

الجمهورية الجزائرية الديمقراطية الشعبية  
People's Democratic Republic of Algeria  
وزارة التعليم العالي والبحث العلمي  
Ministry of higher education and scientific research  
جامعة مولاي الطاهر، سعيدة  
University of SAIDA, Dr MOULAY Tahar



كلية العلوم  
Faculty of Science  
قسم البيولوجيا  
Department of biology  
Specialty: Applied Microbiology

Theme:

## Sustainable bioconversion of organic waste to ethanol

A project submitted in partial fulfillment of the requirements  
For the degree of Master in Biological Science- Startup degree within the  
framework of ministerial resolution 12-75

**Presented by:**

- Ms. ADDA Amani

**Supervisor:** Mr. BENREGUIEG Mokhtar

**Defended on:** October 12<sup>th</sup> 2023

**Committee Members:**

Chair

Examiner

Examiner

Examiner

Supervisor and reporter

BELLIL Yahia

BENABBOU Taha Ahmed

BENZAI Yacine

DJELLOULI Nassima

BENREGUIEG Mokhtar

MCA University of Saida

MCB University of Saida

MCA University of Saida

MCA University of Saida

MCA University of Saida

Academic year 2022/2023

## Notice

This thesis is considered a patented work and is protected under intellectual property laws. Any attempt to reproduce, distribute, or utilize the information contained within this document without proper authorization is subject to legal action. This includes but is not limited to legal accountability in a court of law.

All rights, including copyright and patent rights, are reserved.

Ms. ADDA Amani

## تنويه

تعتبر هذه الأطروحة عملاً محميًا بموجب حقوق الملكية الفكرية وتحظى بحماية كبراءة اختراع. أي محاولة لاستنساخ أو توزيع أو استخدام المعلومات الواردة في هذا الوثيقة دون تصريح صحيح ينتج عنه إجراءات قانونية. ويشمل ذلك ولكن لا يقتصر على المسائلة القانونية أمام المحكمة.

كل الحقوق، بما في ذلك حقوق الطبع والنشر وحقوق الاختراع، محفوظة.

عدة أماني



A decorative gold floral border surrounds the text. It features intricate scrollwork, leaves, and a central flower-like motif at the top and bottom. The border is composed of multiple layers of gold-colored elements, creating a rich, ornate frame.

# Acknowledgment

I am thankful to Almighty ALLAH for giving me the strength, knowledge and ability to undertake this study and my parents for their support and encouragement.

I would like to thank my advisor Dr. benrguig, for providing me with guidance and support throughout this journey.

Thus, I warmly thank the experts who agreed to examine this work, Dr .Bellil Y, Dr Benabbou AT, Dr Benzai Y and Dr Djellouli N.

I express my gratitude to Dr Benzai Y, the director of the business incubator, Dr Djellouli N, Responsible of CATI and to Dr.Mouffok M, Responsible of CDE for their valuable guidance.

I also like to thank the department of biology and the business incubator, University of Saida. Dr Moulay Tahar, Algeria, for the laboratory permissions and the encouragement provided.



# Dedication

Praise be to God who without this work would not have been

To whom dreamt and prayed for this moment, to my dear  
mother

To whom gave his all supporting me, to my beloved father

To my cheares ones who have shown unwavering belief in me  
and encouraged me to strive for excellence through both the  
good times and the challenges, this goes out to Racha, Ilham,  
Wissem, Younes and Mohamed.

To the one who played a significant and crucial role in  
enabling the accomplishment of this task, to Mr. Chohra  
Younes.

And finally to Mimide my cat

I dedicate this humble work.

## **Abstract**

The aims of this study were to provide a business plan for implementing a bioethanol production company, and to determine the utilization of organic wastes: fruit and vegetable wastes (FVW) combination towards bioethanol production with the used of indigenous yeasts as starter. Berries, banana, watermelon, apple, potato, carrot, beetroots and sugar beet wastes were taken as materials from Central market around Saida City. *Saccharomyces cerevisiae* was the indigenous yeast used in the production process, which was isolated from fermented raisins. The FVW were subjected to a pretreatment process that comprises grinding feedstock while simultaneously adding 500ml of water to create a homogenous mixture. Fermentation was conducted at a temperature of 35°C for a duration of 15 days, in an environment with a pH of 6.8. From the results obtained during the study, conclusions drawn were, maximum quantity of fermented broth (undistilled ethanol) produced was 3 litres after fermentation duration of 15 days. The maximum quantity of distilled ethanol after final distillation was found to be 450ml. These findings show/prove that ethanol can be made from the named organic waste and the process is recommended as a means of generating wealth from waste.

**Key words:** bioethanol, organic wastes, fruit and vegetable wastes, *Saccharomyces cerevisiae*, Fermentation, distillation, business plan.

## Résumé

Les objectifs de cette étude étaient de fournir un plan d'affaires pour la mise en œuvre d'une entreprise de production de bioéthanol et de déterminer l'utilisation des déchets organiques : combinaison de déchets de fruits et légumes (FVW) vers la production de bioéthanol avec l'utilisation de levures indigènes comme starter. Des baies, des bananes, des pastèques, des pommes, des pommes de terre, des carottes, des betteraves et des déchets de betterave sucrière ont été récupérés comme matériaux du marché central autour de la ville de Saïda. *Saccharomyces cerevisiae* était la levure indigène utilisée dans le processus de production, isolée de raisins secs fermentés. Les FVW ont été soumis à un processus de prétraitement qui comprend le broyage de la matière première tout en ajoutant simultanément 500 ml d'eau pour créer un mélange homogène. La fermentation a été réalisée à une température de 35°C pendant 15 jours, dans un environnement avec un pH de 6,8. À partir des résultats obtenus au cours de l'étude, les conclusions tirées sont que la quantité maximale de bouillon fermenté (éthanol non distillé) produite était de 3 litres après une durée de fermentation de 15 jours. La quantité maximale d'éthanol distillé après distillation finale s'est avérée être de 450 ml. Ces résultats montrent/prouvent que l'éthanol peut être fabriqué à partir des déchets organiques mentionnés et que le processus est recommandé comme moyen de générer de la richesse à partir des déchets.

**Mots clés :** bioéthanol, déchets organiques, déchets de fruits et légumes, *Saccharomyces cerevisiae*, Fermentation, distillation, plan d'affaires.

## ملخص

كانت أهداف هذه الدراسة هي تقديم خطة عمل لإنشاء شركة لإنتاج الإيثانول الحيوي، وتحديد الاستفادة من النفايات العضوية: مزيج نفايات الفاكهة والخضروات في إنتاج الإيثانول الحيوي مع استخدام الخمائر المحلية كبدائية. وتم أخذ مخلفات التوت والموز والبطيخ والتفاح والبطاطس والجزر والشمندر والشمندر السكري كموا من السوق المركزي في محيط مدينة سعيدة. كانت خميرة الجعة هي الخميرة الأصلية المستخدمة في عملية الإنتاج، والتي تم عزلها من الزبيب المخمر. تم إخضاع مخلفات الفواكه والخضروات لعملية معالجة أولية تشتمل على طحن المواد الأولية مع إضافة 500 مل من الماء في نفس الوقت لتكوين خليط متجانس. تم إجراء التخمير عند درجة حرارة 35 درجة مئوية لمدة 15 يومًا، في بيئة ذات درجة حموضة 6.8. من النتائج التي تم الحصول عليها خلال الدراسة تم التوصل إلى أن الحد الأقصى لكمية المرق المتخمر (الإيثانول غير المقطر) المنتج هو 3 لترات بعد مدة تخمير قدرها 15 يومًا. وجد أن الحد الأقصى لكمية الإيثانول المقطر بعد التقطير النهائي هي 450 مل. تظهر هذه النتائج/تثبت أنه يمكن تصنيع الإيثانول من النفايات العضوية المذكورة ويوصى بهذه العملية كوسيلة لتوليد الثروة من النفايات

**الكلمات المفتاحية:** الإيثانول الحيوي، النفايات العضوية، نفايات الفواكه والخضروات، خميرة الجعة، التخمير، التقطير، خطة العمل

## List of figures

<b>Figure 1:</b> chemical structure of Ethanol .....	4
<b>Figure 2:</b> Different pretreatment methods for fruit and vegetable biomass .....	10
<b>Figure 3:</b> Fractionation process for the pretreatment of FVW waste .....	13
<b>Figure 4:</b> Diversity of fermentation systems for bioethanol production (idealised) .....	15
<b>Figure 5:</b> Design of stirred tank bioreactor detailing the process conditions involved in fermentation process .....	16
<b>Figure 6:</b> Schematic representation of bioethanol production from sugar-based feedstock .....	19
<b>Figure 7:</b> <i>Saccharomyces cerevisiae</i> strain colonies on sabouraud broth. ....	28
<b>Figure 8:</b> Organizational structure. ....	37
<b>Figure 9:</b> Fermentation of fruits and vegetables wastes broth in a sealed plastic jug. ....	44
<b>Figure 10:</b> <i>Saccharomyces cerevisiae</i> colonies on Sabouraud Broth under microscope 1000X. ....	45
<b>Figure 11:</b> filtration process of the resulting mixture.....	46
<b>Figure 12:</b> Bioethanol distillation process.....	47
<b>Figure 13:</b> Our product “Bioethanol”.....	47
<b>Figure 14:</b> Bioethanol flammability test. ....	48



## List of tables

<b>Table 1:</b> Physico-chemical properties of Ethanol.....	5
<b>Table 2:</b> Pretreatment methods.....	11
<b>Table 3:</b> Ethanol Production by Yeast under Different Conditions. ....	14
<b>Table 4:</b> Team’s skills and training courses. ....	24
<b>Table 5:</b> Start-up activities timeline. ....	26
<b>Table 6:</b> Competitor analysis.....	32
<b>Table 7:</b> Business number of bioethanol 99%.....	39
<b>Table 8:</b> Business number of bioethanol 70%.....	40
<b>Table 9:</b> Business number of methanol. ....	40
<b>Table 10:</b> Business number of biofertilizers.....	41
<b>Table 11:</b> Materials used in the production process. ....	42

## Contents

<b>General introduction</b> .....	1
<b>Theoretical background: Bioethanol Production and Properties</b> .....	3
1.0 Introduction to bioethanol .....	3
2.0 Chemistry of Ethanol .....	4
3.0 Fruit and Vegetable Wastes (FVW) as a raw feedstock for bioethanol production.....	6
3.1 Bioethanol from Banana Wastes .....	6
3.2 Bioethanol from Citrus Fruit Wastes .....	7
3.3 Bioethanol from Date Fruit Waste .....	7
3.4 Bioethanol from Potato Processing Waste .....	8
3.5 Bioethanol from Cheese Whey.....	8
4.0 Bioethanol production .....	10
4.1 Pretreatment and detoxification of fruits and vegetables wastes (FVW).....	10
4.2 Fermentation aspects .....	13
4.2.1 Yeast involved in the fermentation process .....	13
4.2.2 Bioreactors for fermentation process .....	15
4.2.3 Fermentation process.....	17
4.3 Ethanol recovery by distillation .....	19
5.0 Advantages of bioethanol.....	20
<b>Business plan</b> .....	21
1.0 Project overview .....	21
1.1 Project idea (proposed solution).....	21
1.2 Value proposition .....	22
1.3 Work team .....	23
1.4 Objectives.....	25

1.5 Project realization timeline.....	25
2.0 Innovative aspects .....	27
2.1 Nature of innovations .....	27
2.2 Fields of innovations .....	29
3.0 Market analysis .....	30
3.1 Market segment display .....	30
3.2 Competitor analysis.....	31
3.3 Marketing strategy.....	33
4.0 Production plan and organization.....	35
4.1 The production process .....	35
4.2 Supply.....	36
4.3 Labor .....	37
4.4 Major partnerships.....	38
5.0 Financial plan .....	39
5.1 Cost and burdens .....	39
5.2 Business number .....	39
5.3 Expected results calculation table .....	41
5.4 Treasury plan.....	41
6.0 Experimental prototype .....	42
6.1 Introduction .....	42
6.2 Materials and methods .....	42
6.2.1 Materials.....	42
6.2.2 Method .....	43
6.3 Results .....	45
6.4 Discussion .....	48

6.5 Conclusion.....	50
<b>General conclusion .....</b>	<b>51</b>
<b>References .....</b>	<b>52</b>
<b>Appendix – Supplementary tables .....</b>	<b>61</b>

**Word count: 18436**

# General introduction



### General introduction

Worldwide, various industries generate waste during the production processes they implement. These wastes range from organic material, mainly fruits and vegetables wastes (such as seeds, skin, bagasse, peels, shells, and scraped portions, among others) to synthetic material (such as plastics). In most cases, it is a serious environmental and health problem; Organic waste can serve as a breeding ground for disease transmission. As these waste materials decompose, they have the potential to attract disease vectors like mosquitoes and rodents, enabling the dissemination of hazardous infections to humans. Moreover, the decomposition of organic waste yields detrimental gases like methane, a potent greenhouse gas, in addition to releasing volatile organic compounds and toxic substances. These emissions contribute to climate change and the deterioration of air quality. However, some plant residues, mainly generated by food industries, have been analyzed due to their high biological potential. A clear example is the use of fruits and vegetables wastes, rich in sugars, to obtain bioethanol. Bioethanol is one of the most interesting biofuels since it has a positive characteristic on the environment. Algeria annually produces 34 million tons of waste, of which 50% is organic waste that can be recycled. It is projected to produce between 70 and 75 million tons by the year 2035 (1), this gives significant importance in the field of the circular economy.

During the last decades, there has been a great interest in the production and use of bioethanol in different industries like pharmaceutical, cosmetics, chemical and petrochemical industry. For this reason, the development of biorefineries and processes, as well as the design of bioreactors, have been fundamental issues to understand this area. Likewise, the study of physiological processes that are carried out by some bioethanol-producing microorganisms is also necessary to know. Yeast, like *Saccharomyces cerevisiae*, have been demonstrated to have a prominent role in bioethanol production. One of the great merits of bioethanol consists in the enormous variety of raw materials, from which it can be produced. The production methods vary depending on whether or not the raw material is rich in fiber. The basic materials for producing bioethanol must have certain features, including high carbon and hydrogen concentrations and low concentrations of oxygen, nitrogen and other organic components (2).

## GENERAL INTRODUCTION

### **Aims and objectives**

The main objectives of this business project is:

- To provide an in-depth understanding of the principles and concepts of bioethanol production.
- To develop a comprehensive business plan for a bioethanol production company.
- To design and develop a working prototype.
- To conduct a feasibility analysis on producing and utilizing bioethanol for small and medium-sized business.

### **Project plan structure**

The business project is structured in a) theoretical background part, where the fundamental concepts and principles of bioethanol and its production from organic waste are discussed in-depth. Followed by, b) the business plan, where detailed suggestions for the incorporation of a bioethanol production company are given, including production plan, financial plan, marketing strategy and experimental prototype, which describes the design, and testing of the product. Finally, c) the conclusion part, which summarizes the main findings of the study followed by concluding remarks about the feasibility of the proposed business.

**Theoretical background: Bioethanol  
Production and Properties**

### **Theoretical background: Bioethanol Production and Properties**

#### **1.0 Introduction to bioethanol**

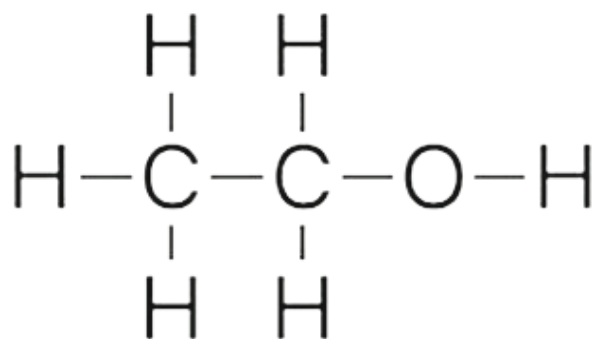
Bioethanol is fermentation alcohol. That is, it refers to ethyl alcohol produced by microbial fermentation processes, as opposed to synthetically produced ethanol from petrochemical sources. It is produced through distillation of the ethanolic wash emanating from fermentation of biomass-derived sugars (3). One of the great merits of bioethanol consists in the enormous variety of raw materials, from which it can be produced. The production methods vary depending on whether or not the raw material is rich in fiber. The basic materials for producing biofuels must have certain features, including high carbon and hydrogen concentrations and low concentrations of oxygen, nitrogen and other organic components (2). Ethanol or bioethanol can be produced from any feedstock containing substantial amounts of sugar or food wastes containing starch or cellulose, which can be converted to sugar and used to produce ethanol. The feedstock for ethanol production includes sugarcane, sugar beet, sorghum, maize, wheat, cassava, and mixed food waste, that is, biomass containing easily fermentable sugars (4).

Today, there are four types of process technology called first, second, third and fourth generation technology. First generation process technology produces bioethanol from sugars (a dimer of the monosaccharides glucose and fructose) and starch-rich (polysaccharides of glucose) crops such as grain and corn. Sugars can be converted to ethanol directly but starches must first be hydrolysed to fermentable sugars by the action of enzymes. The raw material in second generation is lignocellulosic raw materials. These are most abundantly available biomass in the world and are found as leaves, peels, bodies, branches, etc. of almost all the existing plants (2). However, studies have reported that the process of producing bioethanol from lignocellulosic biomass is complex and longer in duration, it is expensive in comparison to starchy crops (5).

Algal biomass is used to make third generation bioethanol. Algae are divided into two categories: microalgae and macroalgae. Because of their high lipid content, microalgae have gotten a lot of attention for biodiesel manufacturing. Macroalgae, on the other hand, are utilized to produce bioethanol (6). For the fourth generation technology, biofuels can be produced from specially engineered plants or biomass that have higher energy yields or lower barriers to cellulosic breakdown or are able to be grown on nonagricultural land or bodies of water (2).

