Fermentative production of bioethanol from a variety of soft date (Ghars)

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Abstract— The aim of this work is to study the bioethanol production from a local date (Ghars), relatively low commercial importance. The fermentable sugar is extracted from this resource using hot water (80°C). Sugar extraction is studied versus the dates initial mass. The must pH is adjusted between 4.2-4.5. The yeast Saccharomyces cerevisiae is used for fermentation. The sugar conversion rate and the alcohol content increases when the yeast dose increases.
Ammonium sulfate and magnesium sulfate are tested as salts nutrients, at concentrations of 2.5 and 3.0 g.L-1, and 1.0, 2.5 and 3.0 g.L-1, respectively. At low magnesium sulfate concentrations, sugars degradation is better, 56.56% is obtained, but the fermentation period is longer. More efficient and higher conversion rates are obtained when specific culture medium is used. The study of pH evolution during fermentation shows that after a decreasing in the first 24 h, this parameter becomes constant.

Keywords: bioethanol fermentation, Ghars, soft dates, ammonium sulfate, magnesium sulfate.

I. INTRODUCTION

Bioethanol is ethyl alcohol. There are two main ways to produce ethanol, namely by synthesis from hydrocarbons and from biomass. Only this second approach deserves the name "bioethanol". The production of bioethanol from biomass uses three categories of agricultural raw materials: simple sugars, starch and lignocelluloses. The availability of raw materials is one of the major problems in the production of bioethanol [1]. Sugar and starch based materials such as sugarcane and grains are two groups of raw materials currently used as the main resources for ethanol production in many countries that these materials are abundant materials [2]. Brazil utilizes sugarcane for bioethanol production while the United States and Europe mainly use starch from corn, and from wheat and barley, respectively [1]. Brazil is the largest single producer of sugarcane with about 31% of global production [3]. The United States is predominantly a producer of bioethanol derived from corn, and production is concentrated in Midwestern states with abundant corn supplies [4].
The third group is lignocellulosic materials, its biochemical conversion through saccharification and fermentation is a major pathway for bioethanol production from biomass.
Date palms are widely spread in Mediterranean areas by Arabs and are cultivated globally [5] Date palm is considered as the central pivot around which life in the Saharan regions. It is of great socio-economic and environmental importance in many countries [6].
Algeria is second biggest producer of dates [7] It was estimated at 49,288 tons in 2006 [8]. The date varieties do not have the same market value. Generally, there are two varieties of dates, and according to their texture which can be hard or soft. The variety Ghars is a soft date category. The number of date palms for the production of soft dates is 221,568 in Algeria [9].

The world economy depends heavily on various fossil energy sources for the production of fuel, electricity and other goods. [10] The increase in fossil fuel consumption is alarmingly, both environmentally and economically on the reduction of the world's natural resources. The environmental impacts are more seen by the ecological effects, the level of greenhouse gases in the atmosphere is one example. Conventional transport fuels contribute to the worsening of the environmental conditions. Renewable sources could serve as an alternative. Biomass can be considered as a future source for the production of significant renewable energy for fuel production. [11] Any oil-based fuels may be replaced by renewable biomass fuel such as bioethanol.

In this study, Ghars is used as substrate for bioethanol production. Indeed, this type of high sugar date can be used as carbon source for fermentation production of this metabolite. In this sense, the use of this biomass is an alternative way to produce a biofuel instead to fossil fuel.

II. MATERIAL AND METHODS

For this study, a soft date's variety, purchased locally, has been chosen on basis of its low market value. Dry yeast, Saccharomyces cerevisiae is used. It is stored at 4°C. This strain has never been used in the fermentation.

A. Preparation of date juice

In fermentation industries, must is a sweet liquid used as raw material [12]. For must preparation, biomass was washed, dried, pitted and crushed. The material obtained is immersed in hot water (80 °C), under stirring, during 1 hour. This maceration in hot water allows better extraction of sugars. After mixture cooling and filtration, filtrate (juice) pH is adjusted in the range of 4.2-4.5 using sulfuric acid (1N), to avoid the growth of bacteria, and promote the proliferation yeast [13]; then sterilized at 120 °C for 20 minutes in an autoclave. This liquid is used as a culture medium.

