 19 U were achieved on the 3rd day when grown on RS, CC, mixture of RS: CC (1:1) and (1:2), respectively. Nevertheless, there was a probability of producing xylanase parallel to cellulase deduced from previous results shown in Fig (1-D).

After reaching maximum cellulolytic activity, incubation was continued after the temperature was shifted to 50°C which was suitable for enzymatic saccharification (Tanara *et al.,* 2015). The saccharification % of CC was significantly higher than that of RS ($LSD_{0.05}$ = 1.154), recording 41.66% and 33.20%, respectively, Fig (2).

As stated by Poludasu *et al.,* (2013) the hemicellulose portion forming holocellulose in treated and untreated rice straw usually smaller than that in treated and untreated corn cobs. On the other hand, as cellulose is characterized by crystallinity that is absent in hemicellulose according to Daniela *et al.,* (2011), it makes hemicellulose more suseptable for enzymatic degradation. Depending on previous findings, the degradation of CC during saccharification predicted to easily release fermentable sugars more than RS, as shown in Fig (3). Worthy to notice that the ratio of hexoses to pentoses which were released after 3 days from the saccharification process of CC and RS was calculated to be (1.9:1) and (3:1), respectively. These results are in agreement with findings previously stated by Poludasu *et al.,* (2013). That was why Choudhary *et al.,* (2014) insisted on the importance of synergistic action of cellulases types gathered with xylanase to efficiently degrade polysaccharide for increasing sugar yields.

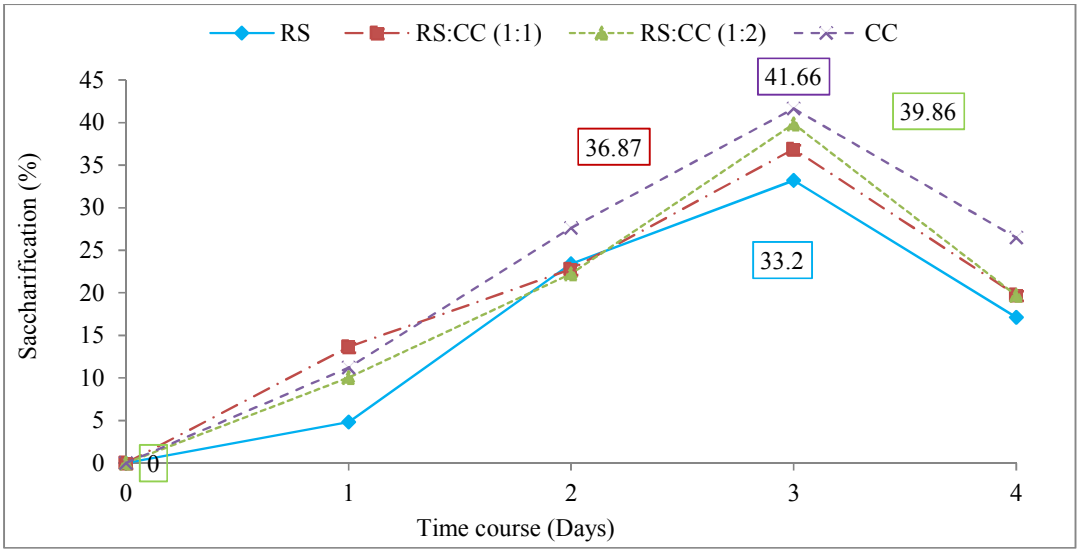

Fig 2: Saccharification % of different agricultural debris by *T. reesei* cellulases ($LSD_{0.05}$ = 1.154).

