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Utilization of Agro-Wastes for Bioethanol Production

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Abstract: Four agro-wastes were evaluated as substrates for bioethanol production. Seven of the pioneer isolates in the production of cellulase, xylanase and laccase enzymes were selected for soluble sugar and bioethanol production. The highest level of soluble sugar was produced on sugar beet pulp followed by corn cobs. The experimental design included using soybean okara and sesame husk as nitrogen sources added to the production medium. Extraordinary soluble sugar yields were obtained on sugar beet pulp or corn cobs in combination with soybean okara or sesame husk at the concentration of (0.3%) of either. Different concentrations of corn cobs or sugar beet (5, 10, 15, g/100 m medium) were mixed with optimal concentrations of nitrogen sources. Results showed that 10 g of sugar beet or corn cobs achieved the highest soluble sugar yield. The efficiency of four strains of *S. cerevisiae* for bioethanol production was investigated, *S.cerevisiae* (AUMC 14720) recorded the highest level of bioethanol on fermentable fungal broth after four days at 30°C.

1 Introduction

Landfilling, open field burning, and incineration are all common methods for dealing with agricultural waste. However, these approaches have raised many environmental problems, including land degradation and air pollution. Moreover, the agricultural waste's potential worth, predominantly lignocellulosic biomass, has not been realized. The bio-based recycling economy is a contemporary civilization trend in which bio-based rubbish may be recycled to generate high-value commodities while minimizing waste. For example, cellulosic biomass has been widely used in the production of bioethanol and platform chemicals (Chen et al 2017), and waste lignin may be used as a feedstock in the production of important aromatic compounds (Chen et al 2017, Cao et al 2019). Agricultural wastes contain carbon and nitrogen elements ideal for fungal development (Arias et al 2016, Ljoma et al 2019). Crop straw is the most prevalent and least expensive source of lignocellulosic agricultural waste (e.g., rice, wheat, or corn). Cellulose, hemicellulose, and lignin are the basic components of lignocellulose, with cellulose and xylan being two of the most essential polysaccharide components of plant cell walls (Zeng et al 2017). In addition to lignocellulose, numerous agricultural wastes; for example, banana skin include sugars that may be easily used by microorganisms or used as a supporting material due to their physical integrity (Osma et al 2007). As a result, a lignocellulosic agricultural waste should first serve as

a support for the creation of ligninolytic enzymes, and then, depending on its components, it can serve as a substrate source for microorganisms. In addition to the usual composition of cellulose, hemicellulose, and lignin; other components such as sugars, crude proteins, and metal ions should be examined before lignocellulosic agricultural waste processing. This is because those components can influence fungal growth and enzyme production.

Fungi, particularly brown-rot and white-rot basidiomycetes, are used in the biological degradation of lignocellulose-containing raw materials. However, this causes extensive application duration since they have a low rate of fungal breakdown using a portion of the plant polysaccharides (Payne et al 2015). For example, *Penicillium oxalicum* and *Trichoderma reesei* generated efficient lignocellulose-degrading enzymes (Qian et al 2016, Song et al 2016).

Furthermore, since we live in the technological era, new resources and technologies for the pretreatment and manufacture of biofuels from lignocellulosic biomass are being developed. As a result, pretreatment technologies have progressed in lockstep with the development of biofuel production systems (Kumari and Singh 2018).

Bioethanol is a renewable energy source made from agricultural wastes. The first generation of ethanol production used maize as a substrate. In the second generation, microorganisms and agro-wastes were used as substrates (Sarangi and Nayak 2021).

The major goal of the research is to improve the biodegradation of lignocellulosic wastes, using fungal cultures to produce bioethanol under optimal conditions.

2 Materials and Methods

2.1 Agro-waste substrates and yeast cultures

Four different local agricultural wastes were collected, namely corn cobs, corn stover (from Shalakan- Al-Qalubeiah governorate), sugarcane bagasse (from a sugar cane juice shop), and sugar beet pulp (from a sugar factory in Kafr El-Sheikh), having different cellulosic, hemicellulosic, and lignin contents as sources of carbon. Furthermore, we obtained soybean okara and sesame husk as nitrogen sources from the Institute of Food and Feed Technology, Agricultural Research Center, and Local Company for manufacturing of Tahina, Egypt, respectively.

Before using these wastes, dry agro-biomass was cleaned thrice with water to remove dust particles before being air-dried at room temperature. The dried biomass was then ground and sieved with a 1 mm mesh. The chemical profiles of the lignocellulosic wastes applied in this research were determined at the Central Laboratory, Faculty of Agriculture, Ain Shams University **(Table 1)**.