B. Preparation of inoculum

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The inoculum preparation phase is the most important in fermentation process. This is to prepare a preculture to inoculate the fermentation medium. This step aims to adapt the yeast strain used in fermentation media. The strain is seeded in the sterilized juice. In a 250 ml Erlenmeyer flask, 100 mL of sterilized juice is mixed with a known mass of the strain, the mixture is introduced into the incubator at 30±2 °C, under continuous stirring at 50 oscillations per minute, for 15 hours.

C. Alcoholic fermentation

The fermentation operation was carried out in anaerobic conditions in Erlenmeyer flasks filled to 2/3 of its capacity. Sterilized juice medium is seeded with inoculums. The fermentation is conducted at a temperature of 30 ± 2°C, under low agitation, during 72 hours. At the end of the fermentation, the date wine is recovered, filtered, then distilled in order to extract the ethanol. The distillation temperature is about 78°C.

D. Analytical techniques

Total sugars concentration is determined by Dubois method, which enables to determine monosaccharide concentration using phenol and sulfuric acid. In the presence of these two reactants, sugars give a creamy yellow color, of which the intensity is proportional to total sugars concentration. The optical density is determined at 490 nm [14]. The reducing sugars concentration is determined by the method based on the reduction of Fehling's solution. The content of alcohol in distillate is determined using aerometer.

III. THEORETICAL CONSIDERATIONS ON ALCOHOLIC FERMENTATION PROCESS

The alcoholic fermentation is a biochemical process by which carbohydrates, mainly glucose, are decomposed under anoxic conditions into ethanol and carbon dioxide with release of energy according to the following reaction scheme:

$$C_6H_{12}O_6 \longrightarrow 2C_2H_6O + 2CO_2 + ATP$$

The fermentation can occur only under certain conditions:

A. Temperature

It may affect the viability and growth of the yeast, and thus the production of ethanol.

B. Oxygen

In the presence of micro-aeration that promotes the development of micro-organisms, the resistance of the strains to the action of ethanol is increased, as is also the efficiency of conversion of sugars into ethanol. Although the fermentation is an anaerobic phenomenon, yeasts require some oxygen for multiply and synthesize sterols that allow a better resistance to ethanol (and thus improved survival).

C. Mineral elements

The needs of microorganisms in minerals elements necessary for their growth and renewal can be made in several ways. Nitrogen phosphorus, potassium and magnesium are supplied in salts. Note that a portion of the nitrogen can be found in the date juice

D. Rate conversion of sugar

Fermentation will be quantified by the percentage of converts sugars calculated according to equation-1, it is the ratio between the amount of sugar converted and the amount of initial sugar.

$$\tau(\%) = \frac{m_0 - m_r}{m_0} .100 \qquad \text{(eq.1)}$$

E. Determination of sucrose mass

After the determination of the total sugars, sucrose rate is deduced according to the formula of Eq-2:

Sucrose = (total sugars – reducing sugars) x 0,95 (eq.2) [15]

IV. RESULTS AND DISCUSSION

A. Effect of the biomass initial mass

It would be interesting to see how the initial amount of dates (m_0) used for the fermentation process affects the amount of sugar extracted, one hand, and on the rate or yield of ethanol, on the other hand. Results are given in Table -1-

Table 1 Extraction conditions and characteristics of juice dates

Extraction step			
m_0(g)	100	200	300
Must density (ρ) (g/ml)	1.012	1.041	1.048
Must characteristic			
Mass of total sugars (g)	13.91	33.93	46.96
mass of reducing sugars (g)	3.00	3.44	5.00
sucrose mass (g)	10.91	30.49	41.96

These results suggest that this type of biomass contains more sucrose than reducing sugars. Note that the rate of sugar is substantially the same, indicating that the yield of extraction is the maximum in the current operating conditions. The evolution of the conversion rate and the alcohol content is given on the same graph (fig -1).

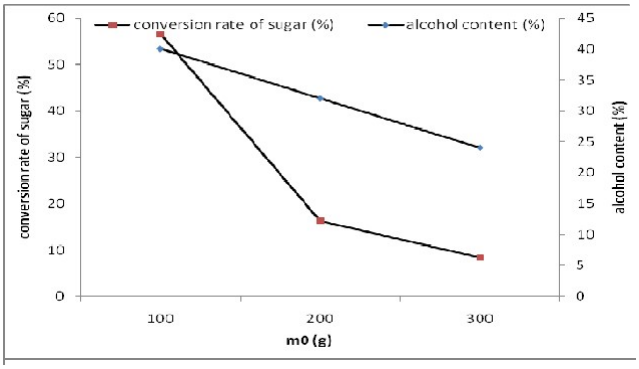

Fig. 1 Evolution of the alcohol content and the conversion rate versus the initial weight of Ghars

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Fig.1 shows that the sugar conversion decreases when the initial mass of the biomass increases, ie when the amount of sugar increases. This can be explained by that the available sugar is not completely consumed by the yeast; this probably is related to the inhibition of yeast growth, caused by the accumulation of toxic substances [16]. Also the conversion decrease is reflected by the decline in bioethanol production.

