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Valorization of *Typha Australis* Stems in Bioethanol Production Using Enzymatic Hydrolysis and Biofermentation

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Abstract

The present study aims to valorize the *Typha australis* stems, through second-generation bioethanol production using enzymatic hydrolysis and fermentation. The monitoring of fermentation kinetics parameters, such as pH, density, length of fermentation, and the Brix, indicated a great variability of these parameters during the fermentation process of the must with three *Saccharomyces cerevisiae* strains, such as *Angel brand Thermal-tolerant alcohol active dry yeast*, *Angel brand super alcohol active dry yeast* and Angel super alcohol active dry yeast in the presence of urea (CON_2H_4) used as a growth factor. The distillation of musts after fermentation has yielded ethanol extraction rate (% v / v at 20 ° C) between 4.95and 44.93 after fractional distillation. The best performance in ethanolic bioconversion was recorded with *Angel brand super alcohol active dry yeast*. This *Saccharomyces cerevisiae* strains could be used as effective ferments, in perspective of intensive production of second-generation bioethanol with *Typha australis* stems.

Keywords: *Typha australis*; *Saccharomyces cerevisiae*; Enzymatic hydrolysis; Fermentation; Bioethanol.

1. Introduction

Belonging to the family of Typhaceae, *Typha australis* is a perennial aquatic plant [1, 2]. It is also used for making baskets, mats and shoes [3]. In folk medicine, the roots of *Typha australis* were used against tumors diseases in Chile [4]. Nowaday, the proliferation of this plant is a real problem for Benin valley rivers, due to the fact that it invaded water bodies of rivers and limited the development of agricultural activities in many areas.

However, several studies have reported that invasive plants could be used in several areas, and particularly in the field of renewable energy production. Thus, according to Henry Ford [5], there is carburant in plants that can be obtained through fermentation. According to Rudolf [6], the use of vegetable fuel in engines is insignificant today. But these oils will soon become as important as petroleum.

Face with the declining of fossil energy resources such as crude oil, and the many climatic disturbances that are causing global warming, biofuels including bioethanol becomes an excellent substitute to gasoline which is the main fuel currently used in the field of transport [7]. Indeed, it is recognized that the use of pure bioethanol instead of gasoline allows a reduction in CO_2 emissions in the order of 90% [8]. Then, researches in the field of bioethanol began last century, and evolved progressively in improving processes. The first bioethanol generations come from food grade plants. This type of bioethanol comes into competition with food and that is why there is need for the second generation bioethanol production. This type of bioethanol production uses plant residues (leaves, stalks,). These resources are one of the most abundant renewable resources on earth, and certainly cheaper.

So, the desire to take advantage from this available and invasive plant resources, led us to assess the potential of *Saccharomyces cerevisiae* strains in the production of bioethanol from *Typha australis* stems by enzymatic hydrolysis and biofermentation.

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2. Material and Methods

2.1. Collection of the Plant

Typha australis stems were collected in swampy areas of Godomey district located in Abomey Calavi (Southern Benin). Once brought to the laboratory, samples were washed, dried at 25 ° C and have mechanically grinding using a grinder.

2.2. Pre-Treatment by Steam Explosion

Pretreatment by steam explosion is a very promising technique for lignocellulosic biomass before their bioconversion process. For the realization of this pre-treatment, 1kg of crushed *Typha australis* stems were weighed and placed in Erlenmeyer flask containing 0,5litre of distilled water. The mixture was steam pretreated in an autoclave for 1 hour at a temperature of 270 ° C [9].

2.3. Enzymatic Hydrolysis

Enzymatic hydrolysis is a process which led to make available fermentable sugar molecules presents in the lignocellulosic material. Thus, after the step of steam pretreatment of the raw material, the enzymatic hydrolysis step is indispensable for obtaining the fermentable sugars. In this study, the completion of the enzymatic hydrolysis of musts obtained from *Typha australis* plants previously pretreated and impregnated with water, was conducted by adding enzymes (cellulase) from *Aspergillus niger* at concentration of 6 g of enzyme in 1kg of *Typha australis* grinding stem pretreated. The mixture was placed in an oven at temperature of 50° C during 144h in order to release the available fermentable sugar molecules in the plant [10].

