

# Production of Bioethanol Fuel from Low-Grade-Date Extract

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**Abstract**—Experiments on production of bioethanol through anaerobic fermentation of sugars extracted from low-quality dates using a wild strain of *Saccharomyces cerevisiae* were conducted at 30°C and 33°C. The effect of the pH during fermentation was insignificant at the operating temperatures. The average ethanol yield for all experiments was greater than 71% of its theoretical value. Experiments in a 1L volume fermentor at 30°C and 120 rpm without controlling the pH during fermentation gave ethanol yields of 91.3%, 68.7% and 54.8 % for the 10, 15 and 20% initial sugar concentrations, respectively. The drop in ethanol yield for 20% sugars could be attributed to probable ethanol inhibition.

**Index Terms**—Bioethanol, *Saccharomyces cerevisiae*, dates, fermentation

## I. INTRODUCTION

Bioethanol production has been of great interest worldwide due to intensive demand for the depleting fossil oil. Also, the high increase of fossil oil prices in the last few years and the increased awareness of environmental problems related to greenhouse gases prompted a serious search for an alternative energy source that is sustainable and economically competitive to be used as a fuel.

The attractiveness of ethanol as a motor fuel derives from the fact that it: has high heating value per gallon (about 2/3 that of gasoline); can be blended up to 10% with gasoline without changing retuning the engine or increasing emission; enhances the octane rating of unleaded gasoline [1]; and could provide a secure source for national energy independence [2].

Production of bioethanol has been continually increasing during the last few years and has reached around 88.7 billion liters in 2011, thus, replacing the need for one million barrels of crude oil per day worldwide; over 90% of the total world bioethanol is produced in America [3], [4] (see Table I).

Uses of bioethanol are not limited to being an energy substitute or a transportation fuel; they extend to include a wide-band of chemical industries, such as production of acetaldehydes and acetic acid and their derivatives. 95% ethanol is used pharmaceutical and medical purposes.

The first generation of bioethanol was produced from natural crops, e.g., cereal crops (wheat and maize) and sugar crops (sugar cane, sweet sorghum and sugar beet); while

lignocellulosic biomass was the raw material for the second generation. A valid argument for the switch relates to competition between food and energy. This situation produces more challenges for the bioethanol industry. Agricultural wastes (agrowastes) were considered one of these sources that serve dual purpose of waste disposal (an environmental concern) and production of biofuel (an energy concern). Rice straw, wheat straw, corn straw and bagasse were considered the four major agrowastes feed-stocks for bioethanol production due to their availability throughout the year [5]. The conventional technique of producing bioethanol is by yeast fermentation of sugars under certain conditions or by hydrolysis of grain to glucose followed by yeast fermentation [6]. Other bio-resources such as apple pomaces [7] and mahula (*Mahuca Latifolia*) flowers [8] have been also used.

TABLE I: WORLD PRODUCTION OF ETHANOL FUEL IN MILLION LITERS

	2006	2007	2008	2009	2010	2011
Europe	1627	1882	2814	3683	4615	5467
Africa	0	49	72	108	165	170
America	35625	45467	60393	66368	77800	79005
Asia/Pacific	1940	2142	2743	2888	3183	4077
World	39192	49540	66022	73047	85763	88719

Source: The Global Renewable Fuels Alliance (GRFA, 2011)

Dates fruit is a suitable resource for bioethanol production. They contain considerable amounts of inverted sugars (glucose and fructose); the two sugars are present in dates in an almost equal amount. The flesh of dates contains about 70 to 75% sugars [9]. A second-grade (or low-grade) dates showed the same sugar content as dates of high quality [10].

Fermentation of sugars is an anaerobic biological process in which sugars are converted to alcohol by the action of microorganisms, usually yeast [11]. *Saccharomyces cerevisiae* is the most popular industrial microorganism used for sugar fermentation to produce bioethanol, because it utilizes cheap materials for growth and production. This organism has been already accepted as non-pathogenic, safe producer, which can be easily manipulated genetically and grown on simple and cheap media compared to that of animal cell cultivation [12].

