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Selection of Date's Microflora with high Alcoholic Fermentation Potential

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بسم الله الرحمن الرحيم

﴿ وَمِن ثَمَرَٰتِ ٱلنَّخِيلِ وَٱلۡأَعۡنَٰبِ تَتَّخِذُونَ مِنۡهُ سَكَرٗا وَرِزۡقًا حَسَنًاۗ إِنَّ فِي ذَٰلِكَ لَأٓيَةٗ لِّقَوۡمٖ يَعۡقِلُونَ ٦٧ ﴾

سورة النحل /67

صدق الله العظيم

Dedication

I dedicate this modest work to:

My dear mother FATMA, who worked for my success, through her love, support, and precious advice

My dear father LAID, for all the sacrifices made and for all his assistance and his presence in my life

To my dear sister: Fatima

To my dear brothers (Mouhammed, Aissa, Rahim, Fattah and Abdallah).

My dears: Ritadj, Sassabil, Ghaith, Loai

My big family M'hammedi

All my classmates from the promotion 2019/2020 especially Nouha

FATHA (فاتحة)

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I would first like to thank ALLAH, the almighty

for giving me the courage, the
will, the love knowledge and especially patience
to be able to produce this modest work.

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orientations, its qualities of order and efficiency
and for the development of this work.

To all those who have helped me from near or
far in the realization of this memory, I express
thanks

Abstract

The date palm is the main crop of Saharan agriculture, which in addition to date fruit, offers a wide range of agricultural by-products. The current study gives an overview about this tree considering its morphological and physicochemical characteristics, as well as the possibilities of valorizing lignocellulosic biomass namely palms and trunks. Currently, similar agricultural products are being used in biotechnological processes as solution of choice to produce bioenergy alternatives to fossil fuels like bioethanol. Through the study we present two studies using fermented palms and common dates to produce ethanol. The first work involves anaerobic fermentation using *Saccharomyces cerevisiae* and *Zygosaccharomyces rouxii* the best records were obtained with Zygosaccharomyces rouxii that showed an osmotolerant ability and YEtOH / S yield around 38%, compared to that of S. cerevisiae, cultured on 17.4 °Brix date broth.

Keywords: Date palm, lignocellulosic biomass, bioethanol, valorization

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ABBREVIATIONS LIST

°Bx	Brix degree
°C	Celsius degree
CBH	Cellobiohydrolase
CBM	Cellulose Binding Module
Cm	Centimetre
DNA	Deoxyribonucleic Acid
DOE	Department of Energy
EG	Endoglucanase
FAO	Food and Agriculture Organization of the United Nations
HVO	Hydrotreated Vegetable Oil
IEA	International Energy Agency
mM	microMole
OEMs	Original Equipment Manufacturers
PCL	Polycaprolactone
PH	Hydrogene Potentiel
UNDP	United Nations Development Programme

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2- Comparison of two procedure of bioethanol production

The date palm (***Phoenix dactylifera L***.) is considered the noble tree of the desert regions characterized by hot and dry climate. Because of its nutritional, ecological, social and economic utilities, the date palm is the most common fruit tree appreciated by the people of the oasis.

The number of date palms nationwide is around 11 million, with an annual date production estimated around one million tones **(Ministry of Agriculture, 2017).**

Date palm lignocellulosic biomass represented by dried palms and trunks are usually considered as a waste that serve as an animal feed or a raw material for some basic use like in construction and artisanal activities. However, a few incomes are generated from such utilisation.

Comparing to the other biomass sources like sugarcane and corn, the phoenicical lignocellulosic biomass is an interesting source for producing valuable products at the industrial level like bioethanol. This is the second generation form of biomass widely available and which requires less treatment process as saccharification and fermentation.

This work comprises three parts:

- A first part relating to the bibliographic study comprising two chapters including; Generalities around date palms than form of biomass valorization. The second chapter tackles the subject of enzymes required for biomass hydrolysis (cellulase, pectinase and lignase), and a section dedicated to industrial production of each enzyme. Than a shift is made on the industrial bioconversion of biomass to bioethanol including the several stages of bioconversions.
- The last part is a comparison between two approaches of ethanol production from date waste and date palm biomass. The techniques are compared in matter of raw material treatment, and ethanol yield.
- Finally a general conclusion sums up the perspectives of this work.

Chapter 01

Date Palm and Biomass Valorization

I-Generalities

1- Phoenix dactylifera:

The date palm is called scientifically *Phoenix dactylifera***,** it is a species among other species belonging to the genus of Phoenix. **(fig 01)**

Botanically it is a flowering plant within the palm family *Arecaceae*. Which is essentially cultivated for its sweet fruits known as "date". The true origin of the tree is uncertain, but probably it was first cultivated in the region situated between Egypt and Mesopotamia. Nowadays the date palm is widely cultivated across the Middle East, North Africa, South Asia, and it has been adapted in many tropical and subtropical areas worldwide **(Munier, 1973).**

The name *Phoenix dactylifera L*., might be seemingly originated from "phoenix"; the Phoenician word which means palm. In the other hand, the noun "*dactylifera*" is derived from the Greek word "daktulos" which means " finger", due to the similarity between the mature fruit shape and the finger form **(Linné, 1734).**

Another interpretation of the word *Phoenix* is referring to the legendary Egyptian bird, "Phoenix" **(Pliny, 1489; Van Zyl, 1983).**

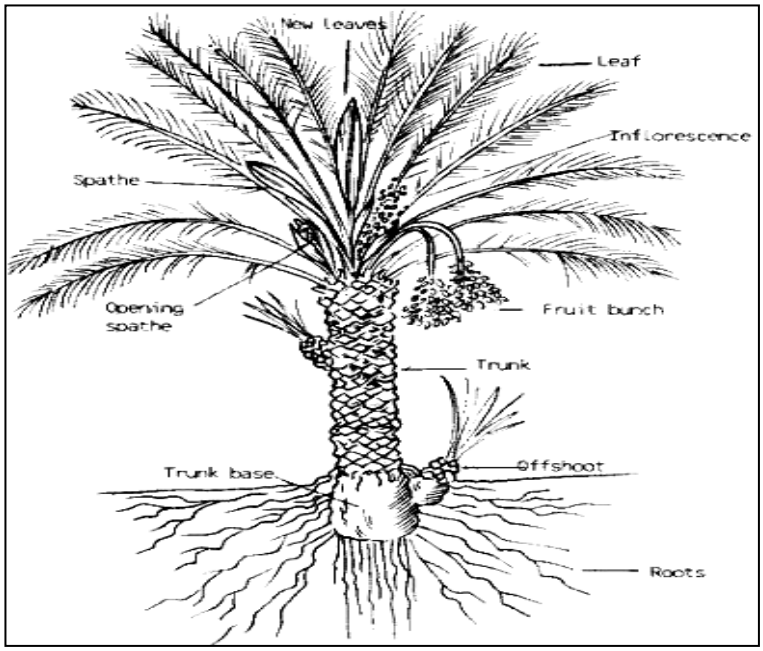


Figure 01: *Phoenix dactylifera L* **(Oihabi; 1991)**

2- Systematic classification

The date palm (*P dactylifera) is b*elonging to the Angiosperms-Monocotyledons Palmaceae *that constitutes* a family of about 200 genera and 1500 species **(Dowson, 1982).**

Phoenix genera contains a dozen species, all of them are native to the tropical or subtropical regions of Africa or Southern Asia, including *Phoenix dactylifera L.* **(Munier, 1973).** According to Dransfield and Uhl, (1986) *P dactylifera* can be classified as following:

- class:	Spadiciflora
- Order:	Palmea
- Family:	Palmaceae
- Sub-family:	Coryphyoideae
- Tribe:	Phoeniceae
- Genus:	Phoenix
- Species:	*Dactylifera L.*

3- Historical background of date palm cultivation

The exact time for cultivation of the date palm *(Phoenix dactylifera* L.) is so far lost in Antiquity. However, many sources indicate that the date palm was cultivated as early as 4000 B.C. Some evidence have been discovered in this regard, like the date palm trunk used in the construction of the temple of the God's moon legend in Southern Iraq - Mesopotamia **(Popenoe, 1913; 1973).**

Further proofs were discovered in Egypt's Nile Valley where date palm was used as a symbol of the Egyptian year in hieroglyphics and its branch as a symbol for a month. However, the culture of date palm didn't become important in Egypt until somewhat later than that of Iraq about 3000 - 2000 B.C **(Danthine, 1937)**,.

The above is confirmed by history, and corroborated by the archaeological research into ancient historical remains of the Sumerians, Akadians and Babylonians. Houses of these very ancient people were roofed with palm tree trunks and fronds. The uses of date for medicinal purposes, in addition to its food value, were also documented **(Bousdira et *al*., 2003).**

Date palm is probably the most ancient cultivated tree in the world. It could be safely assumed that the reason for mentioning dates and date palms in the Jewish, Christian, and Islamic religions was due mainly to the influence of the Prophet Abraham, who was born and raised in the old city of Ur where date palms were grown. Ibrahim's love of the date and date palm left a lasting influence on these religions **(Dowson, 1982).**

4- Botanical description

4-1- Vegetative organs

4-1-1 Root system

Being a monocotyledon, date palm has no tap root. Its root system is fasciculate and roots are fibrous, similar to a maize plant. Secondary roots appear on the primary root which develop directly from the seed. These secondary roots produce lateral roots (tertiary roots and so on) of the same type with approximately the same diameter throughout their length. **(Zaid, A.and P. F. de Wet. 2002.)**

All date palm roots present pneumatics, which are respiratory organs. Roots are found as far as 25 m from the palm and deeper than 6 m, but 85 percent of the roots are distributed in the zone of 2 m deep and 2 m on both lateral sides in a deep loamy soil **(Munier, 1973).** It is worth mentioning that date roots can withstand wet soil for many months, but if such conditions spread over longer periods, they become harmful to the health of the roots and to fruit production **(Bakkaye, 2006)**. The next figure shows a date palm's root system. **(Fig 02)**

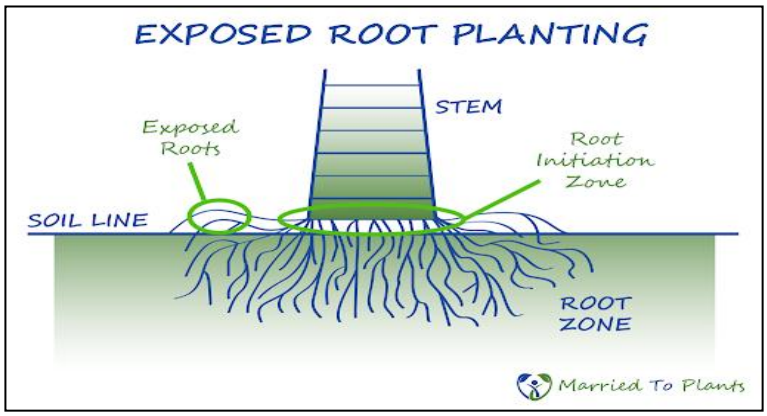


Figure 02: Date palm root system (Married to plants; 2016).

