BIOCONVERSION OF VARIOUS AGRO-INDUSTRIAL BYPRODUCTS INTO BIOETHANOL BY TWO LOCAL YEAST STRAINS USING STATIC CULTURE TECHNIQUE

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Abstract

Some agro-industrial byproducts [sugar cane molasses (SM), corncobs waste (CW), sugar cane bagasse (SB), sawdust (SD), sugar beet pulp (SBP) and fruit juice waste (FJW)] were used as substrates for bioethanol production by *Clavispora lusitaniae* Gr45 and *Saccharomyces cerevisiae* B1 after 3 days incubation at 30°C in static batch culture. Different treatments of these materials as a sole carbon source were applied with or without nitrogen sources (yeast extract or ammonium sulfate). The highest bioethanol concentration when SM is used (13.05 gl^{-1}) was obtained by *Sacch. cerevisiae* at the treatments: [yeast fermentation medium (YFM) -glucose + 4 % SM], (yeast extract + 6 % SM) and (10% SM), which represent about 10.6 % increase comparing to YFM medium (control). The addition of acid CW hydrolyzate as the sole carbon source to YFM medium was the best of the CW treatments by *Cl. lusitaniae* Gr45 which recorded the highest bioethanol concentration, productivity, yield and conversion coefficient being, 13.32 gl^{-1}, 0.18 $gl^{-1}h^{-1}$, 26.64 % and 26.65 %; respectively. These figures were increased to 15.8 gl^{-1}, 0.22 $gl^{-1}h^{-1}$, 31.6 % and 35.9 %, respectively, on YFM medium containing acid SB hydrolyzate as the sole carbon source. All acid SD hydrolyzate treatments increased bioethanol production by *Cl. lusitaniae* Gr45 and decreased it by *Sacch. cerevisiae* B1. The highest bioethanol production obtained on SD was on SD hydrolyzate containing yeast extract followed by SD hydrolyzate only and SD hydrolyzate containing ammonium sulfate being 16.5, 13.76 and 13.54 gl^{-1}; respectively. Using SBP as sole carbon source added to YFM medium decreased significantly the growth and bioethanol production by both strains. Generally, FJW was the best carbon source in YFM medium which increased bioethanol production to about 68 % and 38 % by *Sacch. cerevisiae* B1 and *Cl. lusitaniae* Gr45; respectively using static culture technique.

INTRODUCTION

In recent years, Growing attention has been paid to the conversion of biomass into ethanol, a clean liquid fuel, that considered the alternative to fossil fuels which may be depleted in the next years. Converting lignocellulosic biomass into

fermentable sugars is a major bottleneck for lignocellulose-derived bioethanol production. Plant biomass or lignocellulosic biomass, i. e. agricultural residues, agro-industrial byproducts and energy crops is evaluated worldwide as a potential feedstock for the sustainable production of bioenergy due to its abundance, availability and renewability. On the other hand, using agricultural residues in the production of the biofuel reduce burning of these residues which usually happened by farmers for clearing the field, which subsequently reduce the air pollution and emission of greenhouse gases. (Phitsuwan *et al.,* 2013).

The selection of lignocellulosic crops likely depends on quantity and agronomic considerations including seasons, environment, and regional geography. In Brazil, ethanol from sugar cane has been utilized in the transportation sector since 1975 (Yan *et al*., 2013). In the USA, switchgrass is the leading dedicated energy crop that has been selected for ethanol production (Keshwani and cheng 2009).

Molasses, the byproduct of the sugar-processing and other intermediates from sugar cane processing are very good raw materials for ethanol production due to their high content of fermentable sugars, which can be directly used for fermentation without any modification (Vučurović and Razmovski, 2012). Rodriguez *et al.* (2010) mentioned that grape pomace could be an important agro-industrial waste to be transformed into bioethanol.

Some yeasts use alcoholic fermentation for respiration. Rao *et al.* (2008) isolated a total of 374 yeasts from a variety of rotten fruits and barks of trees. Out of these, 27 yeast strains were able to assimilate xylose and produce 0·12–0·38 g of ethanol per gram of xylose. *Saccharomyces cerevisiae* is the most widely used ethanol producing microorganism. It has attracted considerable attention in recent years for the production of bioethanol from agricultural wastes owing to its higher tolerance to both ethanol and inhibitors present in hydrolysates of lignocellulosic materials (Bettiga *et al.,* 2009).

Freer and Greene *(*1990) demonstrated that *Clavispora lusitaniae* NRRL Y-5394 and *Candida wickerhamii* NRRL Y-2563 had faster initial rates of ethanol production from cellobiose than did the other cellobiose-fermenting yeasts tested. Both yeasts are capable of rapidly fermenting cellobiosesubstrate. Utilization studies showed that *Cl. lusitaniae* could ferment only glucose and cellobiose, while C. *wickerhamii* fermented glucose, cellobiose, and cellodextrins.

The scope of this work was to evaluate the potentiality of using different agro-industrial byproducts as raw materials for the production of bioethanol by fermentation using locally isolated bioethanol producing yeasts *Clavispora lusitaniae* Gr45 and *Saccharomyces cerevisiae* B1.

MATERIALS AND METHODS

Yeasts used

Stock yeast culture (*Saccharomyces cerevisiae* B1 and *Clavispora lusitaniae* Gr 45) slants were obtained kindly from Department of Microbiology, Faculty of Agriculture, Ain Shams Univ. maintained at 5°C on a preservation media after incubation at 30°C for 48-72 h. Standard inoculum was prepared by inoculation of conical flask (250 ml) containing 100 ml of yeast extract peptone medium (YEPM) with a loop of the tested culture. Then, incubated at 30°C for 24 hrs. The content of this flask was used as standard inoculum (O.D, 1.3 and 0.9 for *Sacch. cerevisiae* B1 and *Cl. lusitaniae* Gr45, respectively) for static flask culture experiments.