2.1.1 Source of yeast strains

The yeast strains used for bioethanol production from fermented materials were as follows: *Saccharomyces cerevisiae* AUMC 14720 (from Fungi Center at Assiut University), *S. cerevisiae* MIRCEN 20610 (from Microbiological Resources Center, Cairo MIRCEN), *S. cerevisiae* (DM) (from Microbiology Department, Faculty of Agriculture, Ain Shams University) and *S. cerevisiae* (baker's yeast) local strain isolated from active dry yeast from a local market.

2.2 Qualitative determination of lignocellulolytic enzyme's activity

Fungal strains were isolated from the collected agro-wastes (corn cobs, corn stover, sugar beet pulp and sugarcane bagasse) and compost samples adopting the plate technique using potato dextrose (PDA) and malt agar media. They were purified and maintained on PDA and malt agar media, then morphological examinations were performed to identify fungal genera.

Detection of cellulase, xylanase, and laccase activities of isolated fungal cultures was done using cellulose and hemicelluloses and Czapek-Dox agar medium (Kluepfel 1988) containing 1% carboxymethylcellulose as a substrate for cellulase activity or 1% beechwood as a substrate for xylanase activity (Hölker et al 2004). The medium was adjusted to pH 5.0 and autoclaved. The plates of these media were inoculated with a 6 mm agar disc of activated fungal culture and incubated at 28 °C for six days. After incubation plates were flooded with Congo red (1% aqueous) and left for 30 min. Finally, washed twice with 1M NaCl for 20 min.

A transparent hydrolytic zone was observed when cellulose and xylan were degraded. Hydrolysis capacity was assessed to evaluate the activity of degrading enzymes (cellulase and xylanase) of fungi by the diameters of the clear zones or diameters of the colonies (Florencio et al 2012). The guaiacol agar medium containing 0.02% (w/v) guaiacol, 1% (w/v) yeast extract, and 2% (w/v) agar, was used to detect the laccase activity of fungal isolates indicating lignin degradation at 28°C for six days under dark conditions. The positive reaction is recorded by the diameter of fungal growth (Aslam et al 2012).

Table 1. Chemical profiles of collected agricultural wastes

Parameters (%)	Corn cobs	Corn stover	Sugarcane bagasse	Sugar beet pulp	Soybean okara	Sesame husk
Organic matter	34.19	32.24	36.36	34.19	46.96	46.27
Organic carbon	19.88	19.33	21.14	19.88	27.24	26.84
Nitrogen	0.978	0.630	0.308	1.018	6.04	2.81

2.3 Biodegradation efficiency of agro-wastes

Lignocellulolytic enzyme activity was estimated for the selected fungal isolates using agricultural wastes as carbon sources applying the submerge technique. Erlenmeyer flasks (250 mL) containing 50 g of agro-wastes individually as a carbon source with soybean okara or sesame husk equal to 1% nitrogen and 100 mL of production broth medium that containing (gl-1): 2.0 KH_2PO_4, 1.4 $(NH_4)_2SO_4$, 0.3 $MgSO_4.7H_2O$, 0.3 $CaCl_2.2H_2O$, 0.3 Urea, 1 mL of Tween 80 and 1.0 mL of trace elements were used. The trace element composition consists of (g/l) 0.001, $FeSO_4.7H_2O$; 0.001, $MnSO_4.7H_2O$; 0.001 $ZnSO_4.7H_2O$, and 0.001, $CoCl_2.6\ H_2O$ and adjusted to pH 5 (Ryu and Mandels 1980). The sterilized flasks were inoculated with 6 mm agar discs of selected fungi and incubated for 15 days at 28°C under shaking conditions. After the culture growth, the medium was filtered through filter paper. The filtrated broth was centrifuged at 8000 rpm for 20 min. The clear supernatant was analyzed for soluble sugar.

2.4 Morphological identification of fungal cultures

The morphological identification of fungal isolates was done by comparing colonial characters of pure cultures, microscopic characters, and dimensions of informative characters of each fungal isolate compared to those found in identification references (Gaddeyya et al 2012).

2.5 Effect of nitrogen source on enzyme activity

This experiment was conducted to study the effects of different concentrations of soybean okara or sesame husk (0.2, 0.3, 0.4% N) as nitrogen sources on the biodegradation of corn cobs and sugar beet pulp. The appropriate nitrogen source was selected to replace the original nitrogen source in the productive medium and then incubated at 28°C for 15 days. Finally, fermentable broth was determined for soluble sugars.