It has been established that the root form and their abundance illustrate clearly the properties of the date palm. While, the deep presence of primary roots permits to the date palm to absorb water from underground moisture and consequently, unlike most fruit palms, it can highly resist to water stress and drought conditions. **(Belguedj, M. 2002)**

Date palm root development and distribution depends on soil characteristics, type of culture, depth of the underground water and variety.

4-1-2 Trunk

The date palm trunk, also known as stem or stipe is cylindrical and vertical, of the same girth all the way to the top. The girth stops increasing after the full growth of the canopy of fronds. It has a brown color, lignified but not ramified. Its average circumference is about 1 to 1.10 m.

The trunk is formed of tough, fibrous vascular bundles contained together in a matrix of cellular tissue, which is more lignified next to the outer part of the trunk. The date palm is like the other monocotyledon, without any cambium layer. **(Fig 03)**

The trunk is covered for several years with the bases of the old dry fronds, making it rough, but with age these bases weather and the trunk becomes smoother with visible cicatrices of these bases. Vertical growth of date palm is ensured by its terminal bud, called phyllophor and its height could reach 20 metres. **(E. Small; 2009).**

Figure 03: Date palm trunk (V.Voinakh; 2003)

Horizontal or lateral growth is ensured by an extra fascicular cambium which soon disappears, and which results in a constant and uniform trunk width during the palm's entire life. However, the terminal bud could experience an abnormal growth caused by a nutritional deficiency, which leads to shrinkage of the trunk. This stage is mainly caused by drought conditions. ***(A. Zaid and P.F. de Wet;2002)***

Sometimes date palms show a branching phenomenon which was studied by **Zaid (1987)** and found to be attributed to several causes. The author's findings are summarized as follows:

- Branching in date palm is a result of either dichotomy, axillary bud development, polyembryony or attack by a disease.

- Branched date palms are fertile and can produce as much fruit as a single headed palm.

- There is a need of an analysis of the vascular system of branched date palm by cinematographic techniques. This anatomical study is necessary to show the continuity of growth from the single to the divided state of the shoot.

- It is necessary to study in vitro the regenerating capacity of divided portions of the apical meristem and axillary buds of these specimens in the hope of establishing a rapid mass propagation technique for date palm.

4-1-3 Leaves

Depending on variety, age of a palm and environmental conditions, leaves of a date palm are 3 to 6 m long (4 m average) and have a normal life of 3 to 7 years **(Fig 04)**. The greatest width of the frond midrib attains 0.5 m, but elsewhere it is only half this size and rapidly narrows from the base upwards. **(Nixon and Wedding; 1956).**

The frond midrib or petiole is relatively triangular in cross section with two lateral angles and one dorsal. It is bare of spines for a short distance but full of spines on both sides thereafter. Intermediate zones have spine-like leaflets, also called leaflet-like spines **(Bouguedoura ; 1982).**

Figure 04: Date palm leaves **(Messaid; 2007)**

4-1-4 Fibre, Spines and Leaflets

As well described by **(Dowson; 1982),** the base of the frond is a sheath encircling the palm. This sheath consists of white connective tissue ramified by vascular bundles. As the frond grows upwards, the connective tissue largely disappears leaving the dried, and now brown, vascular bundles as a band of tough, rough fibre attached to the lateral edges of the lower part of the midribs of the fronds and ensheathing the trunk. Varieties differ in the height to which the fibre grows up the central column of unopened fronds, and in the texture of the fibre and also somewhat in color.

Spines, also called thorns, vary from a few cm to 24 cm in length and from a few mm to 1 cm in thickness. They are differentially arranged on the two outer edges of the fronds while their number varies from 10 to about 60. Spines can be single, in groups of two, or in groups of three. **(Randall J. Evans; 1995)**

Leaflets or Pinnae are between 120 to 240 per frond, entirely lanceolate, folded longitudinally and oblically attached to the petiole. Their length ranges from 15 to over 100 cm and in width from 1 to 6.3 cm. Their arrangement depends on variety and could be in groups of 1, 2, 3, 4, or 5 pinnae. **(Zaid. A and P.F. de Wet;2002*)***

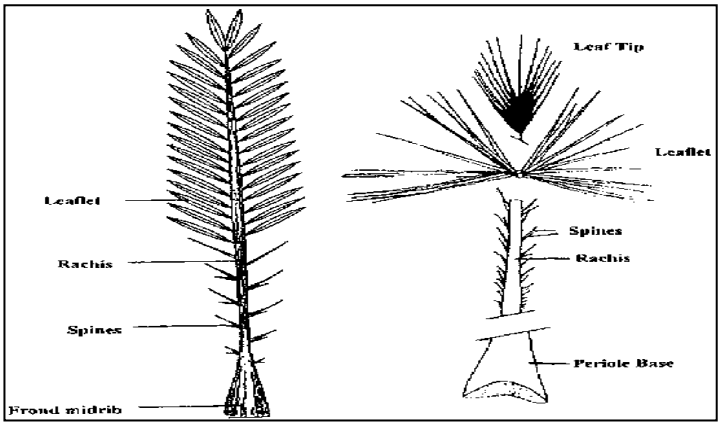


Figure 05: Date palm leaf characteristics **(Munier ; 1973)**

4-1-5 Flowers:

Date palm is a dioecious plant where pistillate and staminate flowers are born on separate plants. Male and female flowers are arranged in strands that attach to a rachis forming an inflorescence called spadix **(fig 06)**. A bract, called spathe, enclosing the immature inflorescence, splits longitudinally at anthesis, which allows for the pollination of mature male and female flowers. **(Uhl and Dransfield; 1987).**

a) Male flower (Wiki Loves Earth ; 2013) **b) Female flower (k. Hess; 2018)**

Figure 06: date palm flowers

4-1-6 Fruits

Called dates, develop from one fertilized ovule forming one carpel, while the other two ovules are aborted but remain visible at the fruit calyx. If no fertilization occurs, three or more carpels develop simultaneously **(Zaid and de Wet 2002)**.

The date fruit develops on the flowering strands and is a berry characterized by a membranous endocarp surrounding a seed. Large variations exist in the shape, size, color, and chemical composition of date fruit, depending largely on varietal differences but also on climate, soil, and growing conditions **(fig 07)**. Similarly, date seeds vary in size and shape, but they are generally ventrally grooved, oblong, and range from 5 to 15 mm with an embryo born in the middle of the seed that is surrounded by the endosperm. **(Al-Yahyai and Kharusi 2011)**

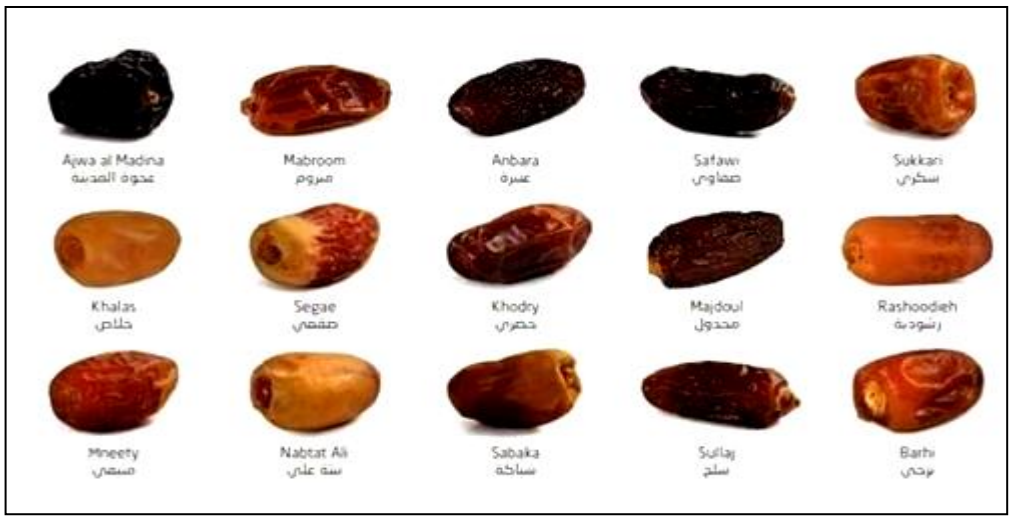


Figure 07: Date palm fruit (Trade India; 2017)

5- Development of date Palm culture in the south of Algeria

Date palm *(Phoenix dactylifera L.)* is the main crop of both traditional and industrial agriculture in the south of Algeria. A small economy in these regions is taking place basing on the use of its fruit by-products such as paste, flour, syrup, vinegar, and confectionery. This provides some income for the oasis inhabitants. All parts of the date palm are used, including the leaves and trunks which are used for house construction.

The fruit is consumed in fresh and dry forms, and sometimes processed to produce syrup or fermented to produce wine and vinegar (**Mimouni and Siboukeur, 2011)**.

However the other by-products like leaflets and seeds are destined to animal feeding. This final usage underestimates the high value of the date palm by-products which can serve as a potential resource for added-value industrial activities. **(Ould El Hadj et al, 2012).**

II- Valorization of agricultural biomass in microbial biotechnology

There is an urgent need to pass from polluting industries to more eco-friendly products in and hence succeeding in the transition from refinery fossil fuel substrates to a biomass-based biorefinery. The attempt to changing the raw materials from hydrocarbons to biological molecules would radically alter the technological foundation of the industry. Although biomass is widely available in large amounts, the available volumes of selected sources are still small compared to coal or crude oil. First and foremost, the experts are seeking for technologies that enable the economical and green processing of complex terrestrial and aquatic biomass and waste. **(Faruk et al., 2012)**

Importantly, date palm biomasses (leaves, trunks,.) are completely biodegradable, renewable, sustainable and recyclable materials, unlike the synthetic composites. Hence, date palms don't contain any toxic elements, which make it environmentally friendly and relatively safe for human health during processing and life cycle. **(Paul et al, 2015).**

By far date fruits (*Phoenix dactylifra. L.*) are one of great importance in human nutrition owing to their rich content of essential nutrients, which include carbohydrates, salts, and minerals, dietary fiber, vitamins, fatty acids, amino acids and protein **(Chandrasekaran and Bahkali, 2013).** Carbohydrates are the major chemical elements of the date, mainly including glucose, fructose and small amounts of cellulose and starch **(Tang et al., 2014).**

The high nutritive and high sugar content of date palm wastes are very good sources for microbial fermentation industry and a promising source of other renewable energy industries because of its abundant organic matters such as vigorous amounts of amino acids, free organic acids, and carbohydrates**(Gupta and Kushwaha, 2011). (Ahmed et al. 2015)**.

The Date palm trees is widely spread over vast geographical areas reaching a total number of more than 150 million trees in more than 30 different countries **(Fig 08)**. More than 100 million date palm trees are counted in Arab countries **(Hassan, 2003)**.