Fermentation of sugar by the yeast *Saccharomyces cerevisiae* was carried out in an immobilized cell reactor (ICR) to improve the performance of the fermentation process for production of ethanol and to facilitate the separation of cells from the final product [13]. Other researchers used different types of microorganisms at different conditions to produce ethanol, e.g., wild-type strains of yeast *Hansenulapolyomorpha* to ferment glucose, cellobiose and xylose to ethanol [14]; bioethanol production from

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*Yarrowialipolytica* biomass [15]; bioethanol production by mangrove-derived marine yeast *Saccharomyces cerevisiae* [16]; production of ethanol from wheat straw hemicellulose acid hydrolysate using an adapted and parent strain of *Pichiastipitis*, [17]; use of strains of *Pichiastipitis* and *Candidashehatae* in fermenting a mixture of glucose and fructose, [18]; and the semi-continuous ethanol production from whey with co-immobilized enzyme and *Saccharomyces cerevisiae* followed by pervaporation of product, [19].

The main objective of this study is the production of bioethanol from low-grade-date extract using wild strain *Saccharomyces cerevisiae* yeast.

## II. MATERIALS AND METHODS

Low grade, unclassified as a dates' variety that is considered as wastes and usually used as animal fodder has been used to prepare the substrate for this study.

Sugars in dates were extracted by using deionized water at 40 °C for two hours to prepare the substrate for the fermentation. Fibers and free suspended solid in date extract were removed by centrifugation at 5000 rpm in a centrifuge (Model VarifugeF- Heraeussepatech D-6072 from Karl-Kolb) for 5 minutes and then filtered through a Whatmann filter paper. Clear date extract was decanted and collected in a 1000 ml Erlenmeyer flask. Three different initial sugar concentrations of 10, 15 and 20 g/100 ml (referred to as 10,15 and 20% respectively in the paper) were prepared and sterilized in autoclave (Astel AMB230N) at 121 °C for 15 minutes.

Wild strain *Saccharomyces cerevisiae* yeast (STAR brand; used in the bakery industry) obtained from the local market was used. The yeast was activated in a Malt Yeast Peptone Glucose broth (MYPG Broth). The MYPG broth was prepared by dissolving 1.5 g malt extract, 1.5 g yeast extract, 2.5 g peptone and 5 g dextrose in 500 ml of deionized water. The pH of the broth was recorded and adjusted to 4.5 by adding few drops of 1N HCl at 25 °C. The MYPG Broth was then sterilized in an autoclave for 15 minutes at 121 °C. 0.2 grams of the yeast, *Saccharomyces cerevisiae* were activated in 100 ml of MYPG broth in a 400 ml Erlenmeyer flask at 30 °C and 120 rpm for 24 hours. A 1L total volume fermentor (Minifore Lambda) supported with FNet software was used in the fermentation experiments.

Ethanol as well as sugars in date extract were analyzed by a High Performance Liquid Chromatograph, HPLC (Agilent 1200 Infinitely series) equipped with RID detector and Aminex® column, 150 × 7.8mm (Cat. #125-0115) from BIO-RAD was used. The column was maintained at 40 °C and 1 mM sulfuric acid solution was used as a mobile phase for the analysis at flow rate of 0.8 ml/min.

Two sets of fermentation experiments were conducted at a constant date extract final concentration of 13.43% at 30 °C and 33 °C to study the effect of pH during the fermentation. The first set was run without controlling the pH during fermentation process while the other set was made with controlling and re-adjusting the pH during fermentation to its initial value. For both sets an initial pH was 4.5.

Different sets of experiments were conducted using the

three different concentrations of substrate (i.e., 10, 15 and 20%). The substrate was loaded in the fermentor vessel and all openings and connections of the vessel were tightly closed. The fermentor vessel with its contents were sterilized at 121 °C for 15 min in autoclave (Astel AMB230N), and then the vessel was connected to the fermentor body. Yeast was then aseptically transferred to the fermentor. Fermentation experiments were performed at 30 °C using a constant agitation speed of 120 rpm. The total working volume was 400 ml.

## III. RESULTS AND DISCUSSIONS

Nutrition requirements for the *Saccharomyces cerevisiae* to generate energy and cellular synthesis such as carbon source, nitrogen source in addition to essential minerals and vitamins are present in the composition of date extracts. Other factors such as pH, temperature and initial sugar concentrations also affect the mechanism of ethanol production through the fermentation process.

