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Correlation between Ph with Saccharification and Ethanol Fermentation of Sugarcane Juice Industrial Wastes by Genotypic Hybrids of *Sacccharomyces cerevisiae* as Ph Determining Factor

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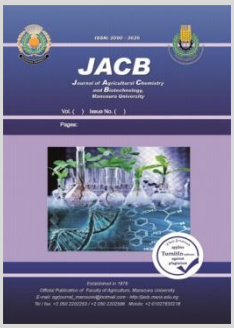

ABSTRACT

In this study five parental strains of *Saccharomyces cerevisiae* and 15 genotypic hybrids resulted from the conjugation between the parental strains harbouring the opposite genetic markers were used to assess the relationships between the parameters under investigation. However, correlation coefficient indicates the direction of relationship between two variables. The results showed that at 0.02 g sugar cane juice wastes, the hybrids resulted from the mating between p1 x p4 showed positive correlation between consumed sugars and pH, as well as, between ethanol production and pH of the fermentation medium. These results clearly demonstrated that if consumed sugars increased the pH medium was also increased as a consequence of ethanol production increased. Meanwhile, the hybrids resulted from the mating between p2 x p5 showed the same trend between the same parameters. At 0.04 g concentration of dissolved solids, the hybrids resulted from the cross between p2 x p5 showed negative correlation between consumed sugars and pH, as well as, positive correlation between ethanol production and pH. This indicated that these hybrids consumed lower concentration of sugars to be converted into higher values of ethanol which increased pH value. At 0.06 of dissolved solids, the hybrids resulted from the crosses between p2 x p5 showed the above trend of negative correlation between consumed sugars and pH, as well as, positive correlation between ethanol production and pH .

Keywords: Correlation, pH , Saccharomyces , consumed sugar , ethanol.

INTRODUCTION

Sugarcane juice by – products are sub – products of juice industry, rich in sucrose and some other sugars like fructose, raffinose and glucose. The use of these cheap sub – products for ethanol production reduces the cost of fuel production and contributes to the circular economy. Due to higher content of sucrose, these sub–products can be used as a substrate for bioethanol production by the yeast *Saccharomyces cerevisiae* (Akbas and Stark 2016). Sucrose like other carbohydrates can be hydrolyzed by acid or by pure enzyme like invertase or enzyme secreted by microbial cells such as *Saccharomyces cerevisiae*. The biological conversions of sucrose was better than those of acid hydrolyzed ones for good purity colour and flavor (Cantarella *et al*. 1989). Sucrose is readily matabloised by baker's yeast, the sub – products containing its promotes more vigorous and prolonged fermentation. Sucrose was hydrolyzed by extracellular invertase secreted by yeast into glucose and fructose monosaccharides which are taken up and utilized (Carlson 1987). The main end – products of yeast fermentation metabolism are ethanol and carbon dioxide. In addition, some other secondary metabolites were also produced in smaller amounts such as glycerol and organic acids which are essential to maintain internal redox balance and to withstand high osmotic pressure (Malony and Foy 2003). In the acidic medium, the organic acids can diffuse through plasma membrane and acidified the cytoplasm leading to reduction the yield of growth (Halm *et al*. 2004). Fermentations with initial pH equal 6.0 produced high ethanol than did fermentations at initial pH equal 5.6 , 5.0 , 4.4 (Wilkins *et al*. 2007). Therefore, Narendranath and Power (2005) found that when the pH medium decreased the lactic acid production by bacteria also decreased. In contrast to the bacteria, the growth rate of yeast was increased when the concentration of dissolved solids in the growth medium increased the PH medium had no significant effect on the growth rate of yeast at any concentration of dissolved solids in the medium. Although, pH medium had a significant effect on the final ethanol content in the medium. A reduction in the final ethanol produced was shown when the medium pH reduced. Therefore, increased the final ethanol concentration was appeared as the increased in concentration of dissolved solids (sugars) in the medium. This leading to more residual sugars observed at pH equal 4.0 than at pH of 5.5 after 48 h of fermentation. During the growth of yeast cells it is important to maintain a constant intracellular pH. Many enzymes in the yeast cells are functional during the growth and metabolism. Each of these enzymes functioning well at optimal pH, which is acidic because of acidophilic environment of yeast cells itself. When the extracellular pH deviates than the optimal value, the cells needs to invest energy to either pump in or pump out hydrogen ions to maintain the intracellular pH at the optimal level (Thomas *et al*. 2002). If the extracellular pH deviates very much than the

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optimal value, it may too difficult for the cell to maintain constant pH intracellular, then the enzymes may not functional normally. When the enzymes were deactivated, the cells will not able to grow and produce ethanol efficiency. This leading to the observed reduction in ethanol productivity when the pH was lowered than 5.5. This phenomenon resulted in the increased residual sugars in fermentation medium at the lower pH value. The value of pH medium do not affect on the saccharification process because normal glucose production was observed at pH value of 4.0 (Narendranath *et al*. 1997). If lowering pH medium reduces the growth and metabolism of the bacterial cells, it also reduces the efficiency of yeast cells to convert sugars into ethanol which leading to reduce ethanol yield (Narendranath and power 2005). Observations that produced ethanol was more toxic for yeast cells if compared with added ethanol (Novak *et al*. 1981).

Heterosis is the tendenay of hybridization individuals to show superior qualities than either of the parents. Agriculturalists used this phenomenon to improve crops and animals. At modern times hybrid versions were used among important crops (maize, sorghum, wheat, sunflower) which containing a major part of human diet (Lippman Zamir 2006). Hybrid vigor or heterosis also accurs in microorganisms such as yeast (Plech *et al*. 2013) and has taken important potential in industrial application. Yeast hybrids have exhibited a level of improved traits including faster fermentation values, more sugar consumption, greater stress tolerance and produces higher values of aroma compounds (Snoek *et al*. 2015). Interspecific hybridization was a powerful technique for strain development of brewing yeast which enables the combination of genotypes from different parental strains. Through, the recombinant hybrids genotypes can be generated without targeted modification, which also help to demonstrate the evolutionary history of industrial hybrid yeast strains (Peris *et al*. 2016). Also, they conferred that several hybrids isolated from fermented beverages like wine and beer appeared that these hybrids are stress tolerant to fermentative environment and yield high quality products that are difficult to show from a single genotype. In some cases, the hybrids shown to outcompete both parents in industrial application demonstrating heterosis (Belloch *et al*. 2008). In this study 15 genotypic hybrids were generated, allowing us to explore whether hybridization in yeast was useful in biofuel research to be used in the industrial application which is a mature biotechnology in this field.