B. Effect of initial yeast dose

Saccharomyces cerevisiae est l'espèce de levures utilisée dans la fermentation alcoolique [17,18]. In this part, the yeast dose (m_y) is the variable parameter of the fermentation process. The sugar conversion and the production of bioethanol are plotted as a function of the initial dose of the yeast (Fig 2). Note that the sugar conversion rate increases with the initial dose of yeast, which can be shown in the production of bioethanol. The curves shapes are consistent and reflecting well the effect of this parameter.

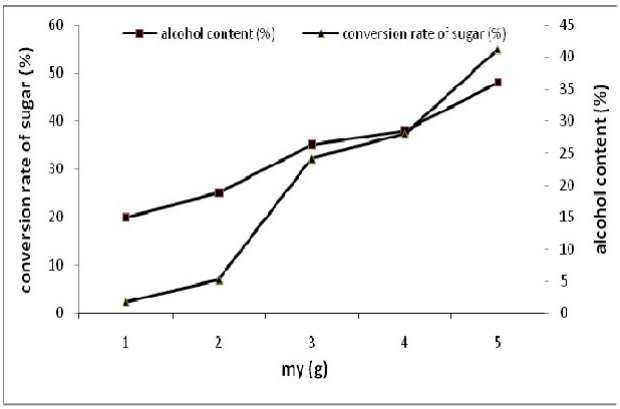

Fig. 2 Alcohol content and conversion rate according to the dose of yeast

C. Effect of nutrient salts concentration on the fermentation

Yeast should be fed into appropriate nutrients. To determine the concentration of nutrient salts which is best adapted to the growth of yeast, and further to find out whether or not an increase in concentration may improve the alcohol production. Several salts and nutrient broth are tested. Each nutrient solution used contained only one salt, except the broth nutrient. The salts tested are ammonium sulfate and magnesium sulfate. The broth nutrient compound peptone, yeast extract, sodium chloride and glucose.

Ammonium sulfate

The ammonium is one of the elements which can be assimilated by the yeast Saccharomyces, ammonium sulfates are used as a nutrient in the fermentation. Two trials were conducted with salt concentration of 2.5 $g.L^{-1}$ and 3 $g.L^{-1}$. The results are given in Table 2.

Table 2. Effect of salt concentration ($(NH_4)_2SO_4$)

C_0 ($g.L^{-1}$)	2.5	3.0
pH	3.42	3.57
Alcool content (%)	24	17
Conversion rate (%)	55.60	32.39
fermentation time (h)	52	49

These results show that small amounts of alcohol are produced and the rate of degradation varies between 55.6% and 32.39%. Comparing these results to those obtained without the addition of salt, where the conversion rate was 31.6%, then there is an optimum concentration at relatively moderate levels, it can be strengthened by the fact that the fermentation time is lower (52h and 49h instead of 72 hours). So we can say that the salt is privileged and quickly assimilated by the yeast, which directly affects the growth of the yeast species during the growth phase.

Magnesium sulfate

Magnesium is one of the elements that can be assimilated by Saccharomyces yeast. The results are gathered in Table 3.

Table 3. Effect of salt concentration (MgSO4)

C_0 ($g.L^{-1}$)	1.0	2.5	3.0
pH	3.62	3.42	3.57
Alcohol content (%)	36	31	32
Conversion rate (%)	56.56	34.59	31.74
fermentation time (h)	70	61	52

When magnesium sulphate concentrations are low, sugars degradation rate is better. These results show that the magnesium concentration may, in certain circumstances, have an effect on the growth of yeast, and therefore on sugars degradation. Indeed, Mg^{2+} ions, with the phospholipids, play an important role in stabilizing the cell membrane and thus contribute to the protection of the cells against various stress factors, mainly ethanol. Conversely, the loss of magnesium coupled to excessive calcium entry increases the sensitivity of yeast to ethanol [19].

