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PRODUCTION OF BIOFUEL FROM SUGARCANE BAGASSE WASTES USING *Saccharomyces cerevisiae*

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ABSTRACT

The dry pulpy fibrous residue that remains after crush of sugarcane stalks is called Bagasse. It is Agroindustrial solid wastes which accumulated each day, causing big environmental problems. *Saccharomyces cerevisiae* is a unicellular fungus and has an interesting role in bioethanol production. In this study, two isolates of *S. cerevisiae* were used for bioethanol production from bagasse. In this study, Sugar cane bagasse were collected, hydrolyzed with concentrated HCl and used as a main carbon source (30 g/l). The growth curves of the two tested strains of *S. cerevisiae* were the same. The effect of temperature and pH levels on the growth, carbohydrates yields, and mainly bioethanol productivity from sugarcane wastes was studied for both *Saccharomyces* strains. The best conditions for bioethanol productivity was in fermentation medium after 3 days of growth at pH 6 and 30°C. In conclusion, *Saccharomyces* can be used for bioethanol production with the lowest possible costs from environmental wastes.

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1 Introduction

As a result of the rapidly increasing consumption of fossil fuel during the past few decades, problems of growing energy dependency, the risk of the energy supply shortage, and the problem of greenhouse gases emissions in the atmosphere are also increased. Increasing the use of bio-energy and decreasing the dependency on fossil can be a possible solution of these problems (Ajanovic, 2011; Ajanovic, 2013). At present, the world's biofuel production is essentially low, but incessantly rising as a result of the fact that several countries around the world have already set targets to replace a certain percentage of their fossil fuel usage with biofuel. For example, the European Union had a target to replace 5.75% of the fossil fuel used in the transportation section with biofuel. The second target of the E.U. is to replace 10% of the fossil energy used for transportation with a renewable energy source by 2020 (EUR, 2016; International Energy Agency, 2016).

Now in these days, the major feedstock used for liquid biofuel production depends on agriculture food crops. For example, corn, wheat, sugar beets, and sugar canes are widely used for bio-ethanol production. For biodiesel production, oil seeds such as soybean, rapeseed, sunflowers, and palm oil are also being used (Demirbas, 2008a). The availability of these feed stocks differs from one region to another as a result of climatic factors. According to Ajanovic (2013), the major feedstock used to produce bioethanol is wheat, which accounts for 70%, whereas the use of barley for bioethanol production accounts for just 15%, followed by 10% for corn, and 5% for rye of the total bioethanol production in the European Union in 2008. Rapeseed is used to produce 79% of the European Union's biodiesel, where 18% of the production comes from soybean, and just 3% is based on sunflowers. Agroindustrial wastes included natural (organic) and non-natural of unwanted materials, produced mainly from agriculture operations (Quintero et al., 2011; Koçar & Civa, 2013; Sarkar et al., 2012; Qiu et al., 2014; Visioli et al., 2014). Solid Agroindustrial wastes are wastes in solid forms such as grape pomace, sugar beet pomace, potato starch residues, raw corn starch, sugarcane bagasse etc (Rabi et al., 2009; Koçar & Civa 2013; Visioli et al., 2014). Furthermore, agricultural wastes can be sugar-containing residue such as sugarcane and starch-containing residues (Koçar & Civa 2013; Visioli et al., 2014). Additionally, Agroindustrial wastes can be either degradable wastes or non-degradable (Rabi et al., 2009).

Biofuels as a source of alternate can be derived from biomass which include cultivation crops, wood, by-product waste of agricultural crops, animal waste, algae, sewage biosolids and food manufacturers waste (Demirbas, 2008 a, Demirbas 2008b; Demirbas, 2009; Demirbas, 2011). Biomass can be converted by using thermo-chemical or biological methods. Thermo-chemical methods include direct combustion providing electricity, heat and mechanical power. Biological conversion methods include fermentation of the biomass to produce energy carries like hydrogen, ethanol and biogas, or extraction of oils from the biomass for biodiesel production (Costa & de Morais, 2011). So the main types of biofuels are Bioethanol, Biodiesel, Biohydrogen and biogas (Uçkun Kiran et al., 2014; Demirbas & Fatih Demirbas, 2011).