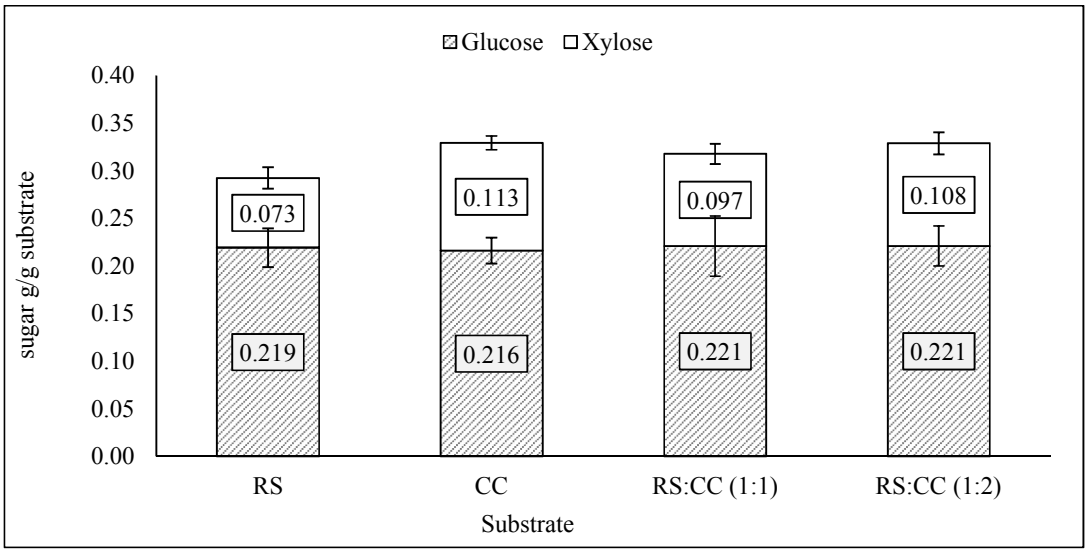

Fig 3: Reducing sugars produced corresponding to best saccharification value (3 days).

Consolidated saccharification and fermentation

Based on previous results, collective processes of cellulase production, saccharification and fermentation were gathered in the same container in a consolidated manner summarized in Table (1). Cellulases reproduced on RS, CC and their mixtures (1:1 and 1:2) had FPase activities of 15, 23, 16 and 21 U after 3 days on 35°C, respectively. Saccharification % of same residual substrates after another 3 days at 50°C recorded 60.75, 81.13, 65.45 and 74.19 %, ranked as CD, A, C and B ($LSD_{0.05}$= 6.792), respectively. Worthy to notice that the fermentable hexoses and pentoses final concentrations were equal to the sum of those released during cellulases production and those released during saccharification that was why the saccharification values increased than previous shown in Fig. 2. For fermentation to take place, temperature was shifted down to 35°C through additional 5 days of incubation as shown in Fig (4).

The fermentation experiment was carried out by *S. cerevisiae* inoculant at first (symbolized by start on Fig. 4) followed by *A. oryzae* inoculant after 48 hours without sterilization to reserve energy. The yeast was used to ferment hexoses (as glucose), while the fungus was used to insure efficient fermentation of resulting pentoses (as xylose) according to Maheswari and Kalaikodi (2011).

During fermentation process, as shown in Fig (4), the ethanol production magnitude fluctuated between high and low after the 2nd and 3rd days, respectively.

Millati *et al.,* (2008) reported that ethanol started being a carbon source for *Mucor indicus* after the depletion of the carbon source from the fermentation medium. Also Srivastava *et al.* (2011) stated that any drop in ethanol accumulated from production might refer to its consumption as carbon source when the main simple carbon source as hexose was nearly consumed.

This might explain in present work the temporary drop in ethanol produced after day 8 (2nd day of fermentation) as shown in Figures (4-B, 4-C and 4-D), when *A. oryzae* was introduced to fermentation process, as it took 24 hours to be adapted for consuming xylose and fermenting it after glucose depletion.

The prolonged ethanol production period up to 5 days and the drop in accumulated ethanol produced during fermentation of hexoses was similarly reported before by Wang *et al.*(2016).They found that white rot fungus *Phlebia sp.* was capable of fermenting most of 20g glucose /L in 2 days giving 10g ethanol in a fluctuating manner. On the other hand *Phanerochaete chrysosporium* was able ferment same quantity in 9 days giving not more than 2 g ethanol/L.

The apparent overlapping happened in Fig (4-C) when glucose fermentation lead to its depletion in the same time xylose fermentation began on days 3 and 4. The overlapping disappeared when CC was increased twice, as in Fig (4-D), making its trend similar to the case of using CC individually, proving the upper hand of CC structure and sugar content than of RS.

As demonstrated in Table (1), the criteria of C6 and C5 (considered as glucose and xylose, respectively) fermentation efficiency % differed according to their ratios, concentrations and type of their saccharified source. Generally, fermentation efficiency increased by increasing C5 sharing in the source.