Media used

- Yeast extract peptone medium (YEPM) (Liang *et al.,* 2008), which has the following composition (g/l): glucose (20), yeast extract (10) peptone (20) agar (20) and completed with distilled water to 1000ml (pH was adjusted to 6.0). This media was used for culture maintainance as preservation media at 5°c.
- Yeast fermentation mediaum (YFM) (Banat and Marchant, 1995) which has the following composition (g/l): KH_2PO_4 (2.0), $MgSO_4.7H_2O$(1.0), $(NH_4)_2\ SO_4$ (2.0), Yeast extract (0.5), $MnSO_4$(0.1), glucose (50) and completed with distilled water to 1000ml (pH was adjusted to 6.0). This media was used in all experiments with amount of 100 ml in 250 ml Erlenmeyer flasks.

Agro-industrial byproducts

Some local agro-industrial byproducts such as corncob waste (CW), sugar cane bagasse (SB), sawdust (SD), sugar beet pulp (SBP) as well as sugar cane molasses (SM) or fruit juice waste (FJW) were used as a carbon source either alone or in combination with other constituents of YFM medium (Banat and Marchant, 1995).

Acid hydrolysis of agro-industrial byproducts

Diluted acid hydrolysis of CW, SB, SD, SBP, using sulphuric acid (2.0 %) was performed in the autoclave at 120°C for 30 min. in 2 L flasks. The solid: liquid final ratio was 5 %. After hydrolysis process, solid materials were removed by filtration. The resulting hydrolyzate was neutralized with calcium carbonate then centrifuged. The filtrates were kept in refrigerator at 5-7°C till use. Each acidic hydrolyzate was used alone or added to YFM medium as a sole carbon source or supplemented with nitrogen source (ammonium sulfate or yeast extract).

Sugar cane molasses

Five concentrations of sugar cane molasses (SM) from 2 to 10 % with 2 % intervals were tested in order to detect the best treatment at the optimum SM concentration for maximum bioethanol production by the tested yeast strains. These SM treatments were using different SM concentrations alone as whole medium, adding to YFM medium as a sole carbon source and adding to nitrogen source only (ammonium sulfate or yeast extract).

Fruit juice waste

Different concentrations of FJW ranging from 80 to 150 $ml l^{-1}$ were used. This experiment was designed to determine the suitable fruit juice waste (FJW) concentration which gives the highest bioethanol production by the tested yeast strains.

Chemical determinations

Total nitrogen (Jackson, 1973) and total sugar (Flood and Preistly, 1973) were determined in SM, acidic hydrolysis of cellulosic agro-industrial byproducts (SD, SB, CW and SBP) and FJW. Residual glucose in fermented culture was determined with glucose kits according to by method of Young and Friedman (2001). Bioethanol in fermented culture was determined colormetrically according to the method of Lau and Luk (1994). Yield of bioethanol and productivity were calculated according to Gamal *et al.* (1991) using the following equations:

$$\text{Yield (\%)} = \frac{\text{Bioethanol produced } (gl^{-1})}{\text{Original sugar concentration } (gl^{-1})} \times 100$$

$$\text{Productivity } (gl^{-1}h^{-1}) = \frac{\text{Bioethanol produced } (gl^{-1})}{\text{Fermentation time (h)}}$$

Conversion coefficient of bioethanol was calculated according to Ramadan *et al.* (1985) as following :

$$\text{Conversion coefficient (\%)} = \frac{\text{Bioethanol produced } (gl^{-1})}{\text{Utilized sugar } (gl^{-1})} \times 100$$

Statistical analysis

The collected data were statistically analyzed using SPSS computer analysis program (Forster, 2001).

RESULTS AND DISCUSSION

Although intensive activity toward the development of bioethanol production is taking place, commercial products are few and costly. One of the important issues related to bioethanol production is the reduction of production costs. Bioconversion of raw materials to available fermentable sugars is probably the most cost effective and environment friendly procedure for raw materials utilization. Therefore, six agro-industrial byproducts, i.e. sugar cane molasses, corncob waste, sugar cane bagasse, sawdust, sugar beet pulp and fruit juice waste were used for bioethanol production by the most efficient bioethanol producing strains *Cl. lusitaniae* Gr45. and *Sacch. cerevisiae* B1 in static batch culture. Most lignocellulosic materials need to be pretreated before used as substrate for bioethanol production. Dilute acid hydrolysis has been successfully developed for pretreatment of different lignocellulosic materials. Dilute sulphuric acid has been the most commonly used so far for converting cellulosic materials into fermentable sugars with high reaction rates, especially as it is a low cost technology (Jeffries and Jin, 2000).

Different treatments of these agro-industrial byproducts were applied as whole medium with or without nitrogen sources (yeast extract or ammonium sulfate) and as a sole carbon source on YFM medium. The used agro-industrial byproducts were varied in sugar and nitrogen content as presented in Table 1. Data revealed that all tested materials were rich in sugar content which ranged from 35.42 (FJW) to 48.3 % (SM). While, total nitrogen was ranged from 0.24 (SD) to 2.5 % (FJW).

1. Sugar cane molasses (SM)

Sugar cane molasses (SM), is a byproduct of sugar industry, contains high sugar concentration and other metals necessary for the fermentation process and is inexpensive. SM has been successfully used for fermentative production of bioethanol. Several microorganisms, including the well-known yeast bioethanol producer, *Sacch. cerevisiae*. In this investigation, SM in different concentrations ranged from 2 to 10 % SM were used for bioethanol production by *Cl. lusitaniae* Gr45 or *Sacch. cerevisiae* B1. Results of different SM treatments were tabulated in Tables 2 and 3. The results indicated that increasing the SM concentrations led to increase the bioethanol production at all treatments and recorded the highest figure by *Cl. lusitaniae* Gr45 at 10% SM (Table 2). All SM treatments at 10 % concentration gave bioethanol concentration lower than YFM medium (12.45 gl^{-1}). These values were 9.8, 8.33, 8.12 and 7.0 gl^{-1} bioethanol for 10 % SM in YFM medium as carbon source, added to yeast extract or ammonium sulfate and as whole medium, respectively. The corresponding figures of bioethanol productivity were 0.13, 0.11, 0.11 and 0.09 $gl^{-1}h^{-1}$, respectively.