The purpose of this study was to estimate the correlation coefficient between the final pH values after fermentation with ethanol productivity and consumed sugars converted into ethanol by the hybrid genotypes of yeast in order to determine the effect of resulted pH on ethanol productivity.

MATERIALS AND METHODS

Microbial strains

Five yeast strains were used in this study. These strains, as well as, their references or sources are listed in Table (1).

Table 1. Yeast strains used in this study.

Yeast strains	Source	Designation
Saccharomyces cerevisiae	Bakers yeast , a block of compressed fresh yeast in its wrapper , The Egyptian Starch, Yeast and Detergents Company.	P_1
Saccharomyces cerevisiae	Microbial Genomics and Bioprocessing Research, United States, Department of Agriculture, USA.	P_2
Saccharomyces cerevisiae	grape juice Fermented	P_3
Saccharomyces cerevisiae	Instant yeast supplier silesaffre 59703 marcq, France	P_4
Saccharomyces cerevisiae	Fermented wheat flour juice having a popular name " Buzza "	P_5

Sugarcane juice sub - products

Sugarcane juice wastes were used in this study with its residual sugars being used as a sole source of carbon instead of glucose added to the fermentation medium. It was collected from the local market of sugarcane juice industry in Mansoura city through October 2018. The white fibers inside the stem were collected after removal of the outer surface of the stem. It was cut to separate parts three cm long. The parts containing residual sugars were used as a sole source of carbon in the fermentation medium of ethanol productivity with different concentrations including 2, 4 and 6%.

Media and growth condition

Yeast extract peptone dextrose medium (YEPD) was used as a complete medium for growth and maintenance of yeast strains according to Chung *et al*. (1995). Pre Sporulation medium was used to stimulate the cells to sporulate according to Bähler *et al*. (1994). Sporulation medium was also used according to Sherman *et al*. (1982). Fermentation medium used for ethanol production was consisted of (g / L), sugarcane juice industrial wastes with the concentration of 2 % or 4 % or 6 % ; peptone, 10g ; yeast extract, 2 g and distilled water up to 1000 ml.

Reagents used for determining ethanol productivity

These reagents were prepared according to Plevaka and Bakoshinskaia (1964). They included potassium dichromate solution (PS), Ferrous ammonium sulphate solution (Titrate solution) and Diphenylamine solution indicator.

Reagents used for determining reducing sugars

These reagents were prepared according to Nelson (1944). They included Nelson's A, Nelson's B and arseno - molybdate reagent.

Antifungal marking agents

Selectable genetic markers are an important tool in the construction of yeast hybrids. Ideally, the antifungal markers allow efficient selection of yeast hybrids without affecting any cellular functions. Antifungal resistance markers are alternative to auxotrophic markers. Thus, nine antifungal drugs were used in this study with different concentrations (µg/ml) for genetically marking yeast strains as shown in Table 2.

Table 2. Antifungal drugs and their concentrations used for genetic marking yeast strains.

Antifungal Agents	Concentration (mg/ml)	Abbreviation
Flocazole	0.01	*Floz*
Flucoral	0.01	*Fluc*
Fungican	0.01	*Func*
Treflucan	0.01	*Tref*
Lamisil	0.5	*Lami*
Fungisafe	0.5	*Funs*
Itracon	0.01	*Itrc*
Itranox	0.01	*Itrn*
Trosyd	0.01	*Tros*

Methods

Yeast isolation

Yeast isolates were recovered from four sources including (i) fermented grapes, (ii) baker's yeast, (iii) fermented wheat flour juice having a popular name " buzza " and (iv) instant yeast . About one gram of each source was used and further serially diluted in conical flask 100 ml using distilled water. About 100 µl of each of the last two serial dillutions was then spread on the top of YEPD medium. Then, the spread yeast cells were further incubated for 72 hours at 30 °C. Single colonies of the expected yeast isolates were picked up and then purified and screened by using a microscope and selective medium (Bonciu *et al*. 2010).

Genetic marking

Antifungal drugs were used in this study for genetic marking of yeast strains. Susceptibility to antifungal drugs was measured by plate diffusion method according to Collins and lyne (1985).

Hybridization technique

This technique was done between the cells carrying the opposite genetic markers until the colonies of cells were appeared on sporulation medium which formed asci. Then each colony formed asci was picked up and grown on YEPD slant agar medium according to Grinsted and Bennett (1990) .

Determination of ethanol

The amount of potassium dichromate solution consumed in oxidation of ethanol (PSC) determination was calculated according to the following equation:

$$PSC = 10 - [\,0.26 \times TS\,]$$

Where, PSC: The amount of potassium dichromate solution consumed in oxidation of ethanol.

TS: The amount of titration solution consumed in the oxidation of ethanol.

The ethanol of unknown sample was determined using a standard curve of ethanol. The standard curve (Fig. 1) was dependent on the amount of PSC in the reaction which related with each concentration of ethanol prepared to be used as a standard (Ciani and Ferraro 1998).

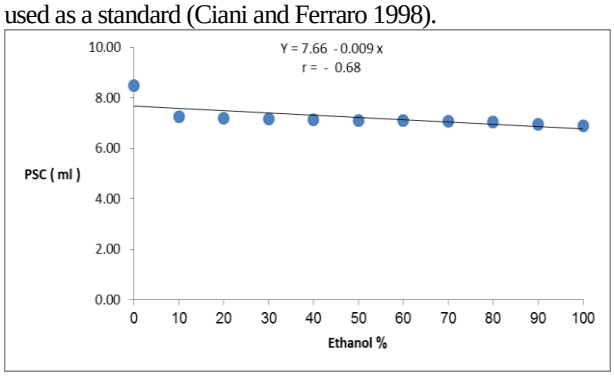

Figure 1. Standard curve for ethanol determination.