Broth nutrient

This is a suitable nutrient broth for the growth of most microorganisms. The objective is to test this medium, already prepared, containing various nutrients, to see its effect on the sugar conversion rates, and therefore the bioethanol production of. Two concentrations were tested, 2.5 $g.L^{-1}$ and 3.0 $g.L^{-1}$. Results obtained are given in Table 4.

Table 4. Effect of broth nutrient

C_0 ($g.L^{-1}$)	2.5	3.0
pH	3.80	4.08
Alcohol content (%)	40	42
Conversion rate (%)	95.42	96.26
fermentation time (h)	70	71

These results (table 4) show that the conversion rate is higher compared to the case of using only salts. The broth allowed a better conversion, and thus better production of bioethanol. This may be related to improved nutrition of yeast which has a longer activity.

D. pH evolution during fermentation

pH is currently used in oenology as an indicator of different aspects: contamination risks, efficiency of sulfating, sensorial properties. As it is quite easy to

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measure, pH could be used not only at the end of the process to qualify the end product but also as an indicator throughout the fermentation. In fact, during the alcoholic fermentation of must, the conversion of substrates (sugars, organic acids, nitrogen) into metabolites such as ethanol and organic acids by the yeasts *Saccharomyces cerevisiae* modifies the thermodynamic equilibrium in the medium and consequently the pH [20]

The medium pH is determined at different fermentation times, figure 3 shows this evolution.

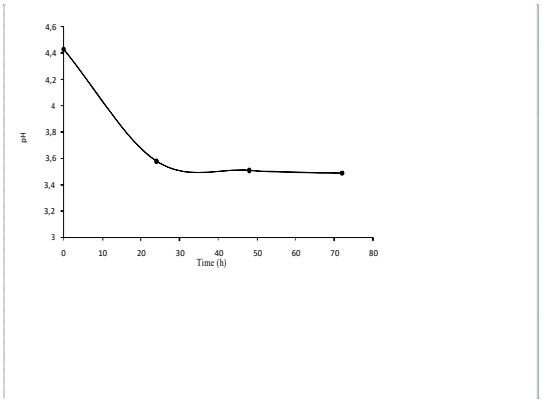

Fig. 3 *Evolution of medium pH during fermentation*

Regarding pH evolution, fermentation can be divided into two phases: during the first 24 h, the pH decreases from 4.45 to 3.55, then it becomes constant during the 46 following hours. The pH decrease is assumed to be correlated to the nitrogen consumption. The nitrogen concentration did not influence the pH itself when it was added in the must. However, it is known that during the fermentation, the consumption of nitrogen by yeasts produces H^+ ions. Won et al. [21] and Sigler et al. [22] also mentioned the same phenomenon. Studies of Hernandez-Orte et al. [23] showed that the main part of the nitrogen source was consumed between 0 and 50 h of alcoholic fermentation.

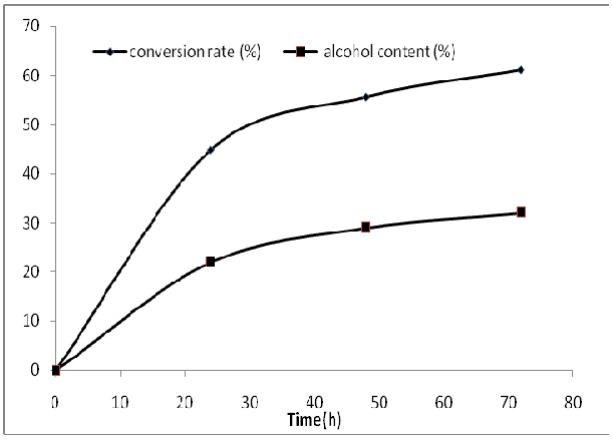

Fig.4. Alcohol content and conversion rate as a function of time

Figure 4 shows that at low conversion rates, the alcohol content is relatively low, but when the conversion rate is high, the alcohol content is so high.

V. CONCLUSIONS

Due to its chemical composition and its relatively high fermentable sugar, this type of dates (Ghars) low-value can be considered as a potential substrate for the production of bioethanol.

The study results open up promising pathways that can help provide locally at least, production of bioethanol from a local product of low commercial value.