## 2.0 Chemistry of Ethanol

Ethanol is a clear colorless, volatile, and flammable liquid that is made by the fermentation of different biological materials (7), just like other alcohols. It has a characteristic odour. Ethanol has psychoactive properties. Other types of alcohol also have such properties, but ethyl alcohol is significantly less toxic to humans as compared to methanol or isopropanol (2). Ethanol is also called ethyl alcohol or grain alcohol (7). Its chemical formula is  $C_2H_6O$ , or can be written as  $C_2H_5OH$  or  $CH_3CH_2OH$ . It has one methyl ( $-CH_3$ ) group, one methylene ( $-CH_2-$ ) group, and one hydroxyl ( $-OH$ ) group (2). It has a characteristic, agreeable odor. In dilute aqueous solution, it has a somewhat sweet flavor, but in more concentrated solutions, it has a burning taste. Ethanol is an alcohol, a group of chemical compounds whose molecules contain a hydroxyl group,  $-OH$ , bonded to a carbon atom (7). It may be shown as:



**Figure 1:** chemical structure of Ethanol

The word alcohol derives from Arabic al-kuhul, which denotes a fine powder of antimony used as an eye makeup. Alcohol originally referred to any fine powder, but medieval alchemists later applied the term to the refined products of distillation, and this led to the current usage. Ethanol melts at  $-114.1\text{ }^\circ\text{C}$ , boils at  $78.5\text{ }^\circ\text{C}$ , and has a density of  $0.789\text{ g/mL}$  at  $20\text{ }^\circ\text{C}$ . Its low freezing point has made it useful as the fluid in thermometers for temperatures below  $-40\text{ }^\circ\text{C}$ , the freezing point of mercury, and for other low-temperature purposes, such as for antifreeze in automobile radiators. (Table 1). The molecular weight is 46.07. One gallon of 190 proof ethanol weighs 6.8 lb. Ethanol has no basic or acidic properties. When burned, ethanol produces a pale blue flame with no residue and considerable energy, making it an ideal fuel. Ethanol mixes readily with water and with most organic solvents. It is also useful as a solvent and as an ingredient when making many other substances including perfumes, paints, lacquer, and explosives. The flash point of ethanol is



## THEORETICAL BACKGROUND

the lowest temperature (i.e. 12.8 °C) where enough fluid can evaporate to form an ignitable concentration of vapour and characterizes the temperature at which ethanol becomes flammable in air. The ignition point of ethanol is the minimum temperature at which it is able to burn independently (i.e.425 °C) (2).

**Table 1:** Physico-chemical properties of Ethanol (3).

Molecular formula	C <sub>2</sub> H <sub>5</sub> OH
Molecular mass	46.07 g/mol
Appearance	Colourless liquid (between -117 °C and 78 °C)
Water solubility	Miscible
Density	0.789 kg/l
Boiling temp	78.5 °C (173 °F)
Freezing point	-117 °C
Flash point	12.8 °C (lowest temperature of ignition)
Ignition temp	425 °C
Explosion limits	Lower 3.5% v/v; upper 19% v/v
Vapour pressure	38 °C) 50 mmHg
Higher heating value (at 20 °C)	29,800 kJ/kg
Lower heating value (at 20 °C)	21,090 kJ/L
Specific heat, Kcal/Kg	60 °C
Acidity (pKa)	15.9
Viscosity	1.200 mPa·s (20 °C)
Carbon (wt)	52.1%
Hydrogen (wt)	13.1%
Oxygen (wt)	34.7%
C/H ratio	4

### 3.0 Fruit and Vegetable Wastes (FVW) as a raw feedstock for bioethanol production

According to the Food and Agriculture Organization (FAO), fruit and vegetable waste occupies a major chunk in food waste. These are biodegradable substances generated in huge quantities; however, maximum wastes are dumped in open land to rot. This type of open dumping creates a lot of issues in and around the area due to emission of foul odor. Moreover, it also attracts birds, rats, pigs, etc., and creates big trouble by ultimately becoming vectors and carriers of various diseases. The rotten items, peels, shells, and scraped portions of vegetables or slurries are the major wastes, and they can be treated through fermentation under controlled conditions for the production of bioethanol (4).

### 3.1 Bioethanol from Banana Wastes

Banana is one of the main fruit crops grown widely around the globe. It is a widely cultivated fruit crop in Asia and America (56% and 26%, respectively). Every year, during the process of post-harvest transporting or grading, a huge portion of banana crop is rejected, wasted or discarded, which are then disposed in the lands which contributes in the environmental pollution. However, these low-cost wastes including rotten bananas and their peels can be utilized for the production of second-generation bioethanol, as they comprise valuable sugars and minerals, which are essential for the process of fermentation (8). Besides glucose, the banana wastes also comprises various other carbohydrates, which can be converted into simple sugars by the process of enzymatic hydrolysis using enzymes like cellulases, hemicellulases and pectinases (9).

In order to produce bioethanol, banana waste is collected, washed, cut into small pieces and sun-dried. Following this, the sun-dried banana waste is crushed, ground well and blended with distilled water. At times, the banana waste is also utilized without drying. However, in such cases, it is essential to wash the waste and mash it well. In either case, the mashed or the powdered mixture of banana waste is required to be autoclaved, and, subsequently, the sterilised feedstock is subjected to further hydrolysis. To the feedstock, 0.5–2.5% (v/v) of diluted sulphuric acid is added, which is kept at 70°C to 110°C for 10–30 min. This mixture is then subjected to enzymatic hydrolysis at pH ranging from 4.5 to 5.5, using enzymes like cellulases, hemicellulases and pectinases (10) and is incubated at 50°C for an hour. Following this process, the sterile hydrolysate is mixed with 18–24 h grown yeast culture and is incubated anaerobically with constant shaking at 35°C, pH 5–5.5 for 3–7 days. During this process, aliquots can be tested to examine the concentration of bioethanol

generated. The bioethanol produced during this process is directly proportional to the water content of the feedstock. On achieving the desired concentration of bioethanol, it is then subjected to the process of distillation (11).

### 3.2 Bioethanol from Citrus Fruit Wastes

Another commonly cultivated crop across the world is citrus fruits. Production of oranges across the globe is nearly 50.2 million tons (12). A huge amount of fruit is wasted if it is not stored, transported and retailed properly. During the production of orange juice, a variety of waste including citrus pulp floater, peels, fibres and internal tissues is generated, which contains high content of sugar, protein, pectin, celluloses and hemicelluloses. The citrus fruit waste when treated in the floatation tank leads to clogging and when disposed in inland sites results in environment pollution. However, the comparatively lower cost of these wastes and the presence of abundant sugars make them a suitable feedstock for bioethanol production (13). Rotten citrus fruits and peels are employed as a feedstock for the second-generation bioethanol production. This waste is fermented either by using a monoculture of yeasts or by co-culturing yeast with another yeast strain called *Candida parapsilosis* NRRLY-12969. The co-culturing of yeasts is an efficacious technique as the unavailable sugars like pentoses can also be fermented by the new yeast strain resulting in enhanced bioethanol production. Bioethanol production from citrus wastes involves collection, washing, surface sterilization and drying in sun or oven. The dried waste is then ground, blended with distilled water and sterilised further. Subsequently, the cooled feedstock is blended with enzymes like cellulases, hemicellulases and pectinases for enzymatic hydrolysis. The enzyme hydrolysate obtained is then subjected to aerobic fermentation using a monoculture of yeast at 35°C for 5 days and, subsequently, anaerobic fermentation from sixth to ninth day or by employing co-culturing technique using *S. cerevisiae* and *C. parapsilosis* (13). The bioethanol produced with this process is then purified by distillation and considered for further use (9).

### 3.3 Bioethanol from Date Fruit Waste

Dates are mostly cultivated in the Gulf countries, including Egypt, Libya, Algeria and Pakistan. Generally, dates are wasted in huge quantity during harvesting, storing and transporting as well as in retail outlets. Since, they are a rich source of carbohydrates, the waste obtained from dates has a high content of biodegradable sugars, and the bioethanol can be generated by bioconverting these sugars using yeasts under anaerobic conditions (14). Bioethanol production from dates waste

involves washing of the waste, immersing it in a water bath and rubbing and rinsing it with water. Date seeds are then removed, ground, and placed in a water bath at 90°C–95°C to extract sugars. The temperature of the date juice obtained from a series of extraction is maintained at about 60°C, in order to avoid contamination of the juice. Following acid hydrolysis, the hydrolysate obtained is fermented using a yeast culture at nearly 32°C for 72 h, following which bioethanol produced is subjected to distillation. On repeating this process at least four times, bioethanol with 90% strength can be generated (9).

### **3.4 Bioethanol from Potato Processing Waste**

Another crop that possesses high-value sugar for the production of bioethanol is potatoes. The potato crop is the third chief food crop that is cultivated worldwide, with 325 million tons yield every year (15). It has been estimated that nearly one-third of the world's total potato crop is “overstock”, which is sufficient to yield 1200–7200 million litres of bioethanol per year (16). Likewise, the gross proportion of potatoes wasted in potato processing has been estimated to be as high as 50%, with 5%–20% wastage during their growth and 18% wastage in the potato chip producing industry. Currently, majority of the waste produced from the potato industry is being employed as animal feed (17). However, the starch present in processing wastewater produced from potato chip industry yields glucose, which can be employed as a constituent for bacterial fermentation for bioethanol production (18). A study was conducted to optimize the hydrolyzing conditions for solid potato waste generated from potato flake production, and the obtained medium was utilized for fermentation to bioethanol (about 31 g/L) using *Saccharomyces cerevisiae* (19). Similarly, another study derived 20 g/L ethanol from medium comprising sugars released from fresh potato peel [12% (w/w)] using enzymes including amylase, pectinase or fungal  $\beta$ -glucanase. Additionally, it was also reported that the combination of potato mash and potato peel to the medium and employing the same three enzymes resulted in augmented bioethanol production (as high as 50 g/L) (20).

### **3.5 Bioethanol from Cheese Whey**

Although, dairy industry has not been correlated to massive environmental issues; however, its interaction with environment must still be taken into account, since pollutants generated from this industry are predominantly organic in nature. Production of dairy products yields a variety of solid and liquid wastes along with certain by-products, which may include damaged or perished

## THEORETICAL BACKGROUND

products, off-specification products, solids, curd, cheese, milk sludge and whey comprising proteins, fats and lactose, making their exploitation necessary (21).

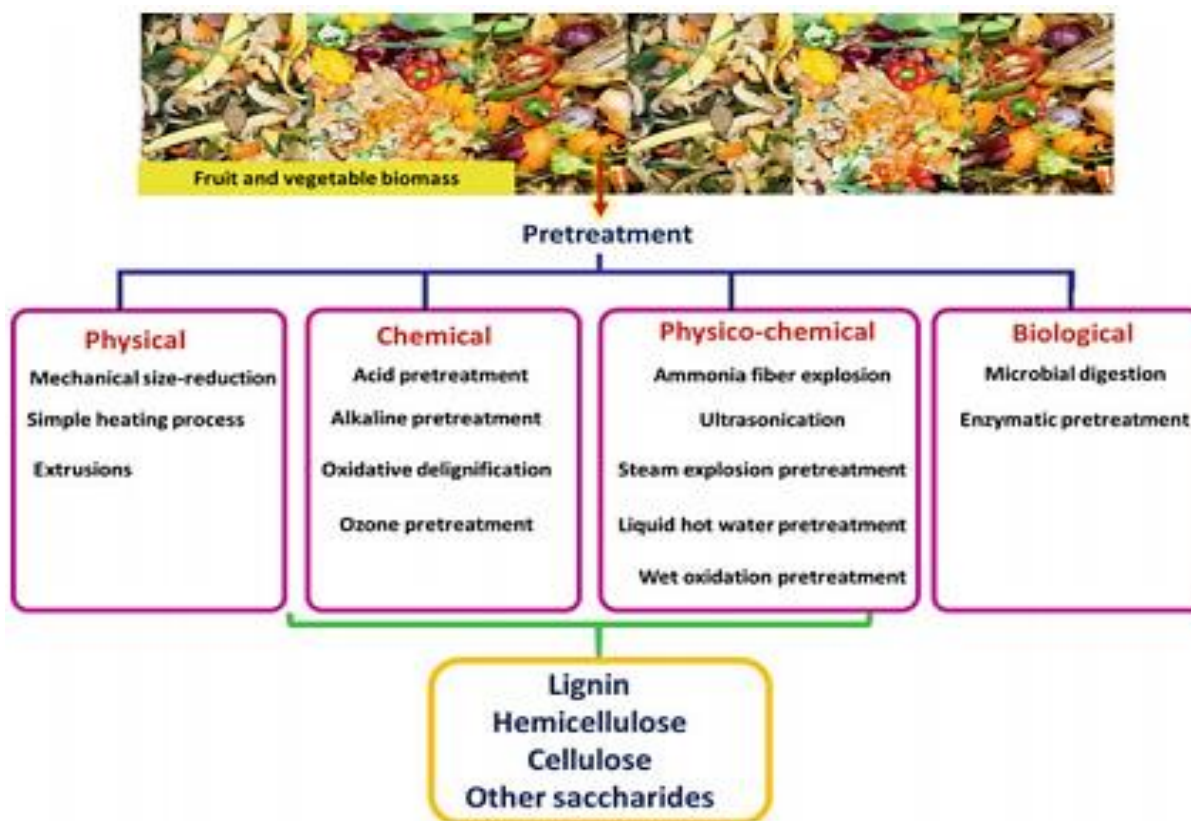
Cheese whey, a by-product of cheese industry, is generated in huge quantity and can result in environmental pollution if disposed into rivers or farmland (22). Every year, more than 160 million tons of whey is produced in the world as a by-product of nearly about 18 million tons of cheese produced (23). Of the total cheese whey produced, 70% is utilized for the production of other products, and nearly 30% is used for feeding pigs or as fertilizer or dispensed in the environment. Whey powder is one of the products that are produced from whey and are utilized as a constituent of several food products for humans and animal feed (24; 23; 25). Although, whey is often disposed, it is a rich source of vital nutrients such as lactose (5–6%), protein (1%), lactic acid (0.1–0.8%) and fat (0.06%) (26). Various researches have been conducted to investigate bioethanol production from cheese whey (23; 27; 28; 29). Several studies conducted in the past reported lower yield of bioethanol from whey; however, efforts are being made for its enhancement. Another challenge faced during the production of bioethanol from cheese whey is the presence of lactose as a form of fermentable sugar, which comprises glucose and galactose. *S. cerevisiae*, a conventional bioethanol producer, is incompetent to deploy lactose due to the absence of  $\beta$ -galactosidase and also because lactose cannot be transported into the cell (30). On the other hand, other yeasts, like *Kluyveromyces fragilis*, also known by other names like *K. marxianus*, *Candida kefyr* or *C. pseudotropicalis*, is capable of fermenting lactose. It has been reported that *K. fragilis* is proficient in fermenting lactose up to an economically feasible concentration of 20% albeit slowly. Furthermore, it has also been revealed that *K. fragilis* is impeded by moderate concentrations of sugar and salt found in whey and has a relatively lower bioethanol tolerance in comparison to *S. cerevisiae*. Another solution to the challenge is pretreating whey with  $\beta$ -galactosidase in order to hydrolyse lactose to galactose and glucose prior to using it as a constituent of fermentation medium. A study reported an Algerian strain of *S. cerevisiae* to be an efficient ethanol producer from pre-hydrolysed whey treated with  $\beta$ -galactosidase (31).



## 4.0 Bioethanol production

### 4.1 Pretreatment and detoxification of fruits and vegetables wastes (FVW)

An economical way of pretreatment procedure should assist in increasing the availability of carbohydrates in the enzymatic hydrolysis step while reducing the loss of simple saccharides for hydrolysis and fermentation (32). Figure 2 lists different pretreatment methods for FVW biomass.



**Figure 2:** Different pretreatment methods for fruit and vegetable biomass (33).

The primary purpose of an efficient pretreatment process is (34) , (1) to obtain simple saccharides by hydrolysis, (2) to avoid loss of simple saccharides formed, (3) to limit the production of inhibitors, (4) to minimize energy requirements, and (5) to reduce bioethanol production cost. Biomass pretreatment procedures are categorized as (34), (1) physical/mechanical pretreatment, (2) chemical pretreatment, (3) physicochemical pretreatment, and (4) biological/enzymatic pretreatment. Table 2 represents various pretreatment methods utilized in waste biomass processing.