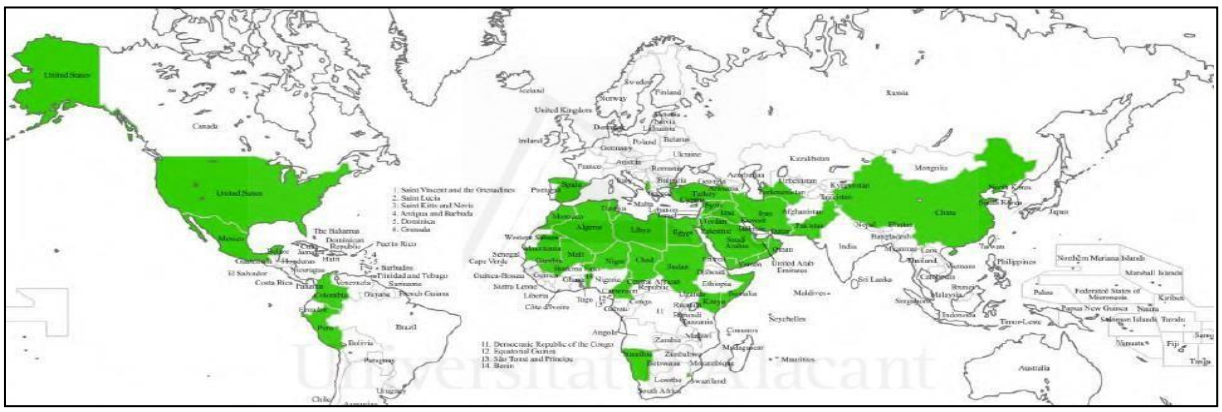


Figure 08: Geographical distribution of the date palm in the world **(Sakin Abdrabo, 2013)**

These trees produce huge quantities of agricultural waste obtained from the seasonal pruning process; they are estimated to be 94.71 thousand tons per year in Tunisia. As an example of the contribution of date palm organs, **(Chandrasekaran and Bahkali 2013)** has measured that each date tree produces about 20 kg of dry leaves annually, unfortunately this production is not valued. **(Mohamed, 2014)**

II-1 Biomass, problems and solutions

The use of bio-based renewable resources holds great potential value for industries in many sectors, including energy, organic chemicals, polymers, fabrics and health-care products. In general, a bio-based economy offers many benefits and opportunities:

• New areas of economic growth and development for the many regions that have plentiful biomass resources.

• Creation of new innovative business sectors and entrepreneurial skills.

• improved energy security, by reducing dependence on non-renewable resources .

• Enhance economic and environmental linkages between the agricultural sector and a more prosperous and sustainable industrial sector.

• Reduction of greenhouse gas emissions.

• Improved health by reducing exposure to harmful substances through substitution of natural bio-based materials for chemical and synthetic materials.

• Job creation and rural development.

At the same time, many issues need to be addressed in order to avoid negative impacts and facilitate a smoother transition to a bio-based economy, such as:

• How to manage competition of land used as raw material for industry with other land uses, especially in relation to food and animal feed.

• Bioethical issues, where genetically modified crops are used or proposed.

• Potential loss of biodiversity through large-scale and/or contract farming.

• Equitable treatment of farmers in their interaction with bio-based companies.

• Expanded research and development efforts, including potential integration of fossil fuel and bio-based approaches.

•Improving transportation and delivery systems, e.g. for raw materials, delivery to/from processing facilities, and final product distribution and use.

Biomass that is produced in tropical and sub-tropical climates has an average productivity that is over five times higher than that of biomass grown in the temperate regions of Europe and North America **(Bassam 1998).**

Since developing countries are located predominantly in the warmer climates and lower latitudes, they have a considerable comparative advantage.

As the role of biomass for energy and industry has become more economically competitive, there is increasing concern as to the impact on food security, especially for countries that are net food importers or those that experience droughts and other disruptions in the food supply.****

There is potential synergy between food and non-food uses, especially as new agro-industrial biotechnology methods are deployed. Where there are potential conflicts, it is crucial that bio-based industrial development is accompanied by investment in greater agricultural productivity and/or due consideration for distributional issues that arise when the agricultural sector and industrial sector compete for the same raw materials.

II-2 Biomass generation rate in phoenicical region

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 "*Phoenicia*" had an important progress in date palms cultivars **(Table 01):** it has 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. **(Ahmed Boulal et al. 2016).**

Algeria produces more than 400 different varieties of dates with an annual production of over 400,000 tons **(http://faostat3.fao.org/home/index.html)**.

Some studies are undergone for developing a real valuable products from the date palm by-products in this region. The raw material include, date of low quality and the palm as well as the trunk. The lignocellulosic nature of the palms makes them biodegradable by enzymes degradation of lignin and cellulose.

Table 01: Varietal inventory (cultivar) in the three phoenicultural regions of Algeria

(Bouguedoura et al., 2010)

Région		Nombre de cultivars	Cultivars les plus courant
Ouest	Atlas	70	*Ghars, 'Asyan, Feggus*
	Tidikelt	60	Tgazza, Taqerbuch, Cheddakh, Aggaz, Ghars
	Saoura	80	Feggus, Hartan, Cherka, Hmira, Deglet Talmine
	Gourara	230	Hmira, Tinnaser, Taqerbuch
	Touat	190	Tgazza, Aghamu, Taqerbuch
Centre	El- Menia	70	Timjuhart, Ghars, Timedwel
	M'Zab	140	Azerza, Ghars, Deglet Nour, Taddela
Est	Ouargla	70	Ghars, Deglet Nour, Degla Beida
	Oued Righ	130	Deglet Nour, Ghars, Degla Beida
	Souf	70	Deglet Nour, Ghars, Degla Beida, Mich Degla
	Zibans	140	Deglet Nour, Ghars, Degla Beida, Mich Degla
	Aures	220	*Buzrur, 'Alig, Buhles, Mich* Degla
	Tassili	180	Tanghimen, Tabanist, Khadaji

II-3 Lignocellulosic biomass

The Lignocelluose, represents the major component of the biomass, it is a renewable organic material found in plants. It is made up of mostly hemicelluloses, cellulose, lignin and small amount of protein and pectin depending on the source**.).** Furthermore, The percentage of lignin in lignocellulosic biomass is around 10–35% and is the second most abundant natural organic polymer. **(Xu, R., Zhang, K., Liu, P., Han, H., Zhao, S., Kakade, A., Khan, A., Du, D., Li, X, 2018.)**

The lignocellulosic wastes are huge generated from various agro-based industries including pulp and paper mill (effluent 150–200m^3/ton and solid 160–450kg/ton), sugarcane molasses-based distilleries (effluent 15 lit/1 lit alcohol production and 7.5 million tons/year), agricultural waste (200 billion tons/year), and food industry (1.3 billion ton/year). **(Chandra R., Abhishek A., Sankhwar M, 2016).**

Increased generation of lignocellulosic wastes from both the industrial and agricultural sectors have continued to pose environmental challenge globally due to, in part, poor waste management. However, the prospect of the valorization of lignocellulosic wastes for value-added products shall suffice as effective waste management strategies. article.

Due to its complexity, the degradation of lignocellulosic waste is a great challenge for sustainable development. (**Ayodeji O. Falade and al.;**)

Chapter 02

Industrial bioconversion of biomass

III- Enzymes used in bioconversion of agricultural biomass

During biomass conversion, the degradation of the raw material is accomplished by a concerted action of several enzymes therefore; there is a renewal of interest in the bioconversion of lignocellulosic biomass using enzymes. Invertase and amylase were the first enzymes to be isolated and produced at an industrial scale. Since then many enzymes are becoming a core tool in industrial fermentation processes. Furthermore, cellulases and pectinases find other usage in food industry including fruit juice clarification. **(Vitolo M, 2019)**

The performance of enzymes used in biomass conversion relies on the properties of the enzyme itself. The most important features are the stability, the amount of product or substrate inhibition, synergism with other enzymes, productive binding to the substrate, solubility of the substrate as well as the composition of the biomass. **(A.K.Patel et al; 2017).**

According to many authors the ideal enzymatic preparation to be used in the biomass hydrolysis must be:

1. highly efficient in the degradation and conversion of the used biomass,
2. should hydrolyze completely the biomass,
3. Functions in a wide value of pH (preferably in mildly acidic conditions),
4. Resistant to the stressful environment of the process,
5. Should be constantly available, economical and cost-effective.

Regardless of all these criteria, the choice of the suitable enzyme for biomass conversion is more reliable on the biomass characteristics rather than on the efficiency of the used enzyme **(Singhania et al, (2010).**

Moreover, the biomass hydrolysis of a mixed enzymes preparation depends mainly on the properties of each enzyme and its ratio within the multi-enzyme preparation.

Enzymes can be obtained from microorganisms and many of them are easily handled to have high specific enzyme production ability.

A strain can produce different types of enzymes, enabling the producer to operate a flexible fermentation plant to either produce different enzymes or direct the production for a

particular enzyme. This helps the biotechnological plant to cope with market fluctuations regarding some enzymes. Moreover, by using microorganisms as enzyme sources, the producer can control all phases (from the source to the final product) of the enzyme production. **(Michele Vitolo**; **2010).**

Protein engineering is an active area of research and involves attempts to create new enzymes with novel properties, either through rational design or in vitro evolution. These efforts have begun to be successful, and a few enzymes have now been designed "from scratch" to catalyze reactions that do not occur in nature **(Sauer J et al; 2000)**. In fact there is many uses of enzyme in a wide variety of industries and applications:

- **In the starch industry**, amylases, amyloglucosidases, and glucoamylases convert starch into glucose and various syrups. Glucose isomerase converts glucose into fructose in production of high-fructose syrups from starchy materials.
- **In the paper industry**, amylases, xylanases, cellulases, and ligninases are used to degrade starch to lower viscosity, aiding sizing and coating paper.
- **In the production of biological detergents**, proteases, produced in an extracellular form from bacteria, are used in pre-soak conditions and direct liquid applications, helping with the removal of protein stains from clothes.
- **In molecular biology**, restriction enzymes, DNA ligase, and polymerases are used to manipulate DNA in genetic engineering, important in pharmacology, agriculture and medicine, and are essential for restriction digestion and the polymerase chain reaction. Molecular biology is also important in forensic science.

III-1 Sources of Enzymes

III-1-1 Microorganism with highly enzyme productivity

Many microorganisms are able to produce enzyme naturally and in industrial level. Enzymes can be obtained from microorganisms and many of them are easily handled to have high specific enzyme production ability.

A strain can produce different types of enzymes, enabling the producer to operate a flexible fermentation plant to either produce different enzymes or direct the production for a particular enzyme. This helps the biotechnological plant to cope with market fluctuations regarding some enzymes.