Determination of reducing sugars

Reducing sugars (RS) were determined as sucrose by the method of Nelson (1944). In this method, one ml of the cultural filtrate was diluted to 10^{-4}. One ml of this diluted sample was added to one ml of Nelson 's alkaline copper reagent. The tubes were heated in a boiling water - bath for exact 20 min., removed simultaneously and cooled to 25°C, then one ml of arsenomolybdate reagent was added and shacked well for 5 min to dissolve Cu_2O and reduce the arsenomolybdate. Seven ml of DW was added to each test tube and thoroughly mixed. The optical density of obtained green color was recorded at 540 nm using Spekal 11 spectrophotometer. The blank was carried out using the fermentation medium without inoculation. The consumed reducing sugars of samples were determined via a standard curve of glucose (Fig. 2) using the following equation:

Consumed sugars (CS) = Initial concentration of sugars (IC) - Residual sugars in the fermentation medium at the end of fermentation time (RSF).

The standard curve was prepared using different concentrations of sucrose ranging from 0 to 10 mg / ml.

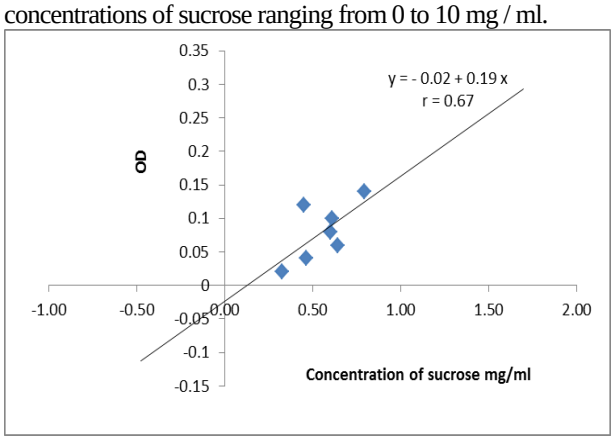

Figure 2. Standard curve for sugars determination.

Testing pH

The pH of the fermentation medium was measured at the end of fermentation time according to (Nasir *et al*. 2017).

Statistical analysis

One of statistical modeling is regression analysis. It was used in this study as a set of statistical estimation of the relationship between two variables such as pH values as a dependent variable and ethanol production, consumed sugar and residual sugar as the independent variables. It helps to show the dependent variable changes for each one of the independent variable (Fox 1997). In addition, correlation was also estimated between the two variables to measure the association between them. (Lindley 1987).

RESULTS AND DISCUSSION

Relationship between pH and 0.02 dissolved solids.

In this section we shall discuss the association between final pH values in the fermentation medium and consumed sugars. Regression analysis assess the relationship between an outcome variable which is also called the response or dependent variable (pH value) and the risk factors called the predicators or independent variable (consumed sugars). Correlation coefficient indicates the direction of association between two variables. A minus value of correlation coefficient indicates a perfect negative correlation, while a plus value indicating a perfect positive correlation. Negative

correlation indicates that as the value of one variable increases, the value of the other variable decreased, and vise versa.

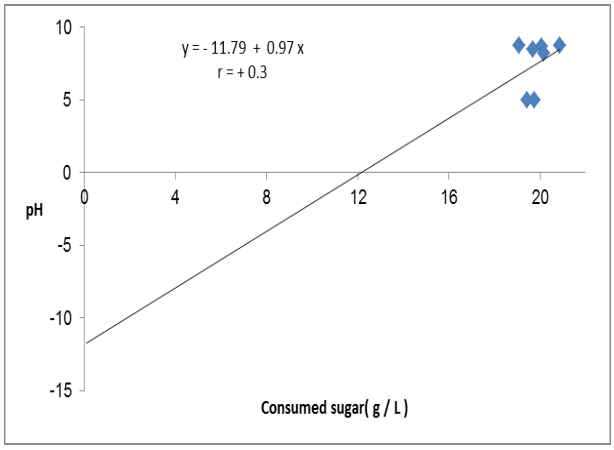

Figure 3. Correlation and regression line between final PH values of fermented 0.02 sugarcane juice industrial wastes and consumed sugars by genotypic hybrids resulted from the mating between P_1 X P_4.

Note: r = +0.3 NS
NS = insignificant differences at 0.05 and 0.01 level of probability.

As shown from the results summarized in (Figures 2 and 3) the hybrids resulted from the mating between p1 x p4 showed positive correlation between consumed sugars, as well as, ethanol production and final pH in the fermentation medium containing 0.02 g sugarcane juice wastes. On the contrary, the same hybrids showed negative correlation between residual sugars and pH. These results demonstrated that when consumed sugars increased the pH medium was also increased due to ethanol production increased. This observation leading to negative correlation obtained between residual sugars and pH value Figure (4). These results agreed with Narendranath and power (2005), who reported that medium pH had a significant impact on the final ethanol concentration in the medium, a reduction in the final ethanol produced was observed as the pH of the medium reduced. However, the increase of ethanol concentration was shown as the dissolved solids (sugars) concentration in the medium increased. Lower extracellular pH is harmful to the yeast. This attributed to the acidification of cytoplasmic pH and subsequent inhibition of cellular function (Kashket 1987). The lowered pH resulted in increased residual sugars. This indicated that the external pH was closer to consumed sugars and ethanol productivity.

The results obtained in Figure (5) showed positive correlation between consumed sugar and pH. In addition, positive correlation was obtained between ethanol production and pH (Figure 6). Meanwhile, negative correlation was shown between residual sugar and pH (Figure 7). These results reflected that ethanol production increased as the consumed sugar increased which leading to increased pH value of the fermentation medium. This indicated that final ethanol concentration had on impact effect on medium pH. An increase of ethanol productivity reduced residual sugars concentration as the pH of the medium increased due to ethanol production. These results agreed with Narendranath and power (2005), who found that residual sugars increased at the lower pH.

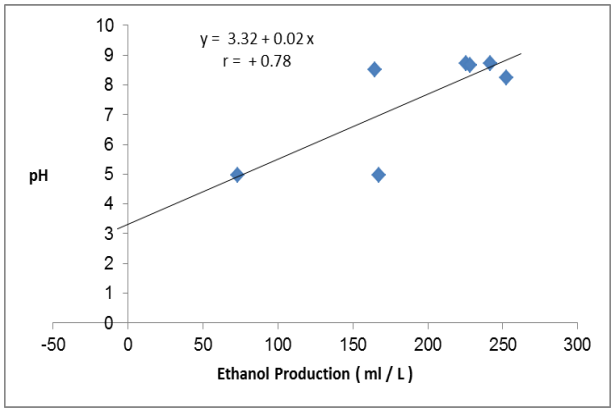

Figure 4. Correlation and regression line between final PH values of fermented 0.02 sugarcane juice industrial wastes and ethanol productivity by genotypic hybrids resulted from the mating between P_1 X P_4.

