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Valorizationof extracts from sorghum stems (*Sorghum saccharatum* L.) by alcoholic bioconversion

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Abstract
The present study aim to investigate the production of bioethanol by fermentation stems extracts from sorghum (*Sorghum saccharatum* L.) by using the *Saccharomyces carlbergensis* yeast. The monitoring of kinetic bioconversion parameters revealed that the average fermentation time of broths is 48 hours, with ethanol yields depending ofinitial concentration of yeast and presence of Urea used as a growth factor. The high yield of alcoholic bioconversion (50.25 ± 0.20 $ml.kg^{-1}$) is obtained from the Urea enriched broth (2$g.L^{-1}$), with yeast initial concentration of 1 $g.L^{-1}$.This study indicated that the valorization of sorghum stems extracts by alcoholic bioconversion is a new way in the exploration of renewable bioenergy production.

Keywords: *Sorghum saccharatum*, yeasts, alcoholic fermentation, bioethanol, Benin

1. Introduction
The demographicincreases of the world population had has led to the massive proliferation of internal combustion engines in order to meet the needs of people. In fifty years of exploitation, one-third of the world's fossil reserves have been exhausted [1]. The abusive use of fossil fuels is at the root of air pollution and global warming. The average concentration of the three main gases responsible for the greenhouse effect in the atmosphere has increased and reached the pre-industrial level of 41% for carbon dioxide, 160% for methane and 20 % for nitrous oxide in 2012 [2]. In 2015, for the first time, the annual mean carbon dioxide content reached 400 ppm. The general increase in temperature resulted in significant evaporation and decreased precipitation in many parts of the world [3]. Rainfall in most parts of the world is disrupted, causing flooding and snowfall in some areas, and drying up of land in other areas.
A NASA reports at the beginning of the millennium indicated that the movement of the axis of rotation of the earth has changed in direction. This change results from changes in water distribution caused by the annual loss of more than 396 million tonnes of ice in Greenland and Western Antarctica. The melting of glaciers at the two poles does not explain the change in the polar axis of the earth. There is the displacement of a large mass due to a large-scale loss of liquid in the soil [4]. The exhaustion of fossil fuels and the numerous problems associated with the massive use of mineral fuels, would have enabled regional and international institutions to seek global solutions based on the use of renewable energies [5].
The biofuel sector has taken off in Europe, United States of America and Brazil do to the policy in favor of biofuels. In Africa in general and in Benin in particular, the biofuels sector remains embryonic. Indeed, biofuel policy remains a low priority in government programs. The production of bioethanol could be misunderstood because of the persistent problems of hunger, health, unemployment and the low income of the population. It may seem difficult to produce bioethanol in a country where people are dying of hunger [6]. Therefore, the use of neglected lignocellulosic resources such as sorghum stems, with a view to their valorization, would be a promising ways. Thus, the present study aims at the valorization of the sorghum stem extracts by alcoholic bioconversion in the production of bioethanol.

2. Material and methods

2.1. Plant collection and extraction

Fresh sorghum stems (*Sorghum saccharatum* L.) were collected in the commune of Segbana (North Benin).They were transported to the laboratory. After chopping, grinding, distilled water adding ($1L.kg^{-1}$) and pressing by using a mechanical press equipped with 0.1 mm of slit filter, the sorghum stems extracts were obtained. The extract was sterilized at 121 °C for 15 minutes and was cooled to room temperature.

2.2. Preparation of fermentation broth

Dry and active yeast of *Saccharomyces carlbergensis* is first revitalized by inoculation in Peptone buffered for 12hours. This solution was added to previoussterilized sorghum stems extract at concentrations of 0; 1; 2 and 3g. L^{-1}. Urea used as growth factor, was also added at different concentrations (0 and 2 g. L^{-1}) to the mixture.

2.3. Fermentation process

The fermentation process used is a batch type. Each inoculated fermentation broth was maintained at 25 °C in laboratory with periodic agitation, for seven [7] days. During the fermentation, periodic samples are collected and analyzes are carried out in order to evaluate the evolution of the fermentation process.

2.4. Kinetic parameters evaluation

The kinetic parameters evaluated during the fermentation process are the pH, the density, the brix degree and the Limit Attenuation (Al). The pH was determined by using a pH-meter (OHAUS ST10) and the brix degree was determined by using the MISCO refractometer "Palm Abbe 201". The density of the fermentation broth is determined according to the methods described by AOAC [7]. The limit attenuation was determined using the method described by Gbohaida *et al*. [8].

2.5. Distillation process

The extraction of the bioethanol from fermented broth was carried out by distillation process with using QUICKFIT vigreux column distiller, (85 cm in length and 4.45 cm in diameter). The temperature is maintained at 79 °C. at the top of the column until the alcohol is completely depleted from the fermented broth.

2.6. Statistical analysis

Experiments were performed in triplicate, and data analyzed are means ± SE subjected to one-way Anova. Means are separated by the Tukey's multiple range test when Anova was significant ($P<0.05$) (SPSS 10.0; Chicago, IL, USA).