Moreover, by using microorganisms as enzyme sources, the producer can control all phases (from the source to the final product) of the enzyme production. (Michele Vitolo;). The filamentous fungi are the major source of cellulases and hemicellulases and the mutant strains of *Trichoderma* including *T. reesei, T. viride* and *T. longibrachium* are the best known producers of the enzyme, (**Kabel et al. 2005).**

Cellulases for biomass conversion could be a blend or enzyme cocktail containing endo- and exo-cellulase, xylanase, β-glucosidase **(Fig 09)**, pectinase, etc. which could vary for different biomass on the basis of their composition analyzed and evaluated the potential of several commercial cellulases for biomass conversion. They performed the standard assays for different enzymes as Filter Paper. **(Nieves et al. 2009 and)**

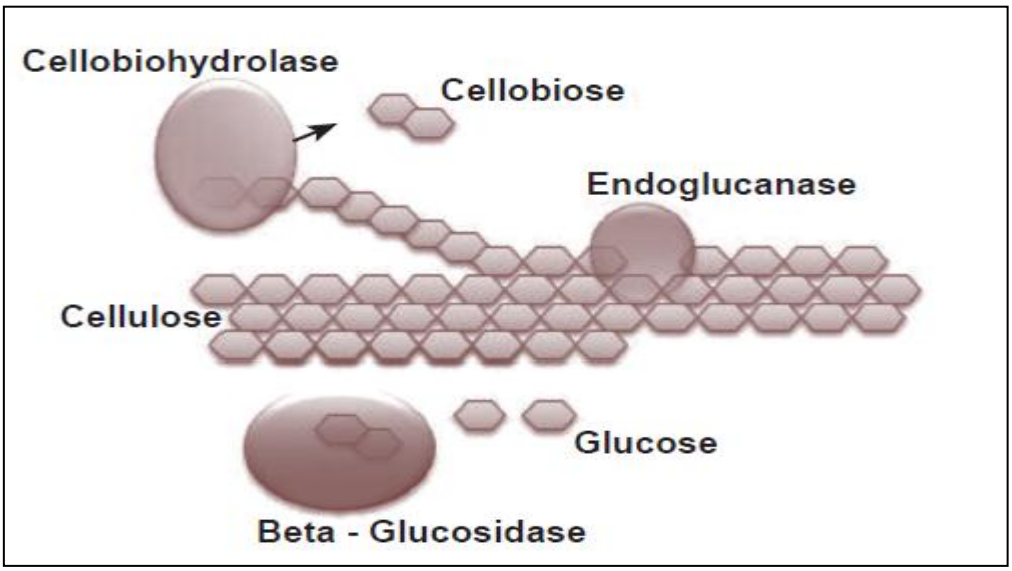


Figure 09: Enzymatic hydrolysis of cellulose schematic diagram showing cellulase synergy **(Anil Kumar Patel et al; 2013).**

III-1-1-1 Cellulase

Microbial cellulases have become the focal biocatalysts due to its wide spread industrial applications. Cellulases are constituted of two separate folding units, called domains. They are structurally and functionally discrete and both forming the cellulases module.**(B. Henrissat, et al ;1998).**

However there is a huge difference between the bacterial and the fungal cellulases, mainly in matter of chemical structure.

- **Fungal cellulases:**

Fungi were long considered more potent in their abilities to degrade cellulose comparing to bacteria and have a much greater capacity for the hydrolysis of cellulose than most bacteria. In addition, fungi have a remarkable capacity for the production of extracellular enzymes facilitating therefore purification of the produced enzymes**. (L. M. J. Carvalho,et al; 2016)**

However, regarding enzymatic system, fungal cellulases are simpler with a catalytic domain (CD) linked to a cellulose binding module (CBM). The latter is associated with a catalytic domain at the N-terminal via a tiny polylinker region.**(Y. H. Percival Zhang, M. E. Himmel, and J. R. Mielenz; 2006)**

- **Bacterial cellulases:**

Bacterial cellulase systems are more complex than fungal enzymes, comprising a multiplicity of activities and usually classified as exo- or endoglucanases **(Bioresource Technology 36, 1991)**

The most advantage of bacteria is their ability to tolerate extreme conditions, which make theme an excellent source for cellulolytic enzymes. Therefore, there is an ongoing research for new bacterial strains from extreme environments; that produce enzymes which are more stable, have higher temperature optima, or have broader or different pH optima **(K.L. Barbosa et al, 2020)**

The **table 02** show us the deferent microorganisms responsible of the production of bacterial and fungal cellulase

Table 02: microorganisms having cellulolytic abilities **(R. C. Kuhad et al; 2011)**

Fungi	Soft rot fungi *Aspergillus niger; A. nidulans; A. oryzae; A. terreus; Fusarium solani; F. oxysporum; Humicola insolens; H. grisea; Melanocarpus albomyces; Penicillium brasilianum; P. occitanis; P. decumbans; Trichoderma reesei; T. longibrachiatum; T. harzianum; Chaetomium cellulyticum; C. thermophilum; Neurospora crassa; P. fumigosum; Thermoascus aurantiacus; Mucor circinelloides; P. janthinellum; Paecilomyces inflatus; P. echinulatum; Trichoderma atroviride* Brown rot fungi *Coniophora puteana; Lanzites trabeum; Poria placenta; Tyromyces palustris; Fomitopsis sp.* White rot fungi *Phanerochaete chrysosporium; Sporotrichum thermophile; Trametes versicolor; Agaricus arvensis; Pleurotus ostreatus; Phlebia gigantea*
Bacteria	Aerobic bacteria *Acinetobacter junii; A. amitratus; Acidothermus cellulolyticus; Anoxybacillus sp.; Bacillus subtilis; B. pumilus; B. amyloliquefaciens; B. licheniformis; B. circulan; B. flexus; Bacteriodes sp.; Cellulomonas biazotea; Cellvibrio gilvus; Eubacterium cellulosolvens; Geobacillus sp.; Microbispora bispora; Paenibacillus curdlanolyticus; Pseudomonas cellulosa; Salinivibrio sp.; Rhodothermus marinus* Anaerobic bacteria *Acetivibrio cellulolyticus; Butyrivibrio fibrisolvens; Clostridium thermocellum; C. cellulolyticum; C. acetobutylium; C. papyrosolvens; Fibrobacter succinogenes; Ruminococcus albus*
Actinomycetes	*Celludomonas fimi; C. bioazotea; C. uda; Streptomyces drozdowiczii; S. lividans; Thermomonospora fusca; T. curvata*

In some bacteria, especially anaerobes, the cellulase system may be produced as, or form, an aggregate. The best characterized system of this type is the cellulosome of C. therrnocelhm. It is a discrete structure, of diameter about 18 nm and Mr of about 2 × 10 c, kDa, comprising at least 15 different polypeptides, many of which have glucanase activity. The cellulosome appears as such in the culture supernatant; it binds to and is active on crystalline cellulose. **(Wu & Demain, 1988).**

The binding appears to be mediated by a polypeptide of M, about 200 kDa which accounts for some 25% of the protein in the cellulosome. it is difficult to disrupt for the purification of its corn. **(Coughlan & Ljungdahl, 1988)**

III-1-1-2 Lignin degradation

Has been extensively studied in wood-rotting organisms, especially white-rot basidiomycetes **(Hatakka, 1994; Leonowicz et al., 1999; Martinez et al., 2004; Wan & Li, 2012),** and most of these studies established white-rot fungi as the most effective "delignifyer" partly as a result of the potent ligninolytic extracellular oxidative enzymes (ligninases) produced **(Glenn, Morgan, Mayfield, Kuwahara, & Gold, 1983; Tien & Kirk, 1983).**

The ligninolytic extracellular oxidative enzymes have been **classified** into :

- phenol oxidases
- heme peroxidases.

Enzymes in the phenol oxidases include laccases (EC 1.10.3.2) while the heme peroxidases include lignin peroxidase (EC 1.11.1.14), manganese peroxidase (EC 1.11.1.13), versatile peroxidase (EC 1.11.1.16), and dyP-type peroxidases (EC 1.11.1.19).

Some accessory enzymes such aryl-alcohol oxidase (EC 1.1.3.7), glyoxal oxidase (EC 1.2.3.5), and glucose 1-oxidase (EC 1.1.3.4) are also implicated in the degradation of lignin and are as which generate the hydrogen peroxide (H_2O_2) required by the peroxidases **(Ander & Marzullo, 1997; Guillen, Martinez, & Martinez, 1992; Kersten & Kirk, 1987).**

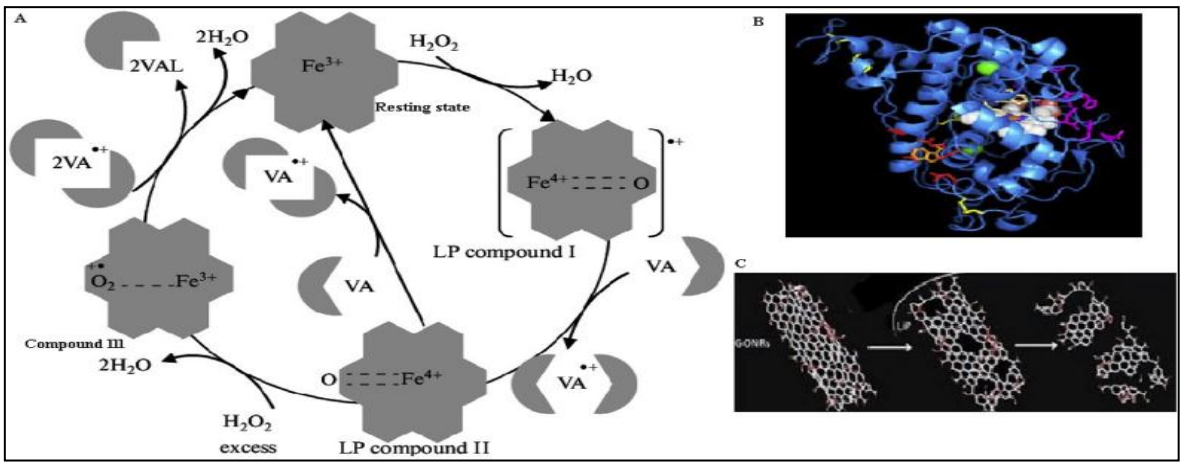
The most used enzyme is lignin peroxidases (EC 1.11.1.14) which is belonging to family oxidoreductase, which degrades lignin and its derivatives in the presence of H2O2. Chemical structure of lignin peroxidases (LiPs) are monomeric glycosylated containing enzyme that has molecular weight (40–68 kDa) with four carbohydrates, 370 water molecules, 343 amino acids residues, two calcium ions, and heme group. **(Choinowski T., Blodig W., Winterhalter K.H., Piontek K,1999.)**

- **Microorganisms producers of Lignase:**

Many members of bacteria, fungi, actinomycetes, and Cyanobacteria have been reported with ability to degrade lignocellulosic waste and other wood containing fibers. **(Chandra R. CRC Press, Taylor & Francis Group; 2015).**

Bacillus sp., *Pseudomonas* sp., *Citrobacter* sp., *Klebsiella pneumonia, Serratia marcescens* were reported to produce an extracellular peroxidases in order to degrade the lignin. **(Anwar F., Hussain S., Ramzan S., Hafeez F., Arshad M., Imran M., Maqbool Z., Abbas N, 2017).**

Within fungal population, *Trametes versicolor, Ganoderma lucidum, T. reesei, A. niger, P. chrysosporium, Penicillium brefeldianum, Trichoderma longibrachiatum, Aspergillus nidulans* are very efficient in lignin degradation during wood decay. **(Kersten P., Cullen D, 2007)**



a) Catalytic mechanisms of lignin peroxidase **b) LiP molecular structure**

c) LiP degradation of the compound structure.