Note: r = +0.78 *
*** = significant differences at 0.05 and 0.01 level of probability.**

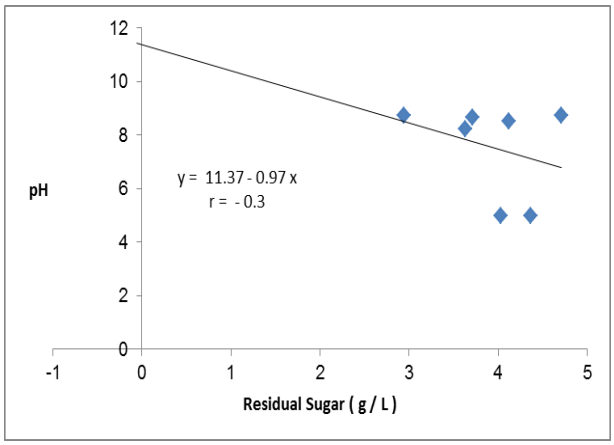

Figure 5. Correlation and regression line between final PH values of fermented 0.02 sugarcane juice industrial wastes and residual sugars by genotypic hybrids resulted from the mating between P_1 X P_4.

Note: r = -0.3 NS
NS = insignificant differences at 0.05 and 0.01 level of probability.

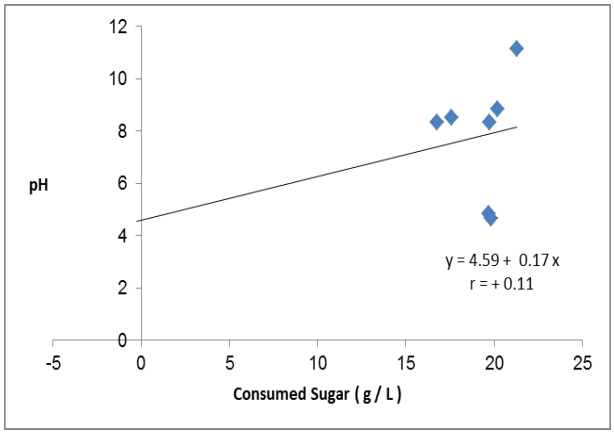

Figure 6. Correlation and regression line between final PH values of fermented 0.02 sugarcane juice industrial wastes and consumed sugars by genotypic hybrids resulted from the mating between P_2 X P_5.

Note: r = +0.11NS
NS = insignificant differences at 0.05 and 0.01 level of probability.

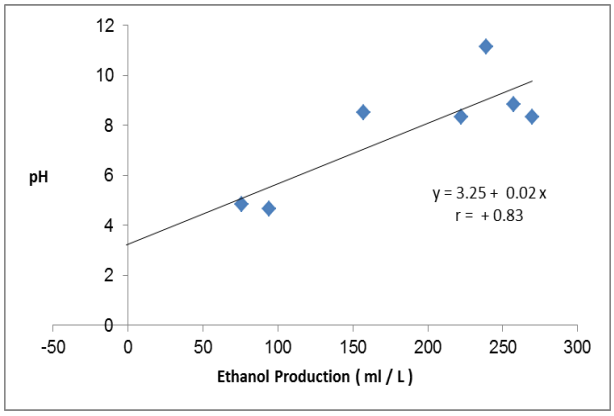

Figure 7. Correlation and regression line between final PH values of fermented 0.02 sugarcane juice industrial wastes and ethanol productivity by genotypic hybrids resulted from the mating between P_2 X P_5.

Note: r = +0.83*

*** = significant differences at 0.05 and 0.01 level of probability.**

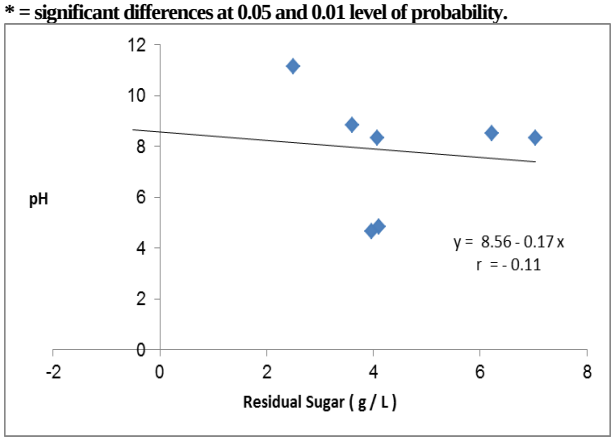

Figure 8. Correlation and regression line between final PH values of fermented 0.02 sugarcane juice industrial wastes and residual sugars by genotypic hybrids resulted from the mating between P_2 X P_5.

Note: r = -0.11NS

NS = insignificant differences at 0.05 and 0.01 level of probability.

The results diagrammatic in Figure (8) showed negative correlation between consumed sugar and pH. Meanwhile, the results presented in Figure (9) showed positive correlation between ethanol production and pH. This indicated that the hybrids resulted from the mating between p3 x p5 consumed less concentration of sugars to produce high values of ethanol which increased pH value. In addition, the residual sugars showed positive correlation with pH value (Figure 10). This resulted from consumed less concentration of sugars which leading to increased residual sugar concentration. The hybrids consumed less sugar concentrations to be produced higher amount of ethanol which increased the pH values. During fermentation process, it is important to consumed sugars for producing ethanol which effect on intracellular pH. There are many enzymes functioning related to fermentation process within the yeast cells during the metabolism of sugars to be converted into ethanol which increased the pH value. Novak *et al.* (1981) showed that produced ethanol was apparently more toxic for yeast than added ethanol. The results obtained in this study agreed with Kumdam *et al.* (2013), who found that the halotolerant yeast Debaryomyces nepalensis was survive at pH 3.0 – 11, while the optimum fermentation pH is 6.0.