This may was in agreement with obtained results of Takeshige and Ouchi (1995). They found that bioethanol production by yeast strain, $X_2$180-1B, was less than half by alcohol yeast, YOY655 in a SM medium containing 30 % sugars, although both strains produced approximately the same amount of bioethanol in a nutrition medium with the same sugar content. They added that the poor fermentation of $X_2$180-1B is not because of its weak fermentation capacity, arises from its susceptibility to some factors in SM other than sugars. Moreover, no significant difference was observed between SM concentrations of 4 % and 6 % used as whole medium or that added to yeast extract on the bioethanol production. Data also clearly show that using 10 % SM was the most appropriate concentration for highest bioethanol production by *Sacch. cerevisiae* B1 if molasses were used without any additives, being 13.05 gl^{-1} (Table 3). The highest bioethanol concentration being 13.05 gl^{-1} was also obtained on SM added to YFM medium without glucose at the concentration of 4 % and on the treatments of 6 % SM containing yeast extract.

2. Corncobs waste (CW)

Corncobs waste (CW) has the potential to produce alcohol components as mentioned by (Reno *et al.,* 2011). Corncobs wastes are produced with huge quantities all over the country. The discarding of these tons of wastes always considered a terrible problem. This is due to the expensive cost of transferring this waste and the pollution from discarding them by burning. In the trials for using this waste as substrate for bioethanol fermentation, data given in Table (4) revealed that YFM medium containing CW waste hydrolyzate as sole carbon source gave the highest bioethanol production (13.32 gl^{-1}) among all other treatments, which increased about 6.98 % comparing to control. In this treatment, the highest figures of bioethanol productivity, bioethanol yield and conversion coefficient being 0.18 $gl^{-1}h^{-1}$, 26.64 % and 26.65 %, respectively were obtained. On the contrary, by *Sacch. cerevisiae* B1 (Table 5), all treatments of CW hydrolyzate didn't enhance bioethanol production. Whereas, YFM complete medium (control) attained the highest bioethanol concentration (11.5 gl^{-1}). Also, it could be noticed that using CW hydrolyzate as a whole medium recorded the lowest bioethanol production by both strains. At all CW treatments, the growth of both strains was lower than attained by YFM medium. Ge *et al.* (2011) obtained 36.49 total reducing sugars (g/l) when treated corncob hydrolysis with 1% (v/v) of sulfuric acid, and when the acid hydrolysate was detoxified with overliming plus activated charcoal, that yielded maximum productivity of 0.152 $gl^{-1}h^{-1}$ and bioethanol yield of 0.31 gg^{-1} .

3. Sugar cane bagasse (SB)

Sugar cane bagasse (SB) is one of the cellulosic feedstock that used for bioethanol production. Different treatments to SB hydrolyzate were prepared as previously conducted. Data given in Tables 6 and 7 indicated to significant effect of SB treatments on the growth and bioethanol production by both tested strains. The highest bioethanol production was obtained by *Cl. lusitaniae* Gr45 on YFM medium containing the SB hydrolyzate as a sole carbon source. This treatment also recorded the highest bioethanol concentration, yield, productivity and conversion coefficient being 15.8 gl^{-1}, 31.6 %, 0.22 $gl^{-1}h^{-1}$ and 35.9 %, respectively. While *Sacch. cerevisiae* B1 on different SB treatments (Table 7) achieved lower bioethanol production than YFM medium. The lowest bioethanol concentrations were obtained by *Sacch. cerevisiae* B1 and *Cl. lusitaniae* Gr45 at SB hydrolyzate as whole treatment being 2.97 and 6.5 gl^{-1}, respectively. In this respect, Gubicza *et al.* (2014) used dilute phosphoric acid and steam explosion as pretreatment for SB. This process has been successfully scaled-up to 80 L fermentations with yields as high as 0.27 gg^{-1}.

4. Sawdust (SD)

Sawdust (SD) can be used as a veritable resource for bioethanol production. Different treatments to SD were prepared as previously conducted. Data presented in Table 8 show that all treatments of SD hydrolyzate recorded by *Cl. lusitaniae* Gr45 attained higher bioethanol production than YFM medium. The highest bioethanol production was obtained at SD hydrolyzate containing yeast extract followed by SD hydrolyzate only and SD hydrolyzate containing ammonium sulfate being 16.5, 13.76 and 13.54 gl^{-1}, respectively. At the first treatment bioethanol productivity, yield, conversion coefficient, were 0.23 $gl^{-1}h^{-1}$, 33.0 % and 33.0 %, respectively. On contrast, all SD treatments gave lower bioethanol production by *Sacch. cerevisiae* B1 than the control (YFM medium) (Table 9). The lowest values of bioethanol concentrations, productivity, yield and conversion coefficient were observed at SD hydrolyzate treatment followed by that containing ammonium sulfate and yeast extract. Moreover, the first treatment recorded the lowest growth of *Cl. lusitaniae* Gr45 and *Sacch. cerevisiae* B1 with decreased about (57.2 %) and (57.9 %) than control, respectively. The final pH range obtained by *Sacch. cerevisiae* B1 (4.8-4.9) was lower than that obtained by *Cl. lusitaniae* Gr45 (5.52-6.65) at different treatments.

5. Sugar beet pulp (SBP)

Data given in Tables 10 and 11 show the bioethanol production by *Cl. lusitaniae* Gr 45 and *Sacch. cerevisiae* B1 after three days incubation period on different

treatments of acid beet waste (SBP) hydrolyzate. It could be noticed that most treatments of SBP hydrolyzates didn't enhance bioethanol production by both tested strains, but YFM complete medium (control) attained the highest bioethanol concentration. Addition of nitrogen source as $(NH_4)_2SO_4$ and yeast extract gave the highest bioethanol production by *Cl. lusitaniae* Gr45 and *Sacch. cerevisiae* B1 being 10.73 and 4.03 gl^{-1} respectively. This result may coincide with what mentioned by Ergun and Mutlu (2000), they stated that there is a digestible nitrogen deficiency of sugar beet molasses, so, addition of ammonium phosphate, ammonium dihydrogen phosphate and ammonium sulfate are usually performed to the fermentation medium for better productivity. In these treatments, the highest figures of bioethanol productivity, bioethanol yield and conversion coefficient were obtained. Using SBP as sole carbon source on YFM medium gave a negative effect on the growth and bioethanol production by both strains to record the lowest figures being 1.0 and 2.01 gl^{-1} for *Sacch. cerevisiae* B1 and 1.08 and 5.5 gl^{-1} for *Cl. lusitaniae* Gr45, respectively. Also, the lowest value of final pH was recorded in this treatment.