Figure 10: lignin peroxidase **(N. A. Kulikova et al ; 2011)**

III-1-1-3 Pectinase:

Pectin is an important component of the middle lamella and primary cell wall of higher plants. Three major pectic polysaccharides groups are recognized, all containing d-galacturonic acid residues to a greater or a lesser extent. They are homogalacturonan (HG), rhamnogalacturonan I (RGI), and rhamnogalacturonan II (RGII)**.(Alkorta, I.; Garbisu, C.; Llama, M.J.; Serra, J.L; 1998).**

Pectinases are a group of enzymes that degrade pectic substance and are classified according to their mechanism of action. For example:

- **Methylesterases** : these enzymes remove methoxy groups from highly or partially esterified galacturonan.
- **Polygalacturonases**: catalyze the hydrolysis of the glycosidic bonds in a random fashion (endopolygalacturonase) or from the nonreducing end of homogalacturonan releasing galacturonic or digalacturunic acid residues (exopoly-glacturonases). **(Cornuault, V.; Posé, S.; Knox, J.P; 2020).**

As with all enzymes, pectinases have an optimum temperature and pH at which they are most active. A commercial pectinase might typically be activated at 45 to 55 °C and work well at a pH of 3.0 to 6.5. **(http://www.enzymeindia.com/enzymes/pectinase.asp).**

Pectinolytic enzymes, or pectinases, are also classified according to their mode of action and their substrate:

- Polygalacturonases, which are sub classified as : endo-polygalacturonases (E.C. 3.2.1.15) and exo-polygalacturonases (E.C. 3.2.1.67);
- Lyases:, which are sub classified into pectatelyases (E.C. 4.2.2.9 and EC. 4.2.2.2) or pectin lyases (E.C. 4.2.2.10); and pectin methylesterases (E.C. 3.1.1.11).

It is recommended to use a combination of different kinds of pectinases, along with other enzymes such as cellulases and hemicelullases, as multiple enzymes can degrade different parts of the polymer, resulting in the maximal degradation of the pectin in various raw materials such as in citrus juice processing. **(Oumer, O.J.; Abate, D; 2018)**.

Fungi are a good sources of pectinolytic enzymes which are excreted to break down the middle lamella in plants so that it can insert fungal hyphae and extract nutrients from the plant. (Gummadi, S.N.; Panda, T. Purification and biochemical properties of microbial pectinases). In addition to fungi, pectinolytic enzymes are naturally produced by many other organisms like bacteria, insects, nematodes, and protozoans For the commercial production of pectinases, Aspergillus spp., Erwinia spp., Bacillus spp., and Penicillium spp. have been extensively used. **(Dhital, R.; Panta, O.P.; Karki, T.B; 2013)**

Pectinases have crucial roles in food industries. These enzymes are useful for fruit juice extraction and wine clarification; tea, cocoa, and coffee concentration and fermentation;

vegetable oil extraction; preparation of jam and jellies; and pickling . Furthermore, these enzymes are used in paper and pulp industries, bleaching of paper, bio-scouring of cotton, retting and degumming of plant fibers, oil extraction, wastewater treatment, poultry feed additives, protoplast fusion technology, and bioenergy production. **(Kubra, K.T.; Ali, S.; Walait, M.; Sundus, H; 2018)**

- **Pectin Properties:**

The molecular weight of pectinase is generally between 20kD and 60kD, and most of them exist as monomeric enzymes. Pectinase activity is usually in the range of pH 3.0 to 9.0, with an isoelectric point of 4.0 to 9.0. Among them, an optimum pH of a hydrolase is 4.0 to 6.5, and its action does not require the participation of Ca^{2+}. The optimum pH of the penctin lyase is 8.0 to 10.0, and its action requires Ca^{2+} involvement. The pectinase produced by different strains has different properties. Common purification methods of pectinase include ammonium sulfate precipitation, acetone precipitation, ion exchange chromatography and gel filtration chromatography.

Pectinases are used to degrade pectin, hemicellulose and lignin in plant fiber raw materials and disperse them into bundle fibers or monofilaments that meet different requirements of the biomass bioconversion. **(Rupinder Tewari , Ram P. Tewari ,Gurinder S. Hoondal; 2005).**

IV-Industrial bioconversion of biomass to bioethanol:

To reduce the dependency on fossil fuels and importations of chemical products **(like ethanol** that varies from 30,000 to 50,000 hl per year) for its proper uses., 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).**

There are a number of technologies for energy conversion from biomass suitable for applications in small and large scales. Considering the convention adopted by the International Energy Agency **(IEA).**

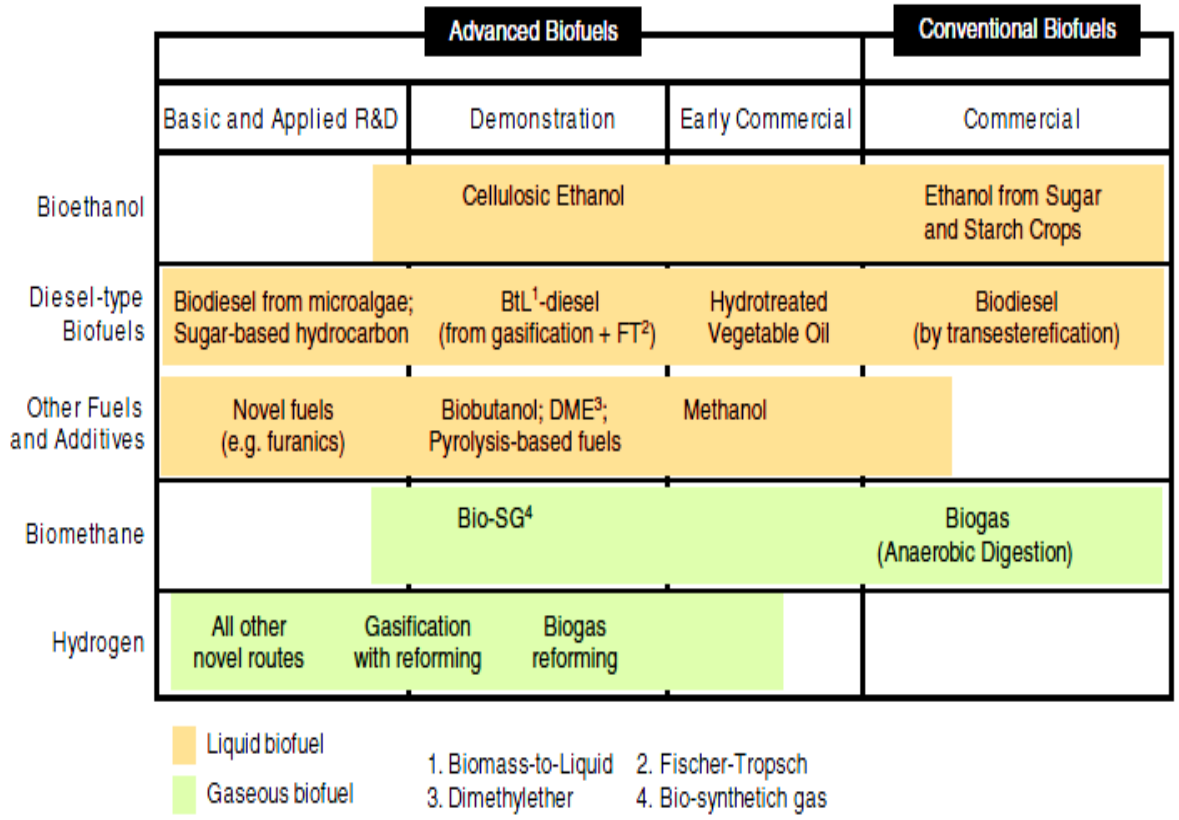


Figure 11: Status of main biofuel technologies based on biofuel technologies includes well-established processes that are producing biofuels at a commercial scale **(P. Bodenes; 2017)**

- **First generation biomass**

These biofuels, commonly referred to as first-generation, include sugar and starch based ethanol, oil-crop based biodiesel, as well as biogas derived from anaerobic digestion of the biomass derived from food product. The first generation ethanol can be used as a pure fuel or can be blended with gasoline and other fuels.

Fermentation of simple sugars from sugar crops and from hydrolysis of starch crops to produce ethanol is a commercial and widely used first generation process **(IEA, 2011)**. Seemingly, the **"*Dactylifera L.*"** fruit has rich content in biodegradable sugars of about 73 to 83% on dry mass basis in two inverted forms, glucose and fructose which are suitable for conversion to bioethanol **(FAO statistics, 2015)**

Table 3: An overview of production technologies of first generation biofuels **(C.R.Soccol ; 2019).**

Biofuel type	Specific name	Feedstock	Conversion technologies
Biodiesel	Biodiesel from energy crops: methyl and ethyl esters of fatty acids	Oil crops (soybean, rapeseed, palm, etc.)	Cold and warm pressing extraction, purification, transesterification
	Biodiesel from waste	Waste, cooking/ frying oil	Hydrogenation
Bioethanol	Conventional ethanol	Sugar beet, sugarcane	Direct fermentation of juice
	Starchy ethanol	Corn, wheat and other grains	Enzymatic hydrolysis, fermentation

- **Second generation biomass**

Due to many pressures at environmental and social level, the experts have shifted to the second generation of biomass, mainly the lignocellulosic biomass as an abundant and available source of feedstock for production of ethanol. In that context, biological methods of delignification have been suggested as promising tool of bioconversion due to its mild reaction conditions, higher yield, and low energy consumption **(Sánchez, Sierra, & Alméciga-Díaz, 2011).**

The main processes for the production of **second generation** biofuels are shown in **(Fig 2.5)** where the biomass conversion is done according to two approaches: thermochemical decomposition including gasification, bio-carbonization, liquefaction and thermal decomposition (pyrolysis) processes; and biological digestion, essentially conducted by microbial digestion and fermentation. **(A.V. Bridgwater, 2012.)**

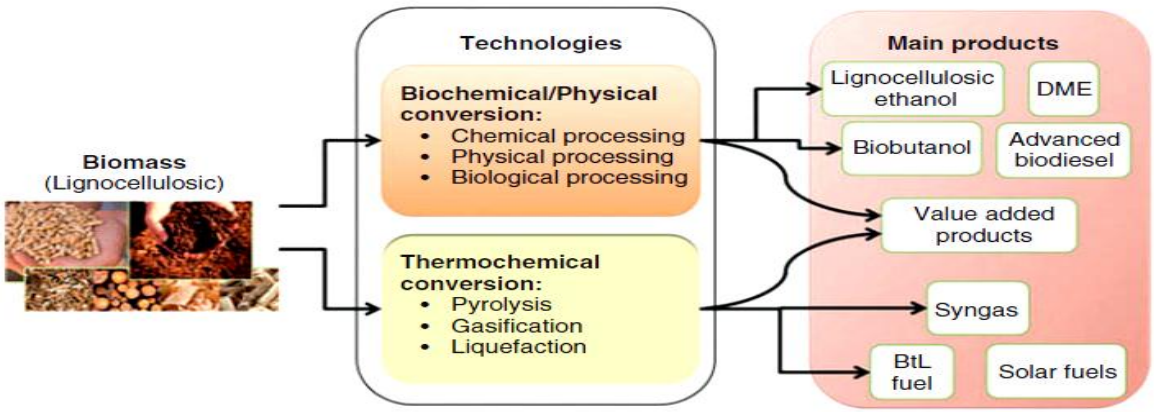


Figure12: Main processes for production of second generation biofuels. Adapted **(D. Bacovsky, D. Michal, M. Worgetter; 2010).**