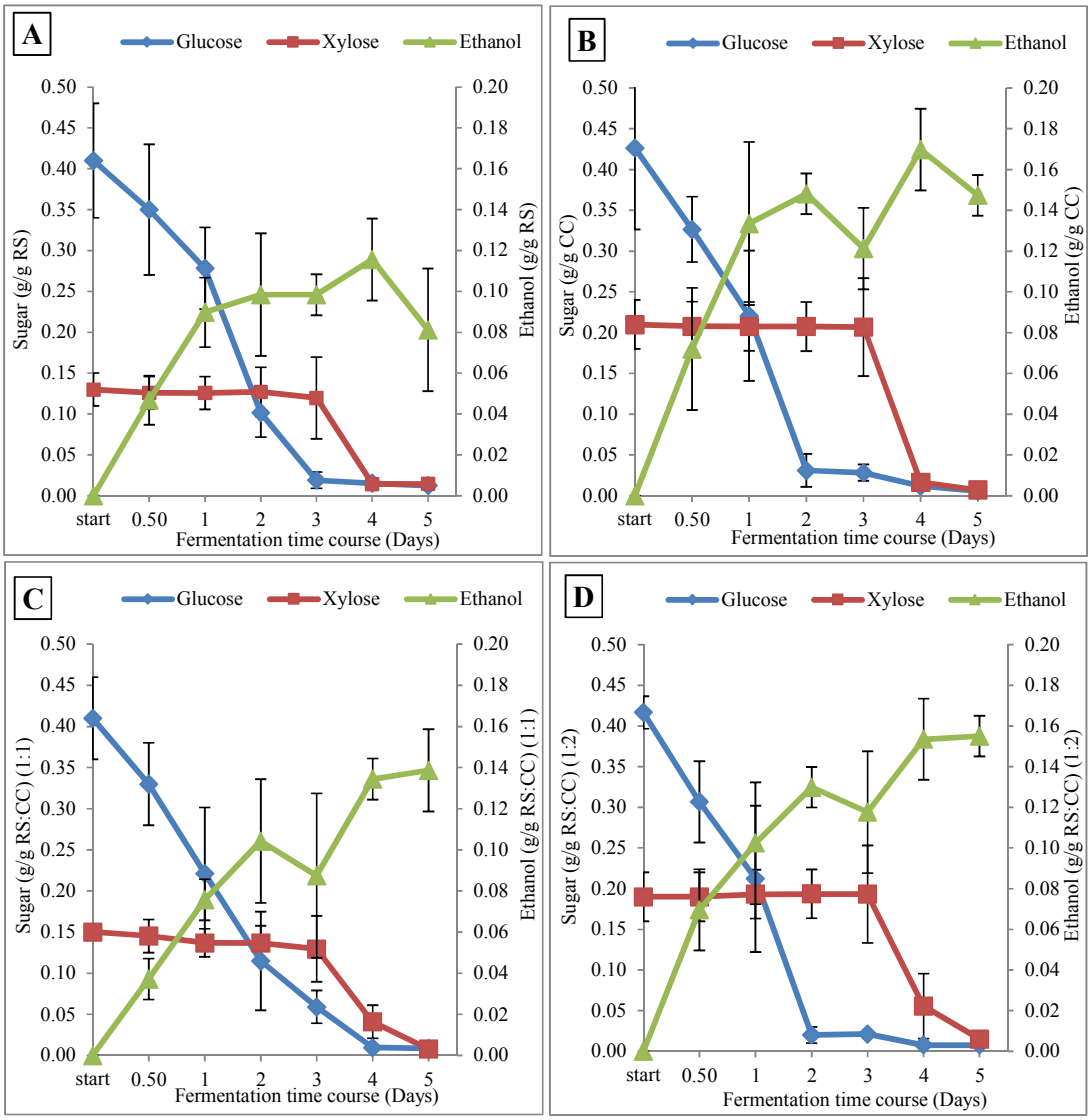

Fig. 4: Ethanol production by *S. cerevisiae* and *A. oryzae* from A) RS, B) CC, C) RS:CC (1:1) and D) RS:CC (1:2) in respect to their residual contents of glucose and xylose.

This was obviously clear in RS (fig. 4A) and CC (fig. 4B) when used individually, as saccharification in CC (81.13%) was higher than in RS (60.75%) making 64% and 54% total reducing sugars (TRS) available for fermentation, respectively. The C5 shared in TRS of RS and CC forming nearly 1/3 and 1/2 that of C6, leading to increased fermentation efficiency for C6 and C5 approximately from 46.92% and 29.62% in up to 67.12% and 45.77%, respectively, with a final fermentation efficiency of 42.69% for RS and 51.15 % for CC.

Table 1: Consolidated cellulase production, saccharification and fermentation using rice straw, corn cobs and their mixtures based on best results for each.