2.4. Alcoholic Fermentation

Three steps were used for alcoholic fermentation process. The first step was yeast inoculum preparation, the second steps was the preparation of fermentation musts, and the last step was the alcoholic fermentation process conduction.

2.4.1. Preparation of Inoculum

Three strains of *Saccharomyces cerevisiae* were used. There are: *Angel brand Thermal-tolerant alcohol active dry yeast, Angel brand super alcohol active dry yeast and Angel super alcohol active dry yeast*. These industrial yeast strains were obtained from Chinese company "*Angel Yeast Co*., Ltd. The inoculum was prepared by introducing 4.0 g of each dry yeast strains (lyophilized) in 9 mL of buffered peptone water [11].

2.4.2. Preparation of Fermentation Musts

At the end of the enzymatic hydrolysis process, the obtained products were pressed and filtered to obtained different musts, while adjusting the volume to 1L with distilled water. A pre-fermentation in batch mode was performed by incorporating the inoculum into 1/10 of the total volume of each fermented musts and put in a closed chamber and in a controlled environment for the purpose of acclimation of yeast strains with the substrate during 24 hours. Then the mixture was added to 9/10 remain of the volume, followed by the addition of 4 g of Urea (NH_2CONH_2), chosen as growth factor because of its importance in the growth of yeasts.

2.4.3. Alcoholic Fermentation Process

To performed alcoholic fermentation process, prepared musts were maintained at 25 ° C under intermittent stirring. The fermentation is batch mode and each fermenter is tightly closed. The alcoholic fermentation of musts took place during 4 days. Periodical samples collection followed by analyses, allowed to follow the evolution of the physicochemical parameters during the fermentation process.

2.5. Distillation

Extraction of ethanol from the fermented musts, was carried out by distillation. The temperature is maintained at 79 ° C at the top of the column until the alcohol is completely depleted in the fermented musts.

2.6. Monitoring of Physicochemical Parameters

The pH of the musts was measured with *OHAUS ST10* pH meter. Acidity was determined according to AOAC [12]. The relative density at 20 °C was determined by the method described by Novidzro [7] and the Brix was determined using a digital refractometer type *Abbe 201 MISCO*. The alcohol content (% v/v) of the musts at the end of the fermentation is determined by the pycnometric method described by AOAC [12].

2.7. Statistical Analysis

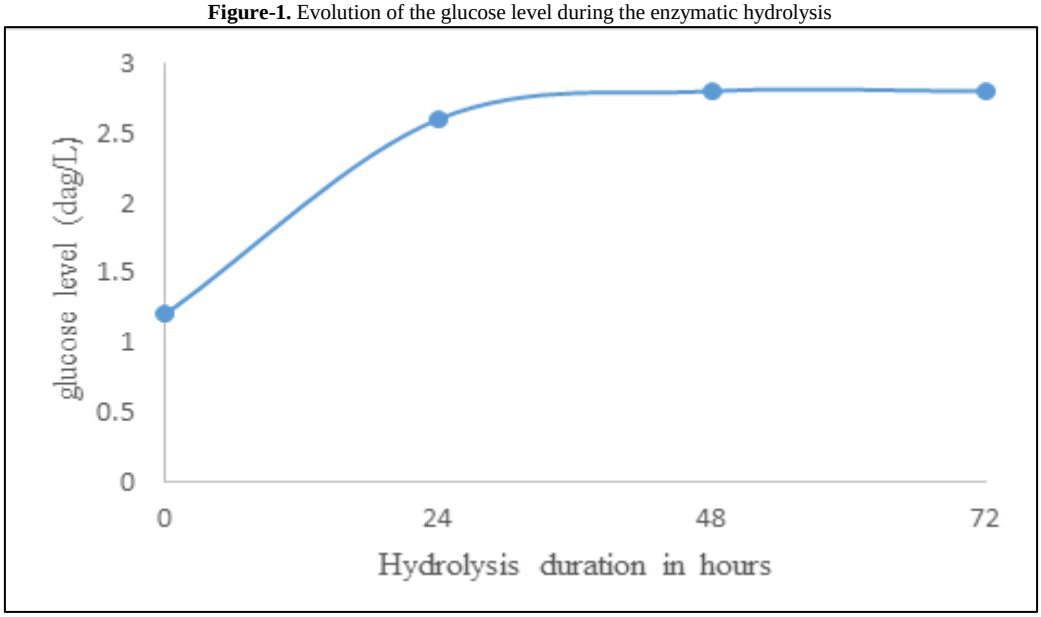
The data were processed using Microsoft Excel 2010. The statistical software data analysis and comparison of the average independent data have been established at the 5% by SPSS 16.0.

