

*Full Length Research Paper*

# Generation of bioethanol from common date by-products, “Teggaza and Lebghel” in Southern Algeria

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**Date by-products constitute the principal food for the oasis populations in Middle East and North Africa. Dates contents consist of 70 to 80% of reducing sugars, and do not require an intensive energy and labour for thermophysical pre-treatment. They can serve as a good feedstock for bioethanol generation through fermentation and distillation. Algeria is among the top sixth producers of dates in the world with more than 250,000 tons/year; from these, more than 30% can be lost for different reasons and may be of low quality. In the laboratory, after an alcoholic fermentation of the substrate of the date varieties, *Teggaza* and *Lebghel* (T & L) using bakery yeast at 30°C for 72 h, the distilled and rectified date juice generated the highest ethanol (88° and 90°) with acceptable productions of 2.5 and 2.78 mL/kg/h, and assessed scale efficiencies of 23.57 and 26.2%. This is unlike the one (ethanol; 50%) directly generated by chemical reaction using the same quantity of sugar. The efficiencies that were obtained seem satisfactory and encourage the great scaling development of bioethanol generation using date waste biomass abundant in Algerian Sahara.**

**Key words:** Algerian Sahara, alcoholic fermentation, bioethanol, bakery yeast, dates by-product, distillation.

## INTRODUCTION

Due to the variability in oil-market and increase in air pollution, there is the need to discover novel alternatives for renewable and sustainable energy sources to ensure energy security in the future. Bioethanol produced from bioenergy feedstock is one of the sustainable, economic and ecologic solutions to these issues. It can be produced from reduced sugars and stiff biomass or from

Lignocellulosic biomass (Sims et al., 2008). Typical alcohol applications include chemicals, food and fuel products, fungicides, laboratory reagents, plastics, antiseptic, preserving solutions, refrigeration, solvents and others (Simo et al., 2009). Bioethanol was suggested as biofuel substitution; it is economical and ecofriendly and can be produced from many biomass sources including wood chips, corn husks, dates and other

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agricultural by-products. Using bioethanol instead of gasoline leads to the reduction of carbon emission by 80% and overall gasoline consumption by more than 30% (Elsanhoty et al., 2012). The production of bioethanol from lignocellulose raw materials requires generally the incorporation of an efficient pretreatment, followed by a scarification of the carbohydrates to obtain satisfactory effectiveness (Acourene and Ammouche, 2012). Compared to the use of gasoline, bioethanol helps to reduce CO<sub>2</sub> emission to about 80% (Li et al., 2008).

"*Dactylifera L.*" constitutes the central harvest and adapted sapling in arid and semi-arid areas of the globe. It constitutes the principal food of their inhabitants and animals since one kilogram of dates offers about 3,000 calories, income and economy sources for Saharan people in middle-East and North Africa (Boufis et al., 2014). The economy of the these regions is based principally on date palm cultivation and the use of its fruit by-products to prepare pasta, flours, syrups, vinegars, alcohols, yeasts, and confectioneries. It provides major sources of revenue for these oasis populations. The date palm is completely used, including its trunks, leaves for basketry and house structure. The date fruit is used in fresh and dry forms, and transformed into syrup (rub of tamr), (Amani et al, 2013), or fermented to produce metabolite (wine and vinegar); its leaflets and seeds are used in animal feed (Abbès et al., 2011). The world's potential production of dates is increasing in some countries like Egypt (17.2%), Saudi Arabia (13.7%), Iran (13%), United Arab Emirates (9.8%), Pakistan (9.6%), Algeria (9%), Iraq (7.2%), Sudan (5.4%), Oman (3.5%) and Libya (2%) (Chandrasekaran and Bahkali, 2013).

Algeria "*Phoenixia*" had an important progress in date-palms cultivars: it got 18,000,000 palms, covering more than 350,000 ha, where 11,000,000 trees are productive (FAO Statistic, 2015). The Algerian harvest attained 500,000 tons. The leftover dates that constitute the common dates reach 250,000 tons, in which 30% are of low quality dates. Only Adrar Province produced 86,500 tons of dates in 2012, coming from 2,000,000 of date-palms. This important production is commercialized in large quantities to foreigners in border countries while a few quantities are locally consumed.

Dates are rich in biodegradable sugars of about 73 to 83% on dry mass basis in two inverted forms, glucose and fructose (FAO statistics, 2015). They have long conservation and constitute a basis for various products, sugar-liquid, juice, alcohol, vinegar etc. Various products can be derived from dates feedstock like bio-polymers (Louhichi et al., 2013), organic and amino-acids (Radwan et al., 2010), bakery-yeast (Qureshi et al., 2012), probiotics and antibiotics (Abd-Alla and El-Enany, 2012), enzymes (Mussatto et al., 2010) and biofuels (hydrogen, butanol) (Nigam and Singh, 2011).