Substrate	FPase (U)	*Sacch. (%)	*TRS g/g Sub	C_6 g/g Sub	C_5 g/g Sub	C6:C5 ratio	Ethanol g/g Sub		Final Ethanol g/g Sub	Ethanol yield %		**Final ethanol yield	Fermentation efficiency %		
							C_6	C_5		C_6	C_5		C_6	C_5	**Final
RS	15^{B}	60.75^{CD}	0.54	0.411^{AB}	0.13^{DE}	3.2:1	0.10	0.02	0.12^{C}	24.4	15.4	22.2	46.92^{C}	29.62^{C}	42.69^{C}
CC	23^{A}	81.13^{A}	0.64	0.429^{A}	0.21^{A}	2.1:1	0.15	0.05	0.17^{A}	34.9	23.8	26.6	67.12^{A}	45.77^{B}	51.15^{A}
RS:CC (1:1)	16^{B}	65.45^{C}	0.56	0.406^{B}	0.15^{C}	2.7:1	0.10	0.05	0.14^{B}	24.4	33.3	25.0	46.92^{C}	64.04^{A}	48.08^{B}
RS:CC (1:2)	21^{A}	74.19^{B}	0.61	0.416^{AB}	0.19^{BC}	2.2:1	0.13	0.03	0.15^{B}	31.0	15.8	24.6	59.62^{B}	30.39^{C}	47.31^{B}
LSD_{005}	2.667	6.792	-	0.0194	0.0183	-	-	-	0.0130	-	-	-	6.733	11.47	2.840

*Saccharification and TRS values based on sum of released sugar during cellulase production at 35°C and during saccharification process at 50°C.
**Final ethanol: The maximum ethanol produced after consuming mostly all available glucose and xylose.
C6: glucose, C5: xylose, TRS: total reducing sugars.

From the sugar type point of view, Millati *et al.*, (2008), clarified that glucose have an inhibitory effect on the xylose conversion and on contrary; the influence of xylose on the glucose conversion was negligible. On the same trend, Santos *et al.,* (2015) revealed that during cultivation in a mixture of xylose and glucose, the fungus didn't assimilate xylose as long as glucose was present in the medium.

This revealed the role of *A. oryzae* in fermenting xylose began after glucose depletion. Nevertheless, the highest ethanol yield in the fermented pretreated corn cobs was due to their high amount of reducing sugars as stated by Poludasu *et al.* (2013).

Guillaume *et al.*, (2007) stated that sugar transport across plasma membrane is the primary step in hexose metabolism. In *Saccharomyces*, the hexose uptake by specific transporter proteins HXT of many types is through gene activation mechanism. HXT gene expression depends on hexose concentration sensed by yeast cells. On the other hand, HXT of high affinity to glucose are induced by small amounts and repressed by large amounts of glucose. On the contrary, the low affinity HXT types behave and in the same time they have higher capacity to support fermentation. Both high and low affinity HXT types have higher affinity for glucose than any different sugar. Also, Itelima *et al.* (2013) stated that the fermentation process was significantly dependent on the effectiveness of sugar transporters of *S. cerevisiae* cells to translocate different sugar types across the cell membrane.

Many known xylose transporters in *S. cerevisiae* are neither selective nor have a higher affinity for xylose, making xylose transporters competitively inhibited by D-glucose (Subtil and Boles, 2012; Farwick *et al*., 2014). Added to that, transport affinity for glucose is often two orders of magnitude higher than for xylose (Leandro *et al.,* 2006; Du *et al.,* 2010 and Young *et al.,* 2011).

Never the less, in *F. oxysporum*, the HXT which had high affinity to glucose was negatively affected by high ethanol concentration as non-competitive inhibitor, as clarified by Ali *et al.*, (2013). Also, they found that the fungal cells compensate reduced activity by increasing HXT transcription and consequently their numbers.

Depending on previous stated findings, in the present work glucose fermentation preceded xylose fermentation by *S. cerevisiae* at first before inoculating *A. oryzae*. Besides, it may be deduced that the increase in pentose shares in the substrate as in CC or RS:CC (1:2) mixture allowed through gene activation mechanism to form and activate the corresponding nonspecific transporters in both yeast and fungi. Accordingly, glucose competed xylose to use its nonspecific transporters, this lead to higher ethanol production from hexose moiety within CC or RS:CC (1:2) more than RS:CC (1:1) or RS as briefed in Table (1).

Conclusion

It is obviously clear that the share existence of pentoses in the cellulosic debris available as fermentable sugar increased the availability of fermentable hexoses to be fermented more efficiently. The consolidated process gathering *T. reesei* cellulase production, cellulosic debris saccharification and fermentation of available released sugars to ethanol by cooperative action of *S. cerevisiae* with *A. oryzae* did reveal the importance of each microorganism in the system besides the effectiveness of mixing more than one source of cellulosic debris. The whole process needs optimization for magnifying the results in further researches.

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