The bioconversion of the lignocellulosic materials to ethanol is more complicated than those from sugar and starches (first generation), as lignocellulosic materials contain more complex sugar polymers, such as cellulose and hemicellulose, which are more difficult to break down **(P. Langan, S. Gnanakaran, K.D. Rector, N. Pawley, D.T. Fox, D.W. Cho and K.E. Hammel. 2011).** Because of that, the second generation bioethanol production from biomass requires additional processing steps **(Figure).**

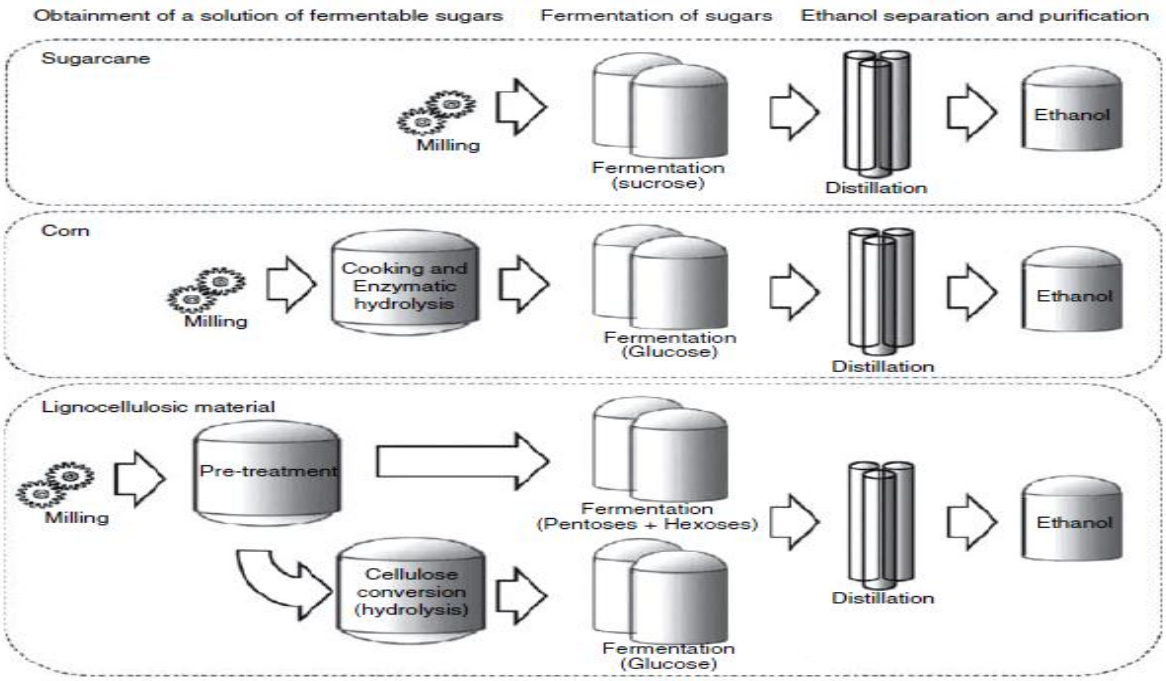


Figure 13: Processing steps for second generation bioethanol production from lignocellulosic biomass compared to first generation processes. **(S.I. Mussato et al; 2010.)**

Biofuels of the third generation come from algae and hydrogen produced from lignocellulosic biomass. The products resulting from their conversion are described as third generation because they do no longer require the use of land.

The microalgae processing for producing biodiesel is comprised of the following steps: microalgae growth, harvest, dewatering and drying. Two methods of algal oil transformation are applied: a two-step method that is divided into oil extraction and oil transesterifi cation; and a single step *in situ* transesterifi cation of algal oils to biodiesel. Three types of conversion methods prevail: chemical, thermochemical and enzymatic. Four major types of catalysts performing this reaction have been applied to algal biodiesel: alkali, acid, lipase, and heterogenous catalysts. **(P. Hallenbeck. 2012).**

The third generation biomass is still in development and many technical problems should be overcame before a wide generalization of the technology In the Algerian current

context the ready available approach is the second generation biomass as far as there is a huge biomass from agricultural origin that need a valorization at the industrial level.

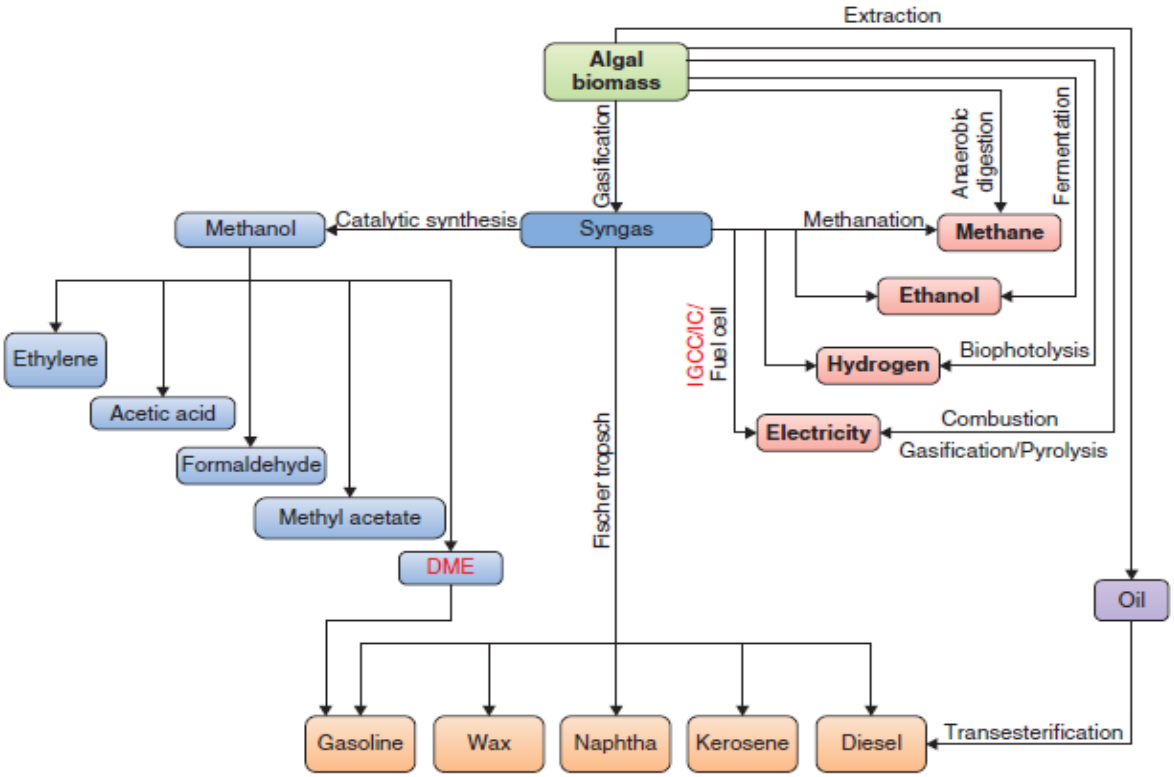


Figure 14: Overview of the main pathways for processing algae biomass.**(T. Forster-Carneiro et al ; 2016)**

Second section

The Technical part

The next part is reserved to the technical approach used in biorefineries to produce biofuel (bioethanol) in which we will shed light on all the stages of the production starting from the treatment of the raw material to the purification and conditioning of the final product.

V- The technical approach used in biorefinery to produce biofuel (bioethanol)

V-1 Bio-refineries

The large majority of chemicals products are issued from petroleum feedstocks. Around 5% of the total oil production is used in chemical manufacturing, but the value of these chemicals is high and contributes with comparable revenue to fuel and energy products. The key feature of the biorefinery concept is the coproduction of fuels, chemicals and energy from different biomass feedstocks **(IEA; 2011).** The concept is analogous to the basic concept of conventional oil refineries: producing a variety of fuels and other products from a certain feedstock **(Fig 15).**

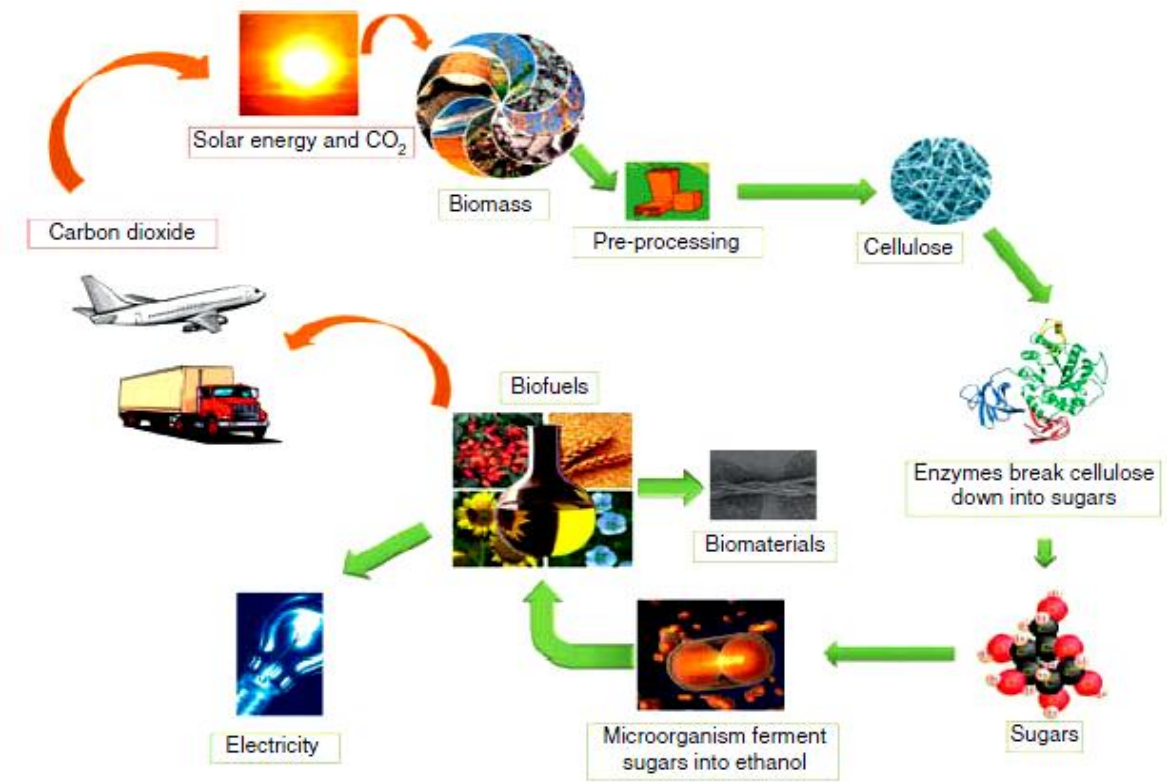


Figure 15: The concept of the biorefinery **(Guohua Jiang ; 2014).**

V-2 Processing of Biomass of second generation to Ethanol

Overall, the whole process is divided into Pretreatment and First Stage Hydrolysis then the second stage which is an enzymatic or chemical hydrolysis. Then a fermentation stage after which the fermented medium undergoes the distillation process in order to separate the ethanol. For further purity, a dehydration step can be envisaged. The residual processing raw material, micro biomass, chemicals and enzymes can be valorized in the production of biogas as a by-product and solid waste residue (**Y. Lin, and S. Tanaka, 2006).**