2.6 Effect of carbon source on enzyme activity

Effects of different concentrations of carbon source (5, 10, 15 g waste /100 mL) in the presence of nitrogen concentration, selected from the previous experiment, were studied by replacing the original carbon source in a productive medium with tested carbon sources as mentioned before. The soluble sugars were determined in fermented broth after incubation at 28°C for 15 days.

2.7 Bioethanol production from agro-waste

The saccharification experiment was performed by the pretreatment of agricultural waste with the potent fungus using the submerge technique for 15 days at 28°C on the optimized medium. The production fermentable broth was sterilized and inoculated with the standard inocula of *S. cerevisiae* AUMC 14720, *S. cerevisiae* MIRCEN 20610, *S. cerevisiae* (DM), or baker's yeast individually for ethanol production. The fermentation medium was incubated at 30°C for 4 days. Bioethanol was determined after the incubation period.

2.8 Analytical technique

2.8.1 Soluble sugar assay

Soluble sugar concentration was determined in the cell-free supernatant using the phenol–sulfuric acid method (DuBois et al 1956).

2.8.2 Bioethanol determination

According to Khalil et al (2015) bioethanol content was estimated calorimetrically using the potassium dichromate technique.

2.9 Statistical analyses

Data were reported as the mean values of three replicates, and standard deviations were statistically examined using the SAS (Rodriguez 2011) and analysis of variance. Duncan's multiple range test (1955) was used to assess mean differences at a significant level of 95% ($p \leq 0.05$).

3 Results and Discussion

The highest fraction of cellulose (49.21%) was observed in sugarcane bagasse, whereas the highest level of hemicellulose was in corn cobs (36.46%). Furthermore, the highest lignin level was in corn cobs (9.99%) **(Table 2)**.

Table 2. Cellulose, hemicellulose, and lignin contents in tested agricultural wastes

Agricultural wastes	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Corn cobs	39.65	36.46	9.99
Corn stover	43.61	27.85	4.45
Sugarcane bagasse	49.21	25.30	7.21
Sugar beet pulp	35.61	22.73	3.91

3.1 Characterization of fungal isolates

A number of 35 fungal isolates were obtained from the various agro-wastes. Isolation was carried out on potato dextrose and malt agar media at 28°C. The fungal isolates belonged to *Fusarium* sp., *Trichoderma* sp., *Aspergillus* sp., *Rhizopus* sp. and *Alternaria* sp. They were characterized based on their morphological and colony characteristics as described by Gaddeyya et al (2012). The screening of these isolates for utilization of agriculture wastes based on their abilities to secret cellulase and xylanase enzymes in the Czapek-Dox medium and the laccase enzyme in the guaiacol agar medium.

3.2 Assessment of lignocellulolytic enzymes activity

Results showed the qualitative efficiency of the most efficient fungal isolates (seven isolates) expressed by hydrolysis capacity, showing high enzyme activity. Qualitative screening of these cultures showed that the six isolates F1, F2, F4, F5, F6, and F7 produced various cellulase levels with hydrolysis capacity ranging from 1.0 to 2.3 **(Table 3)**, F2 and F4 isolates could not produce. Out of the seven isolates, five isolates (F2, F3, F4, F6, and F7) can produce xylanase with hydrolysis capacity ≥ 0.9. The growth diameter of four tested cultures (F2, F3, F4, and F7) ranged from 1.8 to 6.4 cm on the guaiacol agar medium, showing laccase activity. All three major lignocellulolytic enzymes, cellulase, xylanase, and laccase were recorded only by *Trichoderma* sp. (F2), *Aspergillus niger* (F4), *Alternaria* sp. (F7) isolates with highly potent, so were selected for further studies. Moreover, the *Aspergillus* sp. (F4) isolates showed the highest activity levels for cellulase, xylanase and laccase.

The genera *Aspergillus*, *Trichoderma*, and *Penicillium*, are often used microorganisms to produce hydrolytic enzymes (Yadav 2017). Furthermore, soft rot fungi such as *Aspergillus niger* and *Trichoderma reesei* (Xue et al 2017), as well as the white-rot fungus *Phanerochaete chrysosporium*, have been shown to produce significant amounts of cellulase (Manavalan et al 2015).