### A. pH Effect

Two sets of experiments were conducted with and without controlling the pH during fermentation. Results of both sets were illustrated in Fig. 1 and Fig. 2. Results on the effect of pH at 30 °C and 33 °C during fermentation of date extract are illustrated in Table II.

The results show that the ethanol yield at 30 °C was found to be 71.6 and 73.2 % with and without controlling the pH, respectively during the fermentation process with a slight increase in the final ethanol yield and concentration. At 33 °C higher ethanol yields were obtained for the two cases with values of 78.4 and 84.6 %, respectively.

TABLE II: ETHANOL YIELD AND CONCENTRATION OF pH CONTROLLED AND UNCONTROLLED pH AT 30 AND 33 °C FERMENTATION TEMPERATURES

Ethanol	(30 °C) <sup>(a)</sup>	(30 °C) <sup>(b)</sup>	(33 °C) <sup>(a)</sup>	(33 °C) <sup>(b)</sup>
Yield (%)	71.6	73.2	78.4	84.6
Conc. (g/100 ml)	4.90	4.96	4.85	5.36

(a) pH controlled (b) pH Uncontrolled

The final ethanol concentration was found to be lower for experiments with controlled pH due to dilution. On the other hand, ethanol yields during fermentation period showed higher values at 30 °C compared to that at 33 °C (see Fig. 1) although the final yield at the end of fermentation is higher at 33 °C.

Fig. 2 shows sugar consumption profiles during fermentation. Sugars are completely consumed after 24 hours fermentation time at 30 °C. It could be concluded from Table II and from Fig. 1 and Fig. 2, the effect of pH during fermentation at 30 and 33 °C is insignificant. Higher ethanol yields were obtained at 33 °C and insignificant amount of sugars were left unfermented.

### B. Effect of Initial Sugar Concentration

Fermentation experiments at different initial sugar concentrations of 10, 15 and 20% and at 30 °C and 120 rpm were carried out using *Saccharomyces cerevisiae* wild yeast

strain.

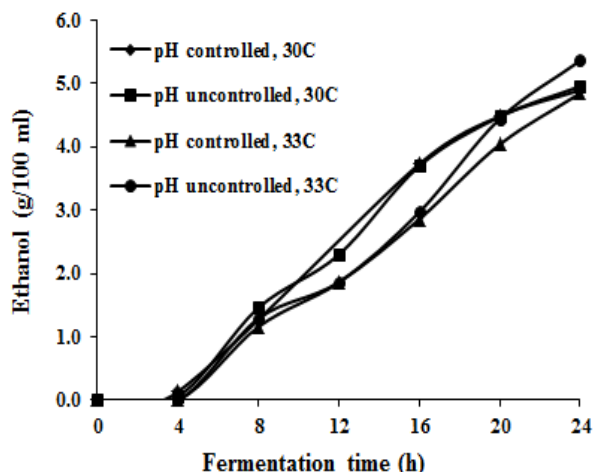


Fig. 1. Ethanol profiles of controlled and non-controlled pH fermentations at 30 °C and 33 °C

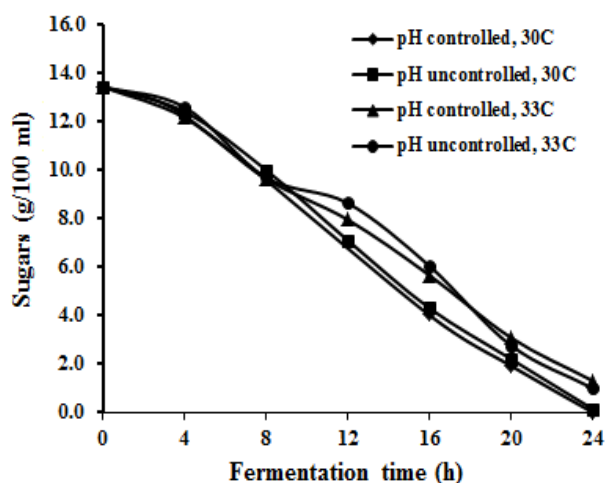


Fig. 2. Sugar consumption profiles of controlled and uncontrolled pH fermentations at 30 °C and 33 °C