Brazil and USA are producing more than 80% of all biofuels production around the world. Raw materials are varied from country to another. In USA, wood, straw, wheat and mainly corn are the principle materials. Sugarcane is the most used raw materials in Brazil (International Energy Agency, 2016; Koçar & Civa, 2013; Kumar et al., 2013; Petrova & Ivanova, 2010).

Sugars can be converted to bioethanol through anaerobic fermentation where one molecule of glucose was converted to 2 molecules of ethanol, 2 molecules of CO_2 and 2 molecules of ATP (Kun, 2003). Bioethanol can be produced from fermentation of simple sugar and convert them into ethanol (Demirbas, 2008b; Demirbas, 2009). Simple sugars which are fermented to one of the biofuels products are generally obtained after biomass pre-treatment using physical methods such as heat and pressure; chemical methods such as acidic and alkali pre-treatment and enzymatic methods by using specific enzymes (Champagne, 2007; Quintero et al., 2011; Schlosser & Blahusiak, 2011; Qiu et al., 2014; Visioli et al., 2014; Khawla et al., 2014).

Fermentable sugars derived from pre-treated cellulose, hemicelluloses and starch can also convert in to ethanol (Binod, et al., 2010; Petrova & Ivanova, 2010; Hahn-Hägerdal & Gorwa-Grauslund, 2010). Furthermore, bioethanol production from fermentable sugars can be preceded by either simultaneous saccharification and fermentation (SSF) or separate enzymatic hydrolysis and fermentation (SHF) processes. SSF is more favoured because of its low potential costs and its production can be enhanced by applying thermo-tolerant microorganisms such as *Kluyveromyces marxianus*, *Candida lusitaniae*, *Zymomonas mobilis* or with the help of mixed culture of microorganisms like *Brettanomyces clausenii* and *Saccharomyces cerevisiae* (Binod et al., 2010; Petrova & Ivanova, 2010; Sheikh et al., 2016).

S. cerevisiae, *Hanseniaspora uvarum*, *Starmerella bacillaris* and *Z. mobilis* can ferment glucose to ethanol with higher yields which known as glucose and starchy based ethanol production microorganisms (Rabi et al., 2009; Petrova & Ivanova, 2010; Sheikh, et al., 2016). Further, *Pichia stipitis*, *Escherichia coli* and *Klebsiella oxytoca* which known as lignocellulosic and mixed-sugars based ethanol production microorganisms also help in the production of biofuel (Sheikh et al., 2016). The aim of this study was determination of the best growth conditions for maximum

biofuel (bioethanol) production by *S. cervesia* (baker's yeast) from local very cheap sugarcane bagasse. The impacts of temperature and pH levels on sugar production were also evaluated during the study.

2 Materials and Methods

2.1 Sugarcane bagasse collection and acid hydrolysis

The sugarcane bagasse wastes were collected from local fresh juices shops, Jeddah, Saudi Arabia. The collected sugarcane bagasse was dried at 80°C for three days and powder by electrical blender. Acid hydrolysis of sugarcane bagasse was carried out by using 0.5% Hydrochloric acid which was added to the obtained powder. This was followed by the autoclaving and prepared autoclaved at 1.5 atmospheres pressure and 121°C for 15 min. Finally, the samples were cooled and glucose concentration was determined. The clear filtrate was adjusted to approximately reach pH 6.5 and preserved until used.

2.2 Used microorganism

Pure culture of *S cerevisiae* was obtained from the Department of Biology, KAU, Saudi Arabia while the commercial formulation of *S. cerevisiae* was purchased from Hyper market, Jeddah, Saudi Arabia.