Fungal isolates were examined for their ability to release extracellular laccase as an indicator of lignin degradation while employing guaiacol as a phenolic substrate. When guaiacol is used as the substrate, extracellular laccase formation by filamentous fungus is indicated by a reddish-brown color on solid media (Aslam et al 2012). The production of extracellular laccase seen in this work is consistent with prior research by Revankar and Lele (2006), Fu et al (2013).

Table 3. Qualitative assay of enzyme activities (cellulase, xylanase, and laccase) represented as hydrolysis capacity at 28°C after six days

Isolate's code		Cellulase hydrolysis capacity*	Xylanase hydrolysis capacity*	Laccase colony diameter
Fusarium sp.	F_1	1.0 ± 0.3	-	-
Trichoderma sp.	F_2	2.1 ± 0.3	1.6 ± 0.4	5.2 ± 0.6
Aspergillus sp.	F_3	-	1.5 ± 0.2	1.8 ± 0.3
	F_4	2.3 ± 0.4	2.1 ± 0.4	6.4 ± 0.5
Rhizopus sp.	F_5	1.2 ± 0.2	-	-
	F_6	1.1 ± 0.2	0.9 ± 0.1	-
Alternaria sp.	F_7	1.8 ± 0.3	1.9 ± 0.3	4.7 ± 0.4

*Hydrolysis capacity = diameter of hydrolysis zone (in cm)/diameter of the colony (in cm)

3.3 Evaluation of biodegradation efficiency of fungal isolates

This study showed the suitability of selected isolates for cellulase, xylanase, and laccase production. Fermentation was done with the addition of 5% corn cobs, corn stover, sugarcane bagasse, and sugar beet pulp separately in the production broth medium. The order of substrate suitability was sugar beet pulp > corn cob > sugarcane bagasse > corn stover, that indicated by the concentration of soluble sugars in fermented broth **(Fig 1)**. The tested isolates exhibited different levels of enzyme activities. Sugar beet pulp yielded the highest titers of 18.05 and 21.79 mg/mL of soluble sugars by *Trichoderma* sp. (F2), *Aspergillus* sp. (F4) isolates, respectively, against the lowest value by *Alternaria* sp. (F7) isolate recorded the lowest soluble sugar values for all tested agro-wastes. However, it was noticed that isolate *Aspergillus* sp. (F4) increased the soluble sugars produced from sugar beet pulp, sugarcane bagasse, corn stover, and corn cobs to 95.0, 71.05, 101.57 and 144.9 folds compared with the control treatment (without fungal inoculum). Microbial enzymes are essential for decomposing lignocellulosic biomass and releasing fermentable sugars.

Wheat straw was selected as a suitable carbon source for the synthesis of xylanase enzyme produced from an anaerobic rumen fungus by Lowe et al (1987), where significant activity levels (0.507 IU/mL) were achieved. Similar work was done by Ravindran et al (2018), who reported a 3-fold increase in xylanase activity and a 1.2-fold increase in cellulase activity by *Aspergillus niger* as the producing microbe when using agriculture wastes.

3.4 Effects of agro-industrial wastes as nitrogen sources

The effects of the agro-industrial wastes (soybean okara or sesame husk) as a nitrogen source when added to corn cobs or sugar beet pulp on soluble sugar production by the tested isolates are illustrated in **Fig 2.** Soybean okara and sesame husk improved the biodegradation of corn cobs and sugar beet pulp by tested isolates compared with production medium containing corn cobs or sugar beet as source carbon with the original nitrogen source (urea and $(NH_4)_2SO_4$). The highest values of soluble sugars were achieved by *Trichoderma* sp. (F2), *Aspergillus* sp. (F4) isolates, and with sugar beet pulp by *Aspergillus* sp. (F4), *Alternaria* sp. (F7) isolates. The corresponding estimates of soluble sugars with corn cobs by *Trichoderma* sp. (F2) and *Aspergillus* sp. (F4) isolates were 19.87 and 23.58 mg/mL in respective order, whereas with sugar beet pulp were 27.11 and 19.34 mg/mL, respectively. Sesame husk as a nitrogen source gave maximum values with corn cobs by *Alternaria* sp. (F7) isolate and with sugar beet pulp by *Trichoderma* sp. (F2) isolate. Corresponding values of soluble sugars were 16.74 mg/mL with corn cobs and 22.85 mg/mL with sugar beet pulp. Among the examined fungal candidates, the *Aspergillus* sp. (F4) was superior concerning the conversion of the experimented agro-wastes to soluble sugars.