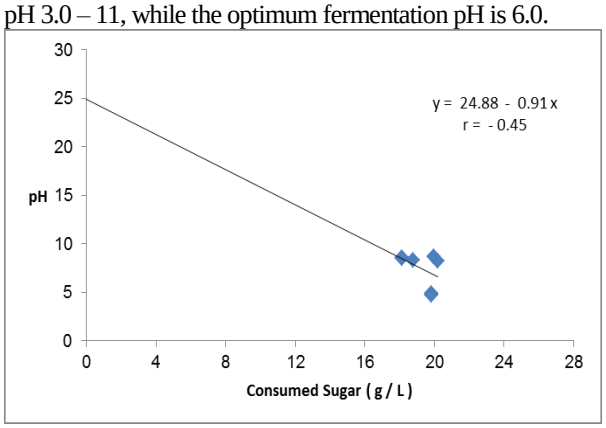

Figure 9. Correlation and regression line between final PH values of fermented 0.02 sugarcane juice industrial wastes and consumed sugars by genotypic hybrids resulted from the mating between P_3 X P_5.

Note: r = -0.45 NS

NS = insignificant differences at 0.05 and 0.01 level of probability.

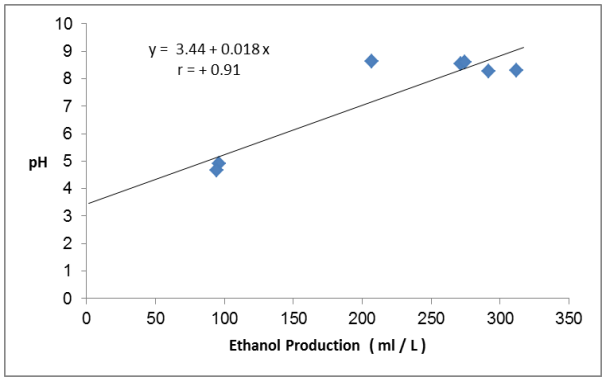

Figure 10. Correlation and regression line between final PH values of fermented 0.02 sugarcane juice industrial wastes and ethanol productivity by genotypic hybrids resulted from the mating between P_3 X P_5.

Note: r = +0.91 NS

NS = insignificant differences at 0.05 and 0.01 level of probability.

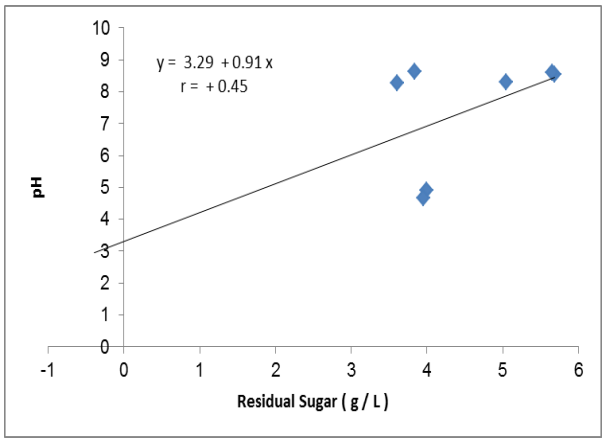

Figure 11. Correlation and regression line between final PH values of fermented 0.02 sugarcane juice industrial wastes and residual sugars by genotypic hybrids resulted from the mating between P_3 X P_5.

Note: r = +0.45 NS

NS = insignificant differences at 0.05 and 0.01 level of probability.

Relationship between pH and 0.04 g dissolved solids.

The results presented in Figure (11) showed negative correlation between consumed sugars and pH. However, the results diagrammatic in Figure (12) showed positive correlation between ethanol production and pH. These results demonstrated that the hybrids resulted from the cross between p2 x p5 consumed lower concentrations of sugars to be converted into higher values of ethanol which increased pH value. This leading to a positive correlation obtained between residual sugars and pH value (Figure 13). These results are in harmony which Helle *et al.* (2003), who found a decrease in ethanol productivity at the presence of acetic acid in the fermentation medium. The impact of media pH was shown in this study.

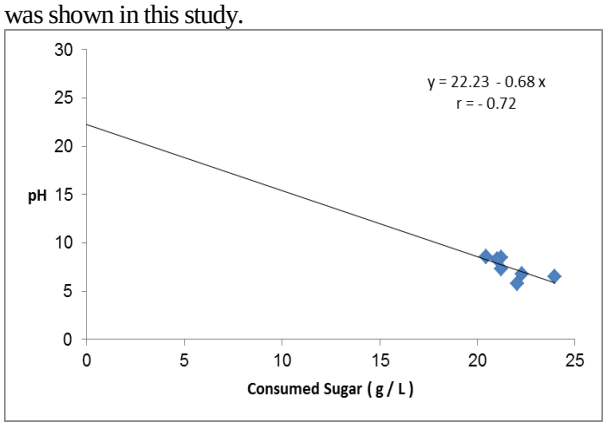

Figure 12. Correlation and regression line between final pH values of fermented 0.04 sugarcane juice industrial wastes and consumed sugars by genotypic hybrids resulted from the mating between P_3 X P_5.

Note: r = -0.72 NS
NS = insignificant differences at 0.05 and 0.01 level of probability.

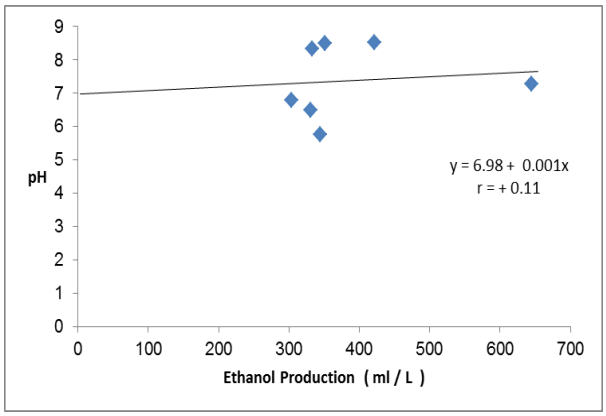

Figure 13. Correlation and regression line between final pH values of fermented 0.04 sugarcane juice industrial wastes and ethanol productivity by genotypic hybrids resulted from the mating between P_3 X P_5.

Note: r = +0.11 NS
NS = insignificant differences at 0.05 and 0.01 level of probability.