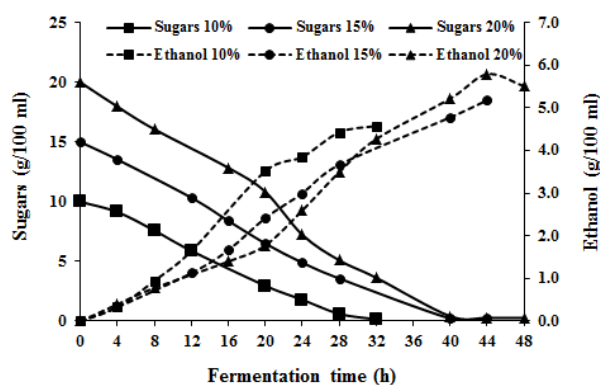


Fig. 3. Sugars and ethanol profiles during fermentation at 30 °C

Sugar profiles as well as the ethanol produced during fermentation were shown in Fig. 3. The 10% initial sugar concentrations date extract was completely fermented in 32 h producing 4.58 g/100ml ethanol with ethanol yield of 91.3% of the theoretical (see Table III). On the other hand, more fermentation time, 44 and 48 h are needed for complete fermentation for initial sugar concentration of date extract of 15 and 20% resulting in final ethanol concentrations of 5.10 and 5.51 g/100 ml and ethanol yields of 68.7 and 54.8%, respectively (see Table III). Lower ethanol yield was obtained for 20% concentration compared to the other two concentrations as shown in Fig. 4. A probable explanation of

this could be related to ethanol inhibition. It is clear that from Fig. 4 that higher ethanol yield rates during fermentation is obtained with date extract concentration of 10%. Working at lower concentration results in higher ethanol yields and less fermentation time.

TABLE III: ETHANOL YIELD AND CONCENTRATION DURING FERMENTATIONS AT 30 °C AND UNCONTROLLED pH OF DIFFERENT INITIAL SUGAR CONCENTRATIONS

Initial sugar concentrations	10%	15%	20%
Ethanol yield, %	91.3	68.7	54.8
Ethanol concentration, g/100 ml	4.58	5.10	5.51

#### IV. CONCLUSIONS

Dates as well as low-grade-dates are rich of sugars, mainly glucose and fructose which can be converted to ethanol and can also serve as essential carbon sources for yeast growth. Other nutrients, minerals and vitamins are also present within date's constituents which enhance the fermentation process to produce bioethanol. Many factors, such as temperature and initial sugar concentrations can affect the whole process. pH, within a reasonable range, was shown to have minor effects bioethanol production. Ethanol yields >71% were obtained at controlled and uncontrolled pH experiments. Ethanol yields of 91.3%, 68.7% and 54.8% were obtained from the 10%, 15% and 20% initial sugar concentration. Ethanol inhibitions could explain the drop of ethanol yields at higher sugar concentrations. More studies on bioethanol production from low-grade-date extracts are recommended to enhance the ethanol yield considering the economics of the process.

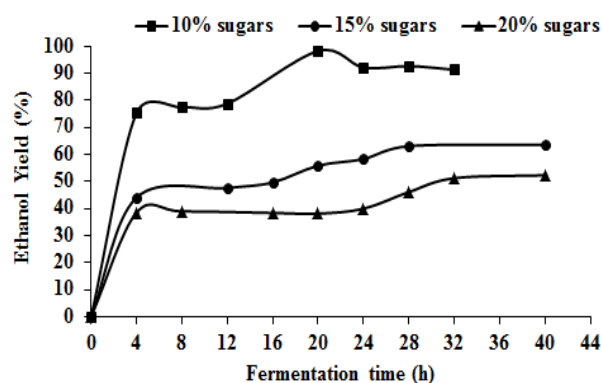


Fig. 4. Ethanol yields at different initial sugar concentrations

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#### REFERENCES

- [1] J. E. Bailey and D. F. Ollis, *Biochemical Engineering Fundamentals*, 2<sup>nd</sup>ed, New York, London: McGraw-Hill 1986.
- [2] M. F. Demirbas and B. Mustafa, "Recent advances on the production and utilization trends of bio-fuels: A global perspective," *Energy Conversion and Management*, vol. 47, issues 15, pp. 2371-238. September 2006.