Algerian economy is based principally on fossil fuel. Algeria imports about 30,000 to 50,000 hl of ethylic alcohol per year for its proper uses. To reduce the dependency on fossil fuels and importations of chemical

products, the Algerian Government has developed a national program from 2011 to 2030 to promote concrete-actions in the fields of energy efficiency and renewable energy (MEM, 2011; Stambouli et al., 2012).

The objective of the present work is to study the feasibility and productivity of generating bioethanol of highest quality in laboratory from the transformation of two common varieties of date by-products, Teggaza and Lebghel (T & L) of low quality in Adrar Province using anaerobic fermentation and distillation processes.

## MATERIALS AND METHODS

### Raw material and microorganisms

The date by-product (Figure 1) used in the present study to generate bioethanol is composed essentially of (T & L) varieties of dates originated from Algerian Sahara. It was obtained from Adrar conditioning unit of dry dates feedstock at the Agronomy Research National Institute of Algeria (INRAA, elmouchir.caci.dz). The dates were dried, kept in bags and stored at room temperature. They were sold in few quantities at local markets or served as feed for animals. The microorganism *S. cerevisiae* used in the fermentation process of date juice was provided by the industrial plant of bakery yeast production, from Oued-Semar in Algeria.

### Bioethanol generation medium

Dates were washed, plunged in water, rubbed, and rinsed to eliminate sand, pebbles, insects and leftover plants. Then the seeds were separated from the coats and then petted (Figure 2a). Dates substrate was imbibed in hot water at 90 to 95°C to facilitate sugars extraction. Then 250 g of dates was diluted into 1 L of tap water, and simultaneously sulfuric acid was added and adjusted to obtain pH between 4.3 and 4.7, to inhibit bacteria and favor overgrowth of yeast (Wei-Hao et al., 2016). The anaerobic medium was inoculated with 1 g/L by *S. cerevisiae* model SII esaffre 59703 which is available in local markets; it was reactivated during 60 to 90 min under 30°C into an aqueous solution in glucose with 12% V/V.

Two bioreactors were prepared for each date variety studied. The first bioreactor is a glass bottle of 3 L capacity and used to follow the fermentation process while the second is a plastic jug with a great capacity of 30 L used to assess the system scaling efficiency. For both bioreactors, the inoculum size is 3% from the active dry yeast. The inoculum was prepared in a 3 L bioreactor containing 2 L of date substrate. It was incubated at 30°C, 10 rpm and air flow rate of 1 vvm for 30 to 60 min and then stored at 4°C.

During the fermentation process, the bioreactor was equipped with a manual agitator shaft (Figure 2b). The main objective of aeration is to provide microorganism growth in submerged cultures with appropriate oxygen for their metabolic needs. Agitation guarantees a homogeneous distribution of microorganisms and nutrients in the broth.

The density of sugars consumed, pH and the alcohol concentration of the date juice are controlled using Dubois method given in DuBois et al. (1956). The pH was measured by a digital pH-meter model Mettler Toledo methods (ISO11289, 1993; AOAC, 98, 1.12) and the date juice temperature during the alcoholic fermentation was recorded using thermocouples K type connected to the data logger model Fluke 2635A. Reducing sugars RS and total sugars TS are assessed by titration using a spectrometer UV (Siddiq et al., 2013). Saccharose content was estimated with the following formula (Reynes et al., 1994).



Figure 1. Date's variety by-product used in bioethanol generation.