The results suggested that increasing media pH can alleviate some of the inhibitory effect of the acetic acid may be found in the fermentation media as caused a decrease in the concentration of undissociated acetic acid, the inhibitory from of acetic acid for *S.cerevisiae* fermentations. The results obtained herein agreed with Hallsworth (1998), who reported that ethanol produced during carbohydrate metabolism considered as a main factor of cell stress and limiting growth. Furthermore, ethanol and carbon dioxide are the major fermentation products of glucose, fructose and sucrose. Diez and Yokoya (1996) mentioned that several by – products may be produced during fermentation of sucrose by *Z. mobils*, such as, phenol, lactic acid higher alcohols, acetaldehyde, methanol and levan. Levan is a polysaccharide of fructose produced during sucrose fermentation (Ananthalakshmy *et al.* 1999). In addition, Doelle and Greenfield (1985) decided that the high initial sugar concentration in the medium leading to long fermentation time to show the efficiency of conversion. However, Tano *et al.* (2000) reported that the use of higher intial concentration of sugar caused of incomplete utilization of sugar. Several mineral compounds containing sugarcane juice and molasses are known to be inhibitors of fermentation by *Z. mobils* (Lawford and Rousseau 1998).

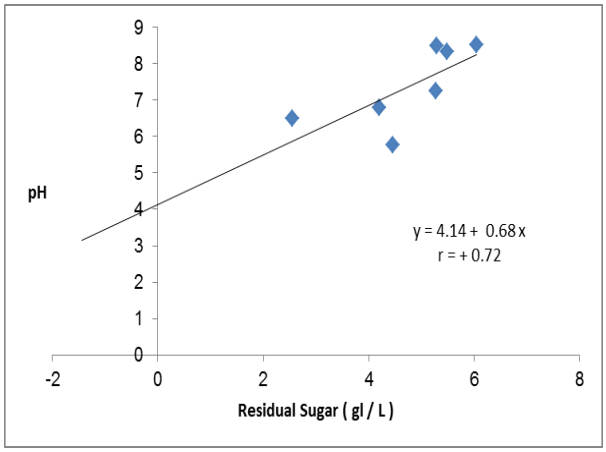

Figure 14. Correlation and regression line between final pH values of fermented 0.04 sugarcane juice industrial wastes and residual sugars by genotypic hybrids resulted from the mating between P_3 X P_5.

Note: r = +0.72*
*** = significant differences at 0.05 and 0.01 level of probability.**

Relationship between pH 0.06 g dissolved solids

The results summarized in Figure (14) showed negative correlation between consumed sugar and pH . However, positive correlation was obtained between ethanol production and pH (Figure15). The negative correlation obtained between consumed sugar and pH leading to obtained positive correlation between residual sugar and pH (Figure16). These results appeared that the hybrids resulted from the mating between p2 x p5 consumed less concentrations of sugars to be converted into higher values of ethanol which increased the value of medium pH. This phenomenon may be due to the higher concentrations of sugars cane juice wastes (0.06 g) in the fermentation medium. The ability of yeast to produce ethanol depending on the initial concentration of sugars in the fermentation medium. Deesuth *et al.* (2015) reported that 1 mol of glucose in the ethanol fermentation medium can be converted into 2 mol of ethanol and 2 mol of carbon dioxide. Therefore, a medium contained a high concentrations of sugars will produce a high ethanol concentration. The higher concentration of sugar in the fermentation medium leading to increase osmotic pressure, which had a negative effects on yeast cells. The results obtained in this study agreed with Bafrncova *et al.*

(1999), who decided that under appropriate and nutritional conditions, *S. cerevisiae* produced and tolerate high concentrations of ethanol. Similar observations were obtained by Zhang *et al.*(2015), who showed that ethanol produced was found to be the primary factor inhibiting yeast growth and fermentation process because the yeast completely stopped growing and fermenting when the exogenous ethanol concentration exceeded 70g / L (˷ 9 % v / v) . During ethanol fermentation by Saccharomyces cerevisiae glycerol is the main by – product. This metabolite regulates osmotic pressure due to high sugar concentration and ethanol in the fermentation medium (Tang *et al*. 2011). Raising the external pH was closer to ethanol production which leading to increased pH value of fermentation medium.

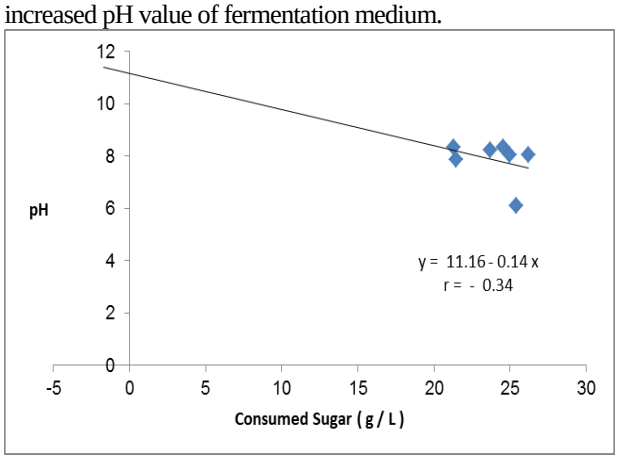

Figure 15. Correlation and regression line between final pH values of fermented 0.06 sugarcane juice industrial wastes and consumed sugars by genotypic hybrids resulted from the mating between P_2 X P_5.

Note: r = -0.34 NS

NS = insignificant differences at 0.05 and 0.01 level of probability.

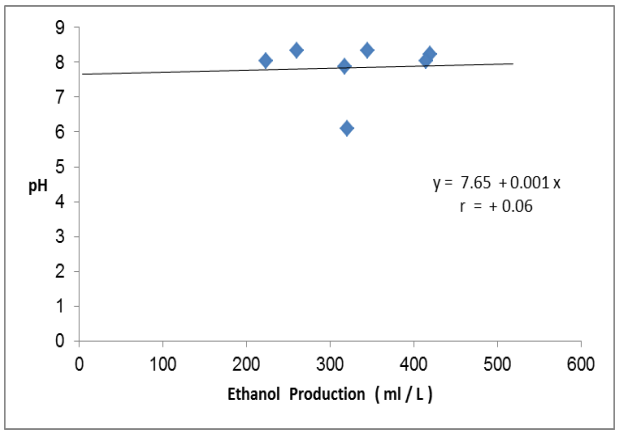

Figure 16. Correlation and regression line between final pH values of fermented 0.06 sugarcane juice industrial wastes and ethanol productivity by genotypic hybrids resulted from the mating between P_2 X P_5.