The antibiotic E-MOX (5 µg/ml) was added to the growth medium during purification of the commercial yeast from contamination by bacteria, and incubation was carried out for 24 hours at 25°C. Stock cultures of both isolates of *S. cerevisiae* were maintained at 4°C on Yeast-Malt (YM) medium which was composed of (g/l): Yeast extract, 3; Malt extract, 3; Peptone, 3; Glucose, 10; Agar, 20; pH 6.5. To maintain good viability for ethanol production, the culture was regenerated after every 3 weeks. The pre-culture was prepared by growing cells obtained from a newly prepared slant of 24 hours old and incubated at 25°C in 250 ml Erlenmeyer flasks containing 50 ml of the YM medium. The flasks were incubated with shaking at 180 rpm for 24 hours at 25°C in an incubator shaker (Model: JSSI 200T-JSR).

Production medium consisted of (g/l): yeast extracts 0.5, K_2HPO_4, 1; KH_2PO_4, 1.2; $MgSO_4.7H_2O$, 0.5; NaCl, 0.5 at pH 7.0 was prepared. Hydrolysed Sugarcane bagasse solution was the principle solution for the medium and the medium was distributed in 30 ml screw caps bottles, autoclaved at 121°C for 15 min and inoculated with 3 ml of the preculture ($4x10^5$ cfu/ml). All tests were done in triplicates and mean value was calculated.

2.3. Effect of different pH levels

Both commercial and standard *S. cerevisiae* isolates were grown in the fermentation medium with different pH values and all bottles were inoculated with 3 ml of inoculums at 25°C for 5 days. Ethanol content in fermented samples was estimated after 24 hours of incubation and continuous till the end of study.

2.4 Effect of different temperature

Growth and bioethanol production was carried out in fermentation medium with 3ml of pre-culture. This mixture was incubated at different temperatures viz. 25, 30, 37 and 40°C for 5 days. Ethanol content in fermented samples was estimated after every 24 hours. .

2.5 Moisture contained of sugarcane bagasse wastes

To determine the moisture of sugarcane bagasse waste, samples were weighed and dried at 105°C in an oven for 48 hours until the weight of samples become stable.

2.6 Growth estimation

Yeast growth was detected by measuring the absorbance at 660nm (A_{660nm}) using UV-10 Shimadzu spectrophotometer and non inoculated fermentation medium was used as a blank. The relationship between A_{660nm} of the yeast suspension and its dry weight was prepared.

2.7 Sugar contained of sugarcane bagasse wastes

The phenol sulphuric acid method was used to estimate the total carbohydrates (Dubois et al., 1956). One ml of sample solution was mixed with 1ml of 5% phenol and the obtained mixture was vortex well. Five ml of concentrated sulphuric acid were added and the mixture was heated in a water bath for 5 min and after 30 min. at room temperature, the absorbance was read at 490 nm. Dinitrosalicylic Acid Reagent was used to measure the amount of reducing sugars as described by Miller et al., (1960).

2.9 Ethanol Determination by Gas Chromatography

In each sample, ethanol production was estimated after fermentation process using gas chromatography (Agilent Model headspace gas chromatography HS-GC-7890A) according to the method described by Doelle & Greenfield (1985) The applied conditions were Poropack Q as a column material, nitrogen as carrier gas (12 ml/min), run time (4 min), column temperature 260°C, detector and injection temperatures were 280°C and 250°C, respectively and the yield of ethanol was calculated.

2.10 Statistical analysis

Three replicates for each experiment were carried out and the mean value was recorded. Student t- test using SPSS version 16 was applied to detect any significant differences between the

commercial *S. cerevisiae* and the standard *S. cerevisiae* at $p < 0.05$.

3 Results and Discussion

Ethanol can produce by *S. cerevisiae* from fermentation of sugarcane bagasse wastes which were used after acidic hydrolysis as a medium for ethanol production. Already few studies were conducted on sugarcane bagasse wastes from local resources, this study also provides information about the fermentation conditions for ethanol production from sugarcane bagasse by two strains of *S. cerevisiae*.