6. Fruit juice waste (FJW)

This experiment was initiated to determine the optimal bioethanol production conditions for high efficiency bioethanol production from the fruit juice waste (FJW) at different concentrations. Results in Tables 12 and 13 proved the significant effect of all FJW concentrations on bioethanol production by *Sacch. cerevisiae* B1 whereas no significant effect could be detected either between 100 and 120 mll^{-1} or between 130 and150 ml l^{-1} concentrations on bioethanol production by *Cl. lusitaniae* Gr45. The highest bioethanol production by *Cl. lusitaniae* Gr45 and *Sacch. cerevisiae* B1 were attained on FJW at 130 and 150 mll^{-1} concentrations being 17.2 and 19.9 gl^{-1}, respectively. These treatments attained productivity and bioethanol yield, being 0.28 $gl^{-1}h^{-1}$ and 37.4 % for *Sacch. cerevisiae* B1 and 0.24 $gl^{-1}h^{-1}$ and 32.33 % for *Cl. lusitaniae* Gr45, respectively.

In this respect, Lin and Tanaka (2006) stated that substrates like fruits wastes, sugar cane, sugar beets, sweet sorghum and molasses already have sugars and once simple sugars are present, enzymes from microorganisms can readily ferment them to bioethanol. Starches must firstly be hydrolyzed to fermentable sugars by the action of enzymes and cellulose must likewise be converted into sugars, generally by the action of mineral acids. Also, Dhillon *et al.* (2013) mentioned that apple-processing industries generate huge quantities of wastes 'apple pomace' (skin, pulp and seeds) and juice. It can be used as a promising raw material for direct extraction of bioactive compounds and bio production of high value-added products, such as enzymes, organic acids and biofuels.

Table 1. Total sugars and nitrogen content of agro-industrial byproducts.

Agro-industrial byproducts	Total sugars (%)	Total Nitrogen (%)
Sawdust	36.8	0.24
Sugar cane Bagasse	45.2	0.45
Corncobs waste	45.6	0.53
Sugar beet pulp	44.5	1.84
Fruit Juice waste	35.4	2.50
Black strap cane molasses	48.3	0.80

Table 2. Effect of different sugar cane molasses treatments at different concentrations on bioethanol production by *Cl. lusitaniae* Gr45 after 3 days incubation at 30°C as a static batch culture.*

Treatments	Bioethanol concentration (gl^{-1})	Bioethanol productivity ($gl^{-1}h^{-1}$)	Final pH
YFM complete medium (control)	12.45 a	0.17	3.5
YFM – glucose + 2 % sugar cane molasses	4.62 k	0.06	4.8
YFM – glucose + 4 % sugar cane molasses	5.11 i	0.07	4.9
YFM – glucose + 6 % sugar cane molasses	7.00 e	0.09	4.6
YFM – glucose + 8 % sugar cane molasses	8.12 d	0.11	4.7
YFM – glucose + 10 % sugar cane molasses	9.80 b	0.13	4.5
Yeast extract + 2 % sugar cane molasses	4.55L	0.06	5.7
Yeast extract + 4 % sugar cane molasses	4.69 J	0.06	5.0
Yeast extract+ 6 % sugar cane molasses	4.69 J	0.06	4.8
Yeast extract + 8 % sugar cane molasses	6.79 F	0.09	4.7
Yeast extract+ 10 % sugar cane molasses	8.33 c	0.11	4.7
$(NH_4)_2SO_4$ + 2 % sugar cane molasses	2.31 o	0.03	5.0
$(NH_4)_2SO_4$ + 4 % sugar cane molasses	5.74 h	0.07	5.0
$(NH_4)_2SO_4$ + 6 % sugar cane molasses	6.68J	0.09	4.7
$(NH_4)_2SO_4$ + 8 % sugar cane molasses	7.00 e	0.09	4.7
$(NH_4)_2SO_4$ + 10 % sugar cane molasses	8.12 d	0.11	4.7
2 % sugar cane molasses	3.78 n	0.05	5.1
4 % sugar cane molasses	4.34 m	0.06	4.8
6 % sugar cane molasses	4.34 m	0.06	4.8
8 % sugar cane molasses	5.88 g	0.08	4.7
10 % sugar cane molasses	7.00 e	0.09	4.6

* The values are mean of three replicates.

Productivity = Bioethanol concentration (gl^{-1}) / fermentation time (h) = $gl^{-1}h^{-1}$ (Gamal *et al.*, 1991).

Values in the same parameter followed by the same latter do not significantly differ from each other, according to Duncan's at 5% level.

Table 3. Effect of different sugar cane molasses treatments at different concentrations on bioethanol production by *Sacch. cerevisiae* B1 after 3 days incubation at 30°C as a static batch culture. *

Treatments	Bioethanol concentration (gl^{-1})	Bioethanol productivity ($gl^{-1}h^{-1}$)	Final pH
YFM complete medium (control)	11.80 d	0.16	3.2
YFM – glucose + 2 % sugar cane molasses	8.70g	0.12	4.6
YFM – glucose + 4 % sugar cane molasses	13.05 a	0.18	4.6
YFM – glucose + 6 % sugar cane molasses	12.43 b	0.17	4.6
YFM – glucose + 8 % sugar cane molasses	11.75 d	0.16	4.6
YFM – glucose + 10 % sugar cane molasses	11.75 d	0.16	4.6
Yeast extract + 2 % sugar cane molasses	9.60f	0.13	4.8
Yeast extract+ 4 % sugar cane molasses	9.60f	0.13	4.6
Yeast extract+ 6 % sugar cane molasses	13.05 a	0.18	4.6
Yeast extract+ 8 % sugar cane molasses	12.43 b	0.17	4.6
Yeast extract + 10 % sugar cane molasses	12.20c	0.16	4.6
$(NH_4)_2SO_4$ + 2 % sugar cane molasses	10.17 e	0.14	4.6
$(NH_4)_2SO_4$ + 4 % sugar cane molasses	10.17 e	0.14	4.6
$(NH_4)_2SO_4$ + 6 % sugar cane molasses	11.75 d	0.16	4.5
$(NH_4)_2SO_4$ + 8 % sugar cane molasses	12.20 c	0.17	4.5
$(NH_4)_2SO_4$ + 10 % sugar cane molasses	12.43 b	0.17	4.5
2 % sugar cane molasses	9.60 f	0.13	4.7
4 % sugar cane molasses	11.75 d	0.16	4.6
6 % sugar cane molasses	12.20 c	0.16	4.7
8 % sugar cane molasses	12.43 b	0.17	4.4
10 % sugar cane molasses	13.05a	0.18	4.6

*The values are mean of three replicates.