This work examined some parameters of the fermentation process. Each factor studied has made its own contribution in terms of its influence on the sugar transformation and bioethanol production.

Further this study demonstrated the feasibility of such batch processing.

References

[1] Balat, M. Production of bioethanol from lignocellulosic materials via the biochemical pathway: A review, Energy Conversion and Management 2011; 52: 858–875.

[2]Talebnia F, Karakashev J, Angelidaki I. Production of bioethanol from wheat straw: An overview on pretreatment, hydrolysis and fermentation, Bioresource Technology 2010;101: 4744–4753.

[3] Hartemink AE, Sugarcane for bioethanol: soil and environmental issues. Adv Agron 2008;99:125–82.]

[4] [Asher A. Opportunities in biofuels creating competitive biofuels markets. Biofuels Australasia conference, Sydney, Australia, 2006 November 20–22.

[5] Pandey, B.P. and Anita, Economic Botany, S.Chand and Co. Ltd., Ram Nagar, New Delhi, India, 1990, p. 7-99.).

[6] Dubost D, Mutation du système de production oasien en Algérie. Ed *CRSTRA. 1990, Alger*)

[7] The report Algeria 2011, by Oxford business group.

[8] Rapport Technique, '*Statistiques Agricoles, Palmier Dattier, Superficies et Production*', Ministère de l'Agriculture et du Développement Rural, D.S.A.S. Ed., 2006, Série B, Alger, Algérie.

[9] I.T.D.A.S., 2001. Statistiques agricoles (Station expérimentale d'Ain-Bennoui, Biskra).

[10] Uihlein A, Schbek L. Environmental impacts of a lignocellulosic feedstock biorefinery system: an assessment. Biomass and Bioenergy 2009, 33:793-802.

[11] Lynd LR, Wang MQ. A product-nonspecific framework for evaluating the potential of biomass-based products to displace fossil fuels. Journal of Industrial Ecology 2003;7:17-32.

[12] J. M. Clement, Ed. Masson, Paris.- Dictionnaires des industries alimentaires, 1978, 108-261.

[13] Guiraud H and Galzi P, Ed. Usine nouvelle, Paris- Analyses microbiologiques dans les industries agro-alimentaires.: 1980, p.70-90.

[14] Dubois M, Gilles K.A, Hamilton J.K., Rebers P.A. & Smith F., 1956. - Colorimetric method for determination of sugar and related substances. *Anal. Chem.,*28, 350-356.

[15] Audigie C., Figiralla J., Zonszain F., 1980.- Manuel d'analyses biochimiques. Ed. Doin, Paris, p270.

[16] A. Meyer, 'cours de microbiologie générale'. 1998, Ed, Doin.

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[17] Torija M.J, Rozès N, José M.P, Guillamón M, Mas A. Effects of fermentation temperature on the strain population of Saccharomyces cerevisiae. International Journal of Food Microbiology 2003; 80: 47 – 53.

[18] Alfenore S,Molina-Jouve C, Guillouet S, Uribelarrea J-L, Goma G, Benbadis L. Improving ethanol production and viability of *Saccharomyces cerevisiae* by a vitamin feeding strategy during fed-batch process. Applied Microbiology and Biotechnology October 2002, Volume 60, Issue 1-2 :67-72.

[19] Brich R.M, Ciani M, Walker G.M, Magnesium, calcium and fermentative metabolism in wine yeasts, journal of wine Research, 2003; 14 (1): 3-15.

[20] Akin H, Brandam C, Meyer X, Strehaiano P. A model for pH determination during alcoholic fermentation of a grape must by *Saccharomyces cerevisiae.* Chemical Engineering and Processing: Process Intensification Volume 47, Issue 11, October 2008, p. 1986–1993.

[21] Won J.I, Yang Y.L, Kim B.G, Choi C.Y. Adaptative control of specific growth rate based on proton production in anaerobic fed-batch culture, Biotechnol. Lett. 1993; 15 (5) : 511–516.

[22] Sigler K, Knotkova A, Kotyk A. Factor governing substrate induced generation and extrusion of proton in yeast, Biochim. Biophys. Acta 643, 1981:572–582.

[23] Hernandez-Orte P, Ibarz M.J, Cacho J, Ferreira V. Addition of acids to grape juice of merlot variety: effect on amino acid uptake and aroma generation during alcoholic fermentation, Food Chem. 98, 2006; 300–310.

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