## THEORETICAL BACKGROUND

**Table 2:** Pretreatment methods (33).

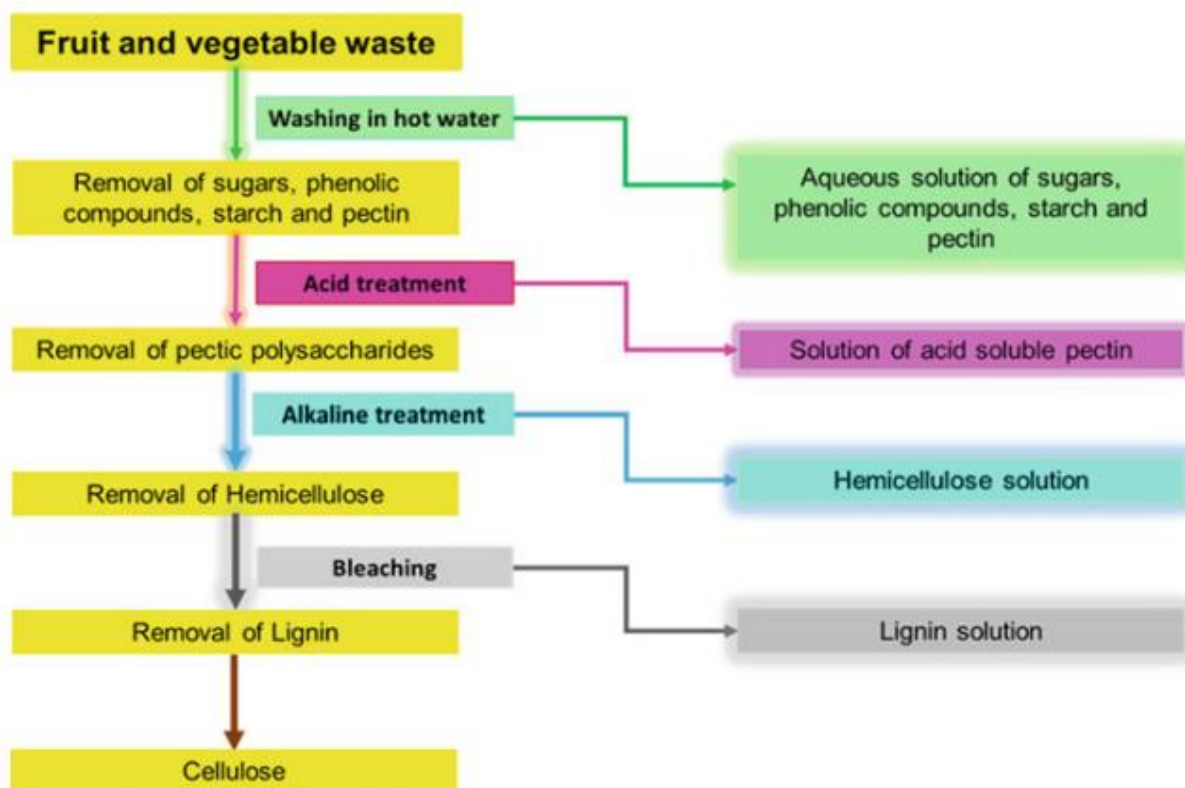
<b>Physical treatment</b>	
Mechanical size reduction	Chipping, grinding, hammer milling, ball milling, disk milling
Simple heating process	Microwave irradiation
Extrusions	Mixing, heating, and shearing of waste biomass
<b>Chemical pretreatment</b>	
Acid pretreatment/ saccharification	High temperature and less acid conc. Low temperature and higher acid conc.
Oxidative delignification	Hydrogen peroxide (H <sub>2</sub> O <sub>2</sub> ) or peracetic acid
Alkaline pretreatment (solubilizing polysaccharides)	NaOH, Ca (OH) <sub>2</sub> , KOH, and NH <sub>3</sub> are most frequently used alkalis for pretreatment
Ozone pretreatment	Ozonolysis of lignocellulosic biomass to increase cellulose biodegradability
<b>Physicochemical pretreatments</b>	
Ammonia fiber explosion pretreatment	Liquid ammonia and the steam explosion process
Cell wall disruption	Ultrasonication
Autohydrolysis or steam explosion pretreatment	Pressurized steam (20–50 bar, at 160–270°C) for a few seconds
Liquid hot water pretreatment (hemicellulose solubilization)	High temperatures and pressure (160–220°C) are used to maintain the liquid state of water and biomass kept in water up to 15 min
Wet oxidation pretreatment	Biomass treated with water involving oxygen at a temperature >120°C and 0.5–2 MPa pressures for <30 min
<b>Biological pretreatment</b>	
Cellulose and lignin digestion	Cellulase producing bacteria and fungi
Enzymatic pretreatment	Cellulases and hemicellulases enzymes

## THEORETICAL BACKGROUND

Pretreatment approaches are chosen on the basis of the type and composition of feedstock used for bioethanol fermentation (34). Among different physical/mechanical pretreatment practices defined in Table 2, extrusion is supposed to be cost-effective and easy to process when combined with mixing, shearing, and heating. It assists in the release of a considerable quantity of simple saccharides. Microwave heating is a heating pretreatment approach that should be carried out at appropriate temperatures based on biomass composition (35). Acid pretreatment is a well-recognized chemical approach generally used for lignocellulosic biomass and convert hemicellulose into its monomers and ultimately enhances bioethanol fermentation. Acids such as hydrochloric acid, acetic acid, formic acid, nitrous acid, nitric acid, maleic acid, phosphoric acid, and sulfuric acid have been utilized for acid pretreatment of lignocellulosic feedstock (36; 37; 38; 39; 40; 41).

Acid and alkali pretreatment was more broadly applicable for the lignocellulosic feedstock.  $\text{NH}_3$ ,  $\text{Ca}(\text{OH})_2$ ,  $\text{KOH}$ , and  $\text{NaOH}$  are primarily used alkalis for pretreatment (42). The most promising microbes for biotic treatment are different white-rot fungi related to the Basidiomycetes class. *P. chrysosporium* is capable of lignin biodegradation and has extreme competence compared to the different acknowledged species of white-rot fungi as of its excessive growth rate (43). For valorization of FVW, a combined way is much effective that include multiple pretreatment methods. In this fractionation method, FVW feedstocks are converted into its component at low cost, and simultaneously it will offer fractions of several valuable by-products. However, cellulose is the final product of FVW pretreatment, which is further used for bioethanol fermentation. In this strategy, FVW was washed in hot water and then kept in several solutions having acid, alkali, and oxidative agent sequentially. Such combined pretreatment methods are advantageous to obtain different fractions of sugar, lignin, pectin, cellulose, hemicellulose, and other bioactive components (44). Figure 3 represents fractionation process for the pretreatment of FVW waste.

## THEORETICAL BACKGROUND



**Figure 3:** Fractionation process for the pretreatment of FVW waste (44).

### 4.2 Fermentation aspects

#### 4.2.1 Yeast involved in the fermentation process

Ethanol-producing yeasts are characterized for producing ethanol and CO<sub>2</sub> in anaerobic conditions using fermentable sugars as substrate (45). The principal attributes for considering a good ethanol-producing yeast for their use in an industry are diverse as high tolerance levels of ethanol concentration, acidity, high temperature, low glycerol formation, capacity for use different sources of carbon and nitrogen. Also, it is important to observe the tolerance and inhibitors of the yeast in production about biomass hydrolyzed (46; 47).

Most yeasts grow quite well in simple nutritional media, which supplies carbon and nitrogen-backbone compounds together with inorganic ions and a few growth factors. The latter are organic compounds required in very low concentrations for specific catalytic or structural roles in yeast, but are not used as energy sources. Growth factors for yeast include vitamins, which serve vital functions as components of coenzymes; purines and pyrimidines; nucleosides and nucleotides;

## THEORETICAL BACKGROUND

amino acids; fatty acids; sterols; and other miscellaneous compounds (e.g., polyamines and choline) (48).

*Saccharomyces cerevisiae* is the more globally used yeast for industrial production of ethanol (49; 50); Brazil, the United States, European Union (EU), and China being the main producers of ethanol (Renewable Fuels Association). Furthermore, *S. cerevisiae* is an ideal yeast for the industry due to easy manipulation with molecular methods such as genetic engineering since its genome has been extensively studied (51; 52). The studies have been characterized for the improvement of the strains using by-products as substrate rich in sugars for the production of ethanol (Table 3). For example, yeasts such as *Schizosaccharomyces pombe*, *Candida krusei*, *Kluyveromyces marxianus*, *Dekkera bruxellensis*, *Pichia striptis*, *Pichia kudriavzevii*, *Wickerhamomyces anomalous*, among others; they have been isolated and identified as producing ethanol with good behavior and tolerant to high concentrations of alcohol (Et OH) (53; 54). The main factors that contribute to the low growth of yeasts in the process and that lead to high ethanol yields characterized in 90–92% of the theoretical conversion of sugar to ethanol are high cell densities, cell recycling, and high ethanol concentration (55) (56).

**Table 3:** Ethanol Production by Yeast under Different Conditions (57).

Yeast (°C)	Substrate (%)	pH	Temperature	Ethanol (% <sub>v/v</sub> )	Condition
<i>Saccharomyces cerevisiae</i> (CDBT2)	Glucose (5)	5.5	30	19.8	Electrochemical cell (4V <sup>1</sup> )
<i>Saccharomyces cerevisiae</i> (UVNR56)	Molasses medium (28)	-	37	10.3	UV-C <sup>2</sup> radiation
<i>Saccharomyces cerevisiae</i> UAF-1	Molasses media (27)	Adjusted 4.0–4.5	-	12.2	VHG <sup>3</sup> technology
<i>Wickerhamomyces anomalous</i> (CDBT7)	Glucose (5)	5.5	30	23.7	Electrochemical cell (4V)



## THEORETICAL BACKGROUND

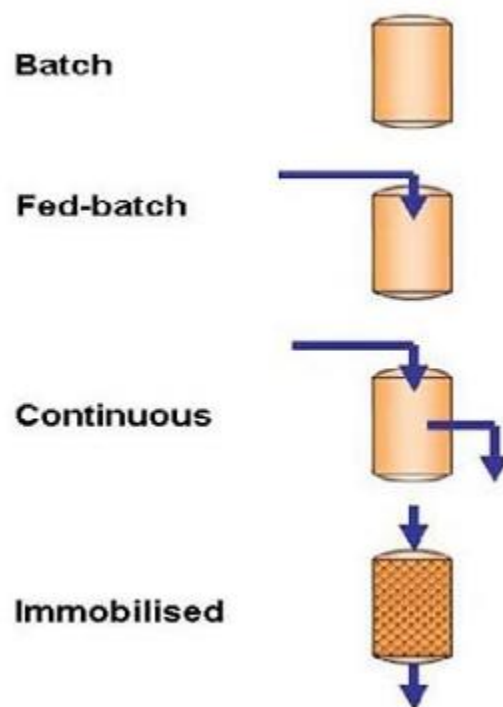
Kluyveromyces marxianus (YZB014)	Xylose (5)	-	45	5.2	Modified strain (recombinant)
Pichia stipitis (PXF58)	Xylose (11.4)	-	30	4.3	UV-mutagenesis4

V = VOLTS; VGH = Very high gravity;

UV-C = Ultraviolet-C; UV-mutagenesis = Ultraviolet mutagenesis.

### 4.2.2 Bioreactors for fermentation process

Regarding industrial bioethanol fermentation processes, several systems may be adopted, including batch, continuous, semi-continuous and immobilised (as summarized in Figure 4) (48).

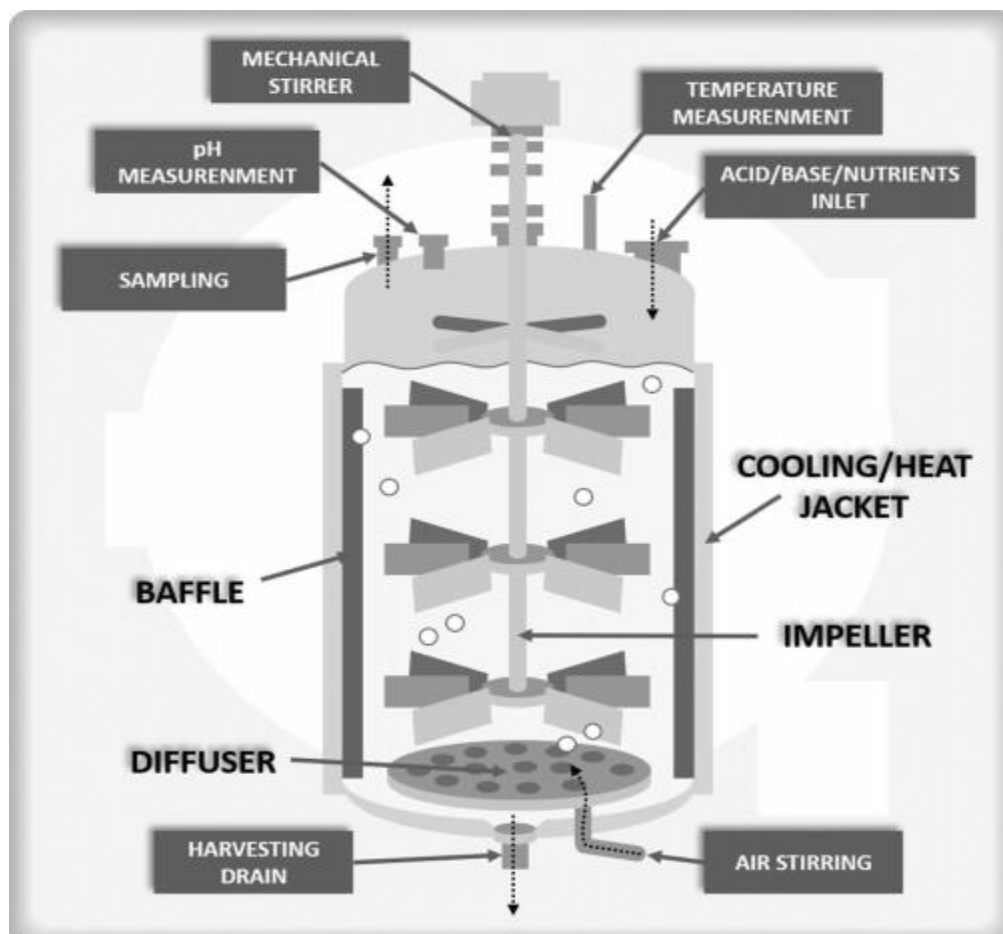


**Figure 4:** Diversity of fermentation systems for bioethanol production (idealised) (48).

The most common bioreactor used for fermentation experiments is a stirred tank operated in batch mode and the ideality is to achieve the continuous mode. Batch configuration is a discontinuous process where the bioreactor is initially loaded with a specific reaction volume that remains

## THEORETICAL BACKGROUND

constant throughout the fermentation. The batch reactor is a simple but efficient from an operational point of view with low-cost, compared with other more sophisticated, usually the main components in batch reactors are the tank, the stirred and mixing system, and several controllers of variables as shown in Figure 5, i.e., in the industry prefer to the steel and concrete to avoid the corrosion issues that are materials expect a long lifetime (58). Additionally, the batch system is the simplest operation mode, which implies the least risk of contamination. Meanwhile, the continuous mode fresh culture medium is continuously added and extracted from the bioreactor (59). The volume of the bioreactor is kept constant because both the inflow and outflow are equal. Normally for this kind of process is used a membrane for filtration of the ethanol (60). In this way is easy to recover a higher amount of ethanol avoiding the inhibition of the ethanol that limits the growth of the microorganism (61).



**Figure 5:** Design of stirred tank bioreactor detailing the process conditions involved in fermentation process (62).

### 4.2.3 Fermentation process

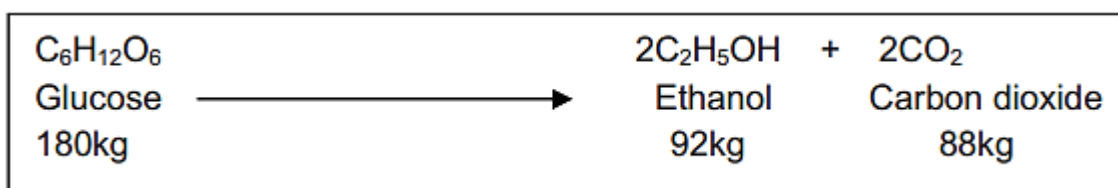
The sugar contained in the feedstocks are converted to ethanol by microbial fermentation. For the industrial production of bioethanol, the microorganisms used must be resistant to environmental stress such as that generated by an acidic pH; high levels of sugars at the beginning of fermentation (which causes hyperosmotic stress) and can grow rapidly on various substrates (63). The most frequently microorganism used is the yeast *Saccharomyces cerevisiae* because it is a microorganism recognized as safe, capable of consuming all types of hexoses and reaching high theoretical ethanol yields, in addition to being able to produce ethanol concentrations of up to 18% (v v<sup>-1</sup>) (64). Other microorganisms also used are *Zymomonas mobilis*, *Pichia stipitis*, *Candida shehatae*, *Candida tropicalis*, *Pachysolan tannophilus* and *Kluyveromyces marxianus* (thermotolerant). Sometimes a co-culture of microorganisms is used to ferment the hexoses and pentoses contained in the fermentation broth. Several process conditions affect the performance of the microorganism into convert the reducing sugars into ethanol. In a first instance, the concentration of cells can be at moderate or high concentration, i.e., a concentration of  $2 \times 10^7$  cell/mL is considered a moderate cell density, meanwhile  $8 \times 10^7$  cell mL<sup>-1</sup> is a high one. The fermentation process should be optimized to find the effective cell concentration taking into account the performance and reaction time (62).

Bioethanol production from biomass broadly involves processes like enzymatic hydrolysis, fermentation and distillation/dehydration. In the first step, the homo and heteropolysaccharide constituents of the biomass utilized for bioethanol production are hydrolysed either enzymatically or by dilute acids into sugars. This process is also known as saccharification, and it yields fermentable sugar-containing solution (9; 5; 65). The obtained solution can be further hydrolysed by yeast-derived invertase to release simple sugars, e.g. glucose and fructose. Subsequently, the simple sugars are fermented further using *Saccharomyces cerevisiae* yeast to yield bioethanol (66; 67; 68; 65). The last step involved in the production of this biofuel is distillation/dehydration, which is applied to the fermented broth to recover and concentrate the bioethanol. This step consumes extensive energy, which accounts for a considerable portion of bioethanol production cost (65). Generally, the fermented broth comprises nearly 12% ethanol, which can be purified up to 96% by the process of distillation. The general steps involved in the production of bioethanol from sugar-based feedstock are represented schematically in figure 6.