Productivity = Bioethanol concentration (gl^{-1}) / fermentation time (h) = $gl^{-1}h^{-1}$ (Gamal *et al.*, 1991).

Values in the same parameter followed by the same latter do not significantly differ from each other, according to Duncan's at 5% level.

Table 4. Effect of different treatments of acid CW hydrolyzate on bioethanol production by *Cl. lusitaniae* Gr 45 after 3 days incubation at 30°C as a static batch culture.*

Treatments	Growth (O.D)	Residual glucose (gl^{-1})	Consumed glucose (gl^{-1})	Bioethanol concentration (gl^{-1})	Bioethanol productivity ($gl^{-1}h^{-1}$)	Bioethanol yield (%)	Conversion coefficient (%)	Final pH
YFM complete medium (control)	2.50 a	0.055	50.00	12.45 b	0.17	24.90	24.90	3.80
YFM – glucose + CW hydrolyzate	1.45 c	0.028	49.97	13.32 a	0.18	26.64	26.65	5.67
YE + CW hydrolyzate	1.52 b	0.041	49.96	5.76 c	0.08	11.52	11.52	6.27
$(NH_4)_2SO_4$ + CW hydrolyzate	1.55 b	0.006	49.99	3.24 d	0.04	6.48	6.48	6.47
CW hydrolyzate	1.46 c	0.018	49.98	0.72 e	0.01	1.44	1.44	6.14

*The values are mean of three replicates.

Productivity = Bioethanol concentration (gl^{-1}) / fermentation time (h) = $gl^{-1}h^{-1}$ (Gamal *et al.*, 1991).

Ethanol yield (%) = [Bioethanol concentration (gl^{-1}) / initial sugars (gl^{-1})] bioethanol x100 (Gamal *et al.*, 1991).

Conversion coefficient (%) = [Bioethanol concentration (gl^{-1}) / consumed sugars (gl^{-1})] x100 (Gamal *et al.*, 1991).

Values in the same parameter followed by the same latter do not significantly differ from each other, according to Duncan's at 5% level.

Table 5. Effect of different treatments of acid CW hydrolyzate on ethanol production by *Sacch. cerevisiae* B1 after 3 days incubation at 30°C as a static batch culture.*

Treatments	Growth (O.D)	Residual glucose (gl^{-1})	Consumed glucose (gl^{-1})	Bioethanol concentration (gl^{-1})	Bioethanol productivity ($gl^{-1}h^{-1}$)	Bioethanol yield (%)	Conversion coefficient (%)	Final pH
YFM complete medium (control)	1.9 a	0.247	49.75	11.50 a	0.16	23.00	23.00	3.0
YFM – glucose + CW hydrolyzate	1.8 b	0.413	49.58	2.03 c	0.03	4.06	4.09	4.6
YE + CW hydrolyzate	1.3 c	0.540	49.46	2.53 b	0.04	5.06	5.11	4.9
$(NH_4)_2SO_4$ + CW hydrolyzate	1.1 d	0.114	49.85	2.01 c	0.03	4.02	4.02	4.9
CW hydrolyzate	1.1d	0.091	49.90	1.50 d	0.02	3.00	3.00	4.9

*The values are mean of three replicates.

Productivity = Bioethanol concentration (gl^{-1}) / fermentation time (h) = $gl^{-1}h^{-1}$ (Gamal *et al.*, 1991).

Bioethanol yield (%) = [Bioethanol concentration (gl^{-1}) / initial sugars (gl^{-1})] x100 (Gamal *et al.*, 1991).

Conversion coefficient (%) = [Bioethanol concentration (gl^{-1}) / consumed sugars (gl^{-1})] x100 (Gamal *et al.*, 1991).

Values in the same parameter followed by the same latter do not significantly differ from each other, according to Duncan's at 5% level.

Table 6. Effect of different treatments of acid sugar cane bagasse (SB) hydrolyzate on bioethanol production by *Cl. lusitaniae* Gr45 after 3 days incubation at 30°C as a static batch culture.*

Treatments	Growth (O.D)	Residual glucose (gl^{-1})	Consumed glucose (gl^{-1})	Bioethanol concentration (gl^{-1})	Bioethanol productivity ($gl^{-1}h^{-1}$)	Bioethanol yield (%)	Conversion coefficient (%)	Final pH
YFM complete medium (control)	2.50 a	0.055	50.00	12.3 b	0.17	24.6	24.6	3.8
YFM – glucose + SB hydrolyzate	1.11 d	0.034	43.97	15.8 a	0.22	31.6	35.9	5.4
YE + SB hydrolyzate	1.76 b	0.024	49.98	9.7 b	0.13	19.4	19.4	6.4
$(NH_4)_2SO_4$ + SB hydrolyzate	1.83 b	0.041	49.96	6.8 c	0.09	13.6	13.6	6.8
SB hydrolyzate	1.22 c	0.021	49.98	6.5 d	0.09	13.0	13.0	6.55

*The values are mean of three replicates
Productivity = Bioethanol concentration (gl^{-1}) / fermentation time (h) = $gl^{-1}h^{-1}$ (Gamal *et al.*, 1991).
Bioethanol yield (%) = [Bioethanol concentration (gl^{-1}) / initial sugars (gl^{-1})] x100 (Gamal *et al.*, 1991).
Conversion coefficient (%) = [Bioethanol concentration (gl^{-1}) / consumed sugars (gl^{-1})] x100 (Gamal *et al.*, 1991).
Values in the same parameter followed by the same latter do not significantly differ from each other, according to Duncan's at 5% level.