3. Results and Discussion

3.1. Effect of the Enzymatic Hydrolysis on the Plant Material

Figure 1 presented the results of the available fermentable sugar (glucose) release from *Typha australis* stems, during enzymatic hydrolysis. These results indicated, a rapid growth of glucose concentration in the ratio of 1.2 to 2.6 dag/L during the first 24 hours of the enzymatic hydrolysis and then a slight increase from 2,6 to 2.8 dag/L during the second 24 hours hydrolysis followed by a stabilization time. These results underlined the efficacy of the enzymatic hydrolysis process, and showed that the complex sugars molecules are broken into simple sugars by the enzymes action. That can be defined by good coordination in the hydrolysis. The three types of enzymes involved in the process are cellulases endo-glucanases-1.4 β and exo-glucanases-1.4 β that hydrolyze cellulose into cellobiose, and β-glucosidases which hydrolyse cellobioses in glucose [13].

Figure-1. Evolution of the glucose level during the enzymatic hydrolysis



3.2. Monitoring of Kinetic Parameters During Fermentation and the Alcoholic Strength of the Distillate

The Figure 2, 3, 4 and 5 respectively showed the results of the evolution of the pH, density, Brix, and the fermentation duration of different musts; and Figure 6 presented the alcoholic rates of distillates. The analysis of results indicated that after 24 hours of fermentation the pH value of the different musts decreased from 3.7 to 3.0 followed by a slight decrease accompanied by a stabilization time in the last two days of fermentation. The highest value of pH on the last day of fermentation which was 3.42 was obtained with the control sample whereas the lowest pHvalue (3.00) is obtained from the must fermented with strains of *Angel brand thermal-tolerant alcohol* and *Angel brand super alcohol active dry yeast* (Figure 2).

The analysis of results presented in Figure 3 indicated, a first reduction phase of the density of different musts. The decline phase duration is 24 hours in fermented musts with *Angel brand thermal-tolerant alcohal dry yeast* and *Angel brand super alcohol dry yeast* strains. However, it is 48 hours with the musts fermented with the strain of *Angel super alcohol dry yeast* and in the control. In the musts enriched with urea, four phases were observed during the fermentation process of musts with *Angel brand super dry alcohol yeast* strain and *Angel alcohol super dry yeast*, which indicated two phases in the evolution of the density. Soro [14], Novidzro [7] and then Gbohaida, *et al.* [15] reported that the fall of the relative density of a musts is closely linked to the reduction in the rate of soluble substances of fermentation medium [14, 15]. These results are closely to that obtained during the evaluation of the Brix of musts (Figure 4). These results are also in accordance with those reported by Mossi, *et al.* [16] on the fermentation of *Sorghum saccharatum* (L.) rods musts from Benin, with final Brix comprises between 1.3 and 1.1 °Bx [16].

Regarding the duration of fermentation (Figure 5) all the fermented musts have obtained the same average fermentation time (72 hours). This mean that the bioconversion of glucose available in this raw plant material (*Typha australis*) does not depend on the yeast strain and the growth factor used. According to Shen, *et al.* [17], Novidzro [7] and Gbohaïda, *et al.* [18] on lignocellulosic biomass in the presence of a strain of *S. cerevisiae*, the good fermentation process duration is between 24 and 144 hours [9-16, 18, 19]. According Gubicza, *et al.* [20] and Appiah, *et al.* [21] the end of the alcoholic fermentation is marked by the cessation of intake of sugars by yeasts and by stopping the falling of the weight of the fermentation medium [20, 21].