Note: r = +0.06 NS

NS = insignificant differences at 0.05 and 0.01 level of probability

The results clearly demonstrate that pH was increased considerably when there is an increase in the concentration of ethanol produced in the fermentation medium. The increased in sugar concentration in the medium from 0.02 g to 0.06 g of sugar cane juice wastes leading some hybrids to consumed little concentration of dissolved solids to be converted into higher values of ethanol concentration. This reflected the negative correlation obtained between pH and consumed sugars, as well as, the positive correlation obtained in the same time between pH and ethanol production. The higher concentration of dissolved solids in the fermentation medium most likely exerts a severe osmotic stress on the yeast cells.

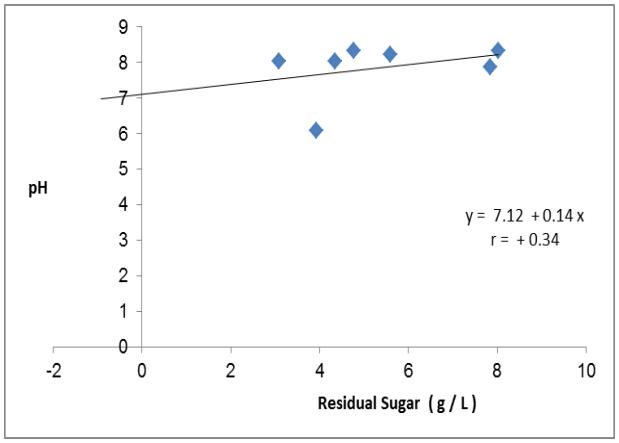

Figure 17. Correlation and regression line between final pH values of fermented 0.06 sugarcane juice industrial wastes and residual sugars by genotypic hybrids resulted from the mating between P_2 X P_5.

Note: r = +0.34 NS

NS = insignificant differences at 0.05 and 0.01 level of probability.

For active bioconversion of sugars into ethanol, the intracellular conditions must remain relatively constant relation to ionic composition, pH and metabolite levels (Csonka and Hanson 1991). Any change in the osmolality of the environment could, therefore, quicly compromise essential cell function and then the yeast cells need to adapt these changes in their environment to survive. In general, *S. cerevisiae* is acidophilic microbe which grows better under acidic conditions. The optimal pH for growing yeast was ranged from 4 to 6 depending on the environmental conditions and the strain genotype (Narendranath and power 2005).

During the growth of yeast cells, it is important of yeast cells operative during growth and metabolism. Each one works well at its optimal pH, which is acidic because of acidophilic nature of yeast cells itself. If the extracellular pH varied than the optimal level, the yeast cells invest energy for either pump in or pump out of hydrogen ions to maintain the optimal level of intracellular pH (Thomas *et al*. 2002). If the extracellular pH varied so much than the optimal level, it may became difficult for the cell to adjust constant intracellular pH, which leading the cell enzymes may not function naturally. If the enzymes disactivated, the yeast cells will not be able to grow and converting sugars into ethanol efficiently. That is the most likely explanation for the observed negative correlation between sugar consumption and pH, meanwhile positive correlation obtained between pH and ethanol production. This also resulted in increased residual sugars when the hybrids consumed little concentration of sugars to be converted into ethanol.

Sugars consumed in the fermentation medium

As shown from the results presented in Table 3 at 0.02 concentration of sugarcane juice wastes some hybrids consumed sugars more than the mid – parents , while some

other hybrids consumed sugars less than the mid – parent without any significant differences. The sugars consumed were ranged between 19.09 – 20.86 g / L. However, at 0.04 concentration most hybrids consumed sugars more than the mid – parents. The sugars consumed were ranged between 18.66 – 24.92 g / L. In addition, at 0.06 concentration all hybrids consumed sugars less than the mid parents. The sugars consumed were ranged between 22.25 – 26.08 g / L . The increase in sugar – rich hydrolysates increased the maximum rate of glucose consumed to be converted into ethanol. Baker's yeast was used since thousands of years for its ability to grow on numerous types of glucose – rich hydrolysates to be produced different types of alcohol. Quite low probability of another organism that can complete with *Saccharomyces cerevisiae* in the fermentation process. Saccharification and fermentation by yeast was faster and low cost process. The risk of contamination in the fermentation medium was lower due to the presence of ethanol.

Table 3. Sugars consumed during ethanol fermentation by the parental yeast strains and hybrids resulted from the cross between P1 X P4.

strains	Concentration of sugarcane juice industrial wastes (g)								
	0.02			0.04			0.06		
	Intial concentration	Consumed	Residual	Intial concentration	Consumed	Residual	Intial concentration	Consumed	Residual
P_1	23.80	19.77	4.03	26.50	24.16	2.34	29.30	26.08	3.22
P_4	23.80	19.43	4.37	26.50	18.66	7.84	29.30	24.28	5.02
MP	23.80	19.60	4.20	26.50	21.41	5.09	29.30	25.18	4.12
H_1	23.80	20.86	2.94	26.50	24.92	1.58	29.30	23.60	5.70
H_2	23.80	19.09	4.71	26.50	24.43	2.07	29.30	23.49	5.81
H_3	23.80	20.09	3.71	26.50	24.37	2.13	29.30	23.16	6.14
H_4	23.80	20.17	3.63	26.50	21.38	5.12	29.30	22.25	7.05
H_5	23.80	19.68	4.12	26.50	24.69	1.81	29.30	22.94	6.36
F - test	NS	**	**	NS	NS	NS	NS	NS	**
LSD 0.05		6.04	3.49						4.95
LSD 0.01		8.38	4.84						6.88

**** = Significance at 0.01 level of probability.** **Intial concentration = g / Litre**

As shown from the results summarized in Table 4 consumed sugars at 0.02 g were ranged between 17.58 - 20.19 g / L. However, at 0.04 g consumed sugars were ranged between 19.70 – 23.95 g / L. Meanwhile, at 0.06 g consumed sugars were ranged between 21.27 – 26.22 g / L. These results indicated that the range of consumed sugars was increased with the initial concentration increased. Additionally, the expression of genes involved in converting sugars into ethanol was increased with the increased of intial sugar concentration. This regulatory mechanism in baker's yeast results from the preferential consumption of glucose over other carbon sources. Therefore, when the yeast was grown in a mixture of glucose and other carbon sources as sucrose, ethanol, galactose and maltose, glucose was metabolized first, wherease the other carbon sources were not metabolized until glucose is exhausted.