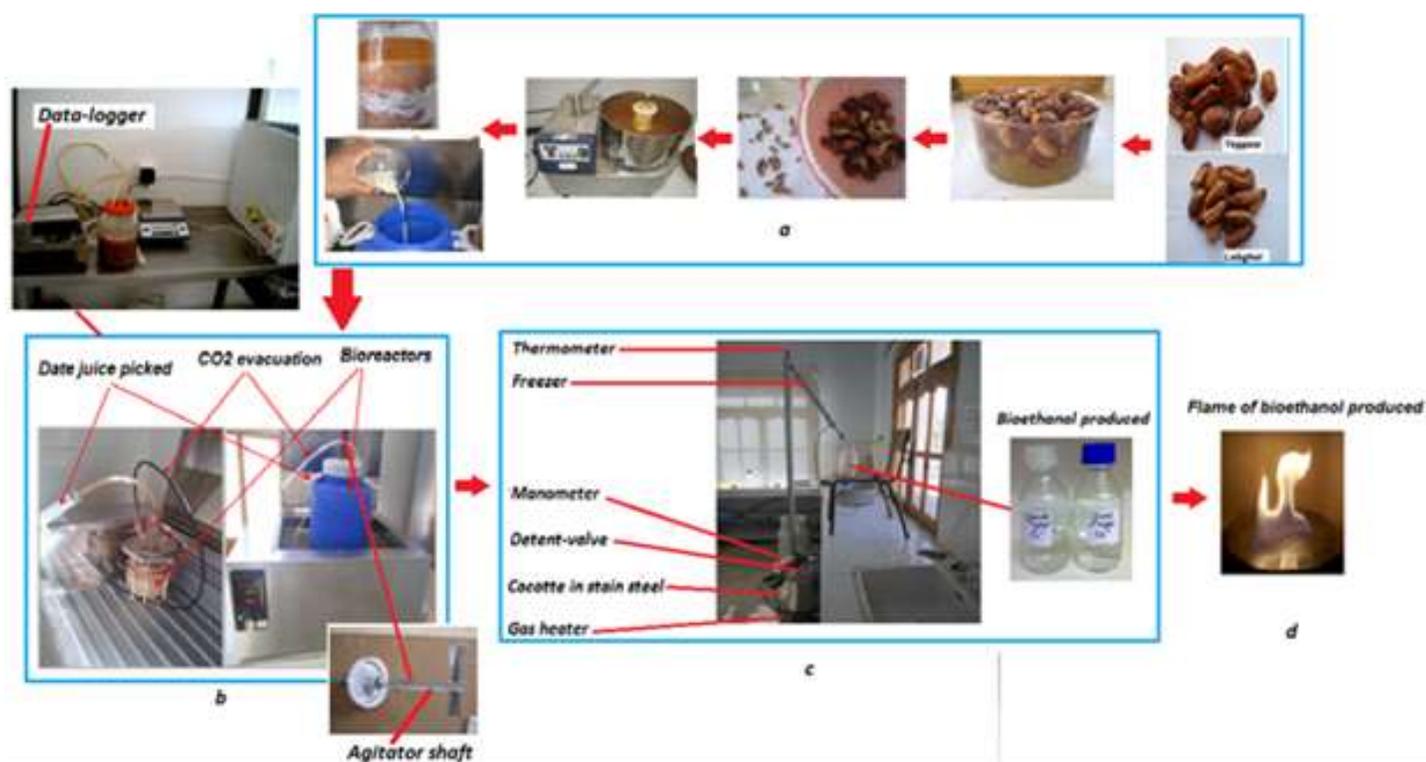


Figure 2. Bioethanol generation process in laboratory.

$$Saccharose (\%) = (TS - 0.95RS) \% \tag{1}$$

Different analyses were performed on dates including water content, sugars content, pH, consistency, protein rate, cinder rate and the concentration of ethanol. The physicochemical properties of the date by-product (T & L) used to produce the bioethanol at URERMS laboratory are summarized in Table 1. The moisture containing MC% in the fresh date was determined as the difference between the fresh mass FM and the dry mass of date DM at 105°C, until a constant mass was attained. This was done with a digital balance model SKU: US-TRADER-PRO UPC: 878285001193 and the

following formula (Siddiq et al., 2013):

$$MC(\%) = \frac{FM - DM}{FM} \times 100 \tag{2}$$

Based on the water content, dates are classified as soft if MC > 30%, dry if MC < 10 and semisoft or semidry if 10% < MC < 30%. The consistency of dates was determined as the ratio of total sugar/water content (Reynes et al., 1994). Dates with consistency up to 3.5 are classified as dry, those between 2 and 3.5 are considered as semisoft or semidry, and those with ratio less than 2 are soft dates

**Table 1.** Physicochemical characteristics of dates studied.

Characteristics	Teggaza	Lebgheh
Color	Brown Reddish	Yalow
Total date mass (g)	6.05	6.2
Seed mass/pulpy mass (%)	15.9	19.46
Date large/date length (%)	55.93	45.65
Pulpy mass/date mass (%)	86.2	83.71
Seed mass /date mass (%)	13.79	16.29
pH	5.44	5.7
Water content (%)	7.46	9.4
Dried substance (%)	92.54	89.6
Cinder rate (%)	3.29	4.38
Organic substance (%)	96.71	95.65
Reduced sugars rate (%)	41.23	41.52
Protein rate (%)	2.19	1.75
Azote rate (%)	0.35	0.28
Consistency	2.3	1.1
Refractive index	1.3409	1.34

(Lakkana et al., 2009).

After 72 h of alcoholic fermentation (Figure 2b), the substrate juice was used to filter the bioethanol (Figure 2c). At the beginning of the distillation process, the degree of alcohol is measured every 30 min, and once the process is slowed, the alcohol is recorded every one hour. The distillation process is stopped when the concentration of the alcohol became very feeble. The distillation temperature was kept at 78°C.