The results on alcohol content obtained after the first fractional distillation of each sample (Figure 6) indicated that the alcohol content of different musts is between 4.95 and 44.93 ° V / V. The highest rate (44.93 ° v / v alcohol) was obtained with the enrich fermented must with *Angel alcohol strain* while the lowest rate (4.95 ° v / v alcohol) has been recorded in the control sample. These alcohol extraction yields are better than those reported in Brasil by

Pacheco, *et al.* [22], on the residues of cashew apple treated with hydrochloric acid (3%) with a delignification by using 2% NaOH solution.

Figure-2. Evolution of different pH musts (enriched (a) and unenriched (b)) during fermentation

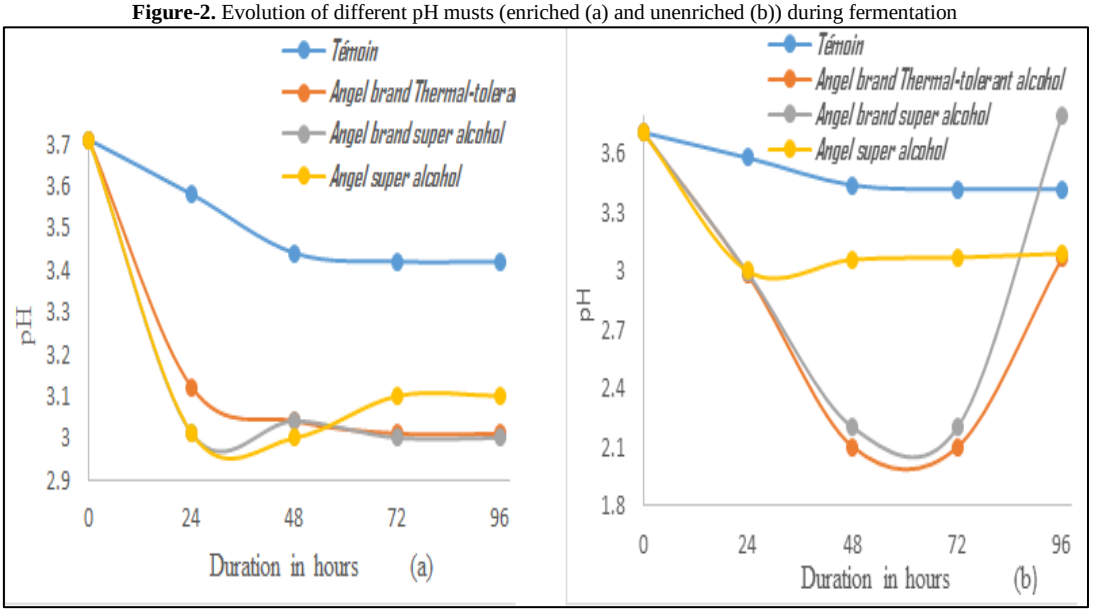


Figure-3. Evolution of the density of the different musts (enriched (a) and unenriched (b)) during fermentation