Table 4. Sugars consumed during ethanol fermentation by the parental yeast strains and hybrids resulted from the cross between P2 X P5.

Strains	Concentration of sugarcane juice industrial wastes (g)								
	0.02			0.04			0.06		
	Intial concentration	Consumed	Residual	Intial concentration	Consumed	Residual	Intial concentration	Consumed	Residual
P_2	23.80	19.69	4.11	26.50	19.70	6.80	29.30	26.22	3.08
P_5	23.80	19.84	3.96	26.50	23.95	2.55	29.30	25.38	3.92
MP	23.80	19.77	4.04	26.50	21.83	4.68	29.30	25.80	3.50
H_6	23.80	17.58	6.22	26.50	19.87	6.63	29.30	23.70	5.60
H_7	23.80	21.30	2.50	26.50	20.22	6.28	29.30	21.45	7.85
H_8	23.80	16.76	7.04	26.50	21.81	4.69	29.30	24.96	4.34
H_9	23.80	19.73	4.07	26.50	21.10	5.40	29.30	21.27	8.03
H_{10}	23.80	20.19	3.61	26.50	21.03	5.47	29.30	24.54	4.76
F - test	NS	NS	NS	NS	NS	NS	NS	**	**
LSD 0.05								7.89	5.57
LSD 0.01								1.09	7.74

**** = Significance at 0.01 level of probability.** **Intial concentration = g / Litre**

The results presented in Table 5 showed that the sugars consumed were ranged between 18.11 – 20.19 g/ L at 0.02 g of sugar cane juice wastes. Meanwhile, at 0.04 g of initial concentration the sugars consumed were ranged between 20.45 – 23.95 g / L. However, at 0.06 g the sugar consumed were ranged between 24.25 – 27.02. These results indicated that the consumed sugars were increased due to the increased of initial sugars concentration. This clearly demonstrated that in the presence of high concentrations of glucose the expression of genes involved in glucose consumption and metabolism were increased. Ethanol industry based on sucrose has traditionally utilized sugar cane juice due to its high sucrose content and wide cultivation. Ethanol produced during carbohydrate metabolism had considered as s major factor of cell stress, limiting growth and ethanol production (Hallsworth 1998).

Table 5. Sugars consumed during ethanol fermentation by the parental yeast strains and hybrids resulted from the cross between P3 X P5.

Strains	Concentration of sugarcane juice industrial wastes (g)								
	0.02			0.04			0.06		
	Intial concentration	Consumed	Residual	Intial concentration	Consumed	Residual	Intial concentration	Consumed	Residual
P_3	23.80	19.80	4.00	26.50	21.22	5.28	29.30	24.25	5.05
P_5	23.80	19.84	3.96	26.50	23.95	2.55	29.30	25.38	3.92
MP	23.80	19.82	3.98	26.50	22.58	3.92	29.30	24.82	4.49
H_{11}	23.80	18.11	5.69	26.50	22.05	4.45	29.30	25.37	3.93
H_{12}	23.80	18.14	5.66	26.50	22.30	4.20	29.30	25.87	3.43
H_{13}	23.80	20.19	3.61	26.50	21.21	5.29	29.30	27.02	2.28
H_{14}	23.80	19.96	3.84	26.50	20.45	6.05	29.30	26.51	2.79
H_{15}	23.80	18.75	5.05	26.50	21.02	5.48	29.30	25.96	3.34
F - test	IS	IS	**	NS	**	**	NS	**	**
LSD 0.05			1.39		4.03	3.62		3.38	2.70
LSD 0.01			1.93		5.60	5.03		4.69	3.75

**** = Significance at 0.01 level of probability.** **Intial concentration = g / Litre**

In conclusion, crosses of a number of yeast stocks produced different outputs of mitochondrial genotypes depending on whether the yeast cells are glucose – repressed or depressed. Though , the availability of *S.cerevisiae* isolates for creation of novel artificial hybrids has the potential to greatly increase the genotypic and phenotypic diversity of yeast genotypes to be available for select the efficient genotypes to be use in the larger – ethanol industry , without resource to genetic manipulation.

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العلاقة بين درجة الأس الأيدروجينى مع كل من عملية التسكر والتخمر الكحولى لمخلفات صناعة قصب السكر بإستخدام تراكيب وراثية هجينة من خميرة الخباز بإعتبار الأس الأيدروجينى عامل محدد

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تم في هذه الدراسة إستخدام خمس سلالات أبوية من خميرة الخباز بالإضافة إلي 15 تركيب وراثي هجين ناتجة عن التهجين بين السلالات الأبوية التى تحمل علامات وراثية متفارقة وذلك لتقدير العلاقة بين الصفات تحت الدراسة . حيث يعتبر معامل الإرتباط دليل علي إتجاه العلاقة بين متغيرين ، فإن النتائج قد أوضحت في حالة إستخدام تركيز 0,02 جرام من مخلفات صناعة عصير قصب السكر كمصدر وحيد للكربون في بيئة التخمر أن الهجن الناتجة عن التزاوج بين السلالات P1 X P4 قد أظهرت إرتباط موجب بين السكر المستهلك و pH ، كذلك بين الإيثانول و pH ، كذلك بين إنتاج الإيثانول و pH بيئة التخمر . إن هذه النتائج تعكس بوضوح أن زيادة إستهلاك السكر يترتب عليه زيادة قيمة pH كنتيجة لزيادة إنتاج الإيثانول . بينما أظهرت الهجن الناتجة عن التهجين بين p2 x p5 نفس الإتجاه بين نفس القياسات . أما عند تركيز 0,04 جرام من مخلفات صناعة قصب السكر فقد أظهرت الهجن الناتجة عن التزاوج بين p2 x p5 إرتباط سالب بين السكر المستهلك و pH . وهذا يعكس أن الخلايا الهجينة قد إستهلكت تركيز منخفض من السكر في حالة إرتفاع تركيزه في البيئة لتحويله إلي إنتاج مرتفع من الإيثانول والذي أدي بالتالي إلي رفع pH بيئة التخمر . بينما عند رفع تركيز مخلفات صناعة قصب السكر في بيئة التخمر إلي 0,06 جرام فقد أظهرت الهجن الناتجة عن التهجين بين P2 X P5 نفس الإتجاه السابق المتمثل في علاقة الإرتباط السالب بين السكر المستهلك و pH ، كذلك الإرتباط الموجب بين إنتاج الإيثانول و pH .