The deposit after distillation (Figure 2c) is composed of a cocotte of 30 L capacity, built in stain steel; its cover is made of a manometer, detent-valve, and a vertical colon tube of 3.5 cm diameter and 1.5 m height built in cooper. The cocotte is rumped at 75% of its capacity with date substrate juice. The liquid mixture is evaporated at 78°C by heating the cocotte at the bottom and the vapor crosses the distillation colon by density gradient. The ethanol vapor is the condensed one traversing the sloped tube retriggered to accelerate the ethanol condensation process. The distillate produced was recuperated in a bottle at the end of the cooling system and rectified in order to increase the alcoholic degree.

## RESULTS AND DISCUSSION

*S. cerevisiae* yeast has an optional anaerobic respiration in the fermentation process. In anaerobic phase, glucose was converted to ethanol by fermentation effect. Firstly, the process is active particularly between 24 and 55 h, where the yeast population reaches 37,719 and 35,088 cells/ $\mu$ L. The ethanol produced increased during the last 40 h of the process and an important degradation of the sugar is observed after 72 h (Figure 3). The density of the date juice (Figure 4) decreased considerably during the fermentation process from 1.07 to 0.99 g/cm<sup>3</sup>, due to the conversion of sugar into alcohol and the loss of mass under CO<sub>2</sub> form. Also, the diminution of the protein rate in the date juice (Figure 5) represents

an additional azotic source for the yeast to grow. In addition, azote has an important role in alcohol transformation; it ensures the transport of sugars to the external of the biologic cells to generate the fermentation process. The typical period of the fermentation process of the date juice varied between 48 and 72 h under similar conditions. The glucose is not consumed entirely due to the cessation of yeast growth caused by accumulation of toxic substances into the date juice, particularly the octane and decane in the date juice (Benziouches, 2011).

The total solvable solids in the date juice (Figure 6) were measured by a handheld refractometer for viniculture, using refractometer, model Atago NAR-3T (°Bx). This corresponds approximately to the total sugar concentration in (g/L) (DuBois et al., 1956). The continuous diminution of the refractive index (Figure 7) indicated the augmentation of the light speed through the date caused by the reduction of the date density. The concentration of the ethanol in the alcohol produced (Figure 8) is performed using liquid chromatography (Benziouches, 2011). After distillation of the date juice, a significant specific production of the bioethanol reached 180, and 200.5 mL/kg of dates at 90°C was obtained, representing bioconversion efficiencies of 23.57 and 26.2% for both variety of dates (T & L) respectively (Ahmed et al., 2016). Finally, the vibration sign of the biofuel produced (Figure 9) is identified using Infra-Red Spectra. It showed different vibrations bands characterized by the following wave numbers:

3339.08, 2974.29, 2888.12 and 1380.91/1380.5, 1087.06/1087.18, 1044.53/1044.66 cm<sup>-1</sup>, corresponding

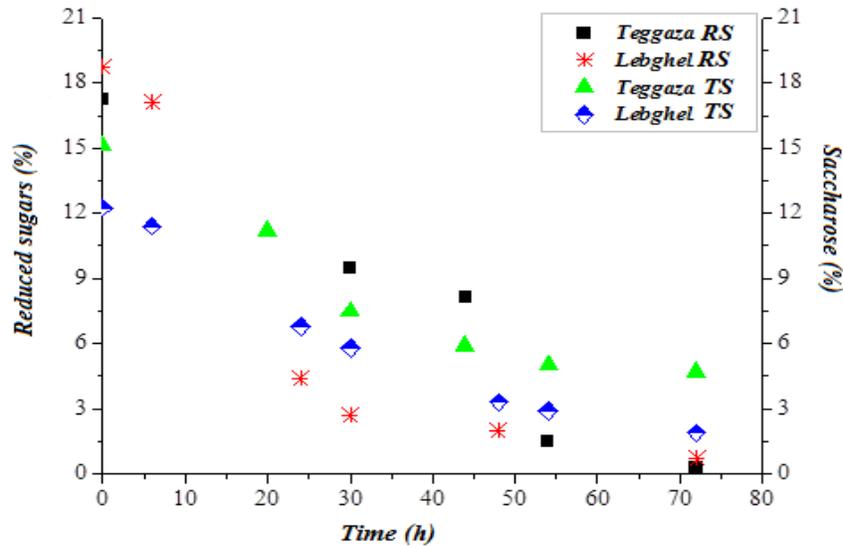


Figure 3. Sugars consumed during the fermentation process.