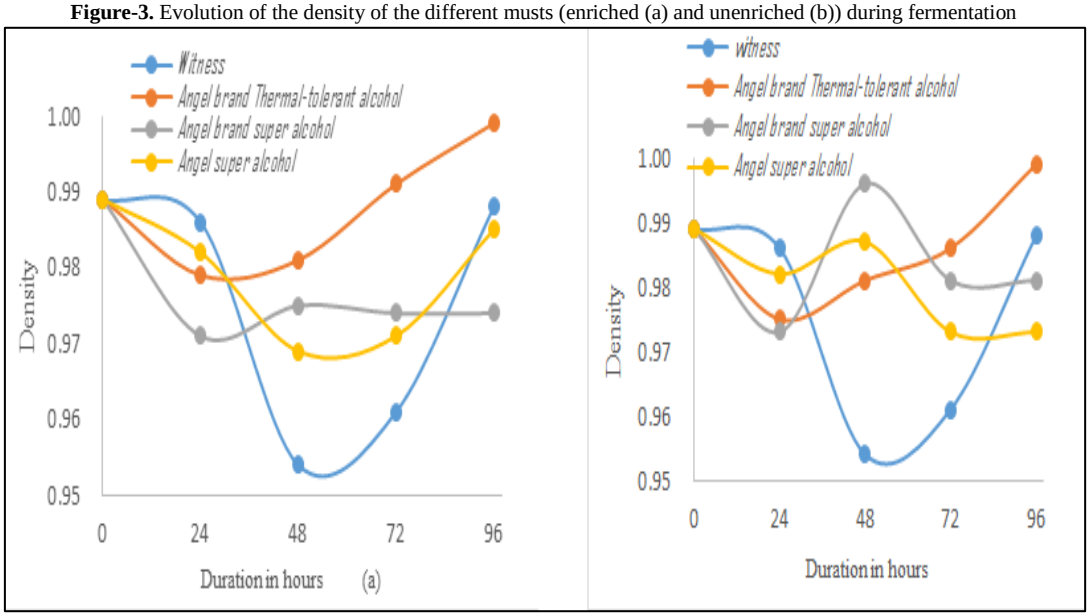


Figure-4. Evolution of the brix different musts (enriched (a) and unenriched (b)) during fermentation

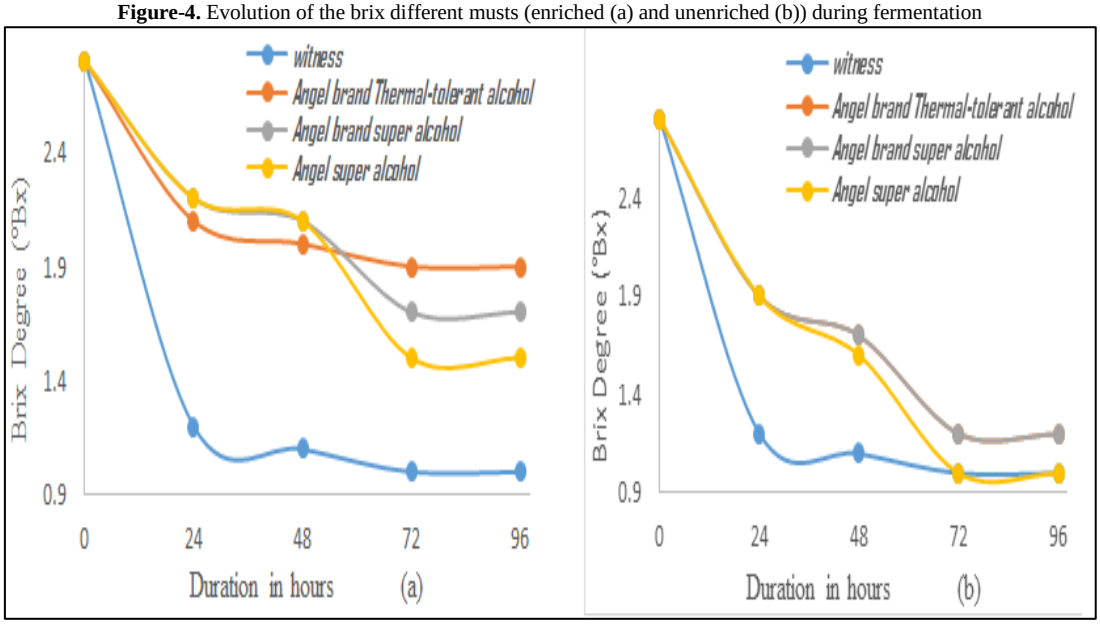


Figure-5. Average length of fermentation musts (enriched and unenriched)