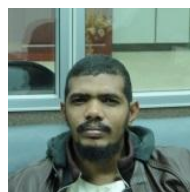
- [3] The Global Renewable Fuels Alliance (GRFA). [Online]. Available: [http://www.globalrfa.org/pr\\_021111.php](http://www.globalrfa.org/pr_021111.php)
- [4] L. Mojovic, D. Pejin, M. Rakin, J. Pejin, S. Nikolic, and A. Djukic-Vukovic, "How to improve the economy of bioethanol production in Serbia," *Renewable and Sustainable Energy Reviews*, vol. 16, pp. 6040–6047, 2012.
- [5] N. Sarkar, S. K. Ghosh, S. Bannerjee, and A. Aikat, "Bioethanol production from Agricultural wastes: An overview," *Renewable Energy*, vol. 37, pp. 19–27, 2012.
- [6] B. Hahn-Hägerdal, M. Galbe, M. F. Gorwa-Grauslund, G. Lidén, and G. Zacchi, "Bio-ethanol – the fuel of tomorrow from the residues of today," *Trends in Biotechnology*, vol. 24, issue 12, pp. 549–556, 2006.
- [7] E. Evcan, C. Tari, and B. Ozen, "Bioethanol production from low cost agro-industrial waste products," *New Biotechnology*, vol. 295, pp. 40 Poster I.1.11., 2012.
- [8] S. K. Mohanty, S. Behera, M. R. Swain, and R. C. Ray, "Bioethanol production from Mahula (*Madhucalatifolia* L.) flowers by solid-state fermentation," *Applied Energy*, vol. 86, pp. 640–644, 2009.
- [9] M. Elleuch, S. Besbes, O. Roiseux, C. Blecher, C. Deroanne, N. Dirar, and H. Attia, "Date flesh: Chemical composition and characteristics of the dietary fibre," *Food Chemistry*, vol. 111, pp. 676–682, 2008.
- [10] S. Besbes, L. Drira, C. Blecher, C. Deronne, and H. Attia, "Adding value to hard date (*Phoenix dactylifera* L.): Compositional, functional and sensory characteristics of date jam," *Food Chemistry*, vol. 112, pp. 406–411, 2009.
- [11] A. Demirbas, "Progress and recent trends in biofuels," *Progress in Energy and Combustion Science*, vol. 33, no. 1, pp. 1–18, 2007.
- [12] P. A. Vrsalovic and D. Vasic-Racki, "Modeling of the alcohol dehydrogenase production in baker's yeast," *Process Biochemistry*, vol. 40, no. 8, pp. 2781–2791, 2005.
- [13] G. Najafpour, H. Younesi, and K. Ismail, "Ethanol fermentation in an immobilized cell reactor using *Saccharomyces cerevisiae*," *Bioresource Technology*, vol. 92, pp. 251–260, 2004.
- [14] O. Ryabov, O. Chmil, and A. Sibirny, "Xylose and cellobiose fermentation to ethanol by the thermotolerant methylotrophic yeast *Hansenulapolyomorpha*," *FEMS Yeast Research*, vol. 4, pp. 157–164, 2003.
- [15] Y. A. Tsigie, W. Chung-Hsan, H. H. Lien, I. Suryadi, and J. Yi-Hsu, (2012). Bioethanol production from *Yarrowialipolytica* Polg biomass. *Bioresour. Technol.* [Online]. Available: <http://dx.doi.org/10.1016/j.biortech.2012.11.091>
- [16] K. Saravanakumar, P. Senthilraja, and K. Kathiresan, "Bioethanol production by mangrove-derived marine yeast, *Saccharomyces cerevisiae*," *Journal of King Saud University – Science*, 2013.
- [17] J. N. Nigam, "Ethanol production from wheat straw hemicellulose hydrolysate by *Pichia stipitis*," *Journal of Biotechnology*, vol. 87, pp. 17–27, 2001.
- [18] H. Sreenatha and T. Jeffries, "Production of ethanol from wood hydrolysate by yeasts," *Bioresource Technology*, vol. 72, pp. 253–260, 2000.
- [19] M. Staniszewski, W. Kujawski, and M. Lewandowska, "Semi-continuous ethanol production from whey with co-immobilized enzyme and yeast cells followed by pervaporative recovery of product – Kinetic model predictions considering glucose repression," *Journal of Food Engineering*, vol. 91, pp. 240–249, 2009.



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