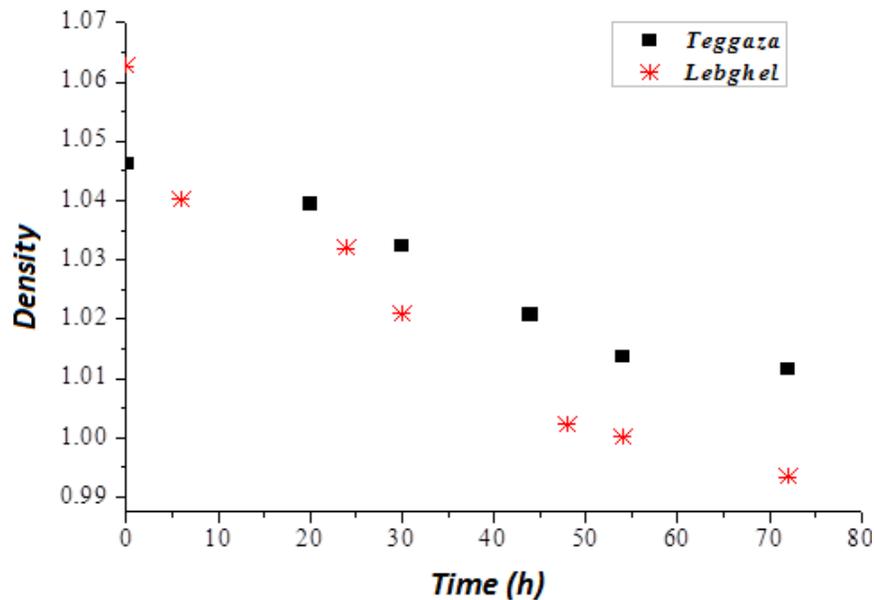


Figure 4. Density of date's juice during the fermentation.

to the molecule groups C-H, O-H and C-O for the two varieties (T & L) respectively. This is compared to the results reported by Khaled and Segni (2014), Ghanim (2013) and Chniti et al., (2014), where 1 kg of date produces 300 to 350 mL of ethanol at 95°. The production obtained in the present study appeared acceptable since the price of 1 L of ethanol at 90° in the worldwide market is about 10€ on web site (<http://www.servilab.fr/>, 2015). The price of 1 kg of date crude is about 0.25€ and when it is transformed into bioethanol its cost is 1€. This

corresponds to an income of about 6€/1L of ethanol produced by fermentation process at 90°, which it is equivalent to a benefit of 60%.

## Conclusion

The current study in laboratory shows that the date by-product of the varieties, T & L, abundant in Algerian Sahara, constitutes an important biomass and a favorable

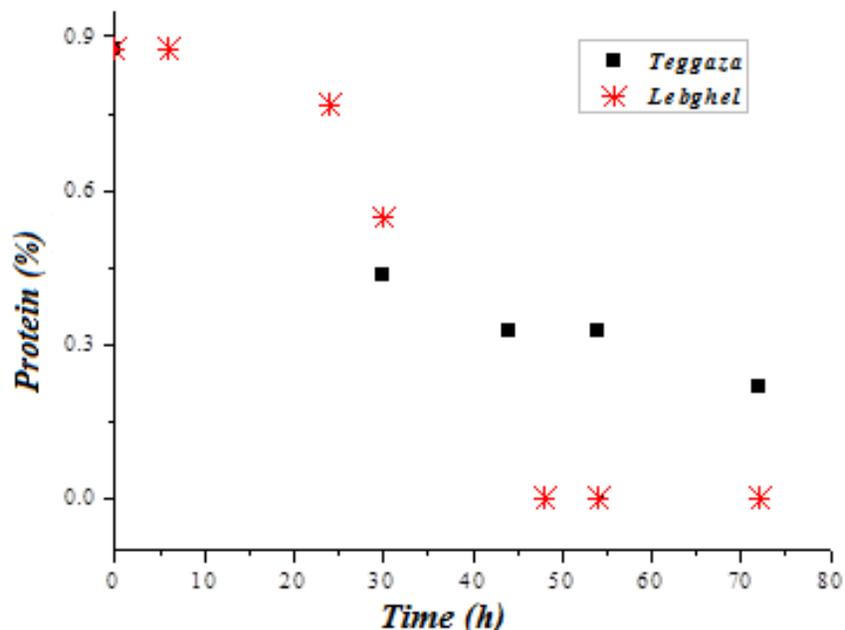


Figure 5. Protein rate during the fermentation process.