## THEORETICAL BACKGROUND

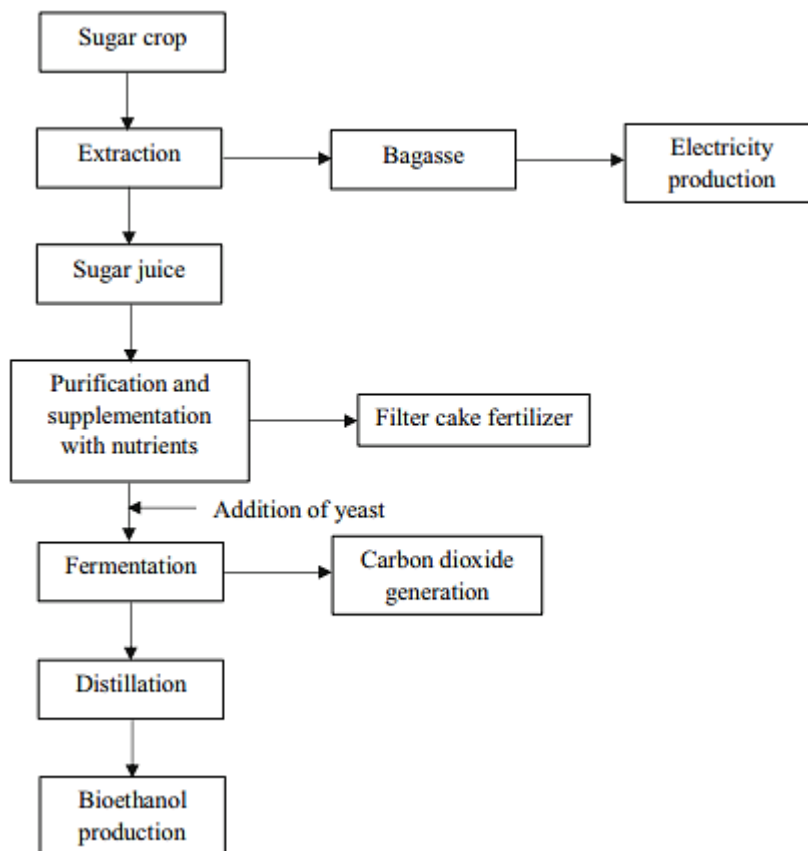
Typical parameters monitored during fermentation include: changes in yeast cell density, sugar consumption, pH, temperature, degree of foaming and alcohol. To ensure consistency of fermentation performance, distilleries not only monitor, but also control several of these parameters, notably temperature and pH. Of particular importance are spirit yield calculations, conversion efficiencies (of sugar to ethanol) and the relationship between initial sugar concentration and final yield of ethanol (3).

In terms of chemical stoichiometry, the theoretical conversion to ethanol from glucose is as follows:



For each kilogram of glucose fermented, around 470g of ethanol can be produced (i.e. <50%) representing a yield of 92% of theoretical maximum. However, in industrial fermentation practice, the best yields obtainable are only around 90% of this theoretical conversion (eg. using sugarcane molasses as feedstock). This is because fermentable carbon is diverted to new yeast biomass and minor fermentation metabolites (organic acids, esters, aldehydes, fusel oils etc) (48).

## THEORETICAL BACKGROUND



**Figure 6:** Schematic representation of bioethanol production from sugar-based feedstock (69).

### 4.3 Ethanol recovery by distillation

After fermentation, downstream processing began with several unit operations, which are performed for bioethanol recovery from the fermentation broth. At first, liquid-solid separation is performed to separate solid fractions (containing residual saccharides) and bioethanol from fermentation broth. Definitely, filtration and centrifugation are the best choice for liquid-solid separation. To minimize the water content of hydrolysate, supernatant is driven to the rotary evaporator. Serial evaporation helps to attain pure condensate with the concentrated syrup. Evaporation is followed by distillation. Condensate consisting bioethanol will be circulated to the distillation unit. Separation of ethanol from condensate is based on the differences in the boiling points of water (100°C) and bioethanol (~78°C) mixture. If water an ethanol solution is very dilute, repeated distillation is preferred to attain >95% of ethanol concentration. Bioethanol recovery using distillation attains 99.6% efficiency to minimize the losses of evaporated portion of bioethanol (70; 71).

### 5.0 Advantages of bioethanol

Bioethanol is safe as it has little side effects on the environment, produced via sugar fermentation by microbial activity, and can be used as a gasoline substitute (bioethanol-gasoline mixture has a higher octane number) (72). Despite its disadvantages (lower vapor pressure, less energy density, and corrosive nature), bioethanol is a sustainable energy source due to its many positive properties (9). Ethanol provides several benefits. It is easily biodegraded in the environment, and produces much less pollutants in internal combustion engines than petroleum fuels (73; 74). It has low toxicity and is miscible with water. Many car makers are producing more vehicles with tolerances to burn high %E fuels more efficiently. Thus, the risk posed by ethanol to the environment is significantly lower than that of fuels produced from petroleum and the demand for ethanol will increase with time as these automobile improvements take place. Ethanol is miscible with gasoline in any proportion, but is found most commonly as 10% ethanol and 85% ethanol. Flex-fuel vehicles (FFVs) can operate on blends of ethanol and gasoline anywhere between 0 and 85% ethanol. Benefits of ethanol include: higher performance; cleaner burning fuel; positive energy balance; currently cheaper than gasoline (after considering subsidies). A notable fact is that fuel ethanol is versatile and can basically be used in two capacities: a fuel additive (E10) and an almost stand-alone fuel (E85). Different issues surround each type of fuel. Any internal combustion engine, including small engines such as those in lawn mowers, can use a blend up to 10% ethanol (2).

# Business plan



## **Business plan**

### **1.0 Project overview**

#### **1.1 Project idea (proposed solution)**

Algeria is currently facing a significant shortfall in ethanol production, with the domestic supply falling far short of the growing demand for this important product. Despite some efforts to boost local production, the market is still facing a significant shortage of ethanol, leading to increased importation and rising costs.

In addition to the shortage of ethanol, Algeria is also grappling with the issue of organic waste management, which poses a significant threat to public health and the environment. In response to these challenges, our project offers a sustainable solution by producing ethanol from organic wastes. Specifically, we focus on producing ethanol from one of the most frequently generated and polluting form of waste “fruits and vegetables waste (FVW)”. This approach can contribute to meeting the national demand for ethanol, reducing the import bill, and promoting sustainable waste management practices in Algeria.

Bioethanol is briefly defined as ethyl alcohol, a clear colorless, volatile, and flammable liquid. The production process we adopted is extremely straightforward, eco-friendly and does not necessitate any lengthy steps. Sugars are extracted from FVW through milling, and the sugars are then fermented in the presence of yeast to produce bioethanol. After that the produced bioethanol is distilled to attain >95% of ethanol concentration.

Bioethanol is an important industrial chemical; it is commonly used in the pharmaceutical industry to produce medications and in the production of cosmetics and personal care products, such as perfumes, lotions, and shampoos. In addition, it is used in the food industry to produce vinegar and flavorings. Furthermore, Bioethanol can be used as a fuel for vehicles either in its pure form (E100) or blended with gasoline in many different concentrations, including E20 (20% bioethanol, 80% gasoline), E5 (5% bioethanol, 95% gasoline) and E2 (2% bioethanol, 98% gasoline). Bioethanol has several advantages; it is a renewable, nontoxic, biodegradable resource and as a fuel it is oxygenated, there by provides the potential to reduce particulate matter and CO<sub>2</sub> emissions from combustion.

### **1.2 Value proposition**

#### **Environmental Sustainability and waste reduction:**

Producing ethanol from organic waste goes beyond mere energy generation; it embodies a comprehensive approach to environmental sustainability and waste reduction. Our innovative process transforms organic waste, which would typically contribute to landfill accumulation and environmental degradation, into a valuable resource. This methodology aligns with the principles of the circular economy, wherein waste materials are diverted from landfills, minimizing their impact on ecosystems and conserving natural resources. Through this approach, we are actively combating the challenges posed by waste accumulation and pollution, fostering cleaner surroundings, and mitigating the negative effects on human health. By incorporating organic waste into the bioethanol production cycle, we are effectively closing the loop on waste management, thereby exemplifying a transformative solution that benefits not only the energy sector but also the broader ecosystem.

#### **Competitive price of ethanol:**

One of the pivotal objectives of our project is to introduce ethanol into the market at a competitive price point, establishing a noteworthy advantage over conventional sources. Our strategic utilization of organic waste as a primary feedstock serves as a pivotal driver in achieving this goal. This innovative approach inherently curtails production expenses through the utilization of abundant and renewable resources. The efficient conversion of organic waste into bioethanol not only aligns with sustainable practices but also results in substantial cost reductions, allowing us to offer ethanol to a diverse array of industries without compromising on quality. This competitive pricing strategy is poised to foster increased market penetration and broader economic viability, positioning our venture as an attractive solution within the ethanol landscape.

### **Customized purity levels:**

Tailoring our approach to meet the distinct demands of various industries, we recognize that ethanol purity holds pivotal significance. Our innovative project introduces a solution of unparalleled flexibility, empowering us to fine-tune the purity levels of ethanol to match the precise requisites of each sector. With an astute grasp of the diverse spectrum of requirements, whether mandating purity levels of 70%, 80%, or 90%, our production process is adeptly calibrated to ensure the delivery of ethanol that aligns seamlessly with the exacting specifications of each industry. This adaptable customization not only underscores our commitment to precision but also fortifies our position as a responsive and agile partner to diverse industrial sectors.

### **1.3 Work team**

The team consists of two final-year master's students in Applied Microbiology with a strong academic background in biochemistry, industrial microbiology, and environmental microbiology. While we may not have extensive professional experience, our academic training and internships has equipped us with the necessary knowledge and skills to carry out the technical aspects of bioethanol production. We are both eager to apply our theoretical knowledge to practical applications and are committed to achieving the project goals with dedication and teamwork.

Regarding our roles in the team, I (student 1) take the lead in developing the project plan and refining the business model, while my teammate (student 2) concentrates on the theoretical aspects of the project .We both equally contributed to the success of the experimental prototype by working collaboratively. Together, we bring a unique blend of technical and business skills (table1) that we believe will be essential for the success of our project.

## BUSINESS PLAN

**Table 4:** Team’s skills and training courses.

	Speciality	Training courses- internship’s	Skills	Planned project tasks
<b>Student 1:</b> ADDA Amani	Applied microbiology	English B2 (CEFR)  <b>Avicenne school of education</b> Qualification - (1)Pharmacist assistant - (2) laboratory assistant - (3) medical assistant (4) pharmacy manager  <b>Central laboratory- public hospital institution, Ahmed Madghari-Saida</b>	Research and analysis skills  Time management skills  Presentation skills	Planning and scheduling  Managing the collection of raw materials  Monitoring the production process  Bank Loans application
<b>Student 2:</b> CHOHRA Younes	Applied microbiology	English B1 (CEFR)  <b>Central laboratory- public hospital institution, Ahmed Madghari-Saida</b>  Quality control laboratory-““GIP lait la source” – <b>Saida</b>	Adaptability  Communication skills  Analytic thinking	Market research and marketing  -Managing the collection of raw materials -Developing and maintaining relationships with suppliers and customers

### 1.4 Objectives

Main objectives for our business are:

- Invite and secure investment for the creation of a small-scale production plant.
- Start production of bioethanol within a year using a functioning biodiesel mini-plant.
- National and international partnerships with companies and organizations working on biofuels.

### 1.5 Project realization timeline

A suggested timeline for the major start-up activities is provided in Table 5. The entire start-up process is anticipated to take approximately 10-11 months.

The start-up activities can be categorized into two phases: The initial phase, which does not involve significant cash flows, and the final phase, where all major cash flows take place.

It is estimated that the duration of initial phase to be at least 4 months. This phase includes:

- Company incorporation with minimum required share capital
- Reaching tentative agreements with agri-food processing companies and bioethanol equipment suppliers (raw materials suppliers)
- Reaching tentative agreements with Pharmaceutical, Cosmetic, Research institutions, universities and chemical companies

After the successful completion of the initial phase, the final phase may start. The final phase is expected to take approximately 7 months, after which the operations may start. This phase includes:

- Increase the company's share capital through additional investments or partnerships.
- Securing funding for working capital needs through bank loans (e.g.: from Algeria Startup Fund).
- Rent offices/warehouse.
- Procuring necessary equipment's.
- Leasing vehicles for feedstocks collection and transportation.
- Hire personnel.
- Finalizing contract agreements with Pharmaceutical, Cosmetic, Research institutions, universities and chemical companies.

## BUSINESS PLAN

- Start-up operations.

**Table 5:** Start-up activities timeline.

	Activity description	Duration	Start	Finish
1	Bioethanol production start-up	370 days	1/10/2023	10/09/2024
2	<b>Company incorporation</b>			
3	Sign Incorporation Agreement	7 days	1/10/2023	8/10/2023
4	Increase Share Capital	2 days	8/10/2023	10/10/2023
5	<b>Bank Loans</b>			
6	Investigate Eligibility Criteria	10 days	10/10/2023	20/10/2023
7	Loan Application & Approval	30 days	20/10/2023	19/11/2023
8	<b>Rent Offices/Warehouse</b>			
9	Investigate Market	30 days	19/11/2023	19/12/2023
10	Rent Offices/Warehouse	1 day	19/12/2023	20/12/2023
11	Revamp Offices/Warehouse	15 days	20/12/2023	3/01/2024
12	<b>Purchase equipment</b>			
13	Investigate Market	10 days	3/01/2024	13/01/2024
14	Purchase Equipment	30 days	13/01/2024	12/02/2024
15	<b>Lease vehicles</b>			
16	Investigate Market	7 days	12/02/2024	19/02/2024
17	Lease Vehicles	7 days	19/02/2024	26/02/2024
18	<b>Collection activities licensing</b>			
19	Investigate Eligibility Criteria	30 days	26/02/2024	25/03/2024
20	Activities Licensing	20 days	25/03/2024	14/03/2024
21	<b>Contractual agreement- Agri-food processing companies and bioethanol equipment suppliers</b>			
22	Investigate eligible Collaborators	30 days	14/03/2024	15/04/2024
23	Sign a tentative agreement	10 days	15/04/2024	25/04/2024
24	Sign a contract agreement	5 days	25/04/2024	30/04/2024
25	<b>Contractual agreement- Pharmaceutical, Cosmetic, Research institutions, universities and chemical companies</b>			
26	Investigate Eligible Collaborators	60 days	30/04/2024	29/06/2024
27	Sign a Tentative agreement	30 days	29/06/2024	28/07/2024
28	Sign a Contract agreement	10 days	28/07/2024	7/08/2024
29	<b>Hire Personnel</b>			
30	Investigate Eligible Employees	30 days	7/08/2024	7/09/2024
31	Hire Accountant	1 day	7/09/2024	8/09/2024
32	Hire Secretary	1 day	8/09/2024	9/09/2024
33	Hire Drivers	1 day	9/09/2024	10/09/2024
34	<b>Start-Up Operations</b>			
35	Start Operations	1 day	10/09/2024	/

### **2.0 Innovative aspects**

The nature of innovation embedded in our project lies in its transformative approach towards sustainability and resource utilization. Unlike traditional methods that often rely on limited resources; our project leverages the vast potential of organic waste, a readily available and abundant source. This innovation stems from the convergence of cutting-edge technologies with environmentally conscious practices, addressing two critical challenges simultaneously: waste management and renewable energy production.

Our project stands at the forefront of innovation by introducing a circular economy model, where organic waste, which would have otherwise been discarded, is ingeniously repurposed into valuable bioethanol. This approach represents a paradigm shift in waste management strategies, transitioning from mere disposal to the creation of a renewable energy source.

### **2.1 Nature of innovations**

When it comes to innovation, there are different types of innovations that can be classified based on their nature. In the case of our project, we believe that it offers a unique blend of incremental and sustainable innovation.

#### **Sustainable innovation:**

Our project seeks out a sustainable approach to energy production by utilizing organic waste, which would otherwise be discarded and contribute to environmental pollution. This sustainable innovation demonstrates a profound commitment to environmental stewardship. Instead of letting organic waste perpetuate pollution, our approach ingeniously transforms it into a valuable products. By harnessing waste as a feedstock, we not only alleviate the strain on landfills but also significantly curtail the emission of harmful pollutants associated with waste degradation.

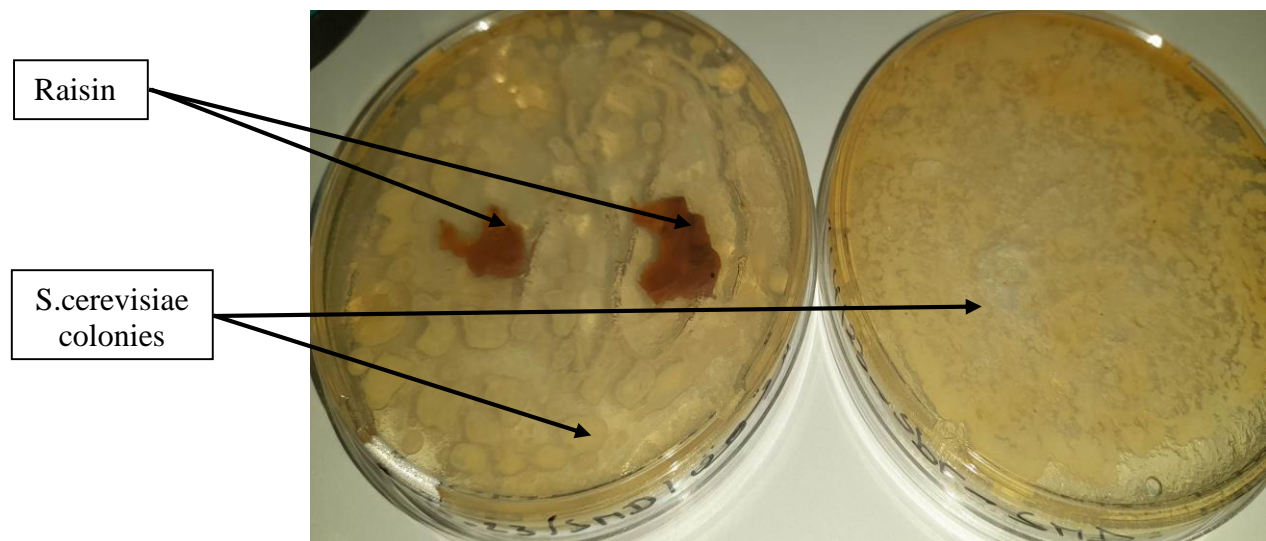
This approach of using waste to generate bioethanol, not only contributes to the reduction of organic waste pollution but also addresses the issue of ethanol shortage, generating a long-term social and environmental benefits while creating economic profits.

### Incremental innovation:

Incremental innovation, as a concept, involves making small, steady improvements to existing processes or technologies. It is about enhancing efficiency, often by optimizing specific elements, without completely redesigning the system. In our bioethanol production project, this philosophy guides our approach to refine the process.

Our project demonstrates incremental innovation as we have focused on enhancing the efficiency of bioethanol production. To accomplish that, we used our own yeast strain isolated from fermented raisins as shown in figure 7, Furthermore, we strategically introduced a new range of feedstock materials that had never been used to produce bioethanol in Algeria, including potato, orange, banana, and watermelon waste. By doing so, we not only broaden the sources of organic material available for bioethanol production but also introduce a diversification strategy that enhances the sustainability and resilience of our process. This innovative selection of feedstock aligns with our project's vision of reducing waste while producing a valuable biofuel, aiming to extract value from organic residues that were once overlooked.