The influence of nitrogen supplies on ligninolytic enzymes generated by various species is highly debatable. Some strains require much nitrogen to produce enzymes, whilst others can only be triggered by a shortage of nitrogen (Jing 2010). According to Aydinoglu and Sargin (2013) and Karp et al (2015) organic nitrogen is more favorable to laccase formation than inorganic nitrogen.

3.5 Effect of different concentrations of agricultural wastes as nitrogen source

Table 4 shows the effects of different nitrogen concentrations (0.2, 0.3, 0.4 N%) from sesame husk or soybean okara as a nitrogen source on utilizing agro-wastes by *Aspergillus* sp. (F4) isolate compared with the control treatment (without fungal inoculum). It was observed that soluble sugars released increased gradually with increasing nitrogen concentration, recording maximum values at 0.3N of soybean okara or sesame husk with corn cobs or sugar beet pulp. For sesame husk with corn cobs treatment, different nitrogen concentrations gave constant values of measured parameters with different nitrogen concentrations but increased the soluble sugars compared with the control treatment. The corresponding values of soluble sugars for soybean okara + corn cobs, sugar beet pulp + soybean okara, and sugar beet pulp + sesame husks were 29.78, 38.89 and 34.97 mg/mL, respectively. At 0.3N, it was noticed that soybean okara increased the soluble sugars 155.6 and 141.8 folds with sugar beet pulp and corn cobs in respective order. In contrast, sesame husk increased 139.9 fold with sugar beet compared with the control treatment. Considerable differences between the values of soluble sugars for different nitrogen concentrations of sesame husk with corn cobs were not observed; hence, the lowest concentration of 0.2N was selected for further experiments.

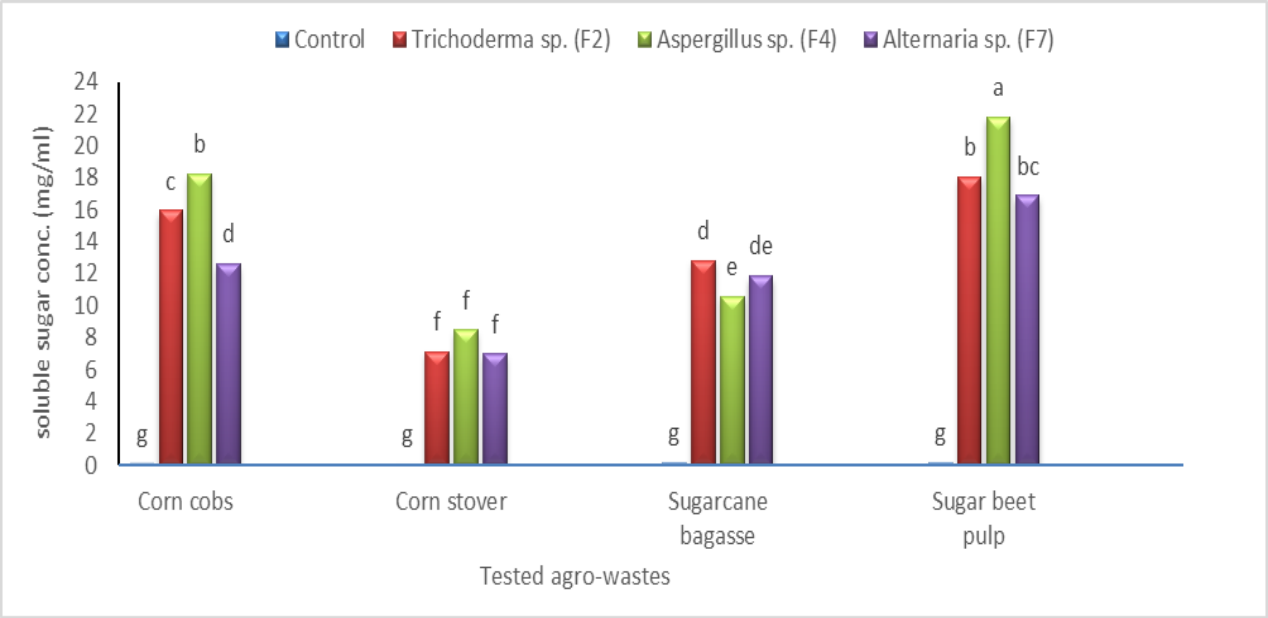

Fig 1. Soluble sugars produced by bioconversion of agricultural wastes using fungal isolates under shaking conditions at 28°C for 15 days
Each value is the mean of three replicates.
Means designated by the same letter are not significant at the 5% level.