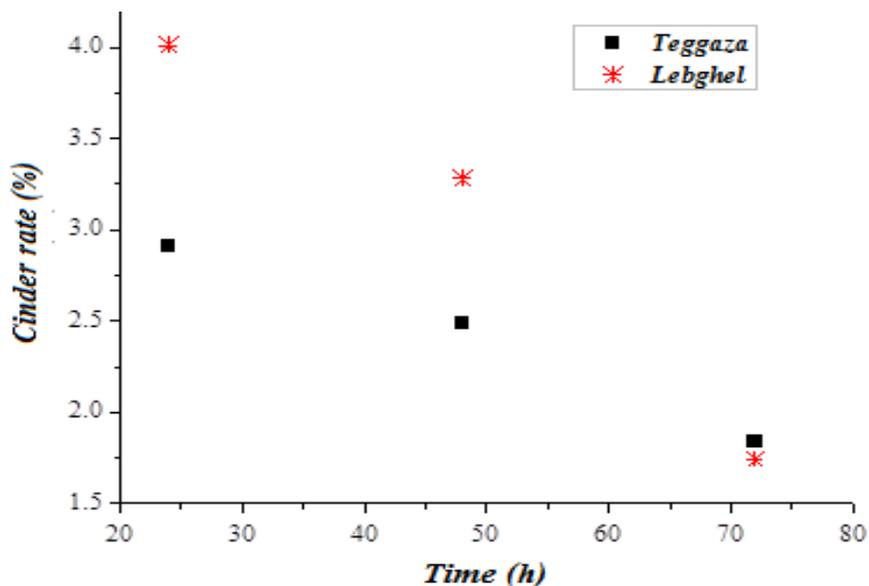


Figure 6. Cinder of dates during the fermentation process.

medium for bakery yeast growth for alcoholic fermentation due to its sugar content, cheap pretreatment and it is an attractive biomass. Dates are used to generate bioethanol at relatively moderate cost, without negative effects on air and water resources. After an alcoholic fermentation, the distilled and rectified date juice generated the highest ethanol concentration of about 88 and 90°; they had an acceptable production of 180 and 200.5 mL or 2.5 and 2.78 mL/kg/h, and assessing scale efficiencies of 23.57

and 26.2% for both varieties studied (T & L) respectively. This is compared to the theoretical ethanol efficiency obtained from a chemical reaction using the same sugar quantity, which is 50%. The present results obtained encourage the continuation of research and development in this clean and sustainable energy field and prospect novel bio-resources, bio-technologies and microorganisms that are more economical and efficient. They are useful because they enhance ethanol productivity, shorten

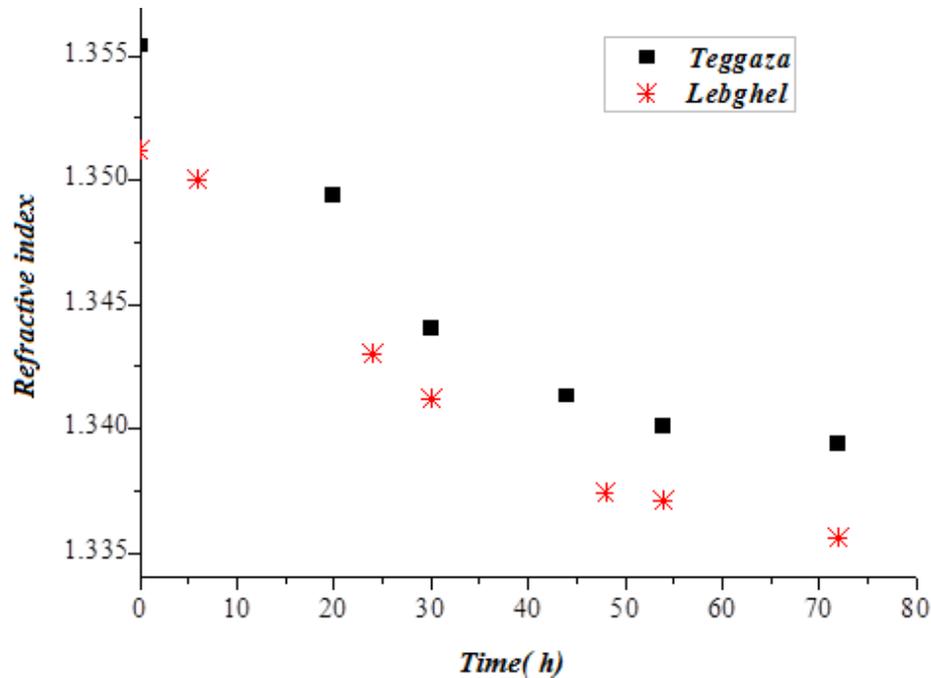


Figure 7. Refractive index during the fermentation process.