The symbiotic interplay of these innovations has significantly elevated our bioethanol output. The tailored yeast strain optimizes fermentation, while diverse feedstock broadens the resource pool. Consequently, our incremental innovation have led to a remarkable surge in bioethanol yield.



**Figure 7:** *Saccharomyces cerevisiae* strain colonies on sabouraud broth.



### **2.2 Fields of innovations**

Our upcoming business venture is committed to providing advanced product attributes and services that meet changing market demands. This includes incorporation new processes, tailored ethanol purity options, and new offers.

#### **New process (additional value):**

In addition to the fact that we use a raw material (organic waste) which is plentiful and readily available at low cost or even, free of charge. Our project integrate new processes that increase profitability by optimizing operations and by diversifying sources of income. Contrary to other ethanol production companies, we have implemented place innovative strategies that go beyond the simple production and sale of ethanol:

- 1- Methanol Capture and Marketing through distillation of after fermentation broth.
- 2- Commercialization of fermentation residue as biofertilizer.

#### **New offers**

In recent years, the importance of sustainable agriculture has become increasingly apparent. In response, we have developed a unique product designed to replace traditional fertilizers with a more environmentally friendly alternative.

Traditionally, fermentation residue has been discarded as waste, contributing to environmental challenges. However, through research and development, we have harnessed the potential of fermentation residue as a powerful resource for Biofertilizer production. Our innovative approach involves repurposing the byproducts of bioethanol production, specifically the remnants of organic feedstocks, into nutrient-rich biofertilizers.

These biofertilizer, once considered waste, are now a key asset in promoting sustainable agriculture. Packed with essential nutrients, organic matter, and beneficial microorganisms, they enhance soil fertility, structure, and nutrient availability. By reintroducing these valuable elements back into the soil, we facilitate healthy plant growth, improved crop yields, and reduced dependence on traditional chemical fertilizers.

### New features

We understand that different industries have specific requirements for ethanol purity. Our project offers the flexibility to customize the purity levels of ethanol according to the needs of each industry. Whether it is 70%, 80%, or 90% purity, we can tailor our production process to deliver the desired ethanol specifications, providing top-quality ethanol suitable for a wide range of applications. This adaptability is amplified by our utilization of distillation techniques, allowing us to finely adjust ethanol purity levels to meet precise customer requirements.

### 3.0 Market analysis

#### 3.1 Market segment display

The company's potential marketing segments will be structured based on the product encompassing Bioethanol, Methanol, and Biofertilizers and their diverse applications. Each of these products serves distinct market segments, tailored to their specific needs and objectives.

Bioethanol: Ethanol finds numerous applications in various industries. In the pharmaceutical industry, it is used as a solvent in the production of medicines and pharmaceutical products. It is also widely used in the personal care and cosmetics industry, where it is used in the manufacture of perfumes, lotions, and shampoos. Furthermore, in the chemical industry, ethanol is a valuable solvent for many chemical reactions and manufacturing processes. It is used in the production of plastics, coatings, and resins. Moreover, ethanol is used in the food industry for various applications, contributing to the creation of alcoholic beverages, natural extracts, food preservatives, and bakery products. In addition to these applications, ethanol can be used in the transportation industry; it is utilized as a fuel for vehicles, available in its pure form (E100) or mixed with gasoline at different concentrations. These blends include E20 (20% ethanol, 80% gasoline), E5 (5% ethanol, 95% gasoline), and E2 (2% ethanol, 98% gasoline).

Methanol: Methanol serves as a vital feedstock for various chemical processes, meeting the requirements of chemical companies. It also serves industries looking to enhance combustion efficiency by using methanol as a fuel additive.

Biofertilizers: In agriculture, our biofertilizers are available to farmers and agricultural companies aiming to enhance soil fertility and boost crop yields naturally. They also align with environmental

solutions, supporting organizations committed to sustainable agriculture and reducing chemical fertilizer use.

The project is to serve selected segments in selected states at the beginning of the operations. The firm will focus on providing ethanol 70% purity level but mostly >95% purity level to regional pharmaceutical and parapharmaceuticals companies (e.g. GeoPharm, Biopharm, Wahran Pharma, SIDAL) and manufacturers of cosmetic products and detergents (e.g. SARL LINATOL, Lingex Algeria, Sabrinel laboratories, Venus) as key target markets.

As we venture out and expand our operations, our aim is to diversify our product offerings to cater to a broader spectrum of industries and clients. Research centers and universities will become a prominent target market, aligning with their scientific experiments and academic requirements. Additionally, we intend to collaborate closely with public hospital establishments, ensuring a reliable supply of ethanol for their needs, contributing to healthcare advancements. We also aim to establish partnerships with significant diesel distributors, such as NAFTAL, as ethanol can serve as an eco-friendly fuel option for vehicles, meeting the growing demand for cleaner transportation alternatives.

To ensure rapid growth, we plan to sell treated fermentation residue as biofertilizer to farmers and agricultural companies, and methanol to chemical companies (e.g. Algeria Chemical Specialties (ACS Group)). The company will have extra income from this additional business activity.

### **3.2 Competitor analysis**

The competitor analysis section of this business plan delves into the existing players within the industry, it is essential to assess the competitive environment thoroughly. Understanding the strengths and weaknesses of existing players in the market is a pivotal step in shaping our strategy for success. In this section, we will conduct a comprehensive competitor analysis, examining four key competitors in the bioethanol industry (detailed in Table 6). Furthermore, we will elucidate the advantages that set our future company apart from these competitors, paving the way for a clear understanding of our unique value proposition in the market.

**Table 6:** Competitor analysis.

<p><b>Competitors and Potential Competitors</b></p>	<p><b>1- Private ethanol production plant from date waste in Biskra, Algeria.</b></p>	<p><b>2- Egyptian Ethanol Company, a subsidiary of El Nasr Co. for Intermediate Chemicals (NCIC), produces ethanol from molasses, which is derived from sugar production.</b></p>
<p><b>Advantage Compared to Competitors</b></p>	<p>1- What sets us apart is, firstly, that we produce ethanol from organic waste (fruit and vegetable waste) that is abundantly available and generated daily, unlike our competitor whose raw material is limited and relies on the deterioration of a portion of the date harvest before production begins. Secondly, we plan to capture and sell the co-products generated during the bioethanol production process (methanol and fermentation residues as biofertilizers) alongside ethanol, which our competitor lacks.</p>	<p>2- Producing ethanol from organic waste offers distinct advantages compared to a competitor relying on molasses as a raw material. Firstly, by using organic waste, we have access to an abundant and renewable resource, ensuring a consistent supply. In contrast, the availability of molasses can be limited and subject to fluctuations. Secondly, ethanol production from organic waste contributes to waste management and environmental sustainability.</p>
<p><b>Competitors and Potential Competitors</b></p>	<p><b>3- An ethanol production plant in Britain (UK) using wheat as feedstock.</b></p>	<p><b>4- An ethanol plant using sugar, located in Cumra- Konya Seker, Turkey.</b></p>
<p><b>Advantage Compared to Competitors</b></p>	<p>3- The potential weakness of this competitor is that they use wheat in their ethanol production process, which is considered an expensive raw material in</p>	<p>4- What sets us apart from this competitor is our choice of raw material. Sugar, considered a vital staple for food security in societies, tends to become</p>

## BUSINESS PLAN

	its cultivation and a sensitive material in food security. In contrast, we utilize a low-cost, readily available, and renewable raw material (fruit and vegetable waste) in our production process.	scarce and expensive due to its extensive use. In contrast, we utilize an inexpensive and renewable raw material. Additionally, our distinctiveness extends to our strategy of maximizing the value of by-products generated during the fermentation process, such as methanol and biofertilizers, which we also plan to sell.
--	---	--

### 3.3 Marketing strategy

Our marketing strategy aims to establish strong distribution channels that ensure stability in production and sales. For this reason, we have planned the following marketing mix:

#### **Placement strategy (distribution)**

Our placement strategy, or distribution strategy, is a critical component of our business model. It ensures that our products reach customers efficiently and reliably.

In the initial phase, we will concentrate on serving local and regional markets, building strong relationships with nearby industries, research centers, and universities. This approach allows us to establish a solid customer base and ensure product consistency. As we expand our production capacity and product offerings, our distribution network will grow accordingly. We will gradually expand to national and international markets, enabling us to reach a broader clientele.

To make our products easily accessible, we will explore various distribution channels; direct and indirect channels, including direct sales through our own company retail stores, sales representatives, third-party distributors and wholesalers who purchase ethanol in bulk and resell it to retailers or end-users, and e-commerce platforms. This multi-channel approach enhances customer convenience.

### **Promotion and sales plan**

Our company has a long-term plan of opening distribution channels all around Saida province, which is why we will deliberately build our brand to be well accepted and recognized in Saida City before venturing out. As a matter of fact, our publicity and advertising strategy is not solely for winning customers over but to effectively communicate our brand. Here are the platforms we intend to leverage for promoting and advertising our company:

- Introduce our business by sending introductory letters and brochures to retailers, factories, facility managers, hotels, households and key stakeholders in and around Saida province.
- Place adverts on community based newspapers, radio and TV stations.
- Encourage the use of word of mouth publicity from our loyal customers.
- Leverage on the internet and social media platforms like; YouTube, Instagram, Facebook, Twitter and other platforms to promote our business.
- Position our banners and billboards in strategic places all around province of Saida.
- Distribute our fliers and handbills to create awareness about our business; these will include information about the company's contact details, web page address, the company's mission and the environmental impact of its activities.
- Brand all our official cars and distribution vans / trucks and ensure that all our staff wear our branded shirt or cap at regular intervals.

### **Pricing strategy**

To establish a profitable pricing strategy for our products, we plan to adopt a cost-plus pricing approach. This method involves calculating the total cost of producing and delivering the products, and adding a reasonable profit margin to determine the final price. The total cost includes the expenses incurred in the production process, such as raw materials, labor, and overhead costs, as well as marketing and distribution expenses. This pricing strategy allows us to cover our costs, earn a reasonable profit, and remain competitive in the market.

An exception is made for bioethanol, which we plan to sell it slightly lower than current market price. This is in order to insure rapid growth, and eliminate market risk. Additionally, we will offer volume discounts for bulk orders and establish long-term contracts with our customers to ensure stability in sales and revenue.

### **4.0 Production plan and organization**

#### **4.1 The production process**

The production of bioethanol is relatively simple from a technical standpoint, also allowing the construction of small-decentralized production units without excessive extra costs. We propose to secure a suitable site in the northwest of Algeria (Saida, Oran or Tlemcen). The ideal site would contain an existing manufacturing plant with equipment that can be adapted for use in bioethanol production. The ideal site would also have rail and truck access and storage suitable for feedstock, process chemicals, finished product and co-products. Our major intention is to keep levels of monthly production, sales, and collections together. For this reason, we planned storage capacities only for one month's production.

#### **Raw materials**

The selection of raw materials is crucial for both efficiency and environmental sustainability. Our primary raw materials include organic waste derived from fruits, vegetables, and agricultural residues. These materials are sourced from local farms, food processing facilities, and agricultural cooperatives. By utilizing organic waste as our raw material, we play a pivotal role in waste reduction and environmental preservation. Our partnerships with these suppliers ensure a steady and reliable source of raw materials.

#### **Manufacturing**

Our manufacturing process is designed to optimize resource utilization and minimize environmental impact. From the moment, organic waste enters our facility; it undergoes a series of carefully planned steps. The waste is first collected, sorted, and prepared, removing any non-organic materials. Then, it is finely ground and blended to create a homogeneous mixture. This step is essential for maximizing the surface area exposed to our proprietary yeast strain during fermentation. Specialized grinders and mixers are employed to achieve the desired consistency and quality of the mixture. Once the waste is properly prepared, it enters the fermentation process. We utilize industrial-scale fermenters equipped with precise temperature and pH control systems. Within these fermenters, our proprietary yeast strain is introduced into the mixture. The subsequent steps involve subjecting the mixture to distillation to separate ethanol from the fermentation broth. Our distillation process is carried out using industrial-grade distillation apparatus, including Alembic distillers.

After distillation, two products are recovered: Ethanol and a fermentation rest or residual product (whole stillage). The ethanol typically being an azeotropic mixture with water is further purified in the separation step by molecular sieving using a separation system. The outcome comprises high-quality ethanol and methanol. The residual product, also known as fermentation rest, undergoes further treatment involving decanting and drying, to end with a material, which is sold as biofertilizers.

### **Packaging**

The packaging for our products is designed to meet specific requirements. For ethanol and methanol, we will use standard industrial drums and containers, ensuring safety and compliance with regulations. These containers will be designed to meet industry standards for chemical products and will be labeled, sealed, and handled securely during transportation and storage.

Biofertilizers, being organic and environmentally friendly, will be packaged in bags or containers, keeping in line with sustainable practices. These packages will emphasize their natural and biodegradable characteristics to appeal to environmentally conscious customers.

### **4.2 Supply**

Our bioethanol production process relies on a dependable network of suppliers who play a crucial role in providing the organic wastes required as our primary raw materials. These suppliers encompass a diverse range of sources, including local agricultural producers, food processing facilities, and municipal waste management companies. Agricultural producers contribute fruit and vegetable waste, while food-processing facilities provide byproducts from their operations. Municipal waste management companies are instrumental in diverting organic waste from landfills to our production facility, ensuring both environmental sustainability and a consistent flow of raw materials. Our established relationships with these suppliers, built on trust and mutual benefit, underpin the foundation of our bioethanol production supply chain.

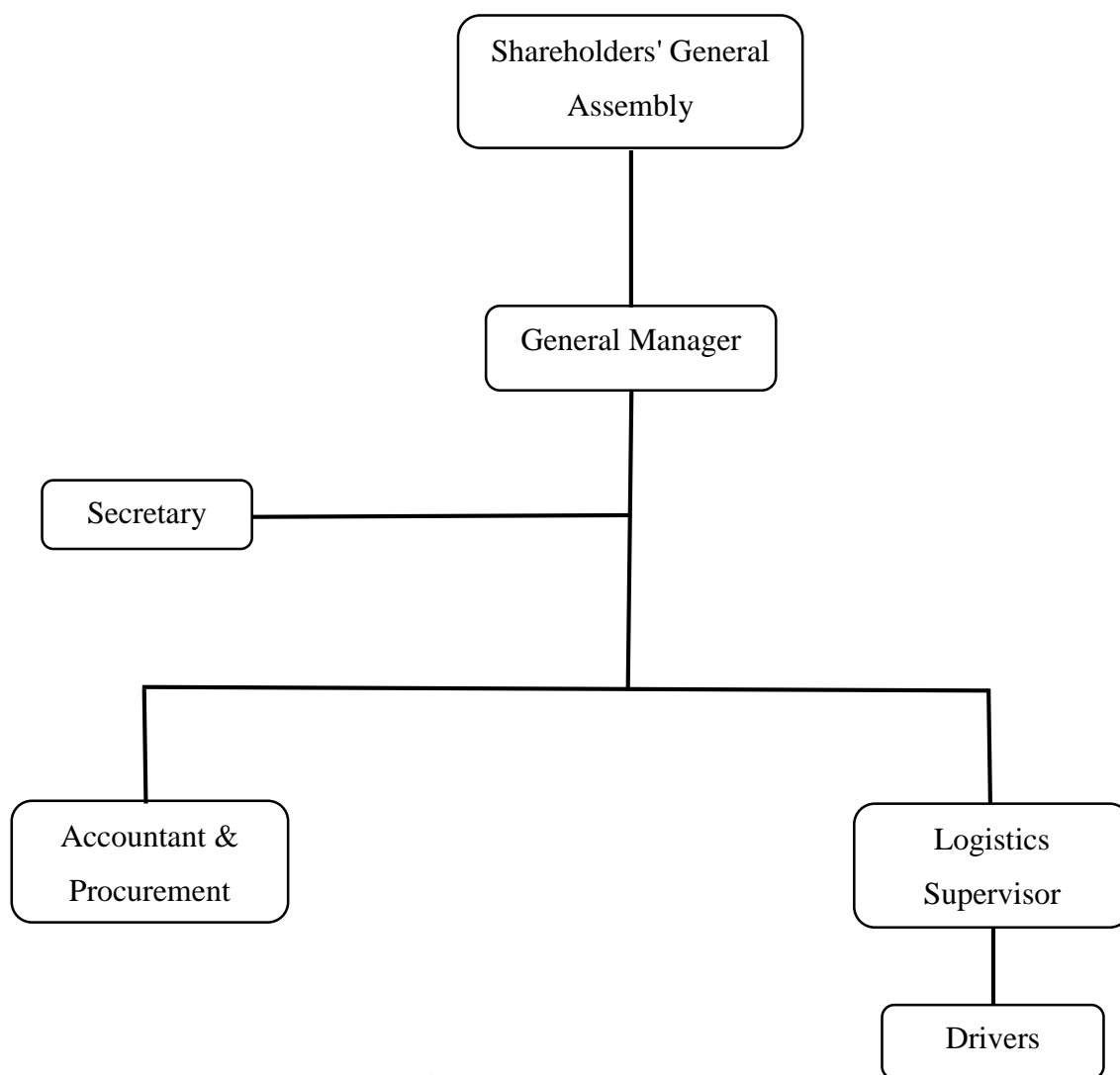
The purchase policy may involve bulk purchasing, contract agreements, and adherence to safety protocols. Payment terms are typically negotiated and can include upfront payments, credit terms, partial prepayments, or other arrangements based on mutual agreement. The payment policies established with these suppliers aim to ensure a smooth supply chain and foster strong



relationships. Factors such as order volume, supplier reputation, delivery schedules and long-term partnership potential are considered in negotiating favorable payment terms.