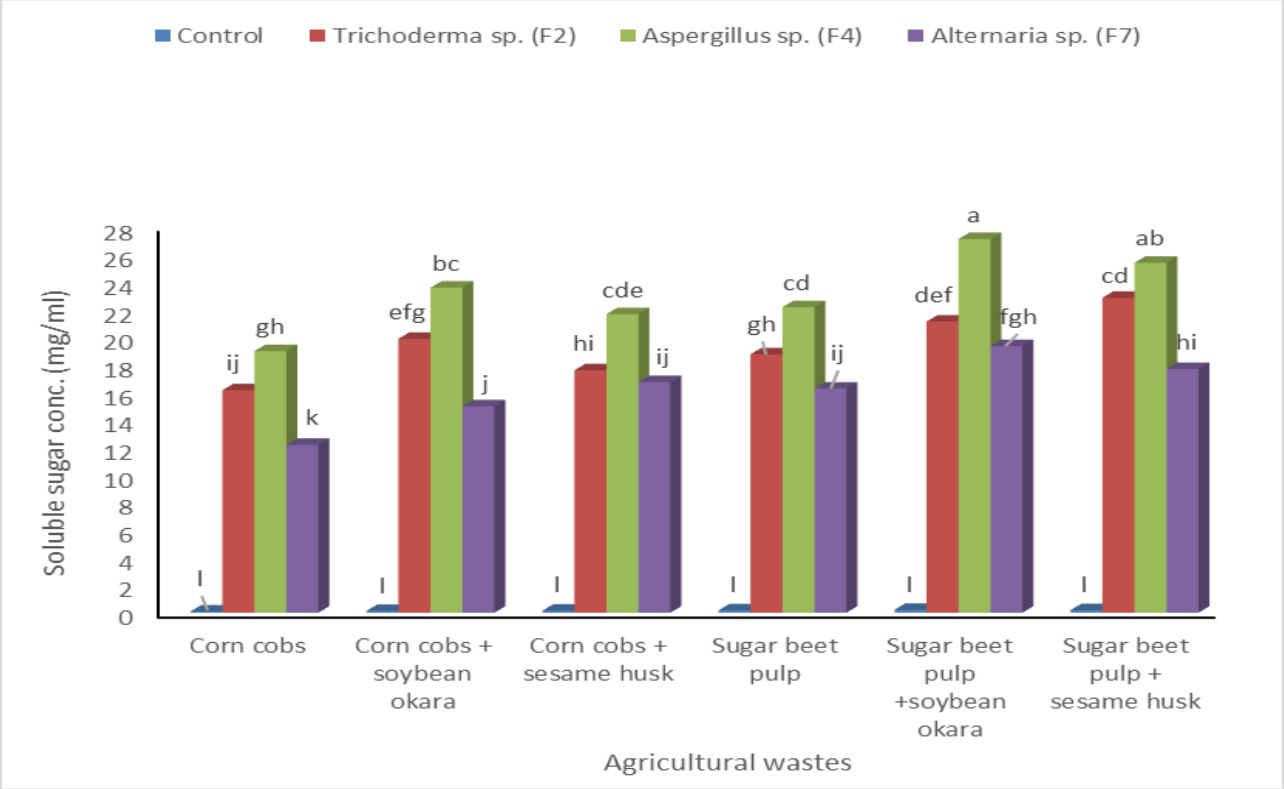

Fig 2. Effects of nitrogen source (concentration 0.1%N) on soluble sugars produced by bioconversion of agricultural wastes using fungal isolates under shaking conditions at 28°C for 15 days
Each value is the mean of three replicates.
Means designated by the same letter are not significant at the 5% level.

Table 4. Effects of different nitrogen concentrations of soybean okara or sesame husk as a nitrogen source on soluble sugars (mg/ml) produced by bioconversion of agricultural wastes using *Aspergillus* sp.(F_4) under shaking conditions at 28°C for 15 days

Isolate's code	Carbon source	Nitrogen source	(Nitrogen concentration %)		
			0.2	**0.3**	**0.4**
Control	Corn Cobs	Soybean okara	0.16f	0.21f	0.21f
		Sesame husk	0.19f	0.25f	0.24f
	Sugar beet pulp	Soybean okara	0.21f	0.25f	0.26f
		Sesame husk	0.22f	0.25f	0.31f
Aspergillus sp. (F_4)	Corn Cobs	Soybean okara	26.17e	29.78d	29.61d
		Sesame husk	25.86e	25.87e	25.81e
	Sugar beet pulp	Soybean okara	32.34c	38.89a	38.65a
		Sesame husk	29.46d	34.97b	33.11c

Each value is the mean of three replicates.
Means designated by the same letter are not significant at the 5% level.