3. Results and discussion

Figures 1, 2 and 3 presented the results of the evolution of the kinetic parameters, in particular the Brix, the pH and the density of the various fermentation broth. The analysis of results indicated that the evolution of these parameters is in agreement with the general kinetics of growth of fermenter microorganisms [9]. Indeed, twenty-four hours after the start of the fermentation process, there is a rapid decrease in the kinetic parameters, in particular the pH, the density and the Brix. These decreases indicate a proliferation of yeasts and a start of the process of bioconversion of the sugars contained in the broth. This phase could therefore correspond to the exponential growth phase of yeasts, which often results in the production of secondary metabolites. The increase in acidity could be due to the production of carbon dioxide or acid compounds by yeasts during fermentation. Carbon dioxide (CO_2) can be dissolved in the liquid medium in the form of carbonic acid (H_2CO_3), which is dissociated into bicarbonate ions (HCO_3^-), carbonates (CO_3^{2-}) and hydrogen (H^+) [10]. After 48 hours of fermentation, a pH stabilization was observed in the broth. This may correspond to the depletion of the medium in fermentable sugars or to the saturation of the medium by secondary metabolites capable of inhibiting the growth of yeasts or slowing their fermentation activity [11].

In fermentation broth enriched with urea, the results obtained revealed that the addition of growth factors to the reaction medium significantly improved the fermentation activity of the yeast strains used. The nutrient intake therefore has a significant influence on the fermentation kinetics. These results are in accordance with the indications on the nutrient requirements of yeasts reported by Al-Obaidi, [12].

The results of the evaluation of the fermented performance of the yeast strain used and its adaptation to the carbohydrate substrate (extract from sorghum stems) indicated that the average duration of bioconversion of the substrate is 48 hours (Table 1), with a limiting attenuation between 72.2 ± 0.1 and 74.4 ± 0.2 gL-1 (Table 2). The yield of ethanol production varied from 20.1 ± 0.1 to 50.25 ± 0.20 $ml.kg^{-1}$ of stems (Table 3). The high yield of alcoholic bioconversion is obtained from the urea enriched broth ($2g.L^{-1}$), with yeast initial concentration of 1 $g.L^{-1}$. Several studies reported that Alcohol fermentation, also known as ethanol fermentation, is the anaerobic pathway carried out by yeasts in which simple sugars are converted to ethanol and carbon dioxide. Yeasts typically function under aerobic conditions, or in the presence of oxygen, but are also capable of functioning under anaerobic conditions, or in the absence of oxygen. When no oxygen is readily available, alcohol fermentation occurs in the cytosol of yeast cells. The basic equation for alcohol fermentation showed that yeast starts with glucose, a type of sugar, and finishes with carbon dioxide and ethanol.

Table 1: Average fermentation time (hour)

	Initial yeast concentrations ($g.L^{-1}$)			
	0	**1**	**2**	**3**
Absence of Urea	24±0,1a	48±0,2a	48±0,1a	48±0,1a
Presence of Urea ($2g.L^{-1}$)	24±0,1a	48±0,2b	48±0,1a	48±0,1a

Values are mean (n = 3) ± SE. The means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests

Table 2: Limit attenuation (%)

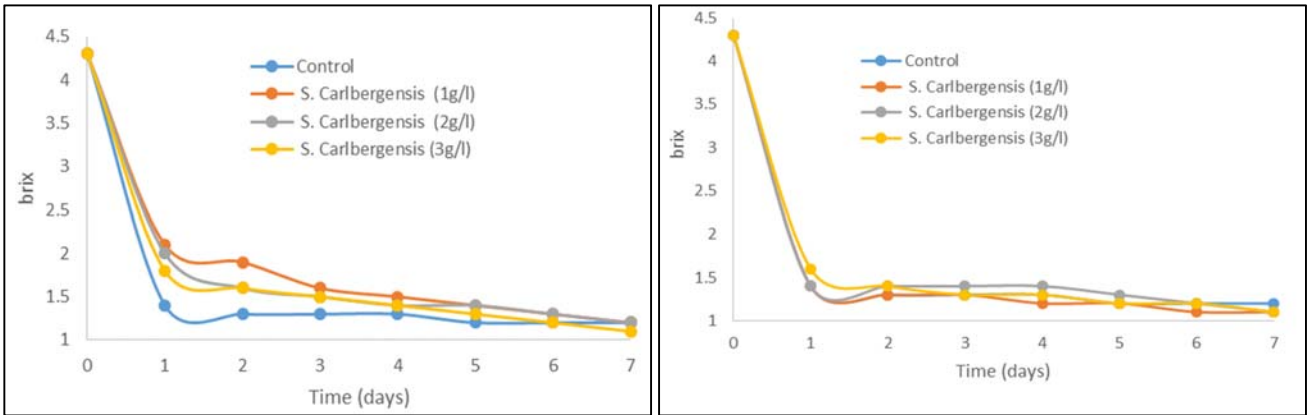
	Initial yeast concentrations ($g.L^{-1}$)			
	0	**1**	**2**	**3**
Absence of Urea	72,1 ±0,1a	74,4±0,2a	74,4±0,2a	74,4±0,1a
Presence of Urea ($2g.L^{-1}$)	72,2±0,2a	72,1±0,1b	74,4±0,2a	72,1±0,1b