**4.3 Labor**

The projected company size is envisioned to be comprised of a workforce consisting of 10 employees. To effectively manage the operations and ensure smooth workflow, a proposed organizational structure is presented in figure 8. This structure delineates the various business units within the company and assigns specific responsibilities and tasks to each unit. The following sections outline the roles and duties associated with each business unit.



**Figure 8:** Organizational structure.

**The Shareholders' General Assembly:** plays a pivotal role in making significant decisions concerning the company's structure, form, the distribution of dividends and significant strategic decisions.

**The General Manager:** oversees the company's day-to-day operations, acts as the company's representative in interactions with authorities, supervises the accountant and procurement officer, and is responsible for managing the company's marketing activities.

**The Logistics Supervisor:** assumes responsibility for the efficient management of the company's logistics on a daily basis. This entails organizing and optimizing logistical processes and overseeing the implementation of certification systems to ensure compliance and quality control.

**The Accountant & Procurement Officer:** maintains accurate financial records using the double-entry accounting method. They manage and handle the company's procurement processes efficiently.

**The Secretary:** will offer comprehensive secretarial support to all mentioned officers and will also oversee the management of the company's call center.

**The Drivers:** will be responsible for the collection, transportation, and delivery of raw materials.

#### **4.4 Major partnerships**

We envision forging partnerships with prospective raw materials suppliers and packaging manufacturers. Regarding raw materials, we anticipate establishing relationships with a network of organic waste suppliers. These suppliers may encompass local agricultural producers, food processing facilities, and waste management entities. Through collaborative agreements, we will secure a consistent and reliable source of organic wastes, essential for our production. Simultaneously, we anticipate establishing a significant partnership with established packaging manufacturers specializing in drums, bags, and containers, designed to meet our specific needs. This collaboration will be pivotal in ensuring the efficient storage and transportation of our products.

The Company also plans to forge partnerships with reputable banks to secure necessary funding through bank loans and financial assistance. These collaborations will enable the company to invest in its production infrastructure, equipment, and working capital.

## 5.0 Financial plan

### 5.1 Cost and burdens

To accurately determine the funding needs for the proposed business initiative, a thorough assessment of expected cash inflows and outflows is essential. This analysis will be conducted by examining a five-year projection outlined in Table B- through table H of the Appendix. By reviewing these tables, a comprehensive estimation of the financial requirements can be made, enabling informed decision-making and effective financial planning for the business venture.

The following funding sources have been considered to cover the start-up investments:

1. Government funding opportunity that are available for start-up companies.
2. Bank loans.
3. Attracting investors.
4. Offering shares.
5. Financial savings.

See table I (Appendix)

### 5.2 Business number

Business number is the table that shows the sales of the total goods sold multiplied per unit selling price.

**Table 7:** Business number of bioethanol 99%.

	<b>Total (DZD)</b>				
	<b>Year</b>				
	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>
Quantity of the items sold (Liters)	162499910	194999910	238332890	324999910	433329900
Selling price per unit	3650	3650	3780	3840	3920
Global Business Figures (A)	593124671500	711749671500	900898324200	1247999654400	1698653208 000

## BUSINESS PLAN

**Table 8:** Business number of bioethanol 70%.

	<b>Total (DZD)</b>				
	<b>Year</b>				
	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>
Quantity of the items sold (Liters)	162499910	194999910	238332890	324999910	433329900
Selling price per unit	1500	1500	1680	1720	1800
Global Business Figures (B)	243749865000	292499865000	400399255200	558999845200	779993820000

**Table 9:** Business number of methanol.

	<b>Total (DZD)</b>				
	<b>Year</b>				
	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>
Quantity of the items sold (Liters)	64999910	77999910	95333100	129999880	173333110
Selling price per unit	800	800	800	800	800
Global Business Figures (C)	51999928000	62399928000	76266480000	103999904000	138666488000

## BUSINESS PLAN

**Table 10:** Business number of biofertilizers.

	<b>Total (DZD)</b>				
	<b>Year</b>				
	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>
Quantity of the items sold (Kg)	584999900	701999900	857999900	116999900	1559999900
Selling price per unit	60	60	70	78	80
Global Business Figures (D)	35099994000	42119994000	60059993000	9125992200	124799992000
Total Global Business Figures (A+B+C+D)	923974458500	1108769458500	1437624052400	1920125395800	2742113508000

### 5.3 Expected results calculation table

It is the summary statement of the charges and products achieved by the firm during the financial year, and it highlights profit/ gain or loss. See table J of the Appendix.

### 5.4 Treasury plan

The Treasury plan outlines the financial management strategies and practices implemented by our company to ensure effective cash flow management and optimal utilization of financial resources. It encompasses various aspects, including cash inflows and outflows, budgeting and liquidity management. The Treasury plan focuses on maintaining a healthy cash position, ensuring timely payments to suppliers and employees, managing debt and financing options, and optimizing investment opportunities. By closely monitoring and managing our company's cash flow, we aim to enhance financial stability, meet financial obligations, and support sustainable growth and development. See Table K of the Appendix.

## 6.0 Experimental prototype

### 6.1 Introduction

Yeast fermentation of organic waste (sugar-based feedstock's) is the method of our choice for producing bioethanol. It is a process in which the yeast performs fermentation to obtain energy by converting sugar into ethanol.

The raw material that was fruits waste (berries, banana, watermelon, apple) and vegetables waste (potato, carrot, beetroots, sugar beet) were collected from whole sale market and our houses, and as a yeast we used our own yeast strain (*Saccharomyces cerevisiae*) isolated from fermented raisins.

### 6.2 Materials and methods

#### 6.2.1 Materials

**Table 11:** Materials used in the production process.

Products	Materials
Fruits and vegetables waste (berries, banana, watermelon, apple, potato, carrot, beetroots, sugar beet wastes)	Distillation apparatus
Yeast strain ( <i>Saccharomyces cerevisiae</i> )	5L Stainless steel pressure cooker (cocotte)
10g Peptone	Copper tube
40g Glucose	Large plastic jug (5L)
15g Agar	Thermometer
	Plastic tubes
	Blender
	Autoclave
	Magnetic stirrer
	Balance

## BUSINESS PLAN

	Erlenmeyer flask
	Glass lab bottles
	Distilled water
	Alcohol (ethanol)
	Bleach
	12 Petri dish
	Platinum loop

### 6.2.2 Method

#### Preparation of Sabouraud Broth for Yeast Culture

The Sabouraud broth, a vital medium for yeast culture, was meticulously prepared to support the growth and vitality of yeast organisms, all equipment used in the making Sabouraud Broth were sterilized in autoclave at 121C° for 20 min. In a controlled and sterilized laboratory environment, 10g of peptone, 40g of glucose, and 15g of agar were accurately measured and combined. This mixture was then introduced into 400ml of distilled water, ensuring a precise blend of the ingredients. To achieve a homogeneous composition and complete dissolution of the ingredients, the concoction was stirred diligently using a magnetic stirrer at a temperature of 70°C for 20 min. Following this, the medium was put in glass lab bottles and allowed to cool down, reaching an optimal temperature suitable for yeast culture and then sterilized in autoclave before being use. The resulting Sabouraud broth was vital for providing the necessary nutrients and environment for the successful cultivation of yeast cells (75).

#### Isolation and Culturing of *Saccharomyces cerevisiae*

The isolation and culturing of *Saccharomyces cerevisiae*, a key step in this study began with the extraction of the yeast strain from fermented raisins. The yeast strain was carefully isolated, and a small sample was introduced into the prepared Sabouraud Broth. The culture was maintained at a controlled temperature of 30°C for an optimal duration of 24 hours, allowing the yeast to proliferate

and establish a robust population. This incubation period facilitated the growth and vitality of *Saccharomyces cerevisiae* (76).

### **Procedure of production: fermentation**

The preparation process of the waste material involves the collection and sorting of 3Kg of fruits and vegetables waste (FVW), with the exclusion of non-organic elements. Subsequently, the waste materials are finely ground and blended, forming a mixture. This blend is then subjected to heat at a temperature of 100°C for 45 minutes, supplemented by the addition of 500 ml of water. Through this process, a broth is generated.

A yeast mixture was prepared, using the previously isolated and cultured *Saccharomyces cerevisiae* strain, the yeast was then introduced into a 400 ml water solution at a temperature of 30-35 C°. The yeast mixture was then enclosed together with the homogenous mixture of FVW within a sealed plastic jug that has been sterilized using bleach followed by ethanol. This enclosure fosters anaerobic conditions, as visually depicted in figure 10. The fermentation was conducted at a temperature of 35°C, in an environment with a pH of 6.8. The progression of fermentation, spanning 15 days or until the cessation of CO<sub>2</sub> gas production is evident. (Note: The CO<sub>2</sub> is directed through a plastic tube into water within a plastic bottle to ensure airtight conditions) (77).



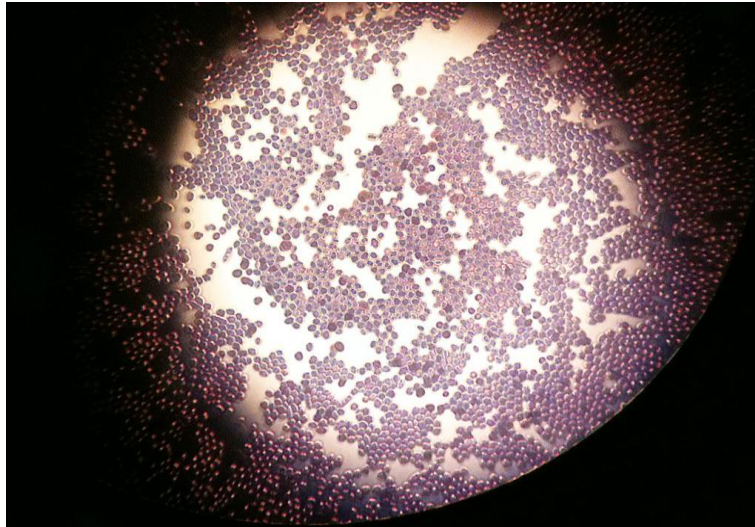
**Figure 9:** Fermentation of fruits and vegetables wastes broth in a sealed plastic jug.



### 6.3 Results

#### Yeast culture observation and characteristics

A portion of the cultured yeast was extracted and subjected to microscopic observation. This detailed analysis revealed essential characteristics of the yeast strain, such as morphology, cellular structure, and growth patterns, providing valuable insights into the cultivated *Saccharomyces cerevisiae* population.



**Figure 10:** *Saccharomyces cerevisiae* colonies on Sabouraud Broth under microscope 1000X.

*Saccharomyces cerevisiae*, a unicellular fungus, possesses distinctive features in morphology, cellular structure, and growth pattern. In terms of morphology, it typically manifests as oval or spherical cells, usually 3-4 micrometers in diameter when viewed under a microscope. On solid media, yeast colonies exhibit a creamy to white color, appearing smooth and round with a defined structure. Yeast cells often aggregate, forming clusters or short chains, particularly during active growth phases. Moving to cellular structure, yeast cells have a robust cell wall comprised of glucans and mannoproteins, imparting structural integrity and protection. Beneath the cell wall lies the cell membrane, a semi-permeable barrier essential for various cellular processes. *Saccharomyces cerevisiae* is a eukaryotic organism, featuring a well-defined nucleus that houses its genetic material (DNA). Concerning the growth pattern, budding is a common mode of reproduction, where a new daughter cell, known as a bud, emerges from the mother cell. The growth rate is influenced by factors like nutrient availability, temperature, and pH, typically following a logarithmic or exponential pattern during the log growth phase. During fermentation,

*Saccharomyces cerevisiae* metabolizes sugars anaerobically, converting them into ethanol and carbon dioxide. Understanding these cellular and growth characteristics is paramount for optimizing yeast growth and fermentation processes in bioethanol production (78; 79; 80).

### **Filtration and distillation procedures for the post-fermentation mixture:**

Upon the completion of the fermentation process, the resulting mixture (post-fermentation) is filtered. This is achieved by passing the mixture through a strainer and subsequently through a filter cloth as shown in figure 11. These filtration steps are essential to eliminate any solids or impurities (fermentation rest) that might be present in the mixture.

Subsequent to filtration, the procedure advances to distillation (see figure 12). The objective here is to segregate ethanol from the mixture. This is achieved by applying heat to the fermented solution, causing ethanol to vaporize. The vaporized ethanol is then collected through a process of condensation. Notably, the vaporization temperature of ethanol stands at 68°C (81).



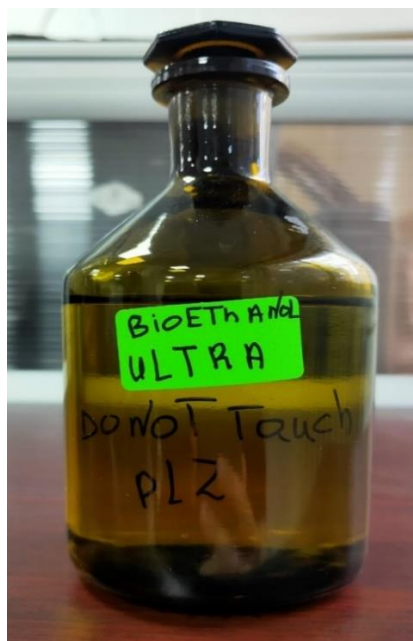
**Figure 11:** filtration process of the resulting mixture.



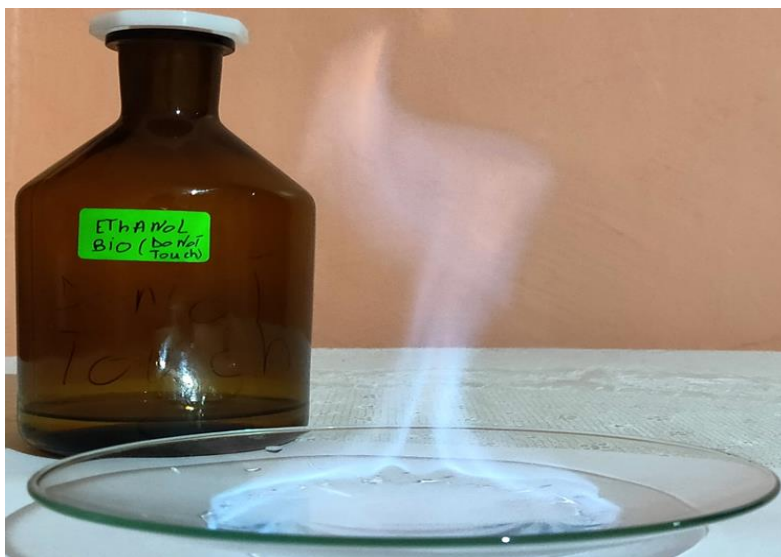
**Figure 12:** Bioethanol distillation process.

Following the processes of filtration and distillation, 450 ml of bioethanol was generated from the preceding experiment; the ethanol yield was calculated by using the formula below, which was found to be 150 g/l. Images depicting the resulting ethanol are presented below (refer to figure 13 and figure 14).

$$Y E/S = \frac{\text{Ethanol produced}}{\text{Substrate}}$$



**Figure 13:** Our product “Bioethanol”.



**Figure 14:** Bioethanol flammability test.

#### **6.4 Discussion**

*Saccharomyces cerevisiae* is chosen for bioethanol production due to its high efficiency in converting sugars to ethanol, ensuring a substantial ethanol yield. It possesses a remarkable tolerance to elevated ethanol concentrations, a critical attribute for maintaining fermentation performance. This yeast is known for its robust and consistent fermentations across diverse conditions, it can thrive in a wide range of conditions, including varying pH levels and temperatures, making it reliable for large-scale bioethanol production. *Saccharomyces cerevisiae* exhibits versatility in utilizing various sugars commonly present in organic waste and agricultural residues. Its track record of successful industrial usage, generating minimal unwanted byproducts, adds to its appeal. In summary, *Saccharomyces cerevisiae* is an ideal microorganism for bioethanol production, highlighted by its excellent fermentative ability, ethanol tolerance, well-understood metabolism, adaptability, and compatibility with industrial-scale processes (77).

The choice of fermentation conditions, specifically a temperature of 35°C and a pH of 6.8, was a critical aspect of our bioethanol production process. Fermentation temperature plays a pivotal role in the metabolic activity of yeast during ethanol production. A temperature of 35°C was chosen as it optimally supports yeast metabolism, enhancing their efficiency in converting sugars from organic waste into ethanol. This temperature strikes a balance, ensuring a robust fermentation process without causing thermal stress on the yeast. Additionally, maintaining a pH of 6.8 in the fermentation environment is vital for yeast health and activity. Yeast exhibits optimal enzymatic

activity and ethanol production around a neutral pH. A pH of 6.8 provides an environment conducive to yeast fermentation, promoting a steady and efficient conversion of sugars into ethanol. These chosen fermentation conditions were based on established literature and preliminary experiments. The aim was to create an environment that maximizes ethanol yield while ensuring the health and activity of the yeast culture throughout the fermentation period.

Our bioethanol yield of 450 ml from 3 kg of organic waste aligns closely with results reported by (82) in their study on bioethanol production from a similar feedstock, which is food wastes. In their research, a comparable yield was obtained using a slightly modified fermentation process. They have conducted fermentation using *Saccharomyces cerevisiae*, at ambient temperature (was not specified). The maximum quantity of distilled ethanol after final distillation was found to be 496 ml after fermentation duration of 14 days, which is closely similar to ours. This suggests that our approach is consistent with established findings in the field, emphasizing the reliability and validity of our results.