Sugarcane bagasse wastes were collected from different places, dried and powdered. The sugars were extracted, hydrolyzed used for production of ethanol by commercial and standard *S. cerevisiae.* Moisture and ash content of sugarcane bagasse wastes were determined (Table 1). The mean moisture value was 42% and ash contained was 4.3%. Generally, *S. cerevisiae* is widely used for most fermentation processes due to the presence of saccharifying enzymes and the obtained glucose was converted to ethanol and ethanol quantity was obtained from the equation (Meenakshi & Kumaresan, 2014):

Glucose (1 mole) $\xrightarrow[\text{Yeast}]{}$ **Ethanol (2 mole) + Carbon dioxide (2 mole) + Heat**

Table 1 Moisture content % and ash % of sugarcane wastes

Moisture and Ash content	sugarcane bagasse waste
Moisture content (%)	42.0±2.44
Ash (%)	4.3±0.27

Fermentation yields which is defined as the amount of ethanol produced in relation to the maximum ethanol that could be produced from the initial sugars. By stoichiometric reaction, 1 mole of glucose could produce 2 moles of ethanol, so 100 g of glucose could produce 46 g of ethanol if the conversion were 100%. Thus, the yields were determined by the following formulas:

EtOH max = Initial glucose × 46 / 100

EtOH max is the maximum production of ethanol obtained from the available glucose (initial glucose concentration), 46 is Gay-Lussac coefficient for glucose.

EtOH yield (%) = Sample EtOH concentration × 46 / % of EtOH max of theoretical yield

Bioethanol production for both commercial and genetically modified species was maximum after 72 hours, thus all experiments were carried out for 72 hours (Figure 1). The initial pH is one of the important factors that affect the performance of

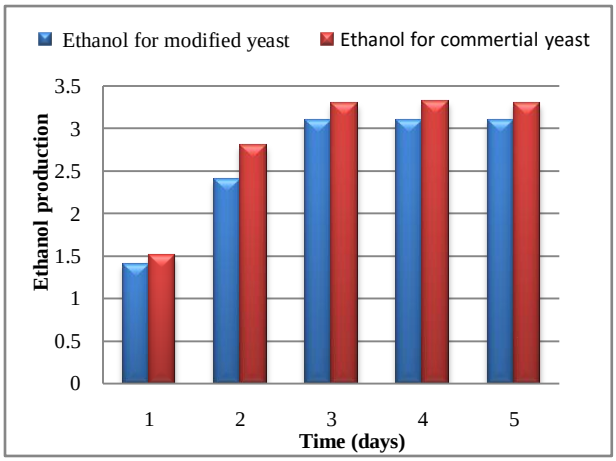

Figure 1 Bioethanol production by commercial and standard *S. cerevisiae* after different incubation period

the microbes. The effect of pH on ethanol fermentation was studied at different pH ranging from pH 4.0 to 8.0 for the two used yeast strains namely commercial *S. cerevisiae* and genetically modified *S. cerevisiae*. The initial carbon source concentration was adjusted to 30 g/l and the medium was inoculated with 3 ml inoculums, age 24 hours old culture and incubation was carried out at 30°C, for 72 hours of fermentation period. Glucose conversion was continuing simultaneously during the fermentation. Theoretically, glucose was consumed at the end of fermentation in both strains completely. As shown in Table (2)

Table 2 Effect of different pH values on sugar content and Ethanol production by two *Saccharomyces* isolates

Initial pH	Commercial *S. cerevisiae* (control)			*S. cerevisiae*		
	Sugars		Ethanol production (%)	Sugars		Ethanol production (%)
	Total	Reducing		Total	Reducing	
4.0	35.6±1.3	31.56±1.4	0.7	35.3±1.2	32.4 ±1.4	0.5*
5.0	34.8±1.4	32.0±1.7	1.9	35.1±1.3	30.0±1.5	1.8*
6.0	32.1±1.7	30.1±1.0	3.7	33.5±1.4	30.3±1.5	3.3*
7.0	33.1±2.3	31.4±2.0	2.7	33.2±1.5	30.2±1.8	2.1*
8.0	33.7±1.9	30.3±2.3	1.9	34.2±1.9	32.8 ±1.7	1.5*