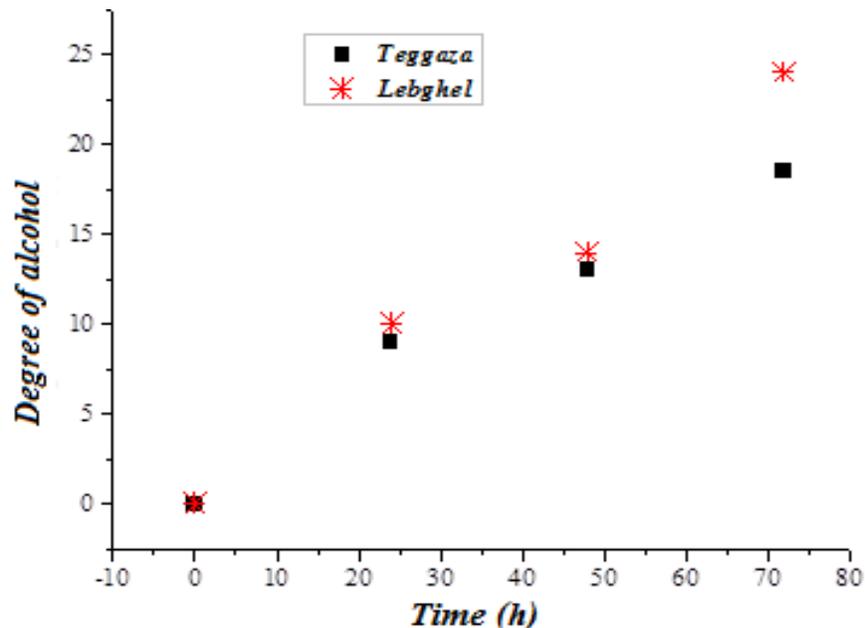


Figure 8. Alcohol concentration during the fermentation process.

ethanol development process, and reduce the energy consumed to lower the final cost of the product. The amount of 250,000 tons/year of date by-product seems favorable for developing a biofuel industry in South Algeria. However, firstly it is necessary to build bioethanol pilot installations to confirm the results obtained in

laboratory before transposing the experience into industrial scales.

#### Conflict of Interests

The authors have not declared any conflict of interests.