A basic representation of **the conversion of lignocellulosic biomass** into ethanol by enzymatic and chemical hydrolysis is shown below **(Fig 16).**

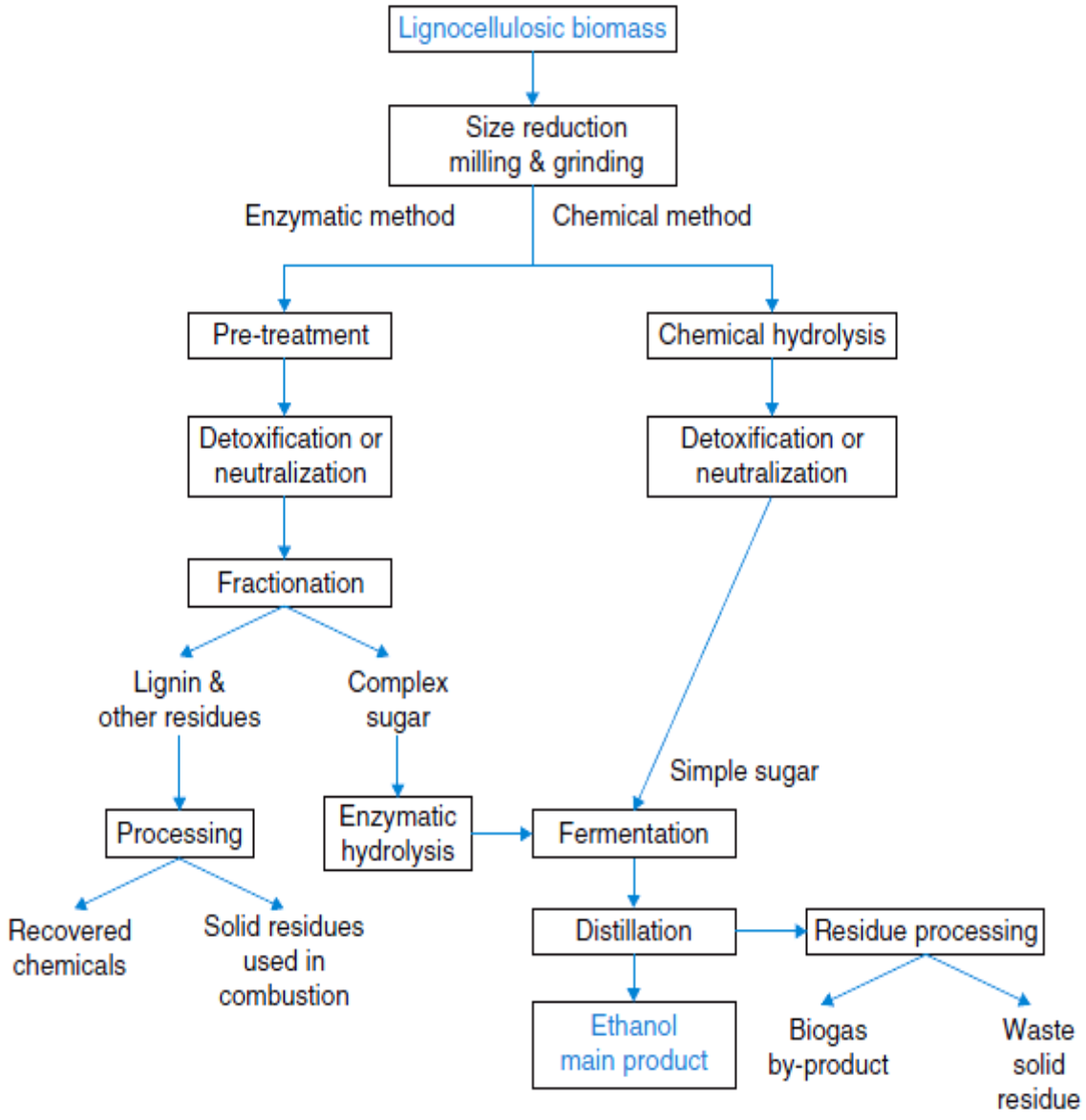


Figure 16: A basic representation of the conversion of ligno-cellulosic biomass into ethanol.

(V. Sikarwar ; 2017)

a) Pretreatment

1- First Stage Hydrolysis

Once the feedstock is delivered to the ethanol plant, it needs to be carefully stored and conditioned to prevent early fermentation and bacterial contamination. Through pretreatment, simple sugars are made available in proportions depending on the type of biomass used and the pretreatment process. **(Y. Sun and J.Cheng, 2002.)**

Pretreatment involves delignification of the feedstock in order to make the polymer more accessible in the hydrolysis step. The most common methods are steam explosion and dilute acid prehydrolysis, which are followed by enzymatic hydrolysis. Sulphuric acid or carbon dioxide is often added in order to reduce the production of inhibitors and improve the solubilisation of hemicellulose (**A. M. Hendriks and G. Zeeman, 2009)**.

Steam explosion has a few limitations :

- the lignin-carbohydrate matrix is not completely broken down;
- some other degradation products are generated that reduce the efficiency of the hydrolysis and fermentation steps;
- portion of the xylan fraction is destroyed.

The use of dilute sulphuric acid (0.5–1%; 433–463 K for 10 minutes) is more suitable resulting in the hydrolysis of the hemicellulose and the releasing of simple sugars (xylose, arabinose, mannose, and galactose).

It is worth mentioning that some the resulting compounds of the cellulosic matrix can inhibit the enzymatic hydrolysis and fermentation. **(E. Tommas, 2008)**

This is why a part of the acetic acid, and much of the sulphuric acid and other inhibitors produced during the degradation of the materials need to be removed, and a neutralization step is needed be performed before fermentation. The cost of the pretreatment is high accounting for approximately 33%of the total cost **(M. Oliva, and M. Ballesteros, 2008).**

2- Second Stage Hydrolysis.

In the second stage hydrolysis, the released cellulose of the biomass is converted into glucose, which is again catalysed by dilute acid, concentrated acid, or preferably by enzymes (mixed enzymes cellulases, pectinases, lignases), either produced in a separate reactor or bought externally from industrial suppliers **(X. H. Cui, X. B. Zhao, J. Zeng, S. K. Loh, Y. M. Choo, and D.H. Liu, 2014).**

The conversion of cellulose and hemicellulose can be expressed by the reaction of glucan (for hexoses) and xylan (for pentose) with water:

$(C_6H_{10}O_5)_n + nH_2O \rightarrow nC_6H_{12}O_6$ **(1)**

$(C_5H_8O_4)_n + nH_2O \rightarrow nC_5H_{10}O_5$ **(2)**

According to many authors the maximum theoretical yield of hexoses and pentoses is 1.136 kg and 1.111 kg per kg of glucan and xylan, respectively. To overcome inhibition by hydrolyte components, membrane techniques have been investigated **(Q. Kang et al; 2014).**

The strategies that have been adopted to detoxify lignocellulosic hydrolysates and their effects on the chemical composition of the hydrolysates to improve the fermentability of lignocellulosics. Hydrolysis of myco- LB (LB after fungal pretreatment) has been recognized as a promising approach to avoid fermentation inhibitors and improve total sugar recovery. Genetic manipulation could modify the metabolic routes to produce bioethanol or other value-added compounds in an efficient manner.

Genetically engineered fungi that produce large volumes of cellulase, xylanase, and hemicellulase enzymes are under investigation. These could convert agricultural residues (e.g., corn stover, straw, and sugar cane bagasse) and energy crops (e.g., switchgrass) into fermentable sugars. **(J. Baeyens, et al; 2015).**

Additional research tried to find microorganisms which can effectively ferment both types of sugars into ethanol with *Escherichia coli*, *Klebsiella oxytoca*, and *Zymomonas mobilis* as promising candidates **(D. Deswal, R. Gupta, P. Nandal, and R. C. Kuhad; 2014).**

b) Fermentation

Fermentation is the biological process to convert the hexoses and pentoses into ethanol by a variety of microorganisms, such as bacteria, yeast, or fungi. The conversion reaction for hexoses (C6) and pentoses (C5) is as follows:

$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$ (3)

$3C_6H_{10}O_5 \rightarrow 5C_2H_5OH + 5CO_2$ (4)

The theoretical maximum yield of broth hexoses and pentoses is :

- 0.511 kg ethanol
- 0.489 kg CO_2 per kg sugar.

The overall theoretical ethanol yield (at 20∘C) hence becomes:

- 0.719 of glucan (and/or other 6C structures)
- 0.736 liters per kg for xylan (and/or other 5C structures),

- *S. cerevisiae*, the yeast commonly used for first generation ethanol production, cannot metabolize xylose. Other yeasts and bacteria are under investigation to ferment xylose and other pentoses into ethanol **(J.Ruchala et al; 2019)**

c) Purification:

This step is foreseen for recuperation of high purity bioethanol. Typical ethanol concentrations are in the range of 3–6 vol% only, very low in comparison with 12 to 15 vol% obtained from 1st generation feedstock. **Q. Kang, L. Appels, J. Baeyens, 2014)**

Due to the higher water content of the broth, additional distillation efforts are required. Different process improvements, including energy pinch, very high gravity fermentation, and hybrid processes, are described in detail by Kang et al. **(R. Dewil, and T. Tan, 2014).**

3- Energy production

a) Steamand Electricity Generation

The bottom product of the first distillation column (stillage) contains mainly lignin and water next to unconverted cellulose and hemicellulose. This insoluble fraction is dewatered by a pressure filter and sent to a fluidized bed combustor system for steam and electricity generation. This system allows the plant to be self-sufficient in energy supply, reduces solid waste disposal cost, and generates additional revenue through sales of excess electricity**.** Burning the solid residues for steam and power production is the most beneficial option and meets the energy demand of the plant. **(F. K. Kazi, J. A. Fortman, R. P. Anex et al., 2010)**

The comparison

In this part, we will make a comparison between two methods of bioethanol production from two different raw materials: dry palms and pedicel (second generation biomass) and waste dates (first generation biomass).

1. Production of bioethanol from dried palm and pedicel (according to A. Boulal, M. Khelafi and K. Kaidi, 2018)

The authors were interested in the by-product of date palms (dry palm and pedicel), that would be suitable for bioethanol conversion due to its richness in cellulose. Firstly, the raw material was submitted to grinding, steam cracking, acid hydrolysis and dilution. Then fermentation was undergone under anaerobic condition followed by a distillation. The detailed protocol of the assay is given below.