*: significant results at $p < 0.05$ compared to control

and Figure (2), the ethanol concentration was increased between pH 4.0 to 7.0 and then decreased marginally below this value when the pH reached 8.0. The maximum ethanol concentration, 3.70% was obtained for commercial *S. cerevisiae* and 3.3 % for local strain culture, grown at pH 6.0. The growth in both cases was increased to about 1.2 g/l for commercial strain while it was about 0.9 g/l for genetically modified strain. These results are in agreement with that obtained by Turhan et al. (2010) who reported that high ethanol production was obtained by using initial pH 5.0 to 6.0. It was also shown that no ethanol production existed below pH 4.0. Roukas (1994) reported that maximum ethanol yield, maximum growth rate and biomass concentration were obtained at pH 5.5 on carob as a medium for ethanol production. Similarly, Osman (2011) tested wide initial pH range and found that at pH 3.0 no growth or ethanol production was observed, while pH 6.0 was the optimum for both biomass and ethanol production. Similar results were obtained by Mohanty et al. (2009).

Bioethanol production for both commercial and genetically modified *S. cerevisiae* species are highly affected by the temperature variation. Under suggested fermentation protocol, both commercial and genetically modified *S. cerevisiae* strains showed diverse results under different temperature conditions (25°C, 30°C, 37°C, 40°C) with regarding to bioethanol production (Table 3, Figure 3). Ethanol production was reported 3.7% for commercial *S. cerevisiae* and 3.3% for genetically modified *S. cerevisiae* at 25°C, while at 30°C, ethanol productions were 4.15 and 4.01% for both commercial and genetically modified *S. cerevisiae*, respectively. However, a slight reduction in ethanol production was occurred at 37°C for both *S. cerevisiae* strains. The ethanol concentrations were 3.64% and 3.48% for both commercial and genetically modified strains, respectively. Consequently, there was no ethanol production was observed at

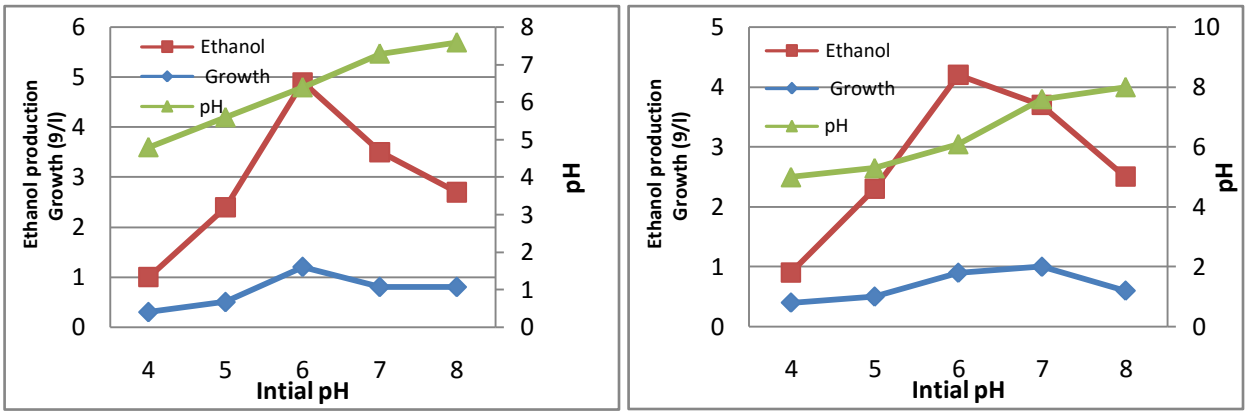

Figure 2 Growth and % of Ethanol production under different pH values using Commercial *S. cerevisiae* (A) and standard *S. cerevisiae* (B)