Values are mean (n = 3) ± SE. The means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests

Table 3: Alcoholic yield (ml/kg of stems)

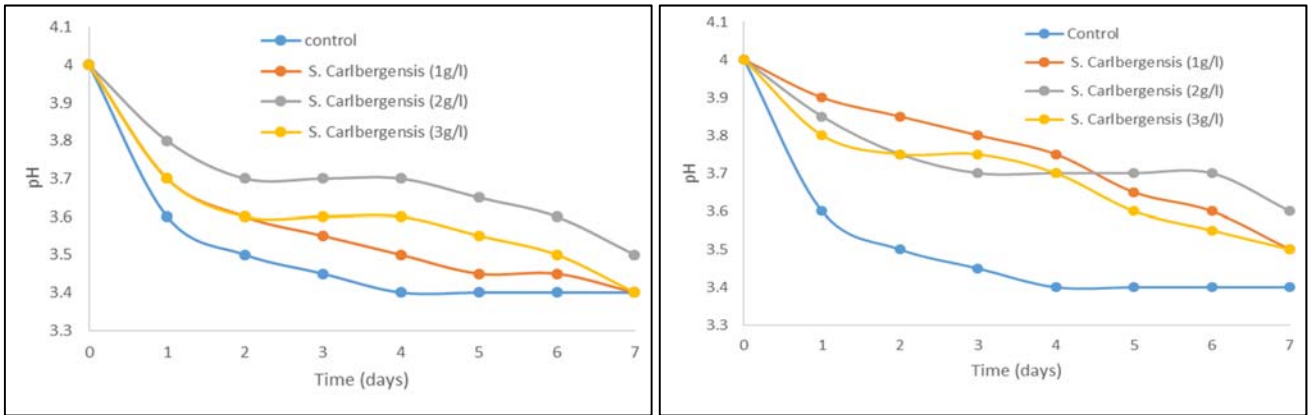
	Initial yeast concentrations (g.L^{-1})			
	0	**1**	**2**	**3**
Absence of Urea	20,1±0,1a	20,1±0,1a	17,4±0,1a	12±0,1a
Presence of Urea (2g.L^{-1})	20,1±0,1a	50,25±0,20b	44,85±0,2b	44,5±0,2b

Values are mean (n = 3) ± SE. The means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests



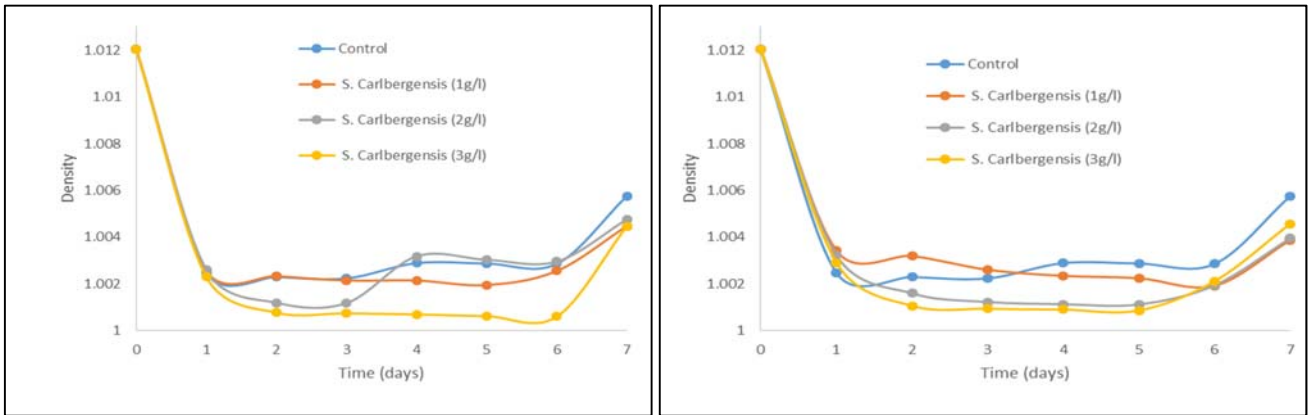
(A) (B)

Fig 1: Evolution of Brix during the fermentation process a) *Fermentation broth without urea; b) Fermentation broth with urea*



(A) (B)

Fig 2: Evolution of pH during the fermentation process *a) Fermentation broth without urea; b) Fermentation broth with urea*



(A) (B)

Fig 3: Evolution of the density during the fermentation process *a) Fermentation broth without urea; b) Fermentation broth with urea*

Conclusion

This work underlined the bioconversion potential of *Saccharomyces carlbergensis* in the production of bioethanol using stem extract of sorghum. Based on this results, the valorization of sorghum stems extracts by alcoholic bioconversion is a new way in the exploration of renewable bioenergy production. Further, more investigations are need

in other to improve the alcoholic bioconversion yield for industrial use.

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