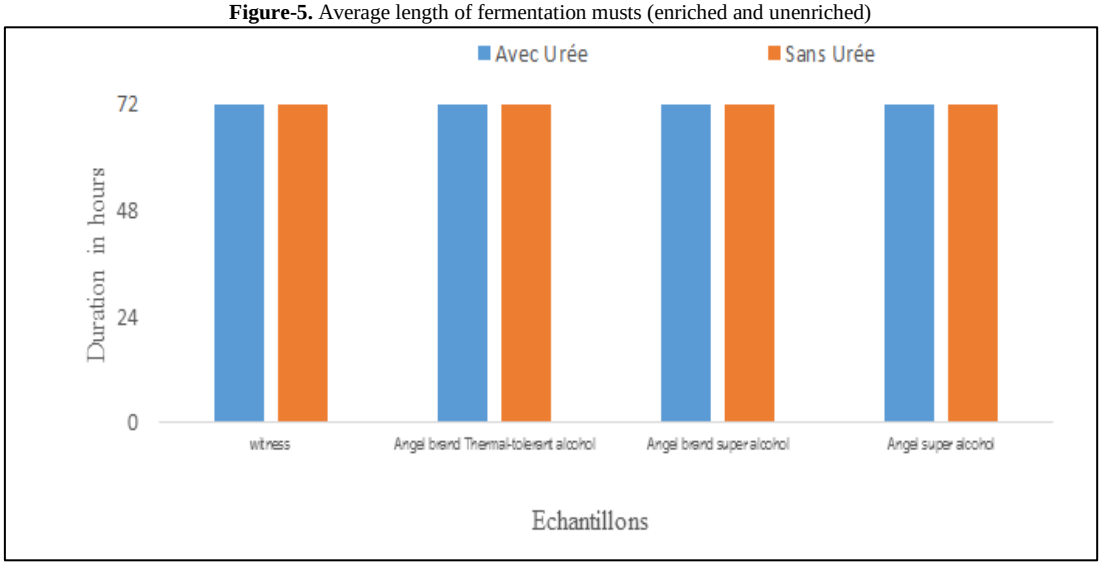
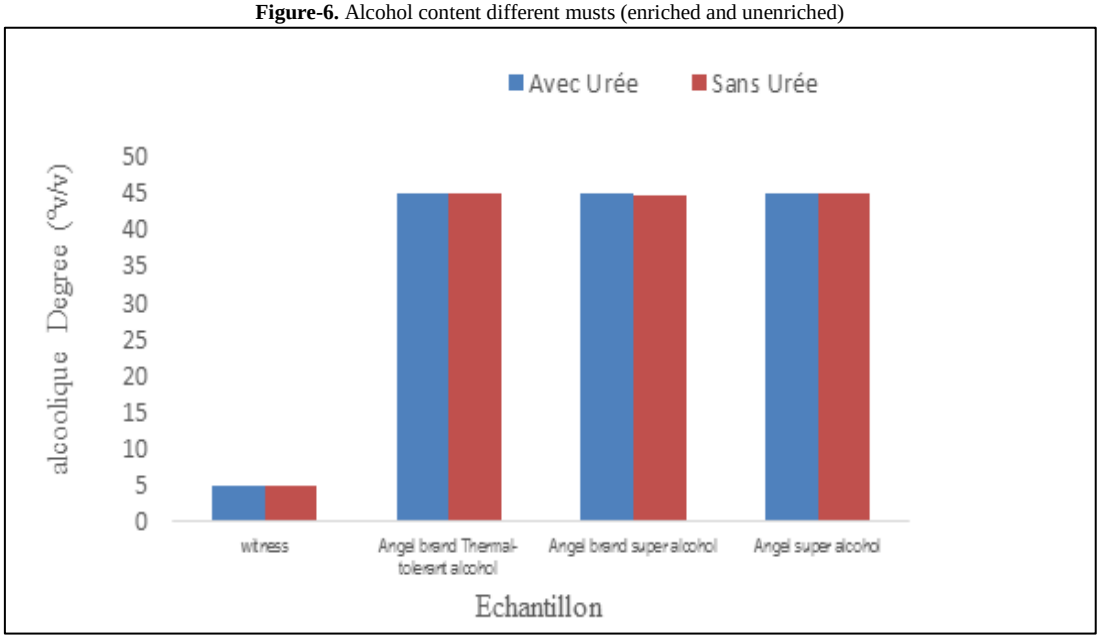


Figure-6. Alcohol content different musts (enriched and unenriched)



4. Conclusion

The present work underlined the bioconversion potential of *Typha australis* stems in second generation of bioethanol production by enzymatic hydrolysis and fermentation processes. These findings could be used in the perspective of intensive production of second-generation bioethanol with lignocellulosic plant materials.

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