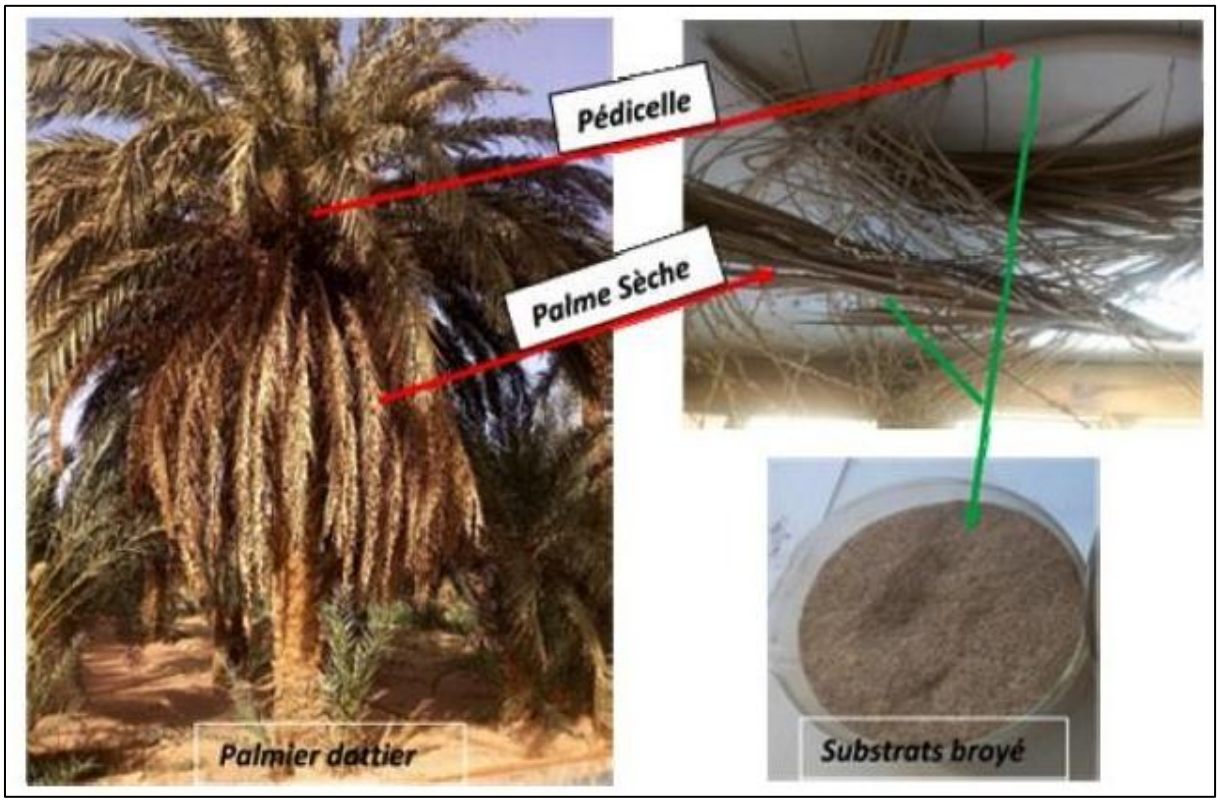


Fig. 17: grinding of the dried palm (Boulal et al; 2018)

1-1. Biological material

The fermentation was tried with the industrial baker's yeast *Saccharomyces cervisiae* obtained from the local market.

Saccharomyces cerevisiae under Microscope X1000

Reactivation of dry yeast

Figure 18: Biological fermentation material (Boulal et al; 2018)

1-2. Detailed protocol

- **Grinding:** to increase its specific surface (developed surface of the powder per unit of mass).
- **Steam / steam explosion pretreatment:** Thermo-mechanochemical process which hydrolyzes lignocellulosic material.
- **Acid hydrolysis:** to break the osidic bonds of the polysaccharidic polymers and to produce simple monomer sugars.
- **Dilution:** Dilution is to create a favorable environment for the growth of microorganisms.
- **Fermentation:** In a bioreactor (a laboratory fermenter), the prepared must was introduced.

  The addition of yeast of the genus *Saccharomyces cerevisiae* (baker's yeast) results in the anaerobic fermentation of the sugars. This reaction took place over a period of around 72 hours. The resulting products are ethanol and carbon dioxide. After the fixed period, the bioreactor was immersed in a 30° C water bath.
- **Distillation:** the fermented date liquor yielded almost 8-15% ethanol. To obtain a pure ethanol, the author set a distillation stage to separate ethanol from the liquor by a multi-column system. The distillation temperature is fixed around 78 ° C.

Yield: The yield of bioethanol produced was calculated taking into account the volume of the must liquor introduced in the bioreactor at the beginning of the fermentation.

Alcohol content: two methods were used to estimate the alcohol content in the distillate:

- With an alcoholmeter (0-100 °).

- With the Dichromate test: In an acidic medium, ethanol (CH3CH2OH) is oxidized by potassium dichromate (K2Cr2O7) to give a green color.
-

1-3. Results and discussion

a) The tonnage of date palm by-products:

Estimation of the date palm biomass suitable for bioconversion**: dry fins, pedicels**

- **Dry fins**

Based on the fact that,

- A leaflet weighs on average 5 g **(A. Chehma et HF. Longo, 2012)**

- A palm has on average 180 leaflets **(A. Boulal, 2013)**,

- A date palm yields an average of 15 palms per year (**(A. Chehma et HF. Longo, 2012)**,

- There are around 18 million date palms in Algeria **(FAO; 2015).**

The tonnage of the dry palms as follows,

05 × 180 = 900 g; i.e. 0.9 kg / palm

0.9 × 15 = 13.5 kg / palm tree / year

13.5 × 18,000,000 = 243,000,000 kg

That is: 243,000 tones of dry palms / year

The weight of the pedicel is 4.5g compared to the weight of dates it carries, of 245 g, represents 1.84% of pedicels for one kg of dates.

Knowing that the Algerian production of dates is estimated at 848,000 tons per year **(CACI; 2015)** , we can estimate the tonnage of date pedicels as follows,

848,000 × 01.84% = 15,603 tons

That is: 15,603 tones of pedicels / year.

- **Result:** they find that the ethanol produced at the laboratory level has the following characteristics: volatile, flammable, clear and possessing a pungent odor. The dichromate test confirms the presence of ethanol in the solution.

- **Performance**: The average alcohol yield is 20% with a degree of (75 °).
- **Physico-chemical characterizations of the final product:**

The results obtained are very acceptable. They are shown in Table 3.

From the comparisons with the commercial ethanol, we can be satisfied of the quality of this product in terms of physicochemical characteristics and the degree of purity. The purity could be enhanced with successive distillation.

	Bioethanol	**Commercial ethanol 96%**
pH	6.2	Neutral (07)
Density (g /cm 3)	0.834	0.789
Refractive index (20°c)	1.367	1.3611
Degree of purity (%)	75	96

2- Production of bioethanol from date waste (According to S. Chniti et al; 2014)

2-1. Date waste (Deglet-Nour)

The fruits used are sorting wastes of dates (fruit with texture defects, very wet fruits, fruits spoiled by microorganisms and insects).

- **Date juice extraction**

Before extraction, the dates were preliminary, washed, seed removed and are then cut into small pieces (0.5 to 1 cm). Afterward, a precise quantity of date pulp were thoroughly mixed with water.

The extraction is carried out in a shaking water bath **(Julabo SW23, Seelbach, Germany)**, for different periods. Once the extraction was completed, the solution was filtered and then stored for physico-chemical analysis.

2-2. Microorganisms and method of preservation

The strains used in this work are *Saccharomyces cerevisiae* 522D, *Zygosaccharomyces rouxii* IP 2021.92 and *Candida pelliculosa* IP 820.63. Working culture are carried out in liquid Sabouraud medium.

- **Culture media**

The date syrup was diluted to tow appropriate proportions (Medium 1: 17.4 ° Brix and medium 2: 35.8 ° Brix), then centrifuged at 5000 rpm for 30 min, in order to separate the cellulosic debris, while the supernatant is used as a source of carbon for the production of ethanol. The medium is then sterilized by autoclaving at 120 ° C. for 20 minutes, after enrichment in mineral salts with NH_4Cl (10 mM), and adjusted to pH 6 by adding 1M KOH.

- **Fermentation process**

The fermentations assays were carried out in 500 ml flasks closed with screw caps, containing 300 ml of the date medium, at 28 ° C under stirring (180 rpm).

The fermentation is monitored by sampling (5 ml) at 0, 18, 24, 48, 66 and 72 h, of the culture. The determination of sugars (glucose, fructose, sucrose) and metabolites (ethanol and glycerol) is carried out by high performance liquid chromatography, 'HPLC' according to the method described by **Djelal et al ….**

The consumption of NH4Cl in the media during fermentation was determined by the method developed by Mann **(L.T.Mann; 1963)**

- **Evolution of biomass**

Monitoring the growth showed that , after 72 h of fermentation, *Z. rouxii* and C. *pelliculosa* recorded more than 50% of growth comparing to S. cerevisiae. The first two strains are more this shows that these yeasts are resistant to stressful factors caused by the high concentrations of sugars in the culture medium (hyperosmolarity).

Table 04: Alcoholic fermentation of the medium based on date syrup (after 72 h of culture).

	Eq Glu ($g.l^{-1}$)*	Eq Glu Consommé	EtOH ($g.l^{-1}$)	Gly ($g.l^{-1}$)	Biomasse (%)
S. cerevisiae	174 349	94 4	63 ND**	10.0 ND**	3.1 2.0
Z. rouxii	169 357	67 41	33 55	4.6 10.0	5.8 4.6
C. pelliculosa	168 356	71 3	41 ND**	4.6 ND**	5.7 2.6

* Concentration of total sugars in glucose equivalents (g/l).

** ND: <Limit of quantification.

Table 3 groups together the different concentrations obtained for the fermentation products. The yields compared to the substrates consumed in ethanol (YEtOH / S), in glycerol (YGly / S) and in biomass (YX / S), for the three strains, over the same culture period (72 h), showed that the ethanol yields are 38, 29 and 34%, for *S. cerevisiae*, *Z. rouxii* and *C. pelliculosa* respectively in the diluted Medium 1 (brix:17.4). However, for the medium at 36 ° Brix, no ethanol was detected for *S. cerevisiae* and *C. pellicula*; and a little yield was recorded for *Z. rouxii* (Table).

Table 05: Product and biomass yield after 72 h of fermentation

	Eq Glu ($g.l^{-1}$)*	$Y_{EtOH/S}$ (%)	$Y_{Gly/S}$ (%)	$Y_{X/S}$ (%)	$Y_{EtOH/X}$ ($g.g^{-1}$)	$Y_{Gly/X}$ ($g.g^{-1}$)
S. cerevisiae	174 349	38 ND**	3 ND**	2 14	20.0 ND**	3.0 ND**
Z. rouxii	169 357	29 38	4 7	5 3	5.7 12.0	0.8 2.2
C. pelliculosa	168 356	34 ND**	4 ND**	4 24	7.0 ND**	0.8 ND**

Conclusion:

According to the results of the two authors, we can assume that date palm is an interesting plant in producing high valued product. In fact, the data collected by **Boulal et al;** have shown that date palm is a potential source for lignocellulosic biomass that would be converted to bioethanol. Also, it has been demonstrated that the resulted bioethanol has a satisfactory degree of purity and density.

Moreover, further optimization of the fermentation condition and the pretreatment of the raw material might improve highly the process of bioethanol production.

In the other hand, a similar conclusion was determined for the wastes of date's sorting inappropriate for direct human consumption. **Chniti et al;** have stated that a good bioethanol rate could be obtained with the osmotolerant yeast *Zygosaccharomyces rouxii*, with concentrated date medium more than that of *S. cerevisiae*.

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