In contrast to our study, which utilized organic waste as the feedstock, a separate study (83) focused on bioethanol production from lignocellulosic feedstock. Sugarcane bagasse, wheat straw, water hyacinth, ragi, and rice straw were selected as lignocellulosic feedstock for producing ethanol. They were pretreated with mild, dilute acid using 2% H<sub>2</sub>SO<sub>4</sub>, followed by enzymatic saccharification using locally isolated fungal species *Aspergillus niger* to convert complex polysaccharide into simple sugar. Then they optimized the reaction condition with the help of chemically predefined media at temperature of 38°C and pH 4.8. Fermentation was carried using yeast *Saccharomyces cerevisiae* at temperature of 35°C under anaerobic conditions. Maximum ethanol yield was obtained with sugarcane bagasse around 11.90 g/l followed by wheat straw 9.56 g/l, rice straw 8.84 g/l, ragi straw 7.01 g/l, and water hyacinth 6.19 g/l of ethanol in fermentation. This study bioethanol yield remained comparatively lower than our yield from organic waste. This discrepancy in yield could be attributed to multiple factors. The inherent composition and structure of organic waste might render it more amenable to fermentation, thus achieving a higher bioethanol yield. Furthermore, the complexity and energy-intensiveness of the pre-treatment process lignocellulosic feedstock could have potentially affected the overall efficiency and yield of bioethanol. This comparison highlights the potential and efficiency of utilizing organic waste as a feedstock for bioethanol production. The relatively simpler pre-

processing requirements and higher bioethanol yield position organic waste as a promising feedstock.

### **6.5 Conclusion**

In conclusion, the utilization of organic wastes for the production of bioethanol emerges as a promising and sustainable approach, effectively addressing both the national demand for ethanol and contributing to solid waste management. Numerous studies within this domain have demonstrated notable success, achieving high ethanol yields within few steps. The simplicity of the entire procedure, from initiation to completion, sets it apart from alternative methods.

With organic wastes serving as a suitable substrate, the recycling of organic matter becomes not only feasible but also environmentally advantageous. Consequently, the bioconversion of organic wastes into ethanol through yeast fermentation presents itself as a viable and uncomplicated option, offering potential scalability for widespread implementation. This innovative approach stands at the intersection of efficient ethanol production and responsible waste management, paving the way for a more sustainable and eco-friendly future.



**General conclusion**

### **General conclusion**

In conclusion, bioethanol represents a crucial stride towards a sustainable energy landscape, presenting itself as a sustainable energy source due to its many positive properties (biodegradability, less toxicity, renewability). As elucidated in the theoretical background, the significance of bioethanol cannot be overstated. Its capacity to mitigate carbon emissions, utilize organic waste as a valuable resource, and contribute to a greener future underscores its vital role in the global pursuit of renewable energy solutions. Transitioning from the theoretical background to practical application, our business plan for a bioethanol production company stands as a testament to the potential of bioethanol as a commercially feasible venture. It envisions not only economic growth through bioethanol sales but also a positive societal and environmental impact. Our targeted market segments, encompassing a wide array of industries, underline the versatility and demand for sustainable products. Financially, our projections illustrate promising returns, signifying the potential viability of the proposed business model. This sustains the notion that environmental sustainability and economic prosperity can indeed coexist and even reinforce each other.

The production of bioethanol from organic waste represents a transformative force with significant societal and economic impact. On the societal front, this project is a leader for waste reduction and community health. By curbing the volume of organic waste and mitigating environmental concerns, it contributes to a cleaner, healthier environment. From an economic perspective, the project stands as an engine of growth. It generates new employment opportunities, fostering job creation in various stages of bioethanol production. Moreover, it nurtures small businesses that rely on ethanol as a fundamental raw material, providing them with the stability and resources needed for expansion. The economic impact extends further, reducing the nation's dependence on imported ethanol. This not only bolsters economic resilience but also holds the potential to trim the import bill, facilitating broader economic growth. In its commitment to sustainability, the project adheres to the principles of a circular economy. It demonstrates how waste can be transformed into a valuable resource.

While this thesis has made significant strides in understanding the production, properties, and market potential of bioethanol, there are avenues for further exploration. Future research could focus on optimizing the production process, investigating new feedstock sources, and exploring emerging markets for bioethanol



# References

## References

1. Algeria ECO. [Online] 01 18, 2022. [Cited: 08 23, 2023.] <https://www.algerie-eco.com/2022/01/18/dechets-le-recyclage-optimal-peut-generer-92-milliards-da-par-an/>.
2. Developments in bioethanol. BAJPAI, Pratima. s.l. : Springer Nature, 2021.
3. Bioethanol: Science and technology of fuel alcohol. WALKER, Graeme M. s.l. : Bookboon, 2010.
4. Food Waste to Green Fuel: Trend & Development. SRIVASTAVA, Neha et MALIK, Maqsood Ahmad (ed). s.l. : Springer, 2022.
5. Bioalcohol as green energy-A review. SHAH, Yatri R. et SEN, Dhruvo Jyoti. no 2 , s.l. : Int J Cur Sci Res, 2011, Vol. 1. p. 57-62.
6. Bioethanol: An Overview of Current Status and Future Direction. AGGARWAL, Neeraj K., KUMAR, Naveen, et MITTAL, Mahak. s.l. : Bioethanol Production: Past and Present, 2022. p. 1-15.
7. Advances in bioethanol. BAJPAI, Pratima. s.l. : Springer Science & Business Media, 2013.
8. Life cycle assessment of second generation ethanol derived from banana agricultural waste: Environmental impacts and energy balance. GUERRERO, Ana Belén et MUÑOZ, Edmundo. p. 710-717, s.l. : Journal of Cleaner Production, 2018, Vol. 174.
9. Bioethanol production from fruit and vegetable wastes. BHUVANESWARI, Meganathan et SIVAKUMAR, Nallusamy. p. 417-427, s.l. : Bioprocessing for biomolecules production, 2019.
10. Effect of pH on ethanol-type acidogenic fermentation of fruit and vegetable waste. WU, Yuanyuan, WANG, Cuiping, ZHENG, Mingyue, et al. p. 158-163, s.l. : Waste Management, 2017, Vol. 60.
11. Pervaporation of ethanol produced from banana waste. BELLO, Roger Hoel, LINZMEYER, Poliana, FRANCO, Cláudia Maria Bueno, et al. no 8, s.l. : Waste management, 2014, Vol. 34. p. 1501-1509.

## REFERENCES

12. High value-added products from the orange juice industry waste. CYPRIANO, Daniela Z., DA SILVA, Lucimara Lopes, et TASIC, Ljubica. p. 71-78, s.l. : Waste Management, 2018, Vol. 79.
13. Estimation of sugar and bio ethanol from different decaying fruits extract. GIRISH, Venkatachalapathy, KUMAR, Krishnappa Ravi, et GIRISHA, Sirangala Thimmappa. no 1, s.l. : Advances in Applied Science Research, 2014, Vol. 5 . p. 106-110.
14. Bioethanol production from date palm fruit waste fermentation using solar energy. BOULAL, Ahmed, KIHAL, Mabrouk, KHELIFI, Cherif, et al. no 30, s.l. : African journal of biotechnology, 2016, Vol. 15. p. 1621-1627.
15. Crops that feed the world 8: potato: are the trends of increased global production sustainable? BIRCH, Paul RJ, BRYAN, Glenn, FENTON, Brian, et al. p. 477-508, s.l. : Food Security, 2012, Vol. 4.
16. Simulation of fuel ethanol production from potato tubers . TASIĆ, Marija B. et VELJKOVIĆ, Vlada B. no 11, s.l. : Computers & chemical engineering, 2011, Vol. 35. p. 2284-2293.
17. Influence of steam-peeled potato-processing waste inclusion level in beef finishing diets: effects on digestion, feedlot performance, and meat quality. RADUNZ, A. E., LARDY, G. P., BAUER, M. L., et al. no 11, s.l. : Journal of animal science, 2003, Vol. 81. p. 2.
18. Enhancement of ethanol production from potato-processing wastewater by engineering *Escherichia coli* using *Vitreoscilla haemoglobin*. ABANOZ, K., STARK, B. C., et AKBAS, M. Y. no 6, s.l. : Letters in applied microbiology, 2012, Vol. 55. p. 436-443.
19. Ethanol production from waste potato mash by using *Saccharomyces cerevisiae*. Izmirlioglu G, Demirci A. pp 1571–1581, s.l. : In: American Society of Agricultural and Biological Engineers Annual, 2010, Vol. 2.
20. Enzymatic hydrolysis and ethanol fermentation of by-products from potato processing plants. YAMADA, Sunao, SHINOMIYA, Noriyuki, OHBA, Kiyoshi, et al. no 6, s.l. : Food science and technology research, 2010, Vol. 15. p. 653-658.
21. Cheese whey management: A review. PRAZERES, Ana R., CARVALHO, Fátima, et RIVAS, Javier. s.l. : Journal of environmental management, 2012, Vol. 110. p. 48-68.

## REFERENCES

22. Whey and whey proteins—From ‘gutter-to-gold. SMITHERS, Geoffrey W. no 7, s.l. : International dairy journal, 2008, Vol. 18. p. 695-704.
23. Fermentation of lactose to bio-ethanol by yeasts as part of integrated solutions for the valorisation of cheese whey. Guimarães PMR, Teixeira JA, Domingues L. no.3, s.l. : Biotechnol Adv, 2010, Vol. 28. P.375–384.
24. Possibilities of whey utilisation. BOŽANIĆ, Rajka, BARUKČIĆ, Irena, et LISAK, Katarina. no 7, s.l. : Austin journal of nutrition and food sciences, 2014, Vol. 2. p. 7.
25. The biotechnological utilization of cheese whey: a review. SISO, MI González. no 1 , s.l. : Bioresource technology , 1996, Vol. 57. p. 1-11.
26. Biofuel production from food wastes. LI, S. et YANG, X. s.l. : In : Handbook of biofuels production. Woodhead Publishing, 2016. p. 617-653.
27. Utilization of cheese whey powder (CWP) for ethanol fermentations: Effects of operating parameters . KARGI, Fikret et OZMIHCI, Serpil. no 5, s.l. : Enzyme and Microbial Technology, 2006, Vol. 38. p. 711-718.
28. Comparison of ethanol production from cheese whey permeate by two yeast strains. KOUSHKI, Mohammadreza, JAFARI, Mojtaba, et AZIZI, Mohammadhosein. s.l. : Journal of Food Science and Technology, 2012, Vol. 49. p. 614-619.
29. Ethanol production from crude whey by *Kluyveromyces marxianus*. ZAFAR, Salman et OWAIS, Mohammad. no 3, s.l. : Biochemical engineering journal, 2006, Vol. 27. p. 295-298.
30. Metabolic engineering of *Saccharomyces cerevisiae* for lactose/whey fermentation. DOMINGUES, Lucília, GUIMARÃES, Pedro MR, et OLIVEIRA, Carla. no 3, 2010, Vol. 1. p. 164-171.
31. Optimization of the bioethanol production on sweet cheese whey by *Saccharomyces cerevisiae* DIV13-Z087C0VS using response surface methodology (RSM). BOUDJEMA, Khaled, FAZOUANE-NAIMI, Fethia, HELLAL, Amina, et al. no 5, s.l. : Romanian Biotechnological Letters, 2015, Vol. 20. p. 10814.

## REFERENCES

32. Review of pretreatment processes for lignocellulosic ethanol production, and development of an innovative method. CHIARAMONTI, David, PRUSSI, Matteo, FERRERO, Simone, et al. p. 25-35, s.l. : Biomass and bioenergy, 2012, Vol. 46.
33. Utilization of Fruit-Vegetable Waste as Lignocellulosic Feedstocks for Bioethanol Fermentation. In : Food Waste to Green Fuel: Trend & Development. VERMA, Manisha et MISHRA, Vishal. s.l. : Singapore : Springer Nature Singapore, 2022. p. 189-211.
34. Pretreatment of lignocellulosic wastes for biofuel production: A critical review. KUMARI, Dolly et SINGH, Radhika. p. 877-891, s.l. : Renewable and Sustainable Energy Reviews, 2018, Vol. 90.
35. Biogas production potential and kinetics of microwave and conventional thermal pretreatment of grass. LI, Lianhua, KONG, Xiaoying, YANG, Fuyu, et al. p. 1183-1191, s.l. : Applied biochemistry and biotechnology, 2012, Vol. 166.
36. Bioethanol production potential of *Andropogon gayanus*. BAGUDO, B. U., DANGOGGO, S. M., USMAN, J., et al. no 1, s.l. : Annals of Biological Research, 2014, Vol. 5. p. 106-111.
37. Methanol production from cow dung. AJAYI, Olusegun A., ADEFILA, Sam S., et al. no 7, s.l. : Journal of Environment and Earth Science, 2012, Vol. 2. p. 2225-0948.
38. Enhanced methane production from algal digestion using free nitrous acid pre-treatment. BAI, Xue, LANT, Paul A., JENSEN, Paul D., et al. s.l. : Renewable energy, 2016, Vol. 88. p. 383-390.
39. Pretreatment conditions of rice straw for simultaneous hydrogen and ethanol fermentation by mixed culture. SEN, Biswarup, CHOU, Yen-Ping, WU, Shu-Yii, et al. no 7, s.l. : international journal of hydrogen energy, 2016, Vol. 41. p. 4421-4428.
40. Feasibility of rice straw as alternate substrate for biobutanol production. RANJAN, Amrita, KHANNA, Swati, et MOHOLKAR, V. S. s.l. : Applied energy, 2013, Vol. 103. p. 32-38.
41. Biobutanol production from 2-year-old willow biomass by acid hydrolysis and acetone-butanol-ethanol fermentation. HAN, S.-H., CHO, D. H., KIM, Yong Hwan, et al. s.l. : Energy, 2013, Vol. 61. p. 13-17.

## REFERENCES

42. Liquid hot water and alkaline pretreatment of soybean straw for improving cellulose digestibility. WAN, Caixia, ZHOU, Yuguang, et LI, Yebo. s.l. : Bioresource technology , 2011, Vol. 102. p. 6254-6259.
43. Biological pretreatment of lignocellulosic biomass—An overview. SINDHU, Raveendran, BINOD, Parameswaran, et PANDEY, Ashok. s.l. : Bioresource technology, 2016, Vol. 199. p. 76-82.
44. Isolation and characterization of cellulose from different fruit and vegetable pomaces. Polymers. SZYMAŃSKA-CHARGOT, Monika, CHYLIŃSKA, Monika, GDULA, Karolina, et al. no 10, 2017, Vol. 9. p. 495.
45. Sugarcane spirits (cachaça) quality assurance and traceability: an analytical perspective. In : Production and management of beverages. SERAFIM, Felipe AT et LANÇAS, Fernando M. s.l. : Woodhead Publishing, 2019. p. 335-359.
46. Ethanol fermentation. In: Santos, F., Caldas, C., & Borém, A., (eds.), Sugarcane: Agricultural Production, Bioenergy, and Ethanol. DE VASCONCELOS, João Nunes. s.l. : Elsevier Inc, 2015. p. 311-340.
47. Improving ethanol and xylitol fermentation at elevated temperature through substitution of xylose reductase in *Kluyveromyces marxianus*. ZHANG, Biao, LI, Lulu, ZHANG, Jia, et al. no 3-4, s.l. : Journal of Industrial Microbiology and Biotechnology, 2013, Vol. 40. p. 305-316.
48. WALKER, Graeme M. Bioethanol: Science and technology of fuel alcohol. s.l. : Bookboon, 2010.
49. Biochemical production of bioethanol. In: Luque R., Campelo, J., & Clark, J., (eds.). Arshadi, M., & Grundberg, H. s.l. : Handbook of Biofuels Production, Woodhead Publishing Limited, 2011. pp. 199–220.
50. Yeast bioprospecting versus synthetic biology—which is better for innovative beverage fermentation? ALPERSTEIN, Lucien, GARDNER, Jennifer M., SUNDSTROM, Joanna F., et al. no 5, s.l. : Applied microbiology and biotechnology, 2020, Vol. 104. p. 1939-1953.

## REFERENCES

51. *Saccharomyces cerevisiae* bio-ethanol production, a sustainable energy alternative. AKHTAR, N., KARNWAL, A., UPADHYAY, A. K., et al. s.l. : Asian J. Microbiol. Biotechnol. Environ. Sci, 2018, Vol. 20. p. S200-S204.
52. Metabolic engineering of *Saccharomyces cerevisiae*: a key cell factory platform for future biorefineries. HONG, Kuk-Ki et NIELSEN, Jens. s.l. : Cellular and Molecular Life Sciences, 2012, Vol. 69. p. 2671-2690.
53. High-temperature ethanol production using thermotolerant yeast newly isolated from Greater Mekong Subregion. TECHAPARIN, Atiya, THANONKEO, Pornthap, et KLANRIT, Preekamol. s.l. : Brazilian journal of Microbiology, 2017, Vol. 48. p. 461-475.
54. Application of the severity factor and HMF removal of red macroalgae *Gracilaria verrucosa* to production of bioethanol by *Pichia stipitis* and *Kluyveromyces marxianus* with adaptive evolution. SUKWONG, Pailin, SUNWOO, In Yung, LEE, Min Ju, et al. s.l. : Applied biochemistry and biotechnology, 2019, Vol. 187. p. 1312-1327.
55. Methods and tools for sustainable chemical process design. In : Assessing and measuring environmental impact and sustainability. GARGALO, Carina L., CHAIRAKWONGSA, Siwanat, QUAGLIA, Alberto, et al. s.l. : Butterworth-Heinemann, 2015. p. 277-321.
56. Improvement of ethanol production by ethanol-tolerant *Saccharomyces cerevisiae* UVNR56. THAMMASITTIRONG, Sutticha Na-Ranong, THIRASAKTANA, Thanawan, THAMMASITTIRONG, Anon, et al. no 1, s.l. : SpringerPlus, 2013, Vol. 2. p. 1-5.
57. Physiology of Ethanol Production by Yeasts. In : Bioethanol. GLORIA, Miriam Soledad Valenzuela, ALVA-SÁNCHEZ, Diana Laura, ESCAREÑO, MP Luévanos, et al. s.l. : Apple Academic Press, 2022. p. 1-20.
58. Design and physical features of sequencing batch reactors . KETCHUM JR, Lloyd H. no 1, s.l. : Water Science and Technology, 1997, Vol. 35. p. 11-18.
59. Progress and challenges in large-scale expansion of human pluripotent stem cells . KROPP, Christina, MASSAI, Diana, et ZWEIGERDT, Robert. s.l. : Process Biochemistry , 2017, Vol. 59. p. 244-254.