Table 7. Effect of different treatments of acid sugar cane bagasse (SB) hydrolyzate on bioethanol production by *Sacch. cerevisiae* B1 after 3 days incubation at 30°C as a static batch culture.*

Treatments	Growth (O.D)	Residual glucose (gl^{-1})	Consumed glucose (gl^{-1})	Bioethanol concentration (gl^{-1})	Bioethanol productivity ($gl^{-1}h^{-1}$)	Bioethanol yield (%)	Conversion coefficient (%)	Final pH
YFM complete medium (control)	1.9 a	0.247	49.75	11.52 a	0.16	23.04	23.10	3.0
YFM – glucose + SB hydrolyzate	1.8 b	0.540	49.46	4.50 b	0.06	9.00	9.03	4.9
YE + SB hydrolyzate	1.7 c	0.450	49.55	4.03 c	0.05	0.05	8.13	5.1
$(NH_4)_2SO_4$ + SB hydrolyzate	1.4 d	0.440	49.56	3.51 d	0.05	0.05	7.08	4.9
SB hydrolyzate	1.4 d	0.200	49.80	2.97 e	0.04	6.00	6.00	5.1

*The values are mean of three replicates.
Productivity = Bioethanol concentration (gl^{-1}) / fermentation time (h) = $gl^{-1}h^{-1}$ (Gamal *et al.*, 1991).
Bioethanol yield (%) = [Bioethanol concentration (gl^{-1}) / initial sugars (gl^{-1})] x100 (Gamal *et al.*, 1991).
Conversion coefficient (%) = [Bioethanol concentration (gl^{-1}) / consumed sugars (gl^{-1})] x100 (Gamal *et al.*, 1991).
Values in the same parameter followed by the same latter do not significantly differ from each other, according to Duncan's at 5% level.

Table 8. Effect of different treatments of acid sawdust (SD) hydrolyzate on bioethanol production by *Cl. lusitaniae* Gr45 after 3 days incubation at 30°C as a static batch culture.*

Treatments	Growth (O.D)	Residual glucose (gl^{-1})	Consumed glucose (gl^{-1})	Bioethanol concentration (gl^{-1})	Bioethanol productivity ($gl^{-1}h^{-1}$)	Bioethanol yield (%)	Conversion coefficient (%)	Final pH
YFM complete medium (control)	2.50 a	0.06	50.00	12.52 e	0.17	25.04	25.04	3.80
YFM – glucose + SD hydrolyzate	1.21 c	0.06	43.94	12.60 d	0.18	25.20	28.68	5.52
YE + SD hydrolyzate	1.65b	0.03	49.98	16.50 a	0.23	33.00	33.00	6.65
$(NH_4)_2SO_4$ + SD hydrolyzate	1.15 c	0.09	49.91	13.54 c	0.19	27.10	27.13	6.27
SD hydrolyzate	1.07d	0.00	50.00	13.76 b	0.19	27.50	27.50	6.43

*The values are mean of three replicates.

Productivity = Bioethanol concentration (gl^{-1}) / fermentation time (h) = $gl^{-1}h^{-1}$ (Gamal *et al.*, 1991).

Bioethanol yield (%) = [Bioethanol concentration (gl^{-1}) / initial sugars (gl^{-1})] x100 (Gamal *et al.*, 1991).

Conversion coefficient (%) = [Bioethanol concentration (gl^{-1}) / consumed sugars (gl^{-1})] x100 (Gamal *et al.*, 1991).

Values in the same parameter followed by the same latter do not significantly differ from each other, according to Duncan's at 5% level.

Table 9. Effect of different treatments of acid sawdust (SD) hydrolyzate on bioethanol production by *Sacch. cerevisiae* B1 after 3 days incubation at 30°C as a static batch culture. *

Treatments of YFM medium	Growth (O.D)	Residual glucose (gl^{-1})	Consumed glucose (gl^{-1})	Bioethanol concentration (gl^{-1})	Bioethanol productivity ($gl^{-1}h^{-1}$)	Bioethanol yield (%)	Conversion coefficient (%)	Final pH
YFM complete medium (control)	1.9 a	0.247	49.75	11.50 a	0.16	23.00	23.11	3.0
YFM – glucose + SD hydrolyzate	1.1 b	0.200	49.80	4.97 b	0.05	9.94	9.98	4.8
YE + SD hydrolyzate	0.9 c	0.280	49.72	2.53 c	0.04	5.06	5.08	4.9
$(NH_4)_2SO_4$ + SD hydrolyzate	0.9 c	0.097	49.90	2.01 d	0.03	4.02	4.02	4.9
SD hydrolyzate	0.8 d	0.091	49.90	1.49 e	0.02	2.98	2.98	4.9

*The values are mean of three replicates.

Productivity = Bioethanol concentration (gl^{-1}) / fermentation time (h) = $gl^{-1}h^{-1}$ (Gamal *et al.*, 1991).

Bioethanol yield (%) = [Bioethanol concentration (gl^{-1}) / initial sugars (gl^{-1})] x100 (Gamal *et al.*, 1991).

Conversion coefficient (%) = [Bioethanol concentration (gl^{-1}) / consumed sugars (gl^{-1})] x100 (Gamal *et al.*, 1991).

Values in the same parameter followed by the same latter do not significantly differ from each other, according to Duncan's at 5% level.

Table 10. Effect of different treatments of sugar beet pulp (SBP) hydrolyzate on bioethanol production by *Cl. lusitaniae* Gr45 after 3 days incubation at 30°C as astatic batch culture.*

Treatments	Growth (O.D)	Residual glucose (gl^{-1})	Consumed glucose (gl^{-1})	Bioethanol concentration (gl^{-1})	Bioethanol productivity ($gl^{-1}h^{-1}$)	Bioethanol yield (%)	Conversion coefficient (%)	Final pH
YFM complete medium (control)	2.50 a	0.055	49.995	12.50 a	0.17	25.00	25.00	3.80
YFM – glucose + SBP extract	1.08 d	0.064	49.936	5.50 e	0.08	11.00	11.00	5.72
YE + SBP extract	1.92 b	0.005	49.995	8.51 c	0.12	17.02	17.02	6.77
$(NH_4)_2SO_4$ + SBP extract	1.70 c	0.158	49.842	10.73 b	0.15	21.46	21.50	6.80
SBP extract	1.73 c	0.248	49.752	7.40 d	0.10	14.80	14.80	6.84

*The values are mean of three replicates.