According to Zhang (2007), the use of soybean okara as the only substrate for the production of edible fungus (mushrooms) with health and therapeutic value prompted a series of investigations in Japan and the United States. Fernandez et al (2017) conducted comprehensive research to improve solid-state fermentation using several agro-industrial wastes using the producer microbe *Rhizopus microsporus var*. *oligosporus*.

3.6 Effects of different concentrations of agricultural wastes as carbon source

Table 5 presented the effects of different concentrations of corn cobs or sugar beet pulp with an optimal concentration of nitrogen source (from the previous experiment) on the biodegradation of agro-waste by *Aspergillus* sp. (F4) isolate compared with the control treatment.

Different concentrations of corn cobs or sugar beet pulp (5, 10, 15 g/100ml medium) were examined taking the soluble sugar parameter as an indication of the efficiency for biodegradation. A gradual increase in soluble sugars in fermented broth was reported with increasing agro-waste concentration up to 10g/100 mL then stabilized or slightly decreased as the waste concentration increased to 15 g/100 mL, for all treatments except corn cobs + sesame husk (0.2N), which reached the maximum values at 15 g/100 mL. Maximum values of soluble sugars were recorded with sugar beet pulp + soybean okara (0.3N) followed by sugar beet pulp + sesame husk (0.3N) at 10 g/100 mL waste medium. Corresponding figures were 44.21 and 38.99 mg/mL for soluble sugars, respectively.

Dhillon et al (2012) investigated the utilization of apple pomace as a carbon source for cellulase production lactose was added as a carbon source. After 48 hours of fermentation, a high carboxymethyl cellulase activity of 172.31 IU per gram of dry substrate was detected. Ravindran et al (2018) used wheat bran, wheat flour type II, soybean meal, and sugarcane bagasse to examine four lignocellulosic substrates as potential carbon sources for amylase synthesis. Wheat bran appears to be the most important substrate for amylase production.

3.7 Bioethanol production

Bioethanol production by *Saccharomyces cerevisiae* strains using the product of waste degradation compared with the synthetic medium recommended by Cheng et al (2009) is presented in **Fig 3**. It was observed that the concentration of ethanol produced from the fermented broth was more than that produced in the synthetic medium. Fermentable broth produced from sugar beet pulp gave a higher bioethanol concentration than corn cobs and synthetic medium. The test culture efficiency order is sugar beet > corn cobs > synthetic media. *Saccharomyces cerevisiae* 14720 produced the maximum ethanol concentration on the fermentable broth of sugar beet, followed by *Saccharomyces cerevisiae* 20610. *Saccharomyces cerevisiae* 14720 increased the bioethanol concentration on the fermentable broth of sugar beet and corn cobs by approximately 1.4 and 1.2 folds, respectively, compared with the synthetic medium.

Table 5. Effects of concentrations of different carbon sources on soluble sugars produced by bioconversion of agricultural waste using *Aspergillus* sp. (F_4) under shaking conditions at 28°C for 15 days

Isolate's code	Treatment		Soluble sugars (mg/mL)		
	Carbon source	Nitrogen source and concentrations (%)	5	10	15
Control	Corn Cobs	Soybean okara (0.3)	0.22h	0.31h	0.36h
		Sesame husk (0.3)	0.29h	0.35h	0.39h
	Sugar beet pulp	Soybean okara (0.3)	0.26h	0.31h	0.36h
		Sesame husk (0.4)	0.33h	0.38h	0.42h
Aspergillus sp. (F_4)	Corn Cobs	Soybean okara (0.3)	30.05e	34.73c	34.85c
		Sesame husk (0.2)	25.61g	28.11f	32.19d
	Sugar beet pulp	Soybean okara (0.3)	39.02b	44.21a	44.21a
		Sesame husk (0.3)	34.55c	38.99b	38.85b

Each value is the mean of three replicates.
Means designated by the same letter are not significant at the 5% level.
*5,10,15 represent the used waste weight