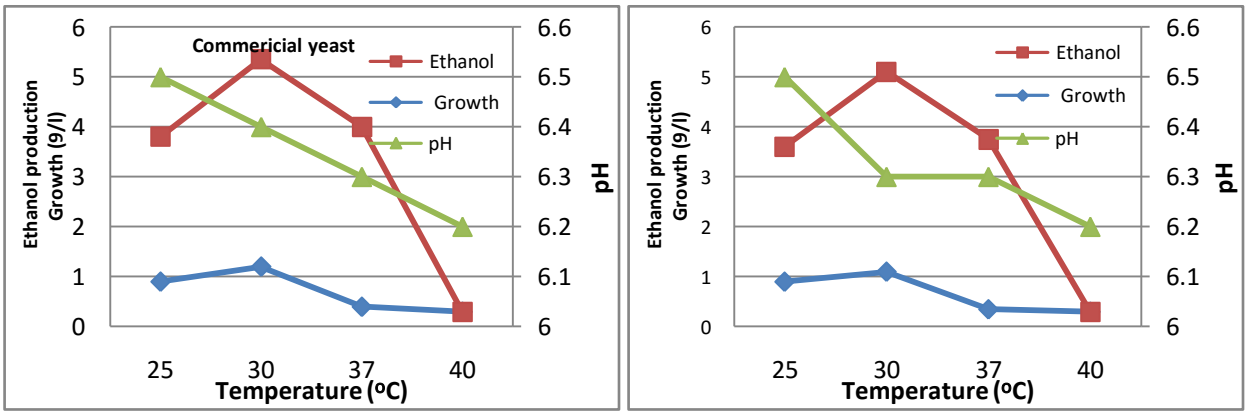

Figure 3 Growth and % of Ethanol production under different temperature using Commercial *S. cerevisiae* (A) and standard *S. cerevisiae* (B)

Table 3 Effect of temperature on sugar content and Ethanol production by two *Saccharomyces* isolates

Temp.(°C)	Commercial *S. cerevisiae* (control)			*S. cerevisia*		
	Sugars		Ethanol production	Sugars		Ethanol production
	Total	Reducing	(%)	Total	Reducing	(%)
25	34.0±1.4	32.5±1.7	3.7	33.7±1.4	30.1±1.0	3.3*
30	32.2±1.0	30.2±1.6	4.15	30.8±1.3	30.1±1.0	4.01
37	35.5±1.0	33.5±3.3	3.68	34.1±1.5	31.3±1.7	3.45
40	36.5±2.0	34.4±2.3	0	35.0±2.3	32.2±1.7	0

*: significant results at $p < 0.05$ compared to control

40℃ for both types of *S. cerevisiae*. These results may be due to the reduction in the growth of *S. cerevisiae* at higher temperature that too decreased the rate of fermentation process.

The data reported here proved that ethanol production for the two tested strains of S. *cerevisiae* was the highest at 30℃ and increasing temperature decreased the production of bioethanol. The difference in ethanol production the two tested strains of *S. cerevisiae* at different temperature were not significant. However, ethanol production at 30℃ showed a massive increase compared to production at 25℃. These results provide clear evidence that the optimum temperature for growth and ethanol production of both commercial and genetically *S. cerevisiae* species is 30℃. This result is against what has been reported by Turhan et al. (2010) but agreed with that obtained by Sheikh et al. (2016).

Conclusion

In conclusion, the two *S. cerevisiae* strains have the ability to produce bioethanol from very cheap carbon source, Sugarcane bagasse wastes. Results of study recommended that fermentation medium should have pH 6 for maximum ethanol production by *S. cerevisiae*. Further, suitable temperature for bioethanol production was noticed at 30℃ which gave highest bioethanol production with both commercial and genetically modified *S. cerevisiae* species. There were no significant differences were reported in bioethanol production between the two types of yeasts at different temperature values but the differences were significant at different pH values. Finally, it can be conclude that commercial and genetically modified *S. cerevisiae* can use sugarcane bagasse wastes for ethanol production and formation process medium and using of 30 g/l sugar at pH 6 and 30℃ for 72 hours yielded the maximum bioethanol production.

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Conflict of Interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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