Productivity = Bioethanol concentration (gl^{-1}) / fermentation time (h) = $gl^{-1}h^{-1}$ (Gamal *et al.*, 1991).

Bioethanol yield (%) = [Bioethanol concentration (gl^{-1}) / initial sugars (gl^{-1})] x100 (Gamal *et al.*, 1991).

Conversion coefficient (%) = [Bioethanol concentration (gl^{-1}) / consumed sugars (gl^{-1})] x100 (Gamal *et al.*, 1991).

Values in the same parameter followed by the same latter do not significantly differ from each other, according to Duncan's at 5% level.

Table 11. Effect of different treatments of sugar beet pulp (SBP) hydrolyzate on bioethanol production by *Sacch. cerevisiae* B1 after 3 days incubation at 30°C as a static batch culture.*

Treatments	Growth (O.D)	Residual glucose (gl^{-1})	Consumed glucose (gl^{-1})	Bioethanol concentration (gl^{-1})	Bioethanol productivity ($gl^{-1}h^{-1}$)	Bioethanol yield (%)	Conversion coefficient (%)	Final pH
YFM complete medium (control)	1.9 a	0.247	49.75	11.40 a	0.16	22.80	22.90	3.0
YFM – glucose + SBP extract	1.0 b	0.449	49.56	2.01 d	0.03	4.02	4.05	5.1
YE + SBP extract	0.8 d	0.131	49.89	4.03 b	0.06	8.06	8.07	5.2
$(NH_4)_2SO_4$ + SBP extract	1.0 b	0.102	49.89	2.53 c	0.04	5.06	5.07	5.2
SBP extract	0.9 c	0.165	49.83	2.01 d	0.03	4.02	4.03	5.2

*The values are mean of three replicates.

Productivity = Bioethanol concentration (gl^{-1}) / fermentation time (h) = $gl^{-1}h^{-1}$ (Gamal *et al.*, 1991).

Bioethanol yield (%) = [Bioethanol concentration (gl^{-1}) / initial sugars (gl^{-1})] x100 (Gamal *et al.*, 1991).

Conversion coefficient (%) = [Bioethanol concentration (gl^{-1}) / consumed sugars (gl^{-1})] x100 (Gamal *et al.*, 1991).

Values in the same parameter followed by the same latter do not significantly differ from each other, according to Duncan's at 5% level.

Table 12. Effect of different concentrations of fruit juice waste (FJW) on bioethanol production by *Cl. lusitaniae* Gr 45 after 3 days incubation at 30°C as a static batch culture. *

FJW concentration (ml l^{-1})	Initial total sugar (gl^{-1})	Bioethanol concentration (gl^{-1})	Bioethanol productivity ($gl^{-1}h^{-1}$)	Bioethanol yield (%)	Final pH
80	28.3	3.50 c	0.049	12.37	4.33
100	35.4	10.01 b	0.140	28.28	4.23
120	42.5	15.88 b	0.220	37.36	4.37
130	46.0	17.20 a	0.240	37.39	4.17
150	53.1	17.20 a	0.240	32.39	4.45

*The values are mean of three replicates.

Productivity = Bioethanol concentration (gl^{-1}) / fermentation time (h) = $gl^{-1}h^{-1}$ (Gamal *et al.*, 1991).

Bioethanol yield (%) = Bioethanol concentration (gl-1) / initial sugars (gl-1) x100 (Gamal *et al.*, 1991).

Values in the same parameter followed by the same latter do not significantly differ from each other, according to Duncan's at 5% level.

Table 13. Effect of different concentrations of fruit juice waste (FJW) on bioethanol production by *Sacch. cerevisiae* B1 after 3 days incubation at 30°C as a static batch culture.*

FJW Concentration (ml l^{-1})	Initial total sugar (g l^{-1})	Bioethanol concentration (gl^{-1})	Bioethanol productivity ($gl^{-1}h^{-1}$)	Bioethanol yield (%)	Final pH
80	28.3	2.10 e	0.029	7.40	4.30
100	35.4	12.06 b	0.170	43.07	4.14
120	42.5	17.96 a	0.250	42.26	4.22
130	46.0	19.40 d	0.270	42.17	4.20
150	53.1	19.90 c	0.280	37.48	4.27

*The values are mean of three replicates.

Productivity = Bioethanol concentration (gl^{-1}) / fermentation time (h) = $gl^{-1}h^{-1}$ (Gamal *et al.*, 1991).

Bioethanol yield (%) = Bioethanol concentration (gl-1) / initial sugars (gl-1) x100 (Gamal *et al.*, 1991).

Values in the same parameter followed by the same latter do not significantly differ from each other, according to Duncan's at 5% level.

Table 14. Comparative data of maximum bioethanol production by *Cl. lusitaniae* Gr 45 and *Sacch. cerevisiae* B1 on YFM medium and agro-industrial byproducts treatments.

Medium	Bioethanol production							
	Cl. lusitaniae Gr 45				*Sacch. cerevisiae* B1			
	Conc. (gl^{-1})	Productivity ($gl^{-1}h^{-1}$)	Yield (%)	Conversion coefficient (%)	Conc. (gl^{-1})	Productivity ($gl^{-1}h^{-1}$)	Yield (%)	Conversion coefficient (%)
YFM	12.45	0.17	25.00	25.00	11.80	0.16	22.80	22.90
10 % SM only	7.00	0.09	-	-	13.05	0.18	-	-
Change (%)	-43.80	-	-	-	10.60	-	-	-
CW as carbon source in YFM medium	13.32	0.18	26.64	26.65	2.03	0.03	4.06	4.09
Change (%)	6.98	-	-	-	-82.80	-	-	-
SB as carbon source in YFM medium	15.80	0.22	31.60	35.90	4.50	0.06	9.00	9.03
Change (%)	26.90	-	-	-	-61.90	-	-	-
SD hydrolysate + yeast extract	16.50	0.23	33.00	33.00	4.97	0.05	9.94	9.98
Change (%)	32.50				-57.80	-	-	-
SBP + $(NH_4)_2SO_4$	10.73	0.15	21.46	21.50	2.53	0.04	5.06	5.07
Change (%)	-13.70	-	-	-	-78.60	-	-	-
FJW	17.20	0.24	37.30	-	19.90	0.28	37.40	-
Change (%)	38.15	-	-	-	68.60	-	-	-