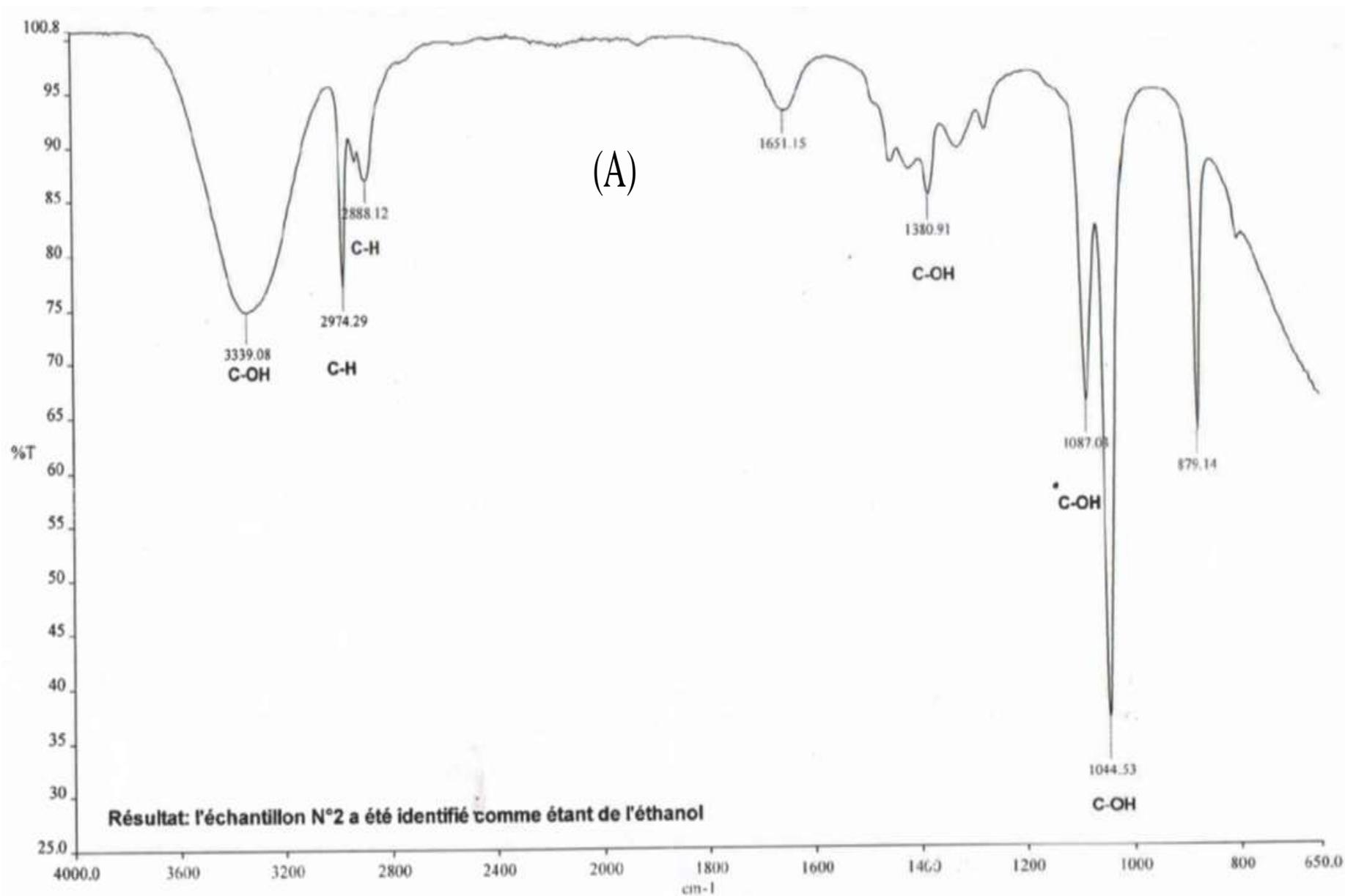


Figure 9. Infra-Red Spectra of the bioethanol produced. (A) Teggaza; (B) Lebghel.

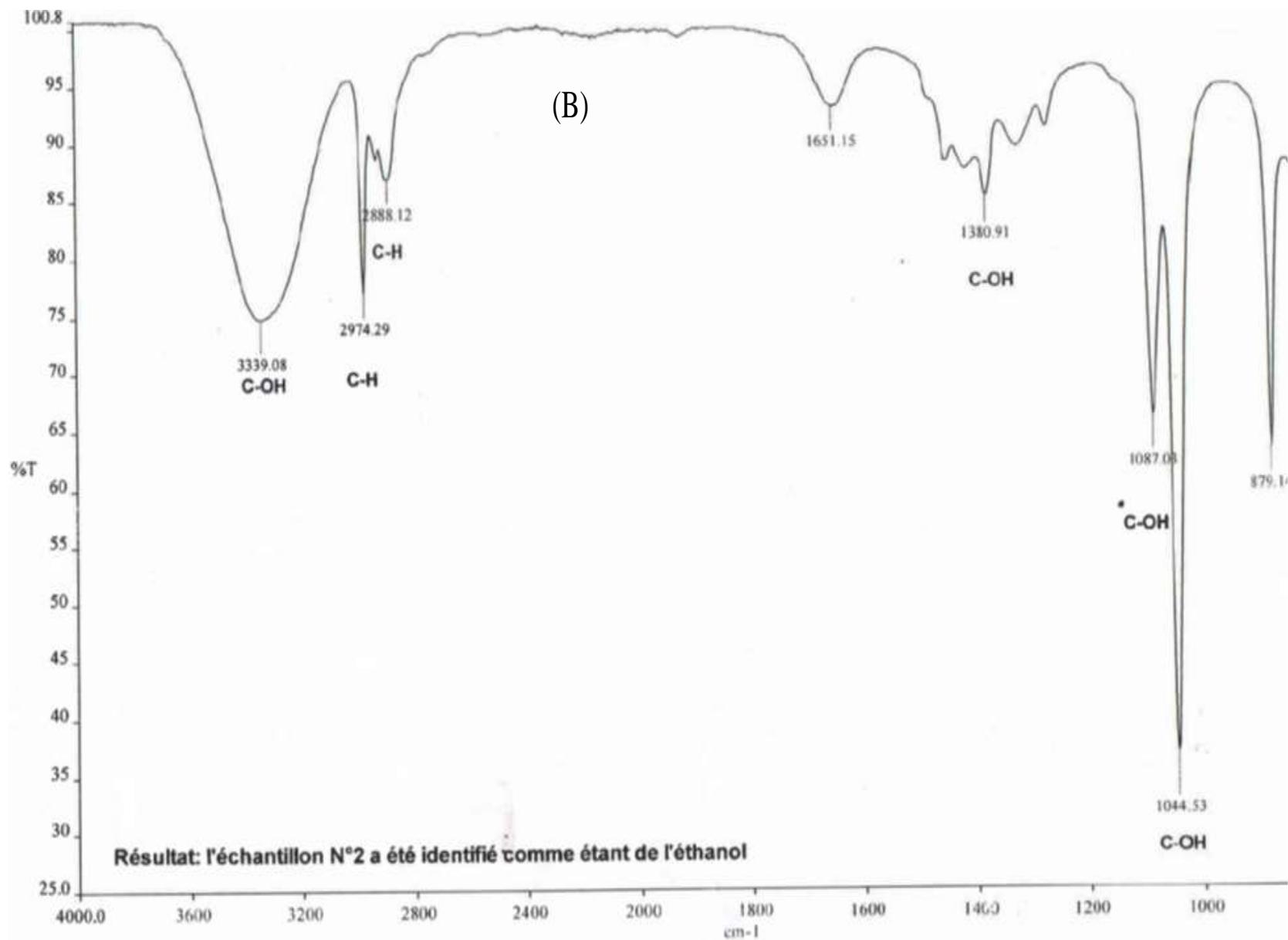


Figure 9. Contd.

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