## REFERENCES

60. Continuous ethanol production with a membrane bioreactor at high acetic acid concentrations. YLITERVO, Päivi, FRANZÉN, Carl Johan, et TAHERZADEH, Mohammad J. no 3, s.l. : Membranes, 2014, Vol. 4. p. 372-387.
61. Ethanol fermentation integrated with PDMS composite membrane: An effective process. FU, Chaohui, CAI, Di, HU, Song, et al. s.l. : Bioresource technology, 2016, Vol. 200. p. 648-657.
62. Design and Engineering Parameters of Bioreactors for Production of Bioethanol. LAFUENTE-RINCÓN, David Francisco, HERNÁNDEZ-DE LIRA, Inty Omar, HERNÁNDEZ-ESCOTO, Héctor, et al. s.l. : Bioethanol: Biochemistry and Biotechnological Advances , 2022. p. 233.
63. Lignocellulosic ethanol: technology and economics . ZHANG, Cheng. s.l. : Alcohol fuels-current technologies and future prospect, 2019.
64. Chapter 16–Fermentation, beer, and biofuels . GODBEY, W. s.l. : An Introduction to Biotechnology, 2014. p. 331-351.
65. Emerging techniques in bioethanol production: from distillation to waste valorization. GAVAHIAN, Mohsen, MUNEKATA, Paulo ES, EŞ, Ismail, et al. no 6, s.l. : Green Chemistry, 2019, Vol. 21. p. 1171-1185.
66. Ethanol, fruit ripening, and the historical origins of human alcoholism in primate frugivory. DUDLEY, Robert. no 4, s.l. : Integrative and comparative biology, 2004, Vol. 44. p. 315-323.
67. Microbial processing of fruit and vegetable wastes into potential biocommodities: a review. PANDA, Sandeep K., RAY, Ramesh C., MISHRA, Swati S., et al. no 1, s.l. : Critical Reviews in Biotechnology, 2018, Vol. 38. p. 1-16.
68. biofuel policy, biofuel economy and global biofuel projections. DEMIRBAS, Ayhan. Biofuels sources. no 8, s.l. : Energy conversion and management, 2008, Vol. 49. p. 2106-2116.
69. Use of biofuels to produce hydrogen (reformation processes). DE LA PISCINA, Pilar Ramírez et HOMS, Narcís. no 11, s.l. : Chemical Society Reviews, 2008, Vol. 37. p. 2459-2467.
70. Dehydration of bioethanol by hybrid process liquid–liquid extraction/extractive distillation. AVILÉS MARTÍNEZ, Adriana, SAUCEDO-LUNA, Jaime, SEGOVIA-HERNANDEZ, Juan Gabriel, et al. no 17, s.l. : Industrial & engineering chemistry research, 2012, Vol. 51. p. 58.



## REFERENCES

71. Production of bioethanol from lignocellulosic materials via the biochemical pathway: a review. BALAT, Mustafa. no 2, s.l. : Energy conversion and management, 2011, Vol. 52. p. 858-875.
72. Fermentation biotechnology. OWEN, P. 1991.
73. Bioethanol. PIRA Technology Report, Smithers PIRA. BAJPAI, P. UK : s.n., 2007.
74. FUELS, Green. Environmental effects of ethanol and gasoline. 1998.
75. technical data. EXODO CIENTIFICA. [Online] [Cited: 10 03, 2023.] [https://exodocientifica.com.br/\\_technical-data/M033.pdf](https://exodocientifica.com.br/_technical-data/M033.pdf).
76. Isolation of yeasts from raisins and palm-juice and ethanol production in molasses medium. SHAMIM, Rahman Shafkat, ISLAM, Sarkar Mohammad Khairul, RAFIQUL, Islam Mohammad, et al. no 12, s.l. : Indian Journal of Science and Technology, 2016, Vol. 9. p. 1-8.
77. Bio-Ethanol Production from Fruit and Vegetable Waste by Using *Saccharomyces cerevisiae*. MONERUZZAMAN KHANDAKER, M., ALIYU ABDULLAHI, U., DOGARA ABDULRAHMAN, M., et al. s.l. : Bioethanol Technologies IntechOpen, 2021.
78. *saccharomyces cerevisiae* characteristics, morphology, life cycle. warbleton council. [Online] 10 01, 2023. [Cited: 10 03, 2023.] <https://warbletoncouncil.org/saccharomyces-cerevisiae-8359>.
79. Cell size and morphological properties of yeast *Saccharomyces cerevisiae* in relation to growth temperature. ZAKHARTSEV, Maksim et REUSS, Matthias. no 6, s.l. : FEMS yeast research, 2018, Vol. 18. p. foy052.
80. Characteristics of *Saccharomyces cerevisiae* yeasts exhibiting rough colonies and pseudohyphal morphology with respect to alcoholic fermentation. REIS, Vanda Renata, BASSI, Ana Paula Guarnieri, SILVA, Jessica Carolina Gomes da, et al. s.l. : Brazilian Journal of Microbiology, 2013, Vol. 44. p. 1121-1131.
81. Bio-Ethanol Production from Fruit and Vegetable Waste by Using *Saccharomyces cerevisiae*. MONERUZZAMAN KHANDAKER, M., ALIYU ABDULLAHI, U., DOGARA ABDULRAHMAN, M., et al. s.l. : Bioethanol Technologies. IntechOpen, 2021.

## REFERENCES

82. Production of Bioethanol from Food Waste. Akash Alwandi, Dr. Shivasharanappa, Dr. Shashikant R. Mise. s.l. : International Research Journal of Engineering and Technology, 2021, Vol. 8.

83. Production of Bioethanol from Lignocellulosic Biomass. C. T. Puttaswamy, Bipin R. Sagar, Udaya Simha, S. Manjappa, C. S. Vinod Kumar. 239-244, s.l. : Indian journal of advances in chemical science, 2016.

## **Appendix – Supplementary tables**

**Appendix – Supplementary tables**

**Supplementary table A: Business model canvas**

<b>Key Partners</b>	<b>Key Activities</b>	<b>Value Propositions</b>	<b>Customer Relationships</b>	<b>Customer Segments</b>
-Government -Packaging manufacturers	-Collection of raw materials (fruit and vegetables waste). -Manufacturing of bioethanol. -Quality tests for ethanol. -Sales and distribution.	-Competitive price (60% less than average price). -Produce High quality product (purity). -Customized: purity. -Organic waste reduction.	-Dedicated personal assistance -Long term focused relation. -Loyalty programs: implement loyalty programs to reward repeat customers and offer exclusive offers.	<u>Industries:</u> Chemicals and petro-chemicals Pharmaceutical and para-Pharmaceutical Detergent Cosmetic and perfumes Food and beverages <u>Research institutions and universities</u> <u>Farmers</u>
	<b>Key Resources</b>		<b>Channels</b>	
	-Human resources quality control technicians...) -Financial ressources (Bank loans) -Intellectuel ressources (patent application)		-Sales representatives. -Free samples for evaluation. -Our own company retail stores. -Distributors. -Website / Social Media.	

APPENDIX – SUPPLEMENTARY TABLES

<b>Cost Structure</b>	<b>Revenue Streams</b>
<ul style="list-style-type: none"> <li>-Raw materials</li> <li>-Materials</li> <li>-Process bill (water and electricity...)</li> <li>-Workers salary</li> <li>-Warehouse rent cost.</li> <li>-Sales and marketing</li> </ul>	<ul style="list-style-type: none"> <li>Selling ethanol (main product).</li> <li>Selling methanol (co-product).</li> <li>Biofertilizer (solid residues).</li> </ul>

**APPENDIX – SUPPLEMENTARY TABLES**

**Table B:** Start-up investment requirements.

	<b>Number of Units</b>	<b>Cost per Unit (DZD)</b>	<b>Total Cost (DZD)</b>
Bioreactor	6	300000	1800000
Alembic (distiller)	1	220000	220000
Fermentation tank	2	80000	160000
Cooling system	1	300000	300000
Separation system	1	400000	400000
Warehouse rent	1	800000	800000
Vehicles Leasing	5	50000	250000
<b>Total Investment Requirements</b>			<b>3930000</b>

**APPENDIX – SUPPLEMENTARY TABLES**

**Table C: Costs & Expenses.**

a) It is Assumed that the distance covered by vehicles is 120 thousands km in aggregate for all 3 vehicles per year

	Number of Units					Price per Unit(DZD)					Total(DZD)				
	Year					Year					Year				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
<b>Costs of goods</b>															
Labour Cost	8	8	8	8	8	30000	30000	30000	30000	30000	240000	240000	240000	240000	240000
Vehicles Operating Leasing	5	6	6	6	8	50000	50000	50000	50000	50000	250000	300000	300000	300000	400000
Transport Fuel Consumption (A)	120	120	120	120	120	29	29	29	29	29	3480	3480	3480	3480	3480
<b>Administrative expenses</b>															
Manager	1	1	1	1	1	40000	40000	45000	45000	50000	40000	40000	45000	45000	50000

**APPENDIX – SUPPLEMENTARY TABLES**

Logistics Supervisor	1	1	2	2	2	40000	40000	45000	45000	50000	40000	40000	90000	90000	100000
Accountant	1	1	2	2	2	30000	30000	33000	33000	35000	30000	30000	60000	60000	70000
Secretary	1	1	2	2	2	25000	25000	28000	28000	30000	25000	25000	56000	56000	60000
<b>Overhead expenses</b>															
Office/ Warehouse Rent	1	1	1	1	1	800000	800000	800000	800000	800000	800000	800000	800000	800000	800000
Electricity/ Heating	1	1	1	1	1	400000	400000	400000	400000	400000	400000	400000	400000	400000	400000
<b>Total of Cost &amp; Expenses</b>											182848	187848	199448	199448	212348
											0	0	0	0	0



**APPENDIX – SUPPLEMENTARY TABLES**

**Table D:** Projected revenues of bioethanol 99%.

	Units (litre)					Price per Unit (DZD)					Total (DZD)				
	Year					Year					Year				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Revenues	16250 0000	195000 000	238333 000	325000 000	43333 0000	3650	3650	3780	3840	3920	59312500 0000	7117500 00000	90089874 0000	1248000 000000	16986536 00000

**Table E:** Projected revenues of bioethanol 70%.

	Units (litre)					Price per Unit (DZD)					Total (DZD)				
	Year					Year					Year				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Revenues	162500 000	195000 000	238333 000	325000 000	43333 3000	1500	1500	1680	1720	1800	24375000 0000	2925000 00000	40039944 0000	5590000 00000	7799994 00000

**APPENDIX – SUPPLEMENTARY TABLES**

**Table F:** Projected revenues of methanol.

	Units (litre)					Price per Unit(DZD)					Total (DZD)				
	Year					Year					Year				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Revenues	6500 0000	78000 000	95333 200	130000 000	173333 200	800	800	800	800	800	52000000 000	62400000 000	76266560 000	10400000 0000	13866656 0000

**Table G:** Projected revenues of biofertilizer.

	Units (Kg)					Price per Unit(DZD)					Total (DZD)				
	Year					Year					Year				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Revenues	58500 0000	70200 0000	85800 0000	11700 0000	156000 0000	60	60	70	78	80	35100000 000	42120000 000	60060000 000	91260000 00	1248000000 00

**APPENDIX – SUPPLEMENTARY TABLES**

**Table H : Profits.**

	<b>Total (DZD)</b>				
	<b>Year</b>				
	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>
Total Revenues	923975000000	1108770000000	1437624740000	1920126000000	2742119560000
Expenses	1828480	1878480	1994480	1994480	2123480
Profit before taxes	923969241520 (A)	1108768121520	1437622745520	1920124005520	2742117436520
Taxes	-	-	-	-	-
<b>Net Profit</b>	923969241520	1108768121520	1437622745520	1920124005520	2742117436520

**Note 1:** The startups have been exempted from taxes for a period of five years, starting from the date of obtaining the start-up enterprise label.

**Note 2: (A):** For the first year only, we have added the total start-up investment needs to the first year expenses.

**APPENDIX – SUPPLEMENTARY TABLES**

**Table I : Startup budget.**

	<b>Year (DZD)</b>				
	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>
<b>Assets</b>					
Building	800000	800000	1000000	1000000	1100000
Other tangible assets (A)	2880000	2304000	1728000	1152000	576000
Clients	80000	70000	85000	95000	100000
Treasury (B)	3930000	3030000	2330000	2430000	2800000
<b>Total Assets</b>	<b>7690000</b>	<b>6204000</b>	<b>5143000</b>	<b>4677000</b>	<b>4576000</b>
<b>Charges</b>					
Equity	900000	1000000	1300000	1500000	2000000
Borrowings and financial debts	3030000	2030000	1030000	930000	800000
Other Debts	400000	500000	600000	500000	300000
<b>Total Charges</b>	<b>4330000</b>	<b>3530000</b>	<b>2930000</b>	<b>2930000</b>	<b>3100000</b>

APPENDIX – SUPPLEMENTARY TABLES

Verification of the Assets/Charges balance (C)	3360000	2674000	2213000	1747000	1476000
--	---------	---------	---------	---------	---------

**Notes:**

A: To find out the rate at which the value of the machine is depreciating, we divided its price (2880000da) by 5, and we get 576000 da. Then, in order to find how much the cost of the machine will be for the next year we only have to do the following : 2880000- 576000 = 2304000 da.

B: (Equity+ Borrowings and financial debts)

C: Total Assets- Total Charges

**APPENDIX – SUPPLEMENTARY TABLES**

**Table J:** Expected results calculation table.

	<b>Year (DZD)</b>				
	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>
Sales and Related Products (A)	923974458500	1108769458500	1437624052400	1920125395800	2742113508000
Financial year production (1)	923974458500	1108769458500	1437624052400	1920125395800	2742113508000
Consumed purchases (B)	800000	1000000	1300000	1600000	2000000
External services and other consumption (C)	1453480	1503480	1503480	1503480	1603480
Consumption during the Financial year (2) = B+C	2253480	2503480	2803480	3103480	3603480
Operating added value (1-2)	923972205020	1108766955020	1437621248920	1920122292320	2742109904520
Staff costs (D)	375000	375000	491000	491000	520000
Gross Operating Surplus (E)	923971830020	1108766580020	1437620757920	1920121801320	2742109384520
Other operating products (F)	87100000000	104520000000	136326560000	113126000000	263466560000

**APPENDIX – SUPPLEMENTARY TABLES**

Amortization expense (G)	3576000	3097600	2775200	2256800	1916400
Operational results (H)	1011068254020	1213283482420	1573944542720	2033245544520	3005574028120
Financial expenses (I)	3181500	2131500	1035150	976500	840000
The net result for the fiscal year	1011071435520	1213285613920	1573945577870	2033246521020	3005574868120

**Notes:**

A: Total Global Business Figures.

B: the price of the consumed raw materials.

C: it includes the vehicles operating leasing, transport fuel consumption, Office/Warehouse Rent and Electricity/Heating costs.

D: is the sum of labor Cost + Manager +Accountant + Logistics Supervisor +Secretary Salaries

E: Operating added value - Staff costs

F: is the projected revenues of the Co-products (methanol and biofertilizers).

G: Amortization rate varies from one installation to another, but in general, institutions use the following rates:

Buildings 2% (e.g. 800000-(800000\*2%) = 784000)

Equipment and tools 10% (e.g. 2880000-(2880000\*10%) = 2592000)

Transportation equipment 20% (e.g.  $250000 - (250000 * 20\%) = 200000$ )

Amortization expenses for the first year =  $784000 + 2592000 + 200000 = 3576000$

H: is Gross Operating Surplus + other operating products - Amortization expense

I: We assume that the interest on loans granted by the Algerian startup fund is 5%, starting from the first year we will have to pay the original borrowed money plus an additional fees (Borrowings and financial debts \* 5%).

Example:  $3030000da + (3030000da * 5\%) = 3181500da$



**APPENDIX – SUPPLEMENTARY TABLES**

**Table K:** Treasury plan.

	<b>Year (DZD)</b>				
	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>
<b>Cash flow from operational activities</b>					
The net result for the fiscal year	1011071435520	1213285613920	1573945577870	2033246521020	3005574868120
+Amortization and provisions (A)	2880000	2304000	1728000	1152000	576000
-change in inventory (B)	4420000	5064000	9000000	10000000	12738000
-change in clients and other receivables (C)	2000000	1900000	2000000	500000	1700000
+change in suppliers and other payables (D)	78246	97808	127151	156493	195616
Cash flow generated by the activity (E)	1011067973766	1213281051728	1573936433021	2033237329513	3005561201736

**APPENDIX – SUPPLEMENTARY TABLES**

Cash flow from operations financing					
Capital increase/ASF share	3030000	2030000	1030000	930000	800000
Capital increase/ startuper share	900000	1000000	1300000	1500000	2000000
ASF capital repayments	-	2424000	1522500	772500	465000
Cash flow from operating activities financing (F)	3930000	606000	807500	1657500	2335000
Change in cash for the period (E+F)	1011071903766	1213281657728	1573937240521	2033238987013	3005563536736
Opening cash (beginning of period) (G)	3930000	3030000	2330000	2430000	2800000
Closing cash (end of period) (H)	923977101520	1108771151520	1437625075520	1920126435520	2742120236520
Change in cash (I)	923973171520	1108768121520	1437622745520	1920124005520	2742117436520

**Notes:**

A: To find out the rate at which the value of the machine is depreciating, we divided its price (2880000da) by 5, and we get 576000 da. Then, in order to find how much the cost of the machine will be for the next year we only have to do the following :  $2880000 - 576000 = 2304000$  da.

B: Total Revenues- expected stock

C: is the rest of money after the customers pay their debts

D: change in suppliers and other payables= forecast annual purchases \* (TVA 19%+1) \* (payment period/365)

E: Through the model of the treasury liquidity schedule in the direct way stipulated by the Algerian legislator, we find that it consists of the following categories:

The net result for the fiscal year + Amortization and provisions - Change in inventory - Change in clients and other receivables (if its balance is positive, it is subtracted) + change in suppliers and other payables (if its change gives a positive balance, it is added, and if it gives a negative balance, it is subtracted).

F: Capital increase/ASF share + Capital increase/ startuper share - ASF capital repayment

G: it's the money we started our production period year with.

H: Opening cash + Total Revenues – Expenses

I: Closing cash - Opening cash