Comparing the bioethanol production by *Cl. lusitaniae* Gr45 and *Sacch. cerevisiae* B1 on YFM medium with that obtained by the tested agro-industrial byproducts (Table 14), it could be noticed that the bioethanol production by the first strain increased about 6.98, 26.9 and 32.5 % by using CW, SB as carbon source on YFM medium and sawdu SD st hydrolyzate containing yeast extract (as nitrogen source) treatments, respectively and decreased by about 43.8 % at 10 % SM treatment whereas the later treatment increased the bioethanol production by the second strain about 10.6 %. Using SBP hydrolyzate containing $(NH_4)_2SO_4$ gave a drastic effect on bioethanol production by both tested strains, whereas using FJW at 130 and 150 $ml l^{-1}$ led to increase the bioethanol production by *Cl. lusitaniae* Gr45 and *Sacch. cerevisiae* B1 about 38 and 68 %, respectively. So, it could be stated that using FJW was the best agro-industrial waste material for bioethanol production by *Sacch. cerevisiae* B1and *Cl. lusitaniae* Gr45 after 3 days incubation period at 30°C using static batch culture technique.

CONCLUSION

Bioethanol production by *Cl. lusitaniae* Gr45 increased about 6.98, 26.9 and 32.5 % on CW, SB as carbon source instead of glucose on YFM medium and SD hydrolyzate containing yeast extract (as nitrogen source) treatments, respectively. Whereas, the later treatment increased the bioethanol production by *Sacch. cerevisiae* B1 to about 10.6 %. Whereas, the FJW at 130 and 150 $ml l^{-1}$ recorded the highest bioethanol production by *Cl. lusitaniae* Gr45 and *Sacch. cerevisiae* B1 which increased by about 38 and 68 %, respectively comparing to production on YFM medium (control). So, it could be deduced that FJW was the best agro-industrial byproduct for bioethanol production by *Sacch. cerevisiae* B1 and *Cl. lusitaniae* Gr45 after 3 days incubation period at 30°C using static batch culture technique. Further studies are needed to increase the efficiency of using the other agro-industrial byproduct for bioethanol production.

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التحويل الحيوي لبعض المخلفات الزراعية و مخلفات التصنيع الزراعي إلى الإيثانول الحيوى بواسطة الخمائر المحلية في البيئات الثابتة

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تم اختبار بعض المخلفات الزراعية و الصناعية (مولاس قصب السكر ، قوالح الذرة ، مصاصة قصب السكر ، نشارة الخشب ، تفل بنجر السكر و مخلف عصير الفاكهة) كمواد خام لانتاج الايثانول الحيوي بواسطة ميكروبات *Clavispora lusitaniae* Gr45 و *Saccharomyces cerevisiae* B1 ذلك بعد ثلاثة ايام من التحضين على درجة حرارة ٣٠ درجة مئوية بمزارع الدفعة الواحدة الثابتة. شملت الدراسة انتاج الايثانول الحيوي على هذه المواد بدون اضافات أو باضافة مصادر نيتروجين (سلفات أمونيوم او مستخلص خميرة). أعلى انتاج من الايثانول الحيوي عند استخدام مولاس قصب السكر (١٣.٠٥ جم/لتر) كان بواسطة *Sacch. cerevisiae* B1مع المعاملات: بيئة YFM – جلوكوز + ٤ % مولاس، مستخلص خميرة + ٦ % مولاس و ١٠% مولاس، و هو ما يمثل زيادة بمقدار ١٠.٦ % مقارنة بالانتاج على بيئة YFM. اضافة ناتج التحليل المائى الحامضى لقوالح الذرة كمصدر كربون وحيد لبيئة YFM باستخدام *Cl. lusitaniae* Gr45 كان افضل معاملات قوالح الذرة. ادت هذه المعاملة الى تحقيق اعلى تركيز للايثانول الحيوي و اعلى انتاجية و محصول و معامل تحويل (١٣.٣٢ جم/لتر ، ٠.١٨ جم/لتر/ساعة ، ٢٦.٦٤ % و ٢٦.٦٤ % على الترتيب). و قد زادت هذه القيم الى ١٥.٨ جم/لتر ، ٠.٢٢ جم/لتر/ساعة ، ٣١.٦ % و ٣٥.٩ % على الترتيب و ذلك على بيئة YFM المحتوية على ناتج التحليل المائى الحامضى لمصاصة قصب السكر كمصدر وحيد للكربون. جميع معاملات ناتج التحليل المائى لنشارة الخشب رفعت انتاجية الايثانول الحيوي بواسطة *Cl. lusitaniae* Gr45 ، فى حين انخفضت الانتاجية فى حالة الانتاج بواسطة *Sacch. cerevisiae* B1. اعلى انتاجية للايثانول الحيوي عند استخدام نشارة الخشب كانت فى حالة التنمية على ناتج التحليل المائى الحامضى لنشارة الخشب مضاف اليها مستخلص الخميرة يلى ذلك استخدام ناتج التحليل المائى لنشارة الخشب بدون اضافات ثم ناتج التحليل المحتوى على سلفات الامونيوم (١٦.٥ ، ١٣.٧٦ ، ١٣.٥٤ جم/لتر على الترتيب). استخدام تفل بنجر السكر بعد اضافته الى بيئة YFM ادى لانخفاض معنوي للنمو و لانتاجية الايثانول الحيوي بواسطة كلا الميكروبين. و بشكل عام، يمكن القول ان مخلف عصير الفاكهة كان أفضل مصدر كربوي عند اضافته الى بيئة YFM حيث ادى الى زيادة انتاجية الايثانول الحيوي الى حوالى ٦٨ % و ٣٨ % بواسطة كل من *Sacch. cerevisiae* B1 و *Cl. lusitaniae* Gr45 على الترتيب.