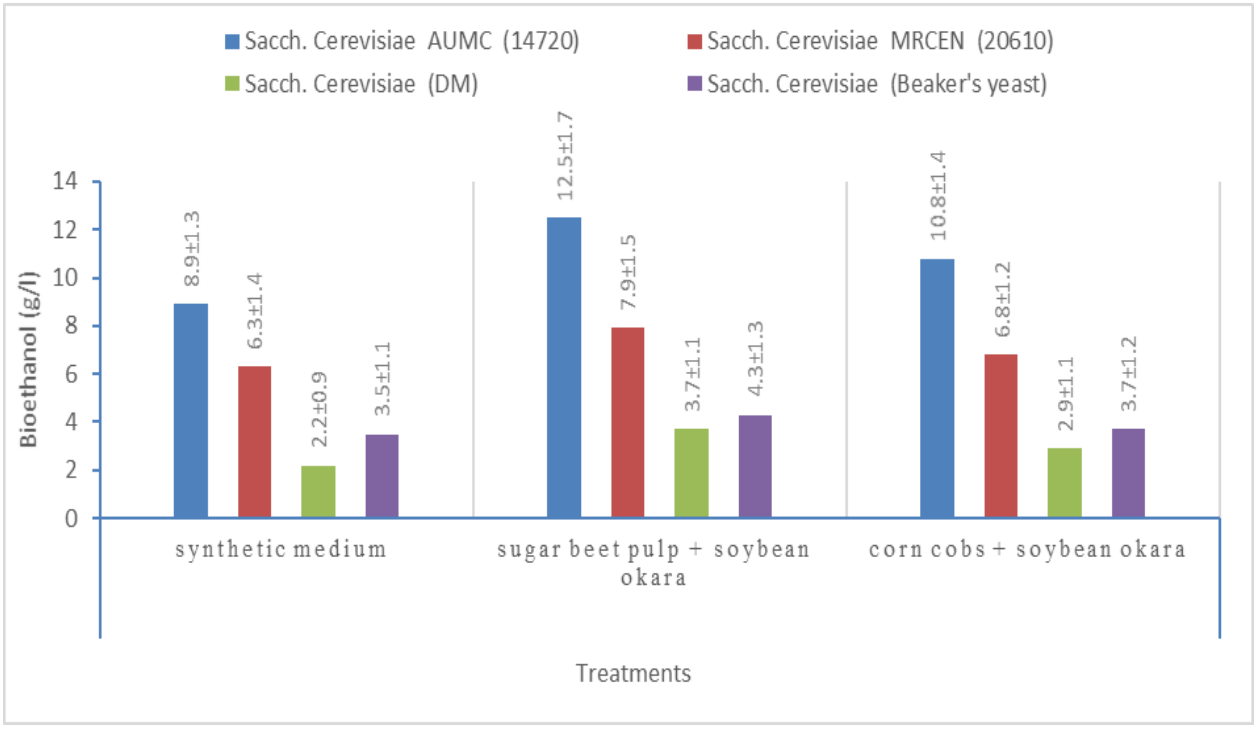

Fig 3. Production of bioethanol (g/L) by *Sacch. cerevisiae* strains were grown on synthetic medium and fermentable broth under static conditions at 30°C for four days
Each value is the mean of three replicates.
The results are presented as the means ± standard error.

Ethanol has historically been produced from glucose-based food crops, such as corn starch, cane sugar, and other starch-rich cereals, by *Saccharomyces* yeasts grown on the glucose obtained from these feedstocks. However, these crops are costly and in limited supply. Bioethanol and related liquid-fuel-based production are currently the only economically feasible activities that employ agro-industry waste as a raw material after fermentation and saccharification (Guan et al 2016). Nevertheless, researchers began exploring potential alternatives for converting agricultural lignocellulosic wastes into raw materials to manufacture different enzymes (Ravindran and Jaiswal 2016). Polysaccharides are abundant in such wastes as cellulose, hemicellulose, starch, pectin, and inulin. *Saccharomyces* cerevisiae, a fermentative yeast, is widely employed to generate ethanol from renewable biomass such as sugar beet molasses or sugar cane (Sanchez and Cardona 2008). These strains of *S. cerevisiae* were chosen as production microorganisms because they are commercially available and have several applications in the food industry.

4 Conclusions

In conclusion, sugar beet pulp, corn cobs, and soybean okara (agro-industrial by-products) are potential cheap substrates for high levels of enzyme and bioethanol production by *Aspergillus* sp. (F_4) after adding soybean okara or sesame husk as a nitrogen source. The use of these residues for bioethanol production would aggregate value to waste and reduce environmental pollution.

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